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Protein Reviews

M. Zouhair Atassi *Editor*

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M. Zouhair Atassi
Biochem and Mol Biol, Baylor College of Medicine, Houston, TX, USA

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M. Zouhair Atassi
Baylor College of Medicine
Houston, TX, USA

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Preface

This volume is number 21 of **Protein Reviews,** which is a book series published by Springer as a sub series of Advances in Experimental Medicine and Biology (https://www.springer.com/series/14330). The authors are selected from active leading experts in academic or industrial organizations. The manuscripts are each reviewed by expert workers in the field. Before they are published in print, the manuscripts are first published online to enhance their availability and usefulness to the scientific community. The printed volumes are each either devoted to a particular theme or contains chapters on varied selected topics. The review articles are designed for active scientists and for advanced graduate research students. Colleagues interested in writing a review or in guest-editing a special thematic issue are encouraged to submit their proposal to the editor for consideration.

The first chapter by Ngee Kiat Chua and Andrew J. Brown deals with the "Degron Architecture of Squalene Monooxygenase and how Specific Lipids Calibrate Levels of This Key Cholesterol Synthesis Enzyme." Squalene monooxygenase (SM) is a highly tuned enzyme, which catalyzes a rate-limiting step in the pathway. A well-characterized mechanism is the cholesterol-mediated degradation of SM. The mechanisms and modes of inhibition by well-known SM inhibitors, some of which have been effective in lowering cholesterol levels in animal models. Certain human cancers have been linked to dysregulation of SM levels and activity emphasizing the relevance of SM in health and disease. **The second chapter is by Stephen J. Fairweather, Nishank Shah, and Stefan Bröer, entitled "Heteromeric Solute Carriers: Function, Structure, Pathology and Pharmacology**." In this chapter the authors present a systematic analysis of the structural and functional aspects of heteromeric solute carriers and conclude with common principles of their functional roles and structural architecture**. The third chapter by Sergey V. Ivanov, Ryan Bauer, Elena N. Pokidysheva, and Sergei P. Boudko deals with "Collagen IV Exploits a Cl- Step Gradient for Scaffold Assembly**." Within the scaffold, the structural integrity of collagen continues to depend on Cl concentration. The authors review recent findings and set future directions for studies on the role of Cl in type IV collagen assembly, function, and disease. **The fourth chapter by Tomoaki Niimi deals with the "Roles of Slit Ligands and Their Roundabout (Robo) Family of Receptors in Bone Remodeling**." Slit guidance ligands (Slits) and their roundabout (Robo) family of receptors are well- known axon guidance molecules that were originally identified in Drosophila mutants with commissural

axon pathfinding defects. **The fifth chapter by Fredrik Noborn and Göran Larson, entitled "Characterization of C. elegans Chondroitin Proteoglycans and Their Large Functional and Structural Heterogeneity: Evolutionary Aspects on Structural Differences Between Humans and the Nematode,"** discusses their recent data in relation to previous knowledge of core proteins and GAG-attachment sites in Chn and CS proteoglycans of C.elegans and humans, and point out both converging and diverging aspects of proteoglycan evolution. **In the sixth chapter, Mitsunori Shiroishi reviews the "Structural Basis of a Conventional Recognition Mode of IGHV1-69 Rheumatoid Factors**." The author summarizes the immunochemical character of the IGHV1-69-derived RFs and then focuses on the recognition mechanism of the IGHV1- 69-derived RFs, referring the structural features of the IGHV1-69-derived neutralizing antibodies. **The seventh chapter by Jin Ye is entitled "Regulated Alternative Translocation: A Mechanism Regulating Transmembrane Proteins Through Topological Inversion**." These observations suggest that topological inversion through RAT could be an emerging mechanism to regulate transmembrane proteins. **The final chapter is by Li Zhou and Viktor Todorovic**. In this chapter, current progress on IL-36 protein and biology is reviewed with a discussion on investigative tools for this novel target.

I want to convey special thanks to the authors of this volume for their special effort in these hard times imposed by the coronavirus pandemic. I hope that this volume will offer the scientific communities a valuable source for dissemination of essential contemporary discoveries on protein molecules and their diverse biological activities.

Houston, TX, USA M. Zouhair Atassi

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The Degron Architecture of Squalene Monooxygenase and How Specific Lipids Calibrate Levels of This Key Cholesterol Synthesis Enzyme

Ngee Kiat Chua and Andrew J. Brown

Abstract

Cholesterol synthesis is a fundamental process that contributes to cellular cholesterol homeostasis. Cells execute transcriptional and post-translational mechanisms to control the abundance of enzymes of the cholesterol synthesis pathway, consequently affecting cholesterol production. One such highly tuned enzyme is squalene monooxygenase (SM), which catalyzes a rate-limiting step in the pathway. A well-characterized mechanism is the cholesterol-mediated degradation of SM. Notably, lipids (cholesterol, plasmalogens, squalene, and unsaturated fatty acids) can act as cellular signals that either promote or reduce SM degradation. The N-terminal region of SM consists of the shortest known cholesterol-responsive degron, characterized by atypical membrane anchoring structures, namely a re-entrant loop and an amphipathic helix. SM also undergoes non-canonical ubiquitination on serine, a relatively uncommon attachment site for ubiquitination. The structure of the catalytic domain of SM has been solved, providing insights into the catalytic mechanisms and modes of inhibition by well-known SM inhibitors, some of which have been effective in lowering cholesterol levels in animal models. Certain human cancers have been linked to dysregulation of SM levels and activity, further emphasizing the relevance of SM in health and disease.

Keywords

Cholesterol synthesis · Degron · Endoplasmic reticulum-associated degradation (ERAD) · Squalene · Squalene monooxygenase · Ubiquitin

1 Introduction

The mevalonate pathway leads to the formation of essential metabolites, including ubiquinone, dolichol and cholesterol (Brown and Sharpe 2016). Cellular regulation of enzymes within the pathway is achieved by control of gene expression and protein turnover; this allows the mevalonate pathway to be less or more active, depending on the metabolic needs of the cell (Brown and Sharpe 2016).

N. K. Chua (✉)
Ubiquitin Signalling Division, The Walter and Eliza Hall Institute for Medical Research, Melbourne, VIC, Australia
e-mail: chua.n@wehi.edu.au

A. J. Brown (✉)
School of Biotechnology and Biomolecular Sciences, University of New South Wales (UNSW), Sydney, NSW, Australia
e-mail: aj.brown@unsw.edu.au

In addition, the pathway is of clinical importance in cardiovascular disease; statins (a class of cholesterol-lowering drugs) inhibit this pathway by inhibiting 3-hydroxy-3-methyl-glutaryl-*coenzyme A* reductase (HMGCR), the first rate-limiting enzyme of the pathway (Buhaescu and Izzedine 2007; Goldstein and Brown 2015). The pathway is also of significant interest for research related to cancers, immunity and Alzheimer's disease (Buhaescu and Izzedine 2007; Mullen et al. 2016).

Genes encoding the enzymes of the mevalonate pathway are transcriptionally controlled by transcription factors known as the Sterol Regulatory Element Binding Proteins (Brown and Sharpe 2016; Shimano and Sato 2017). However, post-translational regulation mechanisms for these enzymes can differ substantially. Sterols which accelerate the degradation of one enzyme may not do so for another enzyme (Brown and Sharpe 2016; Chen et al. 2019). Also, how these enzymes sense sterols are determined by unique intrinsic protein domains (Sever et al. 2003; Howe et al. 2016; Luo et al. 2020). Finally, the protein effectors which mediate the degradation of these enzymes are quite distinct, adding another layer of complexity to the regulation of their half-lives (Zelcer et al. 2014; Menzies et al. 2018).

Here, we focus on squalene monooxygenase (SM, UniProt ID:Q14534, EC 1.14.14.17), a second rate-limiting enzyme located in the endoplasmic reticulum (ER) (Gill et al. 2011). This enzyme has gained increasing attention over the past decade given its link to human diseases [reviewed in (Chua et al. 2020)], including cancers (Stopsack et al. 2017; Cirmena et al. 2018; Garcia-Bermudez et al. 2019), cardiovascular disease (Belter et al. 2011), and Rett Syndrome (Buchovecky et al. 2013). Here, our specific focus is on the unique homeostatic mechanisms employed by cells to regulate SM levels and activity in response to diverse lipids. Thus, the main goal of our review is to describe post-translational mechanisms that regulate SM, encompassing endoplasmic reticulum-associated degradation (ERAD), protein quality control mechanisms, ubiquitination and lipid-regulated protein stability.

2 Advances in Squalene Monooxygenase Research

Inhibitor Development and Protein Structure In the 1990s when mammalian SM was first cloned (Sakakibara et al. 1995), natural and synthetic mammalian SM inhibitors were examined in cell models, enzyme assays, and animals (Belter et al. 2011). These exploratory studies were aimed at lowering cholesterol levels by inhibiting SM, but none of these inhibitors have yet to be tested in humans (Belter et al. 2011; Padyana et al. 2019). Around the same time, terbinafine was developed to inhibit the yeast homolog of SM, known as Erg1p (Ryder 1992). Terbinafine is still used clinically to treat fungal infections. In 2019, the structure of the catalytic domain of SM was finally solved, providing insights into catalytic mechanisms of enzyme-substrate and enzyme-inhibitor interaction (Padyana et al. 2019).

Gene Regulation Transcription of the gene encoding SM, *SQLE*, is controlled by the transcription factors, Sterol Regulatory Element Binding Protein-2, Sp1 and NF-Y (Howe et al. 2017). Independent studies since the early 2000s have mapped the sterol response elements within *SQLE*, identifying which nucleotide positions in the proximal promoter are responsible for the sterol-responsiveness of the *SQLE* gene (Nagai et al. 2002; Howe et al. 2017).

Regulation by Protein Degradation Although there were some suggestions of SM being another rate-limiting enzyme in the 1970s (Gonzalez et al. 1979), it was only in 2011 that SM was formally shown to be regulated post-translationally (Gill et al. 2011). Excess cholesterol accelerates the proteasomal degradation of SM, accumulating the substrate, squalene (Gill et al. 2011), which notably is downstream from the classic rate-limiting enzyme HMGCR (DeBose-Boyd 2008; Goldstein and Brown 2015). Thus, SM positions itself as another rate-limiting step of the pathway, with cholesterol feeding back on its own *de novo* synthesis by signaling the destruction of a later enzyme in the pathway than HMGCR. This

feedback process requires the first 100 amino acids of SM (termed SM N100) (Gill et al. 2011). Unlike mammalian SM, the yeast counterpart, Erg1p, undergoes accelerated degradation by lanosterol and lacks the mammalian SM N100 region (Gill et al. 2011; Foresti et al. 2013). Subsequently, several proteins of the ERAD pathway which facilitate the degradation of SM have been identified, including the E3 ubiquitin ligase MARCHF6 (Foresti et al. 2013; Zelcer et al. 2014), the ubiquitin-conjugating enzyme Ube2J2 (Stefanovic-Barrett et al. 2018; Chua et al. 2019a; Tan et al. 2019), and the ATPase valosin-containing protein (VCP)/p97 which assists the unfolding of ER proteins destined for degradation (Chua et al. 2019b).

3 Architecture of the Squalene Monooxygenase Protein

Broadly, SM is divided into two parts; the first part is the N-terminal region encompassing a regulatory domain (the SM N100 degron), with the second part being the C-terminal catalytic domain constituting the bulk of the protein (Brown et al. 2019; Padyana et al. 2019) (Fig. 1).

The SM N100 degron is found in many eukaryotic organisms such as humans and other mammals (Gill et al. 2011). However, it is absent in plants (such as *Panax ginseng* and *Arabidopsis thaliana*) and lower organisms such as *Saccharomyces cerevisiae* (Gill et al. 2011; Chua et al. 2020). Interestingly, although chicken, zebrafish and lamprey SM contain the SM N100 degron, the ability of the SM N100 degron to be degraded in response to excess cholesterol differs between these species (Chua et al. 2017), which suggests that sequence-specific properties enable this response in mammals, like humans and hamsters (Gill et al. 2011).

The C-terminal catalytic domain contains the binding pocket for FAD (a co-factor), squalene (the substrate) and SM inhibitors (NB-598 and Compound-4″) (Brown et al. 2019; Padyana et al. 2019) (Fig. 1). This entire domain is well-conserved across organisms functionally, but displays subtle amino acid differences that affect inhibitor binding (discussed in Sect. 3.1) (Padyana et al. 2019).

3.1 The Catalytic Domain of Squalene Monooxygenase

SM catalyzes the conversion of squalene to 2,3(*S*)-monooxidosqualene, which can then act again to introduce a second epoxide group, forming 2,3(*S*);22(*S*),23-dioxidosqualene, ultimately generating the oxysterol 24,25(*S*)-epoxycholesterol (Fig. 2). Synthesis of cholesterol and 24,25(*S*)-epoxycholesterol occur in parallel (Fig. 2), and the flux down these two branches is determined by the accumulation of 2,3(*S*)-monooxidosqualene (Gill et al. 2008). Typically, the majority of 2,3(*S*)-monooxidosqualene is converted to lanosterol by lanosterol synthase, directing the pathway towards cholesterol synthesis (Fig. 2). Levels of 24,25(*S*)-epoxycholesterol are usually very low, ranging from 0.1% to 1% with respect to cholesterol, depending on tissue and cell type (Brown 2009). Upon partial inhibition of lanosterol synthase, the reaction catalyzed by SM converting 2,3(*S*)-monooxidosqualene to 2,3(*S*);22(*S*),23-dioxidosqualene is favoured, resulting in greater flux down the shunt pathway to produce 24,25(*S*)-epoxycholesterol (Gill et al. 2008).

To date, no SM recombinant proteins have been successfully co-crystalized with the squalene substrate, but there are structural and biochemical data showing the non-competitive binding mechanism of mammalian SM inhibitors, NB-598 and Compound-4″ (Padyana et al. 2019). Molecular docking analyses of the SM catalytic domain structure demonstrated that squalene binds to the same pocket as these inhibitors (Padyana et al. 2019).

Despite the high conservation of the catalytic domain between mammals and fungal species, terbinafine has more potent inhibitory effects on fungal Erg1p than mammalian SM (Padyana et al. 2019). As reported, this is a consequence of specific amino acid differences within the catalytic domain that are not conserved (Padyana et al.

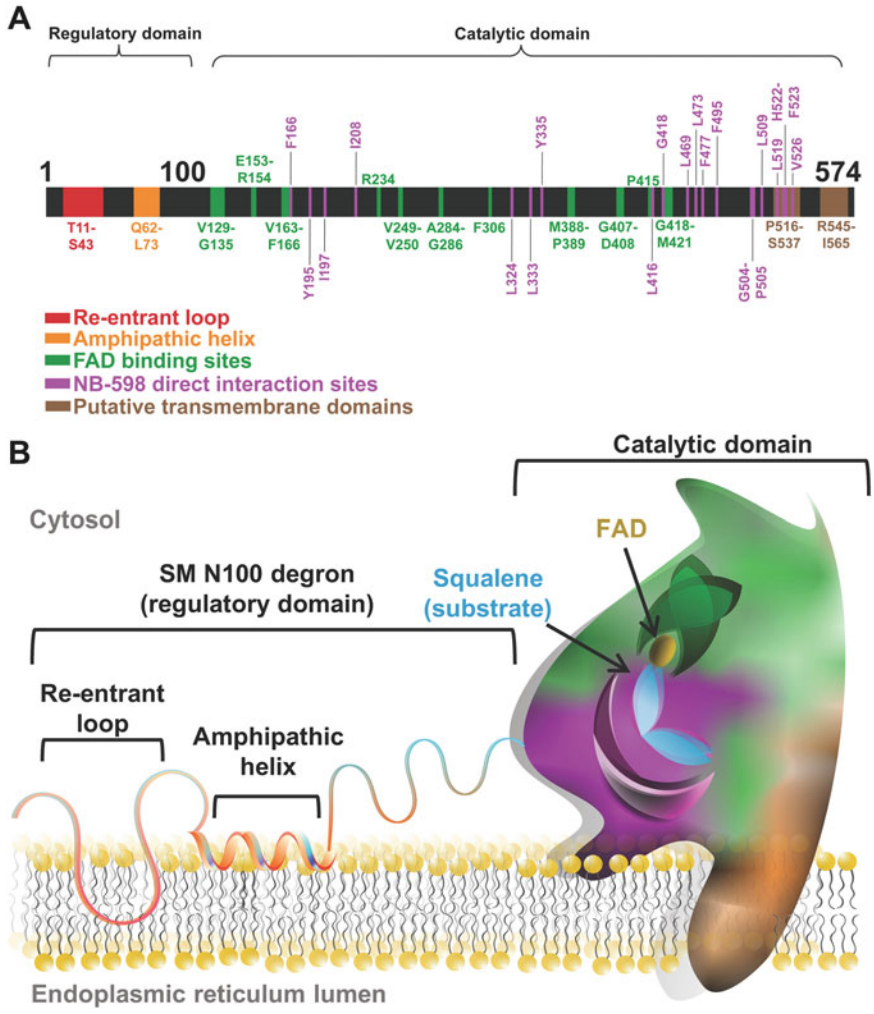

Fig. 1 Primary sequence and illustration of the squalene monooxygenase domains. (**a**) The regulatory domain comprises an amphipathic helix (*orange*) and a re-entrant loop (*red*) capable of sensing cholesterol. Catalytically important residues are those involved in the interaction with the squalene monooxygenase inhibitor, NB-598 (*purple*), and also those that permit FAD binding (*green*). Two putative transmembrane regions are shown in the C-terminal end of the protein (*brown*). (**b**) Schematic of the primary sequence in (**a**) in the context of the endoplasmic reticulum membrane. The cholesterol-sensing membrane bound structures of the regulatory domain, the re-entrant loop and amphipathic helix, are facing the cytosol. The C-terminal region make up the bulk of the protein and have the soluble FAD co-factor binding site and a substrate binding domain. (**a**) was reprinted with slight modifications from Progress in Lipid Research, volume 79, Chua et al., *Squalene monooxygenase: a journey to the heart of cholesterol synthesis* (volume 79), 2020, with permission from Elsevier. Content of (**b**) was originally sourced from an article licensed under a Creative Commons Attribution 4.0 International License published by Brown and colleagues (Brown et al. 2019)

2019). Three human SM amino acids (Phe-166, Ile-197 and Leu-324) are not conserved with fungal Erg1p, which affects the interaction of the binding pocket with the aromatic structure of terbinafine (Padyana et al. 2019). Smaller hydrophobic valine residues of Erg1p replace two human SM amino acids, Ile-197 and Leu-324, enabling optimal interactions between terbinafine and the Erg1p binding pocket (Padyana et al. 2019). Towards the C-terminal

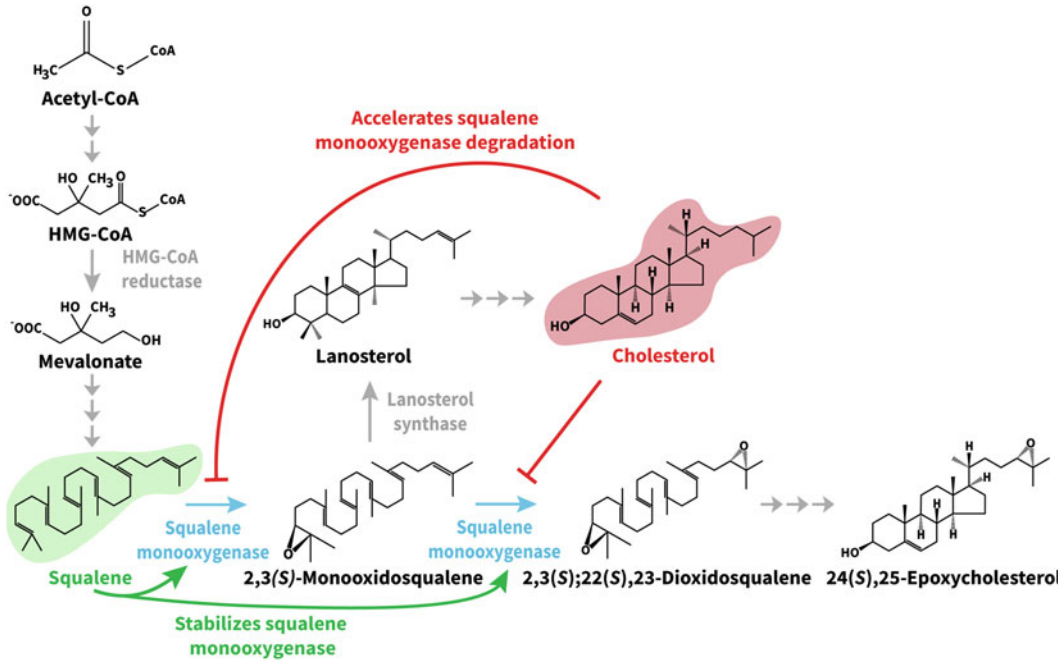

Fig. 2 Simplified schematic of the cholesterol synthesis pathway. Squalene monooxygenase undergoes accelerated degradation upon cholesterol excess in cells. In addition to its role as a substrate of squalene monooxygenase, squalene (shaded *green*) can allosterically stabilize squalene monooxygenase. The pathway highlights the conversion of 2,3(*S*)-monooxidosqualene to either lanosterol or 2,3(*S*);22(*S*),23-dioxidosqualene which represents a divergence of the pathway to produce either cholesterol (shaded *red*) or 24(*S*),25-epoxycholesterol as the end product

end of the catalytic domain are two putative transmembrane domains which have yet to be validated for anchoring SM to ER membranes (Brown et al. 2019; Padyana et al. 2019) (Fig. 1).

3.2 Cholesterol Induces Structural Changes in the Degron of Squalene Monooxygenase

The only validated ER-embedded structures of SM are the re-entrant loop (spanning amino acids Thr-11 to Ser-43) (Howe et al. 2015) and amphipathic helix (amino acids Gln-62 to Leu-73) (Chua et al. 2017) located within the SM N100 degron (Fig. 1). Notably, the hydrophobicity of the SM N100 degron has hampered the expression of recombinant full-length SM in multiple studies (Sakakibara et al. 1995; Gill et al. 2011), including the structural elucidation of the catalytic domain of SM (Padyana et al. 2019).

Both the re-entrant loop and amphipathic helix of SM N100 undergo structural changes upon cholesterol excess (Howe et al. 2015; Chua et al. 2017). The borders of the SM N100 re-entrant loop appear to be more buried in the membrane with cholesterol excess (Howe et al. 2015; Howe and Brown 2017). In comparison, the current model for the amphipathic helix is that it deforms with cholesterol excess, with the helix transitioning into a cytosolically exposed hydrophobic patch that increases protein disorder (Chua et al. 2017). These cholesterol-induced structural changes of the re-entrant loop and the amphipathic helix facilitate the degradation of SM by the ERAD machinery (Fig. 3, further discussed in Sect. 5), representing an example of allosteric misfolding (also termed "mallostery") (Chua and Brown 2018; Wangeline and Hampton 2018).

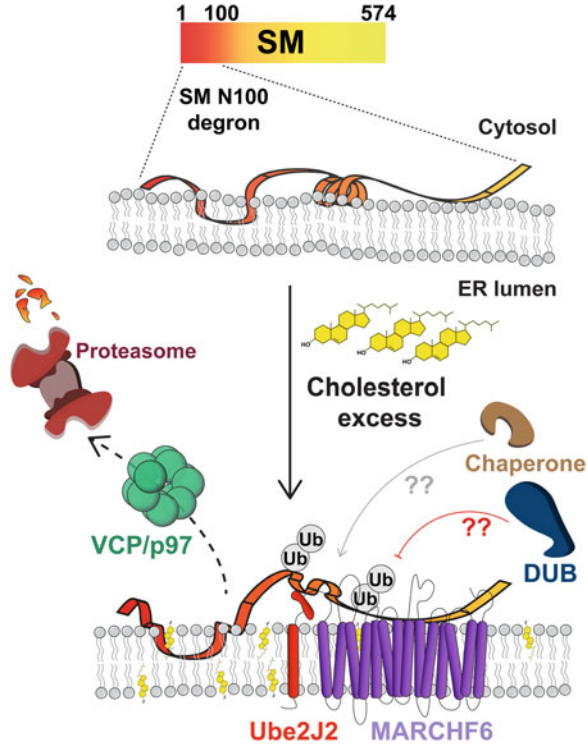

Fig. 3 Model highlighting mechanisms underlying the cholesterol-responsive degron in the endoplasmic reticulum-associated degradation of squalene monooxygenase. Squalene monooxygenase (SM) degradation relies on the cholesterol-responsive SM N100 degron. As cholesterol accumulates, this triggers conformational changes around the borders of the re-entrant loop and deforms the amphipathic helix of the SM N100 degron. This facilitates ubiquitination of serine amino acids near the deformed helix, catalyzed by the E3 ubiquitin ligase MARCHF6 and the E2 ubiquitin conjugating enzyme Ube2J2. Valosin-containing protein (VCP)/p97 acts as an extractor to enable the proteasomal degradation of squalene monooxygenase in the cytosolic compartment. A deubiquitinase (DUB) that could directly deubiquitinate the SM N100 degron and reduce cholesterol-mediated degradation of SM has not yet been identified. In addition, chaperones may also be involved to bind hydrophobic exposed regions of the degron

3.3 Squalene Monooxygenase Senses Cellular Lipid Levels

Evidence supporting direct binding of SM to cholesterol came from photoclickable cholesterol cross-linking experiments, although the precise binding location is still not known (Hulce et al. 2013). This binding likely occurs within the SM N100 degron as this region confers cholesterol-responsiveness to SM. Besides cholesterol, SM protein levels are also post-translationally regulated by other lipids (Honsho et al. 2015; Yoshioka et al. 2020).

Increasing plasmalogen levels in cells reduce cholesterol synthesis by promoting the degradation of SM (Honsho et al. 2015). This phenomenon is observed *in vivo*; mice deficient in plasmalogen synthesis in the cerebellum show higher SM levels (Honsho et al. 2019). These findings provide a link between plasmalogen and cholesterol homeostasis.

Unsaturated fatty acids such as oleate stabilize SM N100-GFP (SM N100 fused to green fluorescent protein as a model cholesterol-responsive degron) and reduces the cholesterol-accelerated degradation of the fusion protein (Stevenson et al. 2014). The substrate of SM, squalene, stabilizes SM and reduces the cholesterol-accelerated degradation of SM (Yoshioka et al. 2020).

How do these lipids exert their effects on SM? This may perhaps occur through membrane effects. For example, cholesterol can thicken the ER membrane (Hung et al. 2007) and induce conformational changes in the SM N100 degron as part of a stepwise process to enable accelerated degradation (Howe et al. 2015; Chua et al. 2017). Another possibility is that cholesterol induces conformational changes by binding directly to the SM N100 degron at a site yet to be determined, which might occur within the membrane embedded re-entrant loop (Howe et al. 2016). Whether the partially ER embedded amphipathic helix can bind cholesterol is still uncertain (Prinz 2017). An enantiomer of the natural form of cholesterol also elicits SM degradation, suggesting that membrane effects are in play (Kristiana et al. 2012).

Squalene binds to both the catalytic domain (Padyana et al. 2019) and the SM N100 degron (Yoshioka et al. 2020). While squalene binding at the catalytic domain permits epoxidation, binding at the membrane bound N-terminal SM N100 degron stabilizes the SM protein, introducing another scarce example of allosteric-mediated stabilization. Squalene excess stabilizes SM by blunting the interaction between the SM N100 degron and the E3 ubiquitin ligase MARCHF6, which reduces overall ubiquitination of SM, hindering SM degradation. It is possible the squalene-bound form may prevent exposure of degradation signals like the misfolded amphipathic helix (Nathan 2020). A plausible rationale for the evolution of squalene-mediated stabilization is that increased SM stability promotes cholesterol synthesis when the demand for cellular cholesterol is high. Thus, SM can be carefully tuned as the SM N100 degron constantly monitors fluctuating levels of lipids, notably the substrate (squalene) and the end-product of the pathway (cholesterol).

4 Non-canonical Ubiquitination of Squalene Monooxygenase

Most proteins that undergo degradation by the proteasome are post-translationally modified by ubiquitin (Hershko and Ciechanover 1998). Conjugated ubiquitin can act as a signal that facilitates proteolytic processing of the protein by the proteasome (Hershko and Ciechanover 1998). Ubiquitin is typically covalently attached to lysine amino acids on substrate proteins, a process catalyzed by E3 ubiquitin ligases (Hershko and Ciechanover 1998; McClellan et al. 2019). However, there are examples where ubiquitin can be conjugated to other amino acids such as serine, cysteine, and threonine (McClellan et al. 2019).

SM is ubiquitinated by MARCHF6, an E3 ubiquitin ligase which is stabilized by cholesterol (Sharpe et al. 2019). This is an efficient mechanism whereby the same lipid, cholesterol, reduces cholesterol synthesis through dual actions. Firstly, cholesterol favors a more unstable form of SM that is prone to degradation by ERAD (Chua et al. 2017), and secondly a cholesterol-stabilized form of MARCHF6 likely enables increased SM ubiquitination and subsequent degradation (Sharpe et al. 2019).

The SM N100 degron contains five lysine residues (Lys-15, Lys-16, Lys-82, Lys-90 and Lys-100) that could act as ubiquitination sites, but the degron still enables cholesterol-accelerated degradation despite having all lysines replaced with arginines (Gill et al. 2011). An alternative site for ubiquitination is the free N-terminus (McDowell and Philpott 2013), but blocking the N-terminus of the native SM N100 degron with a bulky mCherry reporter did not stabilize the SM N100 degron (Chua et al. 2019a).

From a series of systematic mutageneses, four serine residues (Ser-59, Ser-61, Ser-83 and Ser-87) were identified as essential for cholesterol-mediated degradation of the SM N100 degron, suggesting a possible role for non-canonical ubiquitination in the cholesterol-responsiveness of SM. Mass spectrometry confirmed serine 83 is ubiquitinated (Chua et al. 2019a). Interestingly, three of the four serines (Ser-59, Ser-61 and Ser-87) are not conserved in the chicken SM N100 degron. Introducing these human residues into the chicken SM N100 degron destabilized the protein, further supporting the involvement of non-canonical ubiquitination in SM degradation (Chua et al.

2019a). A number of other proteins also appear to be ubiquitinated on multiple amino acids (McDowell and Philpott 2013; McClellan et al. 2019), reflecting the versatility of E3 ubiquitin ligases to carry out their function (Mattiroli and Sixma 2014).

MARCHF6 and its yeast homolog, Doa10p, have been implicated in serine and threonine ubiquitination of substrate proteins (Weber et al. 2016; Chua et al. 2019a; McClellan et al. 2019). Furthermore, the E2 ubiquitin conjugating enzymes associated with MARCHF6 and Doa10p function, Ube2J2 and Ubc6p, are also linked to non-canonical ubiquitination of ERAD substrates, including SM (Wang et al. 2009; Weber et al. 2016; Chua et al. 2019a; McClellan et al. 2019) (Fig. 3).

Non-canonical ubiquitination examples are still limited and the list continues to expand slowly (McDowell and Philpott 2013; McClellan et al. 2019). Interestingly, the current examples are mostly represented by ERAD substrates (McClellan et al. 2019). Whether this indicates that non-canonical ubiquitination is common for ERAD substrates remains to be determined. Are other cholesterol synthesis enzymes ubiquitinated on serine and threonine residues? And if so, does this involve the MARCHF6/Ube2J2 pair, given its frequent involvement reported in other studies (McClellan et al. 2019)?

To date, it is unclear why the thermodynamically unfavorable process of serine and threonine ubiquitination occurs instead of lysine ubiquitination (McClellan et al. 2019). On the other hand, it is well known that serine and threonine residues can be phosphorylation sites. Further structural and biochemical studies are necessary to examine this aspect. The SM N100 degron represents a well-defined region targeted by MARCHF6 and Ube2J2, which could serve as a useful tool for studying non-canonical ubiquitination.

5 Protein Effectors in the Degradation of Squalene Monooxygenase

Like many other proteins in the ER, SM degradation by ERAD is carefully coordinated by a sequence of processes. A central process of ERAD is ubiquitination (Ruggiano et al. 2014; Christianson and Ye 2014). As discussed, SM ubiquitination requires MARCHF6 and Ube2J2 (Sect. 4). Additional proteins other than the ubiquitination machinery are needed to act on SM to drive ERAD as ubiquitination itself does not suffice to remove SM from the ER. VCP/p97 is a protein that forms a part of the ERAD process, specifically extraction, which dislocates SM from the ER (Chua et al. 2019b) (Fig. 3). VCP/p97 has the capacity to bind to ubiquitin on ERAD substrates via co-factors such as Ufd1 and Npl4 (Stach and Freemont 2017). Previous observations showed that these co-factors can bind Lys48-linked polyubiquitin chains (van den Boom and Meyer 2018). While not all ubiquitin-proteasome system substrates require VCP/p97 as part of their degradation, this requirement seems to stem from the fact that some substrates (like ERAD substrates) are embedded in organelles like the ER membrane (van den Boom and Meyer 2018).

An unexplored mechanism is whether there is an intermediate stage between SM ubiquitination by MARCHF6 and extraction by VCP/p97. The SM N100 degron is relatively hydrophobic, having a propensity to aggregate. The current idea is that cholesterol promotes ubiquitination by inducing conformational changes in SM N100 at the re-entrant loop and the amphipathic helix (Howe et al. 2015; Chua et al. 2017) (Fig. 3). This facilitates ubiquitination of accessible serine residues adjacent to the misfolded amphipathic helix (Chua et al. 2019a; Fig. 3). How are misfolded hydrophobic patches shielded from the aqueous cytosol in the interim? Yeast studies demonstrated that hydrophobic misfolded regions of ERAD substrates are shielded by a Hsp70 chaperone, prior to Doa10p-mediated ubiquitination and extraction by Cdc48p (yeast homolog of VCP) (Nakatsukasa et al. 2008; Needham et al. 2019). Perhaps such a chaperoning mechanism occurs during the ERAD of SM but has yet to be tested.

Finally, deubiquitination is one of the most unexplored processes of ubiquitin biology, and the general role for deubiquitinases in ERAD remain understudied (Sowa et al. 2009; Zhang et al. 2013; Christianson and Ye 2014). Protein

levels for SM and the degron fusion protein, SM N100-GFP, increased upon treatment of cells by independent deubiquitinase inhibitors, PR-619 (broad-spectrum deubiquitinase inhibitor) or WP-1130 (targets five deubiquitinases) (Chua et al. 2019b). Inhibiting deubiquitinases should preserve ubiquitination and promote degradation, but SM showed the opposite effect whereby its levels decreased (Chua et al. 2019b). Such findings are difficult to explain given the inhibitors themselves likely perturb ubiquitination and degradation of the proteins mediating the ERAD of SM, such as MARCHF6, which also undergoes autoubiquitination and deubiquitination, thus affecting MARCHF6 stability (Hassink et al. 2005; Nakamura et al. 2014; Zattas et al. 2016).

6 Conclusions and Perspective for the Future

The past decade has seen major advances in our understanding of SM [as reviewed in (Chua et al. 2020)], but challenging questions remain.

How exactly does the SM N100 degron operate in three-dimensions? Does it associate with the putative transmembrane domains of the catalytic domain? Current improved structural biology methods may reveal new mechanisms underlying the regulatory actions of the SM N100 degron in the context of the full-length protein.

How can we map the interaction sites between the cholesterol-responsive SM N100 degron with cholesterol, or with other lipids that regulate it? Even if a structure of the SM N100 degron is obtained, there is no guarantee that lipid binding can be observed directly. Perhaps molecular dynamics would be necessary, as in the case of the crystal structure study where squalene binding to the catalytic domain of SM had to be modeled (Padyana et al. 2019).

Uncovering the in-depth mechanisms of how SM is degraded may offer strategies to degrade SM where it is implicated in diseases (Cirmena et al. 2018; Chua et al. 2020). The idea of degrading proteins for therapeutic purposes has been on the rise in recent years (Schapira et al. 2019). In the context of controlling cholesterol levels, this proposition has been tested with HMGCR, whereby a sterol-analog could potently induce HMGCR degradation, preventing atherosclerosis and lowering cholesterol levels in mice (Jiang et al. 2018).

Given that ubiquitination is reversible, what are the DUBs that deubiquitinate SM? And do they counteract or promote the cholesterol-mediated degradation of SM? While the role of ubiquitination in cholesterol homeostasis is already appreciated (Sharpe et al. 2014; Stevenson et al. 2016; van den Boomen et al. 2020), the fine balance between making or breaking ubiquitin chains in the context of cholesterol homeostasis remains largely unknown. This could form a complex mode of regulation to control cholesterol levels as exemplified by the regulation of low-density lipoprotein uptake which involves regulation of the low-density lipoprotein receptor by the DUB, USP2 and the E3 ubiquitin ligase, IDOL (Nelson et al. 2016).

With the advent of CRISPR screens, cryogenic electron microscopy and chemical biology tools as discovery frameworks, we anticipate answers to these challenging unanswered questions. We look forward to a wealth of further discoveries that will deepen our understanding of SM, an enzyme of cholesterol biosynthesis with far-reaching impacts in biotechnology and human health and disease.

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Conflicts of Interest The authors declare that they have no conflicts of interest.

References

Belter A, Skupinska M, Giel-Pietraszuk M et al (2011) Squalene monooxygenase – a target for hypercholesterolemic therapy. Biol Chem 392:1053–1075. https://doi.org/10.1515/BC.2011.195

Brown AJ (2009) 24(S),25-Epoxycholesterol: a messenger for cholesterol homeostasis. Int J Biochem Cell Biol

41:744–747. https://doi.org/10.1016/j.biocel.2008.05.029

Brown AJ, Sharpe LJ (2016) Chapter 11 – Cholesterol synthesis. In: Ridgway ND, McLeod RS (eds) Biochemistry of lipids, lipoproteins and membranes, 6th edn. Elsevier, Boston, pp 327–358

Brown AJ, Chua NK, Yan N (2019) The shape of human squalene epoxidase expands the arsenal against cancer. Nat Commun 10:888. https://doi.org/10.1038/s41467-019-08866-y

Buchovecky CM, Turley SD, Brown HM et al (2013) A suppressor screen in Mecp2 mutant mice implicates cholesterol metabolism in Rett syndrome. Nat Genet 45:1013–1020. https://doi.org/10.1038/ng.2714

Buhaescu I, Izzedine H (2007) Mevalonate pathway: a review of clinical and therapeutical implications. Clin Biochem 40:575–584. https://doi.org/10.1016/j.clinbiochem.2007.03.016

Chen L, Ma M-Y, Sun M et al (2019) Endogenous sterol intermediates of the mevalonate pathway regulate HMGCR degradation and SREBP-2 processing. J Lipid Res 60:1765–1775. https://doi.org/10.1194/jlr.RA119000201

Christianson JC, Ye Y (2014) Cleaning up in the endoplasmic reticulum: ubiquitin in charge. Nat Struct Mol Biol 21:325–335. https://doi.org/10.1038/nsmb.2793

Chua NK, Brown AJ (2018) Mallostery: filling a niche between quality and metabolic control. J Biol Chem 293:14951–14952. https://doi.org/10.1074/jbc.H118.005031

Chua NK, Howe V, Jatana N et al (2017) A conserved degron containing an amphipathic helix regulates the cholesterol-mediated turnover of human squalene monooxygenase, a rate-limiting enzyme in cholesterol synthesis. J Biol Chem 292:19959–19973. https://doi.org/10.1074/jbc.M117.794230

Chua NK, Hart-Smith G, Brown AJ (2019a) Non-canonical ubiquitination of the cholesterol-regulated degron of squalene monooxygenase. J Biol Chem 294:8134–8147. https://doi.org/10.1074/jbc.RA119.007798

Chua NK, Scott NA, Brown AJ (2019b) Valosin-containing protein mediates the ERAD of squalene monooxygenase and its cholesterol-responsive degron. Biochem J 476:2545–2560. https://doi.org/10.1042/BCJ20190418

Chua NK, Coates HW, Brown AJ (2020) Squalene monooxygenase: a journey to the heart of cholesterol synthesis. Prog Lipid Res 79:101033. https://doi.org/10.1016/j.plipres.2020.101033

Cirmena G, Franceschelli P, Isnaldi E et al (2018) Squalene epoxidase as a promising metabolic target in cancer treatment. Cancer Lett 425:13–20. https://doi.org/10.1016/j.canlet.2018.03.034

DeBose-Boyd RA (2008) Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG CoA reductase. Cell Res 18:609–621. https://doi.org/10.1038/cr.2008.61

Foresti O, Ruggiano A, Hannibal-Bach HK et al (2013) Sterol homeostasis requires regulated degradation of squalene monooxygenase by the ubiquitin ligase Doa10/Teb4. eLife 2:e00953. https://doi.org/10.7554/eLife.00953

Garcia-Bermudez J, Baudrier L, Bayraktar EC et al (2019) Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. Nature 567:118–122. https://doi.org/10.1038/s41586-019-0945-5

Gill S, Chow R, Brown A (2008) Sterol regulators of cholesterol homeostasis and beyond: the oxysterol hypothesis revisited and revised. Prog Lipid Res 47:391–404. https://doi.org/10.1016/j.plipres.2008.04.002

Gill S, Stevenson J, Kristiana I, Brown AJ (2011) Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. Cell Metab 13:260–273. https://doi.org/10.1016/j.cmet.2011.01.015

Goldstein JL, Brown MS (2015) A century of cholesterol and coronaries: from plaques to genes to statins. Cell 161:161–172. https://doi.org/10.1016/j.cell.2015.01.036

Gonzalez R, Carlson JP, Dempsey ME (1979) Two major regulatory steps in cholesterol synthesis by human renal cancer cells. Arch Biochem Biophys 196:574–580. https://doi.org/10.1016/0003-9861(79)90310-2

Hassink G, Kikkert M, van Voorden S et al (2005) TEB4 is a C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum. Biochem J 388:647–655. https://doi.org/10.1042/BJ20041241

Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479. https://doi.org/10.1146/annurev.biochem.67.1.425

Honsho M, Abe Y, Fujiki Y (2015) Dysregulation of plasmalogen homeostasis impairs cholesterol biosynthesis. J Biol Chem 290:28822–28833. https://doi.org/10.1074/jbc.M115.656983

Honsho M, Dorninger F, Abe Y et al (2019) Impaired plasmalogen synthesis dysregulates liver X receptor-dependent transcription in cerebellum. J Biochem (Tokyo) 166:353–361. https://doi.org/10.1093/jb/mvz043

Howe V, Brown AJ (2017) Determining the topology of membrane-bound proteins using PEGylation. In: Gelissen IC, Brown AJ (eds) Cholesterol homeostasis. Springer, New York, pp 201–210

Howe V, Chua NK, Stevenson J, Brown AJ (2015) The regulatory domain of squalene monooxygenase contains a re-entrant loop and senses cholesterol via a conformational change. J Biol Chem 290:27533–27544. https://doi.org/10.1074/jbc.M115.675181

Howe V, Sharpe LJ, Alexopoulos SJ et al (2016) Cholesterol homeostasis: how do cells sense sterol excess?

Chem Phys Lipids 199:170–178. https://doi.org/10.1016/j.chemphyslip.2016.02.011

Howe V, Sharpe LJ, Prabhu AV, Brown AJ (2017) New insights into cellular cholesterol acquisition: promoter analysis of human HMGCR and SQLE, two key control enzymes in cholesterol synthesis. Biochim Biophys Acta Mol Cell Biol Lipids 1862:647–657. https://doi.org/10.1016/j.bbalip.2017.03.009

Hulce JJ, Cognetta AB, Niphakis MJ et al (2013) Proteome-wide mapping of cholesterol-interacting proteins in mammalian cells. Nat Methods 10:259–264. https://doi.org/10.1038/nmeth.2368

Hung W-C, Lee M-T, Chen F-Y, Huang HW (2007) The condensing effect of cholesterol in lipid bilayers. Biophys J 92:3960–3967. https://doi.org/10.1529/biophysj.106.099234

Jiang S-Y, Li H, Tang J-J et al (2018) Discovery of a potent HMG-CoA reductase degrader that eliminates statin-induced reductase accumulation and lowers cholesterol. Nat Commun 9:5138. https://doi.org/10.1038/s41467-018-07590-3

Kristiana I, Luu W, Stevenson J et al (2012) Cholesterol through the looking glass: ability of its enantiomer also to elicit homeostatic responses. J Biol Chem 287:33897–33904. https://doi.org/10.1074/jbc.M112.360537

Luo J, Yang H, Song B-L (2020) Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol 21:225–245. https://doi.org/10.1038/s41580-019-0190-7

Mattiroli F, Sixma TK (2014) Lysine-targeting specificity in ubiquitin and ubiquitin-like modification pathways. Nat Struct Mol Biol 21:308–316. https://doi.org/10.1038/nsmb.2792

McClellan AJ, Laugesen SH, Ellgaard L (2019) Cellular functions and molecular mechanisms of non-lysine ubiquitination. Open Biol 9:190147. https://doi.org/10.1098/rsob.190147

McDowell GS, Philpott A (2013) Non-canonical ubiquitylation: mechanisms and consequences. Int J Biochem Cell Biol 45:1833–1842. https://doi.org/10.1016/j.biocel.2013.05.026

Menzies SA, Volkmar N, van den Boomen DJ et al (2018) The sterol-responsive RNF145 E3 ubiquitin ligase mediates the degradation of HMG-CoA reductase together with gp78 and Hrd1. eLife 7:e40009. https://doi.org/10.7554/eLife.40009

Mullen PJ, Yu R, Longo J et al (2016) The interplay between cell signalling and the mevalonate pathway in cancer. Nat Rev Cancer 16:718–731. https://doi.org/10.1038/nrc.2016.76

Nagai M, Sakakibara J, Nakamura Y et al (2002) SREBP-2 and NF-Y are involved in the transcriptional regulation of squalene epoxidase. Biochem Biophys Res Commun 295:74–80. https://doi.org/10.1016/S0006-291X(02)00623-X

Nakamura N, Harada K, Kato M, Hirose S (2014) Ubiquitin-specific protease 19 regulates the stability of the E3 ubiquitin ligase MARCH6. Exp Cell Res 328:207–216. https://doi.org/10.1016/j.yexcr.2014.07.025

Nakatsukasa K, Huyer G, Michaelis S, Brodsky JL (2008) Dissecting the ER-associated degradation of a misfolded polytopic membrane protein. Cell 132:101–112. https://doi.org/10.1016/j.cell.2007.11.023

Nathan JA (2020) Squalene and cholesterol in the balance at the ER membrane. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.2003388117

Needham PG, Guerriero CJ, Brodsky JL (2019) Chaperoning endoplasmic reticulum–associated degradation (ERAD) and protein conformational diseases. Cold Spring Harb Perspect Biol 11:a033928. https://doi.org/10.1101/cshperspect.a033928

Nelson JK, Sorrentino V, Avagliano Trezza R et al (2016) The deubiquitylase USP2 regulates the LDLR pathway by counteracting the E3-ubiquitin ligase IDOL. Circ Res 118:410–419. https://doi.org/10.1161/CIRCRESAHA.115.307298

Padyana AK, Gross S, Jin L et al (2019) Structure and inhibition mechanism of the catalytic domain of human squalene epoxidase. Nat Commun 10:97. https://doi.org/10.1038/s41467-018-07928-x

Prinz WA (2017) A cholesterol-sensing mechanism unfolds. J Biol Chem 292:19974–19975. https://doi.org/10.1074/jbc.H117.794230

Ruggiano A, Foresti O, Carvalho P (2014) ER-associated degradation: protein quality control and beyond. J Cell Biol 204:869–879. https://doi.org/10.1083/jcb.201312042

Ryder NS (1992) Terbinafine: mode of action and properties of the squalene epoxidase inhibition. Br J Dermatol 126:2–7. https://doi.org/10.1111/j.1365-2133.1992.tb00001.x

Sakakibara J, Watanabe R, Kanai Y, Ono T (1995) Molecular cloning and expression of rat squalene epoxidase. J Biol Chem 270:17–20. https://doi.org/10.1074/jbc.270.1.17

Schapira M, Calabrese MF, Bullock AN, Crews CM (2019) Targeted protein degradation: expanding the toolbox. Nat Rev Drug Discov 18:949–963. https://doi.org/10.1038/s41573-019-0047-y

Sever N, Yang T, Brown MS et al (2003) Accelerated degradation of HMG CoA reductase mediated by binding of insig-1 to its sterol-sensing domain. Mol Cell 11:25–33. https://doi.org/10.1016/s1097-2765(02)00822-5

Sharpe LJ, Cook ECL, Zelcer N, Brown AJ (2014) The UPS and downs of cholesterol homeostasis. Trends Biochem Sci 39:527–535. https://doi.org/10.1016/j.tibs.2014.08.008

Sharpe LJ, Howe V, Scott NA et al (2019) Cholesterol increases protein levels of the E3 ligase MARCH6 and thereby stimulates protein degradation. J Biol Chem 294:2436–2448. https://doi.org/10.1074/jbc.RA118.005069

Shimano H, Sato R (2017) SREBP-regulated lipid metabolism: convergent physiology—divergent

pathophysiology. Nat Rev Endocrinol 13:710–730. https://doi.org/10.1038/nrendo.2017.91

Sowa ME, Bennett EJ, Gygi SP, Harper JW (2009) Defining the human deubiquitinating enzyme interaction landscape. Cell 138:389–403. https://doi.org/10.1016/j.cell.2009.04.042

Stach L, Freemont PS (2017) The AAA+ ATPase p97, a cellular multitool. Biochem J 474:2953–2976. https://doi.org/10.1042/BCJ20160783

Stefanovic-Barrett S, Dickson AS, Burr SP et al (2018) MARCH6 and TRC8 facilitate the quality control of cytosolic and tail-anchored proteins. EMBO Rep 19. https://doi.org/10.15252/embr.201745603

Stevenson J, Luu W, Kristiana I, Brown AJ (2014) Squalene mono-oxygenase, a key enzyme in cholesterol synthesis, is stabilized by unsaturated fatty acids. Biochem J 461:435–442. https://doi.org/10.1042/BJ20131404

Stevenson J, Huang EY, Olzmann JA (2016) Endoplasmic reticulum-associated degradation and lipid homeostasis. Annu Rev Nutr 36:511–542. https://doi.org/10.1146/annurev-nutr-071715-051030

Stopsack KH, Gerke TA, Andrén O et al (2017) Cholesterol uptake and regulation in high-grade and lethal prostate cancers. Carcinogenesis 38:806–811. https://doi.org/10.1093/carcin/bgx058

Tan JME, Cook ECL, van den Berg M et al (2019) Differential use of E2 ubiquitin conjugating enzymes for regulated degradation of the rate-limiting enzymes HMGCR and SQLE in cholesterol biosynthesis. Atherosclerosis 281:137–142. https://doi.org/10.1016/j.atherosclerosis.2018.12.008

van den Boom J, Meyer H (2018) VCP/p97-mediated unfolding as a principle in protein homeostasis and signaling. Mol Cell 69:182–194. https://doi.org/10.1016/j.molcel.2017.10.028

van den Boomen DJH, Volkmar N, Lehner PJ (2020) Ubiquitin-mediated regulation of sterol homeostasis. Curr Opin Cell Biol 65:103–111. https://doi.org/10.1016/j.ceb.2020.04.010

Wang X, Herr RA, Rabelink M et al (2009) Ube2j2 ubiquitinates hydroxylated amino acids on ER-associated degradation substrates. J Cell Biol 187:655–668. https://doi.org/10.1083/jcb.200908036

Wangeline MA, Hampton RY (2018) "Mallostery"—ligand-dependent protein misfolding enables physiological regulation by ERAD. J Biol Chem 293:14937–14950. https://doi.org/10.1074/jbc.RA118.001808

Weber A, Cohen I, Popp O et al (2016) Sequential poly-ubiquitylation by specialized conjugating enzymes expands the versatility of a quality control ubiquitin ligase. Mol Cell 63:827–839. https://doi.org/10.1016/j.molcel.2016.07.020

Yoshioka H, Coates HW, Chua NK et al (2020) A key mammalian cholesterol synthesis enzyme, squalene monooxygenase, is allosterically stabilized by its substrate. Proc Natl Acad Sci 117:7150–7158. https://doi.org/10.1073/pnas.1915923117

Zattas D, Berk JM, Kreft SG, Hochstrasser M (2016) A conserved C-terminal element in the yeast Doa10 and human MARCH6 ubiquitin ligases required for selective substrate degradation. J Biol Chem 291:12105–12118. https://doi.org/10.1074/jbc.M116.726877

Zelcer N, Sharpe LJ, Loregger A et al (2014) The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methylglutaryl coenzyme a reductase and the cholesterol synthesis pathway. Mol Cell Biol 34:1262–1270. https://doi.org/10.1128/MCB.01140-13

Zhang Z-R, Bonifacino JS, Hegde RS (2013) Deubiquitinases sharpen substrate discrimination during membrane protein degradation from the ER. Cell 154:609–622. https://doi.org/10.1016/j.cell.2013.06.038

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Heteromeric Solute Carriers: Function, Structure, Pathology and Pharmacology

Stephen J. Fairweather, Nishank Shah, and Stefan Bröer

Abstract

Solute carriers form one of three major superfamilies of membrane transporters in humans, and include uniporters, exchangers and symporters. Following several decades of molecular characterisation, multiple solute carriers that form obligatory heteromers with unrelated subunits are emerging as a distinctive principle of membrane transporter assembly. Here we comprehensively review experimentally established heteromeric solute carriers: SLC3-SLC7 amino acid exchangers, SLC16 monocarboxylate/H^+ symporters and basigin/embigin, SLC4A1 (AE1) and glycophorin A exchanger, SLC51 heteromer Ost α-Ost β uniporter, and SLC6 heteromeric symporters. The review covers the history of the heteromer discovery, transporter physiology, structure, disease associations and pharmacology – all with a focus on the heteromeric assembly. The cellular locations, requirements for complex formation, and the functional role of dimerization are extensively detailed, including analysis of the first complete heteromer structures, the SLC7-SLC3 family transporters LAT1-4F2hc, $b^{0,+}$AT-rBAT and the SLC6 family heteromer B^0AT1-ACE2. We present a systematic analysis of the structural and functional aspects of heteromeric solute carriers and conclude with common principles of their functional roles and structural architecture.

Keywords

Heteromeric solute carriers · Membrane transporters · Solute carriers · SLC · Membrane physiology · Ancillary proteins · Auxillary proteins · Molecular chaperones · LAT1-4F2hc structure · CD98 · SLC6 · SLC7 · SLC3 · AE1 · Band 3 · CD147 · Glycophorin A · Syntaxin 1A · Ost α-Ost β · B^0AT1 · ACE2 · Collectrin · Blood group antigens · SLC16 · SLC4 · SLC51 · Basigin · Embigin

S. J. Fairweather (✉)
Research School of Biology, Australian National University, Canberra, ACT, Australia

Resarch School of Chemistry, Australian National University, Canberra, ACT, Australia
e-mail: Stephen.fairweather@anu.edu.au

N. Shah · S. Bröer (✉)
Research School of Biology, Australian National University, Canberra, ACT, Australia
e-mail: stefan.broeer@anu.edu.au

Abbreviations

AA	Amino Acids
APC	Amino Acid-Polyamine-Organocation Transporter Family
APN	Aminopeptidase N
ACE2	Angiotensin Converting Enzyme 2
AE1	Anion Exchanger 1
mAbs	monoclonal Antibodies

ADHD	Attention Deficit Hyperactivity Disorder
ASD	Autism Spectrum Disorder
BCH	2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid
$B^0AT1/3$	Broad Neutral Amino acid Transporter 1/3
CA	Carbonic Anhydrase
CD	Cluster of Differentiation
CHS	Cholesterol hemisuccinate
DHEAS	Dehydroepiandrosterone
DAT	Dopamine Transporter
EC	Extracellular
ER	Endoplasmic Reticulum
ERAD	Endoplasmic Reticulum Associated Degradation
FGF	Fibroblast Growth Factor
FXR	Farnesoid X receptor
GAT	GABA Transporter
GSH	Glutathione
GLYT1/2	Glycine Transporter 1/2
GPA/B/C	Glycophorin A/B/C
HS	Hereditary Spherocytosis
HMM	Hidden Markov Model
ISF	Insulin Sensitivity Factor
KO	Knock-out
L-DOPA	L-3,4-dihydroxyphenylalanine
LPI	Lysinuria Protein Intolerance
MeAIB	Methylaminoisobutyric acid
MCT	Monocarboxylate Transporter
NFPS	N-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)-propyl] sarcosine
NET	Noradrenaline Epinephrine Transporter
NSS	Neurotransmitter Sodium Symporter
OMIM	Online Mendelian Inheritance in Man
pCMBS	p-chloromercuribenzene sulphonate
PPI	Protein-Protein Interaction
RBC	Red Blood Cell
dRTA	distal Renal Tubular Acidosis
RPE	Retinal Pigment Epithelium
RdCVF	Rod-derived Cone Viability Factor
SERT	Serotonin Transporter
SAO	Southeast Asian Ovalcytosis
SAR	Stramenopiles [heterokonts] Alveolates and Rhizaria supergroup
TCDB	Transporter Classification Database
TM	Transmembrane
T_3	Triiodothyronine
T_4	Thyroxine
TEM	Transmission Electron Microscopy
VDW	Van der Waals
YFP	Yellow Fluorescent Protein

1 Introduction

Membrane transporters are the primary mediators of metabolite flux across cell membranes and are ubiquitous to all kingdoms of life (Hoglund et al. 2011). Human transporters are divided into three superfamilies, which reflect their separate evolutionary origin and thermodynamic basis for translocation of substrates across biological membranes. One superfamily comprises ATP–Binding Cassette (ABC) transporters, which utilise ATP hydrolysis to drive substrate efflux and intra-cellular organelle uptake and consists of 51 members in humans (ter Beek et al. 2014; Rees et al. 2009; Dean and Annilo 2005; Dean et al. 2001a, b; Dassa and Bouige 2001; Darby et al. 2011). The second superfamily are P-type ATPases, which mediate the transport of ions and phospholipids across membranes (Bublitz et al. 2011). The third superfamily is known collectively as Solute Carriers (Hediger et al. 2013a, b; Saier 2016) including passive and secondary active transporters, which are classified as either uniporters, symporters or antiporters. They utilise substrate and ion gradients to drive substrate transport (Hediger et al. 2013a, b; Chen and Lui 2019). In this review solute carriers will be referred to by their Human Genome (HUGO)/Solute Carrier (SLC) nomenclature (http://slc.bioparadigms.org/) (Wain et al. 2002) and their common names, as both are in frequent usage. Currently, there are 65 classified SLC families containing approximately 430 transporter genes (Perland and Fredriksson 2017). Several other common classification systems for membrane transporters include the Transporter Classification Database (TCDB) and Pfam (Saier et al. 2014, 2016; Chiang et al. 2015; Perland and Fredriksson 2017).

Since the 1980s the function, pharmacology, and, more recently, structural biology of numerous mammalian solute carrier families have been extensively studied (reviewed in (Hediger et al. 2013a, b; Quistgaard et al. 2016; Drew and Boudker 2016; Bai et al. 2017, 2018; Garibsingh and Schlessinger 2019)). To date, approximately 85% of genome annotated putative solute carriers in humans have been functionally characterised. Traditionally, the focus of solute carrier research has been on SLC families that underlie human pathophysiologies. For example, the Neurotransmitter Sodium Symporters (NSS) of the SLC6 family have been intensively studied due to their role in synaptic neurotransmitter clearance and as targets of numerous psychostimulants, neuropsychiatric and analgesic drugs (reviewed in (Broer and Gether 2012; Hog et al. 2006; Clausen et al. 2006; Kristensen et al. 2011; Gether et al. 2006; Iversen 2006; Vandenberg et al. 2014)). Notwithstanding such well-characterised examples, large numbers of solute carrier families and significant aspects of their biology have received much less attention (Cesar-Razquin et al. 2015). This neglect is often unwarranted given that at least 25% of human solute carriers have known roles in human genetic diseases (reviewed in (Rives et al. 2017; Cesar-Razquin et al. 2015; Lin et al. 2015; Bhutia and Ganapathy 2015; Broer and Palacin 2011; Broer 2011; Kampen 2011; Bosshart and Fotiadis 2019; Yahyaoui and Pérez-Frías 2019; Schumann et al. 2020; Wagner et al. 2019)). Moreover, they are over-represented as essential genes and potential drug targets in species parasitic to humans such as apicomplexans, trypanosomes and invertebrate disease vectors (Bushell et al. 2017; Meier et al. 2018; Schmidt et al. 2018; Zhang et al. 2018; Pereira et al. 2014; Cowell et al. 2018; Boudko 2012).

Before reviewing heteromeric solute carriers it is important to define what is meant by this term and what sort of protein complexes we do not define as such. Heteromeric solute carriers are formed by two independently encoded cistrons. They are composed, in all examples to date, of two subunits, namely a translocating subunit, encoded by a multi-pass, SLC transporter gene, and an often smaller, single-pass, α-helical ancillary subunit (Fig. 1 and Table 1). In some cases the ancillary subunits are also given an SLC classification, but universally they belong to different membrane protein families. This definition excludes interactions with universal elements of secretory apparatus such as the SNARE, COPI and II machinery, Rab GTPases, or proteins required for secretory and transient post-translational modifications (PTMs). The overarching qualification criterion is the demonstration that carrier-ancillary interaction is sufficient and necessary or vitally important for physiologically relevant function. However, by necessity of space, we must also exclude some well-established translocator-ancillary interactions that are mediate by membrane-attached rather than integral membrane proteins. For example, the well-characterised interaction between Na^+/H^+ exchanger (NHE) and calcineurin B homologous proteins (CHPs) is excluded despite the fact it fits all other biochemical criteria as an obligatory modulator of translocator function and expression (Allman et al. 2016; Wakabayashi et al. 2013; Pedersen and Counillon 2019).

There is no known biological or phylogenetic pattern with which to predict the identity and formation of unknown heteromeric solute carriers. This is illustrated by the diversity of research approaches that have led to the discovery of novel complexes. Heteromers have been variously identified through systematic exploration of gene expression libraries (Wang et al. 2001), by uncovering heteromer roles in medical phenomena (reviewed (Cordat and Reithmeier 2014; Poole 2000; Bruce and Tanner 1996; Tanner 1993; Shayakul and Alper 2004)), or through the discovery that ancillaries could induce membrane transport where the translocator partner was already endogenously expressed (Wells et al. 1992; Wells and Hediger 1992; Tate et al. 1992; Bertran et al. 1992a, b, c; Kanai et al. 1998; Mastroberardino et al. 1998). Whatever the approach, the discovery of heteromeric solute carriers has been non-systematic. As a result, the underlying principles for complex formation and function are not well understood. Only now, following significant molecular characterisation coupled with the widespread availability of large

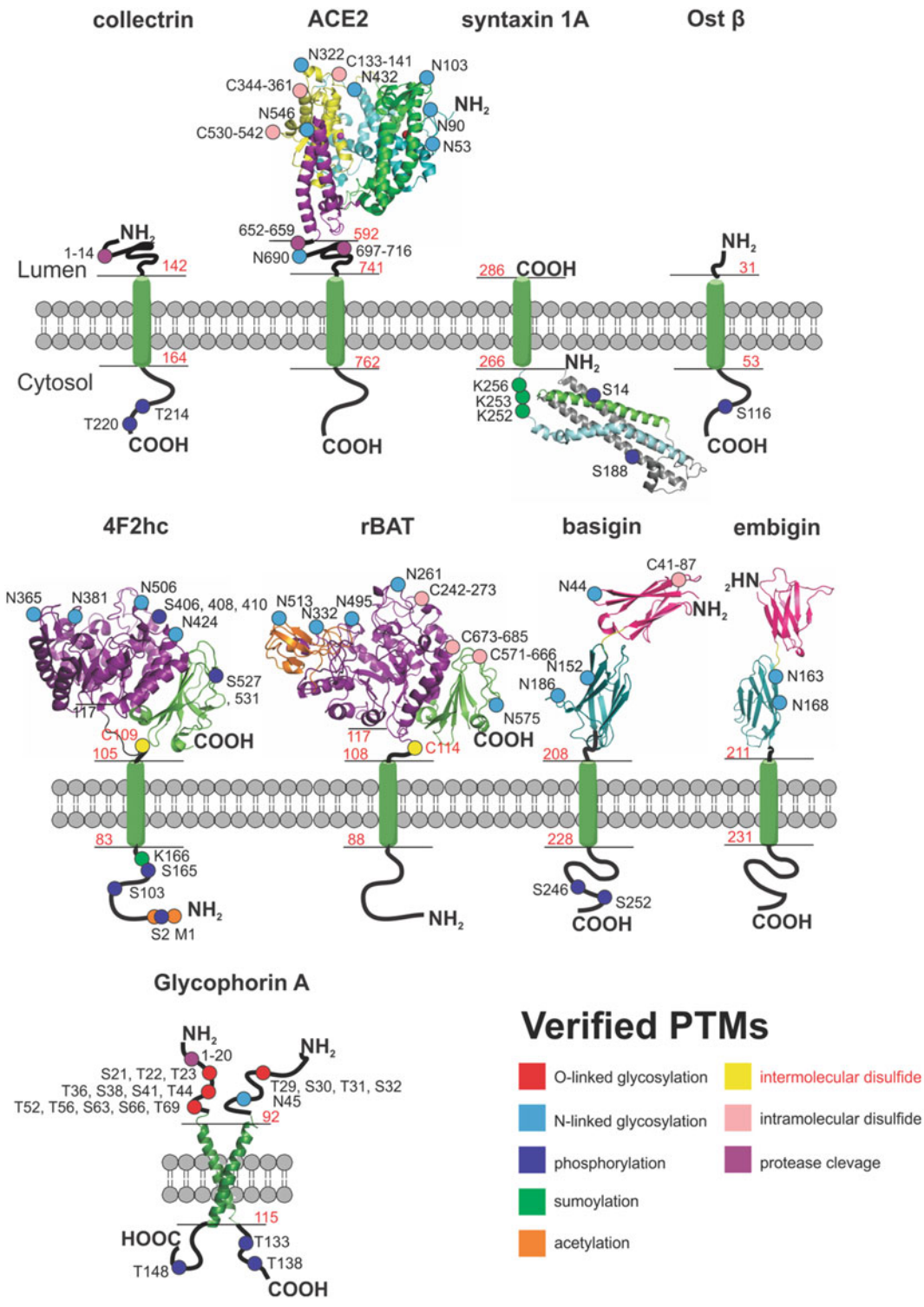

Fig. 1 Topology and domain architecture of solute carrier ancillary subunits

Tertiary structures of solute carrier ancillary monomers are presented as a mixture of x-ray crystal structures, homology/comparative models and cartoons. Human ACE2 (PDB ID 1R42), 4F2hc (2DH2), basigin (3B5H) extracellular domains have been crystallised and are shown coloured. ACE2: subdomains I (cyan) II (yellow)

protein databases, bioinformatics tools, and the first elucidated human structures (Yan et al. 2019, 2020a, c; Lee et al. 2019) are we in a position to understand some underlying principles governing heteromeric solute carrier biology.

Interactions of secondary active transporters with other proteins can serve several purposes:

1. Tissue-specific distribution.
2. Trafficking of the transporter to the final membrane compartment (apical, basolateral, organellar).
3. Folding and quality control of the transporter complex.
4. Tethering of the transporter and formation of larger complexes.
5. Catalytic modulation.

The individual sections of this review give an overview of the biology and physiology of the most well-established heteromeric solute carriers, with a focus on the role of ancillary proteins in the five functions listed above.

2 Heteromeric Solute Carriers: Function and Structure

2.1 Heteromeric Amino Acid Transporters Formed by the SLC3 and SLC7 Families

2.1.1 General Properties

The most well characterised heteromeric solute carriers are the amino acid transporters of the SLC3 and SLC7 families (reviewed in (Palacin et al. 2016; Fotiadis et al. 2013; Broer and Palacin 2011; Verrey et al. 2009; Scalise et al. 2018; Kandasamy et al. 2018)). These heteromeric solute carriers represent one example where both subunits are supplied with an SLC designation. Each heteromeric transporter consists of a SLC7 substrate-translocating 'light' subunit, and a SLC3 ancillary 'heavy' subunit, covalently linked by a disulfide bridge. There are two heavy subunits, namely rBAT (SLC3A1) and 4F2hc (SLC3A2). The 4F2hc subunit is also commonly

Fig. 1 (continued) III (green) IV (purple), the neck, linker and TM domains (cartoon). Zinc (pink) and chloride (red) ions show the approximate location of the ACE2 catalytic site. 4F2hc domains: cytosolic-TM-linker domain (cartoon), domain A (β/α)8 TIM barrel (purple), domain C anti-parallel β8 sandwich (green), C109, which forms a disulfide bond with the holotransporters, is labelled red; basigin: cytosolic-TM-linker domain (cartoon), domain Ig1 (pink), domain Ig2 (cyan) and the Ig domain-TM linker (cartoon). Rat syntaxin 1A (PDB ID 3C98, chain A) cytosolic domains and human TM domain of human glycophorin A (5EH4) have also been crystallised. Syntaxin 1A domains include a 3 anti-parallel helix N-terminal regulatory domain (green and grey), a coiled-coil T-SNARE H3 domain (cyan), and a short intracellular and TM domain (green). Glycophorin A is represented as a homodimer, the TM domains (green, PDB ID 5HE4) and extracellular and cytosolic domains (cartoons). A comparative model of human rBAT extracellular domain structures were built using Phyre2 (Kelley et al. 2015) using α-amylase/glucosidase family enzymes: cytosolic-TM-linker domain (cartoon), domain A $(\beta/\alpha)_8$ TIM barrel (purple), domain B an α2β3 loop (orange), domain C anti-parallel β8 sandwich (green); domain B lies between β3 and α3 of domain A. C114, which forms a disulfide bond with the holotransporters, is labelled red. A homology model of human embigin (isoform 1) using human basigin (isoform 2) as a template (3B5H) was constructed using the HHPred server. The boundaries between TM domains and water-soluble domains are indicated by a black line and the position numbered in red. Other experimentally verified post-translation modifications (PTMs) are coloured and labelled according to the key. References for verified PTMs are as follows: collectrin (Huttlin et al. 2010), ACE2 (Towler et al. 2004; Kristiansen et al. 2004; Shulla et al. 2011; Yan et al. 2020b, d), syntaxin 1A (Tian et al. 2003; Craig et al. 2015; DeGiorgis et al. 2005), Ost β (Huttlin et al. 2010), 4F2hc (Rigbolt et al. 2011; Nguyen et al. 2008; Zhou et al. 2013; Mayya et al. 2009; Impens et al. 2014; Bienvenut et al. 2009), rBAT (Rius and Chillaron 2012; Rius et al. 2016; Bartoccioni et al. 2008; Peter et al. 2000), basigin (Redzic et al. 2011; Olsen et al. 2006, 2010; Yu et al. 2008; Luo et al. 2009; Wright et al. 2014; Rigbolt et al. 2011; Zhou et al. 2013; Bian et al. 2014), embigin (Wollscheid et al. 2009), Glycophorin A (Tomita and Marchesi 1975; Pisano et al. 1993; Bian et al. 2014). The numbering for protease cleavage sites indicates the peptide segment removed

Table 1 Overview of mammalian heteromeric solute carriers: classification, physiology, and expression

Translocator gene (SLC)	Translocator protein	Ancillary gene	Ancillary protein[a]	Substrates and stoichiometry[b]	Transport mode[c]
SLC7A9	$b^{0,+}$AT	SLC3A1	rBAT	Cystine, L-AA^+ (EC): L-AA^0 (IC)	E, 1:1
SLC7A13	AGT1			Cystine, L-Asp, L-Glu	E, 1:1
SLC7A5	LAT1	SLC3A2	4F2hc, CD98	Large L-AA^0, L-DOPA, BCH, T_3, T_4, gabapentin, diverse AA analogues	E, 1:1
SLC7A8	LAT2			L-AA^0, L-DOPA, BCH, T_3, T_4	E, 1:1
SLC7A7	y^+LAT1			L-AA^0/Na^+ (EC): L-AA^+ (IC)	E, 1:1
SLC7A6	y^+LAT2			L-AA^0/Na^+ (EC): L-AA^+ (IC)	E, 1:1
SLC7A10	Asc-1			Small L- and D-AA^0, β-ala, α-aminobutyrate:	E, 1:1, (preferred), U
SLC7A11	xCT			Anionic Cystine (EC): L-Glu (IC)	E, 1:1
SLC7A12[d]	Asc-2	Unknown	Unknown	Small L-AA^0	Unknown
SLC7A15[d]	ArpAT	Unknown	Unknown	L-Tyr, L-DOPA, AA^0, L-Arg, Cystine	E, 1:1
SLC6A1	GAT1	STX1A	Syntaxin 1A	GABA: $2Na^+$: Cl^-	S, 1:2:1
SLC6A2	NET			Noradrenaline, dopamine: Na^+: Cl^-	S, 1:1:1
SLC6A3	DAT			Dopamine, noradrenaline: $2Na^+$: Cl^-	S, 1:2:1[e]
SLC6A4	SERT			Serotonin: $2Na^+$: Cl^-: K^+	S, E (K^+) 1:2:1:1
SLC6A5	GlyT2			Glycine: 3 Na^+: Cl^-	S, 1:3:1
SLC6A9	GlyT1			Glycine/sarcosine: 2 Na^+: Cl^-	S, 1:2:1
SLC6A19	B^0AT1	TMEM27	Collectrin	AA^0: Na^+	S, 1:1
SLC6A18[d]	B^0AT3			Small AA^0: $2Na^+$: Cl^-	S, 1:2:1
SLC6A19	B^0AT1	ACE2	Angiotensin converting enzyme 2	AA^0: Na^+	S, 1:1
SLC6A18[d]	B^0AT3			Small AA^0: $2Na^+$: Cl^-	S, 1:2:1
SLC6A20	IMINO			Pro, OH-Pro: $2Na^+$: Cl^-	S, 1:2:1
SLC4A1	AE1	GYPA	Glycophorin A	HCO_3^-: Cl^-	E, 1:1
SLC16A1	MCT1	BSG	Basigin	L-lactate, pyruvate, ketone bodies (L-D-enantiomers) γ-hydroxybutyrate, ketoacids, R-/S- chloropropionate, 3-bromopyruvate: H^+	S, 1:1
SLC16A8	MCT3			L-lactate[1]	
SLAC16A3	MCT4			L-lactate, pyruvate, ketone bodies, ketoacids[2]: H^+	S, 1:1
SLC16A11	MCT11			Pyruvate: H^+	Unknown
SLC6A12	MCT12			Creatine	U
SLC16A7	MCT2	EMB	Embigin	L-lactate, pyruvate, ketone bodies, ketoacids	S
SLC51A	Ost α	SLC51B	Ost β	Bile salts	U

[a]Not including nomenclature no longer in common usage, see (Chillaron et al. 2001; Palacin et al. 1998a) for older names
[b]Transport Mode and stoichiometry: *IC* intracellular, *EC* extracellular (demonstrating asymmetric substrate specificity for exchangers)
[c]Mode of transport: *U* uniporter, *S* symporter, *E* exchanger
[d]Non-human protein interactions only – silenced genes or non-functional protein in humans/primates
[e]This transport stoichiometry is disputed, with an alternative 1:3:1 stoichiometry proposed, see (Eskandari et al. 2017; Willford et al. 2015)
[1]*In vitro* testing in yeast only
[2]Substrate affinities are much lower compared to MCT1 and 2

referred to by its Cluster of Differentiation (CD) identifier CD98hc. The rBAT subunit heterodimerises with SLC7 light subunits $b^{0,+}$AT (SLC7A9), and AGT1 (SLC7A13) in humans (Feliubadalo et al. 1999; Nagamori et al. 2016; Matsuo et al. 2002; Pfeiffer et al.

1999a; Fernandez et al. 2002). The 4F2hc subunit heterodimerises with one of six SLC7 subunits: LAT1 (SLC7A5), LAT2 (SLC7A8), y^+LAT1 (SLC7A7), y^+LAT2 (SLC7A6), Asc-1 (SLC7A10), and xCT (SLC7A11) (Rossier et al. 1999; Mastroberardino et al. 1998; Pfeiffer et al. 1999a, b; Pineda et al. 1999; Palacin et al. 1998b; Torrents et al. 1998; Estevez et al. 1998; Sato et al. 1999; Fukasawa et al. 2000; Napolitano et al. 2015). Two other SLC7 subunits, ArpAT (SLC7A15) and Asc-2 (SLC7A12) have been characterised in mice but are non-functional in humans. Mouse ArpAT interacts with human rBAT and 4F2hc *in vitro*, and is slightly active in transfected HeLa cells without the heavy subunits but the human and chimpanzee homologues are pseudogenes (Fernandez et al. 2005; Casals et al. 2008). Heterologous expression of Asc-2 with 4F2hc or rBAT elicits no amino acid transport activity, but chimeric fusion of Asc-2 with either of these two does (Chairoungdua et al. 2001). To explain these observations, it has been hypothesised that Asc-2 forms a complex with an unidentified disulfide-linked ancillary protein (Chairoungdua et al. 2001).

The discovery of SLC3/SLC7 heteromers is interesting as it was the ancillary proteins rBAT and 4F2hc which first elicited amino acid transport activity in heterologous systems, and hence were given Solute Carrier (SLC) designations (Wells et al. 1992; Wells and Hediger 1992; Tate et al. 1992; Bertran et al. 1992a, b, c, 1993; Broer et al. 1995; Kanai et al. 1992; Magagnin et al. 1992; Furriols et al. 1993; Markovich et al. 1993). The unlikelihood of a single TM-containing protein transporting substrate by themselves was recognised immediately (Palacin 1994; Broer et al. 1995, 1997). The involvement of a second endogenous protein was deduced from experiments in *X. laevis* oocytes using a C109S mutant of 4F2hc (Estevez et al. 1998) and from earlier work in immune cells using reducing and non-reducing gels (Hemler and Strominger 1982). A flurry of subsequent research confirmed the identity of the various translocator partners for 4F2hc and rBAT (Bassi et al. 1999, 2001; Bridges et al. 2001; Nakauchi et al. 2000; Fukasawa et al. 2000; Chairoungdua et al. 1999; Feliubadalo et al. 1999; Rajan et al. 1999, 2000; Pfeiffer et al. 1999a; Pineda et al. 1999; Rossier et al. 1999; Sato et al. 1999, 2000; Segawa et al. 1999; Fernandez et al. 2002, 2003, 2005; Kanai et al. 1998; Matsuo et al. 2002; Borsani et al. 1999), the substrate specificity of heterodimers (Kanai et al. 1998, 2000a; Mastroberardino et al. 1998; Pineda et al. 1999; Rossier et al. 1999; Segawa et al. 1999; Fukasawa et al. 2000; Nakauchi et al. 2000; Bassi et al. 2001; Sato et al. 1999, 2000; Pfeiffer et al. 1999a, b; Torrents et al. 1998; Chairoungdua et al. 1999; Feliubadalo et al. 1999; Fernandez et al. 2002, 2003; Mizoguchi et al. 2001; Khunweeraphong et al. 2012), their obligatory exchange activity (Broer et al. 2000; Bassi et al. 2001; Mastroberardino et al. 1998; Pfeiffer et al. 1999b; Pineda et al. 1999; Sato et al. 2000; Segawa et al. 1999; Mizoguchi et al. 2001; Coady et al. 1994, 1996; Busch et al. 1994; Chillaron et al. 1996; Matsuo et al. 2002) and the interaction specificity of light chains with heavy chains both *in vitro* and *in vivo* (Pfeiffer et al. 1998, 1999b; Rossier et al. 1999; Mastroberardino et al. 1998; Prasad et al. 1999; Torrents et al. 1998; Nakamura et al. 1999; Broer et al. 2001; Mannion et al. 1998; Nagamori et al. 2016; Fukasawa et al. 2000; Nakauchi et al. 2000). Numerous older reviews comprehensively detail the discovery and physiological characterisation of SLC7-SLC3 heteromers (Palacin et al. 2005; Verrey et al. 2004; Palacin and Kanai 2004; Chillaron et al. 2001; Fotiadis et al. 2013; Kanai et al. 2000b; Broer 2008a; Broer and Wagner 2002; Wagner et al. 2001; Verrey et al. 1999; Kanai and Endou 2001). There is also an extensive literature outlining specific biological roles of SLC7-SLC3 heteromers beyond the scope of this review (Table 2).

Functionally, SLC3/SLC7 heteromeric transporters are amino acid exchangers, with the exception of Asc-1-4F2hc which can function as a uniporter, although the preferred transport mode is also exchange (Fukasawa et al. 2000; Nakauchi et al. 2000). Some display asymmetric ion dependence and vary in their relative affinity for the different classes of amino acids at extracellular and intracellular faces (reviewed in (Fotiadis et al. 2013; Verrey et al. 2004; Chillaron et al. 2001; Scalise et al. 2018), Table 1). A general trend

Table 2 Summary of SLC3-SLC7 heteromeric solute carrier biological functions

Ancillary		Translocator	Gene	Biological function	References
rBAT	SLC3A1	$b^{0,+}AT$	SLC7A9	Renal reuptake of cystine and cationic amino acids in proximal tubule S1 and S2	Chairoungdua et al. (1999), Feliubadalo et al. (1999), Palacin et al. (1998b) and Calonge et al. (1994)
		AGT1	SLC7A13	Renal uptake of anionic amino acids and cystine in proximal tubule S3	Nagamori et al. (2016)
		Unknown		Tumorigenesis	Jiang et al. (2017), Haraguchi et al. (2006) and Konstantinopoulos et al. (2011)
4F2hc	SLC3A2	LAT1	SLC7A5	Thyroid hormone transport	Hennemann et al. (2001), Friesema et al. (2001), Kinne et al. (2011), Braun et al. (2011) and Ritchie and Taylor (2001)
				Immune, cardiomyocyte, placental and cancer cell growth, proliferation, migration mediated by mTOR signalling	Weng et al. (2018), de la Ballina et al. (2016), Cormerais et al. (2016), Broer and Broer (2017), Cantor and Ginsberg (2012), Cantor et al. (2009), Fogelstrand et al. (2009), Baumer et al. (2017), Nicklin et al. (2009), Ablack et al. (2015), Cantor et al. (2011), Yu et al. (2018), Rosario et al. (2013), Uosaki et al. (2015), Almutairi et al. (2019) and Jensen et al. (2017)
				Tumorigenesis, tumour growth and tumour prognostics (breast, lung, glioma, bile duct, tongue, laryngeal, liver)	Broer and Palacin (2011), Fotiadis et al. (2013), Scalise et al. (2018), Chillaron et al. (2001), Cormerais et al. (2016), Parks et al. (2016), El Ansari et al. (2018a, b), Furuya et al. (2012), Kaira et al. (2009), Imai et al. (2009), Yanagisawa et al. (2014), Nakada et al. (2014), Haining et al. (2012), Toyoda et al. (2014), Asano et al. (2007), Nikkuni et al. (2015), Kobayashi et al. (2008), Honjo et al. (2016), Ohkame et al. (2001), Tamai et al. (2001), Kim et al. (2004) and Kaira et al. (2018)
				Small intestine, notochord, and eye development	Ohno et al. (2009) and Katada and Sakurai (2019)
				Blood-brain barrier drug exchange and CNS drug delivery	Dickens et al. (2013), Tomi et al. (2005), Zur et al. (2016), Ylikangas et al. (2013, 2014), Peura et al. (2011, 2013), Puris et al. (2017) and Gynther et al. (2008, 2010)
				Tumour radiotherapy resistance	Digomann et al. (2019)

(continued)

Table 2 (continued)

Ancillary		Translocator	Gene	Biological function	References
		LAT2	SLC7A8	Thyroid hormone transport	Kinne et al. (2011), Braun et al. (2011), Kurayama et al. (2011), Krause and Hinz (2017) and Hinz et al. (2017)
				Tertiary amino acid transporter function with TAT1 (SLC16A10)	Broer and Broer (2017), de la Ballina et al. (2016), Knopfel et al. (2019) and Vilches et al. (2018)
		y^+LAT1	SLC7A7	Renal and intestinal absorption of cationic amino acids, nitric oxide synthesis, macrophage-induced inflammation	Brunini et al. (2003), Arancibia-Garavilla et al. (2003), Rotoli et al. (2005), Bodoy et al. (2019), Rotoli et al. (2018), Rotoli et al. (2004) and Kurko et al. 2015)
		y^+LAT2	SLC7A6	Brain ammonia toxicity and nitric oxide synthesis	Milewski et al. (2017) and Zielinska et al. (2011, 2012, 2015)
		Acs-1	SLC7A10	D-serine regulation of glutamatergic/glycinergic synapses	Xie et al. (2005), Rosenberg et al. (2013), Sason et al. (2017) and Mesuret et al. (2018)
		xCT	SLC7A11	Redox balance and oxidative stress in non-neuronal tissue	Bridges et al. (2001), Seib et al. (2011), de la Ballina et al. (2016), Mandal et al. (2010), Lewerenz et al. (2013) and Guo et al. (2011)
				Cancer-related neurodegeneration, metastasis and diagnostics	Toyoda et al. (2014), Kaira et al. (2009), Nabeyama et al. (2010), Suina et al. (2018), Guo et al. (2011), Chen et al. (2009b), Shin et al. (2018), Lyons et al. (2007), Savaskan et al. (2008), Tsuchihashi et al. (2016) and Shen et al. (2018)
				Protection from neuronal degeneration via oxidative glutamate stress and role in addiction, epilepsy and cognitive ability	Savaskan et al. (2008), Nabeyama et al. (2010), Mandal et al. (2010), Massie et al. (2015), Lewerenz et al. (2009, 2012a, 2012b, 2013), Albrecht et al. (2010), Lewerenz and Maher (2009), Baker et al. (2003), Albertini et al. (2018), De Bundel et al. (2011), Massie et al. (2011), Moran et al. (2005), Melendez et al. (2005), Kau et al. (2008), Moussawi et al. (2009) and McQueen et al. (2018)
		Unknown		β-integrin signalling and cell adhesion	Estrach et al. (2014), Prager et al. (2007), Feral et al. (2005, 2007), Ohgimoto et al. (1995); Takesono et al. (2012), Fenczik et al. (1997) and Henderson et al. (2004)
		Unknown		ER stress response	Liu et al. (2016, 2018) and Kropski and Blackwell (2018)

appears to be that substrate affinity of the cytosol facing binding site is much lower than the outside facing binding site in the transporters that were tested e.g. LAT1-4F2hc, y^+LAT2-4F2hc (Reig et al. 2002; Lahoutte et al. 2004; Broer et al. 2000; Verrey 2003; Meier et al. 2002; Napolitano et al. 2015; Errasti-Murugarren et al. 2019). For example, LAT1-4F2hc affinity for extracellular neutral L-amino acid varies from 12.7–47.2 μM (Yanagida et al. 2001), while cytosolic affinities are 100- to 1000-fold higher (Meier et al. 2002; Napolitano et al. 2015, 2017). However, it must be noted that affinity constants for the same substrate on the same side of the membrane can also vary between different expression systems e.g. for leucine (Yan et al. 2019; Kim et al. 2002a; Kanai et al. 1998) and histidine (Napolitano et al. 2015; Kanai et al. 1998). For some exchangers, such as $b^{0,+}$AT-rBAT, the inward negative membrane potential contributes to asymmetric behaviour, because the uptake of a cationic amino acid can be coupled to the efflux of a neutral amino acid (Pfeiffer et al. 1999a; Chillaron et al. 1996; Busch et al. 1994). Moreover, the y^+LAT1-4F2hc and y^+LAT2-4F2hc heteromers display Na^+ dependence when translocating neutral amino acids but not when substrates are cationic amino acids (Broer et al. 2000; Torrents et al. 1998; Pfeiffer et al. 1999b; Kanai et al. 2000a). Transporters of cystine xCT-4F2hc and $b^{0,+}$AT-rBAT display a pH-dependent asymmetry (Bassi et al. 2001; Meier et al. 2002; Chillaron et al. 1996; Sato et al. 2000; Pfeiffer et al. 1999a; Moschen et al. 2002). An elegant demonstration of the potential molecular basis of this asymmetrical exchange was demonstrated using BasC, a homologue of all human SLC7 translocators (Errasti-Murugarren et al. 2019; Bartoccioni et al. 2019). The structure of BasC bound with a nanobody (Nb74) specific to the cytoplasmic face was combined with functional assays to identify a conserved Lys residue, mutation to Ala of which dramatically reduced extracellular affinity without affecting intracellular affinity for alanine. This Lys is conserved in all human translocators, with the K191E mutation in y^+LAT1 a cause of the rare inheritable aminoaciduria Lysinuric Protein Intolerance (LPI) (Mauhin et al. 2017) (Sect. 2.1.6). The absence of an ancillary subunit in BasC and the mutational analysis suggests that asymmetric affinity resides in the translocator subunit and is not a specific feature of heteromeric transporters.

The SLC3 members rBAT and 4F2hc share about 30% sequence identity, but both are type II, N-glycoproteins with M.W.s of ~94 and ~85 kDa, respectively (Broer et al. 1995; Teixeira et al. 1987; Parmacek et al. 1989; Quackenbush et al. 1987; Palacin et al. 1998a; Zhang et al. 2003; Wollscheid et al. 2009; Yan et al. 2019; Lee et al. 2019) (Fig. 1). They contain a short intracellular N-terminal, a single α-helix TM domain and a large extracellular domain (Fort et al. 2007). The extracellular domains of human rBAT and 4F2hc have ~20% homology to, and share a common ancestry with, the bacterial GH13 α-amylases and insect maltases (Wells and Hediger 1992; Gabrisko and Janecek 2009). Accordingly, structures of the human 4F2hc extracellular domain in isolation (PDB ID 2DH2) and in complex with LAT1 (PDB ID 6IRS, 6IRT, 6JMQ, and 6JMR) show a $(\beta/\alpha)_8$ TIM barrel with an attached anti-parallel β_8 sandwich characteristic of the α-amylase/glucosidase fold (Yan et al. 2019; Fort et al. 2007; Lee et al. 2019), designated as domains A and C, respectively, of the ancillary subunit (Fig. 1) (Gabrisko and Janecek 2009; Fort et al. 2007). In addition, rBAT features domain B between the β_3 strand and α_3 helix of domain A containing the non-functional catalytic site for hexose hydrolysis and a 31 residue C-terminal tail bearing no homology to any known protein (Fort et al. 2007; Gabrisko and Janecek 2009; Yan et al. 2020a). 4F2hc lacks the glucosidase domain B and C-terminal extension (Janecek 1997). These significant structural changes have been traced to a last common ancestor with basal metazoans (cnidarian) (Gabrisko and Janecek 2009) and as a result no enzymatic activity has been demonstrated for either rBAT or 4F2hc EC domains (Fort et al. 2007; Wells et al. 1992). Four splice variants of human 4F2hc and seven of human rBAT have been annotated in protein databases (see UniProtKB entries P08195 and

Q07837). The sequence numbering for human 4F2hc splice variant 2 is used throughout this review (except where noted) as this isoform has predominated in the elucidation of ancillary roles in SLC3-SLC7 solute carriers.[1]

2.1.2 Structural Aspects – Human LAT1-4F2hc and $b^{0,+}$AT-rBAT, the First Complete Heteromeric Transporter Structures

The first complete structures of a heteromeric solute carrier, human LAT1-4F2hc, were solved in an inward open conformation with the binding site exposed to the cytosol using single-particle cryo-EM (Yan et al. 2019; Lee et al. 2019). Two structures of the human LAT1-4F2hc complex are bound to the LAT1 inhibitors BCH (PDB ID 6IRT) and JPH203 (6IRS) with RMSDs of 3.3 and 3.5 Å, respectively (Yan et al. 2019). Both inhibitor-bound structures contain an unplanned mutation A36E in LAT1, which appears not to affect the transport function or overall structure. Two further WT apo structures of LAT1-4F2hc at the lower resolution of 4 Å were also solved with the ancillary stabilised by monoclonal antibodies MEM108 (6JMQ, 3.44 Å RMSD) or HBJ127 (6JMR, 4.19 Å RMSD) (Lee et al. 2019). These antibodies have anti-tumour effects in cell culture models (Hayes et al. 2015) and recognise epitopes on 4F2hc without disrupting the heteromeric interaction (Lee et al. 2019). The isoform solved in all 4 complex structures was the longer 4F2hc, the amino acid numbering of which is used in the following structural description.

The LAT1 translocation subunit contains the LeuT/APC fold common to many functionally and phylogenetically diverse solute carrier proteins. The fold name is derived from the neutral amino acid transporter of the SLC6-related family from *Aquifex aeolicus*, which represented the eponymous structure of the classification (Yamashita et al. 2005). The LeuT fold is the common structure of the Amino acid-Polyamine-organoCation (APC) superfamily in the Pfam/TCDB nomenclature, which contains 20 distinct families (https://pfam.xfam.org/clan/APC#tabview=tab0) (http://www.tcdb.org/superfamily.php) (Vastermark et al. 2014; Wong et al. 2012b; Vastermark and Saier 2014). To date over 200 crystal and cryo-EM structures of numerous eukaryote and prokaryote transporters containing the LeuT/APC fold have been determined, including those representing SLC4 (Huynh et al. 2018; Reithmeier et al. 2016; Arakawa et al. 2015; Yu et al. 2017; Coudray et al. 2017; Wahlgren et al. 2018; Faham et al. 2008) SLC5 (Faham et al. 2008; Watanabe et al. 2010), SLC6 (Coleman et al. 2016; Wang et al. 2013a, 2015; Penmatsa et al. 2013; Wang and Gouaux 2012; Krishnamurthy and Gouaux 2012; Piscitelli and Gouaux 2012; Piscitelli et al. 2010; Singh et al. 2007, 2008; Yamashita et al. 2005; Kantcheva et al. 2013; Malinauskaite et al. 2014, 2016; Quick et al. 2009; Forrest et al. 2007; Perez et al. 2012, 2014; Ressl et al. 2009; Coleman and Gouaux 2018; Yan et al. 2020a, b), SLC7 (Gao et al. 2010; Shaffer et al. 2009; Jungnickel et al. 2018; Ilgu et al. 2016), SLC11 (Ehrnstorfer et al. 2014, 2017; Bozzi et al. 2016, 2019; Lee et al. 2019; Yan et al. 2019, 2020a; Wu et al. 2020), SLC23 and 26 (Geertsma et al. 2015, 2016; Yu et al. 2017; Lu et al. 2011) families. Solved structures in numerous conformations of LeuT itself (Coleman et al. 2016; Krishnamurthy and Gouaux 2012; Kantcheva et al. 2013; Singh et al. 2008; Piscitelli and Gouaux 2012; Piscitelli et al. 2010), MhsT from *B. halodurans* (Malinauskaite et al. 2014; Quick and Javitch 2007), Mhp1 from *M. liquefaciens* (Weyand et al. 2008, 2011; Shimamura et al. 2010; Simmons et al. 2014), BetP from *C. glutmicum* (Perez et al. 2012, 2014; Ressl et al. 2009; Tsai et al. 2011), the *D. melanogaster* dopamine (DAT) (Penmatsa et al. 2013, 2015; Wang et al. 2015), human serotonin (SERT) (Coleman et al. 2016; Coleman and Gouaux 2018), prokaryotic SLC11 (NRAMP) divalent cation transporters (Ehrnstorfer et al. 2014; Bozzi et al. 2016, 2019), and the AdiC bacterial arginine/agmatine exchanger (Kowalczyk et al. 2011; Gao et al. 2009, 2010; Ilgu et al. 2016; Fang et al. 2009; Casagrande et al. 2008), among others, have led to a proposed LeuT fold

[1] This distinction is important as numerous publications use the residue numbering of either isoform 1 or 2 without distinguishing between them. Isoform 2 (UniPROT P08195-2) differs from isoform 1 (UniPROT P08195-1) by having cytosolic, N-terminal residues 1–101 missing.

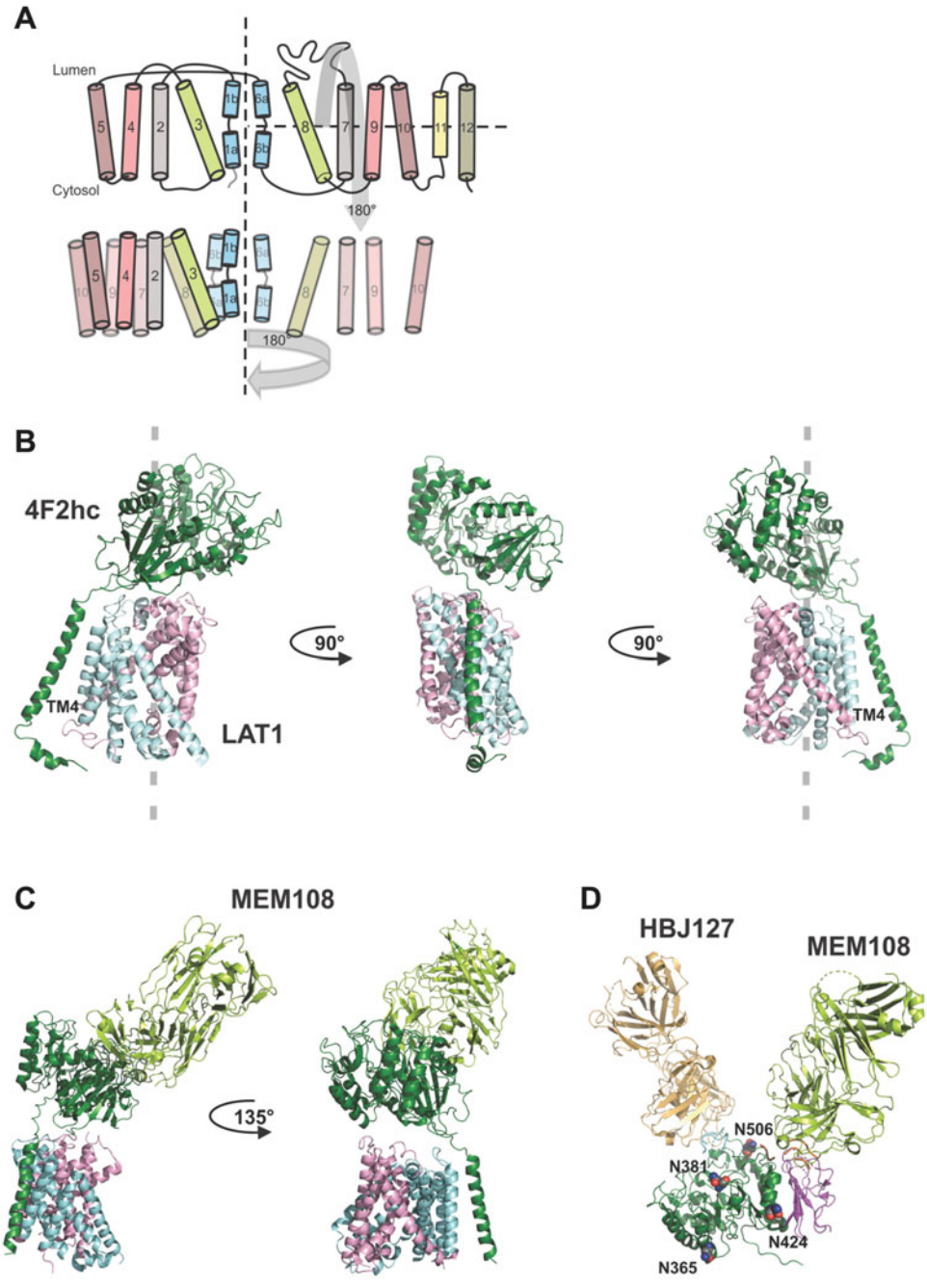

Fig. 2 Topology and quaternary structure of human LAT1-4F2hc

The human LAT1-4F2hc heterodimer structures complexed with the inhibitor JPH203 (**b**) (PDB 6IRT) or with the mAbs MEM108 (**c**) (6JMQ) or MEM108/HBJ127 (**d**) (6JMR) to the 4F2hc EC domain and solved to an average of 3.3 Å, 3.44 Å, or 4.19 Å RMSD, respectively. (**a**) **Upper**: The membrane topology and orientation of the LeuT/APC fold LAT1 subunit. Symmetric helices are paired by colour: 1 and 6 (blue), 2 and 7 (grey), 3 and 8 (green), 4 and 9 (pink), 5 and 10 (maroon). Helices 11 and 12 do not form part of the pseudo twofold inverted repeat motif. Helices 1 and 6 are separated into two halves by short unwound regions in the middle of the membrane, which forms part of the binding pocket in LeuT/APC fold transporters. Helices 1, 6, 2, 7, and 5 form the 'bundle'

transport cycle (Malinauskaite et al. 2014, 2016; Navratna and Gouaux 2019; Bozzi et al. 2019; Fotiadis and Jeckelmann 2019; Simmons et al. 2014; Kazmier et al. 2017; Li et al. 2019). Like all LeuT fold transporters, human LAT1 contains a pseudo twofold inverted axis of symmetry (Fig. 2a). The axis of symmetry demonstrates that helices 1–5 and 6–10 represent a repeated fold, which can be superimposed by inverting and overlaying them. Helices 11 and 12 are not included in the core 5 + 5 inverted-repeat architecture due to variation in their number across LeuT fold transporters (Navratna and Gouaux 2019; Bai et al. 2017). The architecture of the LeuT fold is characterised by two domains; the 'bundle' subdomain is made up of the first two helices of each repeat motif (helices 1/6 and 2/7) (Fig. 2b, c). A second 'hash' subdomain is formed by the third and fourth helices in each inverted repeat (helices 3/8 and 4/9) and constitutes a rigid scaffold around the bundle. Helices 5 and 10 are considered part of these conserved sub-domains and are referred to as the 'arms'. The bundle bends at unwound regions in the middle of TM helices 1 and 6 (Krishnamurthy and Gouaux 2012; Navratna and Gouaux 2019; Penmatsa and Gouaux 2014; Shimamura et al. 2010). This unwound part of TM 1 and TM6 binds the α-amino carboxyl moiety of the substrates or competitive inhibitors of the LAT transporters as demonstrated in GkApcT for L-Ala (187), BasC with 2-AIB substrate (137) and LAT1-4F2hc with competitive inhibitors (e.g., BCH) (66). The unwinding allows for a 'rocking' or hinge-bending movement of TM1 and 6 that occurs during the outward to inward conformation transitions. The inner membrane halves TM1a and 6b move outwards into the bilayer in a gap formed between TM helices 5 and 7 and allow for access to the cytosol at the same time as the outer membrane halves TM1b and 6a significantly narrow the vestibular entry and, with TM 3, 10, EL2 and EL4 close off extracellular access to the binding sites (Forrest et al. 2011; Forrest and Rudnick 2009; Krishnamurthy and Gouaux 2012; Bracher et al. 2016; Shimamura et al. 2010). The TM 5 and TM10 arms are involved in more subtle lateral movements in the membrane (Shimamura et al. 2010). This proposed transport cycle and the structural evidence supporting it are outlined in recent reviews (Navratna and Gouaux 2019; Drew and Boudker 2016). Indeed, twofold inverted-repeats seem to be an almost universal topological design for all solute carriers, despite displaying different structural architectures and transport mechanisms (Fenollar-Ferrer et al. 2014; Forrest 2013; Radestock and Forrest 2011; Vergara-Jaque et al. 2015; Drew and Boudker 2016; Bai et al. 2017; Saier 2016;

Fig. 2 (continued) subdomain, while helices 3, 8, 4, 9 and 10 form the 'hash' subdomain. **Lower**: The second repeat motif (faded) is first rotated 180° about the membrane plane (RHS) and then rotated 180° again about the axis of symmetry (LHS) and overlaid on the first repeat motif to demonstrate the pseudo twofold axis of asymmetry. The dashed lines represent the two axis of symmetry about which the two repeated 5 × 5 inverted halves of the transporter are rotated. (**b**) The structural architecture of the LAT1-4F2hc heteromer (6IRT) demonstrated through 2 × 90° rotations. LAT1 is split into the bundle (pink), and the hash (cyan) subdomains, while the 4F2hc ancillary subunit (green) sits adjacent to TM4 of LAT1. Residues 1–61 are missing from 4F2hc, whereas LAT1 is missing the cytosolic N-terminal residues 1–50. TM helices 11 and 12 have been deleted to assist visualisation of the core structural folds. The bound inhibitor JPH203 is not shown. The dashed grey line in both lateral views of LAT-4F2hc represent the rotational centre of LAT1, demonstrating the ~20 Å deviation from centre of the 4F2hc EC domain. (**c**) The same structural architecture of the LAT1-4F2hc heteromer (6JMQ) but with the mAb MEM108 (lemon) bound to the top of the 4F2hc EC domain (green) through a 135° rotation. The bundle and hash subdomains are coloured as for panel B. The TM domain of 4F2hc interacts at the same position as for the 6IRT structure. The entire intracellular C-terminal of 4F2hc (1–179) is missing, whereas LAT1 is missing the cytosolic N-terminal residues 1–49. (**d**) The 4F2hc EC domain (green) bound by the mAbs MEM108 (lemon) and HBJ127 (orange) at different epitopes of the EC domain. The positions of the 4 N-linked glycan-attached asparagines are shown as spheres

Vastermark and Saier 2014; Abramson and Wright 2009; Shi 2013; Khafizov et al. 2010). The specific sequence of substrate binding and release, variation in the conserved S1 and ion binding sites, and the link of these to transport cycle dynamics in LeuT/APC transporters is a focus of intense research but beyond the scope of this review (see Bosshart and Fotiadis 2019; Bai et al. 2018; Carland et al. 2018; Penmatsa and Gouaux 2014; Navratna and Gouaux 2019; Broer and Gether 2012; Errasti-Murugarren et al. 2019; Bartoccioni et al. 2019; Jungnickel et al. 2018; Ilgu et al. 2016; Kowalczyk et al. 2011; Gao et al. 2009, 2010; Newstead 2019; Fotiadis and Jeckelmann 2019; LeVine et al. 2018, 2019; Singh et al. 2008; LeVine and Weinstein 2014; Khelashvili et al. 2015b; Razavi et al. 2017; Zhao et al. 2011, 2012; Terry et al. 2018; Paz et al. 2018; Wang et al. 2015; Li et al. 2019; Quick et al. 2018; Ponzoni et al. 2018; Gur et al. 2015; Edwards et al. 2018; Kazmier et al. 2014a, b; Malinauskaite et al. 2014; Adelman et al. 2011). The LAT1-4F2hc structure retains all of these fundamental structural features of the LeuT fold, including the hash/bundle subdomain structure and hinges at the centre of TM1 and 6 (Fig. 2b, c). The inhibitor and mAb-bound structures all exhibited strong conservation of the amino acid binding site and gating residues with its bacterial ancestors (Yan et al. 2019; Jungnickel et al. 2018; Faham et al. 2008; Lee et al. 2019). Interestingly, an additional 4F2hc EC domain structure demonstrated simultaneous binding of the two mAbs MEM108 and HBJ127 to separate epitopes (Fig. 2d). Four previously predicted N-glycan sites in 4F2hc were confirmed in this structure, with all sites distal from the LAT1-4F2hc interface (see also Fig. 1).

The structure confirmed extensive and dispersed interaction points between 4F2hc and LAT1, consistent with previous biochemical research ((Rosell et al. 2014; Broer et al. 2001; Jeckelmann and Fotiadis 2019; Fenczik et al. 2001) Sect. 2.1.5) (Fig. 3). They are restricted to the scaffold domain of LAT1, allowing the rocker-switch motion of helix 1 and 6 to occur without hindrance. The position of the intermolecular disulfide bond at C210 in 4F2hc[2] with C164 of LAT1, and van der Waal interactions between the TM domain of 4F2hc and TM4 of LAT1 were also confirmed in LAT1-4F2hc structures (Yan et al. 2019; Lee et al. 2019) (Fig. 3). The pose of the ancillary was predicted reasonably accurately by a previous LAT2-4F2hc model utilising negative-stain TEM (21–13 Å RMSD) of the 4F2hc ectodomain (Rosell et al. 2014; Meury et al. 2014; Fort et al. 2007; Palacin et al. 2016) and low-resolution cryo-EM structure of LAT2 (Gao et al. 2010; Kowalczyk et al. 2011; Jeckelmann and Fotiadis 2019). In all structures the 4F2hc EC domain is shifted a significant distance from the geometrical centre of LAT1 e.g. 20 Å in 6JMQ (Fig. 2b (Lee et al. 2019)). Lee et al. (2019) have provided a comprehensive analysis of the LAT1-4F2hc interactions, identifying 4 main regions of contact between the two subunits: TM4 of LAT1 to the TM domain of 4F2hc and three separate regions of the 4F2hc EC domain-LAT1 interface (Fig. 3). The 3 distinct interaction regions at the heterodimer interface are between β2, β3, β8 sheets/linker of 4F2hc domain C with EL2 of LAT1; the α8 helix of 4F2hc domain A and EL4 of LAT1; and the α1/α2 helices of 4F2hc domain A and LAT1 EL3. The Cβ2/Cβ3/Cβ8-linker-EL2 interface includes the C210-C164 disulfide bond, an electrostatic-hydrogen bond between 4F2hc R534 and LAT1 T163 and an interaction network between LAT1 EL2 and 4F2hc residues D540, P208 and Y628 (not shown). The Aα8-EL4 interface forms an ionic and hydrogen bonding network between R534/K532 on 4F2hc and E303/Q304 on LAT1 EL4. The Aα1/Aα2-EL3 interface seems to be dominated by a K299 to D223

[2] Note that the numbering used in both structure publications are derived from UniPROT designated isoform 1 of human 4F2hc (P08195-1). However, there are also minor mistakes in each manuscript: Nureki and colleagues have mislabelled 4F2hc R434 as R511 when describing the Cβ2/Cβ3/Cβ8-linker-EL2 interaction network, while Yan and colleagues have miss-assigned all 4F2hc residues by an increase of 1 in their manuscript.

salt bridge but could also involve other ionic and H-bond interactions with K255/K300 and other charged residues on LAT1. This Aα1/Aα2-EL3 interaction region was not noted by Yan et al. (Yan et al. 2019), probably due to the poor resolution of this region in the published structures, suggesting these interactions are less stable than others at the LAT1-4F2hc EC interface and perhaps involved in conformational dynamics during the transport cycle. None of these extensive interactions by themselves would confer heterodimerisation between LAT1 and 4F2hc (excepting the disulfide bond) but many would yield significant bond energy stabilisation at distances of 4.2 Å (T163 hydroxyl to R534 guanidino), 6.2 Å (Q304 amide oxygen to R534 guanidino), 4.7 and 5.9 Å (E303 carboxylate oxygens to the charged primary amine of K532), and 4.9 Å between the side chain amine of K299 and carboxylate oxygen of D223 in LAT1. Mutation to K532E (4F2hc) and E303K (LAT1) separately reduced transport activity by 35% and 60%, respectively (Yan et al. 2019), which could be restored using charge-swapped mutations. That these 4F2hc charged residues may play a universal role in 4F2hc-mediated heteromer formation with various translocators is supported by their strict conservation in all species from humans to zebrafish (Yan et al. 2019). In contrast, the LAT1 residues paired with these 4F2hc charged residues are not as strictly conserved, although they do conserve several predicted structural features. For example, the EL2 loop of LAT1 which contains the R534-T163 ionic-hydrogen bond is predicted to maintain a 9 residue length in homologues and begin with a pair of proline residues (P159 and 167) at the protein-bilayer interface (Lee et al. 2019). As the interacting residues in LAT1 diverge widely in both orthologs and paralogs, these locations may also contribute towards the specificity in complex formation between the various heteromer pairings. Apart from the Cβ2/Cβ3/Cβ8-linker-EL2 interaction network, direct contacts are actually quite few over the bulk of the extracellular interface surface area, leading to a substantial distance between the two subunits extracellular domains and a gap which encloses a volume of 8020 $Å^3$ (Fig. 3a, lower panel). This would allow for the passage of large neutral amino acids between the two subunits to access the substrate binding site if such a distance and volume is maintained at the outward open conformation of the heteromer – a verification which awaits further structural evidence. A surface potential calculation of the LAT1-4F2hc heterodimer shows an asymmetric charge distribution at the extracellular interface, with the 4F2hc EC domain displays a highly negatively surface potential, while the LAT1 EC interface shows less widespread regions of positive surface potential (Fig. 3b).

The 4F2hc TM domain tilts across TM4 of LAT1 and forms extensive hydrophobic interactions with this helix and the C-terminal intracellular H4 domain (Fig. 3c, d). The 4F2hc TM domain is also tilted with respect to the vertical membrane axis such that its extracellular half is in closer contact with LAT1 TM4 than its intracellular half (Fig. 3c). As a result, contact between the two helices at the inner membrane leaflet is minimal and distance between the two helices increases towards the cytosol, culminating at 21.2 Å (6IRS, Fig. 3d) to 18.6 Å (6JMQ, Fig. 3c) Cα distances between the last intracellular residues of the two TM helices, N186 of LAT1 TM4 and W183 of 4F2hc. Nureki et al. hypothesise this is due to in-plane opposition of hydrophobic side chains F189, W190 and W193 on 4F2hc to the side chains of L180 and L181 from LAT1 TM4 (Lee et al. 2019). This gap is filled by an electron density modelled as a sterol molecule in the 6JMQ structure (Lee et al. 2019) or by large lipid molecules (probably phosphatidic acids) in heteromer structure 6IRT (Yan et al. 2019) (Fig. 3c, d). Rather than being pushed apart by hydrophobic side chains it is more plausible that 4F2hc TM and LAT1 TM4 form binding pockets for the lipids. The more extensive structural analysis carried out see (Yan et al. 2019) (Fig. 4) demonstrated the charge/polar phosphate and ester phosphatidyl moieties of the proposed lipids would bind into two cation-rich pockets on the intracellular side of the membrane (Fig. 3d). One lipid binding pocket is formed by residues from the 4F2hc TM domain along with residues from TM4, TM9, and

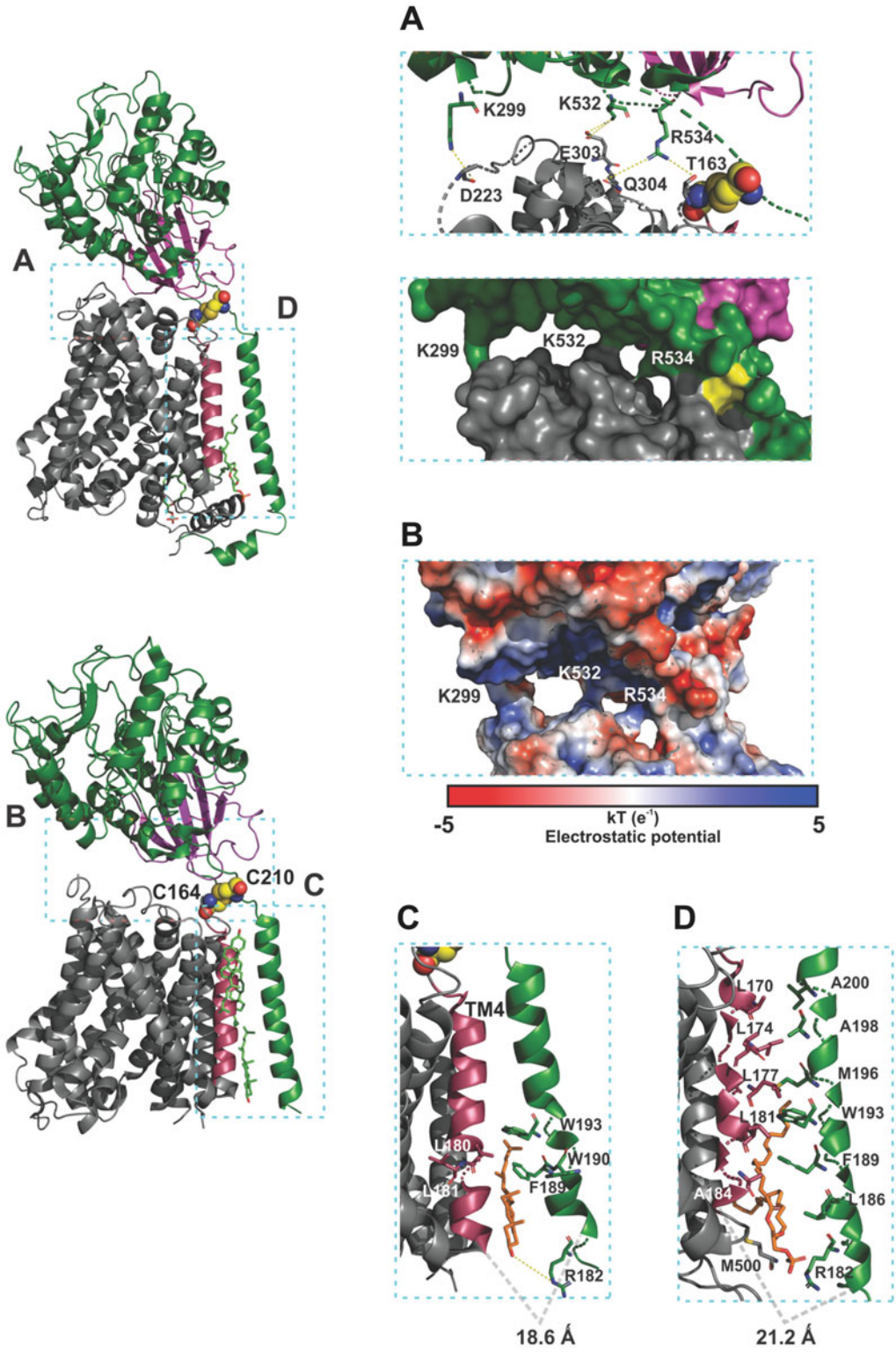

Fig. 3 Interactions of human LAT1 with 4F2hc
Overview and foci of LAT1 and 4F2hc contact points. **Upper** (6IRT) and **lower** (6JMQ) left hand side panels are overview cartoons: The two subunits are linked by a conserved disulfide bridge between Cys210 (4F2hc) and Cys164 (LAT1), (sphere models, C atoms, grey; O atoms, red; N atoms, blue; S atoms, yellow). The 4F2hc-ED is divided into its two subdomains homologous to bacterial α-glycosidases: a TIM-barrel $(\beta/\alpha)_8$ domain A (dark green) and the eight antiparallel β-sheets domain C

the C-terminal intracellular helix H4 of LAT1. Residue R182 from the TM domain of 4F2hc interacts with the lipid head-group. A second sterol binding pocket is formed by the intracellular ends of LAT1 TM 8 and 12 along with the $IL_{8–9}$ and the N-terminal end of the TM4. The functional relevance of the first lipid binding site was demonstrated by mutation to R182 and deletion of the H4 helix, which almost entirely abolished transport activity. The two sites extensively overlap such that the modelled sterol hydroxyl moiety of the second binding pocket is interacting with the same R182 that forms the binding pocket for the phospholipid head group (Fig. 3c). These results together strongly suggests that polar head-groups and moieties of stably bound lipid molecules mediate the TM domain interactions of the heteromer.

Recently the structure of a second SLC7-SLC3 heteromeric amino acid transporter $b^{0,+}$AT-rBAT was also solved by cryo-EM (Yan et al. 2020a; Wu et al. 2020). Unlike LAT1-4F2hc but similar to the structure of B^0AT1-ACE2 (Sect. 2.5.4) the $b^{0,+}$AT-rBAT structures have been solved as a dimer of heterodimers (Fig. 4a), two with empty binding sites (PDB ID 6LID, 2.7 Å RMSD and 6YUP, 2.8 Å RMSD) and another with arginine substrate bound (6LI9, 2.3 Å RMSD). As with LAT1-4F2hc structures, $b^{0,+}$AT-rBAT displays an inward open conformation in both apo- and substrate-bound structures. Five or four N-linked glycosylation sites were identified at N261, N332, N495, N513, N575, all facing outward to the extracellular solution. Three major interaction regions of the transporter with is ancillary rBAT were identified in each heterodimer, reminiscent with respect to both region and the extensive nature of its bonding networks to those in LAT1-4F2hc (Fig. 4b, c). Due to this similarity the analysis of these interacting regions will not be covered in detail but rather differences with LAT1-4F2hc will be noted. The first interaction region is focused on the EC domain (residues 116 to 685) of rBAT facing the EC loops of the $b^{0,+}$AT subunit, which, in contrast to LAT1-4F2hc, provides a smaller gap and intermolecular volume for access by substrate (compare Fig. 4a to Fig. 3b). Interactions in this region are mediated by numerous polar intermolecular bonds in close proximity the single $b^{0,+}$AT-rBAT disulfide bond (C114 in rBAT, C144 in $b^{0,+}$AT) itself located at the intersection of the linker and rBAT EC domains. This linker region, shorter than the 4F2hc linker at 13 Å, stretches from residues 115 to 109. Considerable contacts are made between the rBAT linker and EL2 of $b^{0,+}$AT, along with an extension of rBAT α4/β5 into a cleft between EL2 and EL4 of $b^{0,+}$AT (Yan et al. 2020a; Wu et al. 2020). The second interaction region is mediated by a network of hydrophobic interactions and lies at the interface of the rBAT TM domain with TM4 and the extracellular

Fig. 3 (continued) (magenta). The two contacting TM spanning α-helices are coloured red (LAT1 TM4) and green (4F2hc). The lipids mediating LAT1 TM4-4F2hc TM interactions at the inner membrane leaflet are also coloured green (stick models, C atoms, green; O atoms, red; N atoms, blue). **Insert a**) Outlines the specific interactions between 4F2hc-EC domain and the extracellular face of LAT1 (**upper**). Direct salt-bridge or ionic-dipole bonds are shown by dash lines (yellow), interacting residues as stick models or as spheres for the disulfide bond (oxygens red; nitrogen blue; sulfur atoms, yellow). Certain regions of the secondary structure from both subunits have been shown as dash lines for ease of viewing. A surface representation of the same cut-out (**lower**) shows the considerable volume lying between the residues of subunit extracellular domains. Residues from 4F2hc with overlapping interactions with the EC surface of LAT1 are labelled. **Insert b**) Surface representation with surface formal charge potential of the same 4F2hc-LAT1 extracelullar interface as in (**a**) but from the structure 6JMQ, the bound mAb MEM108 has been removed from the top of the 4F2hc EC domain. Positive surface potential is shown as red, negative as blue, neutral as white (scale from +5 to −5 kT/e). Residues from 4F2hc with overlapping interactions with the EC surface of LAT1 are labelled. **Insert**s **c**) and **d**) shows the extensive interaction network of residues from the 4F2hc TM helix (green), the LAT1 TM4 (red) and adjacent TM 8 (grey), and the predicted lipid molecule (orange) mediating interactions between the helices at the membrane inner leaflet. Electron density suggest a sterol in the mAb MEM108-bound structure (**c**), while the inhibitor JPH203 bound structure (**d**) electron density suggests a phospholipid. A large distance separates the ends of the interacting helices in both structures. Individual residues are represented and coloured according to the same scheme as in the **overview panels**. LAT1 residues are numbered based on sequence entry Q01650-1 isoform 1 (UniProt), while 4F2hc numbering is based on isoform 1 (entry P08150-1)

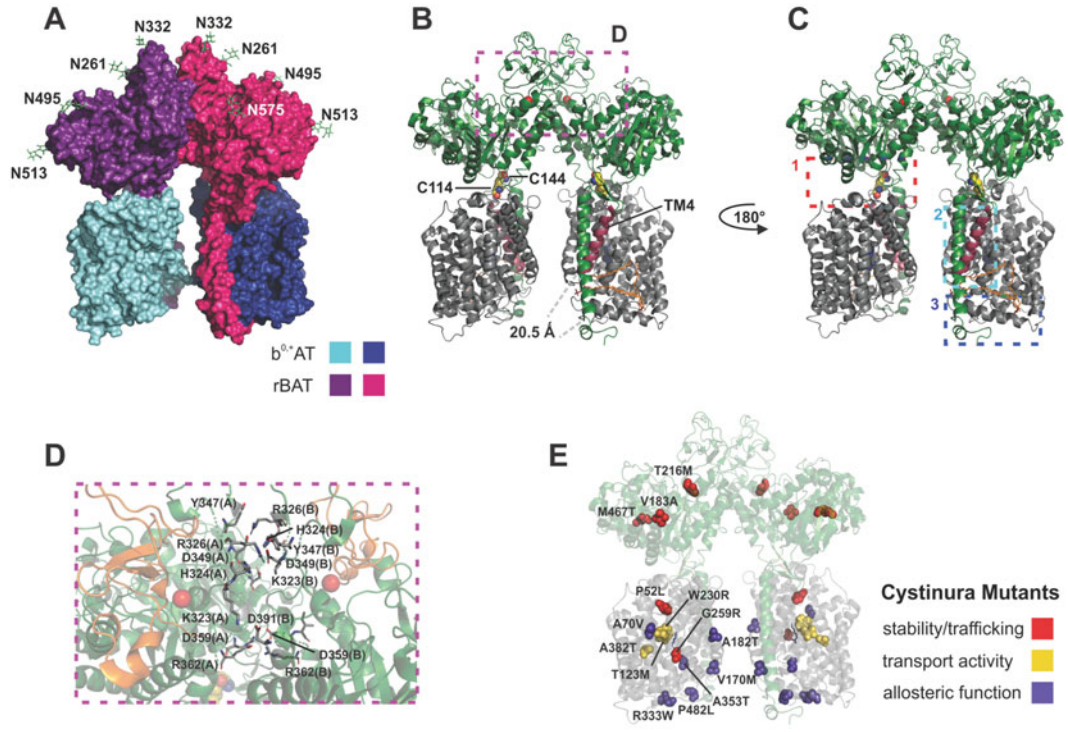

Fig. 4 Structure, interactions and cystinuria mutants of $b^{0,+}$AT-rBAT

The human $b^{0,+}$AT-rBAT complex is formed by a dimer of heterodimers with each dimer formed by one $b^{0,+}$AT translocator subunit and one rBAT ancillary subunit. All structures show are bound by Arg in an inward open conformation (PDB 6LI9) and solved to an average of 2.3 Å RMSD. (**a**) The subunit composition of the $b^{0,+}$AT-rBAT dimer of heterodimers is shown with subunits rendered as a surface (van der Waal) representation and coloured according to the key. N-linked glycosylation sites in rBAT are labelled. (**b** and **c**) The structural architecture of the $b^{0,+}$AT-rBAT heteromer (6IRT) demonstrated through a single 180° rotation. The two subunits are linked by a conserved disulfide bridge between Cys114 ($b^{0,+}$AT) and Cys144 (rBAT), (sphere models, C atoms, grey; O atoms, red; N atoms, blue; S atoms, yellow). The rBAT subunits are coloured green, while the two contacting TM spanning α-helices are coloured red ($b^{0,+}$AT, TM4) and green (rBAT). The lipids mediating $b^{0,+}$AT H4 and rBAT intracellular helix interactions at the inner membrane leaflet are coloured orange. A calcium ion is located in each rBAT subunit in the EC domain (red). The coloured boxes labelled 1 (red, EC interface), 2 (cyan, $b^{0,+}$AT TM4-rBAT TM), and 3 (blue, $b^{0,+}$AT intracellular H4 with rBAT intracellular domain) outline the 3 regions of interaction between individual heteromer subunits (**c**). The magenta box (**b**) shows the interface between the two rBAT subunits which mediate the formation of the dimer of heterodimers. **Insert d**) The extensive network of polar interactions at interface between the two rBAT subunits. Residues involved in polar interactions are shown as sticks and coloured grey. The domain B of rBAT (orange) is highlighted to contrast it with the regions conserved with the other SLC3 ancillary 4F2hc and homologous to bacterial α-glycosidases. (**e**) Common cystinuria causing mutations rendered as VDW spheres and coloured (see key) according to their functional role in the heteromeric solute carrier $b^{0,+}$AT-rBAT. Residues are shown in both heterodimers but labelled only in the right-hand. The Arg substrate is rendered as a stick (blue)

side of TM3 from $b^{0,+}$AT1. As with the 4F2hc TM domain, the rBAT TM domain tilts away from $b^{0,+}$AT TM4 at the intracellular side creating a significant gap of 20.5 Å. In addition, the head groups of several phosphoslipid molecules mediate interactions across this wide gap at the intracellular side. A third interaction region is formed at the intracellular membrane by the rBAT H4 domain, which interacts via multiple hydrogen and ionic-hydrogen bonds with C-terminal intracellular helix of $b^{0,+}$AT. A fourth contact point in the unique C-terminal of rBAT was noted in one apo structure (6YUP) as making van der Waals contacts with EL2 of $b^{0,+}$AT (Wu et al. 2020). In contrast to LAT1-4F2 structures, a second phosphoslipid also interacts with the end of the C-terminal helix closer to TM12 and does not appear to be involved in stabilising the

heteromeric complex. Interestingly, the rBAT EC domains of heteromeric dimers bind or indicate the presence a calcium ion. Although there is no evidence for a role of calcium in any known rBAT related function, the related GH13 glycoside hydrolase family also bind calcium ions in the region homologous to the subdomain B of rBAT. This raises the possibility that calcium binding is a unique feature of rBAT heteromers conferred by presence of subdomain B, which is absent from 4F2hc (see Fig. 1).

The dimerization interface of the $b^{0,+}$AT-rBAT hertodimers is mediated through several regions of contact in the EC domain of the rBAT molecules (Fig. 4d). No interactions are observed between the heterodimers in the membrane or between the $b^{0,+}$AT subunits (Fig. 4a), which is also reminiscent of another LeuT fold heteromeric solute carrier B^0AT1-ACE2 (Sect. 2.5.4). This interface and the dimerization of heterodimers appears to be very stable, such that mutations in these regions were unable to disrupt the rBAT subunit interactions but only overall complex expression. The specific interactions which contribute most to the dimerization interface are a cation-π stack of R326 between H324 and Y347, hydrogen bonding and salt bridge between R326 and D349, polar bonds between K323 and D391, hydrogen bond network D359 and R362 from both protomers, and a series of hydrophobic interactions throughout the interface. Most interestingly, these interactions occur in regions of rBAT either in or significantly displaced by domain B of rBAT, which is an inserted region absent from the conserved α-glycosidase domains in 4F2hc. This insertion gives a straightforward explanation for the rBAT-mediated dimerization that is absent in the LAT1-4F2hc structures. The authors also provide a putative transport mechanism based on the conservation among rBAT orthologues and MD simulations of two binding pockets observed in $b^{0,+}$AT. The second of these binding pockets, so-called 'pocket 2', is occupied by water and occluded in the Arg-bound inward open structure (6LI9) but, interestingly, is not formed in LAT1-4F2hc.

Four broad functions can be ascribed to the role of rBAT and 4F2hc in heterodimer complex formation, which will be discussed in the following sections: tissue specificity, trafficking, quality control and stability, integration into larger complexes. These diverse roles are likely to be mediated by different domains of ancillaries. When mapped onto models and cryo-EM structures of LAT1-4F2hc (Yan et al. 2019; Lee et al. 2019), $b^{0,+}$AT1-rBAT (Yan et al. 2020a; Wu et al. 2020), or LAT2-4F2hc extracellular domains (Fort et al. 2007; Rosell et al. 2014) the interactions between ancillaries and translocation subunits are dispersed over large regions of the heteromers, with the TM and EC domains seeming to always play a vital role. This is consistent with accumulated biochemical evidence which is outlined below (Sect. 2.1.4).

2.1.3 Tissue Distribution

Heteromeric amino acid transporters display a distinct patterning in their tissue and cellular distribution (Fig. 5). The 4F2hc-associated heteromers are generally localised to the basolateral membrane in epithelial cells but also have a wider tissue expression, being vital amino acid uptake systems in all human tissues (Fotiadis et al. 2013). Reflective of this widespread distribution, 4F2hc mRNA expression has been detected in all tissues assayed (Fagerberg et al. 2014). However, this epithelial polarised expression is not observed in all cases, with LAT1 being localised to both apical and basolateral membrane at the blood-brain barrier (Duelli et al. 2000). The rBAT heteromers are localised to the apical membranes of the small intestine and renal epithelium (Fernandez et al. 2002; Nagamori et al. 2016; Lee et al. 1993; Bertran et al. 1993). This suggests, at least in these organs, the trafficking destination of heteromers is likely to be specified by ancillary subunits (Reig et al. 2002; Furriols et al. 1993; Bertran et al. 1992b; Broer and Palacin 2011). Tissue expression of the two ancillaries overlaps closely with their SLC7 partners, with rBAT expression highest in renal and small intestine epithelium, pancreas and liver (Lee et al. 1993; Bertran et al. 1993; Fernandez et al. 2002; Dave et al. 2004). Indeed, the principle that overlapping expression can be used to identify tissue-specific heteromer pairings led to

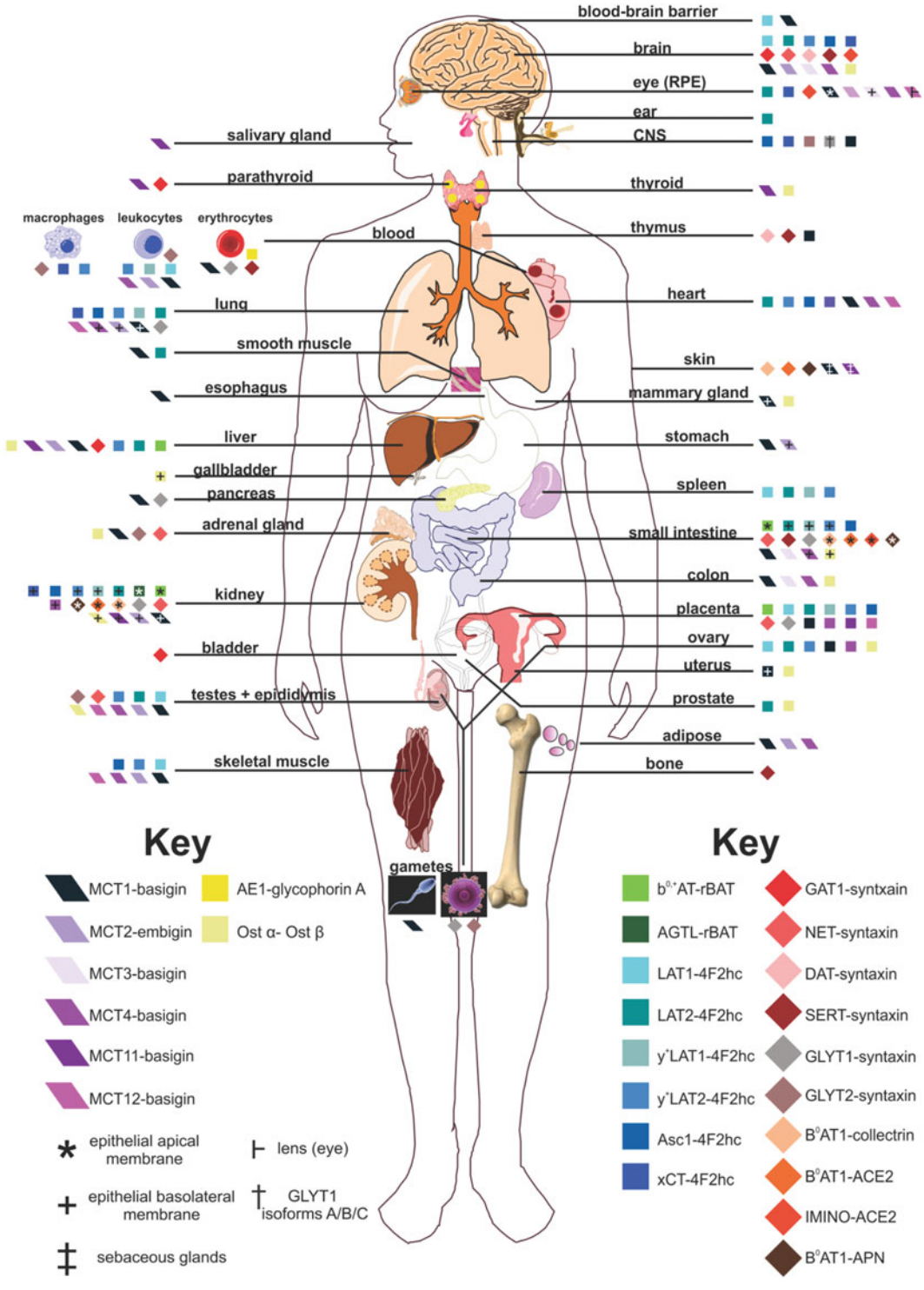

Fig. 5 Tissue distribution of heteromeric solute carriers in humans

The tissue and organ distribution of human heteromeric solute carriers is colour coded according to the key. Only expression demonstrated at the protein level is displayed. As a result the diagram is not exhaustive of expression

the discovery of AGT1-rBAT as the missing renal brush border cystine:anion exchanger (Nagamori et al. 2016; Fernandez et al. 2002). This explained why rBAT is highly expressed in the late proximal tubules (S3 segment) (Kanai et al. 1992; Furriols et al. 1993), whereas $b^{0,+}$AT expression was highest in the early proximal tubules (S1 segment) (Chairoungdua et al. 1999; Pfeiffer et al. 1999a). As a result of this poorly overlapping expression an unknown second partner of rBAT was proposed to account for cystine uptake in the S3 segment and the presence of rBAT-containing heterodimers (Chairoungdua et al. 2001; Feliubadalo et al. 2003; Chillaron et al. 2001; Verrey et al. 2000; Fernandez et al. 2002), subsequently identified as AGT1 (Nagamori et al. 2016).

As befits the ubiquitous expression of its translocation partners, 4F2hc has a wide distribution and is found in most human tissues, including major immuno-protective barriers and many carcinomas as a partner to LAT1 (Quackenbush et al. 1987; Lumadue et al. 1987; Gottesdiener et al. 1988; Yanagida et al. 2001; Ritchie and Taylor 2001; Okamoto et al. 2002; Arancibia-Garavilla et al. 2003; Liu et al. 2003; Kim et al. 2006; Tomi et al. 2005; Nawashiro et al. 2006; Dave et al. 2004; Helboe et al. 2003; Fukasawa et al. 2000; Nakauchi et al. 2000; Bassi et al. 2001; Sato et al. 1999; Nakada et al. 2014) (Fig. 5). Expression of the corresponding light subunit partners correlate relatively well (Fagerberg et al. 2014). For example, LAT1 expression is high in testis, bone marrow, placenta, and brain with highest 4F2hc expression seen in testis, bone marrow, placenta and kidney (Fagerberg et al. 2014). However, co-expression levels have not been systematically quantified for matching profiles.

2.1.4 Trafficking, Protein Folding, Quality Control, and Catalytic Modulation

Either rBAT or 4F2hc are essential for trafficking of the SLC7 translocators to the plasma membrane ((Bassi et al. 2001; Mykkanen et al. 2000; Pineda et al. 1999; Pfeiffer et al. 1998; Mastroberardino et al. 1998; Nakamura et al. 1999; Broer et al. 2001), reviewed in (Palacin and Kanai 2004; Verrey et al. 2004; Broer and Wagner 2002)), and thus can be viewed as affecting both the surface protein expression and its functional outcome, namely the V_{max} of the translocating light subunits. A key interaction between subunits occurs in the ER, where dimerization is concurrent with their biogenesis and trafficking (Fig. 6). The SLC3/SLC7 pairings are the only known heteromeric solute carriers whose interaction is mediated by a single disulphide linkage (Estevez et al. 1998; Palacin et al. 2016; Wang and Tate 1995) (Figs. 3a and 4b). Despite the necessity of the inter-molecular disulphide bond in all SLC3/SLC7 heterodimers, it is not sufficient for correct complex formation and plasma membrane trafficking (Fotiadis et al. 2013; Deora et al. 1998; Pfeiffer et al. 1998; Torrents et al. 1998). This notion is supported by the role of other disulfide bonds in the ER biogenesis of rBAT (see below) and the important role of other domains from both rBAT and 4F2hc in ER exit and trafficking of the heteromer (Palacin et al. 2016).

Reconstitution of purified $b^{0,+}$AT alone results in a fully functional transporter, suggesting that rBAT has no apparent effect on catalytic activity

Fig. 5 (continued) demonstrated by RNA detection methods (e.g. ESTs) and readers should consult RNA and protein tissue expression databases Human Protein Atlas (https://www.proteinatlas.org/), Bgee (https://bgee.org/), Expression Atlas (https://www.ebi.ac.uk/gxa/home) for a comprehensive overview. For expression levels in human tumours see the depmap.org database. In addition to the colour scheme the symbols inside boxes represent the sub-cellular localisation within the tissue/organ indicated, including apical or basolateral membranes for epithelial cells. The localisation of MCT1 in the small intestine epithelium is disputed (Iwanaga and Kishimoto 2015). See text for details and specific references. All GLYT1 expression refers to the GLYT1A isoform except where indicated by the symbol from the key. Abbreviation: RPE (Retinal Pigment Epithelium), CNS (Central Nervous System)

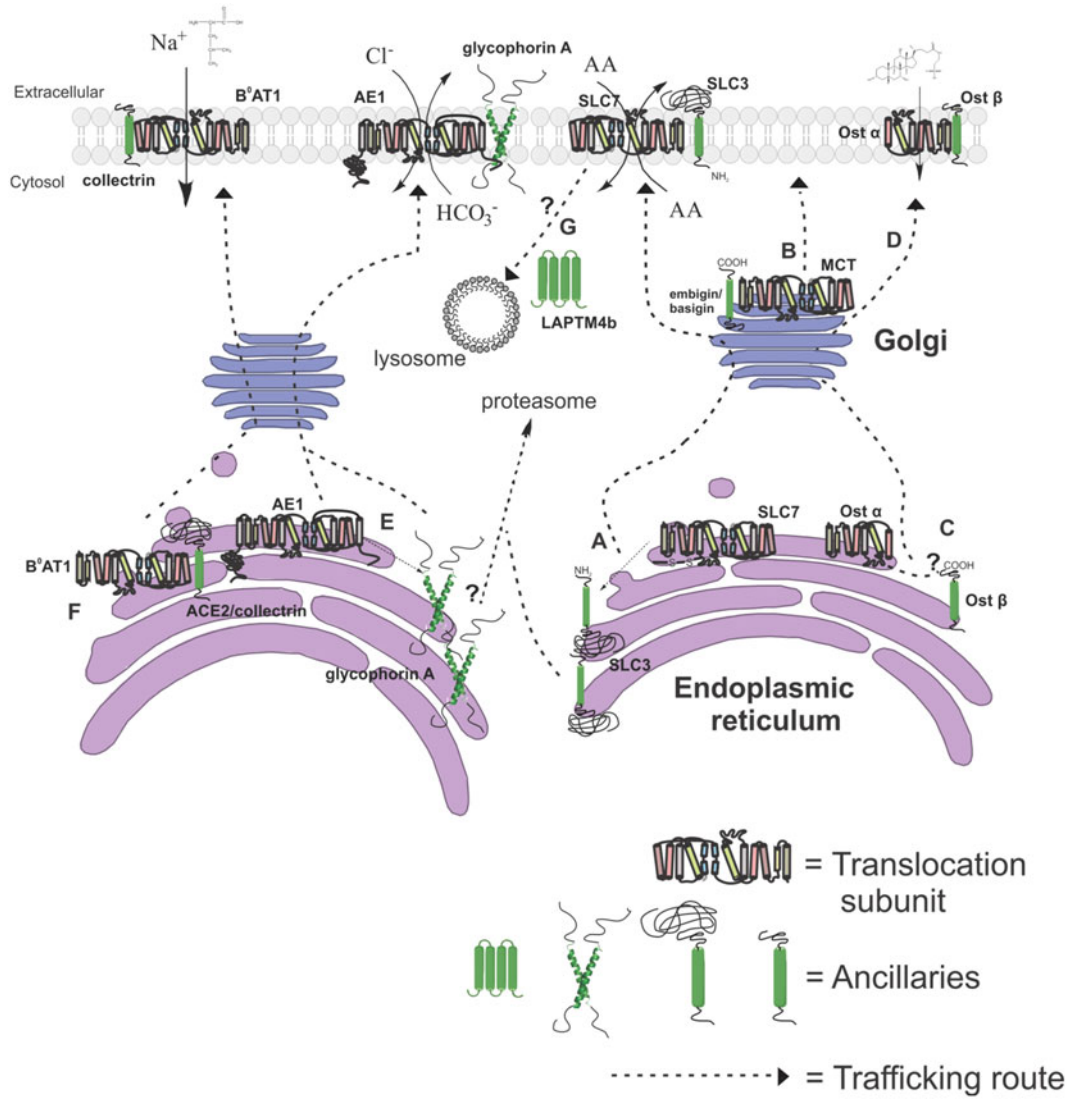

Fig. 6 Selected solute carrier heteromer actions in the secretory pathway

Solute carrier heterodimer modes of action in the secretory pathway are either necessary for trafficking from various organelles to the plasma membrane, for catalytic activation of transport or for both. The location of the heteromer in the diagram represents a known mode of action between the respective subunits. Dash arrows represent essential interactions both before trafficking occurs (between subunits) and for trafficking to the plasma membrane. The question mark (**?**) next to arrows represents an unknown interaction or biological processing. Heteromers shown at the plasma membrane represent an interaction required for activating or significantly modulating the catalytic activity of the translocating subunit. (**a**) The SLC3 (rBAT/4F2hc) requires a SLC7 heteromer partner for both stability and trafficking from the ER; otherwise it is degraded by the proteasome. (**b**) In contrast, SLC16 transporter subunits require embigin and/or basigin for Golgi exit (shown) as well as ER exit (not shown). The 7 TM containing transporter SLC51A (Ost α) requires its ancillary SLC51B (Ost β) for both trafficking to the plasma membrane (**c**) and for substrate translocation at the plasma membrane (**d**). However, the point at which SLC51B controls trafficking is unknown (?). (**e**) AE1 (SLC4A1) does not require glycophorin A to traffic to the membrane in erythrocytes but the reverse is not the case. It is unknown if glycophorin A without AE1 is subject to proteasome degradation. Glycophorin A modulates the kinetic properties of both monovalent and divalent anion transport at the plasma membrane. The ancillary is shown as a homodimer as it is

of the translocating subunit (Bartoccioni et al. 2008). However, rBAT may play more subtle functional roles in catalytic modulation as a cystinuria-causing R365W mutant (Sect. 2.1.6) causes an arginine efflux defect without altering arginine substrate affinity or influx (Pineda et al. 2004). The translocator is also highly stable, probably in the ER, without rBAT (Reig et al. 2002). In contrast, unassociated rBAT is degraded by the proteasome due to the role of $b^{0,+}$AT in the correct ER folding of the extracellular domain of rBAT (Reig et al. 2002; Rius and Chillaron 2012; Bartoccioni et al. 2008). This $b^{0,+}$AT-dependent folding occurs with the consecutive formation of 3 intramolecular disulfide bonds in human rBAT: C242-C273, C571-C666, and C673-C685 – these oxidative links are located in domain B, link domain C with the C-terminal, or in the C-terminal loop, respectively (Rius and Chillaron 2012) (Fig. 1). Mutation of the first two disulphides leads to loss of stability and the inability to leave the ER (Rius and Chillaron 2012; Peter et al. 2000). A double mutant of the C673-C685 disulphide bond retained WT stability but single mutants showed minor disruptions to stability and trafficking (Rius and Chillaron 2012). Deletion of residues C673 to C685, causes heteromer retention and degradation in the ER (Rius et al. 2016). Recent $b^{0,+}$AT-rBAT structures implicated the rBAT C-terminal region and EL2 of $b^{0,+}$AT in heteromer formation (Yan et al. 2020a; Wu et al. 2020) thus providing a rationale for why this deletion may be so disruptive (Sect. 2.1.2). The C242-C273 bond is thought to be co-translationally formed, while the other two are post-translationally formed in the ER lumen (Rius and Chillaron 2012). Once $b^{0,+}$AT-mediated folding is correct, membrane trafficking is rapid and the degradation of rBAT prevented (Rius and Chillaron 2012; Bartoccioni et al. 2008). Thus, in addition to trafficking, ancillary subunits appear to assist in indirect quality control of the light subunit. Formation of the heteromeric complex only occurs if both units are properly folded and exit from the ER only occurs when the complex has formed.

The large extracellular and trans-membrane domains of rBAT play a significant role in complex formation with $b^{0,+}$AT beyond the intramolecular disulfide bonds. Early studies using systematic deletion of the glucosidase-like C-terminal demonstrated rBAT extracellular domains B and C were required for $b^{0,+}$AT-mediated amino acid transport (Deora et al. 1998; Miyamoto et al. 1996; Estevez et al. 1998). However, pre-dating the discovery of the mammalian $b^{0,+}$AT, these findings could be alternatively interpreted as demonstrating the domain specificity for interaction with an unknown endogenous *X.laevis* $b^{0,+}$AT subunit(s) (Chillaron et al. 2001). As mutations in rBAT are the cause of most type I cystinuria cases (Sect. 2.1.6) studying the molecular effects of type I cystinuria mutations has been instructive in understanding $b^{0,+}$AT-rBAT heteromer formation (Chillaron et al. 2010; Sumorok and Goldfarb 2013). The effects of some frequent type I cystinuria-associated mutation of rBAT, located in the TM and extracellular domains, have been studied in some detail (Bartoccioni et al. 2008; Chillaron et al. 1997) including in the recent structure of $b^{0,+}$AT-rBAT (Yan et al. 2020a) (Fig. 4e). Multiple rBAT extracellular domain mutants, including two of the three most common cystinuria-causing mutations, M467T and T216M, will assemble with $b^{0,+}$AT in the ER but are not trafficked. In the $b^{0,+}$AT-rBAT structures these and another rBAT cystinuria mutation V183A impaired the synthesis of protein (Yan et al. 2020a; Wu et al. 2020). By contrast, the rBAT TM domain mutant L89P does not assemble with $b^{0,+}$AT in the ER (Bartoccioni

Fig. 6 (continued) constituted *in vivo*. (**f**) B^0AT1 and other SLC6 heteromers with collectrin, ACE2 or APN are thought to form in the ER, although this has not been established experimentally. Ancillaries are involved in trafficking the translocation subunit to the plasma membrane but are required, at least for collectrin, for catalytic activation of the transporter. (**g**) More recently SLC7-SLC3 heteromers (specifically LAT1-4F2hc) have been shown to be re-directed by LAPTM4b to lysosomes to facilitate leucine uptake for mTORC1 pathway activation. It is not known if this interaction is obligatory for heteromer activity or just for re-direction to lysosomes

et al. 2008), consistent with evidence showing that heterodimerisation requires the TM domain (Franca et al. 2005). Moreover, it appears likely that multiple extracellular domain residues are involved in protein folding (Chillaron et al. 2010), which is consistent with the recovery of heterodimer transport activity in the rBAT mutant R365W when the growth temperature was decreased from 37 °C to 33 °C (Pineda et al. 2004). Although $b^{0,+}$AT-rBAT and 4F2hc-associated transporters are fully functional as heterodimers, $b^{0,+}$AT-rBAT also forms heterotetramers of two disulphide-linked heterodimers held together by non-covalent association *in vivo* (Fernandez et al. 2006). Heterotetramers are not required for translocation of substrate, suggesting the larger oligomeric structure of SLC7-SLC3 transporters may serve other biological functions. For example, several type I cystinuria-linked rBAT mutants demonstrate ER retention independent of $b^{0,+}$AT-rBAT heterodimer formation but correlated with heterotetramer formation (Bartoccioni et al. 2008; Chillaron et al. 2010). Of five verified N-linked glycosylation sites in human rBAT (Fig. 4a) (Yan et al. 2020a), only N575 in the ectodomain appears to be necessary and sufficient for correct stability and trafficking of the heterodimer by delaying the kinetics of protein maturation but not overall protein synthesis efficiency (Rius et al. 2016). It was hypothesised that N575 interacts with residue N679 during maturation; the two residues being in proximity due to the C-terminal intramolecular disulfide bond between C571 and C666 (Rius et al. 2016). A second N-linked glycosylation site at N214 has been speculated to impair folding of rBAT in the ER due to its proximity to the cystinuria causing mutant T216M (Rius et al. 2016), however N-glycans were not detected at this site in cryo-EM resolved structures of rBAT (Yan et al. 2020a). Degradation of the numerous cystinuria- and intramolecular disulfide-associated mutants of rBAT is at least in part mediated via the ERAD pathway. Thus post-assembly proof reading of correctly formed heteromers requires the calnexin chaperone system (Bartoccioni et al. 2008; Rius and Chillaron 2012). There is also a strong indication that $b^{0,+}$AT assists the correct folding of rBAT following the initial contact between the subunits (Rius et al. 2016; Rius and Chillaron 2012; Bartoccioni et al. 2008). It is tempting to speculate that the calnexin system requires intensive contacts with the rBAT protein and glycosylation sites, which is difficult to achieve in the case of integral membrane proteins with small inter-helical loops and lack of glycosylation such as $b^{0,+}$AT. Assembly into larger complexes with large ectodomains could facilitate quality control in the ER. Although it is generally assumed that rBAT plays little role in substrate translocation this has never been investigated in context where the trafficking/stabilisation of the complex has been isolated from substrate transport.

In contrast to rBAT, the role of the extracellular domains of 4F2hc in heteromer formation is less clear and may be partner specific. For LAT1 initial biochemical results were conflicting; deletion of the 4F2hc extracellular domain has been variously shown to slow light subunit trafficking without affecting total membrane expression and function (Broer et al. 2001), to be totally replaceable and unnecessary (Franca et al. 2005) or be required for LAT1 function (Fenczik et al. 2001; Yan et al. 2019). These discrepancies have been greatly clarified by the recent elucidation of several single-particle cryo-EM structures of the human LAT1-4F2hc complex (Lee et al. 2019; Yan et al. 2019) where interactions between the subunits seem to be necessary and extensive between the 4F2hc EC domain and LAT1 EC loops (Sect. 2.1.2). By contrast, the loss of even small sections of the 4F2hc ectodomain results in absolute loss of membrane expression and activity when co-expressed LAT2 and y^{+}LAT2 (Broer et al. 2001). Likewise, the disruption of the 4F2hc-light chain intermolecular disulfide bond has shown translocator and species specific results. A C109S mutation in human 4F2hc results in the loss of y^{+}LAT1-4F2hc expression and transport activity (Kolesnikova et al. 2001; Torrents et al. 1998), while also reducing N-glycosylation, protein maturation, and overall expression of the ancillary (Fort et al. 2007). By contrast, the equivalent mutant of rabbit 4F2hc

has no effect on transporter activity of LAT1 (Boado et al. 2005). Where they have been studied, the N-terminal and TM domains of 4F2hc were also found to be essential for interaction and functioning of the translocation subunits, as, for example, with LAT1 (Broer et al. 2001; Franca et al. 2005). Human 4F2hc contains a second cysteine at C330, the mutation of which has no effect on heteromer activity (Boado et al. 2005). 4F2hc is, therefore, unlikely to show similar dependence on intramolecular Cys-Cys oxidation for biogenesis as rBAT (Rosell et al. 2014).

The primary role of 4F2hc is trafficking of the translocator complex to the surface (e.g. see (Scalise et al. 2018)). Limited information is available whether it has additional function in transport catalysis. Pfeiffer et al. (Pfeiffer et al. 1998) demonstrated that both human and *Schistosoma mansoni* y^+LAT2 homologues with disrupted intermolecular disulfide bonds to human 4F2hc have little reduction in plasma membrane protein levels but 30–80% reductions in maximal transport rates of leucine and arginine. Evidence from purified LAT1-4F2hc heterodimers with the ancillary removed is contradictory: when purified from HEK293F cells the complex is entirely non-functional in the absence of 4F2hc (Yan et al. 2019), yet in liposomes reconstituted from SiHa squamous cell carcinoma cells LAT1 alone displayed the same transport properties as the heteromer (Napolitano et al. 2015). Further clarification of the role of 4F2hc in LAT1 and other light chain transport activity is therefore necessary, but one important difference in these results is the lipid composition of the reconstituted proteoliposomes. Yan et al. (2019) reconstituted LAT1 from HEK293F cells into liposomes with 10% (wt %) cholesterol, whereas Nepolitano et al. (Napolitano et al. 2015; Scalise et al. 2013) do not appear to have added cholesterol to SiHa cell-derived purifications. The potential importance of cholesterol as an allosteric modulator of LAT1-4F2hc has been demonstrated by depleting membrane cholesterol using methyl-β-cyclodextrin which decreases the V_{max} (but not the K_m) of L-DOPA uptake. Addition of 10% cholesterol increased the transport activity of LAT1-4F2hc (Yan et al. 2019), while the cholesterol analogue cholesteryl hemisuccinate (CHS) was required for stable purification of LAT1 with 4F2hc (Dickens et al. 2017). Evidence from LAT2-4F2hc purified into detergent micelles showed that functional reconstitution of human LAT2/4F2hc, but not of human LAT2 alone, was possible (Rosell et al. 2014). In these experiments LAT2/4F2hc but not LAT2 was incorporated into the liposomes. The idea behind this experiment is that LAT2 alone was too unstable to be incorporated in the liposomes and suggests the ancillary subunit as a whole, or its ectodomain, plays a role in LAT2 protein stability during biogenesis rather than directly in functional modulation. This result from LAT2-4F2hc raises the possibility that the reconstitution of LAT1 in the absence of 4F2hc by Yan et al. (2019) did not occur and that, therefore, the authors' interpretation that LAT1 alone was non-functional is not necessarily correct. The incorporation of LAT1 into liposomes in experiments that showed no LAT1 transport activity following reconstitution was not specifically confirmed and, therefore, the alternative explanation cannot be ruled out.

Surprisingly little research has been conducted on trafficking determinants of the translocating subunits themselves, with investigations focused on the effect of a few of the most frequent cystinuria-causing mutations. Cystinuria (OMIM 220100) is caused by homozygous mutations in either subunit of the $b^{0,+}$AT-rBAT heteromer (Sect. 2.1.5). The most common cystinuria-causing mutation in $b^{0,+}$AT (G105R) is located at the boundary of TM1 and IL1 loop of the transporter distal to any known heteromer interacting regions (see Fig. 4c) (Yan et al. 2020a). The strong evolutionary conservation of this residue through all known LeuT/APC transporters and other related families (Shaffer et al. 2009; Reig et al. 2002), suggests it has a conserved role intrinsic to the translocation subunit rather than heteromer formation. Function of another common cystinuria-causing mutant A182T, can be recovered if $b^{0,+}$AT is reconstituted into proteoliposomes, suggesting defective trafficking and a possible role in interacting with rBAT (Reig et al. 2002). Almost

50% of $b^{0,+}$AT cystinuria-associated mutants demonstrate no transport activity but normal plasma membrane localisation (Chillaron et al. 2010). Based on the position of many of these mutants within the highly conserved binding site of the recently elucidated $b^{0,+}$AT1 structures it is likely they represent functional mutants not involved in heteromer formation (Yan et al. 2020a) (Fig. 4e). Cystinuria mutants T123M, W230R, and A382T were located in the substrate binding pockets or transport pathway of the $b^{0,+}$AT subunit. Other $b^{0,+}$AT cystinuria mutants including A70V, V170M, A182T, R333W, A354T, and P482L were dispersed away from the binding site/translocation pathway but retained none or minimal functionality – implying allosteric effects in heteromer transport activity. A third class of $b^{0,+}$AT cystinuria mutants P52L and G259R are not stable, indicating these residues are involved in heteromer biogenesis. A novel V480-P-P482 C-terminal motif has been discovered in $b^{0,+}$AT that was essential to traffic the $b^{0,+}$AT-rBAT heteromer from the ER to the cis-Golgi (Ganapathy 2009; Sakamoto et al. 2009). Mutation of this motif did not affect assembly of the complex in the ER. As this C-terminal motif is entirely conserved across all SLC7 translocators, the authors hypothesised it may represent a universal quality control locus for post-assembly trafficking. Indeed, these residues are located in the IL loop between TM9 and 10 of $b^{0,+}$AT, in close proximity to the truncated intracellular terminal of rBAT in the cryo-EM structures of the complex (Yan et al. 2020a).

2.1.5 Integration into Larger Complexes

4F2hc sits at the centre of a regulatory nexus integrating cellular metabolism via mTORC1 signalling and cell growth due to its role as an activator that potentiates β1 integrin signalling upon ligand binding (Boulter et al. 2018; Bertero et al. 2018; de la Ballina et al. 2016; Estrach et al. 2014; Rainero et al. 2015; Ata and Antonescu 2017; Feral et al. 2007; Kolesnikova et al. 2001; Henderson et al. 2004). Activated lymphocytes undergo clonal expansion to provide a large set of cells that can combat specific pathogens. This clonal expansion critically depends on the ability of 4F2hc to potentiate β1 and β3 integrin signalling, and much less on the ability of 4F2hc to associate with transporter light chains. Amplification of integrin signalling via 4F2hc is also critical for cancer cell proliferation and metastasis (Cantor and Ginsberg 2012). 4F2hc binding to LAT1 is mainly via its extracellular domain, while interaction with integrin β_1 and β_3 is mediated through the TM/cytosolic domains (Fenczik et al. 2001; Henderson et al. 2004; Prager et al. 2007). The 4F2hc protein is part of an even larger metabolic activation complex, which includes LAT1, integrins, Na^+,K^+-ATPase, CD147 and the monocarboxylate transporter 1 (MCT1) (Xu and Hemler 2005). A 4F2hc-xCT metabolon appears to be essential for the post-entry stage of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) infection (OMIM 148000) (Veettil et al. 2008; Kaleeba and Berger 2006). The virus envelope glycoproteins gB and gpK8.1A recognise a macromolecular complex including both xCT and 4F2hc, along with multiple integrin α/β dimers (Veettil et al. 2008). Interestingly, mutation of 4F2hc disulfide bond acceptor (C109S) causes a loss of β-integrin association, suggesting the formation of 4F2hc-β-integrin metabolons are dependent on full heteromeric transporter formation (Kolesnikova et al. 2001). Ingeniously, the virus induces xCT expression via microRNA-led suppression of a transcriptional anti-oxidant control and other cell-cycle networks to facilitate cell entry, metastasis and tumour angiogenesis; thereby simultaneously enhancing new infections and virus survival to spread infected cells (Qin et al. 2010; Dai et al. 2017; Li et al. 2017). A LAT2-4F2hc-specific macromolecular complex has been proposed which binds the cell adhesion molecule ICAM-1 at the C-terminal of the translocator (Yan et al. 2008; Liu et al. 2003). The compact structure of the translocator domain largely prevents complex interactions with more soluble proteins, such as components of signal transduction cascades. Thus, ancillary proteins allow integration into larger complexes and metabolons. Where they have been studied, signalling-associated post-translational

modification have been located largely in ancillaries, such as serine phosphorylation in the case of y^+LAT1-4F2hc/y^+LAT2-4F2hc and tyrosine phosphorylation of 4F2hc (Rotmann et al. 2007; D'Agostino et al. 2018).

The quaternary structure of 4F2hc-containing complexes may change depending on the specific combination of light and heavy subunits but the evidence for several transporters is complicated by the methodology used to conclude larger complex stoichiometry. When investigated using Blue Native gel electrophoresis, xCT-4F2hc appears to be a single heteromer in contrast to $b^{0,+}$AT-rBAT, where the evidence supports that rBAT drives the formation of dimers of heterodimers (Fernandez et al. 2006). This suggested that oligomerisation of SLC3-SLC7 into higher complexes may be specified by the heavy chains – a conclusion further supported by inducing hetero-oligomerisation following the swap of the biological partner of xCT (4F2hc) for rBAT in a xCT-rBAT chimera (Fernandez et al. 2006). The evidence from LAT1 and LAT2 for this hypothesis is, however, more ambiguous. The multiple recent LAT2-4F2hc (Rosell et al. 2014) or LAT1-4F2hc (Yan et al. 2019; Lee et al. 2019) structures solved as single heteromers suggest LAT-composed complexes are defined by interaction with 4F2hc. However, functional homodimerisation of LAT1 occurs when the transporter is purified without 4F2hc co-expression from *E. coli* (Napolitano et al. 2017). Similarly, homodimers of the recombinant EC domain of 4F2hc have been identified from protein purified in mammalian HeLa cells (Fort et al. 2007). A simple structural explanation for whether SLC7-SLC3 heteromers formation is ancillary subunit specified was provided by the recent cryo-EM structure of $b^{0,+}$AT-rBAT as a dimer of heterodimers in contrast to the single heteromer structures of 4F2hc-dependent complexes (Yan et al. 2019, 2020a; Lee et al. 2019; Wu et al. 2020). The dimerization of $b^{0,+}$AT-rBAT heteromdimers was mediated wholly by interactions between the two rBAT EC domains, particularly in and close to domain B, which is absent from 4F2hc.

More recently, additional ancillaries of LAT1 have been uncovered, which appear to re-direct the LAT1-4F2hc heteromer to lysosomes (Milkereit et al. 2015; Weng et al. 2018). The 4TM domain lysosomal LAPTM4b and actin-associated protein Girdin both re-direct LAT1-4F2hc from plasma membrane to lysosomes in order to mediate amino acid flux and to regulate mTOR signalling (Fig. 6). The interaction appears to occur between TM3 of LAPTM4b and 4F2hc and is facilitated by the sphingolipid ceramide (Zhou et al. 2018). It is unknown to what extent these ancillaries represent obligatory ancillary subunits as part of a larger SLC7-SLC3 metabolon complex or represent a conditional complex for re-direction to lysosomes when leucine is needed for mTORC1 activation. It is noteworthy, however, that LAPTM4a has also been shown to interact with and control the endosomal recycling of additional solute carriers such as the organic cation transporter OCT2 (SLC22A2) (Grabner et al. 2011). This processing and sub-cellular distribution of transporters more generally seems to be controlled by larger multi-protein complexes involving multiple 4 TM domain proteins or 'tetraspanins', perhaps giving insight into wider protein complex networks that control SLC7-SLC3 expression and recycling (Schulze et al. 2017; Snieder et al. 2019).

2.1.6 Pathophysiological Aspects

Heteromer formation of SLC3/SLC7 transporters is illustrated by the rare human disorder cystinuria (Table 4). Cystinuria (OMIM 220100) is a disease characterised by renal excretion of cystine, the poor solubility of which typically results in the formation of renal calculi (kidney stones) causing obstruction, infection and, in severe cases, chronic kidney disease (1–2% of all cases) (Chillaron et al. 2010; Rosenberg et al. 1966). The disease is caused by 176 single-point mutations, deletions, insertions, splice variants and exon duplications, in rBAT (over 600 mutant alleles) and 114 in $b^{0,+}$AT (over 500 alleles) with mutations in both subunits estimated to present in nearly 85% of patients ((Nagamori et al. 2016; Rius et al. 2016; Pineda et al. 2004; Font et al. 2001; Mizoguchi et al. 2001; Palacin et al. 1998b, 2000; Pfeiffer et al. 1999a; Rajan et al. 1999; Chairoungdua et al. 1999; Feliubadalo et al.

1999; Chillaron et al. 1997; Gaildrat et al. 2017; Wong et al. 2015; Bisceglia et al. 2010; Bartoccioni et al. 2008; Font-Llitjos et al. 2005; Calonge et al. 1994; Harnevik et al. 2001, 2003; Barbosa et al. 2012; Eggermann et al. 2007; Dello Strologo et al. 2002; Gasparini et al. 1995; Shigeta et al. 2006; Skopkova et al. 2005; Botzenhart et al. 2002; Gitomer et al. 2000; Miyamoto et al. 1995; Pras et al. 1995; Mahdavi et al. 2018; Markazi et al. 2016) reviewed in (Chillaron et al. 2010; Sahota et al. 2019; Palacin et al. 2005)). These mutations lead to defective transport of cystine and dibasic amino acids across the apical membrane of epithelial cells of the renal proximal tubule and the small intestine (Feliubadalo et al. 1999; Calonge et al. 1994). Cystinuria is classified into two types, type A (mutations in both rBAT alleles), type B (mutation in both alleles of $b^{0,+}$AT). A mixed type A/B can occur, but does not cause cystinuria when heterozygous in both genes, instead mixed type cystinuria is typically caused by AAB alleles or ABB alleles (Chillaron et al. 2010; Feliubadalo et al. 1999). Another nomenclature based on aminoaciduria in heterozygotes is still in use designated as type I or non-type I (Dello Strologo et al. 2002). The majority of type I individuals have homozygous mutations in the SLC3A1 gene encoding rBAT (<20% caused by SLC7A9 mutations) and heterozygotes display normal urinary excretion of amino acids. In non-type I cystinuria most mutations lie in the SLC7A9 gene (<5% in SLC3A1), heterozygotes in this cohort display hyper-excretion of cystine and dibasic amino acids, and, occasionally, kidney stones. A mysterious lack of mutations in either rBAT or $b^{0,+}$AT of some cystinuria sufferers is not due to mutations in the other renal cysteine transporter light chain AGT1 and remains of unknown molecular cause (Olschok et al. 2018).

Loss of function mutations in y^+LAT1 (SLC7A7) result in the rare, recessive disorder Lysinuric Protein Intolerance (LPI, OMIM 222700) (Torrents et al. 1999; Bassi et al. 1999; Borsani et al. 1999), which was first diagnosed in 1965 (Torrents et al. 1999; Perheentupa and Visakorpi 1965). In y^+LAT1 59 mutations from over 120 independent families (181 individuals) have been identified as causing LPI (Palacin et al. 2004; Mykkanen et al. 2000; Torrents et al. 1998, 1999; Sperandeo et al. 2008; Zhang and Cao 2017; Noguchi et al. 2016; Carpentieri et al. 2015; Ko et al. 2012; Font-Llitjos et al. 2009; Shoji et al. 2002; Borsani et al. 1999). No mutations associated with LPI are found in 4F2hc (SLC3A2), the heavy subunit associated with y^+LAT1. This can be explained by the embryonic lethal phenotype of 4F2hc ko mice and is probably due to the ancillary being the obligatory partner for numerous SLC7 translocators (Tsumura et al. 2003). Numerous other disorders are caused by mutations in the SLC7 subunits of the SLC7-SLC3 heteromers but these do not appear to disrupt complex formation (Table 4).

2.2 SLC16 Monocarboxylate/H^+ Heteromeric Carriers with Basigin and Embigin

2.2.1 General Properties and Structural Aspects

The monocarboxylate transporter (MCT) family comprises 14 members in humans and has been identified in all eukaryotic species sequenced (Halestrap 2013b; Price et al. 1998). All members are part of the large Major Facilitator Superfamily (MFS) and have 12 TM α-helical domains. Based on solved MFS structures, MCTs undergo a "rocking switch" type translocation cycle suggested by the MFS fold and repeated twofold pseudo-symmetry (Quistgaard et al. 2016; Manoharan et al. 2006; Bai et al. 2018; Drew and Boudker 2016; Bosshart and Fotiadis 2019; Yan 2015). Eight human members have physiological substrate specificities and/or mechanisms reported: MCT1 (SLC16A1) (Garcia et al. 1994; Carpenter and Halestrap 1994; Broer et al. 1998; Poole and Halestrap 1992, 1994), MCT2 (SLC16A7) (Garcia et al. 1995; Broer et al. 1999), MCT3 (SLC16A8) (Grollman et al. 2000; Yoon et al. 1997; Price et al. 1998), MCT4 (SLC16A3) (Manning Fox et al. 2000; Dimmer et al. 2000; Price et al. 1998), MCT8 (SLC16A2) (Friesema et al. 2003; Lafreniere et al. 1994), MCT10/TAT1

(SLC16A10) (Friesema et al. 2003; Friesema et al. 2006), MCT11 (SLC16A11) (Rusu et al. 2017) and MCT12 (SLC16A12) (Abplanalp et al. 2013). MCTs 1-4 are all proton-dependent monocarboxylate symporters with a H^+: substrate stoichiometry of 1:1 (see reviews (Halestrap 2013a, b; Halestrap and Wilson 2012; Halestrap 2012; Adijanto and Philp 2012) and Table 1). MCT11 has been reported as a pyruvate-H^+ cotransporter based on a single indirect assay (Rusu et al. 2017). The remaining transporters are uniporters or remain ill-characterised: MCT12 is a kidney basolateral membrane and retinal creatine uniporter (Abplanalp et al. 2013); MCT8 is a high affinity thyroid hormone uniporter (Visser et al. 2011; Schwartz and Stevenson 2007); and TAT1 (MCT10) is a kidney and small intestine basolateral uniporter for aromatic amino acids (Kim et al. 2002b). MCT6 has been shown to transport the organic anions nateglinide, probenecid, and bumentanide in a pH- and membrane potential-dependent manner that has led to the speculation it being a generic intestinal xenobiotic transporter (Murakami et al. 2005; Kohyama et al. 2013; Jones et al. 2017). In addition, zebrafish MCT7 (SLC16A6) has been implicated as a ketone body transporter (Hugo et al. 2012), and MCT9 (SLC16A9) as a carnitine transporter based on serum level changes in a single nucleotide polymorphism (SNP) of the encoding gene (Suhre et al. 2011). Some researchers have chosen to divide MCT transporters into two subfamilies (e.g. (Rusu et al. 2017)), namely H^+-symporters and uniporters. However, this classification is based on transport mode and is not entirely consistent with phylogenetic analysis of the MCT family (Rusu et al. 2017; Halestrap 2013b; Liu et al. 2008). For example, MCT11 is phylogenetically of equal distance to both MCT10/MCT8 and MCTs1-4, while MCT6 is closer to the MCT1-4 grouping – yet in both cases the transporters have been suggested to be H^+-dependent.

Monocarboxylate transporters 1–4, 11 and 12 require either basigin (BSG, CD147, EMMPRIN) or embigin (EMB) as ancillary proteins for correct plasma membrane expression ((Halestrap 2013a; Rusu et al. 2017; Abplanalp et al. 2013; Castorino et al. 2011b; Kirk et al. 2000; Wilson et al. 2005; Poole and Halestrap 1997; Wilson et al. 2013), Fig. 6). Both embigin and basigin are members of the Immunoglobulin (Ig)-like or BSG family within the overall Immunoglobulin superfamily (Muramatsu 2016; Iacono et al. 2007). A third member of the same family neuroplastin has been identified *in vitro* as an ancillary for MCT2 (Wilson et al. 2013). Embigin, basigin and neuroplastin are all type I single pass membrane proteins containing a short intracellular C-terminal, a single TM α-helix, and larger extracellular glycosylated N-terminal domain with 2 or 3 immunoglobulin folds depending on the proteins and their splice variants (Muramatsu 2016; Halestrap 2013b, 2012; Iacono et al. 2007) (Fig. 1). Genomic analysis shows they are closely related genes of the same immunoglobulin superfamily branch (Tachikui et al. 1999; Ozawa et al. 1988). The short isoform of basigin, known as basigin-2 (UniProt P35613-2), is ubiquitously expressed and contains two immunoglobulin-like domains Ig1 and Ig2. The long isoform basigin-1 (P35613-1) is retina-specific and contains an additional Ig0 domain at the N-terminal more distal from the membrane (Ochrietor and Linser 2004; Ochrietor et al. 2003; Redzic et al. 2011). Basigin was discovered through several, functionally independent, research streams and, as a result, has been known by numerous names, including gp42, EMMPRIN, OX-47, HT7, neurothelin, M6, HAb18G, and, as a leukocyte differentiating antigen, by its cluster of differentiation (CD) identifier, CD147. The human BSG gene encodes the basigin protein on chromosome 19 p13.3 using 10 exons. Embigin also has two expressed isoforms; isoform 1 (UniProt Q6PCB8-1) with 327 residues is longer than isoform 2 with 277 residues (Q6PCB8-2). Both isoforms are predicted to contain two Ig domains, and unlike basigin, the longer isoform 1 does not contain an additional Ig domain. Isoform 1 of embigin was first identified from mouse teratocarcinoma and early embryonic cell lines (Huang et al. 1993; Ozawa et al. 1988; Tachikui et al. 1999). Genomic analysis showed that the embigin ORF contains 9 exons located on chromosome 5 in humans and chromosome 13 in mouse (Tachikui et al. 1999).

Neuroplastin (NPTN, SDR1) exists as two major isoforms analogous to the basigin isoforms: a longer 3-Ig domain isoform 2 (Q9Y639-2) and a shorter 2-Ig domain isoform 1 (Q9Y639-1), missing the N-terminal Ig domain (Langnaese et al. 1997; Hill et al. 1988). Both neuroplastin isoforms facilitate increased MCT2 surface expression (Wilson et al. 2013). Several reviews covering the discovery, functions, genetics, and pathologies of basigin (Muramatsu 2016; Iacono et al. 2007; Muramatsu 2012; Grass et al. 2014; Muramatsu and Miyauchi 2003) and neuroplastin (Beesley et al. 2014a; Owczarek and Berezin 2012) are available. Embigin has not been covered in depth by any recent reviews, although older reviews covering the BSG family are available (Iacono et al. 2007).

2.2.2 Tissue-Specific Expression

Tissue expression of MCT1 is ubiquitous and for MCTs 2 and 4 is widespread. For MCT3 expression is restricted mainly to the retinal pigment epithelium (RPE) and a few other tissues (Philp et al. 2003b). The reader is referred to Fig. 5 and a review comprehensively summarising human MCT protein expression (Fisel et al. 2018). Several hormones have been shown to increase surface expression of MCT1 and MCT4 in rats. Testosterone induces plasma membrane expression and lactate transport of MCT1 and MCT4 in rat skeletal muscle *in vivo*, while Triiodothyronine (T_3) increased only MCT4 surface expression and transport rate (Enoki et al. 2006; Wang et al. 2003b). Testosterone also increased rat heart MCT1 and MCT4 transport activity despite the absence of any concurrent increase of plasma membrane expression (Enoki et al. 2006). This increased activity without change of protein expression points to either allosteric modulation by testosterone directly or, possibly, testosterone-dependent modulation via ancillary proteins. Interestingly, MCT11 expression is very high in numerous endocrinal glands (Fig. 5).

Basigin expression is ubiquitous, befitting its wide-ranging functions in reproduction, development, the survival of retinal photoreceptors (long isoform), hippocampal/amygdala function, synaptic vesicle release, activation of T-lymphocytes, and enhancing the proliferation and invasion properties of many cancer types ((Riethdorf et al. 2006; Le Floch et al. 2011; Jiang et al. 2001; Marieb et al. 2004; Su et al. 2009) reviewed in (Muramatsu 2016; Iacono et al. 2007; Hanahan and Weinberg 2011; Weidle et al. 2010)). Many of these functional roles overlap with the heavy reliance on MCTs (especially MCT4), particularly in the release of lactate from rapidly proliferating and glycolytic cells (Baba et al. 2008; Le Floch et al. 2011). Embigin expression is more restricted, being limited to embryonic cells, prostate and mammary glands, and various carcinomas (Fan et al. 1998; Guenette et al. 1997; Huang et al. 1990, 1993). Neuroplastin, is widely expressed at synapses and is involved in cell-cell adhesion, neurite growth (Owczarek et al. 2011) and long-term synaptic plasticity (Smalla et al. 2000) in the nervous system (reviewed in (Beesley et al. 2014b; Owczarek and Berezin 2012)). Thus MCT2 is likely to have different partners in different tissues. The expression of the longer neuroplastin isoform 2 protein is restricted to the brain cortex and cerebellum, whereas isoform 1 displays ubiquitous expression, although rodent and human brains show significant differences in tissue distribution of the two isoforms (Bernstein et al. 2007; Kreutz et al. 2001; Marzban et al. 2003; Owczarek and Berezin 2012; Beesley et al. 2014a).

2.2.3 Trafficking, Protein Folding and Quality Control

Embigin and basigin are essential for correct trafficking to the plasma membrane but less likely for protein stability as MCTs are expressed intracellularly without ancillary association (Wilson et al. 2005; Kirk et al. 2000; Philp et al. 2003a; Poole and Halestrap 1997). Both ancillaries have been shown to control trafficking from the Golgi and ER ((Wilson et al. 2005; Kirk et al. 2000), Fig. 6). Concomitant expression or interaction of MCTs 1, 3, and/or 4 with basigin has been confirmed in spermatozoa (Mannowetz et al. 2012), erythrocytes (Poole and Halestrap 1997), heart (Kirk et al. 2000), retina (Philp et al. 2003a; Hori et al. 2000), pancreas (acinar cells) (Zhao

et al. 2001), the brain (Medin et al. 2019; Ideno et al. 2018) and numerous carcinomas (Le Floch et al. 2011; Slomiany et al. 2009; Pinheiro et al. 2008, 2009a, b; Schneiderhan et al. 2009; Bovenzi et al. 2015; Gallagher et al. 2007). Embigin interaction with MCT2 has been established in rat erythrocytes (Wilson et al. 2005), murine spermatozoa (Mannowetz et al. 2012), various mammalian transfected cell lines, and in *X. laevis* oocytes (Ovens et al. 2010b). There is no indication, unlike SLC7-SLC3 heteromers, that MCT ancillaries regulate localisation in polarised epithelial cells. Rather, localisation to apical and basolateral membranes is controlled by sorting signals on the translocation subunits as, for example, on MCT3 and 4 in RPE cells (Castorino et al. 2011a). Similarly, MCT1 and MCT3 both require basigin for plasma membrane expression in RPE cells but are localised to opposite membranes (Philp et al. 1998, 2003a; Iwanaga and Kishimoto 2015; Bergersen et al. 1999; Chidlow et al. 2005; Gerhart et al. 1999). A combination of exogenous expression, co-immunoprecipitation, FRET, and mouse basigin knockout experiments confirmed that basigin is the physiological partner of MCT1, 3, and 4, and is required for full translocator stability at the plasma membrane; MCT2 shows specificity for embigin (Ovens et al. 2010b; Wilson et al. 2002, 2005; Kirk et al. 2000; Philp et al. 2003a; Poole and Halestrap 1997) or neuroplastin (Wilson et al. 2013). Ancillaries are, however, somewhat promiscuous in their interactions. For example, both MCT1 and 2 interact with basigin in mouse spermatozoa (Mannowetz et al. 2012). Furthermore, embigin will interact with MCT1 when basigin is absent in rat erythrocytes, despite its preference for MCT2 in other species (Poole and Halestrap 1997; Ovens et al. 2010b; Wilson et al. 2005). MCT11 and 12 interactions with basigin have been demonstrated *in vitro* (Rusu et al. 2017; Castorino et al. 2011b). In HEK293 cells, MCT12 co-immunoprecipitated with basigin and siRNA of basigin also reduced MCT12 surface expression (Castorino et al. 2011b). The ancillary also partially rescues age-related cataract-associated mutations in MCT12 by enhancing membrane expression (Staubli et al. 2017). Basigin provides an alternative mechanism to enhanced plasma membrane expression of MCT11 by preventing its ubiquitin-guided degradation (Rusu et al. 2017). Interestingly, research on neuroplastin suggests the use of *X. laevis* oocytes for studying MCT-ancillary interactions may be masking other possible interactions. The authors demonstrated that an endogenous oocyte neuroplastin was mediating MCT membrane expression in absence of heterologous neuroplastin expression (Wilson et al. 2013).

The TM and intracellular N-terminal domains of basigin mediate trafficking of MCT1 and 4 to the membrane ((Kirk et al. 2000), Table 3). Replacement of the basigin immunoglobulin domains with those of the Ig surface receptor CD2 in a basigin-CD2 chimera, demonstrated that IgG domains were not required for trafficking. Association of ancillary TM domains has been mapped to a putative binding site adjacent to the C-terminus and TMs 3/6 of MCTs (Manoharan et al. 2006; Wilson et al. 2005, 2009). Charge-swap mutagenesis suggested that E216 in the TM domain of the short human basigin isoform forms an electrostatic bond with R86 in TM3 of MCT1 (Manoharan et al. 2006). Lipid-facing charged residues such as R86 in TM domains are rare and normally thermodynamically unstable. They are often utilised to promote TM-TM interactions or oligomerisation in membrane proteins (Weiner et al. 1989). The basigin TM domain glutamate (E216) is conserved in all three BSG ancillaries (Fossum et al. 1991), raising the possibility that it forms part of a conserved SLC16 heteromer binding site. Modelling of the MCT1-basigin complex shows TM domains 3 and 6 of MCT1 lie adjacent to each other and interact with the TM domain of basigin (Bergersen et al. 1999; Halestrap 2012). Although TM 3 and 6 in MCTs appear to represent a conserved binding site in MCT1, 3, and 4, there is some evidence of both ancillary- and transporter-specific binding regions. For example, removal of the MCT2 intracellular C-terminal ablated trafficking to the plasma membrane by basigin but not by embigin (Ovens et al. 2010b), while MCT1 interactions with both

Table 3 Mammalian heteromeric solute carrier interactions

Translocator gene (SLC)	Translocator	Ancillary gene	Ancillary	Translocating subunit interactions[a]	Ancillary subunit Interactions[b]	Quaternary structure and stoichiometry (T_nA_n)[c]
SLC7 + SLC3 family						
SLC7A9	$b^{0,+}$AT	SLC3A1	rBAT	Extensive contacts between a) rBAT linker and EL2 of $b^{0,+}$AT b) rBAT α4/β5 with the cleft formed between EL2 and EL4 of $b^{0,+}$AT, c) rBAT TM domain with $b^{0,+}$AT TM4 and the EC side of TM3, d) rBAT intracellular H4 domain and the $b^{0,+}$AT C-terminal intracellular helix.	C114 intermolecular disulfide with $b^{0,+}$AT (not studied); Asn575 (delayed maturation); Cys673-Cys685,Cys571-Cys666, Cys242-Cys273 disulfides (trafficking and ER stability); M467T, T216M prevent trafficking; TM domain L89P cause complex degradation	T_2A_2[d] (dimer of heterodimers) (Yan et al. 2020a; Wu et al. 2020)
SLC7A13	AGT1			Unknown	Unknown	Unknown
SLC7A5	LAT1	SLC3A2	4F2hc/CD98hc	The Cryo-EM structure of human LAT1-4F2hc important interacting regions are between: 4F2hc TM domain and LAT1 TM4, 8, and bound phospholipids; salt bridges LAT1E303-4F2hcK431 and LAT1D223-4F2hcK198; H-bond-cationic bonds from 4F2hc R433 to Q304/T163 of LAT1.	C109 intermolecular disulfide (decreased stability); domains essential for heteromer formation are disputed: ectodomain slows biogenesis but not trafficking or final function (Broer et al. 2001), is essential for function (Fenczik et al. 2001), or is unnecessary (Franca et al. 2005); TM and N-terminal domains are essential for trafficking (Franca et al. 2005). Whether 4F2hc is necessary for substrate translocation is disputed (see (Yan et al. 2019; Napolitano et al. 2015)).	T_1A_1 (Yan et al. 2019; Lee et al. 2019)
SLC7A8	LAT2			Large contact surface area (1735 $Å^2$) with extensive hydrophobic and aromatic-based interactions between ectodomain with extracellular loops of translocator.	Heteromer is not trafficked if any one of many ED regions are deleted	T_1A_1 (Rosell et al. 2014; Meury et al. 2014)
SLC7A7	y^+LAT1			E36 (N-terminal/TM1) and F152 (TM4) disrupt activity but not surface expression	Unknown	Unknown, but LPI mutations E36del and F152L possibly involved in larger complex formation with 4F2hc/y^+LAT2

SLC7A6	y^+LAT2			Unknown	Heteromer is not trafficked if any one ectodomain regions are deleted	Unknown
SLC7A10	Asc-1			Unknown	Unknown	Unknown
SLC7A11	xCT			Unknown	Unknown	T_1A_1
SLC7A12	Asc-2	Unknown	Unknown	Unknown	Unknown	Unknown
SLC7A15	ArpAT	Unknown	Unknown	Unknown	Unknown	Unknown
SLC6 family						
SLC6A1	GAT1	STX1A	Syntaxin 1A	N-terminal aspartate residues (D40, D43, D45). Syntaxin 1A disrupts inhibitory interaction between N-terminal and IL4	Coiled coil H3 (ID, 188–266) and TM domains (266–288)	Unknown
SLC6A2	NET			N-terminal (residues 28–47) for surface expression	H3-TM domain not required	Unknown
SLC6A3	DAT			Amphetamine-modulated DAT dynamics regulated by N-terminal controlled syntaxin 1A and phosphorylation (R51W, T62D), N-terminal (1–33) for dopamine efflux	H3-TM domains not required; N-terminal ID R26Q disrupts interaction; phosphorylation at S14 also modulated DAT dynamics	Unknown
SLC6A4	SERT			N-terminal anionic residues (E16, D17, E19, D20, and E23) in substrate-ion coupling	H3 domain (188–266) essential for interaction, TM domain is not required	Unknown
SLC6A5	GlyT2			Unknown	Unknown	Unknown
SLC6A9	GlyT1			Unknown	Unknown	Unknown
SLC6A19	B^0AT1	TMEM27	Collectrin	TM5, TM7, N-terminal	Unknown	T_2A_2 (Yan et al. 2020c)
SLC6A18	B^0AT3				TM domain, ED (15–40), ID (163–178)	Unknown
SLC6A19	B^0AT1	ACE2	Angiotensin converting enzyme 2	TM7 (R240)	ED carboxypeptidase domain (2–592) not required; extended EC TM7 and EL4 of B^0AT1 interact with neck and linker of ACE2, TM4, TM3 and TM9 of B^0AT1 interacts with ACE2 TM domain	T_2A_2 (dimer of heterodimers) (Yan et al. 2020d)
SLC6A18	B^0AT3			TM7 (R225)		Unknown
SLC6A20	IMINO			Unknown	Unknown	Unknown
SLC6A19	B^0AT1	APN	Aminopeptidase N	Unknown	Unknown	$>T_1A_1$

(continued)

Table 3 (continued)

Translocator gene (SLC)	Translocator	Ancillary gene	Ancillary	Translocating subunit interactions[a]	Ancillary subunit Interactions[b]	Quaternary structure and stoichiometry (T_nA_n)[c]
SLC4 family						
SLC4A1	AE1	GYPA	Glycophorin A	TM8, EL4 (E658, Wr^b antigen, S667), IL4, TM13, TM1 (SAO deletion residues 400–408)	ED TM-adjacent (87–89, R80, A84) TM domain and ID (90–150) for trafficking and modulation of catalytic activity	T_2 and T_4 complexes of the isolated translocator. Possible long chains of T_2A_2 also form (Kalli and Reithmeier 2018)
SLC16 family						
SLC16A1	MCT1	BSG	Basigin	TM3, TM6 (Arg86, MCT1), TM5 (G153V, disrupts trafficking)	TM domain (Glu218, basigin)	T_1A_1 & T_2
SLC16A8	MCT3			Unknown	Unknown	Unknown
SLC16A3	MCT4			TM3, TM6	TM domain and ID (N-terminal)	Unknown
SLC16A11	MCT11			4 mutant haplotype: TM3 (V113/D127), TM10 (G340), C-terminal (P443)	Unknown	Unknown
SLC6A12	MCT12			EL4 (G205V, disrupts trafficking)	Unknown	Unknown
SLC16A7	MCT2	EMB	Embigin	TM3, TM6, C-terminal (basigin-specific)	Unknown	Unknown
SLC51 family						
SLC51A	Ost α	SLC51B	Ost β	N-terminal first 50 residues (ID) required for ER exit	TM (W34-N35) for catalytic activity; ID (C-term, R54/R55) for protein stability and trafficking; ED (N-term 1–27) for Ost β stability and transport activity; residues 20–72 critical for transport activity with Ost α	T_2 complex is stable

[a]Interacting domains of translocating subunit: *IL* intracellular loop, *EL* extracellular loop, *TM* transmembrane
[b]Interacting domains of ancillary subunit: *ID* intracellular domain, *ED* extracellular domain, *TM* transmembrane
[c]T_n = # of translocator subunits, A_n = # of ancillary subunits

basigin and embigin are independent of its C-terminal (Ovens et al. 2010b). It is also probable that the ancillary binding site in MCT1 is larger and involves many interactions as demonstrated by the mapping of trafficking mutant G153V to a MCT family signature sequence in TM5 (Galic et al. 2003). The more recently discovered MCT11-basigin interaction also suggests that heteromer formation may occur more broadly in the SLC16 family (Rusu et al. 2017).

There is speculation that the MCT translocator subunits are not post-translationally glycosylated, thereby providing a hypothesis for the role of their ancillaries in protein stabilisation and ER quality control (Fisel et al. 2018). However, this is based on limited analysis of N-glycosylation in mouse and Chinese hamster MCT1, chicken MCT3 and sequence prediction conducted on human MCT 3, 4, 5, 6 (Price et al. 1998; Yoon et al. 1997; Carpenter et al. 1996). Early isolation of MCT isoforms noted both inter- and intra-species differences in apparent molecular weights not consistent with expected divergence based on native protein sequences – a common feature of differential glycosylation (e.g. see (Poole and Halestrap 1992)). One explanation is that translocation subunits undergo alternative, non-N-linked, glycosylation. The ancillary proteins, by contrast, are heavily glycosylated proteins. Extensive N-glycosylation of embigin is likely the cause of the difference between the predicted molecular weight of 36 kDa and the observed molecular weight of ~70 kDa (Halestrap 2013b). Basigin is less heavily glycosylated, with 3 predicted N-linked glycosylation sites in the ubiquitously-expressed shorter isoform and 4 in the longer retina-specific isoform (Muramatsu 2016) (Fig. 1). The heavy glycosylation of ancillaries and its contrasting absence in translocator subunits implies that glycosylation from ancillaries maybe the critical component in heteromer formation and quality control (Fisel et al. 2018). In breast cancer cell lines decreased basigin glycosylation is accompanied by a decrease in MCT1 surface expression (Azevedo-Silva et al. 2015). Furthermore, glycosylated basigin itself is unable to reach the plasma membrane in the absence of MCT4 – implying mutual dependency for plasma membrane expression of both proteins (Gallagher et al. 2007). However, caution is required as glycosylation of MCTs cannot be ruled out (see above) and, to the authors' knowledge, no systematic assessment of glycosylation of both proteins and its effects on heteromer stability and trafficking has been conducted. Basigin may also present non-canonical glycosylation patterns as speculated from immunoblots comparing changes in its molecular weight between cancerous and non-cancerous cell lines (Kendrick et al. 2017; Riethdorf et al. 2006). The recent solving of the first MCT family member structures, from the bacterium *Syntrophobacter fumaroxidans* did little to further understanding of the heteromer as this transporter was not crystallised with an ancillary and does not seem to require one (Bosshart et al. 2019).

2.2.4 Integration into Larger Complexes

Basigin (CD147) has also been associated with other solute carriers. It stimulates the activity of the hexose uniporter GLUT1 (SLC2A1) in retinal photoreceptors (Ait-Ali et al. 2015), and also co-immunoprecipitates with GLUT1 in melanoma A375 cells (Su et al. 2016). However, GLUT1 stimulation is probably mediated in photoreceptors through basigin receptor substrate RdCVF, rather than direct interaction with GLUT1 (Ait-Ali et al. 2015). Moreover, basigin has been identified in larger complexes comprising amino acid transporters 4F2hc/LAT1 and ASCT2 (SLC1A5), the Na^+/K^+-ATPase, and regulators of cell adhesion such as EpCAM and Integrin β1 (Xu and Hemler 2005). Silencing of basigin in the context of this larger membrane 'metabolon' was shown to reduce surface expression of 4F2hc and cell proliferation (Xu and Hemler 2005). The reduction in cell proliferation might be due to the abrogation of glycolysis due to MCT functional ablation or to the concomitant decreased expression of 4F2hc that compromises mTOR activation and GLUT1 expression, resulting in nucleotide shortage and cell cycle block (Cano-Crespo et al. 2019). Whatever their exact role, there is growing evidence suggesting

multiple nutrient transporters form large integrated metabolic activation complexes.

Monocarboxylate transporters 1, 2 and 4 form specific complexes with carbonic anhydrases II, IV, IX (CAII/IV/IX) and catalytically inactive CA-related proteins (Becker et al. 2014; Ames et al. 2020; Pinheiro et al. 2011; Jamali et al. 2015; Aspatwar et al. 2019; Becker and Deitmer 2008; Becker et al. 2005; Klier et al. 2011). For CAIX at least, this interaction is mediated via the Ig1 domain of basigin for MCT1 and 4 (Jamali et al. 2015; Ames et al. 2020). These complexes have been termed 'metabolons' due to their role in cellular pH regulation involving multiple transporters mediating acid/base fluxes, including, in addition to MCTs, the chloride/bicarbonate exchanger (AE), sodium-bicarbonate cotransporter (NBC) and sodium/hydrogen exchanger (NHE) (reviewed in (Becker et al. 2014; Becker and Deitmer 2020; Becker 2020)). However, in contrast to these other transporters, functional interactions of CAs with MCTs are independent of CA catalytic activity (Aspatwar et al. 2019; Noor et al. 2018; Becker et al. 2010, 2011; Klier et al. 2014). For example, the intramolecular proton shuttle of CAIV facilitates proton monocarboxylate cotransport via MCT2 without requiring carbonic anhydrase activity (Klier et al. 2011, 2014). Furthermore, the interactions seem to be isoform specific, with MCT1 and 4 interacting with intracellular CAII and CAIX at the transporter C-termini, while MCT2 interacts with CAII (Klier et al. 2011; Noor et al. 2015). These complex formations are mediated by a conserved histidine residue, H88 residue in CAIV, H64 in CAII, and H200 in CAIX, which is also the central residue of the enzyme's intramolecular proton shuttle (Noor et al. 2015; Jamali et al. 2015). The binding site of this histidine appears to determine the isoform specificity of complex formation, with acidic clusters in the C-termini of MCTs 1, 4 being responsible for CAII and CAIX interaction but not MCT2-CAIV interaction (Noor et al. 2015; Stridh et al. 2012). The augmentation of MCT activity is also dependent on the expression of the correct trimeric MCT-ancillary-CA combination, with MCT2 requiring embigin to see augmented CAIV-mediated transport (Klier et al. 2011). In particular, the formation of MCT1/4-CAIX-basigin metabolons in breast cancer has gained recent attention, where their role in facilitating the MCT-mediated efflux of lactate and protons results in a reliance on anaerobic respiration that seems particularly essential to cell survival (Ames et al. 2020; Becker 2020; Mboge et al. 2019; Jamali et al. 2015; Pinheiro et al. 2011). The targeting of the CAIX interaction with the Ig1 domain of basigin by an antibody slowing cancer cell respiration and cancer progression is also noteworthy (Ames et al. 2020).

2.2.5 Catalytic Modulation

Ancillaries seem to be essential for transport activity, as MCT1, 2, and 4 are non-functional unless associated with their respective ancillaries (Wilson et al. 2005). The more recent discoveries that MCT11 (Rusu et al. 2017) and MCT12 (Castorino et al. 2011b) require basigin as an essential subunit suggested the possibility that the remaining orphan SLC16 transporters may also require ancillary proteins for functional expression.

Although the choice of heteromer partner does not modulate the substrate kinetic properties of MCTs *per se*, they do seem to influence the sensitivity of MCTs to certain chemical inhibitors. For example, inhibition by p-chloromercuribenzene sulphonate (pCMBS) is specific to MCT1 and MCT4 (Manning Fox et al. 2000; Garcia et al. 1994; Carpenter and Halestrap 1994) while MCT2 is refractory (Broer et al. 1999; Garcia et al. 1995). Furthermore, inhibition only occurs when MCT1 is co-expressed with basigin and not in rat erythrocytes, where MCT1 interacts with embigin (Wilson et al. 2005). These results suggested that the inhibitors target was the ancillary-translocator interactions and disrupting this interaction was modulating transport kinetics. Indeed this was confirmed when the basigin Ig1 domain was identified as the likely target for pCMBS action. Mutations in TM 3 and 6 domains of MCT4, which are involved in complex formation with basigin, also increased this transporters sensitivity to pCMBS inhibition (Wilson et al. 2005). Similarly, the sensitivity of MCT2 to the

inhibitor AR-C155858 is greatly reduced when the transporter is co-expressed with embigin but not with basigin (Ovens et al. 2010b). Given that the *in vivo* ancillary partner of MCT2 is embigin rather than basigin, this result suggests that embigin also regulates the kinetic properties of the transporter. By contrast, the sensitivity of MCT1 to AR-C155858 inhibition is similar whether it is associated with its preferred ancillary basigin, or with embigin. Interaction with transmembrane domains (TMs) 3 and 6 of MCT2, which are known to be the point of interaction between the MCTs and their ancillaries, are shown to be important for AR-C155858 sensitivity (Manoharan et al. 2006; Ovens et al. 2010b). This points towards an interesting heteromer specific inhibitor interaction. This is in contrast to data showing the binding site for AR-C155858 is contained within the C-terminal half of MCT1, and involves TM (transmembrane) domains 7–10, which are involved in the more central substrate binding cavity of the transporter (Ovens et al. 2010a). It is to be noted that where mechanistic insight is available, none of the inhibitors mentioned above seem to inhibit solute carrier activity by means of disrupting direct binding with its ancillary, despite the fact that inhibition is ancillary-dependent. Therefore, the interaction between MCT2 and its inhibitor AR-C155858 must involve allosteric regulation if activity by binding to either subunit but only when the complex itself has formed (Manoharan et al. 2006; Ovens et al. 2010b).

As noted above CAII and CAIV facilitate removal of protons from MCTs by a shuttle system comprised of several acidic residues. This facilitates proton cotransport and augments the transport activity, via catalytic modulation (Becker et al. 2005, 2010; Beck and Deitmer 2008, 2020; Jamali et al. 2015; Klier et al. 2011, 2014).

2.2.6 Pathophysiological Aspects

Induction of lactate transport to combat hypoxia and instigation of metastasis/migration appear to be independent functions of MCT1- and MCT4-basigin heteromers. Simulating glucose-starvation conditions, De Saedeleer et al. (2014) found tumour cell migration was directly dependent on MCT1, while Payen et al. and others (Payen et al. 2017; Gray et al. 2016) found MCT1 expression promotes invasiveness/metastasis independent of lactate transport. These results can be explained if the two subunits are mutually dependent on each other for upregulation in cancer cells, which, indeed, they are (Walters et al. 2013). Basigin also activates extracellular metalloproteinases that play a key role in tumour cell proliferation, migration and invasiveness, apparently independently of MCT subunits (Su et al. 2009; Muramatsu and Miyauchi 2003).

Basigin interactions were disrupted and surface expression of MCT11 decreased in a 4-residue type II diabetes-associated mutant haplotype of MCT11 (Rusu et al. 2017). The residues mutated in the diabetes-associated haplotype, are located in distant locations on the transporter at TM3 (V113/D127), TM10 (G340) and the cytoplasmic C-terminus (P443) – thereby giving little indication as to the possible site of basigin binding (Rusu et al. 2017; Williams et al. 2014). Likewise, cataract-associated mutations in MCT12, which could be rescued by basigin co-expression, provide little insight into the potential presence of a conserved SLC16 heteromer binding site (Staubli et al. 2017). The trafficking mutant, G205V, for example, is located in EL4 of MCT12 (adjacent to TM6) but rather than disrupting heteromer formation this mutant is partially rescued by basigin. The stoichiometry of the SLC16 heteromeric complexes remains ill investigated but an association of a single basigin with a MCT1 homodimer has been proposed (Wilson et al. 2002). Mutations in MCT8 cause Allan-Herndon-Dudley syndrome and related clinically diverse mental retardation (reviewed in (Groeneweg et al. 2017; Visser 2013; Bernal et al. 2015; Ramos 2014; Fu and Dumitrescu 2014; van der Deure et al. 2010; Friesema et al. 2010; Schwartz and Stevenson 2007)). In contrast to MCT1-4, expression of MCT8 is independent of embigin, basigin or neuroplastin (Visser et al. 2009). As a result, the

associated mutations cannot lead to insights into heteromer formation, unless an unknown protein is involved.

2.3 Anion Exchanger 1 (AE1, SLC4A1) and Glycophorin A

2.3.1 General Properties

Among the first heteromeric carriers to be discovered was the complex formed by Anion Exchanger 1 (AE1 or band 3 anion transport protein, SLC4A1) with its ancillary glycophorin A. These two proteins are the most abundant red blood cell (RBC) integral proteins, with AE1 abundance calculated at ~10^6 molecules/cell, equivalent to ~25% of total RBC protein (Fairbanks et al. 1971). Glycophorin A has been calculated at 0.5–1 × 10^6 molecules/cell (Blanchard 1990), corresponding to 1.6% of total human RBC protein (Furthmayr 1978). AE1 is the founding member of the SLC4 family of bicarbonate transporters (reviewed in (Cordat and Reithmeier 2014; Reithmeier et al. 2016)). The SLC4A1 gene was mapped to chromosome 17q21–22 and consists of 20 exons (Showe et al. 1987; Schofield et al. 1994; Sahr et al. 1994). Although initially characterised in erythrocytes, AE1 also plays a major role at the basolateral membrane of acid-secreting α-intercalcated cells of the kidney epithelium. Its physiology is closely linked with carbonic anhydrase and haemoglobin, and the subsequent tight regulation of blood/urine CO_2 and pH via its electro-neutral 1:1 exchange of the base equivalent HCO_3^- (produced from CO_2) with chloride (Table 1). The secretion of HCO_3^- into the blood serum in exchange for chloride is among the fastest recorded turnover rates (k_{cat}) for a carrier at between 4 × 10^4 and 5 × 10^4 s^{-1} (Brahm 1977; Passow 1986). Such a fast exchange is required in order to maintain and co-ordinate bicarbonate efflux with the equally rapid conversion of CO_2 and H_2O into protons and HCO_3^- by carbonic anhydrase. Extensive reviews on the biology, physiology, and structural aspects of AE1 can be found elsewhere (Aoki 2017; Cordat and Reithmeier 2014; Machnicka et al. 2014; Parker and Boron 2013; Romero et al. 2013; Batlle and Haque 2012; Thornell and Bevensee 2015; Skelton et al. 2010; Reithmeier et al. 2016).

Erythrocytes and renal epithelial cells express two different isoforms of AE1: the erythrocyte specific isoform 1 (UniProt P02730-1, eAE1) is longer at 911 residues; the kidney isoform (P02730-2, kAE1) is missing the first 65 N-terminal residues (Brosius 3rd et al. 1989; Kollert-Jons et al. 1993). The human erythrocyte carrier contains a long N-terminal intracellular domain (residues 88–329) and the 14 TM bicarbonate transporter domain (residues 372–839), with a short intracellular linking region between the two and a short C-terminus (Kopito and Lodish 1985a, b; Arakawa et al. 2015; Drickamer 1978; Yannoukakos et al. 1989). Similar to the structural design of other transporters, crystallised AE1 consists of two repeating halves (TM 1–7 and 8–14), inverted to form a pseudo-twofold axis of symmetry in the plane of the membrane (Arakawa et al. 2015) (Sect. 2.1.2, Fig. 2a). The repeated TM domains 3 and 10 form loops containing disordered regions in the membrane. A single N-linked glycosylation site is located at Asn642, in the middle of a large, structurally unresolved, extracellular loop between TM domains 7 and 8 (Drickamer 1978; Reithmeier et al. 2016). The 65 missing N-terminal residues of kAE1 are thought to be disordered between residues 1–54, while also containing the first 11 residues of the intracellular globular domain (β_1 strand) which follows (Zhang et al. 2000). The long N-terminal of AE1 contains six phosphorylation sites (Y8, Y21, Y46, S185, S350, S359) and a C-terminal one at Y904 (Brunati et al. 2000; Yannoukakos et al. 1991). Other experimentally verified PTMs include a C-terminal palmitoylation site (C853) and acetylation at M1 (Okubo et al. 1991; Yannoukakos et al. 1989; Drickamer 1978) (Fig. 1).

A total of 5 glycophorins (A–E) are known, ordered according to their abundance in red cell membranes. Glycophorin A and B, together with glycophorin E, form a cluster at chromosome 4q31.21 in humans and share over 95% sequence homology (Onda and Fukuda 1995). They are thought to have arisen from gene duplication of

glycophorin A in combination with a downstream pseudogene (Cartron and Rahuel 1992, 1995; Onda et al. 1993; Blumenfeld and Huang 1997). Glycophorin A also forms the rare Wr(a+b−) (Wright group a positive b negative) antigens with AE1 (Huang et al. 1996; Holman 1953), a link vital to the discovery of a heteromeric complex between the two proteins (see below).

Human glycophorin A is a type 1 single-pass membrane protein that is heavily sialoglycosylated on its extracellular domain by a single N-linked and 16 O-linked oligosaccharide chains (Fig. 1) (Tomita and Marchesi 1975; Pisano et al. 1993). The canonical isoform of the protein (UniProt ID P02724) contains 150 residues, divided into this heavily glycosylated extracellular domain (residues 1–91), the TM domain (92–114), and a short C-terminal domain (115–150) (Tomita et al. 1978; Furthmayr et al. 1978; MacKenzie et al. 1997). The 150 residues of pro-glycophorin A are reduced to 131 with removal of an N-terminal signal sequence that is cleaved during co-translational insertion into ER membranes (Tomita and Marchesi 1975; Jokinen et al. 1981; Jokinen et al. 1979). Glycophorin A can form homodimers, or even trimers, *in vivo* (Bruce et al. 2004). The structure of the TM domain and small adjacent regions of glycophorin A has been solved by NMR and x-ray crystallography (MacKenzie et al. 1997; Mineev et al. 2011; Trenker et al. 2015), which confirmed that homodimer formation is mediated by TM-TM interactions (Treutlein et al. 1992; Marchesi et al. 1976; Ji 1974; Trenker et al. 2015). A large scale analysis of the human liver phosphoproteome has identified 3 phosphorylation sites in the intracellular, C-terminal of glycophorin A at T133, S138, and S148 (Bian et al. 2014). The high sialic acid content of extracellular oligosaccharides contributes to the glycocalyx of erythrocyte membranes and its negative surface charge, thereby preventing erythrocyte aggregation (Rogers et al. 1992).

2.3.2 Tissue-Specific Expression

AE1 forms a stable complex with glycophorin A in RBCs, while kAE1 does not due to the lack of glycophorin A expression in α-intercalated cells of the distal tubules ((Wu et al. 2010) reviewed in (Thornell and Bevensee 2015)). Together, protein variants of glycophorin A and B form the MNS blood group antigen system for RBCs, which is responsible for 46 identified specific antigens (Reid 2009; Palacajornsuk 2006; Anstee 2011; Poole 2000; Finning and Daniels 2014; Storry et al. 2014). The different antigens arise from single-nucleotide substitutions or allelic variation in the extracellular domain of the 2 glycophorin genes, often at the sites of O-linked sialoglycosylation. The evidence for the formation of a stable heteromeric complex between AE1 and glycophorin A was revealed through the molecular origin of two MNS members, the Wr^a and Wr^b blood group antigens (detailed in (Poole 2000) pp. 33–34, (Huang et al. 1996)). The Wr^b antigen was initially assigned to glycophorin A based on subjects displaying the En(a−) MSN system antigen, which lacked glycophorin A, and also being negative for Wr^b (Adams et al. 1971; Issitt et al. 1975; Tokunaga et al. 1979; Miller et al. 1977). However, sequencing of the ancillary in a Wr^b-containing individual revealed no difference with normal glycophorin A serotypes (Dahr et al. 1986). Furthermore, familial inheritance studies also showed the gene containing Wr^b did not co-segregate with other glycophorin A serotypes (Dahr et al. 1986; Pavone et al. 1977). In contrast, biochemical investigations demonstrated that isoforms of glycophorin A missing TM domain adjacent regions also lacked antibody activity against the Wr^b antigen. This suggested that Wr^b antigen detection was entirely dependent on the TM domain of glycophorin A (Langley et al. 1981; Vengelen-Tyler et al. 1981; Ridgwell et al. 1983, 1984). A plausible resolution to these discrepancies poses that the Wr^b antigen was glycophorin A-dependent but not actually part of the protein – that is, glycophorin A was necessary but not sufficient for Wr^b antibody detection (Rearden 1985). This hypothesis was confirmed using monoclonal antibodies against glycophorin A which co-precipitated AE1 – suggesting that the bicarbonate exchanger was also required for Wr^b antigen detection (Dahr et al. 1986; Rearden et al. 1985; Rearden 1985; Telen and Chasis 1990; Ring et al. 1994).

Sequencing of glycophorin A and AE1 in one of only two known Wr^a (a+b−) individuals, demonstrated homozygosity for a E658K mutation in AE1, in the presence of normal glycophorin A (Bruce et al. 1995). Further individuals, positive for both Wr^a and Wr^b (a+b+) antigens were heterozygotes at AE1 position 658, with one allele encoding glutamate and the other lysine. The common Wr^b (a−b+) RBC phenotype contained only E658 in AE1. Thus, the Wr^a and Wr^b antigens are determined by the identity of residue 658 in AE1. Confirmation of stable complex formation at the erythrocyte membrane between AE1 and glycophorin A was subsequently established using various means (Che and Cherry 1995; Nigg et al. 1980; Telen and Chasis 1990; Ring et al. 1994; Bruce et al. 1995; Knowles et al. 1994; Paulitschke et al. 1995). The frequency of the Wr(a+) antigen hetero- and homozygotes is estimated about 3 in 10,000 for European populations (OMIM 112050), with homozygotes described in only a single family to date (2 individuals) (Bruce et al. 1995; Pang and Reithmeier 2009). While expression of the AE1-glycophorin A complex is restricted to erythrocytes, expression of the translocator is not itself dependent on the ancillary as kAE1 is fully trafficked and functional in its absence. It might be speculated that the missing 65 N-terminal residues of the kAE1 isoform impart the specificity for interaction with glycophorin A. However, even in erythrocytes the functional effect of glycophorin A on AE1 is more subtle and took some time to tease out.

2.3.3 Trafficking, Protein Folding and Quality Control

The effects of glycophorin A and AE1 on their reciprocal secretory pathway processing and the final membrane expression of the heteromer are varied. The co-localisation of wild-type AE1 and glycophorin A has been confirmed in the ER. Hereditary disease-causing mutations in AE1 suggest that this is where the complex initially forms (Auffray et al. 2001; Pang and Reithmeier 2009; Young et al. 2000). Moreover, the dependence on the AE1 G701A mutant for Golgi to plasma membrane trafficking suggests the ancillary is required throughout secretion (Bruce et al. 2000; Tanphaichitr et al. 1998). Despite this, AE1 expression in mature erythrocyte membranes is normal in red blood cells lacking glycophorin A (Pang and Reithmeier 2009; Tanner et al. 1976; Tanner and Anstee 1976a). These results also confirmed earlier findings that humans lacking the Wr^b antigen due to an absence of glycophorin displayed neither detrimental phenotype nor abnormalities in RBCs (Holman 1953; Issitt et al. 1976; Vengelen-Tyler et al. 1981; Tokunaga et al. 1979). In contrast, *X. laevis* oocyte experiments showed glycophorin A increased AE1 plasma membrane expression and the rate of trafficking (Groves and Tanner 1992, 1994a). The two sets of results are incongruous, and results from erythrocytes have been interpreted as demonstrating glycophorin A is not required for trafficking during erythropoiesis (Cordat and Reithmeier 2014). Alternatively, they could be interpreted as suggesting that other, redundant, chaperones may facilitate AE1 trafficking during red cell maturation in the absence of glycophorin A (Groves and Tanner 1992) and that glycophorin A represents a fail-safe mechanism via ancillary-mediated enhancement of AE1 surface expression. The later hypothesis is supported by *in vitro* evidence showing glycophorin A enhances the plasma membrane expression of selected AE1 disease-associated mutants to rescue them (e.g. (Tanphaichitr et al. 1998), see below). The surface expression of glycophorin A is dependent on the presence of AE1, as demonstrated *in vitro* and using mouse AE1-null red cells (Hassoun et al. 1998; Young et al. 2000; Bruce et al. 2004). The establishment of a stable complex between both proteins also helped explaining aspects of AE1 biochemistry. For example, the extent of AE1 N-glycosylation was inversely correlated to the expression of glycophorin A (Tanner and Anstee 1976a, b; Gahmberg et al. 1976; Dahr et al. 1987; Bruce et al. 2004; Miller et al. 1977). As a result individuals who lack glycophorin A display a more heavily glycosylated AE1, while overexpression of glycophorin A causes AE1 to become less glycosylated (Gahmberg et al. 1976; Tanner et al. 1976; Bruce et al. 1994). It

is thought that this change in glycosylation occurs at the single AE1 N642 glycosylation site, although this has not been confirmed experimentally. As mentioned above, enhanced glycosylation on AE1 appears to exist as a potential compensation for the loss of glycophorin A as the absence of the ancillary doesn't appear to have any absolute effect on AE1 membrane expression in reticulocytes (Pang and Reithmeier 2009). Furthermore, AE1 is protected from proteolytic cleavage in oocytes when glycophorin A is absent (Groves and Tanner 1994b, 1992). There is no evidence that other glycophorin isoforms, B or C interact with AE1 (Bruce et al. 2004; Bruce et al. 1994; Groves and Tanner 1992; Young and Tanner 2003).

The interaction region facilitating AE1-glycophorin A heteromer formation was confirmed by experiments showing the dependence of Wr^b antigen on the presence of both proteins as described above ((Bruce et al. 1995), Table 3). The interaction site was mapped to E658 of AE1, which is exposed to the extracellular solution adjacent to TM domain 8 and the nearby single N-linked glycosylation site of the transporter (Reithmeier et al. 2016; Arakawa et al. 2015). This gave rise to speculation that heterodimerisation occurs between TM 8 of AE1 and the TM domain of glycophorin A (Reithmeier et al. 2016). Subsequently, the extracellular region adjacent to the TM domain, the TM domain itself, and cytosolic domains of glycophorin A have all been shown to play a role as a chaperone of AE1 and in the catalytic activity of the heteromer.[3] The C-terminal region, in particular, enhances trafficking of AE1 to the cell surface, while residues 68–72 (F87, S88, P90, E91) are involved in anion transport (Young and Tanner 2003). Using chimeras constructed by fusing AE1 with non-interacting glycophorins B and C, it was shown that the extracellular region 59–70 (78–89) from glycophorin A was required for Wr^b antigen detection and complex formation (Bruce et al. 1995; Ridgwell et al. 1984; Dahr et al. 1986; Poole et al. 1999). Specifically, AE1 E658 forms a salt bridge with R61 (R80) from glycophorin A (Bruce et al. 1995), while A65P (A84P) was also shown to disrupt complex formation (Poole et al. 1999). However, an MSN antigen variant called SAT, expressing a hybrid A/B glycophorin and containing only the EC domain of glycophorin A 1–71 (20–90), was negative for the Wr^b antigen (Huang et al. 1996); thus demonstrating the absence of heteromer formation and additional essential roles for the TM and intracellular domains in complex formation. Whilst the TM domain of glycophorin seems essential for interaction with AE1, mutations to TM domain residues (L94, I95, G98, G102) which mediate homodimerisation of glycophorin itself have no effect on AE1 (Young et al. 2000). Indeed, both glycophorin A dimers or monomers can facilitate the trafficking of wild-type AE1 to the plasma membrane *in vitro* (Groves and Tanner 1992; Young and Tanner 2003; Young et al. 2000) (Fig. 6).

The interaction of glycophorin A with AE1 is also evidenced by the ability of the ancillary to rescue the phenotype of a homozygous S667F mutant in TM8 of AE1 causing hereditary spherocytosis (HS) (Toye et al. 2008). However, the rescue of other disease-causing AE1 protein variants rather supports multiple interactions with spatially dispersed regions of AE1. These include the heterozygous deletion (residues 400–408, TM1) causing Southeast Asian ovalocytosis (SAO) (Patterson et al. 2009; Beckmann et al. 2001; Groves et al. 1993), and homozygous distal renal tubular acidosis (dRTA) mutants G701A (IL4), ΔV850 ((Toye et al. 2008; Young et al. 2000; Bruce et al. 2000; Tanphaichitr et al. 1998) reviewed in (Williamson and Toye 2008)). All of these mutants can at least be partially recovered in heterologous expression systems by co-expression with glycophorin A. The S667F and G701A mutants are located close to the Wr^b antigen formation site E658 in AE1. Both G701D and ΔV850 mutants impair AE1 trafficking. G701D is rescued from the Golgi in HEK293 cells transfected with mutant AE1 G701D by

[3] Note: the residue numbering quoted for glycophorin A in publications cited here was based on the protein sequence less residues 1–19, the signal peptide (i.e. 131 residues). The correct numbering of the full 150 residue glycophorin A amino acid sequence is given in brackets.

either monomeric or dimeric glycophorin A (Tanphaichitr et al. 1998; Bruce et al. 2000). The fact that many mutations cause dRTA but not HS is thought to be evidence that glycophorin A rescues most of these mutants in erythrocytes, but not in the kidney where it is absent (Williamson and Toye 2008). The location of the SAO deletion in TM1 (Δ400–408) causes significant disruption to AE1 folding and trafficking from the ER as TM1 contains the internal signal anchor for ER-bound translation and translocation of the nascent polypeptide (Fowler et al. 2017; Kanki et al. 2003). Although TM1 is not located near the supposed AE1 TM8 interaction site (Arakawa et al. 2015), glycophorin A still rescues SAO-affected AE1 proteins in transfected cells (Patterson et al. 2009; Pang and Reithmeier 2009). This is also true of WT AE1, which rescues SAO AE1 from ER degradation by homodimerising and trafficking to the plasma membrane (Cheung et al. 2005; Chernova et al. 1995). The abundant membrane expression of AE1 in RBCs of SAO sufferers indicates that both homodimerisation of AE1 and/or complex formation with glycophorin A can rescue aberrant ER insertion and trafficking of the translocator. Interestingly, the SAO deletion seems to have prevailed as an evolutionary protective mechanism against *Plasmodium* (malaria) parasite infection in some human populations (Jajosky et al. 2017; Paquette et al. 2015).

Homodimerisation and heteromer formation with glycophorin A are facilitated by different binding sites on the translocator subunit (Toye et al. 2008; Williamson and Toye 2008). The large distance between TM8 and TM1 of AE1 may also indicate that multiple copies of AE1 and glycophorin are needed to mediate large multimeric complex formation, not simply heterodimerisation. AE1 from both erythrocytes and kidney exists predominately as a dimer in the membrane and when isolated in detergent micelles (Reithmeier et al. 2016; Arakawa et al. 2015; Hirai and Yamaguchi 2015; Wang et al. 1993, 1994; Reithmeier 1979; Zhang et al. 2000; Casey and Reithmeier 1991; Jennings 1984). An MD study of AE1 and glycophorin A dimers in a physiologically relevant bilayer confirmed stable complexes are formed following salt bridge formation between E658 of AE1 and R61 of glycophorin A. This same study also indicated that glycophorin A dimers can bridge in between AE1 dimers and form very large chains of alternating subunit dimers (Kalli and Reithmeier 2018). These interactions were also facilitated by the formation of a PIP_2, PE and cholesterol enriched lipid annulus around the complexes.

2.3.4 Integration into Larger Complexes

Populations of AE1 and glycophorin A exist in complex with numerous other cytoskeletal and membrane proteins, with only a minority population of AE1 forming a stable complex with glycophorin A (Kodippili et al. 2012; Machnicka et al. 2014; Giger et al. 2016). Erythrocytic AE1 interacts in at least 2 larger macromolecular complexes which include a combination of glycophorin A, glycophorin B, CD47, spectrin, actin, ankyrin, and protein 4.2 (Toye et al. 2005; Satchwell et al. 2011). Protein 4.2 is a transglutaminase superfamily protein anchored through N-glycine myristoylation to the plasma membrane and playing an important role in RBC mechanical stability (Machnicka et al. 2014; Risinger et al. 1992). Mutations in protein 4.2 cause hereditary spherocytosis (HS) type 5 (OMIM 612690) through an impaired ability to interact with the cytosolic N-terminal of AE1. The wild-type protein 4.2 also increased AE1 chloride transport at the cell surface (Toye et al. 2005). The effects of protein 4.2 on AE1 in erythrocytes are almost certainly mediated via the macromolecular complex (reviewed in (van den Akker et al. 2010; Machnicka et al. 2014)). The kidney isoform of AE1 does not interact with glycophorin A as the ancillary is not expressed in α-intercalated cells of the distal tubules ((Wu et al. 2010) reviewed in (Thornell and Bevensee 2015)). However, kAE1 does form *in vitro* membrane complexes with at least two other single-pass membrane proteins, namely nephrin and TMEM139. Nephrin is a large single TM-domain-containing protein essential for regulating glomerular vascular permeability and perhaps anchoring the glomerular podocyte diaphragm to the cell cytoskeleton (Wu et al. 2010).

A homozygous nephrin mutation was shown to further reduce the already low levels of kAE1 expression in glomerular podocytes but no effect on the more abundant distal tubule expression of kAE1 was observed (Wu et al. 2010). TMEM139, another single pass type I transmembrane protein has also been identified *in vitro* as interacting with the C-terminal of kAE1 and altering the surface expression of the translocator in a dose-dependent fashion – indicative of acting as an ancillary subunit of the translocator (Nuiplot et al. 2015). Further confirmation is required before either nephrin or TMEM139 can be considered stable and physiologically relevant heteromeric partners of AE1. AE1 occurs as both homodimers and homotetramers in detergent micelles and membranes (Casey and Reithmeier 1991; Jennings 1984) and as a homodimer when purified (Zhang et al. 2000; Low 1986; Reithmeier 1979). The formation of tetramers requires interaction between the cytoplasmic domain and the cytoskeletal attachment protein ankyrin (Van Dort et al. 1998). Little research has been conducted into the heteromultimeric composition and structure of any larger *in vivo* AE1-glycophorin A complexes.

2.3.5 Catalytic Modulation

Glycophorin A has a marked effect on the anion uptake properties in RBCs without effecting total plasma membrane expression of WT AE1. In the erythrocytes of glycophorin A-deficient individuals, AE1 substrate uptake and binding affinity was variously reduced or unchanged, depending on the anion species being measured (Bruce et al. 1994; Bruce et al. 2004). This led to an interpretation that the transporter has two different binding modes, which are affected differentially by the absence of glycophorin A; specifically divalent anion (SO_4^{2-}) transport affinity is reduced whereas monovalent anion (Cl^-or I^-) affinity is unchanged in the absence of the ancillary (Bruce et al. 1994; Bruce et al. 2004). The V_{max} of monovalent anion uptake is also decreased in the absence of the ancillary, an effect not due to a reduction in AE1 plasma membrane expression (Bruce et al. 2004). Furthermore, a homozygous hereditary spherocytosis type 4 (HS-4) AE1 mutant (S667F) is recovered by glycophorin A through the enhancement of transporter activity at the plasma membrane, not by rescuing trafficking (Toye et al. 2008). The molecular mechanism underlying these changes in glycophorin A-dependent transport kinetics has not been investigated.

2.3.6 Pathophysiological Aspects

Heteromer formation of AE1 is illustrated by the rescue of disease causing mutations in AE1. South East Asian Ovalocystosis (SAO) is caused by homozygous mutations in AE1, but heterozygous individuals are rescued by glycophorin A. The role of glycophorin A in the rescue of the SAO heterozygotes probably involves stabilisation of the mutated AE1 translocator subunit and ensures the disease is largely asymptomatic (Patterson et al. 2009). The homozygous carriers of the SAO allele are very rare and not viable without an intensive antenatal care (Moulin et al. 2017; Liu et al. 1994; Picard et al. 2014). Glycophorin A rescue is also observed in distal renal tubular acidosis (dRTA), which can be inherited in a dominant or recessive mode but almost always as heterozygotes so that symptoms, while not usually lethal, can vary widely. Sufferers of dRTA are typically heterozygous for one or more of at least 8 different mutations in the SLC4A1, the most common of which are R598, G609, S613, P773, A858 (dominant) R602, ΔV850, G701 (recessive) (Karet et al. 1998; Bruce et al. 1997; Chu et al. 2010; Kittanakom et al. 2004). These mutations lead to aberrant trafficking of AE1 to the apical membrane of the α-intercalated cells in the distal nephron, instead of their normal destination in the basolateral membrane; in recessive mutations this leads to retention in the ER or Golgi and retrograde trafficking (Rungroj et al. 2004; Devonald et al. 2003; Toye 2005; Shayakul and Alper 2004; Duangtum et al. 2017; Chu et al. 2014). As the molecular basis for several AE1-glycophorin based disorders outlined here is the same, dRTA is simply characterised phenotypically by renal acidosis, caused largely by this mislocalisation in the kidney. However, comorbidity with SAO and even spherocytosis is often

diagnosed due to mutations in these diseases causing additional transport/activity RBC defects that can lead to haemolysis (Bruce et al. 2000). In addition to the inability to acidify urine due to this aberrant trafficking of kAE1, subjects can also present with metabolic acidosis, nephrocalcinosis, and may develop nephrolithiasis with age (Bruce et al. 1997; Reithmeier et al. 2016; Chu et al. 2010). Homozygous mutations or compound heterozygotes causing dRTA are rare and severe (Chu et al. 2010; Fawaz et al. 2012; Deejai et al. 2019). Recessive dRTA cases are often characterised by the presence of multiple (compound) heterozygous mutants, many of which are benign (Tanphaichitr et al. 1998). The G701D mutant has been reported to be homozygous in several carriers but it is rescued by glycophorin A (Tanphaichitr et al. 1998; Bruce et al. 2000; Stewart et al. 2011) (see above), along with several other heterozygous AE1 mutants.

2.4 The Sterol Transporter Family SLC51 Heteromer Ost α-Ost β

2.4.1 General Properties

The SLC51 family heterodimer Ost α-Ost β, is a unique example of a heteromeric solute carrier where both subunits were identified simultaneously (Wang et al. 2001). By functionally screening a cDNA library from little skate (*Raja erinacea*) Wang et al. 2001 identified both Ost α (SLC51A) and Ost β subunits (SLC51B) as essential components of a taurocholate transport system. Human and mouse Ost α-Ost β heteromers mediate facilitated diffusion (uniport) of taurine- and glycine-conjugated bile acids, other biological or synthetic sterols such as neurosteroids, dehydroepiandrosterone (DHEAS or androstenolone), pregnenolone sulfate, prostaglandin E_2 and other eicosanoids (Seward et al. 2003; Fang et al. 2010; Wang et al. 2001; Ballatori et al. 2005). The major physiologically relevant substrates in the intestine are bile acids and conjugated bile acids, such as taurocholate – the major component of bile released from the gall bladder. Generally, taurine-conjugated bile acids make better substrates than glycine-conjugated ones (Ballatori et al. 2005; Wang et al. 2001), while sulfate-conjugated sterols also strongly inhibit the uptake of other conjugated sterols (Seward et al. 2003), suggesting they are also potential substrates. The heteromer facilitates the basolateral efflux of anionic sterols in epithelial cells, where the inside negative membrane potential allows for the active efflux of anionic sterols into the portal vein (Ballatori et al. 2005). Due to its expression in numerous endocrine glands, it has also been speculated that Ost α-Ost β is involved in the transport of bile acid and other sterol derivatives into endocrine cells, where they are agonists for a wide variety of nuclear receptors (Ballatori 2011). Arachidonic acid has been shown to induce Ost α and Ost β mRNA in a fish liver cell line, but any wider biological relevance of this result has not been demonstrated (Hwang et al. 2008). Several reviews of Ost α-Ost β heterodimer expression, function, regulation and pathophysiology are available (Ballatori et al. 2013; Xue et al. 2019; Dawson et al. 2010; Klaassen and Aleksunes 2010; Beaudoin et al. 2020).

Ost α-Ost β plays a major role as a sterol transporter at various basolateral membrane barriers during bile acid circulation. Approximately 90–95% of bile acids are recycled from intestinal absorption back to the liver (enterohepatic circulation). From hepatocytes, they are transported across the canalicular membrane into the common bile duct. From there they are released back into the duodenum via the gall bladder where emulsification of dietary fats and lipid xenobiotics occurs (Ballatori et al. 2013). The role of Ost α-Ost β in this process has been confirmed by the phenotype of an Ost α −/− mouse, which displays disruptions to small intestine sterol reabsorption leading to decreased serum sterol levels and gall bladder bile acid pool (Ballatori et al. 2008; Rao et al. 2008). The Ost α −/− mouse seem to display little overall detrimental phenotype, enjoying a normal lifespan despite a 10–30% reduction of bile acid pools compared to WT mice (Ballatori et al. 2008; Rao et al. 2008). The lack of a detrimental Ost α −/− phenotype may be attributed to the

presence of multiple sterol transport pathways at each membrane barrier in the bile acid recycling process and regulation of bile acid metabolism through farnesoid X nuclear receptor (FXR) transcriptional regulation. In the liver FXR activation by bile acid ligands at the nuclear envelope downregulates sterol synthesis but upregulates carriers involved in sterol removal (Makishima et al. 1999; Parks et al. 1999). Liver-gall bladder accumulation of bile acids leads to an upregulation of Ost α and Ost β (and several other carriers) in a FXR-dependent process to help alleviate potential sterol cytotoxicity (Boyer et al. 2006; Lan et al. 2011). This process is reversed by inhibition of FXR in Ost α −/− mice and demonstrates that the transporter plays an important role in the available cytosolic pool of FXR ligands (Rao et al. 2008; Ballatori et al. 2008; Lan et al. 2011). The lack of expected toxicity in the liver of the global Ost α −/− mouse are thought to arise from a compensatory increase in urinary bile excretion driven by upregulation of alternative sterol transporters in the liver itself and in the kidney proximal tubule (Soroka et al. 2010, 2011; Vaquero et al. 2013). The overall reduction of the bile acid pool suggests a prominent role of Ost α-Ost β in the intestine and explains the mild phenotype of the global knock-out. A tissue-specific ablation of Ost α in the small intestine in mice does lead to a minor post-natal growth deficiency and minor cytotoxic changes in the ileum due to bile acid accumulation (Ferrebee et al. 2018; Rao et al. 2008; Ballatori et al. 2008). A more severe phenotype is avoided by intra-organ FXR signalling, where FXR-induced FGF15 release in the ileum causes distal increased FXR liver activation, thereby ameliorating the cytotoxic accumulation of bile acids in enterocytes (Boyer et al. 2006; Lefebvre et al. 2009).

2.4.2 Tissue Specific Expression

There are no apparent alternatives of the ancillary Ost β in different tissues. The reported overlap between expression of the two subunits is very strong (Al-Abdulla et al. 2019; Wheeler et al. 2014; Ballatori 2005, 2011; Ballatori et al. 2005, 2009) (Fig. 5). Both subunits co-localise and are expressed in the basolateral membrane of distal small intestine (ileum) epithelial cells, where they mediate the efflux of bile acids and steroids into circulation (Dawson et al. 2005; Ballatori et al. 2005). Ost α-Ost β protein expression has also been reported in basolateral membranes of the renal proximal tubules, hepatocytes and bile duct cholangiocyte cells (Ballatori et al. 2005; Dawson et al. 2005), cerebellar Purkinje cells hippocampal CA neurons, and ovaries (Fang et al. 2010). Its mRNA distribution suggests an even wider expression including multiple endocrinal glands (thyroid, adrenal, mammary, testes, ovary), the colon, prostate and uterus (Seward et al. 2003).

2.4.3 Trafficking, Protein Folding and Quality Control

Human Ost α is an N-glycosylated, 340 residue α-helical membrane protein containing 7 TM domains and constitutes the substrate translocation subunit of the Ost α-Ost β heterodimer (Ballatori et al. 2013). Human Ost β is predicted to be a 128 residue 14.3 kD, single-pass type I integral membrane protein (Fig. 1). Heteromerisation of the two proteins is essential for delivery of a functional sterol transporter to the basolateral membrane ((Li et al. 2007; Seward et al. 2003; Wang et al. 2001; Sun et al. 2007) and Fig. 6). The heteromerisation of both human and mouse Ost α-Ost β has been confirmed by co-immunoprecipitation, bimolecular fluorescence complementation, and mammalian two-hybrid experiments (Sun et al. 2007; Li et al. 2007). There is some evidence suggesting that the two subunits are interdependent for protein stability. For example, Ost β protein is undetectable in Ost α −/− mice while mRNA levels are unchanged (Li et al. 2007). Similarly, the deletion of a R54/R55 motif at the intracellular-TM domain interface of Ost β *in vitro* led to the absence of Ost α protein (Christian et al. 2012). Together these results imply the mutual presence of both subunits for their stability. However, the absence of Ost α *in vitro* did not seem to affect the stability of truncated Ost β (Christian et al. 2012; Christian and Hinkle 2017). Mouse Ost α forms a complex of a size consistent with

homodimerisation (~80 kDa) when expressed heterologously in HEK293 cells along with a protein consistent with being a monomer (~40 kDa) (Li et al. 2007). The larger complex was resistant to Sodium Dodecyl Sulfate (SDS) treatment and did not contain Ost β (Li et al. 2007), suggesting the homodimer of Ost α is, unlike the monomer, stable. No additional evidence of SDS-resistant homodimerisation for human Ost α has been reported (see (Sun et al. 2007)). However, both human and mouse Ost α monomers are detected at two distinct molecular weights (see (Sun et al. 2007) Fig. 3b and (Li et al. 2007) Fig. 1). In the case of the murine Ost α monomer, the slightly larger molecular weight (~40 kDa) is present on immunoblots only when co-expressed with the full Ost β protein (Li et al. 2007; Christian and Hinkle 2017). In the absence of Ost β, or if either the N- or C-terminal of Ost β are replaced, only the smaller monomer of Ost α is present (~35 kDa) (Christian and Hinkle 2017; Li et al. 2007). Both the predicted monomer and homodimer of mouse Ost α are sensitive to N-glycosidase F (PNGase) treatment (Li et al. 2007), which reduces the ~40 kDa monomer band intensity and causes the appearance of the smaller monomer band (Li et al. 2007). The most parsimonious conclusion from these experiments is that N-glycosylation of Ost α requires the presence of Ost β, but the non-glycosylated form is also stable at the plasma membrane and further implying N-linked glycosylation is not necessary for membrane trafficking (Li et al. 2007; Dawson et al. 2005; Sun et al. 2007; Christian and Hinkle 2017). Mouse Ost β is insensitive to N-glycosidase F treatment and appears as a single 19 kDa band in all immunoblots. Ost α-Ost β also exists as a heteromultimer (Li et al. 2007), however, fold-changes in both mRNA and protein levels for Ost α-Ost β through FXR-induction are not uniform, as might be expected in a heteromer with a 1:1 subunit stoichiometry (Chen et al. 2008; Boyer et al. 2006; Guo et al. 2018). These changes showed more copies of Ost β are synthesised than Ost α and indicating a larger transporter complex may utilise several copies of Ost β for each Ost α subunit.

The TM domain, extracellular N-terminal domain, and C-terminal domain of mouse Ost β all play a role in the trafficking and catalytic activity of the translocation subunit at the cell surface (Table 2). A truncation of all but residues 29–53 of Ost β established the TM domain as vital for Ost α-Ost β formation but not sufficient for trafficking as the complex retained in the ER (Christian et al. 2012). Mutation of a conserved residue pair W34A/N35A at the predicted extracellular-TM domain interface of Ost β also disrupts Ost α transporter activity but only when both mutants are present (Christian et al. 2012). Although the plasma membrane expression of Ost β was unaffected in this W34A-N35A mutant, Ost α surface expression was decreased (compare (Christian et al. 2012) Fig. 3b, d). Truncation of the extracellular N-terminal domain (residues 1–27) of Ost β resulted in total loss of heteromer transport activity but also some reduction of ancillary surface expression. Truncation of the cytosolic C-terminal domain (residues 54–128) also resulted in total loss of membrane expression and transport activity. When tagged with YFP the same Ost β C-terminal truncated protein demonstrated plasma membrane expression and full activity, suggesting that this region supports formation of the correct protein topology. Systematic truncations of Ost β C-terminal domain clarified these discrepancies by demonstrating that re-introduction of di-arginine R54/R55 residues into the C-terminal deletion restored Ost α-Ost β complex formation and Ost α stability. The importance of the Ost β TM domain and R54/R55 was affirmed by demonstrating that residues 19–68 were required to retain full taurocholate transporter activity and heteromer formation of with human Ost β (Christian and Hinkle 2017). Replacement of either the N- and C-terminal of Ost β individually had little effect on transport activity and only affected the expression of the higher (N-glycosylated) isoform of Ost α (Christian and Hinkle 2017). In contrast to Ost β, Ost α regions responsible for heteromer formation remain poorly investigated. Removal of the first 50 residues of the Ost α intracellular N-terminal led to accumulation of the complex

in the ER and Golgi (Sun et al. 2007). These combined results suggest that the TM domain and adjacent N- and C-terminal regions of Ost β form a binding site with, at least, the intracellular N-terminal and unknown TM domains of Ost α.

2.5 SLC6 Transporter Complexes with Syntaxin 1A, Collectrin ACE2

2.5.1 General Properties

The SLC6 family contains 20 members responsible for the transport of neurotransmitters, amino acids and their metabolites including osmolytes, and creatine (Broer and Gether 2012; Rudnick et al. 2014; Pramod et al. 2013). It is known as the Neurotransmitter Sodium Symporter (NSS) family and is part of the APC superfamily in the TCDB and Pfam (Chiang et al. 2015). The family comprises four branches: GABA transporters, monoamine transporters, and amino acid transporters I and II, a classification which has been confirmed by phylogenetic analysis (Broer and Gether 2012). The GABA transporter branch comprises transporters for GABA (GAT1, GAT2 and GAT3) and transporters of structurally related metabolites taurine (TauT), betaine (BGT1) and creatine (CT1). The monoamine transporter branch comprises transporters for dopamine (DAT), serotonin (SERT) and noradrenalin (NET). The substrate specificity and pharmacology of monoamine transporters are overlapping. The amino acid transporter branch I, comprises transporters for neurotransmitter glycine (GlyT1, GlyT2), the proline transporter PROT and the general amino acid transporter $ATB^{0,+}$. The most common transport coupling mode is the symport of 1 substrate with 2 Na^+ and 1 Cl^- ions (Table 1). Notable exceptions are NET (substrate/1 Na^+/1 Cl^-), GlyT2 and BGT1 (substrate/3 Na^+/1 Cl^-) (Broer and Gether 2012; Supplisson and Roux 2002; Pramod et al. 2013; Rudnick et al. 2014; Subramanian et al. 2016a, b). In some cases this transport and its mechanistic details are still disputed (Eskandari et al. 2017; Subramanian et al. 2016a). Notably, monoamine transporters can assume a channel/uniporter-like transport mode (Galli et al. 1996), which can also be triggered by the drug amphetamine (Sitte et al. 1998). The amino acid transporter branch II comprises general amino acid transporters (B^0AT1, B^0AT2, B^0AT3, NTT4 and SIT), NTT5 remains an orphan transporter in this branch. With the exception of SIT, transporters in this branch are chloride-independent (Broer and Palacin 2011; Broer et al. 2006a; Broer 2006, 2008a, b, 2013a, b, 2018; Verrey et al. 2005; Makrides et al. 2014; Verrey et al. 2009; Broer and Broer 2017). SLC6 transporters are involved in a wide range of physiological processes, such as neurotransmitter re-uptake (Guastella et al. 1990, 1992; Pacholczyk et al. 1991; Kilty et al. 1991; Blakely et al. 1988, 1991; Hoffman et al. 1991; Usdin et al. 1991; Liu et al. 1992a, b, 1993; Lopez-Corcuera et al. 1992; Smith et al. 1992a, b; Rudnick 1977; Sacher et al. 2002), synaptic vesicle loading and uptake (Zaia and Reimer 2009; Parra et al. 2008), oocyte/embryonic development (Anas et al. 2008; Baltz and Tartia 2010; Steeves and Baltz 2005; Steeves et al. 2003; Tartia et al. 2009; Van Winkle 1988), kidney metabolite reabsorption (Singer et al. 2009; Romeo et al. 2006; Kleta et al. 2004; Bohmer et al. 2005; Kowalczuk et al. 2005; Broer et al. 2005; Seow et al. 2004; Verrey et al. 2005; Oppedisano et al. 2011; Vanslambrouck et al. 2010; Takanaga et al. 2005), intestinal nutrient absorption (Jiang et al. 2015; Broer et al. 2005, 2006b, 2011; Romeo et al. 2006; Kowalczuk et al. 2005; Camargo et al. 2005; Kleta et al. 2004), lung epithelial amino acid clearance (Sloan et al. 2003; Sloan and Mager 1999), erythropoiesis/thrombosis/blood pressure regulation (Davis et al. 2012; Watts et al. 2012; Brenner et al. 2007; Winter et al. 2016; Ziu et al. 2012; Mercado and Kilic 2010) cell volume regulation (Ramamoorthy et al. 1994; Borden et al. 1995; Smith et al. 1992b; Rasola et al. 1995; Matskevitch et al. 1999), ATP replenishment (Christie 2007; Nash et al. 1994; Wallimann et al. 2011), DNA synthesis (Pitt et al. 1994), apoptosis (Warskulat et al. 2007), and placental trophoblast transport (Ramamoorthy et al. 1993, 1994). They play a diverse role in many human pathologies, including: Hartnup disorder,

immunoglycinuria, clinical depression, addiction, anxiety, ADHD, Parkinson's disease, autism, schizophrenia, suicide, OCD, alcoholism (reviewed in (Hahn and Blakely 2007; Owens and Nemeroff 1994; Romei and Raiteri 2016; Harvey and Yee 2013)), platelet aggregation and dysfunction (Jedlitschky et al. 2012; Maurer-Spurej et al. 2004), muscle wasting (Christie 2007), and irritable bowel syndrome (Colucci et al. 2008; Camilleri 2009) (Table 4). Numerous excellent reviews of the SLC6 family cover their tissue distribution, localisation, physiology, pharmacology, disease associations and biophysical properties (Rudnick et al. 2014; Broer 2013a; Broer and Gether 2012; Pramod et al. 2013; Kristensen et al. 2011; Rives et al. 2017; Navratna and Gouaux 2019; Broer and Fairweather 2018; Joseph et al. 2019). The overall protein fold of the SLC6 family is the LeuT-fold. Numerous structures of bacterial, invertebrate and mammalian SLC6-related transporters exist, see Sect. 2.1.2 for a more detailed description of this fold.

2.5.2 Interaction of the SLC6 Neurotransmitter Transporters with Syntaxin 1A

Transporters from all four branches interact with single TM domain-containing ancillary proteins syntaxin 1A, collectrin, and Angiotensin Converting Enzyme 2 (ACE2). Syntaxin 1A forms complexes with SLC6 transporters NET, DAT, SERT, GAT1, GLYT1, GLYT2 (Table 3) (de Wit 2010; Carvelli et al. 2008; Quick 2002, 2003, 2006; Binda et al. 2006; Sung et al. 2003; Lopez-Corcuera et al. 2001; Blakely and Sung 2000; Haase et al. 2001; Cervinski et al. 2010; Fairweather et al. 2015; Geerlings et al. 2000, 2001), in addition to multiple ion channels and receptors (reviewed in (Carbone et al. 2014; Kilisch et al. 2015; Tang et al. 2011; Weiss and Zamponi 2012; Leung et al. 2007; Saxena et al. 2000; Planells-Cases and Ferrer-Montiel 2007; Condliffe et al. 2004)). Syntaxin 1A can also modulate the activity of B^0AT3 and B^0AT1 although there is no overlap in tissue expression between the proteins (Fairweather et al. 2015), suggesting structural conservation of the translocator ancillary interaction. Syntaxin 1A plays a primary role as a T-SNARE protein in vesicular fusion events of the CNS and extensive reviews cover its canonical biological functions (Planells-Cases and Ferrer-Montiel 2007; Han et al. 2017; Rizo and Xu 2015). The ancillary is a 288 residue type IV membrane protein[4] regulating synaptic vesicular fusion via formation of coiled-coil bundles with other SNARE proteins (Fig. 1) (Lang and Jahn 2008; Sorensen 2005). The interaction between the γ-aminobutyric acid (GABA) transporter GAT1 and syntaxin-1A was the first to be identified (Quick 2006; Inoue et al. 1998; Quick et al. 1997) and was quickly followed by demonstration of the interaction and functional modulation of SERT (Haase et al. 2001), GLYT1/2 (Geerlings et al. 2000), DAT (Lee et al. 2004) and NET (Sung et al. 2003). Syntaxin 1A modulates GAT1-mediated GABA uptake, efflux and exchange, in addition to expression of GAT1 in a substrate-dependent manner via sub-cellular redistribution (Hansra et al. 2004; Wang et al. 2003a; Quick 2002; Horton and Quick 2001; Deken et al. 2000; Fan et al. 2006). The net result of these molecular interactions is an approximately 75% reduction in the turn-over rate of GAT1, strangely concurrent with a simultaneous increase in surface expression (Quick 2006; Deken et al. 2000; Beckman et al. 1998; Quick et al. 1997; Horton and Quick 2001). Increased expression is observed in other syntaxin 1A-coupled heteromers with the exception of SERT, the surface expression of which is decreased *in vitro* and *in vivo* by syntaxin 1A (Quick 2002, 2006). Syntaxin 1A can also alter the sub-cellular redistribution and localisation of translocators, including GAT1 (Horton and Quick 2001) and NET, which localises to syntaxin 1A-rich synaptic bouton micro-domains (Matthies et al. 2009). This overall inhibitory effect of syntaxin 1A is replicated with other SLC6 neuronal translocators, including GLYT1 and 2 (Geerlings et al. 2000), DAT (Cervinski et al. 2010; Binda et al. 2008), NET (Sung et al. 2003) and SERT (Quick 2002, 2003) though not necessarily

[4] Type IV have the same orientation as type II except the C-terminal TM domain is a signal anchor.

through the same mechanisms as is implied by the differential effects on expression and localisation. The net result of reduced substrate uptake for most SLC6 heteromers is a combination of the ancillary's numerous roles in co-ordinating and optimising neurotransmitter flux in diverse physiological contexts. Some of these roles involve increases of surface expression (Dipace et al. 2007; Cervinski et al. 2010; Sung et al. 2003; Quick 2002), substrate efflux (Binda et al. 2008; Wang et al. 2003a), modulating transport stoichiometry/substrate coupling (Quick 2003; Binda et al. 2006), and integrating substrate turnover and leak currents for optimised synaptic clearance (Carvelli et al. 2008; Quick 2003; Binda et al. 2006; Sung et al. 2003). The rapid time scale of functional modulation for translocators by syntaxin 1A also suggests that the complex formation is important for physiologically relevant regulation of extracellular neurotransmitter levels (Horton and Quick 2001). However, whether this regulation is coincident with SNARE complex formation or facilitated by a separate pool of syntaxin 1A remains unresolved. For example, GAT1 inhibition by syntaxin 1A depends on interaction with other SNARE machinery proteins (Fan et al. 2006), while GLYT1 and 2 inhibition by syntaxin 1A was reversed by the presence of the SNARE regulatory protein Munc-18 (Geerlings et al. 2000). *In silico* simulations using the SERT N-terminal, suggests that binding occurs via coiled-coil domains as it does during SNARE-mediated vesicular fusion events (Fenollar-Ferrer et al. 2014).

The evidence from various SLC6 transporters suggests a common interaction site with syntaxin 1A at the translocator N-terminal intracellular domain (Fig. 1 and Table 3). Systematic domain deletion of syntaxin-1A demonstrated inhibition of GAT1 transport requires the membrane-adjacent H3 and TM domains (residues 188–288) of the ancillary (Deken et al. 2000). Both H3 and TM domains are necessary for this inhibition, which also appears to be synergistic. The binding is mediated through direct interaction with cytoplasmic N-terminal aspartate residues of GAT1 (Deken et al. 2000) and is also reversible/competitive with a cluster of arginine residues in IL4 of the translocator subunit (Hansra et al. 2004). Similarly, the cytosolic N-terminus of both the norepinephrine (NET, SLC6A2), and dopamine (DAT, SLC6A3) transporters also directly interact with syntaxin 1A (Sung et al. 2003; Binda et al. 2008; Lee et al. 2004; Binda et al. 2006). Residues 28–47 in the N-terminal of NET are vital for controlling plasma membrane expression and distribution, while deletion of residues 1–42 modulates ion selectivity and the magnitude of leak currents (Binda et al. 2006; Dipace et al. 2007; Sung et al. 2003). For DAT, N-terminal residues 1–33 appear to be vital for dopamine efflux (Binda et al. 2008). In contrast to syntaxin 1A-GAT1 interactions, however, NET and DAT immunoprecipitations with syntaxin 1A did not require the syntaxin TM domain (Binda et al. 2008; Sung et al. 2003). The syntaxin 1A-SERT interaction is also dependent on the translocators N-terminal between residues 11–30 (Haase et al. 2001; Quick 2002, 2003), with the association disrupted by mutations to SERT N-terminal anionic residues: E16, D17, E19, D20, and E23 (Quick 2003), which form part of a predicted, stable α-helix (Fenollar-Ferrer et al. 2014). However, in contrast to GAT1, syntaxin 1A binding to SERT only required the H3 domain (188–266) of the ancillary and not both the H3 and TM (266–288) domains (Quick 2003).

This cumulative evidence points to a common binding region composed of a negative charge cluster in the N-terminals of SLC6 subunits. However, further analysis of DAT and NET has raised the possibility that syntaxin 1A binding is controlled by smaller N-terminal sub-domains, which correlate to different functional modulations of translocators. The conformation equilibrium of DAT induced to facilitate net dopamine efflux is activated by amphetamines and regulated by DAT N-terminal interaction with syntaxin 1A (Guptaroy et al. 2009; Kern et al. 2017; Sucic et al. 2010; Binda et al. 2008). Further modulation of efflux is achieved by various kinases and interactions with negatively charged phospholipids at the membrane inner leaflet (Beckman et al. 1998; Ciccone et al. 2008; Binda et al. 2008; Cervinski et al. 2010; Sung

et al. 2003; Dipace et al. 2007; Lee et al. 2004). Indeed, the initial recognition of syntaxin 1A as a binding partner of SLC6 neurotransmitter transporters occurred in the context of their regulation by PKC and other kinases (Ramamoorthy et al. 1998; Qian et al. 1997). A coordinated mechanism for this regulatory nexus proposes that the different functional states of DAT are mediated by different regions of the translocator N-terminals, certain functions of which (e.g. efflux) require interaction with syntaxin 1A (Khelashvili and Weinstein 2015; Khelashvili et al. 2015a; Mondal et al. 2014; Dipace et al. 2007; Razavi et al. 2018). This modular domain hypothesis of DAT-syntaxin 1A interactions is supported by evidence from both DAT- and syntaxin 1A-associated mutants. For example, two autism-associated mutations in the N-terminal of syntaxin 1A (R26Q) and DAT (R51W) display similar reduction in amphetamine-induced dopamine efflux (Cartier et al. 2015). As arginine residues in both subunits would be mutually repulsive, it is unlikely that they represent complementary binding domains for interaction. Instead, DAT R51 is located further towards the membrane in the translocator N-terminal, of which residues 1–33 had previously been identified as important for dopamine efflux. The two mutants could represent separate modular sites of interaction between subunits required for different stages in the dopamine efflux cycle or for different functions related to priming the translocator for efflux. The introduction of an extra negative charge to the same N-terminal region (T62D mutant) stimulates syntaxin 1A binding and increases dopamine efflux, supporting this region as specific to this particular role of DAT (Guptaroy et al. 2009). The potential of different domains of syntaxin 1A or DAT to modulate functional outcomes may be encoded by the ancillary-dependent recruitment of different kinases to the complex. For example, catecholamine efflux for both DAT and NET amphetamine-induced activation of Ca^{2+}/Calmodulin Kinase II (CaMKII) requires syntaxin 1A binding (Binda et al. 2008; Dipace et al. 2007). *In silico* simulations have established the role of PIP_2 membrane microdomain segregation in regulating syntaxin 1A residue S14 exposure to CK2-mediated phosphorylation (Khelashvili et al. 2012, 2015a; Khelashvili and Weinstein 2015). In yet another example, the interaction between NET and syntaxin 1A is disrupted and NET activity downregulated by PKC in an ancillary-dependent fashion (Sung et al. 2003). These mechanisms for DAT or NET functional modulation are dependent on residues at the N-terminal of syntaxin 1A (e.g. S14, R26) – the opposite end of the protein from the H3-TM domain implicated in mediating GAT1 and SERT interactions (Wang et al. 2003a; Quick 2002). One explanation is that domain-specific (modular) interactions are encoded equally by syntaxin 1A and translocators via the pairing of particular regions with the broadly identified interaction regions. In summary, it has been suggested that the various effects of syntaxin 1A are designed to optimise carrier function by engaging separate domains of the ancillary, depending on the particular functional requirement (Quick 2006; Han et al. 2017). This model would envision syntaxin-mediated DAT efflux as optimising neurotransmitter release at the same time as vesicular fusion occurs (Guptaroy et al. 2009; Binda et al. 2008), while the suppression of leak currents and tightening of ion-substrate coupling induced by syntaxin 1A is a product of interactions with different ancillary domains designed to optimise neurotransmitter reuptake, as has been demonstrated with SERT.

In addition to syntaxin 1A, syntaxin 3 may represent another ancillary partner for SLC6 translocators. Syntaxin 3 interacts *in vitro* with both intestinal SERT and the intestinal neutral AA SLC6 translocator B^0AT1 (Nazir et al. 2015; Fairweather et al. 2015). In intestinal CaCo2 cells SERT-syntaxin 3 interaction was upregulated by TGF-β1 in a PI3K-dependent manner, leading to increased plasma membrane expression (Nazir et al. 2015). In contrast, the surface expression of B^0AT1 (Sect. 2.5.5) was inhibited by syntaxin 3 and syntaxin 1A (Fairweather et al. 2015). No evidence of syntaxin 3 as a physiologically important ancillary subunit has been established.

2.5.3 Integration into Larger Complexes

Large scale proteomic (Hadlock et al. 2011; Maiya et al. 2007; Hiller-Sturmhöfel et al. 2008; Gorini et al. 2010, 2014; Haase et al. 2017) and

protein-specific interaction studies have identified additional SLC6 membrane-bound ancillaries, especially synaptic and neuronal proteins such as VAMP2 (Muller et al. 2014; Zhong et al. 2012), synucleins (Lee et al. 2001; Wersinger and Sidhu 2003; Jeannotte and Sidhu 2007; Fountaine and Wade-Martins 2007; Moszczynska et al. 2007; Dauer et al. 2002; Chandra et al. 2004; Bellucci et al. 2011; Longhena et al. 2018; Wersinger et al. 2006; Wersinger and Sidhu 2009), synaptogyrin 3 (Egaña et al. 2009), syntenin-1 (Ohno et al. 2004), Kv2.1 potassium voltage-gated channel (Lebowitz et al. 2019), melatonin receptors (Benleulmi-Chaachoua et al. 2018), GPCR βγ subunits (Mauna et al. 2019; Garcia-Olivares et al. 2017; Garcia-Olivares et al. 2013) and single-pass proteins TMEM17 and TMEM216 (Gupta et al. 2015). Although these interactions are too numerous to cover adequately here, common themes emerge which bear resemblance with the better characterised syntaxin 1A interactions. These include mediation of interactions through the N- and C-termini of the translocators, lipid-raft association and involvement in regulatory nexus linking translocator specific functions with PTMs and brain region-specific expression (Bolland et al. 2019; Eriksen et al. 2010; Torres 2006; Muller et al. 2006; Ozaslan et al. 2003; Zhong et al. 2012; Fenollar-Ferrer et al. 2014). The homo-oligomerisation of SLC6 (Scholze et al. 2002; Torres et al. 2003; Farhan et al. 2006; Sitte et al. 2004; Anderluh et al. 2014) appears to control ER retention export, and localisation of neurotransmitter transporters and is dependent on the scaffold (hash) domain of the LeuT fold (Fig. 2a) (Jayaraman et al. 2018). Regulation of homo-oligomers and heteromers in both DAT and SERT also involves common elements such as PIP2 (Das et al. 2019; Anderluh et al. 2017; Buchmayer et al. 2013), pointing to the potential of common mechanisms controlling the formation of larger NSS transporter metabolons. Several less well characterised TM-domain containing ancillaries warrant a more detailed review.

Both SERT and DAT surface expression and activity are downregulated by Secretory Carrier Associated Membrane Protein 2 (SCAMP2) via redistribution away from the plasma membrane (Muller et al. 2006; Fjorback et al. 2011). SCAMP2 is a 4TM domain-containing chaperone controlling trans-Golgi to surface trafficking or endosomal recycling and is often found on exocytotic or recycling vesicles (Lin et al. 2005). SCAMP2 also interacts with numerous other solute carriers, including the Na^+/Cl^- symporter NKCC2 (SLC12A1) (Zaarour et al. 2011) and the Na^+/H^+ exchangers NHE5 (SLC9A5) (Diering et al. 2009) and NHE7 (SLC9A7) (Lin et al. 2005). The residue C201 in the SCAMP2 E peptide domain (IL1) domain seems to be a critical point for interactions with multiple solute carriers, raising the possibility it is a widespread recycling and redistribution regulator of many transporters. SERT co-localises with SCAMP2 in lipid-rafts and a C201A mutation in SCAMP2 abolished the functional downregulation of SERT, but not the physical interaction of the two proteins (Muller et al. 2006). Deletion of scamp gene homologs from Drosophila exhibited neuronal abnormalities including odour-associated memory and physical climbing consistent with a role in neurotransmitter regulation (Zheng et al. 2014). Based on *in vivo* data from a single publication, we also include neuroligin 2 as an additional potential ancillary subunit for SERT (Ye et al. 2014a, b, 2015). Neuroligin 2 (NLGN2, Q8NFZ4) is type 1 integral synaptic adhesion protein possessing a large extracellular cholinesterase domain, a single TM domain and a short intracellular PDZ-binding domain (Koehnke et al. 2008; Arac et al. 2007). It forms protein complexes with pre-synaptic neurexins for the assembly of post-synaptic $GABA_A$ ionotropic receptors (Graf et al. 2004; Poulopoulos et al. 2009) and also at dopaminergic synapses (Uchigashima et al. 2016). Neuroligin 2 and SERT were co-localised and co-immunoprecipitated from mouse midbrain. A neuroligin 2 null mouse displayed significant decreases in SERT hippocampal and midbrain expression and function, in addition to serotonin-mediated behavioural changes (Ye et al. 2015).

2.5.4 SLC6 Amino Acid Transporter Interactions with Collectrin and ACE2

B^0AT1 and B^0AT3 are unique among SLC6 members in having heteromeric interactions with ancillary proteins collectrin (aka TMEM27, NX17) and ACE2 that are necessary for full plasma membrane expression (Danilczyk et al. 2006; Vanslambrouck et al. 2010; Kowalczuk et al. 2008; Malakauskas et al. 2007; Singer et al. 2009; Fairweather et al. 2012, 2015). B^0AT1, however, retains a small amount of activity and plasma membrane expression in *Xenopus laevis* oocytes without collectrin or ACE2, possibly as a result of lower affinity interactions with endogenous ancillary protein homologues (Kowalczuk et al. 2008; Bohmer et al. 2005; Camargo et al. 2005; Seow et al. 2004). Both ancillaries are type I single TM domain proteins, with collectrin predicted to consist of a signal peptide (residues 1–14), N-terminal extracellular domain (15–141), TM domain (142–164), and intracellular C-terminus (165–222) (Fig. 1). ACE2 is a 805 residue protein consisting of 2 structural domains: the N-terminal extracellular zinc metallopeptidase domain (residues 1–592), a neck/linker (593–741), and TM and short cytosolic domain (742–805) (Hooper 1994; Yan et al. 2020c, d) (Fig. 1). The N-terminal is also predicted to contain a signal peptide from residues 1–17. The ACE2 neck, linker and TM domains residues 592–805 are homologous to collectrin, while the catalytic domain is homologous to the catalytic domain of the ACE2 paralog Angiotensin Converting Enzyme I (ACE) (Turner and Hooper 2002; Zhang et al. 2001). As a result ACE2 is speculated to be a chimera arising from a gene fusion of collectrin and with ACE (Mount 2007; Zhang et al. 2001; Kuba et al. 2013; Turner et al. 2002). Collectrin and ACE2 are located on chromosome X (Xp22.2) adjacent to each other within ~64 kilobases and are transcribed from the same strand, collectrin containing 6 exons, ACE2 18.

Collectrin is involved in several distinct physiological roles: amino acid transport in kidney proximal tubules (Malakauskas et al. 2007; Danilczyk et al. 2006), epithelial cell polarity and growth (Zhang et al. 1999, 2001, 2007; Pasquali et al. 2009; McCoy et al. 2008; Zhang et al. 2004; Li et al. 2003a), hypertension (Chu and Le 2014; Egan 2013; Cechova et al. 2013; Yasuhara et al. 2008; Bril and Feletou 2014), insulin secretion (Saisho et al. 2009; Malakauskas et al. 2009; Akpinar et al. 2005; Fukui et al. 2005; Esterhazy et al. 2012; Pepaj et al. 2014), as a biomarker for the pathogenesis of type II diabetes (Pepaj et al. 2014; Vats et al. 2012; Altirriba et al. 2010) or its role in Turner's syndrome (Pasquali et al. 2009). Originally characterised as upregulated following partial kidney nephrectomy (Li et al. 2003a; Zhang et al. 1999, 2001), mouse knock-outs of collectrin demonstrated the collectrin-dependence of B^0AT1 and B^0AT3 in mouse proximal tubules (Danilczyk et al. 2006; Malakauskas et al. 2007; Singer et al. 2009, 2012). Collectrin has also been associated with indirect upregulation of other unrelated renal brush border transporters in salt-sensitive hypertensive mice (Yasuhara et al. 2008; Danilczyk et al. 2006). Tissue expression is highest in the S1, S2, and S3 segments of kidney proximal tubules (Zhang et al. 2001) with lower mRNA and protein expression in pancreatic β-islet cells, β-islet clonal cells lines (Akpinar et al. 2005; Fukui et al. 2005), vascular endothelium (Cechova et al. 2013) and rat retinal pigment epithelium (Gu et al. 2012) (Fig. 5). The predicted M.W. of collectrin at just over 25 kDa is at odds with its commonly observed M.W. of ~45 kDa on western blots, which may be explained by O- and N-linked glycosylation in both β-islet cells and the proximal tubule (Zhang et al. 2007; Akpinar et al. 2005; Fukui et al. 2005; Malakauskas et al. 2007; Danilczyk et al. 2006).

ACE2 was identified simultaneously from an EST database (Tipnis et al. 2000) and human heart failure 5′ cDNA library (Donoghue et al. 2000). Protein and mRNA expression has been noted in human heart, kidney and testis (Tipnis et al. 2000; Donoghue et al. 2000), lung (Imai et al. 2005; Kuba et al. 2005; To and Lo 2004; Hamming et al. 2004), liver (Paizis et al. 2005), small intestine (Kowalczuk et al. 2008; To and Lo

2004; Hamming et al. 2004), venous endothelium (Hamming et al. 2004), arterial smooth muscle (Hamming et al. 2004), and brain (Doobay et al. 2007) (Fig. 5). ACE2 has been the subject of intense research interest due to its multiple physiological roles in blood pressure regulation, heart disease, glycemic control, foetal growth, diabetic nephropathy, ischemia-reperfusion injury in lung/kidney, intestinal inflammation (Hashimoto et al. 2012) and as the SARS-associated corona virus receptor (Kirchdoerfer et al. 2018a, b; Imai et al. 2005; Kuba et al. 2005; Itoyama et al. 2005; Jeffers et al. 2004; Li et al. 2003b) (for reviews see (Patel et al. 2014; Parajuli et al. 2014; Chamsi-Pasha et al. 2014; Jiang et al. 2014; Chappell et al. 2014; Kuba et al. 2013; Perlot and Penninger 2013; Chhabra et al. 2013; Wang et al. 2013b; Imai et al. 2008a, b, 2010; Ortiz-Melo and Gurley 2016; Santos et al. 2018; Alenina and Bader 2019; Williams and Scholey 2018; Gilbert et al. 2019; He et al. 2018)). ACE2 hydrolyses the C-terminal of peptides with proline and hydrophobic residues in the penultimate and terminal positions, respectively (Pro-X) (Guy et al. 2003; Vickers et al. 2002). This unique specificity amongst brush border peptidases suggests it also plays a significant role in protein digestion, which has subsequently been confirmed (Vuille-dit-Bille et al. 2015; Singer et al. 2012; Hashimoto et al. 2012; Kowalczuk et al. 2008). The specificity of ACE2 for C-terminal neutral amino acid matches the specificity of its heteromer partners, the broad neutral amino acid transporters of the small intestine (Rella et al. 2006; Guy et al. 2003; Vickers et al. 2002; Bohmer et al. 2005; Camargo et al. 2005) (see below). Protein mutagenesis and elucidation of the ACE2 structure revealed peptide binding is initiated by formation of an electro-static bond between R273 of ACE2 and C-terminal peptide carboxylate in the active site, resulting in a 'hinge-bend' movement of catalytic subdomains I and II to close the binding pocket (Guy et al. 2003, 2005; Rella et al. 2006; Towler et al. 2004) (Fig. 1). Divalent zinc is also essential for substrate coordination at the active site and protein stability (Towler et al. 2004; Vickers et al. 2002). The main biological role of ACE2 is the hydrolysis of angiotensin II (1–8) to angiotensin 1–7 (Tipnis et al. 2000; Donoghue et al. 2000; Vickers et al. 2002) with a K_m of 2 μM (Vickers et al. 2002). ACE2 also hydrolyses other vasoregulatory peptides (Lambert et al. 2008; Kalea and Batlle 2010; Vickers et al. 2002) and can convert angiotensin I to angiotensin 1–9, a peptide upregulated during heart failure of unknown function but thought to effect the cardiovascular system and arterial thrombosis (Donoghue et al. 2000; Ocaranza et al. 2006; Flores-Muñoz et al. 2011; Kokkonen et al. 1997; Mogielnicki et al. 2014; Kramkowski et al. 2010). As a result of its role in the biogenesis of vasodilatory angiotensin 1–7 ACE2 has been investigated as a potential treatment for hypertension and heart failure prevention through application of the protein or its activation by agonists (Patel et al. 2014; Parajuli et al. 2014; Chamsi-Pasha et al. 2014; Jiang et al. 2014; Qaradakhi et al. 2020; Kittana 2018) (Table 4).

Initial indications that collectrin was involved in kidney nutrient uptake came from collectrin null mice[5] which displayed extensive renal neutral aminoaciduria replicating the Hartnup disorder phenotype, a condition characterised by high levels of intestinal and renal neutral aminoaciduria (Malakauskas et al. 2007; Danilczyk et al. 2006). Expression of B^0AT1 and B^0AT3 was significantly reduced in these mice and direct interaction between collectrin and B^0AT1 in the kidney was confirmed in WT tissue (Danilczyk et al. 2006). Human collectrin gene deletions recapitulate the aminoaciduria phenotype of Hartnup disorder, further demonstrating the *in vivo* relevance of interaction (Pillai et al. 2019). Dependence of B^0AT1 activity on ACE2 co-expression was also demonstrated, along with intestinal co-localisation, whereas co-localisation in the kidney was minimal (Kowalczuk et al. 2008). Direct interaction between ACE2 and B^0AT1 was confirmed by co-immunoprecipitation and reduction of

[5] As collectrin and ACE2 are located on the X chromosome, the male collectrin/ACE2 null mouse is a (−/*y*) genotype while the female is a collectrin/ACE2 mouse (−/−).

Table 4 Overview of mammalian heteromeric solute carrier disease associations and pharmacology

Ancillary		Translocator		Disease associated	Known specific inhibitors
rBAT	SLC3A1	$b^{0,+}$AT	SLC7A9	Renal aminoaciduria, cystinuria (Feliubadalo et al. 1999; Calonge et al. 1994), Hypotonia-Cystinuria Syndrome (Parvari et al. 2005; Parvari et al. 2001)	None
		AGT1	SLC7A13	None	None
4F2hc	SLC3A2	LAT1	SLC7A5	Cancer cells, activated lymphocytes over-express, rheumatoid arthritis, Autism Spectrum Disorder (ASD) (Lo et al. 2008; Yu et al. 2018; Tarlungeanu et al. 2016; Cascio et al. 2019)	JPH203/KYT-0353 (Cormerais et al. 2016; Oda et al. 2010; Hafliger et al. 2018; Okano et al. 2018); anti-human 4F2hc mAbs HBJ127/MEM-108/ KHK2898/IGN523 (Ueda et al. 2019; Yagita et al. 1986; Hayes et al. 2015; Saito et al. 2014); anti-human LAT1 mAbs SOL22, SOL69, Ab1-6 (Ohno et al. 2008; Ikotun et al. 2013; Ueda et al. 2018, 2019)
		LAT2	SLC7A8	Age-Related Hearing Loss (ARHL), lens cataract development, crescentic glomerulonephritis (Espino Gaurch et al. 2018; Knopfel et al. 2019; Kurayama et al. 2011), ASD (Cascio et al. 2019)	None
		y^+LAT1	SLC7A7	Lysinuric Protein Intolerance (LPI) (Torrents et al. 1999; Bodoy et al. 2019)	None
		y^+LAT2	SLC7A6	None	None
		Acs-1	SLC7A10	None	Lu AE00527, (+)-amino (1-(3,5-dichlorophenyl)-3,5-dimethyl-1H-pyrazol-4-yl)acetic acid (ACPP), BMS-466442 (Sason et al. 2017; Brown et al. 2014; Sakimura et al. 2016)
		xCT	SLC7A11	Kaposi's sarcoma-associated Herpesvirus infection, cocaine relapse, Cancer cells and activated lymphocytes over-express (Veettil et al. 2008; Baker et al. 2003) (Lewerenz et al. 2013)	R/S-4-[4′-carboxyphenyl]-phenylglycine, 2-thiopheneglycine-5-sulfonic acid and (S)-4-carboxy-3-hydroxyphenylglycine (sulfasalazine; S-4CPG), erastin (Patel et al. 2004; Etoga et al. 2010; Gout et al. 2001)
Basigin	BSG	MCT1	SLC16A1	Exercise-induced hyperinsulinemia (EIHI), Erythrocyte lactate	AZD3965, AR-C177977, AR-C122982, p-chloromercuribenzene

(continued)

Table 4 (continued)

Ancillary		Translocator		Disease associated	Known specific inhibitors
				transporter defect (Merezhinskaya et al. 2000; Fishbein 1986), Monocarboxylate Transporter 1 Deficiency (MCT1D) (van Hasselt et al. 2014) chronic fatigue syndromes (Otonkoski et al. 2007; Halestrap and Wilson 2012)	sulfonate, 7-Aminocarboxycoumarin derivatives (7-ACCs, e.g. AR-C155858 (Ovens et al. 2010a; Draoui et al. 2013; Noble et al. 2017; Polanski et al. 2014; Wilson et al. 2005)
		MCT2	SLC16A7	Chronic fatigue syndromes; Tumour cell proliferation, migration and invasiveness (Halestrap and Wilson 2012; Pinheiro et al. 2008, 2009a, 2010a, b)	AR-C177977, AR-C122982, AR-C155858 (Ovens et al. 2010a)
		MCT4	SLC16A8		Bindarit, acriflavine, p-chloromercuribenzene sulfonate, 7ACCs (Draoui et al. 2013; Futagi et al. 2018; Voss et al. 2017; Wilson et al. 2005)
Embigin	EMB	MCT3	SLC16A3		None
Basigin	BSG	MCT11	SLC16A11	Type II diabetes (Rusu et al. 2017; Williams et al. 2014)	None
		MCT12	SLC16A12	juvenile cataracts with microcornea (cataract 47) (Kloeckener-Gruissem et al. 2008)	None
Glycophorin A	GYPA	AE1	SLC4A1	Hereditary spherocytosis (HS), Southeast Asian ovalocytosis (SAO), acanthocytosis, and distal renal tubular acidosis (dRTA) (Toye et al. 2008; Fawaz et al. 2012; Picard et al. 2014; Kager et al. 2017; Yang et al. 2018).	DIDS, H_2DIDS (Falke and Chan 1986a, b, c; Garcia and Lodish 1989)
OSTα	SLC51A	OSTβ	SLC51B	Unknown	None
Syntaxin-1A	STX1A	GAT1	SLC6A1	Epilepsy (Krogsgaard-Larsen et al. 2000)	Tiagabine, NNC-711, SKF89976A, CI-966 (Nielsen et al. 1991; Andersen et al. 1993; Borden et al. 1994)
		NET	SLC6A2	Depression, anxiety, ADHD, addiction (Nemeroff and Owens 2002; Azima and Vispo 1958; Brown and Gershon 1993; Eshleman et al. 1999; Sery et al. 2015)	Reboxetine, Atomoxetine, Nomifensine, Mazindol, Nisoxetine, Talopram, Talsupram (Eshleman et al. 1999; Tatsumi et al. 1997; Andersen et al. 2009)
		DAT	SLC6A3		Modafinil, Armodafinil, GBR12935, Benztropine, JHW 007 (Zolkowska et al. 2009; Hauck et al. 2008)
		SERT	SLC6A4		Sertraline, Escitalopram, Paroxetine, Fluoxetine, Fluvoxamine (Andersen

(continued)

Table 4 (continued)

Ancillary		Translocator		Disease associated	Known specific inhibitors
					et al. 2010; Tavoulari et al. 2009)
		GLYT2	SLC6A5	Hyperekplexia, neuropathic pain (Caulfield et al. 2001; Harvey and Yee 2013; Vuong et al. 2008)	ORG 25543, ALX1393 *N*-arachidonoyl glycine, oleoyl-L-carnatine (Caulfield et al. 2001; Carland et al. 2014; Vandenberg et al. 2014; Harvey and Yee 2013; Zeilhofer et al. 2018; Burstein 2018; Jeong et al. 2010; Vuong et al. 2008; Mostyn et al. 2019; Schumann-Gillett and O'Mara 2019)
		GLYT1	SLC6A9	Glycine transporter 1 encephalopathy, Schizophrenia, OCD, depression, chronic pain, anxiety, allodynia, β-thalassemia (Cioffi 2018; Alfallaj and Alfadhel 2019; Javitt et al. 1994)	Sarcosine-based inhibitors (e.g. ALX5407/NFPS, ALX-5407, LY2365109, AMG 747, Org25935), methylphenidates (e.g. SSR5044734, threo methylphenidate), alkyl- and heteroaromatic-substituted sulphonamides/ sulfones (e.g. DCCCyB), heteroaryl amides (e.g. PF-3463275), benzoylpiperazines (e.g. bitopertin), (Cioffi 2018; Atkinson et al. 2001; Aubrey and Vandenberg 2001; Pinard et al. 2010; Morita et al. 2008; Hirayasu et al. 2016; Harvey and Yee 2013)
Collectrin or angiotensin converting enzyme 2	TMEM27 or ACE2	B^0AT1	SLC6A19	Hartnup disorder, type II diabetes, phenylketonuria (Broer et al. 2005; Jiang et al. 2015; Belanger et al. 2018)	Cinromide, Benztropine, NSC22789, Nimesulide, diarylmethine derivatives (Cheng et al. 2017; Pochini et al. 2014; Danthi et al. 2018; Yadav et al. 2020)
		B^0AT3	SLC6A18	Aminoaciduria (Singer et al. 2009; Vanslambrouck et al. 2010)	None
Angiotensin converting enzyme 2	ACE2	IMINO	SLC6A20	Iminoaciduria, iminoglycinuria (Kowalczuk et al. 2005; Takanaga et al. 2005; Broer et al. 2008)	None

isoleucine uptake in ACE2 null mice intestine *ex vivo* (Camargo et al. 2009; Singer et al. 2012). Functional interaction between both collectrin and ACE2 with murine B^0AT3 has also been established (Singer et al. 2009; Vanslambrouck et al. 2010; Fairweather et al. 2015). ACE2 modestly increases the surface expression and proline uptake by the IMINO

transporter (SLC6A20), while an ACE2 knockout mouse increased levels of faecal proline (Vuille-dit-Bille et al. 2015; Singer et al. 2012). However, IMINO surface expression in *Xenopus laevis* oocytes and activity are robust without ACE2 co-expression (Vuille-dit-Bille et al. 2015; Kowalcczuk et al. 2005).

The exact nature, stoichiometry, initiation, and composition of B^0AT1 and B^0AT3 interactions with ACE2 and collectrin remained poorly understood until the recent publication of the human B^0AT1-ACE2 complex structure elucidated by cryo-EM (Yan et al. 2020c, d). The full complex formed as a dimer of B^0AT1-ACE2 heterodimers with the interface between dimers exclusively mediated by polar interactions in the 'neck' and lesser contacts in the peptidase domains of the ACE2 molecules (Fig. 7a). The two dimers of heterodimers are related by a 180° rotational symmetry around a vertical axis between the two. This arrangement means that the two B^0AT1 molecules in the complex face away from each other and no contacts occur between heterodimers in the membrane. This means that the translocator plays no role in the formation of the B^0AT1-ACE2 dimers, a situation reminiscent of the $b^{0,+}AT1$-rBAT dimer of heterodimers (Sect. 2.1.2). As previously predicted (Danilczyk et al. 2006) the complex is not mediated by a cys-cys disulphide bond but contains 5 N-glycosylation sites at N158, 182, 258, 354, and 368 in B^0AT1 with a further 7 observed on each ACE2, some of which were previously known (Chen et al. 2009a; Towler et al. 2004; Kristiansen et al. 2004). Three separate cryo-EM structures were reconstructed of which two represent distinct conformation states of the heteromer, designated as an 'open' conformation with no substrates bound (PDB ID 6M1D, 2.9 Å RMSD), and a 'closed' conformation with leucine bound to B^0AT1 and a zinc ion at the active site of ACE2 (6M17, 2.9 Å RMSD) (Fig. 7b, c). A third complex structure appears in some type of hybrid state with zinc bound to ACE2 only (6M18) (not shown). The closed, substrate-bound complex forms contacts through the ACE2 peptidase and neck domains, while in the open substrate-free conformation heteromer dimerization is mediated by contacts only between neck domains, resulting in a 22.1 Å gap between the ACE2 peptidase domains in the open conformation. By comparison the B^0AT1 moves very little between the open and closed conformers, possibly indicating that conformational changes between subunits are coupled and substrate binding in either or both subunits is required to drive the transport cycle. However, it is noteworthy that the closed structure does not have sodium bound along with leucine in the B^0AT1 binding site, which is essential for substrate translocation (Bohmer et al. 2005; Camargo et al. 2005). The closed complex structure ACE2 EC peptidase domain is also bound by the Receptor Binding Domain (RBD) of the SARS-CoV-2 virus S1 protein, which was reconstituted with the purified protein at high concentration (Yan et al. 2020c). Understandably this has generated a large amount of current interest in the B^0AT1-ACE2 structure but lies outside the scope of this review. The reader is directed to additional recent publications of the S1 protein bound to ACE2 for an extensive analysis of the viral protein recognition and binding of its human receptor (Wang et al. 2020; Wrapp et al. 2020; Lan et al. 2020b; Shang et al. 2020; Chen et al. 2020). The formation of the B^0AT1-ACE2 complex is mediated by interactions in two distinct regions. The first of these is a polar interaction network formed where the neck domain of ACE2 meets an unexpectedly long extracellular extension of B^0AT1 TM7 helix domain, which forms the N-terminal half of the EL4 domain (Fig. 7d). The EL4 extension is a signature feature of the AA2 branch of the SLC6 family containing B^0AT1, B^0AT3, IMINO and other amino acid transporters and extends over 70 residues longer than in other SLC6 transporters (Broer and Gether 2012; Broer 2006; Broer et al. 2006b; Singer et al. 2009; Zaia and Reimer 2009; Parra et al. 2008; Fairweather 2017). The TM7 helix extends ~30 Å distance into the EC solution in order to contact the ACE2 neck domain through this series of hydrogen bonds and electrostatic contacts centred on K619, K676, and R678 from ACE2 and N346, D349, and E352 in B^0AT1. Even more significant, these key B^0AT1 residues are conserved in the other AA2 branch (or B^0-like) transporters

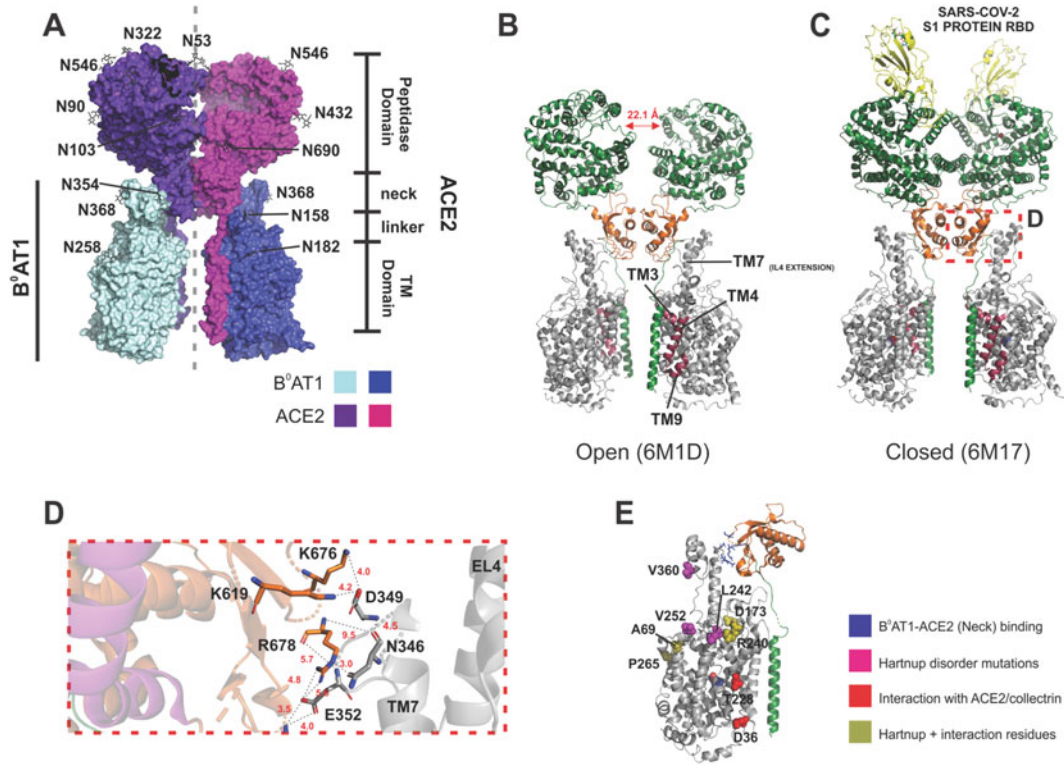

Fig. 7 Ancillary binding and interacting residues in B^0-like SLC6 transporters
The human B^0AT1-ACE2 complex is formed by a dimer of heterodimers with each dimer formed by one B^0AT1 translocator subunit and one ACE2 ancillary subunit. (**a**) The subunit composition of the B^0AT1-ACE2 dimer of heterodimers with subunits rendered as a surface (van der Waals) representation and coloured according to the key. N-linked glycosylation sites in both subunits are labelled. The dashed vertical line represents the 180° rotational axis of symmetry by which the two dimers are related. The structural architecture of the B^0AT1-ACE2 heteromer in an 'open' apo complex (6M1D, RMSD 2.9 Å) (**b**) and in a 'closed' substrate-bound complex (6M17, RMSD 2.9 Å) is rendered as cartoon. The ACE2 subunit peptidase, TM, and linker domains are coloured green, the neck domain is orange. The B^0AT1 subunit is coloured grey with TM spanning α-helices 4, 3, and 9 interacting with ACE2 are coloured purple. The two SARS-CoV-2 S1 (spike protein 1) Receptor Binding Domains (RBD) bound to the EC peptidase domain of ACE2 are coloured yellow. The leucine substrate bound to B^0AT1 subunits (**c**) are coloured blue (sphere models, C atoms, grey; O atoms, red; N atoms, blue; S atoms, yellow). Zinc atoms located in each ACE2 subunit active sight (red) are rendered by their ionic radius (sphere). **Insert d**) The polar interactions at interface between the neck domain of ACE2 and the TM7 extension of B^0AT1 at the N-terminal of the EL4 loop. The ACE2 neck domain (orange), TMPRSS2 protease cleavage site (purple) and B^0AT1 (grey) are rendered as cartoons. Residues involved in polar interactions are shown as sticks and coloured according to the subunit they originate in. (**e**) Common Hatrnup disorder causing and other mutations rendered as VDW spheres and coloured (see key) according to their functional role in the heteromeric solute carrier B^0AT1-ACE2

IMINO (SLC6A20) and B^0AT3 (SLC6A18) whose function is augmented or dependent on ACE2, but not those which do not depend on the ancillary e.g. B^0AT2 (SLC6A15) and NTT4 (SLC6A17). Furthermore, these two ACE2-dependent or non-dependent sub-branches also display a clear tissue expression difference, with B^0AT1 and other ancillary-dependent transporters predominate in epithelial organs, whereas non-dependent transporters are neuronal (Fig. 5). The neck domain of ACE2 also contains the protein hydrolysis site for the serine protease TMPRSS2 (residues 697–716) in the α-helices of its neck domain at the dimeric interface of the two ACE2 molecules rather than in the B^0AT1 binding site (Fig. 7d). Cleavage of ACE2 at this site

by TMPRSS2 facilitates S-protein driven entry of SARS-CoV viruses (Shulla et al. 2011; Heurich et al. 2014) and it has been speculated that the presence of B^0AT1 could block access to this site (Yan et al. 2020c). The second region of contact involves the ACE2 TM domain which aligns on a tilted axis with the membrane and forms a van der Waals network with TM4 and TM3 of B^0AT1, terminating on the cytosolic side of the membrane in a salt bridge between ACE2 Arg768 and Glu456 on TM9 of B^0AT1 (not shown). A third region involves interactions between the linker of ACE2 and the extended EL4 of B^0AT1. However this region has low electron density and as such is unresolved to atomic level in these structures.

Consistent with the recent B^0AT1-ACE2 structures (Yan et al. 2020c) and unlike SLC7-SLC3 heteromeric solute carriers, the interaction between the B^0-like translocators does not require the large peptidase domain of ACE2. A deletion of the ACE2 catalytic domain (Δ2–592) corresponding to the entire metallopeptidase domain (subdomains I to IV, see Fig. 1), showed no effect on B^0AT1 activity (Fairweather et al. 2015). The remaining truncated region of ACE2 is homologous to collectrin and contains the membrane proximal neck/linker domains which mediate interactions with the extended TM7 and EL4 regions of B^0AT1 (Fig. 7d). Together this data strongly suggesting B^0-like transporter heteromer formation is mediated by the collectrin domain of ACE2 and collectrin. It has been suggested that mutations in B^0AT1 causing Hartnup disorder may demonstrate the existence of multiple or different binding sites for different ancillaries of B^0AT1 (Kowalczuk et al. 2008; Camargo et al. 2009) (Fig. 7e). For example, B^0AT1R240Q and A69T are both ACE2 and collectrin dependent, while the Hartnup mutations B^0AT1D173N and B^0AT1P265L show ancillary-dependent differences in their functional effect (Camargo et al. 2009; Kowalczuk et al. 2008). However, this discrimination is very slight and the overall effect of both D173N and P265L is a loss of >90% of transport activity when expressed with either ancillary. In contrast to the hypothesis that B^0-like translocators form stable complexes with their ancillaries, B^0AT1 also mediates collectrin-independent neutral amino acid uptake when purified from kidney (Oppedisano et al. 2011; Oppedisano and Indiveri 2008). Furthermore, B^0AT1 retains a small amount of activity (~5%) and plasma membrane expression when expressed alone in *Xenopus laevis* oocytes – explaining its initial characterisation without collectrin or ACE2 (Kowalczuk et al. 2008; Bohmer et al. 2005; Camargo et al. 2005; Seow et al. 2004). It is unknown whether these results represent small amounts of ancillary-independent trafficking and activity by B^0AT1, or point to transient heteromer formation required for as yet undiscovered post-translational processing. The B^0AT3 translocator does not show ancillary-independent surface expression or activity (Fairweather et al. 2015; Singer et al. 2009).

The collectrin N-terminal and TM domains are also necessary for heteromer formation, consistent with the B^0AT1-ACE cryo-EM structure (Yan et al. 2020c) showing interaction with the transporter over large sections of ACE2 homolgous to collectrin. By contrast the C-terminal of collectrin, except the TM-adjacent region 163–178, is dispensable (Fairweather 2017) and the homologous region of ACE2 mediates no interactions within the B^0AT1-ACE2 structure. Rather the collectrin C-terminal intracellular domain appears to be responsible for functional interactions with other proteins; for example, binding to snapin, a SNAP-25 binding protein in pancreatic β-cells, where it is proposed to play a role in insulin secretion (Fukui et al. 2005). Collectrin dimerization in pancreatic β-cells is also mediated by a C-terminal Cys186 residue (Esterhazy et al. 2012). B^0AT1 expression in β-cells is minimal (Broer et al. 2004; Kleta et al. 2004) suggesting collectrin dimerization plays no significant role in heteromer interaction in this cell type. As both collectrin and ACE2 display a wide-range of tissue specific functions these combined results suggest the formation of solute carrier heteromers are mediated by different domains of the ancillary protein. Furthermore, the different domains of ACE2 also display functional specificity, with recent publications demonstrating that the receptor binding domain of the SARS-CoV-2 spike protein and the B^0AT1-interacting

domains are formed at different parts of ACE2 (see (Yan et al. 2020d; Lan et al. 2020a)).

There remains some incongruence between the B^0AT1-ACE2 structures (Yan et al. 2020c) and the position of some mutations in B^0AT1 causing Hartnup disorder, which are located some distance apart (Broer and Fairweather 2018; Broer 2013a). This is unexpected as several of these Hartnup mutations have been shown to specifically involve trafficking of the complex to the membrane – supposedly one of the main functions of complex formations. Many Hartnup-causing mutations lie some distance from of the conserved SLC6 substrate/ion binding sites (Fairweather et al. 2015; Kowalczuk et al. 2008; Camargo et al. 2009; O'Mara et al. 2006) but also distal from the interaction regions demonstrated in the recent B^0AT1-ACE2 complex structures (Yan et al. 2020c, d) (Fig. 7e). This includes the B^0AT1 mutation R240Q which is required specifically for ACE2-mediated trafficking to the plasma membrane (Kowalczuk et al. 2008; Fairweather et al. 2015) but lies at the extracellular-membrane interface of TM5/EL3 of B^0AT1 – distal to the interaction sites between B^0AT1 and ACE2. The locations of B^0AT1 and B^0AT3 mutations involved in ancillary-dependent functions but distal to the cryo-EM elucidated ancillary binding site has also been investigated. Using a MCT1-B^0AT3 tandem construct which reaches the cell surface using MCT1 ancillaries (Sect. 2.2.3), collectrin-dependent trafficking mutations clustered to TM domains 7, 5 and 1α of B^0AT3 (Fairweather et al. 2015). This region forms a groove in both experimental structures and homology models of eukaryotic SLC6 transporters for hydrophobic molecular binding (e.g. see (Coleman et al. 2016; Penmatsa et al. 2013) and (Fairweather et al. 2015; Laursen et al. 2018; Jungnickel et al. 2018; Dickens et al. 2017; Mostyn et al. 2019)). Residues in TM5, TM7 and TM1α were found to be involved in the catalysis of substrate translocation in B^0AT3 by collectrin or both trafficking and catalysis (Fairweather et al. 2015) (Fig. 7b). Orthologues of collectrin are also able to cross-react (activate) orthologs of B^0AT1 from species as diverse as human, mouse, salmon and sea bass (Margheritis et al. 2016; Fairweather 2017). The species cross-reactivity and domain conservation of collectrin/ACE2 interactions with B^0AT1 and B^0AT3 strongly suggests they involve a common molecular basis. Enough ancillaries and sterol molecules have been found modelled or as bound to the same general TM7-TM5 region of LeuT-fold transporters to suggest this region represents a common hydrophobic binding site. However, with the exception of the EC region of TM7, none of these regions correspond to the B^0AT1-ACE2 interaction site identified in the recent cryo-EM structures of the complex (Yan et al. 2020b, d). Indeed, the ACE2 TM helix appears to bind relatively close to the same site as the ancillary TM domains do in the recently published LAT1-4F2hc and $b^{0,+}AT$-rBAT structures (Sect. 2.1.2). A brief discussion of the potential for conserved ancillary/lipid binding sites in LeuT/APC fold transporters is given in Sect. 3.

2.5.5 Integration into Larger Complexes

B^0AT1 has been shown to form protein complexes with another single-pass membrane protein Aminopeptidase N (APN or CD13), which is, like ACE2, one of 11 mammalian membrane–anchored intestinal peptidases (Bai 1994a, b; Alpers 1987) and is abundantly expressed in both the small intestine and renal brush borders (Riemann et al. 1999; Fairweather et al. 2012). It is responsible for the hydrolysis of angiotensin III to angiotensin IV in the renin-angiotensin system (Chansel et al. 1998; Ward et al. 1990), as well as numerous other bio-active peptides. It has been implicated in cellular endocytosis, cell adhesion, tumour-cell invasion, proliferation, apoptosis, and as a receptor for several viruses and, notably, the human coronavirus 229E (reviewed in (Danziger 2008; Mina-Osorio 2008)). The tissue distribution of APN and B^0AT1 overlap in the small intestine and S1/S2 segments of the proximal tubules. APN is a zinc metalloprotease which homodimerises *in vivo* and hydrolyses N-terminal amino acids at the brush-border membrane except when a proline lies in the penultimate position (Mentlein 2004; Mina-Osorio 2008; Wong et al. 2012a).

APN belongs to the gluzincin metalloprotease family with two consensus zinc-binding sequences, **HE**XX**H** and B**X**L**X**E (zinc-binding residues in bold, B = bulky side-chain, X = any residue) (Sjostrom et al. 2000). A third consensus site **GXMEN** is an exopeptidase substrate-binding sequence also common to all aminopeptidases (Hooper 1994). Human APN is a type II TM protein with a 2 domain topology: an extracellular amino peptidase domain and a membrane-anchoring domain (Wong et al. 2012a, 2017; Santiago et al. 2017) (Fig. 1). The membrane-anchoring domain contains the first 3 subdomains formed by the short cytosolic N-terminal, a TM α-helix, and an extracellular anchoring domain, which links to the extracellular peptidase domain (4 subdomains) (Sjostrom et al. 2000; Olsen et al. 1997; Wong et al. 2012a; Santiago et al. 2017). APN has a broad specificity for neutral amino acids in the order Ala > Phe > Tyr > Leu, overlapping with the substrate preference of B^0AT1 (Leu > Gln > Ala > Phe) (Bohmer et al. 2005; Mina-Osorio 2008; Camargo et al. 2005).

The removal of APN from bovine brush border membrane vesicles (BBMV) by papain treatment, reduced Na^+ dependent alanine transport, suggesting a link with neutral amino acid transport (Plakidou-Dymock et al. 1993). Both the Vmax and Km of alanine uptake were affected by APN removal and an antibody against the partially purified Na^+-dependent alanine transporter also recognised APN, suggesting a close *in situ* proximity of the proteins (Plakidou-Dymock and McGivan 1993). The identity of the neutral amino acid transporters in epithelial vesicles corresponding to system B^0 was unknown at the time (Plakidou-Dymock and McGivan 1993). Subsequently, APN was co-immunoprecipitated with B^0AT1 and shown to increase both surface expression and substrate affinity of the carrier in *X. laevis* oocytes (Fairweather et al. 2012). ACE2 also increases the substrate affinity of B^0AT3 but not of B^0AT1 as indicated by decreased apparent K_m values (Fairweather et al. 2015). All three proteins have been co-localised and isolated in protein complexes from mouse intestine apical membrane using blue-native electrophoresis and in glycolipid rafts (Fairweather et al. 2012). In contrast, brush-border complexes isolated and analysed using blue-native electrophoresis and LC-MS/MS found no evidence of B^0AT1, or ACE2 in APN-containing complexes (Babusiak et al. 2007) despite the high abundance of all three proteins from both intestinal and renal apical membrane isolations from multiple species (McConnell et al. 2011; Gilbert et al. 2010; Cutillas et al. 2005). This data and the recent publication of the human B^0AT1-ACE2 dimers of heterodimer complexes substantiates the idea that the B^0-like transporters may form large complexes *in vivo* (Yan et al. 2020b, d). The lack of a complete APN structure including the linker and TM domains also raises the possibility the B^0AT1-APN complex may retain the same extended TM7-neck architecture as seen for B^0AT1-ACE2.

3 Common Binding Sites for SLC6 and SLC7 Ancillaries

Our review has demonstrated the combined structural and biochemical evidence for the possible presence of two ancillary subunit/cholesterol binding sites in SLC7-SLC3 and SLC6 heteromeric solute carriers. These seem to form two distinct binding regions and have been identified from numerous LeuT/APC-fold transporters that have been structurally elucidated or modelled with ancillary proteins and/or lipids in either the same general TM1α-TM7-TM5 groove or adjacent to TM4 of translocator subunits (Fig. 8). The TM1α-TM7-TM5 groove, for example, was identified in the bacterial SLC7 homologue GkApcT, which was crystallised with the single TM ancillary MgtS and a cholesterol molecule adjacent to TM5 (Jungnickel et al. 2018) (Fig. 8a). Other examples of transporters with lipids (usually cholesterol) bound to the same TM1α-TM7-TM5 region include *C. glutamicum* BetP, *Drosophila* DAT, human LAT1, SERT and GLYT2 (Wang et al. 2015; Penmatsa et al. 2013; Laursen et al. 2018; Dickens et al. 2017; Jungnickel et al. 2018;

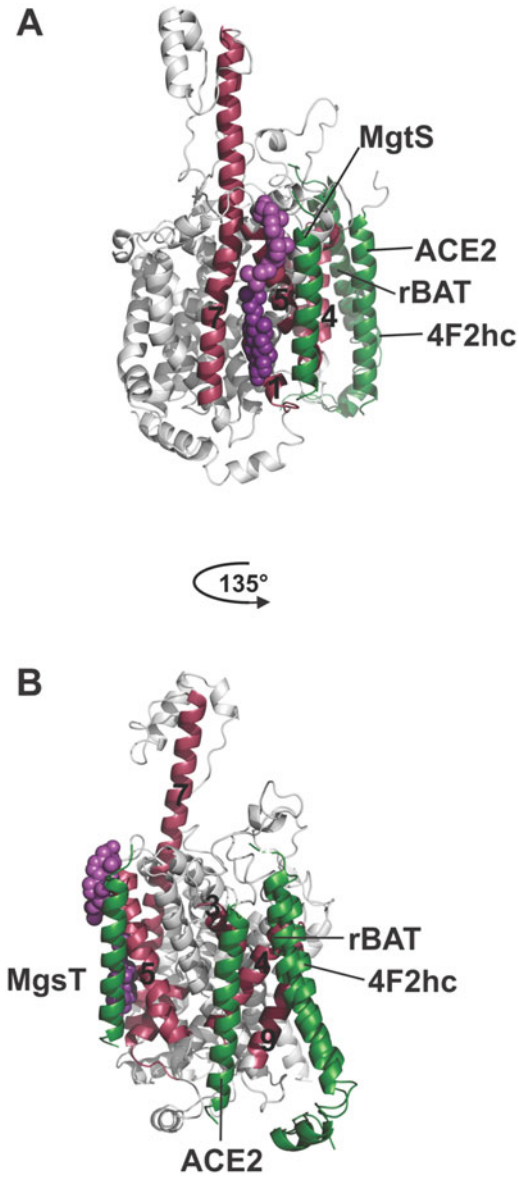

Fig. 8 Proposed conserved binding sites for LeuT fold heteromeric carriers
Multiple transporter structures were superimposed along the protein backbone of Drosophila dopamine transporter (dDAT) (PDB 4M48) (Penmatsa et al. 2013). The superimposed and aligned structures used are: *Geobacillus kaustophilus* GkApcT with the single TM-spanning *E. coli* protein MgtS (5OQT) and bound cholesterol (Jungnickel et al. 2018); *D. melanogaster* dopamine transporter DAT (4XP1) with bound cholesterol molecule (Wang et al. 2015); the human LAT1-4F2hc heteromeric solute carrier (6IRT) (Yan et al. 2019; Lee et al. 2019); the human $b^{0,+}$AT-rBAT heteromeric solute carrier (6LI9) (Yan et al. 2020a); the human B^0AT1-ACE2 heteromeric solute carrier (6M17) (Yan et al. 2020c). The translocator subunit shown is B^0AT1 (grey), all other translocators, including the template structure dDAT, have been deleted for ease of viewing while the ancillary subunit TM domains (green) and cholesterol molecules (purples) are displayed. The large extracellular domains of 4F2hc (residues 163–207, isoform 1), rBAT (111–685), and ACE2 (21–740) have also been deleted to emphasise membrane interactions. In each case the alignment was done with the translocator subunits against dDAT to minimise RMSD of the C_α atoms. The TM domains of translocator subunits (red) interacting with ancillaries, cholesterol and lipid molecules and hypothesised to form conserved LeuT fold TM binding sites are numbered. The TM5-TM7 binding site (**a**) shows the cholesterol of DAT, the cholesterol and ancillary TM domains of MgtS. The aligned structure is rotated 135° to show the 4F2hc, rBAT and ACE2 TM domain binding sites (**b**). The various binding sites were super-imposed onto the structure of dDAT by and aligning all the translocation subunit structures using the Multi-align tool in VMD

Schumann-Gillett et al. 2018; Yan et al. 2019; Coleman and Gouaux 2018; Mostyn et al. 2019; Schumann-Gillett and O'Mara 2019; Koshy et al. 2013; Coleman et al. 2016). Functional modulation by cholesterol or cholesterol analogues, usually inducing substrate efflux or inhibition of uptake, has been extensively demonstrated for both SLC6 and SLC7 heteromeric solute carriers (Dickens et al. 2017; Rahbek-Clemmensen et al. 2017; Laursen et al. 2018; Khelashvili and Weinstein 2015; Carland et al. 2013; Edington et al. 2009; Koshy et al. 2013; Hong and Amara 2010). For several of these transporters including GkApcT, *Drosophila* DAT and SERT, the cholesterol molecules have been captured with crystal structures (Fig. 8a). By contrast, both human LAT1-4F2hc, $b^{0,+}$AT-rBAT, and B^0AT1-ACE2 structures interact with what seems to be primarily a TM4 binding site sometimes facilitated by a phospholipid molecules (Fig. 8b). As with cholesterol and MgtS in the TM7-TM5 site, the 4F2hc, rBAT and ACE2 TM domain poses adopted in this TM4 binding site are not identical. While the rBAT TM domain overlays very closely with the 4F2hc TM domain, the ACE TM domain differs in binding at a somewhat different site and is less tilted with respect to the translocator TM helices with which it interacts.

Both 4F2hc and rBAT TM domains lean significantly such that the distance to the translocator TM4 and TM9 increases from the extracellular to intracellular side. This seems to mediate, at the intracellular leaflet, the interaction between ancillary and translocator TM domains by lipid molecules; lipids not present in the ACE2-B^0AT1 TM4 helical interactions (compare Figs. 3d and 4c with Fig. 7). As a result of this differential tilting 4F2hc TM domain also interacts with TM8, whereas ACE2 TM interacts on the extracellular side with TM3 and on the intracellular side with TM9. It is worth noting the TM1α-TM7-TM5 site is formed within the bundle (dynamic) domain of the LeuT fold, whereas the TM4 binding site is formed by the hash (scaffold) domain (Sect. 2.1.2). As a result we can speculate that 4F2hc, rBAT, and ACE2 are not greatly involved in the conformational dynamics during the transport cycle, whereas cholesterol in the TM5-TM7 site maybe. It is not known if these two general TM binding sites constitute 2 independent but required binding sites or if TM7-TM5 site represents predominately a sterol/lipid binding site modulating the ancillary-dependent function of these heteromeric solute carriers. One possibility is the TM7-TM5 region is allosterically linked to the ancillary binding site at TM4 and transfers information to facilitate substrate-induced conformational changes during the transport cycle. These conformational changes may not involve dynamic movement of the ancillaries at the TM4 region directly, which is largely rigid, but where binding nonetheless is required to activate conformational transitions via the TM1α-TM7-TM5 interactions. However, caution is advised in extrapolating too much from recent structural data as each heteromer is represented by a single conformation or, for B^0AT1-ACE2, with none bound by a full physiologically necessary complement of substrates.

Together this growing body of research evidence suggests heteromeric solute carrier complex formation is mediated by cholesterol/lipid molecular binding in many cases and should be more carefully considered in studying solute carriers (Schumann-Gillett et al. 2018; Koshy and Ziegler 2015) especially in the context of heteromeric interactions. Aligning the translocation subunits of recently determined structures as we have done here shows that ancillary and cholesterol binding sites are possibly conserved across and within heteromeric solute carrier families (Fig. 8). These promising advances facilitated by the 'cryo-EM revolution' represents a favourable outlook for the transport cycle and mechanism of these complexes to be understood at a fundamental level in the near future.

4 Conclusions

Several themes emerge for the role of heteromer formation by transporters:

1. Alternate ancillaries to allow tissue-specific regulation and trafficking.
2. Improved fidelity of protein folding and structure proof-reading.
3. Formation of metabolons and signalling complexes integrating multiple biological functions.
4. Optimisation of physiological function, by modulating the catalytic cycle.

Molecular dynamics simulations show that any two proteins in the membrane are likely to interact, because of weak interactions and the hydrophobic effect. It is possible to envision how two proteins that initially interact only passively, may become interdependent over time in evolution. Depending on abundance, different partners may emerge in different tissues. Integral membrane proteins have few surfaces available to check correct folding. An ancillary binding partner could form a mould, particularly for the scaffolding part of the translocator. Only when the complex is stable enough, it is released from the ER. This would lend itself to the co-dependency and subsequent co-evolution of both partners. The lack of hydrophilic surfaces also limits the formation of larger complexes with soluble proteins. Some transporters have developed very large and complex N- and C-terminals, while others as described here have adopted complex

formation as a solution. Another notable evolutionary trend we have inferred from our analysis of heteromeric solute carriers is the comparatively recent Last Common Ancestor (LCA) of ancillary subunits compared with the very ancient LCA inferred for the translocators (unpublished data). Furthermore, ancillary subunits have a tendency to show multiple large structural transformations such as domain deletion, domain appearance and domain swapping. Overall ancillaries seem to be more susceptible and tolerant of protein variation and mutation. By contrast, the LeuT fold and domain architecture of the carriers themselves has been remarkably conserved across all prokaryotic and eukaryotic phyla, with the notable exception, as noted, of significant hydrophilic domain extension i.e. termini and loop regions. These evolutionary trends will be presented and expanded upon in a forthcoming research publication. Overall, heteromeric complexes offer considerable evolutionary advantages, plausibly explaining their regular occurrence in the human proteome when the thermodynamic cost of synthesising two proteins to fulfil the function usually carried out by one is substantial.

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References

Ablack JN, Metz PJ, Chang JT, Cantor JM, Ginsberg MH (2015) Ubiquitylation of CD98 limits cell proliferation and clonal expansion. J Cell Sci 128(23):4273–4278. https://doi.org/10.1242/jcs.178129

Abplanalp J, Laczko E, Philp NJ, Neidhardt J, Zuercher J, Braun P, Schorderet DF, Munier FL, Verrey F, Berger W, Camargo SM, Kloeckener-Gruissem B (2013) The cataract and glucosuria associated monocarboxylate transporter MCT12 is a new creatine transporter. Hum Mol Genet 22(16):3218–3226. https://doi.org/10.1093/hmg/ddt175

Abramson J, Wright EM (2009) Structure and function of Na(+)-symporters with inverted repeats. Curr Opin Struct Biol 19(4):425–432. https://doi.org/10.1016/j.sbi.2009.06.002

Adams J, Broviac M, Brooks W, Johnson NR, Issitt PD (1971) An antibody, in the serum of a Wr(a+) individual, reacting with an antigen of very high frequency. Transfusion 11(5):290–291

Adelman JL, Dale AL, Zwier MC, Bhatt D, Chong LT, Zuckerman DM, Grabe M (2011) Simulations of the alternating access mechanism of the sodium symporter Mhp1. Biophys J 101(10):2399–2407. https://doi.org/10.1016/j.bpj.2011.09.061

Adijanto J, Philp NJ (2012) The SLC16A family of monocarboxylate transporters (MCTs) – physiology and function in cellular metabolism, pH homeostasis, and fluid transport. Curr Top Membr 70:275–311. https://doi.org/10.1016/b978-0-12-394316-3.00009-0

Ait-Ali N, Fridlich R, Millet-Puel G, Clerin E, Delalande F, Jaillard C, Blond F, Perrocheau L, Reichman S, Byrne LC, Olivier-Bandini A, Bellalou J, Moyse E, Bouillaud F, Nicol X, Dalkara D, van Dorsselaer A, Sahel JA, Leveillard T (2015) Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. Cell 161(4):817–832. https://doi.org/10.1016/j.cell.2015.03.023

Akpinar P, Kuwajima S, Krutzfeldt J, Stoffel M (2005) Tmem27: a cleaved and shed plasma membrane protein that stimulates pancreatic beta cell proliferation. Cell Metab 2(6):385–397. https://doi.org/10.1016/j.cmet.2005.11.001

Al-Abdulla R, Perez-Silva L, Abete L, Romero MR, Briz O, Marin JJG (2019) Unraveling 'The Cancer Genome Atlas' information on the role of SLC transporters in anticancer drug uptake. Expert Rev Clin Pharmacol 12(4):329–341. https://doi.org/10.1080/17512433.2019.1581605

Albertini G, Deneyer L, Ottestad-Hansen S, Zhou Y, Ates G, Walrave L, Demuyser T, Bentea E, Sato H, De Bundel D, Danbolt NC, Massie A, Smolders I (2018) Genetic deletion of xCT attenuates peripheral and central inflammation and mitigates LPS-induced sickness and depressive-like behavior in mice. Glia 66(9):1845–1861. https://doi.org/10.1002/glia.23343

Albrecht P, Lewerenz J, Dittmer S, Noack R, Maher P, Methner A (2010) Mechanisms of oxidative glutamate toxicity: the glutamate/cystine antiporter system xc- as a neuroprotective drug target. CNS Neurol Disord Drug Targets 9(3):373–382

Alenina N, Bader M (2019) ACE2 in brain physiology and pathophysiology: evidence from transgenic animal models. Neurochem Res 44(6):1323–1329. https://doi.org/10.1007/s11064-018-2679-4

Alfallaj R, Alfadhel M (2019) Glycine transporter 1 encephalopathy from biochemical pathway to clinical disease: review. Child Neurol Open

6:2329048x19831486. https://doi.org/10.1177/2329048x19831486

Allman E, Wang Q, Walker RL, Austen M, Peters MA, Nehrke K (2016) Calcineurin homologous proteins regulate the membrane localization and activity of sodium/proton exchangers in C. elegans. Am J Physiol Cell Physiol 310(3):C233–C242. https://doi.org/10.1152/ajpcell.00291.2015

Almutairi SM, Ali AK, He W, Yang DS, Ghorbani P, Wang L, Fullerton MD, Lee SH (2019) Interleukin-18 up-regulates amino acid transporters and facilitates amino acid-induced mTORC1 activation in natural killer cells. J Biol Chem 294(12):4644–4655. https://doi.org/10.1074/jbc.RA118.005892

Alpers DH (1987) Digestion and Absorption of carbohydrates and proteins. In: Johnson LR (ed) Physiology of the gastro-intestinal tract, vol 2. Raven Press, New York, pp 1469–1487

Altirriba J, Gasa R, Casas S, Ramirez-Bajo MJ, Ros S, Gutierrez-Dalmau A, Ruiz de Villa MC, Barbera A, Gomis R (2010) The role of transmembrane protein 27 (TMEM27) in islet physiology and its potential use as a beta cell mass biomarker. Diabetologia 53 (7):1406–1414. https://doi.org/10.1007/s00125-010-1728-6

Ames S, Andring JT, McKenna R, Becker HM (2020) CAIX forms a transport metabolon with monocarboxylate transporters in human breast cancer cells. Oncogene 39(8):1710–1723. https://doi.org/10.1038/s41388-019-1098-6

Anas MK, Lee MB, Zhou C, Hammer MA, Slow S, Karmouch J, Liu XJ, Broer S, Lever M, Baltz JM (2008) SIT1 is a betaine/proline transporter that is activated in mouse eggs after fertilization and functions until the 2-cell stage. Development 135 (24):4123–4130. https://doi.org/10.1242/dev.026575

Anderluh A, Klotzsch E, Reismann AW, Brameshuber M, Kudlacek O, Newman AH, Sitte HH, Schutz GJ (2014) Single molecule analysis reveals coexistence of stable serotonin transporter monomers and oligomers in the live cell plasma membrane. J Biol Chem 289 (7):4387–4394. https://doi.org/10.1074/jbc.M113.531632

Anderluh A, Hofmaier T, Klotzsch E, Kudlacek O, Stockner T, Sitte HH, Schütz GJ (2017) Direct PIP(2) binding mediates stable oligomer formation of the serotonin transporter. Nat Commun 8:14089. https://doi.org/10.1038/ncomms14089

Andersen KE, Braestrup C, Gronwald FC, Jorgensen AS, Nielsen EB, Sonnewald U, Sorensen PO, Suzdak PD, Knutsen LJ (1993) The synthesis of novel GABA uptake inhibitors. 1. Elucidation of the structure-activity studies leading to the choice of (R)-1-[4,4-bis (3-methyl-2-thienyl)-3-butenyl]-3-piperidine-carboxylic acid (tiagabine) as an anticonvulsant drug candidate. J Med Chem 36(12):1716–1725

Andersen J, Kristensen AS, Bang-Andersen B, Stromgaard K (2009) Recent advances in the understanding of the interaction of antidepressant drugs with serotonin and norepinephrine transporters. Chem Commun (Camb) 25:3677–3692. https://doi.org/10.1039/b903035m

Andersen J, Olsen L, Hansen KB, Taboureau O, Jorgensen FS, Jorgensen AM, Bang-Andersen B, Egebjerg J, Stromgaard K, Kristensen AS (2010) Mutational mapping and modeling of the binding site for (S)-citalopram in the human serotonin transporter. J Biol Chem 285(3):2051–2063. https://doi.org/10.1074/jbc.M109.072587

Anstee DJ (2011) The functional importance of blood group-active molecules in human red blood cells. Vox Sang 100(1):140–149. https://doi.org/10.1111/j.1423-0410.2010.01388.x

Aoki T (2017) A comprehensive review of our current understanding of Red Blood Cell (RBC) glycoproteins. Membranes (Basel) 7(4). https://doi.org/10.3390/membranes7040056

Arac D, Boucard AA, Ozkan E, Strop P, Newell E, Sudhof TC, Brunger AT (2007) Structures of neuroligin-1 and the neuroligin-1/neurexin-1 beta complex reveal specific protein-protein and protein-Ca2+ interactions. Neuron 56(6):992–1003. https://doi.org/10.1016/j.neuron.2007.12.002

Arakawa T, Kobayashi-Yurugi T, Alguel Y, Iwanari H, Hatae H, Iwata M, Abe Y, Hino T, Ikeda-Suno C, Kuma H, Kang D, Murata T, Hamakubo T, Cameron AD, Kobayashi T, Hamasaki N, Iwata S (2015) Crystal structure of the anion exchanger domain of human erythrocyte band 3. Science 350(6261):680–684. https://doi.org/10.1126/science.aaa4335

Arancibia-Garavilla Y, Toledo F, Casanello P, Sobrevia L (2003) Nitric oxide synthesis requires activity of the cationic and neutral amino acid transport system y+L in human umbilical vein endothelium. Exp Physiol 88 (6):699–710

Asano S, Kameyama M, Oura A, Morisato A, Sakai H, Tabuchi Y, Chairoungdua A, Endou H, Kanai Y (2007) L-type amino acid transporter-1 expressed in human astrocytomas, U343MGa. Biol Pharm Bull 30 (3):415–422

Aspatwar A, Tolvanen MEE, Schneider HP, Becker HM, Narkilahti S, Parkkila S, Deitmer JW (2019) Catalytically inactive carbonic anhydrase-related proteins enhance transport of lactate by MCT1. FEBS Open Bio 9(7):1204–1211. https://doi.org/10.1002/2211-5463.12647

Ata R, Antonescu CN (2017) Integrins and cell metabolism: an intimate relationship impacting cancer. Int J Mol Sci 18(1). https://doi.org/10.3390/ijms18010189

Atkinson BN, Bell SC, De Vivo M, Kowalski LR, Lechner SM, Ognyanov VI, Tham CS, Tsai C, Jia J, Ashton D, Klitenick MA (2001) ALX 5407: a potent, selective inhibitor of the hGlyT1 glycine transporter. Mol Pharmacol 60(6):1414–1420

Aubrey KR, Vandenberg RJ (2001) N[3-(4-′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS) is a selective persistent inhibitor of glycine

transport. Br J Pharmacol 134(7):1429–1436. https://doi.org/10.1038/sj.bjp.0704381

Auffray I, Marfatia S, de Jong K, Lee G, Huang CH, Paszty C, Tanner MJ, Mohandas N, Chasis JA (2001) Glycophorin A dimerization and band 3 interaction during erythroid membrane biogenesis: in vivo studies in human glycophorin A transgenic mice. Blood 97 (9):2872–2878

Azevedo-Silva J, Queiros O, Ribeiro A, Baltazar F, Young KH, Pedersen PL, Preto A, Casal M (2015) The cytotoxicity of 3-bromopyruvate in breast cancer cells depends on extracellular pH. Biochem J 467 (2):247–258. https://doi.org/10.1042/bj20140921

Azima H, Vispo RH (1958) Imipramine; a potent new antidepressant compound. Am J Psychiatry 115 (3):245–246. https://doi.org/10.1176/ajp.115.3.245

Baba M, Inoue M, Itoh K, Nishizawa Y (2008) Blocking CD147 induces cell death in cancer cells through impairment of glycolytic energy metabolism. Biochem Biophys Res Commun 374(1):111–116. https://doi.org/10.1016/j.bbrc.2008.06.122

Babusiak M, Man P, Petrak J, Vyoral D (2007) Native proteomic analysis of protein complexes in murine intestinal brush border membranes. Proteomics 7 (1):121–129. https://doi.org/10.1002/pmic.200600382

Bai JP (1994a) Comparison of distribution of brush-border exo- and endopeptidases in rat and rabbit intestine. J Pharm Pharmacol 46(11):928–930

Bai JP (1994b) Distribution of brush-border membrane peptidases along the rat intestine. Pharm Res 11 (6):897–900

Bai X, Moraes TF, Reithmeier RAF (2017) Structural biology of solute carrier (SLC) membrane transport proteins. Mol Membr Biol 34(1–2):1–32. https://doi.org/10.1080/09687688.2018.1448123

Bai X, Moraes TF, Reithmeier RAF (2018) Structural biology of solute carrier (SLC) membrane transport proteins. Mol Membr Biol:1–32. https://doi.org/10.1080/09687688.2018.1448123

Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, Kalivas PW (2003) Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat Neurosci 6(7):743–749. https://doi.org/10.1038/nn1069

Ballatori N (2005) Biology of a novel organic solute and steroid transporter, OSTalpha-OSTbeta. Exp Biol Med (Maywood) 230(10):689–698

Ballatori N (2011) Pleiotropic functions of the organic solute transporter Ostalpha-Ostbeta. Dig Dis 29 (1):13–17. https://doi.org/10.1159/000324123

Ballatori N, Christian WV, Lee JY, Dawson PA, Soroka CJ, Boyer JL, Madejczyk MS, Li N (2005) OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. Hepatology 42(6):1270–1279. https://doi.org/10.1002/hep.20961

Ballatori N, Fang F, Christian WV, Li N, Hammond CL (2008) Ostalpha-Ostbeta is required for bile acid and conjugated steroid disposition in the intestine, kidney, and liver. Am J Physiol Gastrointest Liver Physiol 295 (1):G179–g186. https://doi.org/10.1152/ajpgi.90319.2008

Ballatori N, Li N, Fang F, Boyer JL, Christian WV, Hammond CL (2009) OST alpha-OST beta: a key membrane transporter of bile acids and conjugated steroids. Front Biosci (Landmark Ed) 14:2829–2844

Ballatori N, Christian WV, Wheeler SG, Hammond CL (2013) The heteromeric organic solute transporter, OSTalpha-OSTbeta/SLC51: a transporter for steroid-derived molecules. Mol Aspects Med 34 (2–3):683–692. https://doi.org/10.1016/j.mam.2012.11.005

Baltz JM, Tartia AP (2010) Cell volume regulation in oocytes and early embryos: connecting physiology to successful culture media. Hum Reprod Update 16 (2):166–176. https://doi.org/10.1093/humupd/dmp045

Barbosa M, Lopes A, Mota C, Martins E, Oliveira J, Alves S, De Bonis P, Mota Mdo C, Dias C, Rodrigues-Santos P, Fortuna AM, Quelhas D, Lacerda L, Bisceglia L, Cardoso ML (2012) Clinical, biochemical and molecular characterization of cystinuria in a cohort of 12 patients. Clin Genet 81(1):47–55. https://doi.org/10.1111/j.1399-0004.2011.01638.x

Bartoccioni P, Rius M, Zorzano A, Palacin M, Chillaron J (2008) Distinct classes of trafficking rBAT mutants cause the type I cystinuria phenotype. Hum Mol Genet 17(12):1845–1854. https://doi.org/10.1093/hmg/ddn080

Bartoccioni P, Fort J, Zorzano A, Errasti-Murugarren E, Palacin M (2019) Functional characterization of the alanine-serine-cysteine exchanger of Carnobacterium sp AT7. J Gen Physiol 151(4):505–517. https://doi.org/10.1085/jgp.201812195

Bassi MT, Sperandeo MP, Incerti B, Bulfone A, Pepe A, Surace EM, Gattuso C, De Grandi A, Buoninconti A, Riboni M, Manzoni M, Andria G, Ballabio A, Borsani G, Sebastio G (1999) SLC7A8, a gene mapping within the lysinuric protein intolerance critical region, encodes a new member of the glycoprotein-associated amino acid transporter family. Genomics 62 (2):297–303. https://doi.org/10.1006/geno.1999.5978

Bassi MT, Gasol E, Manzoni M, Pineda M, Riboni M, Martin R, Zorzano A, Borsani G, Palacin M (2001) Identification and characterisation of human xCT that co-expresses, with 4F2 heavy chain, the amino acid transport activity system xc. Pflug Arch 442 (2):286–296

Batlle D, Haque SK (2012) Genetic causes and mechanisms of distal renal tubular acidosis. Nephrol Dial Transplant 27(10):3691–3704. https://doi.org/10.1093/ndt/gfs442

Baumer Y, McCurdy S, Alcala M, Mehta N, Lee BH, Ginsberg MH, Boisvert WA (2017) CD98 regulates vascular smooth muscle cell proliferation in atherosclerosis. Atherosclerosis 256:105–114. https://doi.org/10.1016/j.atherosclerosis.2016.11.017

Beaudoin JJ, Brouwer KLR, Malinen MM (2020) Novel insights into the organic solute transporter alpha/beta,

OSTα/β: from the bench to the bedside. Pharmacol Ther 211:107542. https://doi.org/10.1016/j.pharmthera.2020.107542

Becker HM (2020) Carbonic anhydrase IX and acid transport in cancer. Br J Cancer 122(2):157–167. https://doi.org/10.1038/s41416-019-0642-z

Becker HM, Deitmer JW (2008) Nonenzymatic proton handling by carbonic anhydrase II during H+-lactate cotransport via monocarboxylate transporter 1. J Biol Chem 283(31):21655–21667. https://doi.org/10.1074/jbc.m802134200

Becker HM, Deitmer JW (2020) Transport metabolons and acid/base balance in tumor cells. Cancers (Basel) 12(4). https://doi.org/10.3390/cancers12040899

Becker HM, Hirnet D, Fecher-Trost C, Sultemeyer D, Deitmer JW (2005) Transport activity of MCT1 expressed in Xenopus oocytes is increased by interaction with carbonic anhydrase. J Biol Chem 280 (48):39882–39889. https://doi.org/10.1074/jbc.M503081200

Becker HM, Klier M, Deitmer JW (2010) Nonenzymatic augmentation of lactate transport via monocarboxylate transporter isoform 4 by carbonic anhydrase II. J Membr Biol 234(2):125–135. https://doi.org/10.1007/s00232-010-9240-y

Becker HM, Klier M, Schuler C, McKenna R, Deitmer JW (2011) Intramolecular proton shuttle supports not only catalytic but also noncatalytic function of carbonic anhydrase II. Proc Natl Acad Sci U S A 108 (7):3071–3076. https://doi.org/10.1073/pnas.1014293108

Becker HM, Klier M, Deitmer JW (2014) Carbonic anhydrases and their interplay with acid/base-coupled membrane transporters. Subcell Biochem 75:105–134. https://doi.org/10.1007/978-94-007-7359-2_7

Beckman ML, Bernstein EM, Quick MW (1998) Protein kinase C regulates the interaction between a GABA transporter and syntaxin 1A. J Neurosci 18 (16):6103–6112

Beckmann R, Smythe JS, Anstee DJ, Tanner MJ (2001) Coexpression of band 3 mutants and Rh polypeptides: differential effects of band 3 on the expression of the Rh complex containing D polypeptide and the Rh complex containing CcEe polypeptide. Blood 97 (8):2496–2505

Beesley P, Kraus M, Parolaro N (2014a) The neuroplastins: multifunctional neuronal adhesion molecules – involvement in behaviour and disease. Adv Neurobiol 8:61–89

Beesley PW, Herrera-Molina R, Smalla KH, Seidenbecher C (2014b) The Neuroplastin adhesion molecules: key regulators of neuronal plasticity and synaptic function. J Neurochem 131(3):268–283. https://doi.org/10.1111/jnc.12816

Belanger AM, Przybylska M, Gefteas E, Furgerson M, Geller S, Kloss A, Cheng SH, Zhu Y, Yew NS (2018) Inhibiting neutral amino acid transport for the treatment of phenylketonuria. JCI Insight 3(14). https://doi.org/10.1172/jci.insight.121762

Bellucci A, Navarria L, Falarti E, Zaltieri M, Bono F, Collo G, Spillantini MG, Missale C, Spano P (2011) Redistribution of DAT/α-synuclein complexes visualized by "in situ" proximity ligation assay in transgenic mice modelling early Parkinson's disease. PLoS One 6(12):e27959-e27959. https://doi.org/10.1371/journal.pone.0027959

Benleulmi-Chaachoua A, Hegron A, Le Boulch M, Karamitri A, Wierzbicka M, Wong V, Stagljar I, Delagrange P, Ahmad R, Jockers R (2018) Melatonin receptors limit dopamine reuptake by regulating dopamine transporter cell-surface exposure. Cell Mol Life Sci 75(23):4357–4370. https://doi.org/10.1007/s00018-018-2876-y

Bergersen L, Johannsson E, Veruki ML, Nagelhus EA, Halestrap A, Sejersted OM, Ottersen OP (1999) Cellular and subcellular expression of monocarboxylate transporters in the pigment epithelium and retina of the rat. Neuroscience 90(1):319–331

Bernal J, Guadano-Ferraz A, Morte B (2015) Thyroid hormone transporters – functions and clinical implications. Nat Rev Endocrinol 11(7):406–417. https://doi.org/10.1038/nrendo.2015.66

Bernstein HG, Smalla KH, Bogerts B, Gordon-Weeks PR, Beesley PW, Gundelfinger ED, Kreutz MR (2007) The immunolocalization of the synaptic glycoprotein neuroplastin differs substantially between the human and the rodent brain. Brain Res 1134(1):107–112. https://doi.org/10.1016/j.brainres.2006.11.090

Bertero T, Oldham WM, Grasset EM, Bourget I, Boulter E, Pisano S, Hofman P, Bellvert F, Meneguzzi G, Bulavin DV, Estrach S, Feral CC, Chan SY, Bozec A, Gaggioli C (2018) Tumor-stroma mechanics coordinate amino acid availability to sustain tumor growth and malignancy. Cell Metab. https://doi.org/10.1016/j.cmet.2018.09.012

Bertran J, Magagnin S, Werner A, Markovich D, Biber J, Testar X, Zorzano A, Kuhn LC, Palacin M, Murer H (1992a) Stimulation of system y(+)-like amino acid transport by the heavy chain of human 4F2 surface antigen in Xenopus laevis oocytes. Proc Natl Acad Sci U S A 89(12):5606–5610

Bertran J, Werner A, Moore ML, Stange G, Markovich D, Biber J, Testar X, Zorzano A, Palacin M, Murer H (1992b) Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids. Proc Natl Acad Sci U S A 89(12):5601–5605

Bertran J, Werner A, Stange G, Markovich D, Biber J, Testar X, Zorzano A, Palacin M, Murer H (1992c) Expression of Na(+)-independent amino acid transport in Xenopus laevis oocytes by injection of rabbit kidney cortex mRNA. Biochem J 281(Pt 3):717–723

Bertran J, Werner A, Chillaron J, Nunes V, Biber J, Testar X, Zorzano A, Estivill X, Murer H, Palacin M (1993) Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in Xenopus oocytes. J Biol Chem 268(20):14842–14849

Bhutia YD, Ganapathy V (2015) Glutamine transporters in mammalian cells and their functions in physiology and cancer. Biochim Biophys Acta. https://doi.org/10.1016/j.bbamcr.2015.12.017

Bian Y, Song C, Cheng K, Dong M, Wang F, Huang J, Sun D, Wang L, Ye M, Zou H (2014) An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. J Proteomics 96:253–262. https://doi.org/10.1016/j.jprot.2013.11.014

Bienvenut WVL, Lao L, Ryan KL (2009) Acetylation at Met-1, identification by mass spectrometry. https://www.uniprot.org/uniprot/P08195#ptm_processing. Accessed 13-01-2020

Binda F, Lute BJ, Dipace C, Blakely RD, Galli A (2006) The N-terminus of the norepinephrine transporter regulates the magnitude and selectivity of the transporter-associated leak current. Neuropharmacology 50(3):354–361. https://doi.org/10.1016/j.neuropharm.2005.09.012

Binda F, Dipace C, Bowton E, Robertson SD, Lute BJ, Fog JU, Zhang M, Sen N, Colbran RJ, Gnegy ME, Gether U, Javitch JA, Erreger K, Galli A (2008) Syntaxin 1A interaction with the dopamine transporter promotes amphetamine-induced dopamine efflux. Mol Pharmacol 74(4):1101–1108. https://doi.org/10.1124/mol.108.048447

Bisceglia L, Fischetti L, Bonis PD, Palumbo O, Augello B, Stanziale P, Carella M, Zelante L (2010) Large rearrangements detected by MLPA, point mutations, and survey of the frequency of mutations within the SLC3A1 and SLC7A9 genes in a cohort of 172 cystinuric Italian patients. Mol Genet Metab 99 (1):42–52. https://doi.org/10.1016/j.ymgme.2009.09.001

Blakely RD, Sung U (2000) SNARE-ing neurotransmitter transporters. Nat Neurosci 3(10):969–971. https://doi.org/10.1038/79898

Blakely RD, Robinson MB, Amara SG (1988) Expression of neurotransmitter transport from rat brain mRNA in Xenopus laevis oocytes. Proc Natl Acad Sci U S A 85 (24):9846–9850

Blakely RD, Berson HE, Fremeau RT Jr, Caron MG, Peek MM, Prince HK, Bradley CC (1991) Cloning and expression of a functional serotonin transporter from rat brain. Nature 354(6348):66–70. https://doi.org/10.1038/354066a0

Blanchard D (1990) Human red cell glycophorins: biochemical and antigenic properties. Transfus Med Rev 4 (3):170–186

Blumenfeld OO, Huang CH (1997) Molecular genetics of glycophorin MNS variants. Transfus Clin Biol 4 (4):357–365

Boado RJ, Li JY, Chu C, Ogoshi F, Wise P, Pardridge WM (2005) Site-directed mutagenesis of cysteine residues of large neutral amino acid transporter LAT1. Biochim Biophys Acta 1715(2):104–110. https://doi.org/10.1016/j.bbamem.2005.07.007

Bodoy S, Sotillo F, Espino-Guarch M, Sperandeo MP, Ormazabal A, Zorzano A, Sebastio G, Artuch R, Palacin M (2019) Inducible Slc7a7 knockout mouse model recapitulates lysinuric protein intolerance disease. Int J Mol Sci 20(21). https://doi.org/10.3390/ijms20215294

Bohmer C, Broer A, Munzinger M, Kowalczuk S, Rasko JE, Lang F, Broer S (2005) Characterization of mouse amino acid transporter B0AT1 (slc6a19). Biochem J 389(Pt 3):745–751. https://doi.org/10.1042/bj20050083

Bolland DE, Moritz AE, Stanislowski DJ, Vaughan RA, Foster JD (2019) Palmitoylation by multiple DHHC enzymes enhances dopamine transporter function and stability. ACS Chem Neurosci 10(6):2707–2717. https://doi.org/10.1021/acschemneuro.8b00558

Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowski C (1994) Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. Eur J Pharmacol 269(2):219–224

Borden LA, Smith KE, Gustafson EL, Branchek TA, Weinshank RL (1995) Cloning and expression of a betaine/GABA transporter from human brain. J Neurochem 64(3):977–984

Borsani G, Bassi MT, Sperandeo MP, De Grandi A, Buoninconti A, Riboni M, Manzoni M, Incerti B, Pepe A, Andria G, Ballabio A, Sebastio G (1999) SLC7A7, encoding a putative permease-related protein, is mutated in patients with lysinuric protein intolerance. Nat Genet 21(3):297–301. https://doi.org/10.1038/6815

Bosshart PD, Fotiadis D (2019) Secondary active transporters. Subcell Biochem 92:275–299. https://doi.org/10.1007/978-3-030-18768-2_9

Bosshart PD, Kalbermatter D, Bonetti S, Fotiadis D (2019) Mechanistic basis of L-lactate transport in the SLC16 solute carrier family. Nat Commun 10(1):2649. https://doi.org/10.1038/s41467-019-10566-6

Botzenhart E, Vester U, Schmidt C, Hesse A, Halber M, Wagner C, Lang F, Hoyer P, Zerres K, Eggermann T (2002) Cystinuria in children: distribution and frequencies of mutations in the SLC3A1 and SLC7A9 genes. Kidney Int 62(4):1136–1142. https://doi.org/10.1111/j.1523-1755.2002.kid552.x

Boudko DY (2012) Molecular basis of essential amino acid transport from studies of insect nutrient amino acid transporters of the SLC6 family (NAT-SLC6). J Insect Physiol 58(4):433–449. https://doi.org/10.1016/j.jinsphys.2011.12.018

Boulter E, Estrach S, Tissot FS, Hennrich ML, Tosello L, Cailleteau L, de la Ballina LR, Pisano S, Gavin AC, Feral CC (2018) Cell metabolism regulates integrin mechanosensing via an SLC3A2-dependent sphingolipid biosynthesis pathway. Nat Commun 9 (1):4862. https://doi.org/10.1038/s41467-018-07268-w

Bovenzi CD, Hamilton J, Tassone P, Johnson J, Cognetti DM, Luginbuhl A, Keane WM, Zhan T, Tuluc M,

Bar-Ad V, Martinez-Outschoorn U, Curry JM (2015) Prognostic indications of elevated MCT4 and CD147 across cancer types: a meta-analysis. Biomed Res Int 2015:242437. https://doi.org/10.1155/2015/242437

Boyer JL, Trauner M, Mennone A, Soroka CJ, Cai SY, Moustafa T, Zollner G, Lee JY, Ballatori N (2006) Upregulation of a basolateral FXR-dependent bile acid efflux transporter OSTalpha-OSTbeta in cholestasis in humans and rodents. Am J Physiol Gastrointest Liver Physiol 290(6):G1124–G1130. https://doi.org/10.1152/ajpgi.00539.2005

Bozzi AT, Bane LB, Weihofen WA, Singharoy A, Guillen ER, Ploegh HL, Schulten K, Gaudet R (2016) Crystal structure and conformational change mechanism of a bacterial nramp-family divalent metal transporter. Structure 24(12):2102–2114. https://doi.org/10.1016/j.str.2016.09.017

Bozzi AT, Zimanyi CM, Nicoludis JM, Lee BK, Zhang CH, Gaudet R (2019) Structures in multiple conformations reveal distinct transition metal and proton pathways in an Nramp transporter. Elife 8. https://doi.org/10.7554/eLife.41124

Bracher S, Guérin K, Polyhach Y, Jeschke G, Dittmer S, Frey S, Böhm M, Jung H (2016) Glu-311 in external loop 4 of the sodium/proline transporter PutP is crucial for external gate closure. J Biol Chem 291(10):4998–5008. https://doi.org/10.1074/jbc.M115.675306

Brahm J (1977) Temperature-dependent changes of chloride transport kinetics in human red cells. J Gen Physiol 70(3):283–306

Braun D, Kinne A, Brauer AU, Sapin R, Klein MO, Kohrle J, Wirth EK, Schweizer U (2011) Developmental and cell type-specific expression of thyroid hormone transporters in the mouse brain and in primary brain cells. Glia 59(3):463–471. https://doi.org/10.1002/glia.21116

Brenner B, Harney JT, Ahmed BA, Jeffus BC, Unal R, Mehta JL, Kilic F (2007) Plasma serotonin levels and the platelet serotonin transporter. J Neurochem 102 (1):206–215. https://doi.org/10.1111/j.1471-4159.2007.04542.x

Bridges CC, Kekuda R, Wang H, Prasad PD, Mehta P, Huang W, Smith SB, Ganapathy V (2001) Structure, function, and regulation of human cystine/glutamate transporter in retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 42(1):47–54

Bril A, Feletou M (2014) Collectrin: a new component of the renin-angiotensin system? Med Sci (Paris) 30 (2):136–139. https://doi.org/10.1051/medsci/20143002007

Broer S (2006) The SLC6 orphans are forming a family of amino acid transporters. Neurochem Int 48 (6–7):559–567. https://doi.org/10.1016/j.neuint.2005.11.021

Broer S (2008a) Amino acid transport across mammalian intestinal and renal epithelia. Physiol Rev 88 (1):249–286. https://doi.org/10.1152/physrev.00018.2006

Broer S (2008b) Apical transporters for neutral amino acids: physiology and pathophysiology. Physiology (Bethesda) 23:95–103. https://doi.org/10.1152/physiol.00045.2007

Broer S (2011) Targeting tumour cells at the entrance. Biochem J 439(2):e1–e2. https://doi.org/10.1042/bj20111484

Broer S (2013a) Diseases associated with general amino acid transporters of the solute carrier 6 family (SLC6). Curr Mol Pharmacol 6(2):74–87

Broer S (2013b) Epithelial neutral amino acid transporters: lessons from mouse models. Curr Opin Nephrol Hypertens 22(5):539–544. https://doi.org/10.1097/MNH.0b013e328363fff6

Broer S (2018) Amino acid transporters as disease modifiers and drug targets. SLAS Discov 23 (4):303–320. https://doi.org/10.1177/2472555218755629

Broer S, Broer A (2017) Amino acid homeostasis and signalling in mammalian cells and organisms. Biochem J 474(12):1935–1963. https://doi.org/10.1042/bcj20160822

Broer S, Fairweather SJ (2018) Amino acid transport across the mammalian intestine. Compr Physiol 9 (1):343–373. https://doi.org/10.1002/cphy.c170041

Broer S, Gether U (2012) The solute carrier 6 family of transporters. Br J Pharmacol 167(2):256–278. https://doi.org/10.1111/j.1476-5381.2012.01975.x

Broer S, Palacin M (2011) The role of amino acid transporters in inherited and acquired diseases. Biochem J 436(2):193–211. https://doi.org/10.1042/bj20101912

Broer S, Wagner CA (2002) Structure-function relationships of heterodimeric amino acid transporters. Cell Biochem Biophys 36(2–3):155–168. https://doi.org/10.1385/cbb:36:2-3:155

Broer S, Broer A, Hamprecht B (1995) The 4F2hc surface antigen is necessary for expression of system L-like neutral amino acid-transport activity in C6-BU-1 rat glioma cells: evidence from expression studies in Xenopus laevis oocytes. Biochem J 312(Pt 3):863–870

Broer S, Broer A, Hamprecht B (1997) Expression of the surface antigen 4F2hc affects system-L-like neutral-amino-acid-transport activity in mammalian cells. Biochem J 324(Pt 2):535–541

Broer S, Schneider HP, Broer A, Rahman B, Hamprecht B, Deitmer JW (1998) Characterization of the monocarboxylate transporter 1 expressed in Xenopus laevis oocytes by changes in cytosolic pH. Biochem J 333(Pt 1):167–174

Broer S, Broer A, Schneider HP, Stegen C, Halestrap AP, Deitmer JW (1999) Characterization of the high-affinity monocarboxylate transporter MCT2 in Xenopus laevis oocytes. Biochem J 341(3):529–535

Broer A, Wagner CA, Lang F, Broer S (2000) The heterodimeric amino acid transporter 4F2hc/y+LAT2 mediates arginine efflux in exchange with glutamine. Biochem J 349(Pt 3):787–795

Broer A, Friedrich B, Wagner CA, Fillon S, Ganapathy V, Lang F, Broer S (2001) Association of 4F2hc with light chains LAT1, LAT2 or y+LAT2 requires different domains. Biochem J 355(Pt 3):725–731

Broer A, Klingel K, Kowalczuk S, Rasko JE, Cavanaugh J, Broer S (2004) Molecular cloning of mouse amino acid transport system B0, a neutral amino acid transporter related to Hartnup disorder. J Biol Chem 279(23):24467–24476. https://doi.org/10.1074/jbc.M400904200

Broer S, Cavanaugh JA, Rasko JE (2005) Neutral amino acid transport in epithelial cells and its malfunction in Hartnup disorder. Biochem Soc Trans 33 (Pt 1):233–236. https://doi.org/10.1042/bst0330233

Broer A, Cavanaugh JA, Rasko JE, Broer S (2006a) The molecular basis of neutral aminoacidurias. Pflugers Arch 451(4):511–517. https://doi.org/10.1007/s00424-005-1481-8

Broer A, Tietze N, Kowalczuk S, Chubb S, Munzinger M, Bak LK, Broer S (2006b) The orphan transporter v7-3 (slc6a15) is a Na+-dependent neutral amino acid transporter (B0AT2). Biochem J 393(Pt 1):421–430. https://doi.org/10.1042/bj20051273

Broer S, Bailey CG, Kowalczuk S, Ng C, Vanslambrouck JM, Rodgers H, Auray-Blais C, Cavanaugh JA, Broer A, Rasko JE (2008) Iminoglycinuria and hyperglycinuria are discrete human phenotypes resulting from complex mutations in proline and glycine transporters. J Clin Invest. https://doi.org/10.1172/JCI36625

Broer A, Juelich T, Vanslambrouck JM, Tietze N, Solomon PS, Holst J, Bailey CG, Rasko JE, Broer S (2011) Impaired nutrient signaling and body weight control in an Na+ neutral amino acid cotransporter (Slc6a19) deficient mouse. J Biol Chem. https://doi.org/10.1074/jbc.m111.241323

Brosius FC 3rd, Alper SL, Garcia AM, Lodish HF (1989) The major kidney band 3 gene transcript predicts an amino-terminal truncated band 3 polypeptide. J Biol Chem 264(14):7784–7787

Brown AS, Gershon S (1993) Dopamine and depression. J Neural Transm Gen Sect 91(2–3):75–109

Brown JM, Hunihan L, Prack MM, Harden DG, Bronson J, Dzierba CD, Gentles RG, Hendricson A, Krause R, Macor JE, Westphal RS (2014) In vitro Characterization of a small molecule inhibitor of the alanine serine cysteine transporter -1 (SLC7A10). J Neurochem 129(2):275–283. https://doi.org/10.1111/jnc.12618

Bruce LJ, Tanner MJ (1996) Structure-function relationships of band 3 variants. Cell Mol Biol (Noisy-le-grand) 42(7):953–973

Bruce LJ, Groves JD, Okubo Y, Thilaganathan B, Tanner MJ (1994) Altered band 3 structure and function in glycophorin A- and B-deficient (MkMk) red blood cells. Blood 84(3):916–922

Bruce LJ, Ring SM, Anstee DJ, Reid ME, Wilkinson S, Tanner MJ (1995) Changes in the blood group Wright antigens are associated with a mutation at amino acid 658 in human erythrocyte band 3: a site of interaction between band 3 and glycophorin A under certain conditions. Blood 85(2):541–547

Bruce LJ, Cope DL, Jones GK, Schofield AE, Burley M, Povey S, Unwin RJ, Wrong O, Tanner MJ (1997) Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. J Clin Invest 100(7):1693–1707. https://doi.org/10.1172/jci119694

Bruce LJ, Wrong O, Toye AM, Young MT, Ogle G, Ismail Z, Sinha AK, McMaster P, Hwaihwanje I, Nash GB, Hart S, Lavu E, Palmer R, Othman A, Unwin RJ, Tanner MJ (2000) Band 3 mutations, renal tubular acidosis and South-East Asian ovalocytosis in Malaysia and Papua New Guinea: loss of up to 95% band 3 transport in red cells. Biochem J 350(Pt 1):41–51

Bruce LJ, Pan RJ, Cope DL, Uchikawa M, Gunn RB, Cherry RJ, Tanner MJ (2004) Altered structure and anion transport properties of band 3 (AE1, SLC4A1) in human red cells lacking glycophorin A. J Biol Chem 279(4):2414–2420. https://doi.org/10.1074/jbc.M309826200

Brunati AM, Bordin L, Clari G, James P, Quadroni M, Baritono E, Pinna LA, Donella-Deana A (2000) Sequential phosphorylation of protein band 3 by Syk and Lyn tyrosine kinases in intact human erythrocytes: identification of primary and secondary phosphorylation sites. Blood 96(4):1550–1557

Brunini TM, Yaqoob MM, Novaes Malagris LE, Ellory JC, Mann GE, Mendes Ribeiro AC (2003) Increased nitric oxide synthesis in uraemic platelets is dependent on L-arginine transport via system y(+)L. Pflugers Arch 445(5):547–550. https://doi.org/10.1007/s00424-002-0978-7

Bublitz M, Morth JP, Nissen P (2011) P-type ATPases at a glance. J Cell Sci 124(Pt 15):2515–2519. https://doi.org/10.1242/jcs.088716

Buchmayer F, Schicker K, Steinkellner T, Geier P, Stübiger G, Hamilton PJ, Jurik A, Stockner T, Yang JW, Montgomery T, Holy M, Hofmaier T, Kudlacek O, Matthies HJ, Ecker GF, Bochkov V, Galli A, Boehm S, Sitte HH (2013) Amphetamine actions at the serotonin transporter rely on the availability of phosphatidylinositol-4,5-bisphosphate. Proc Natl Acad Sci U S A 110(28):11642–11647. https://doi.org/10.1073/pnas.1220552110

Burstein SH (2018) N-acyl amino acids (elmiric acids): endogenous signaling molecules with therapeutic potential. Mol Pharmacol 93(3):228–238. https://doi.org/10.1124/mol.117.110841

Busch AE, Herzer T, Waldegger S, Schmidt F, Palacin M, Biber J, Markovich D, Murer H, Lang F (1994) Opposite directed currents induced by the transport of dibasic and neutral amino acids in Xenopus oocytes expressing the protein rBAT. J Biol Chem 269 (41):25581–25586

Bushell E, Gomes AR, Sanderson T, Anar B, Girling G, Herd C, Metcalf T, Modrzynska K, Schwach F, Martin

RE, Mather MW, McFadden GI, Parts L, Rutledge GG, Vaidya AB, Wengelnik K, Rayner JC, Billker O (2017) Functional profiling of a plasmodium genome reveals an abundance of essential genes. Cell 170 (2):260–272.e268. https://doi.org/10.1016/j.cell.2017.06.030

Calonge MJ, Gasparini P, Chillaron J, Chillon M, Gallucci M, Rousaud F, Zelante L, Testar X, Dallapiccola B, Di Silverio F et al (1994) Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. Nat Genet 6(4):420–425. https://doi.org/10.1038/ng0494-420

Camargo SM, Makrides V, Virkki LV, Forster IC, Verrey F (2005) Steady-state kinetic characterization of the mouse B(0)AT1 sodium-dependent neutral amino acid transporter. Pflug Arch 451(2):338–348. https://doi.org/10.1007/s00424-005-1455-x

Camargo SM, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, Kuba K, Danilczyk U, Skovby F, Kleta R, Penninger JM, Verrey F (2009) Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations. Gastroenterology 136(3):872–882. https://doi.org/10.1053/j.gastro.2008.10.055

Camilleri M (2009) Serotonin in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes 16(1):53–59

Cano-Crespo S, Chillaron J, Junza A, Fernandez-Miranda-G, Garcia J, Polte C, RdlB L, Ignatova Z, Yanes O, Zorzano A, Stephan-Otto Attolini C, Palacin M (2019) CD98hc (SLC3A2) sustains amino acid and nucleotide availability for cell cycle progression. Sci Rep 9 (1):14065. https://doi.org/10.1038/s41598-019-50547-9

Cantor JM, Ginsberg MH (2012) CD98 at the crossroads of adaptive immunity and cancer. J Cell Sci 125 (Pt 6):1373–1382. https://doi.org/10.1242/jcs.096040

Cantor J, Browne CD, Ruppert R, Feral CC, Fassler R, Rickert RC, Ginsberg MH (2009) CD98hc facilitates B cell proliferation and adaptive humoral immunity. Nat Immunol 10(4):412–419. https://doi.org/10.1038/ni.1712

Cantor J, Slepak M, Ege N, Chang JT, Ginsberg MH (2011) Loss of T cell CD98 H chain specifically ablates T cell clonal expansion and protects from autoimmunity. J Immunol 187(2):851–860. https://doi.org/10.4049/jimmunol.1100002

Carbone E, Calorio C, Vandael DH (2014) T-type channel-mediated neurotransmitter release. Pflug Arch 466 (4):677–687. https://doi.org/10.1007/s00424-014-1489-z

Carland JE, Mansfield RE, Ryan RM, Vandenberg RJ (2013) Oleoyl-L-carnitine inhibits glycine transport by GlyT2. Br J Pharmacol 168(4):891–902. https://doi.org/10.1111/j.1476-5381.2012.02213.x

Carland JE, Handford CA, Ryan RM, Vandenberg RJ (2014) Lipid inhibitors of high affinity glycine transporters: identification of a novel class of analgesics. Neurochem Int 73:211–216. https://doi.org/10.1016/j.neuint.2013.08.012

Carland JE, Thomas M, Mostyn SN, Subramanian N, O'Mara ML, Ryan RM, Vandenberg RJ (2018) Molecular determinants for substrate interactions with the glycine transporter GlyT2. ACS Chem Neurosci 9 (3):603–614. https://doi.org/10.1021/acschemneuro.7b00407

Carpenter L, Halestrap AP (1994) The kinetics, substrate and inhibitor specificity of the lactate transporter of Ehrlich-Lettre tumour cells studied with the intracellular pH indicator BCECF. Biochem J 304 (Pt 3):751–760

Carpenter L, Poole RC, Halestrap AP (1996) Cloning and sequencing of the monocarboxylate transporter from mouse Ehrlich Lettre tumour cell confirms its identity as MCT1 and demonstrates that glycosylation is not required for MCT1 function. Biochim Biophys Acta 1279(2):157–163

Carpentieri D, Barnhart MF, Aleck K, Miloh T, deMello D (2015) Lysinuric protein intolerance in a family of Mexican ancestry with a novel SLC7A7 gene deletion. Case report and review of the literature. Mol Genet Metab Rep 2:47–50. https://doi.org/10.1016/j.ymgmr.2014.12.005

Cartier E, Hamilton PJ, Belovich AN, Shekar A, Campbell NG, Saunders C, Andreassen TF, Gether U, Veenstra-Vanderweele J, Sutcliffe JS, Ulery-Reynolds PG, Erreger K, Matthies HJ, Galli A (2015) Rare autism-associated variants implicate syntaxin 1 (STX1 R26Q) phosphorylation and the dopamine transporter (hDAT R51W) in dopamine neurotransmission and behaviors. EBioMedicine 2(2):135–146. https://doi.org/10.1016/j.ebiom.2015.01.007

Cartron JP, Rahuel C (1992) Human erythrocyte glycophorins: protein and gene structure analyses. Transfus Med Rev 6(2):63–92

Cartron JP, Rahuel C (1995) MNSs and major glycophorins of human erythrocytes. Transfus Clin Biol 2(4):251–258

Carvelli L, Blakely RD, DeFelice LJ (2008) Dopamine transporter/syntaxin 1A interactions regulate transporter channel activity and dopaminergic synaptic transmission. Proc Natl Acad Sci U S A 105 (37):14192–14197. https://doi.org/10.1073/pnas.0802214105

Casagrande F, Ratera M, Schenk AD, Chami M, Valencia E, Lopez JM, Torrents D, Engel A, Palacin M, Fotiadis D (2008) Projection structure of a member of the amino acid/polyamine/organocation transporter superfamily. J Biol Chem 283 (48):33240–33248. https://doi.org/10.1074/jbc.M806917200

Casals F, Ferrer-Admetlla A, Chillaron J, Torrents D, Palacin M, Bertranpetit J (2008) Is there selection for the pace of successive inactivation of the arpAT gene in primates? J Mol Evol 67(1):23–28. https://doi.org/10.1007/s00239-008-9120-6

Cascio L, Chen C-F, Pauly R, Srikanth S, Jones K, Skinner CD, Stevenson RE, Schwartz CE, Boccuto L (2019) Abnormalities in the genes that encode Large Amino

Acid Transporters increase the risk of Autism Spectrum Disorder. Mol Genet Genomic Med:e1036–e1036. https://doi.org/10.1002/mgg3.1036

Casey JR, Reithmeier RA (1991) Analysis of the oligomeric state of Band 3, the anion transport protein of the human erythrocyte membrane, by size exclusion high performance liquid chromatography. Oligomeric stability and origin of heterogeneity. J Biol Chem 266 (24):15726–15737

Castorino JJ, Deborde S, Deora A, Schreiner R, Gallagher-Colombo SM, Rodriguez-Boulan E, Philp NJ (2011a) Basolateral sorting signals regulating tissue-specific polarity of heteromeric monocarboxylate transporters in epithelia. Traffic 12(4):483–498. https://doi.org/10.1111/j.1600-0854.2010.01155.x

Castorino JJ, Gallagher-Colombo SM, Levin AV, Fitzgerald PG, Polishook J, Kloeckener-Gruissem B, Ostertag E, Philp NJ (2011b) Juvenile cataract-associated mutation of solute carrier SLC16A12 impairs trafficking of the protein to the plasma membrane. Invest Ophthalmol Vis Sci 52(9):6774–6784. https://doi.org/10.1167/iovs.10-6579

Caulfield WL, Collie IT, Dickins RS, Epemolu O, McGuire R, Hill DR, McVey G, Morphy JR, Rankovic Z, Sundaram H (2001) The first potent and selective inhibitors of the glycine transporter type 2. J Med Chem 44(17):2679–2682

Cechova S, Zeng Q, Billaud M, Mutchler S, Rudy CK, Straub AC, Chi L, Chan FR, Hu J, Griffiths R, Howell NL, Madsen K, Jensen BL, Palmer LA, Carey RM, Sung SS, Malakauskas SM, Isakson BE, Le TH (2013) Loss of collectrin, an angiotensin-converting enzyme 2 homolog, uncouples endothelial nitric oxide synthase and causes hypertension and vascular dysfunction. Circulation 128(16):1770–1780. https://doi.org/10.1161/circulationaha.113.003301

Cervinski MA, Foster JD, Vaughan RA (2010) Syntaxin 1A regulates dopamine transporter activity, phosphorylation and surface expression. Neuroscience 170 (2):408–416. https://doi.org/10.1016/j.neuroscience.2010.07.025

Cesar-Razquin A, Snijder B, Frappier-Brinton T, Isserlin R, Gyimesi G, Bai X, Reithmeier RA, Hepworth D, Hediger MA, Edwards AM, Superti-Furga G (2015) A call for systematic research on solute carriers. Cell 162(3):478–487. https://doi.org/10.1016/j.cell.2015.07.022

Chairoungdua A, Segawa H, Kim JY, Miyamoto K, Haga H, Fukui Y, Mizoguchi K, Ito H, Takeda E, Endou H, Kanai Y (1999) Identification of an amino acid transporter associated with the cystinuria-related type II membrane glycoprotein. J Biol Chem 274 (41):28845–28848

Chairoungdua A, Kanai Y, Matsuo H, Inatomi J, Kim DK, Endou H (2001) Identification and characterization of a novel member of the heterodimeric amino acid transporter family presumed to be associated with an unknown heavy chain. J Biol Chem 276 (52):49390–49399. https://doi.org/10.1074/jbc.M107517200

Chamsi-Pasha MA, Shao Z, Tang WH (2014) Angiotensin-converting enzyme 2 as a therapeutic target for heart failure. Curr Heart Fail Rep 11(1):58–63. https://doi.org/10.1007/s11897-013-0178-0

Chandra S, Fornai F, Kwon H-B, Yazdani U, Atasoy D, Liu X, Hammer RE, Battaglia G, German DC, Castillo PE, Südhof TC (2004) Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. Proc Natl Acad Sci U S A 101 (41):14966–14971. https://doi.org/10.1073/pnas.0406283101

Chansel D, Czekalski S, Vandermeersch S, Ruffet E, Fournie-Zaluski MC, Ardaillou R (1998) Characterization of angiotensin IV-degrading enzymes and receptors on rat mesangial cells. Am J Physiol 275 (4 Pt 2):F535–F542

Chappell MC, Marshall AC, Alzayadneh EM, Shaltout HA, Diz DI (2014) Update on the Angiotensin converting enzyme 2-Angiotensin (1-7)-MAS receptor axis: fetal programing, sex differences, and intracellular pathways. Front Endocrinol (Lausanne) 4:201. https://doi.org/10.3389/fendo.2013.00201

Che A, Cherry RJ (1995) Loss of rotational mobility of band 3 proteins in human erythrocyte membranes induced by antibodies to glycophorin A. Biophys J 68(5):1881–1887. https://doi.org/10.1016/s0006-3495(95)80365-6

Chen I, Lui F (2019) Physiology, active transport. In: StatPearls. StatPearls Publishing, Treasure Island

Chen HL, Liu YJ, Chen HL, Wu SH, Ni YH, Ho MC, Lai HS, Hsu WM, Hsu HY, Tseng HC, Jeng YM, Chang MH (2008) Expression of hepatocyte transporters and nuclear receptors in children with early and late-stage biliary atresia. Pediatr Res 63(6):667–673. https://doi.org/10.1203/PDR.0b013e318170a6b5

Chen R, Jiang X, Sun D, Han G, Wang F, Ye M, Wang L, Zou H (2009a) Glycoproteomics analysis of human liver tissue by combination of multiple enzyme digestion and hydrazide chemistry. J Proteome Res 8 (2):651–661. https://doi.org/10.1021/pr8008012

Chen RS, Song YM, Zhou ZY, Tong T, Li Y, Fu M, Guo XL, Dong LJ, He X, Qiao HX, Zhan QM, Li W (2009b) Disruption of xCT inhibits cancer cell metastasis via the caveolin-1/beta-catenin pathway. Oncogene 28(4):599–609. https://doi.org/10.1038/onc.2008.414

Chen Y, Guo Y, Pan Y, Zhao ZJ (2020) Structure analysis of the receptor binding of 2019-nCoV. Biochem Biophys Res Commun. https://doi.org/10.1016/j.bbrc.2020.02.071

Cheng Q, Shah N, Broer A, Fairweather S, Jiang Y, Schmoll D, Corry B, Broer S (2017) Identification of novel inhibitors of the amino acid transporter B(0) AT1 (SLC6A19), a potential target to induce protein restriction and to treat type 2 diabetes. Br J Pharmacol 174 (6):468–482. https://doi.org/10.1111/bph.13711

Chernova MN, Jarolim P, Palek J, Alper SL (1995) Overexpression of AE1 Prague, but not of AE1 SAO, inhibits wild-type AE1 trafficking in Xenopus oocytes. J Membr Biol 148(2):203–210

Cheung JC, Cordat E, Reithmeier RA (2005) Trafficking defects of the Southeast Asian ovalocytosis deletion mutant of anion exchanger 1 membrane proteins. Biochem J 392(Pt 3):425–434. https://doi.org/10.1042/bj20051076

Chhabra KH, Chodavarapu H, Lazartigues E (2013) Angiotensin converting enzyme 2: a new important player in the regulation of glycemia. IUBMB Life 65 (9):731–738. https://doi.org/10.1002/iub.1190

Chiang Z, Vastermark A, Punta M, Coggill PC, Mistry J, Finn RD, Saier MH Jr (2015) The complexity, challenges and benefits of comparing two transporter classification systems in TCDB and Pfam. Brief Bioinform 16(5):865–872. https://doi.org/10.1093/bib/bbu053

Chidlow G, Wood JP, Graham M, Osborne NN (2005) Expression of monocarboxylate transporters in rat ocular tissues. Am J Physiol Cell Physiol 288(2):C416–C428. https://doi.org/10.1152/ajpcell.00037.2004

Chillaron J, Estevez R, Mora C, Wagner CA, Suessbrich H, Lang F, Gelpi JL, Testar X, Busch AE, Zorzano A, Palacin M (1996) Obligatory amino acid exchange via systems bo,+-like and y+L-like. A tertiary active transport mechanism for renal reabsorption of cystine and dibasic amino acids. J Biol Chem 271 (30):17761–17770

Chillaron J, Estevez R, Samarzija I, Waldegger S, Testar X, Lang F, Zorzano A, Busch A, Palacin M (1997) An intracellular trafficking defect in type I cystinuria rBAT mutants M467T and M467K. J Biol Chem 272(14):9543–9549

Chillaron J, Roca R, Valencia A, Zorzano A, Palacin M (2001) Heteromeric amino acid transporters: biochemistry, genetics, and physiology. Am J Physiol Renal Physiol 281(6):F995–1018. https://doi.org/10.1152/ajprenal.2001.281.6.F995

Chillaron J, Font-Llitjos M, Fort J, Zorzano A, Goldfarb DS, Nunes V, Palacin M (2010) Pathophysiology and treatment of cystinuria. Nat Rev Nephrol 6 (7):424–434. https://doi.org/10.1038/nrneph.2010.69

Christian WV, Hinkle PM (2017) Global functions of extracellular, transmembrane and cytoplasmic domains of organic solute transporter beta-subunit. Biochem J 474(12):1981–1992. https://doi.org/10.1042/bcj20161093

Christian WV, Li N, Hinkle PM, Ballatori N (2012) beta-Subunit of the Ostalpha-Ostbeta organic solute transporter is required not only for heterodimerization and trafficking but also for function. J Biol Chem 287 (25):21233–21243. https://doi.org/10.1074/jbc.M112.352245

Christie DL (2007) Functional insights into the creatine transporter. Subcell Biochem 46:99–118

Chu PL, Le TH (2014) Role of collectrin, an ACE2 homologue, in blood pressure homeostasis. Curr Hypertens Rep 16(11):490. https://doi.org/10.1007/s11906-014-0490-4

Chu C, Woods N, Sawasdee N, Guizouarn H, Pellissier B, Borgese F, Yenchitsomanus PT, Gowrishankar M, Cordat E (2010) Band 3 Edmonton I, a novel mutant of the anion exchanger 1 causing spherocytosis and distal renal tubular acidosis. Biochem J 426 (3):379–388. https://doi.org/10.1042/bj20091525

Chu CY, King J, Berrini M, Rumley AC, Apaja PM, Lukacs GL, Alexander RT, Cordat E (2014) Degradation mechanism of a Golgi-retained distal renal tubular acidosis mutant of the kidney anion exchanger 1 in renal cells. Am J Physiol Cell Physiol 307(3):C296–C307. https://doi.org/10.1152/ajpcell.00310.2013

Ciccone MA, Timmons M, Phillips A, Quick MW (2008) Calcium/calmodulin-dependent kinase II regulates the interaction between the serotonin transporter and syntaxin 1A. Neuropharmacology 55(5):763–770. https://doi.org/10.1016/j.neuropharm.2008.06.018

Cioffi CL (2018) Glycine transporter-1 inhibitors: a patent review (2011–2016). Expert Opin Ther Pat 28 (3):197–210. https://doi.org/10.1080/13543776.2018.1429408

Clausen RP, Madsen K, Larsson OM, Frolund B, Krogsgaard-Larsen P, Schousboe A (2006) Structure-activity relationship and pharmacology of gamma-aminobutyric acid (GABA) transport inhibitors. Adv Pharmacol 54:265–284

Coady MJ, Jalal F, Chen X, Lemay G, Berteloot A, Lapointe JY (1994) Electrogenic amino acid exchange via the rBAT transporter. FEBS Lett 356 (2–3):174–178

Coady MJ, Chen XZ, Lapointe JY (1996) rBAT is an amino acid exchanger with variable stoichiometry. J Membr Biol 149(1):1–8

Coleman JA, Gouaux E (2018) Structural basis for recognition of diverse antidepressants by the human serotonin transporter. Nat Struct Mol Biol 25(2):170–175. https://doi.org/10.1038/s41594-018-0026-8

Coleman JA, Green EM, Gouaux E (2016) X-ray structures and mechanism of the human serotonin transporter. Nature 532(7599):334–339. https://doi.org/10.1038/nature17629

Colucci R, Blandizzi C, Bellini M, Ghisu N, Tonini M, Del Tacca M (2008) The genetics of the serotonin transporter and irritable bowel syndrome. Trends Mol Med 14(7):295–304. https://doi.org/10.1016/j.molmed.2008.05.001

Condliffe SB, Zhang H, Frizzell RA (2004) Syntaxin 1A regulates ENaC channel activity. J Biol Chem 279 (11):10085–10092. https://doi.org/10.1074/jbc.M313592200

Cordat E, Reithmeier RA (2014) Structure, function, and trafficking of SLC4 and SLC26 anion transporters. Curr Top Membr 73:1–67. https://doi.org/10.1016/b978-0-12-800223-0.00001-3

Cormerais Y, Giuliano S, LeFloch R, Front B, Durivault J, Tambutte E, Massard PA, de la Ballina LR, Endou H,

Wempe MF, Palacin M, Parks SK, Pouyssegur J (2016) Genetic disruption of the multifunctional CD98/LAT1 complex demonstrates the key role of essential amino acid transport in the control of mTORC1 and tumor growth. Cancer Res 76 (15):4481–4492. https://doi.org/10.1158/0008-5472.can-15-3376

Coudray N, S LS, Lasala R, Zhang Z, Clark KM, Dumont ME, Rohou A, Beckstein O, Stokes DL (2017) Structure of the SLC4 transporter Bor1p in an inward-facing conformation. Protein Sci 26(1):130–145. https://doi.org/10.1002/pro.3061

Cowell AN, Istvan ES, Lukens AK, Gomez-Lorenzo MG, Vanaerschot M, Sakata-Kato T, Flannery EL, Magistrado P, Owen E, Abraham M, LaMonte G, Painter HJ, Williams RM, Franco V, Linares M, Arriaga I, Bopp S, Corey VC, Gnadig NF, Coburn-Flynn O, Reimer C, Gupta P, Murithi JM, Moura PA, Fuchs O, Sasaki E, Kim SW, Teng CH, Wang LT, Akidil A, Adjalley S, Willis PA, Siegel D, Tanaseichuk O, Zhong Y, Zhou Y, Llinas M, Ottilie S, Gamo FJ, Lee MCS, Goldberg DE, Fidock DA, Wirth DF, Winzeler EA (2018) Mapping the malaria parasite druggable genome by using in vitro evolution and chemogenomics. Science 359 (6372):191–199. https://doi.org/10.1126/science.aan4472

Craig TJ, Anderson D, Evans AJ, Girach F, Henley JM (2015) SUMOylation of Syntaxin1A regulates presynaptic endocytosis. Sci Rep 5:17669. https://doi.org/10.1038/srep17669

Cutillas PR, Biber J, Marks J, Jacob R, Stieger B, Cramer R, Waterfield M, Burlingame AL, Unwin RJ (2005) Proteomic analysis of plasma membrane vesicles isolated from the rat renal cortex. Proteomics 5(1):101–112. https://doi.org/10.1002/pmic.200400886

D'Agostino S, Lanzillotta D, Varano M, Botta C, Baldrini A, Bilotta A, Scalise S, Dattilo V, Amato R, Gaudio E, Paduano F, Palmieri C, Iuliano R, Perrotti N, Indiveri C, Fusco A, Gaspari M, Trapasso F (2018) The receptor protein tyrosine phosphatase PTPRJ negatively modulates the CD98hc oncoprotein in lung cancer cells. Oncotarget 9(34):23334–23348. https://doi.org/10.18632/oncotarget.25101

Dahr W, Wilkinson S, Issitt PD, Beyreuther K, Hummel M, Morel P (1986) High frequency antigens of human erythrocyte membrane sialoglycoproteins, III. Studies on the EnaFR, Wrb and Wra antigens. Biol Chem Hoppe Seyler 367(10):1033–1045

Dahr W, Moulds J, Unger P, Kordowicz M (1987) The Dantu erythrocyte phenotype of the NE variety. I. Dodecylsulfate polyacrylamide gel electrophoretic studies. Blut 55(1):19–31

Dai L, Cao Y, Jiang W, Zabaleta J, Liu Z, Qiao J, Qin Z (2017) KSHV co-infection down-regulates HPV16 E6 and E7 from cervical cancer cells. Oncotarget 8 (22):35792–35803. https://doi.org/10.18632/oncotarget.16207

Danilczyk U, Sarao R, Remy C, Benabbas C, Stange G, Richter A, Arya S, Pospisilik JA, Singer D, Camargo SM, Makrides V, Ramadan T, Verrey F, Wagner CA, Penninger JM (2006) Essential role for collectrin in renal amino acid transport. Nature 444 (7122):1088–1091. https://doi.org/10.1038/nature05475

Danthi SJ, Liang B, Smicker O, Coupland B, Gregory J, Gefteas E, Tietz D, Klodnitsky H, Randall K, Belanger A, Kuntzweiler TA (2018) Identification and characterization of inhibitors of a neutral amino acid transporter, SLC6A19, using two functional cell-based assays. SLAS Discov:2472555218794627. https://doi.org/10.1177/2472555218794627

Danziger RS (2008) Aminopeptidase N in arterial hypertension. Heart Fail Rev 13(3):293–298

Darby RA, Callaghan R, McMahon RM (2011) P-glycoprotein inhibition: the past, the present and the future. Curr Drug Metab 12(8):722–731

Das AK, Kudlacek O, Baumgart F, Jaentsch K, Stockner T, Sitte HH, Schütz GJ (2019) Dopamine transporter forms stable dimers in the live cell plasma membrane in a phosphatidylinositol 4,5-bisphosphate-independent manner. J Biol Chem 294(14):5632–5642. https://doi.org/10.1074/jbc.RA118.006178

Dassa E, Bouige P (2001) The ABC of ABCS: a phylogenetic and functional classification of ABC systems in living organisms. Res Microbiol 152(3–4):211–229

Dauer W, Kholodilov N, Vila M, Trillat A-C, Goodchild R, Larsen KE, Staal R, Tieu K, Schmitz Y, Yuan CA, Rocha M, Jackson-Lewis V, Hersch S, Sulzer D, Przedborski S, Burke R, Hen R (2002) Resistance of alpha -synuclein null mice to the parkinsonian neurotoxin MPTP. Proc Natl Acad Sci U S A 99(22):14524–14529. https://doi.org/10.1073/pnas.172514599

Dave MH, Schulz N, Zecevic M, Wagner CA, Verrey F (2004) Expression of heteromeric amino acid transporters along the murine intestine. J Physiol 558 (Pt 2):597–610. https://doi.org/10.1113/jphysiol.2004.065037

Davis RP, Pattison J, Thompson JM, Tiniakov R, Scrogin KE, Watts SW (2012) 5-hydroxytryptamine (5-HT) reduces total peripheral resistance during chronic infusion: direct arterial mesenteric relaxation is not involved. BMC Pharmacol 12:4. https://doi.org/10.1186/1471-2210-12-4

Dawson PA, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, Ballatori N (2005) The heteromeric organic solute transporter alpha-beta, Ostalpha-Ostbeta, is an ileal basolateral bile acid transporter. J Biol Chem 280(8):6960–6968. https://doi.org/10.1074/jbc.M412752200

Dawson PA, Hubbert ML, Rao A (2010) Getting the mOST from OST: role of organic solute transporter, OSTalpha-OSTbeta, in bile acid and steroid metabolism. Biochim Biophys Acta 1801(9):994–1004. https://doi.org/10.1016/j.bbalip.2010.06.002

De Bundel D, Schallier A, Loyens E, Fernando R, Miyashita H, Van Liefferinge J, Vermoesen K, Bannai S, Sato H, Michotte Y, Smolders I, Massie A (2011) Loss of system x(c)- does not induce oxidative stress but decreases extracellular glutamate in hippocampus and influences spatial working memory and limbic seizure susceptibility. J Neurosci 31 (15):5792–5803. https://doi.org/10.1523/jneurosci.5465-10.2011

de la Ballina LR, Cano-Crespo S, Gonzalez-Munoz E, Bial S, Estrach S, Cailleteau L, Tissot F, Daniel H, Zorzano A, Ginsberg MH, Palacin M, Feral CC (2016) Amino acid transport associated to cluster of differentiation 98 heavy chain (CD98hc) is at the cross-road of oxidative stress and amino acid availability. J Biol Chem 291(18):9700–9711. https://doi.org/10.1074/jbc.M115.704254

De Saedeleer CJ, Porporato PE, Copetti T, Perez-Escuredo J, Payen VL, Brisson L, Feron O, Sonveaux P (2014) Glucose deprivation increases monocarboxylate transporter 1 (MCT1) expression and MCT1-dependent tumor cell migration. Oncogene 33(31):4060–4068. https://doi.org/10.1038/onc.2013.454

de Wit H (2010) Molecular mechanism of secretory vesicle docking. Biochem Soc Trans 38(Pt 1):192–198. https://doi.org/10.1042/BST0380192

Dean M, Annilo T (2005) Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu Rev Genomics Hum Genet 6:123–142. https://doi.org/10.1146/annurev.genom.6.080604.162122

Dean M, Hamon Y, Chimini G (2001a) The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res 42(7):1007–1017

Dean M, Rzhetsky A, Allikmets R (2001b) The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 11(7):1156–1166. https://doi.org/10.1101/gr.184901

Deejai N, Wisanuyotin S, Nettuwakul C, Khositseth S, Sawasdee N, Saetai K, Yenchitsomanus PT, Rungroj N (2019) Molecular diagnosis of solute carrier family 4 member 1 (SLC4A1) mutation-related autosomal recessive distal renal tubular acidosis. Lab Med 50 (1):78–86. https://doi.org/10.1093/labmed/lmy051

DeGiorgis JA, Jaffe H, Moreira JE, Carlotti CG Jr, Leite JP, Pant HC, Dosemeci A (2005) Phosphoproteomic analysis of synaptosomes from human cerebral cortex. J Proteome Res 4(2):306–315. https://doi.org/10.1021/pr0498436

Deken SL, Beckman ML, Boos L, Quick MW (2000) Transport rates of GABA transporters: regulation by the N-terminal domain and syntaxin 1A. Nat Neurosci 3(10):998–1003. https://doi.org/10.1038/79939

Dello Strologo L, Pras E, Pontesilli C, Beccia E, Ricci-Barbini V, de Sanctis L, Ponzone A, Gallucci M, Bisceglia L, Zelante L, Jimenez-Vidal M, Font M, Zorzano A, Rousaud F, Nunes V, Gasparini P, Palacin M, Rizzoni G (2002) Comparison between SLC3A1 and SLC7A9 cystinuria patients and carriers: a need for a new classification. J Am Soc Nephrol 13 (10):2547–2553

Deora AB, Ghosh RN, Tate SS (1998) Progressive C-terminal deletions of the renal cystine transporter, NBAT, reveal a novel bimodal pattern of functional expression. J Biol Chem 273(49):32980–32987

Devonald MA, Smith AN, Poon JP, Ihrke G, Karet FE (2003) Non-polarized targeting of AE1 causes autosomal dominant distal renal tubular acidosis. Nat Genet 33(2):125–127. https://doi.org/10.1038/ng1082

Dickens D, Webb SD, Antonyuk S, Giannoudis A, Owen A, Radisch S, Hasnain SS, Pirmohamed M (2013) Transport of gabapentin by LAT1 (SLC7A5). Biochem Pharmacol 85(11):1672–1683. https://doi.org/10.1016/j.bcp.2013.03.022

Dickens D, Chiduza GN, Wright GS, Pirmohamed M, Antonyuk SV, Hasnain SS (2017) Modulation of LAT1 (SLC7A5) transporter activity and stability by membrane cholesterol. Sci Rep 7:43580. https://doi.org/10.1038/srep43580

Diering GH, Church J, Numata M (2009) Secretory carrier membrane protein 2 regulates cell-surface targeting of brain-enriched Na+/H+ exchanger NHE5. J Biol Chem 284(20):13892–13903. https://doi.org/10.1074/jbc.M807055200

Digomann D, Kurth I, Tyutyunnykova A, Chen O, Löck S, Gorodetska I, Peitzsch C, Skvortsova I-I, Negro G, Aschenbrenner B, Eisenhofer G, Richter S, Heiden S, Porrmann J, Klink B, Schwager C, Dowle AA, Hein L, Kunz-Schughart LA, Abdollahi A, Lohaus F, Krause M, Baumann M, Linge A, Dubrovska A (2019) The CD98 heavy chain is a marker and regulator of head and neck squamous cell carcinoma radiosensitivity. Clin Cancer Res 25(10):3152–3163. https://doi.org/10.1158/1078-0432.CCR-18-2951

Dimmer KS, Friedrich B, Lang F, Deitmer JW, Broer S (2000) The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochem J 350(Pt 1):219–227

Dipace C, Sung U, Binda F, Blakely RD, Galli A (2007) Amphetamine induces a calcium/calmodulin-dependent protein kinase II-dependent reduction in norepinephrine transporter surface expression linked to changes in syntaxin 1A/transporter complexes. Mol Pharmacol 71(1):230–239. https://doi.org/10.1124/mol.106.026690

Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 87(5):E1–E9

Doobay MF, Talman LS, Obr TD, Tian X, Davisson RL, Lazartigues E (2007) Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 292(1):R373–R381. https://doi.org/10.1152/ajpregu.00292.2006

Draoui N, Schicke O, Fernandes A, Drozak X, Nahra F, Dumont A, Douxfils J, Hermans E, Dogne JM, Corbau R, Marchand A, Chaltin P, Sonveaux P, Feron O, Riant O (2013) Synthesis and pharmacological evaluation of carboxycoumarins as a new antitumor treatment targeting lactate transport in cancer cells. Bioorg Med Chem 21(22):7107–7117. https://doi.org/10.1016/j.bmc.2013.09.010

Drew D, Boudker O (2016) Shared molecular mechanisms of membrane transporters. Annu Rev Biochem 85:543–572. https://doi.org/10.1146/annurev-biochem-060815-014520

Drickamer LK (1978) Orientation of the band 3 polypeptide from human erythrocyte membranes. Identification of NH2-terminal sequence and site of carbohydrate attachment. J Biol Chem 253 (20):7242–7248

Duangtum N, Junking M, Phadngam S, Sawasdee N, Castiglioni A, Charngkaew K, Limjindaporn T, Isidoro C, Yenchitsomanus PT (2017) gamma-COPI mediates the retention of kAE1 G701D protein in Golgi apparatus – a mechanistic explanation of distal renal tubular acidosis associated with the G701D mutation. Biochem J 474(15):2573–2584. https://doi.org/10.1042/bcj20170088

Duelli R, Enerson BE, Gerhart DZ, Drewes LR (2000) Expression of large amino acid transporter LAT1 in rat brain endothelium. J Cereb Blood Flow Metab 20 (11):1557–1562. https://doi.org/10.1097/00004647-200011000-00005

Edington AR, McKinzie AA, Reynolds AJ, Kassiou M, Ryan RM, Vandenberg RJ (2009) Extracellular loops 2 and 4 of GLYT2 are required for N-arachidonylglycine inhibition of glycine transport. J Biol Chem 284(52):36424–36430. https://doi.org/10.1074/jbc.M109.017509

Edwards N, Anderson CMH, Conlon NJ, Watson AK, Hall RJ, Cheek TR, Embley TM, Thwaites DT (2018) Resculpting the binding pocket of APC superfamily LeuT-fold amino acid transporters. Cell Mol Life Sci 75(5):921–938. https://doi.org/10.1007/s00018-017-2677-8

Egan BM (2013) Collectrin, an X-linked, angiotensin converting enzyme 2 homolog, causes hypertension in a rat strain through gene-gene and gene-environment interactions: relevance to human hypertension. Circulation 128(16):1727–1728. https://doi.org/10.1161/CIRCULATIONAHA.113.005695

Egaña LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S, Peña K, Quiroz M, Hong WC, Dorostkar MM, Janz R, Sitte HH, Torres GE (2009) Physical and functional interaction between the dopamine transporter and the synaptic vesicle protein synaptogyrin-3. J Neurosci 29(14):4592–4604. https://doi.org/10.1523/JNEUROSCI.4559-08.2009

Eggermann T, Elbracht M, Haverkamp F, Schmidt C, Zerres K (2007) Isolated cystinuria (OMIM 238200) is not a separate entity but is caused by a mutation in the cystinuria gene SLC7A9. Clin Genet 71 (6):597–598. https://doi.org/10.1111/j.1399-0004.2007.00797.x

Ehrnstorfer IA, Geertsma ER, Pardon E, Steyaert J, Dutzler R (2014) Crystal structure of a SLC11 (NRAMP) transporter reveals the basis for transition-metal ion transport. Nat Struct Mol Biol 21 (11):990–996. https://doi.org/10.1038/nsmb.2904

Ehrnstorfer IA, Manatschal C, Arnold FM, Laederach J, Dutzler R (2017) Structural and mechanistic basis of proton-coupled metal ion transport in the SLC11/NRAMP family. Nat Commun 8:14033. https://doi.org/10.1038/ncomms14033

El Ansari R, Craze ML, Diez-Rodriguez M, Nolan CC, Ellis IO, Rakha EA, Green AR (2018a) The multifunctional solute carrier 3A2 (SLC3A2) confers a poor prognosis in the highly proliferative breast cancer subtypes. Br J Cancer 118(8):1115–1122. https://doi.org/10.1038/s41416-018-0038-5

El Ansari R, Craze ML, Miligy I, Diez-Rodriguez M, Nolan CC, Ellis IO, Rakha EA, Green AR (2018b) The amino acid transporter SLC7A5 confers a poor prognosis in the highly proliferative breast cancer subtypes and is a key therapeutic target in luminal B tumours. Breast Cancer Res 20(1):21. https://doi.org/10.1186/s13058-018-0946-6

Enoki T, Yoshida Y, Lally J, Hatta H, Bonen A (2006) Testosterone increases lactate transport, monocarboxylate transporter (MCT) 1 and MCT4 in rat skeletal muscle. J Physiol 577(Pt 1):433–443. https://doi.org/10.1113/jphysiol.2006.115436

Eriksen J, Jorgensen TN, Gether U (2010) Regulation of dopamine transporter function by protein-protein interactions: new discoveries and methodological challenges. J Neurochem 113(1):27–41. https://doi.org/10.1111/j.1471-4159.2010.06599.x

Errasti-Murugarren E, Fort J, Bartoccioni P, Diaz L, Pardon E, Carpena X, Espino-Guarch M, Zorzano A, Ziegler C, Steyaert J, Fernandez-Recio J, Fita I, Palacin M (2019) L amino acid transporter structure and molecular bases for the asymmetry of substrate interaction. Nat Commun 10(1):1807. https://doi.org/10.1038/s41467-019-09837-z

Eshleman AJ, Carmolli M, Cumbay M, Martens CR, Neve KA, Janowsky A (1999) Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. J Pharmacol Exp Ther 289(2):877–885

Eskandari S, Willford SL, Anderson CM (2017) Revised ion/substrate coupling stoichiometry of GABA transporters. Adv Neurobiol 16:85–116. https://doi.org/10.1007/978-3-319-55769-4_5

Espino Gaurch M, Font-Llitjos M, Murillo-Cuesta S, Errasti-Murugarren E, Celaya AM, Girotto G, Vuckovic D, Mezzavilla M, Vilches C, Bodoy S, Sahun I, Gonzalez L, Prat E, Zorzano A, Dierssen M, Varela-Nieto I, Gasparini P, Palacin M, Nunes V (2018) Mutations in L-type amino acid transporter-2-support SLC7A8 as a novel gene involved in

Age-Related Hearing Loss. Elife 7. https://doi.org/10.7554/eLife.31511

Esterhazy D, Akpinar P, Stoffel M (2012) Tmem27 dimerization, deglycosylation, plasma membrane depletion, and the extracellular Phe-Phe motif are negative regulators of cleavage by Bace2. Biol Chem 393 (6):473–484. https://doi.org/10.1515/hsz-2012-0104

Estevez R, Camps M, Rojas AM, Testar X, Deves R, Hediger MA, Zorzano A, Palacin M (1998) The amino acid transport system y+L/4F2hc is a heteromultimeric complex. FASEB J 12 (13):1319–1329

Estrach S, Lee SA, Boulter E, Pisano S, Errante A, Tissot FS, Cailleteau L, Pons C, Ginsberg MH, Feral CC (2014) CD98hc (SLC3A2) loss protects against ras-driven tumorigenesis by modulating integrin-mediated mechanotransduction. Cancer Res 74 (23):6878–6889. https://doi.org/10.1158/0008-5472.can-14-0579

Etoga JL, Ahmed SK, Patel S, Bridges RJ, Thompson CM (2010) Conformationally-restricted amino acid analogues bearing a distal sulfonic acid show selective inhibition of system x(c)(-) over the vesicular glutamate transporter. Bioorg Med Chem Lett 20(8):2680–2683. https://doi.org/10.1016/j.bmcl.2009.10.020

Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjöstedt E, Lundberg E, Szigyarto CA-K, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlén M (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 13(2):397–406. https://doi.org/10.1074/mcp.M113.035600

Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, Abramson J (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport. Science 321(5890):810–814. https://doi.org/10.1126/science.1160406

Fairbanks G, Steck TL, Wallach DF (1971) Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10 (13):2606–2617

Fairweather SJ (2017) Protein interactions of epithelial neutral amino acid transporters. Doctoral thesis. Australian National University, Canberra. https://openresearch-repository.anu.edu.au/handle/1885/135817

Fairweather SJ, Broer A, O'Mara ML, Broer S (2012) Intestinal peptidases form functional complexes with the neutral amino acid transporter B(0)AT1. Biochem J 446(1):135–148. https://doi.org/10.1042/bj20120307

Fairweather SJ, Broer A, Subramanian N, Tumer E, Cheng Q, Schmoll D, O'Mara ML, Broer S (2015) Molecular basis for the interaction of the mammalian amino acid transporters B0AT1 and B0AT3 with their ancillary protein collectrin. J Biol Chem 290 (40):24308–24325. https://doi.org/10.1074/jbc.M115.648519

Falke JJ, Chan SI (1986a) Molecular mechanisms of band 3 inhibitors. 1. Transport site inhibitors. Biochemistry 25(24):7888–7894

Falke JJ, Chan SI (1986b) Molecular mechanisms of band 3 inhibitors. 2. Channel blockers. Biochemistry 25 (24):7895–7898

Falke JJ, Chan SI (1986c) Molecular mechanisms of band 3 inhibitors. 3. Translocation inhibitors. Biochemistry 25(24):7899–7906

Fan QW, Kadomatsu K, Uchimura K, Muramatsu T (1998) Embigin/basigin subgroup of the immunoglobulin superfamily: different modes of expression during mouse embryogenesis and correlated expression with carbohydrate antigenic markers. Dev Growth Differ 40 (3):277–286

Fan HP, Fan FJ, Bao L, Pei G (2006) SNAP-25/syntaxin 1A complex functionally modulates neurotransmitter gamma-aminobutyric acid reuptake. J Biol Chem 281 (38):28174–28184. https://doi.org/10.1074/jbc.M601382200

Fang Y, Jayaram H, Shane T, Kolmakova-Partensky L, Wu F, Williams C, Xiong Y, Miller C (2009) Structure of a prokaryotic virtual proton pump at 3.2 A resolution. Nature 460(7258):1040–1043. https://doi.org/10.1038/nature08201

Fang F, Christian WV, Gorman SG, Cui M, Huang J, Tieu K, Ballatori N (2010) Neurosteroid transport by the organic solute transporter OSTalpha-OSTbeta. J Neurochem 115(1):220–233. https://doi.org/10.1111/j.1471-4159.2010.06920.x

Farhan H, Freissmuth M, Sitte HH (2006) Oligomerization of neurotransmitter transporters: a ticket from the endoplasmic reticulum to the plasma membrane. Handb Exp Pharmacol 175:233–249. https://doi.org/10.1007/3-540-29784-7_12

Fawaz NA, Beshlawi IO, Al Zadjali S, Al Ghaithi HK, Elnaggari MA, Elnour I, Wali YA, Al-Said BB, Rehman JU, Pathare AV, Knox-Macaulay H, Alkindi SS (2012) dRTA and hemolytic anemia: first detailed description of SLC4A1 A858D mutation in homozygous state. Eur J Haematol 88(4):350–355. https://doi.org/10.1111/j.1600-0609.2011.01739.x

Feliubadalo L, Font M, Purroy J, Rousaud F, Estivill X, Nunes V, Golomb E, Centola M, Aksentijevich I, Kreiss Y, Goldman B, Pras M, Kastner DL, Pras E, Gasparini P, Bisceglia L, Beccia E, Gallucci M, de Sanctis L, Ponzone A, Rizzoni GF, Zelante L, Bassi MT, George AL Jr, Manzoni M, De Grandi A, Riboni M, Endsley JK, Ballabio A, Borsani G, Reig N, Fernandez E, Estevez R, Pineda M, Torrents D, Camps M, Lloberas J, Zorzano A, Palacin M (1999) Non-type I cystinuria caused by mutations in SLC7A9, encoding a subunit (bo,+AT) of rBAT. Nat Genet 23(1):52–57. https://doi.org/10.1038/12652

Feliubadalo L, Arbones ML, Manas S, Chillaron J, Visa J, Rodes M, Rousaud F, Zorzano A, Palacin M, Nunes V (2003) Slc7a9-deficient mice develop cystinuria non-I

and cystine urolithiasis. Hum Mol Genet 12 (17):2097–2108. https://doi.org/10.1093/hmg/ddg228

Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH (1997) Complementation of dominant suppression implicates CD98 in integrin activation. Nature 390 (6655):81–85. https://doi.org/10.1038/36349

Fenczik CA, Zent R, Dellos M, Calderwood DA, Satriano J, Kelly C, Ginsberg MH (2001) Distinct domains of CD98hc regulate integrins and amino acid transport. J Biol Chem 276(12):8746–8752. https://doi.org/10.1074/jbc.M011239200

Fenollar-Ferrer C, Stockner T, Schwarz TC, Pal A, Gotovina J, Hofmaier T, Jayaraman K, Adhikary S, Kudlacek O, Mehdipour AR, Tavoulari S, Rudnick G, Singh SK, Konrat R, Sitte HH, Forrest LR (2014) Structure and regulatory interactions of the cytoplasmic terminal domains of serotonin transporter. Biochemistry 53(33):5444–5460. https://doi.org/10.1021/bi500637f

Feral CC, Nishiya N, Fenczik CA, Stuhlmann H, Slepak M, Ginsberg MH (2005) CD98hc (SLC3A2) mediates integrin signaling. Proc Natl Acad Sci U S A 102(2):355–360. https://doi.org/10.1073/pnas.0404852102

Feral CC, Zijlstra A, Tkachenko E, Prager G, Gardel ML, Slepak M, Ginsberg MH (2007) CD98hc (SLC3A2) participates in fibronectin matrix assembly by mediating integrin signaling. J Cell Biol 178 (4):701–711. https://doi.org/10.1083/jcb.200705090

Fernandez E, Carrascal M, Rousaud F, Abian J, Zorzano A, Palacin M, Chillaron J (2002) rBAT-b(0,+)AT heterodimer is the main apical reabsorption system for cystine in the kidney. Am J Physiol Renal Physiol 283(3):F540–F548. https://doi.org/10.1152/ajprenal.00071.2002

Fernandez E, Torrents D, Chillaron J, Martin Del Rio R, Zorzano A, Palacin M (2003) Basolateral LAT-2 has a major role in the transepithelial flux of L-cystine in the renal proximal tubule cell line OK. J Am Soc Nephrol 14(4):837–847

Fernandez E, Torrents D, Zorzano A, Palacin M, Chillaron J (2005) Identification and functional characterization of a novel low affinity aromatic-preferring amino acid transporter (arpAT). One of the few proteins silenced during primate evolution. J Biol Chem 280 (19):19364–19372. https://doi.org/10.1074/jbc.M412516200

Fernandez E, Jimenez-Vidal M, Calvo M, Zorzano A, Tebar F, Palacin M, Chillaron J (2006) The structural and functional units of heteromeric amino acid transporters. The heavy subunit rBAT dictates oligomerization of the heteromeric amino acid transporters. J Biol Chem 281(36):26552–26561. https://doi.org/10.1074/jbc.M604049200

Ferrebee CB, Li J, Haywood J, Pachura K, Robinson BS, Hinrichs BH, Jones RM, Rao A, Dawson PA (2018) OSTα-OSTβ guards the ileal enterocyte from the accumulation of toxic levels of bile acids. Cell Mol Gastroenterol Hepatol 5(4):499–522. https://doi.org/10.1016/j.jcmgh.2018.01.006

Finning K, Daniels G (2014) Molecular blood grouping. Transfus Apher Sci 50(2):146–147. https://doi.org/10.1016/j.transci.2014.02.010

Fisel P, Schaeffeler E, Schwab M (2018) Clinical and functional relevance of the monocarboxylate transporter family in disease pathophysiology and drug therapy. Clin Transl Sci 11(4):352–364. https://doi.org/10.1111/cts.12551

Fishbein WN (1986) Lactate transporter defect: a new disease of muscle. Science 234(4781):1254–1256

Fjorback AW, Müller HK, Haase J, Raarup MK, Wiborg O (2011) Modulation of the dopamine transporter by interaction with Secretory Carrier Membrane Protein 2. Biochem Biophys Res Commun 406(2):165–170. https://doi.org/10.1016/j.bbrc.2011.01.069

Flores-Muñoz M, Smith NJ, Haggerty C, Milligan G, Nicklin SA (2011) Angiotensin1-9 antagonises pro-hypertrophic signalling in cardiomyocytes via the angiotensin type 2 receptor. J Physiol 589 (Pt 4):939–951. https://doi.org/10.1113/jphysiol.2010.203075

Fogelstrand P, Feral CC, Zargham R, Ginsberg MH (2009) Dependence of proliferative vascular smooth muscle cells on CD98hc (4F2hc, SLC3A2). J Exp Med 206 (11):2397–2406. https://doi.org/10.1084/jem.20082845

Font MA, Feliubadalo L, Estivill X, Nunes V, Golomb E, Kreiss Y, Pras E, Bisceglia L, d'Adamo AP, Zelante L, Gasparini P, Bassi MT, George AL Jr, Manzoni M, Riboni M, Ballabio A, Borsani G, Reig N, Fernandez E, Zorzano A, Bertran J, Palacin M (2001) Functional analysis of mutations in SLC7A9, and genotype-phenotype correlation in non-Type I cystinuria. Hum Mol Genet 10(4):305–316

Font-Llitjos M, Jimenez-Vidal M, Bisceglia L, Di Perna M, de Sanctis L, Rousaud F, Zelante L, Palacin M, Nunes V (2005) New insights into cystinuria: 40 new mutations, genotype-phenotype correlation, and digenic inheritance causing partial phenotype. J Med Genet 42(1):58–68. https://doi.org/10.1136/jmg.2004.022244

Font-Llitjos M, Rodriguez-Santiago B, Espino M, Sillue R, Manas S, Gomez L, Perez-Jurado LA, Palacin M, Nunes V (2009) Novel SLC7A7 large rearrangements in lysinuric protein intolerance patients involving the same AluY repeat. Eur J Hum Genet 17 (1):71–79. https://doi.org/10.1038/ejhg.2008.145

Forrest LR (2013) Structural biology. (Pseudo-) symmetrical transport. Science 339(6118):399–401. https://doi.org/10.1126/science.1228465

Forrest LR, Rudnick G (2009) The rocking bundle: a mechanism for ion-coupled solute flux by symmetrical transporters. Physiology (Bethesda) 24:377–386. https://doi.org/10.1152/physiol.00030.2009

Forrest LR, Tavoulari S, Zhang YW, Rudnick G, Honig B (2007) Identification of a chloride ion binding site in Na+/Cl -dependent transporters. Proc Natl Acad Sci U S A 104(31):12761–12766. https://doi.org/10.1073/pnas.0705600104

Forrest LR, Kramer R, Ziegler C (2011) The structural basis of secondary active transport mechanisms.

Biochim Biophys Acta 1807(2):167–188. https://doi.org/10.1016/j.bbabio.2010.10.014

Fort J, de la Ballina LR, Burghardt HE, Ferrer-Costa C, Turnay J, Ferrer-Orta C, Uson I, Zorzano A, Fernandez-Recio J, Orozco M, Lizarbe MA, Fita I, Palacin M (2007) The structure of human 4F2hc ectodomain provides a model for homodimerization and electrostatic interaction with plasma membrane. J Biol Chem 282(43):31444–31452. https://doi.org/10.1074/jbc.M704524200

Fossum S, Mallett S, Barclay AN (1991) The MRC OX-47 antigen is a member of the immunoglobulin superfamily with an unusual transmembrane sequence. Eur J Immunol 21(3):671–679. https://doi.org/10.1002/eji.1830210320

Fotiadis D, Jeckelmann JM (2019) The structure of the human 4F2hc-LAT1 heteromeric amino acid transporter. Sci China Life Sci 62(6):848–850. https://doi.org/10.1007/s11427-019-9558-5

Fotiadis D, Kanai Y, Palacin M (2013) The SLC3 and SLC7 families of amino acid transporters. Mol Aspects Med 34(2–3):139–158. https://doi.org/10.1016/j.mam.2012.10.007

Fountaine TM, Wade-Martins R (2007) RNA interference-mediated knockdown of alpha-synuclein protects human dopaminergic neuroblastoma cells from MPP (+) toxicity and reduces dopamine transport. J Neurosci Res 85(2):351–363. https://doi.org/10.1002/jnr.21125

Fowler PW, Sansom MS, Reithmeier RA (2017) Effect of the Southeast Asian ovalocytosis deletion on the Conformational dynamics of signal-anchor transmembrane segment 1 of red cell anion exchanger 1 (AE1, Band 3, or SLC4A1). Biochemistry 56(5):712–722. https://doi.org/10.1021/acs.biochem.6b00966

Franca R, Veljkovic E, Walter S, Wagner CA, Verrey F (2005) Heterodimeric amino acid transporter glycoprotein domains determining functional subunit association. Biochem J 388(Pt 2):435–443. https://doi.org/10.1042/bj20050021

Friesema EC, Docter R, Moerings EP, Verrey F, Krenning EP, Hennemann G, Visser TJ (2001) Thyroid hormone transport by the heterodimeric human system L amino acid transporter. Endocrinology 142(10):4339–4348. https://doi.org/10.1210/endo.142.10.8418

Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ (2003) Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 278(41):40128–40135. https://doi.org/10.1074/jbc.M300909200

Friesema EC, Kuiper GG, Jansen J, Visser TJ, Kester MH (2006) Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism. Mol Endocrinol 20(11):2761–2772. https://doi.org/10.1210/me.2005-0256

Friesema EC, Visser WE, Visser TJ (2010) Genetics and phenomics of thyroid hormone transport by MCT8. Mol Cell Endocrinol 322(1–2):107–113. https://doi.org/10.1016/j.mce.2010.01.016

Fu J, Dumitrescu AM (2014) Inherited defects in thyroid hormone cell-membrane transport and metabolism. Best Pract Res Clin Endocrinol Metab 28(2):189–201. https://doi.org/10.1016/j.beem.2013.05.014

Fukasawa Y, Segawa H, Kim JY, Chairoungdua A, Kim DK, Matsuo H, Cha SH, Endou H, Kanai Y (2000) Identification and characterization of a Na(+)-independent neutral amino acid transporter that associates with the 4F2 heavy chain and exhibits substrate selectivity for small neutral D- and L-amino acids. J Biol Chem 275(13):9690–9698

Fukui K, Yang Q, Cao Y, Takahashi N, Hatakeyama H, Wang H, Wada J, Zhang Y, Marselli L, Nammo T, Yoneda K, Onishi M, Higashiyama S, Matsuzawa Y, Gonzalez FJ, Weir GC, Kasai H, Shimomura I, Miyagawa J, Wollheim CB, Yamagata K (2005) The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. Cell Metab 2(6):373–384. https://doi.org/10.1016/j.cmet.2005.11.003

Furriols M, Chillaron J, Mora C, Castello A, Bertran J, Camps M, Testar X, Vilaro S, Zorzano A, Palacin M (1993) rBAT, related to L-cysteine transport, is localized to the microvilli of proximal straight tubules, and its expression is regulated in kidney by development. J Biol Chem 268(36):27060–27068

Furthmayr H (1978) Glycophorins A, B, and C: a family of sialoglycoproteins. Isolation and preliminary characterization of trypsin derived peptides. J Supramol Struct 9(1):79–95. https://doi.org/10.1002/jss.400090109

Furthmayr H, Galardy RE, Tomita M, Marchesi VT (1978) The intramembranous segment of human erythrocyte glycophorin A. Arch Biochem Biophys 185(1):21–29

Furuya M, Horiguchi J, Nakajima H, Kanai Y, Oyama T (2012) Correlation of L-type amino acid transporter 1 and CD98 expression with triple negative breast cancer prognosis. Cancer Sci 103(2):382–389. https://doi.org/10.1111/j.1349-7006.2011.02151.x

Futagi Y, Kobayashi M, Narumi K, Furugen A, Iseki K (2018) Identification of a selective inhibitor of human monocarboxylate transporter 4. Biochem Biophys Res Commun 495(1):427–432. https://doi.org/10.1016/j.bbrc.2017.10.025

Gabrisko M, Janecek S (2009) Looking for the ancestry of the heavy-chain subunits of heteromeric amino acid transporters rBAT and 4F2hc within the GH13 alpha-amylase family. FEBS J 276(24):7265–7278. https://doi.org/10.1111/j.1742-4658.2009.07434.x

Gahmberg CG, Myllyla G, Leikola J, Pirkola A, Nordling S (1976) Absence of the major sialoglycoprotein in the membrane of human En(a--) erythrocytes and increased glycosylation of band 3. J Biol Chem 251(19):6108–6116

Gaildrat P, Lebbah S, Tebani A, Sudrie-Arnaud B, Tostivint I, Bollee G, Tubeuf H, Charles T, Bertholet-Thomas A, Goldenberg A, Barbey F, Martins A,

Saugier-Veber P, Frebourg T, Knebelmann B, Bekri S (2017) Clinical and molecular characterization of cystinuria in a French cohort: relevance of assessing large-scale rearrangements and splicing variants. Mol Genet Genomic Med 5(4):373–389. https://doi.org/10.1002/mgg3.294

Galic S, Schneider HP, Broer A, Deitmer JW, Broer S (2003) The loop between helix 4 and helix 5 in the monocarboxylate transporter MCT1 is important for substrate selection and protein stability. Biochem J 376(Pt 2):413–422. https://doi.org/10.1042/BJ20030799

Gallagher SM, Castorino JJ, Wang D, Philp NJ (2007) Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. Cancer Res 67(9):4182–4189. https://doi.org/10.1158/0008-5472.can-06-3184

Galli A, Blakely RD, DeFelice LJ (1996) Norepinephrine transporters have channel modes of conduction. Proc Natl Acad Sci U S A 93(16):8671–8676. https://doi.org/10.1073/pnas.93.16.8671

Ganapathy V (2009) A traffic signal for heterodimeric amino acid transporters to transfer from the ER to the Golgi. Biochem J 417(2):e9–11. https://doi.org/10.1042/bj20082246

Gao X, Lu F, Zhou L, Dang S, Sun L, Li X, Wang J, Shi Y (2009) Structure and mechanism of an amino acid antiporter. Science 324(5934):1565–1568. https://doi.org/10.1126/science.1173654

Gao X, Zhou L, Jiao X, Lu F, Yan C, Zeng X, Wang J, Shi Y (2010) Mechanism of substrate recognition and transport by an amino acid antiporter. Nature 463(7282):828–832. https://doi.org/10.1038/nature08741

Garcia AM, Lodish HF (1989) Lysine 539 of human band 3 is not essential for ion transport or inhibition by stilbene disulfonates. J Biol Chem 264(33):19607–19613

Garcia CK, Goldstein JL, Pathak RK, Anderson RG, Brown MS (1994) Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. Cell 76(5):865–873

Garcia CK, Brown MS, Pathak RK, Goldstein JL (1995) cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. J Biol Chem 270(4):1843–1849

Garcia-Olivares J, Torres-Salazar D, Owens WA, Baust T, Siderovski DP, Amara SG, Zhu J, Daws LC, Torres GE (2013) Inhibition of dopamine transporter activity by G protein βγ subunits. PLoS One 8(3):e59788–e59788. https://doi.org/10.1371/journal.pone.0059788

Garcia-Olivares J, Baust T, Harris S, Hamilton P, Galli A, Amara SG, Torres GE (2017) Gβγ subunit activation promotes dopamine efflux through the dopamine transporter. Mol Psychiatry 22(12):1673–1679. https://doi.org/10.1038/mp.2017.176

Garibsingh R-AA, Schlessinger A (2019) Advances and challenges in rational drug design for SLCs. Trends Pharmacol Sci 40(10):790–800. https://doi.org/10.1016/j.tips.2019.08.006

Gasparini P, Calonge MJ, Bisceglia L, Purroy J, Dianzani I, Notarangelo A, Rousaud F, Gallucci M, Testar X, Ponzone A et al (1995) Molecular genetics of cystinuria: identification of four new mutations and seven polymorphisms, and evidence for genetic heterogeneity. Am J Hum Genet 57(4):781–788

Geerlings A, Lopez-Corcuera B, Aragon C (2000) Characterization of the interactions between the glycine transporters GLYT1 and GLYT2 and the SNARE protein syntaxin 1A. FEBS Lett 470(1):51–54

Geerlings A, Nunez E, Lopez-Corcuera B, Aragon C (2001) Calcium- and syntaxin 1-mediated trafficking of the neuronal glycine transporter GLYT2. J Biol Chem 276(20):17584–17590. https://doi.org/10.1074/jbc.M010602200

Geertsma ER, Chang YN, Shaik FR, Neldner Y, Pardon E, Steyaert J, Dutzler R (2015) Structure of a prokaryotic fumarate transporter reveals the architecture of the SLC26 family. Nat Struct Mol Biol 22(10):803–808. https://doi.org/10.1038/nsmb.3091

Geertsma ER, Chang YN, Shaik FR, Neldner Y, Pardon E, Steyaert J, Dutzler R (2016) Corrigendum: structure of a prokaryotic fumarate transporter reveals the architecture of the SLC26 family. Nat Struct Mol Biol 23(5):462. https://doi.org/10.1038/nsmb0516-462

Gerhart DZ, Leino RL, Drewes LR (1999) Distribution of monocarboxylate transporters MCT1 and MCT2 in rat retina. Neuroscience 92(1):367–375

Gether U, Andersen PH, Larsson OM, Schousboe A (2006) Neurotransmitter transporters: molecular function of important drug targets. Trends Pharmacol Sci 27(7):375–383. https://doi.org/10.1016/j.tips.2006.05.003

Giger K, Habib I, Ritchie K, Low PS (2016) Diffusion of glycophorin A in human erythrocytes. Biochim Biophys Acta 1858(11):2839–2845. https://doi.org/10.1016/j.bbamem.2016.08.012

Gilbert ER, Williams PM, Ray WK, Li H, Emmerson DA, Wong EA, Webb KE Jr (2010) Proteomic evaluation of chicken brush-border membrane during the early posthatch period. J Proteome Res 9(9):4628–4639. https://doi.org/10.1021/pr1003533

Gilbert A, Liu J, Cheng G, An C, Deo K, Gorret AM, Qin X (2019) A review of urinary angiotensin converting enzyme 2 in diabetes and diabetic nephropathy. Biochem Med (Zagreb) 29(1):010501–010501. https://doi.org/10.11613/BM.2019.010501

Gitomer WL, Reed BY, Pak CY (2000) Identification of two novel mutations [P122S (364C>T) and 1601delAC] in the SLC3A1 gene in type I cystinurics. Hum Mutat 15(4):390. https://doi.org/10.1002/(sici)1098-1004(200004)15:4<390::Aid-humu33>3.0.Co;2-k

Gorini G, Ponomareva O, Shores KS, Person MD, Harris RA, Mayfield RD (2010) Dynamin-1 co-associates with native mouse brain BKCa channels: proteomics analysis of synaptic protein complexes. FEBS Lett 584

(5):845–851. https://doi.org/10.1016/j.febslet.2009.12.061

Gorini G, Harris RA, Mayfield RD (2014) Proteomic approaches and identification of novel therapeutic targets for alcoholism. Neuropsychopharmacology 39 (1):104–130. https://doi.org/10.1038/npp.2013.182

Gottesdiener KM, Karpinski BA, Lindsten T, Strominger JL, Jones NH, Thompson CB, Leiden JM (1988) Isolation and structural characterization of the human 4F2 heavy-chain gene, an inducible gene involved in T-lymphocyte activation. Mol Cell Biol 8 (9):3809–3819

Gout PW, Buckley AR, Simms CR, Bruchovsky N (2001) Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x(c)- cystine transporter: a new action for an old drug. Leukemia 15(10):1633–1640. https://doi.org/10.1038/sj.leu.2402238

Grabner A, Brast S, Sucic S, Bierer S, Hirsch B, Pavenstädt H, Sitte HH, Schlatter E, Ciarimboli G (2011) LAPTM4A interacts with hOCT2 and regulates its endocytotic recruitment. Cell Mol Life Sci 68 (24):4079–4090. https://doi.org/10.1007/s00018-011-0694-6

Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell 119(7):1013–1026. https://doi.org/10.1016/j.cell.2004.11.035

Grass GD, Dai L, Qin Z, Parsons C, Toole BP (2014) CD147: regulator of hyaluronan signaling in invasiveness and chemoresistance. Adv Cancer Res 123:351–373. https://doi.org/10.1016/b978-0-12-800092-2.00013-7

Gray AL, Coleman DT, Shi R, Cardelli JA (2016) Monocarboxylate transporter 1 contributes to growth factor-induced tumor cell migration independent of transporter activity. Oncotarget 7(22):32695–32706. https://doi.org/10.18632/oncotarget.9016

Groeneweg S, Visser WE, Visser TJ (2017) Disorder of thyroid hormone transport into the tissues. Best Pract Res Clin Endocrinol Metab 31(2):241–253. https://doi.org/10.1016/j.beem.2017.05.001

Grollman EF, Philp NJ, McPhie P, Ward RD, Sauer B (2000) Determination of transport kinetics of chick MCT3 monocarboxylate transporter from retinal pigment epithelium by expression in genetically modified yeast. Biochemistry 39(31):9351–9357

Groves JD, Tanner MJ (1992) Glycophorin A facilitates the expression of human band 3-mediated anion transport in Xenopus oocytes. J Biol Chem 267 (31):22163–22170

Groves JD, Tanner MJ (1994a) The effects of glycophorin A on the expression of the human red cell anion transporter (band 3) in Xenopus oocytes. J Membr Biol 140 (1):81–88

Groves JD, Tanner MJ (1994b) Role of N-glycosylation in the expression of human band 3-mediated anion transport. Mol Membr Biol 11(1):31–38

Groves JD, Ring SM, Schofield AE, Tanner MJ (1993) The expression of the abnormal human red cell anion transporter from South-East Asian ovalocytes (band 3 SAO) in Xenopus oocytes. FEBS Lett 330 (2):186–190

Gu X, Neric NJ, Crabb JS, Crabb JW, Bhattacharya SK, Rayborn ME, Hollyfield JG, Bonilha VL (2012) Age-related changes in the retinal pigment epithelium (RPE). PLoS One 7(6):e38673. https://doi.org/10.1371/journal.pone.0038673

Guastella J, Nelson N, Nelson H, Czyzyk L, Keynan S, Miedel MC, Davidson N, Lester HA, Kanner BI (1990) Cloning and expression of a rat brain GABA transporter. Science 249(4974):1303–1306

Guastella J, Brecha N, Weigmann C, Lester HA, Davidson N (1992) Cloning, expression, and localization of a rat brain high-affinity glycine transporter. Proc Natl Acad Sci U S A 89(15):7189–7193

Guenette RS, Sridhar S, Herley M, Mooibroek M, Wong P, Tenniswood M (1997) Embigin, a developmentally expressed member of the immunoglobulin super family, is also expressed during regression of prostate and mammary gland. Dev Genet 21 (4):268–278. https://doi.org/10.1002/(sici)1520-6408(1997)21:4<268::aid-dvg4>3.0.co;2-5

Guo W, Zhao Y, Zhang Z, Tan N, Zhao F, Ge C, Liang L, Jia D, Chen T, Yao M, Li J, He X (2011) Disruption of xCT inhibits cell growth via the ROS/autophagy pathway in hepatocellular carcinoma. Cancer Lett 312 (1):55–61. https://doi.org/10.1016/j.canlet.2011.07.024

Guo C, LaCerte C, Edwards JE, Brouwer KR, Brouwer KLR (2018) Farnesoid X receptor agonists obeticholic acid and chenodeoxycholic acid increase bile acid efflux in sandwich-cultured human hepatocytes: functional evidence and mechanisms. J Pharmacol Exp Ther 365(2):413–421. https://doi.org/10.1124/jpet.117.246033

Gupta GD, Coyaud E, Goncalves J, Mojarad BA, Liu Y, Wu Q, Gheiratmand L, Comartin D, Tkach JM, Cheung SW, Bashkurov M, Hasegan M, Knight JD, Lin ZY, Schueler M, Hildebrandt F, Moffat J, Gingras AC, Raught B, Pelletier L (2015) A dynamic protein interaction landscape of the human centrosome-cilium interface. Cell 163(6):1484–1499. https://doi.org/10.1016/j.cell.2015.10.065

Guptaroy B, Zhang M, Bowton E, Binda F, Shi L, Weinstein H, Galli A, Javitch JA, Neubig RR, Gnegy ME (2009) A juxtamembrane mutation in the N terminus of the dopamine transporter induces preference for an inward-facing conformation. Mol Pharmacol 75 (3):514–524. https://doi.org/10.1124/mol.108.048744

Gur M, Zomot E, Cheng MH, Bahar I (2015) Energy landscape of LeuT from molecular simulations. J Chem Phys 143(24):243134. https://doi.org/10.1063/1.4936133

Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ (2003) Angiotensin-converting

enzyme-2 (ACE2): comparative modeling of the active site, specificity requirements, and chloride dependence. Biochemistry 42(45):13185–13192. https://doi.org/10.1021/bi035268s

Guy JL, Jackson RM, Jensen HA, Hooper NM, Turner AJ (2005) Identification of critical active-site residues in angiotensin-converting enzyme-2 (ACE2) by site-directed mutagenesis. FEBS J 272(14):3512–3520. https://doi.org/10.1111/j.1742-4658.2005.04756.x

Gynther M, Laine K, Ropponen J, Leppanen J, Mannila A, Nevalainen T, Savolainen J, Jarvinen T, Rautio J (2008) Large neutral amino acid transporter enables brain drug delivery via prodrugs. J Med Chem 51 (4):932–936. https://doi.org/10.1021/jm701175d

Gynther M, Jalkanen A, Lehtonen M, Forsberg M, Laine K, Ropponen J, Leppanen J, Knuuti J, Rautio J (2010) Brain uptake of ketoprofen-lysine prodrug in rats. Int J Pharm 399(1–2):121–128. https://doi.org/10.1016/j.ijpharm.2010.08.019

Haase J, Killian AM, Magnani F, Williams C (2001) Regulation of the serotonin transporter by interacting proteins. Biochem Soc Trans 29(Pt 6):722–728

Haase J, Grudzinska-Goebel J, Müller HK, Münster-Wandowski A, Chow E, Wynne K, Farsi Z, Zander J-F, Ahnert-Hilger G (2017) Serotonin transporter associated protein complexes are enriched in synaptic vesicle proteins and proteins involved in energy metabolism and ion homeostasis. ACS Chem Neurosci 8 (5):1101–1116. https://doi.org/10.1021/acschemneuro.6b00437

Hadlock GC, Nelson CC, Baucum AJ 2nd, Hanson GR, Fleckenstein AE (2011) Ex vivo identification of protein-protein interactions involving the dopamine transporter. J Neurosci Methods 196(2):303–307. https://doi.org/10.1016/j.jneumeth.2011.01.023

Hafliger P, Graff J, Rubin M, Stooss A, Dettmer MS, Altmann KH, Gertsch J, Charles RP (2018) The LAT1 inhibitor JPH203 reduces growth of thyroid carcinoma in a fully immunocompetent mouse model. J Exp Clin Cancer Res 37(1):234. https://doi.org/10.1186/s13046-018-0907-z

Hahn MK, Blakely RD (2007) The functional impact of SLC6 transporter genetic variation. Annu Rev Pharmacol Toxicol 47:401–441. https://doi.org/10.1146/annurev.pharmtox.47.120505.105242

Haining Z, Kawai N, Miyake K, Okada M, Okubo S, Zhang X, Fei Z, Tamiya T (2012) Relation of LAT1/4F2hc expression with pathological grade, proliferation and angiogenesis in human gliomas. BMC Clin Pathol 12:4. https://doi.org/10.1186/1472-6890-12-4

Halestrap AP (2012) The monocarboxylate transporter family – structure and functional characterization. IUBMB Life 64(1):1–9. https://doi.org/10.1002/iub.573

Halestrap AP (2013a) Monocarboxylic acid transport. Compr Physiol 3(4):1611–1643. https://doi.org/10.1002/cphy.c130008

Halestrap AP (2013b) The SLC16 gene family – structure, role and regulation in health and disease. Mol Aspects Med 34(2–3):337–349. https://doi.org/10.1016/j.mam.2012.05.003

Halestrap AP, Wilson MC (2012) The monocarboxylate transporter family – role and regulation. IUBMB Life 64(2):109–119. https://doi.org/10.1002/iub.572

Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203(2):631–637. https://doi.org/10.1002/path.1570

Han J, Pluhackova K, Bockmann RA (2017) The multifaceted role of SNARE proteins in membrane fusion. Front Physiol 8:5. https://doi.org/10.3389/fphys.2017.00005

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hansra N, Arya S, Quick MW (2004) Intracellular domains of a rat brain GABA transporter that govern transport. J Neurosci 24(16):4082–4087. https://doi.org/10.1523/jneurosci.0664-04.2004

Haraguchi N, Inoue H, Tanaka F, Mimori K, Utsunomiya T, Sasaki A, Mori M (2006) Cancer stem cells in human gastrointestinal cancers. Hum Cell 19 (1):24–29. https://doi.org/10.1111/j.1749-0774.2005.00004.x

Harnevik L, Fjellstedt E, Molbaek A, Tiselius HG, Denneberg T, Soderkvist P (2001) Identification of 12 novel mutations in the SLC3A1 gene in Swedish cystinuria patients. Hum Mutat 18(6):516–525. https://doi.org/10.1002/humu.1228

Harnevik L, Fjellstedt E, Molbaek A, Denneberg T, Soderkvist P (2003) Mutation analysis of SLC7A9 in cystinuria patients in Sweden. Genet Test 7(1):13–20. https://doi.org/10.1089/109065703321560886

Harvey RJ, Yee BK (2013) Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. Nat Rev Drug Discov 12 (11):866–885. https://doi.org/10.1038/nrd3893

Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 487(7408):477–481. https://doi.org/10.1038/nature11228

Hassoun H, Hanada T, Lutchman M, Sahr KE, Palek J, Hanspal M, Chishti AH (1998) Complete deficiency of glycophorin A in red blood cells from mice with targeted inactivation of the band 3 (AE1) gene. Blood 91(6):2146–2151

Hauck W, Adam P, Bobier C, Landmesser N (2008) Use of large-scale chromatography in the preparation of armodafinil. Chirality 20(8):896–899. https://doi.org/10.1002/chir.20564

Hayes GM, Chinn L, Cantor JM, Cairns B, Levashova Z, Tran H, Velilla T, Duey D, Lippincott J, Zachwieja J,

Ginsberg MH, HvdH E (2015) Antitumor activity of an anti-CD98 antibody. Int J Cancer 137(3):710–720. https://doi.org/10.1002/ijc.29415

He F, Wu C, Li P, Li N, Zhang D, Zhu Q, Ren W, Peng Y (2018) Functions and signaling pathways of amino acids in intestinal inflammation. Biomed Res Int 2018:9171905–9171905. https://doi.org/10.1155/2018/9171905

Hediger MA, Clemencon B, Burrier RE, Bruford EA (2013a) The ABCs of membrane transporters in health and disease (SLC series). Mol Aspects Med 34 (2–3):95–752

Hediger MA, Clemencon B, Burrier RE, Bruford EA (2013b) The ABCs of membrane transporters in health and disease (SLC series): introduction. Mol Aspects Med 34(2–3):95–107. https://doi.org/10.1016/j.mam.2012.12.009

Helboe L, Egebjerg J, Moller M, Thomsen C (2003) Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain. Eur J Neurosci 18 (8):2227–2238

Hemler ME, Strominger JL (1982) Characterization of antigen recognized by the monoclonal antibody (4F2): different molecular forms on human T and B lymphoblastoid cell lines. J Immunol 129(2):623–628

Henderson NC, Collis EA, Mackinnon AC, Simpson KJ, Haslett C, Zent R, Ginsberg M, Sethi T (2004) CD98hc (SLC3A2) interaction with beta 1 integrins is required for transformation. J Biol Chem 279(52):54731–54741. https://doi.org/10.1074/jbc.M408700200

Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ (2001) Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev 22(4):451–476. https://doi.org/10.1210/edrv.22.4.0435

Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S (2014) TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. J Virol 88(2):1293–1307. https://doi.org/10.1128/jvi.02202-13

Hill IE, Selkirk CP, Hawkes RB, Beesley PW (1988) Characterization of novel glycoprotein components of synaptic membranes and postsynaptic densities, gp65 and gp55, with a monoclonal antibody. Brain Res 461 (1):27–43

Hiller-Sturmhöfel S, Sobin J, Mayfield RD (2008) Proteomic approaches for studying alcoholism and alcohol-induced organ damage. Alcohol Res Health 31(1):36–48

Hinz KM, Neef D, Rutz C, Furkert J, Kohrle J, Schulein R, Krause G (2017) Molecular features of the L-type amino acid transporter 2 determine different import and export profiles for thyroid hormones and amino acids. Mol Cell Endocrinol 443:163–174. https://doi.org/10.1016/j.mce.2017.01.024

Hirai T, Yamaguchi T (2015) Structure of human erythrocyte band 3: two-dimensional crystallographic analysis of the membrane domain. Rinsho Ketsueki 56 (7):831–836. https://doi.org/10.11406/rinketsu.56.831

Hirayasu Y, Sato S, Takahashi H, Iida S, Shuto N, Yoshida S, Funatogawa T, Yamada T, Higuchi T (2016) A double-blind randomized study assessing safety and efficacy following one-year adjunctive treatment with bitopertin, a glycine reuptake inhibitor, in Japanese patients with schizophrenia. BMC Psychiatry 16:66. https://doi.org/10.1186/s12888-016-0778-9

Hoffman BJ, Mezey E, Brownstein MJ (1991) Cloning of a serotonin transporter affected by antidepressants. Science 254(5031):579–580

Hog S, Greenwood JR, Madsen KB, Larsson OM, Frolund B, Schousboe A, Krogsgaard-Larsen P, Clausen RP (2006) Structure-activity relationships of selective GABA uptake inhibitors. Curr Top Med Chem 6(17):1861–1882

Hoglund PJ, Nordstrom KJ, Schioth HB, Fredriksson R (2011) The solute carrier families have a remarkably long evolutionary history with the majority of the human families present before divergence of Bilaterian species. Mol Biol Evol 28(4):1531–1541. https://doi.org/10.1093/molbev/msq350

Holman CA (1953) A new rare human bloodgroup antigen (Wra). Lancet 265(6777):119–120

Hong WC, Amara SG (2010) Membrane cholesterol modulates the outward facing conformation of the dopamine transporter and alters cocaine binding. J Biol Chem 285(42):32616–32626. https://doi.org/10.1074/jbc.M110.150565

Honjo H, Kaira K, Miyazaki T, Yokobori T, Kanai Y, Nagamori S, Oyama T, Asao T, Kuwano H (2016) Clinicopathological significance of LAT1 and ASCT2 in patients with surgically resected esophageal squamous cell carcinoma. J Surg Oncol 113(4):381–389. https://doi.org/10.1002/jso.24160

Hooper NM (1994) Families of zinc metalloproteases. FEBS Lett 354:1):1–1):6. https://doi.org/10.1016/0014-5793(94)01079-x

Hori K, Katayama N, Kachi S, Kondo M, Kadomatsu K, Usukura J, Muramatsu T, Mori S, Miyake Y (2000) Retinal dysfunction in basigin deficiency. Invest Ophthalmol Vis Sci 41(10):3128–3133

Horton N, Quick MW (2001) Syntaxin 1A up-regulates GABA transporter expression by subcellular redistribution. Mol Membr Biol 18(1):39–44

Huang RP, Ozawa M, Kadomatsu K, Muramatsu T (1990) Developmentally regulated expression of embigin, a member of the immunoglobulin superfamily found in embryonal carcinoma cells. Differentiation 45 (2):76–83

Huang RP, Ozawa M, Kadomatsu K, Muramatsu T (1993) Embigin, a member of the immunoglobulin superfamily expressed in embryonic cells, enhances cell-substratum adhesion. Dev Biol 155(2):307–314. https://doi.org/10.1006/dbio.1993.1030

Huang CH, Reid ME, Xie SS, Blumenfeld OO (1996) Human red blood cell Wright antigens: a genetic and evolutionary perspective on glycophorin A-band 3 interaction. Blood 87(9):3942–3947

Hugo SE, Cruz-Garcia L, Karanth S, Anderson RM, Stainier DY, Schlegel A (2012) A monocarboxylate transporter required for hepatocyte secretion of ketone bodies during fasting. Genes Dev 26(3):282–293. https://doi.org/10.1101/gad.180968.111

Huttlin EL, Jedrychowski MP, Elias JE, Goswami T, Rad R, Beausoleil SA, Villen J, Haas W, Sowa ME, Gygi SP (2010) A tissue-specific atlas of mouse protein phosphorylation and expression. Cell 143 (7):1174–1189. https://doi.org/10.1016/j.cell.2010.12.001

Huynh KW, Jiang J, Abuladze N, Tsirulnikov K, Kao L, Shao X, Newman D, Azimov R, Pushkin A, Zhou ZH, Kurtz I (2018) CryoEM structure of the human SLC4A4 sodium-coupled acid-base transporter NBCe1. Nat Commun 9(1):900. https://doi.org/10.1038/s41467-018-03271-3

Hwang JH, Parton A, Czechanski A, Ballatori N, Barnes D (2008) Arachidonic acid-induced expression of the organic solute and steroid transporter-beta (Ost-beta) in a cartilaginous fish cell line. Comp Biochem Physiol C Toxicol Pharmacol 148(1):39–47. https://doi.org/10.1016/j.cbpc.2008.03.005

Iacono KT, Brown AL, Greene MI, Saouaf SJ (2007) CD147 immunoglobulin superfamily receptor function and role in pathology. Exp Mol Pathol 83(3):283–295. https://doi.org/10.1016/j.yexmp.2007.08.014

Ideno M, Kobayashi M, Sasaki S, Futagi Y, Narumi K, Furugen A, Iseki K (2018) Involvement of monocarboxylate transporter 1 (SLC16A1) in the uptake of l-lactate in human astrocytes. Life Sci 192:110–114. https://doi.org/10.1016/j.lfs.2017.10.022

Ikotun OF, Marquez BV, Huang C, Masuko K, Daiji M, Masuko T, McConathy J, Lapi SE (2013) Imaging the L-type amino acid transporter-1 (LAT1) with Zr-89 immunoPET. PLoS One 8(10):e77476. https://doi.org/10.1371/journal.pone.0077476

Ilgu H, Jeckelmann JM, Gapsys V, Ucurum Z, de Groot BL, Fotiadis D (2016) Insights into the molecular basis for substrate binding and specificity of the wild-type L-arginine/agmatine antiporter AdiC. Proc Natl Acad Sci U S A 113(37):10358–10363. https://doi.org/10.1073/pnas.1605442113

Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436(7047):112–116. https://doi.org/10.1038/nature03712

Imai Y, Kuba K, Penninger JM (2008a) The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Exp Physiol 93(5):543–548

Imai Y, Kuba K, Penninger JM (2008b) Lessons from SARS: a new potential therapy for acute respiratory distress syndrome (ARDS) with angiotensin converting enzyme 2 (ACE2). Masui 57(3):302–310

Imai H, Kaira K, Oriuchi N, Yanagitani N, Sunaga N, Ishizuka T, Kanai Y, Endou H, Nakajima T, Mori M (2009) L-type amino acid transporter 1 expression is a prognostic marker in patients with surgically resected stage I non-small cell lung cancer. Histopathology 54 (7):804–813. https://doi.org/10.1111/j.1365-2559.2009.03300.x

Imai Y, Kuba K, Ohto-Nakanishi T, Penninger JM (2010) Angiotensin-converting enzyme 2 (ACE2) in disease pathogenesis. Circ J 74(3):405–410

Impens F, Radoshevich L, Cossart P, Ribet D (2014) Mapping of SUMO sites and analysis of SUMOylation changes induced by external stimuli. Proc Natl Acad Sci U S A 111(34):12432–12437. https://doi.org/10.1073/pnas.1413825111

Inoue T, Kanzaki A, Kaku M, Yawata A, Takezono M, Okamoto N, Wada H, Sugihara T, Yamada O, Katayama Y, Nagata N, Yawata Y (1998) Homozygous missense mutation (band 3 Fukuoka: G130R): a mild form of hereditary spherocytosis with near-normal band 3 content and minimal changes of membrane ultrastructure despite moderate protein 4.2 deficiency. Br J Haematol 102(4):932–939

Issitt PD, Pavone BG, Goldfinger D, Zwicker H (1975) An En(a-) red cell sample that types as Wr(a-b-). Transfusion 15(4):353–355

Issitt PD, Pavone BG, Wagstaff W, Goldfinger D (1976) The phenotypes En(a-), Wr(a-b-), and En(a+), Wr(a+b-), and further studies on the Wright and En blood group systems. Transfusion 16(5):396–407

Itoyama S, Keicho N, Hijikata M, Quy T, Phi NC, Long HT, Ha LD, Ban VV, Matsushita I, Yanai H, Kirikae F, Kirikae T, Kuratsuji T, Sasazuki T (2005) Identification of an alternative 5′-untranslated exon and new polymorphisms of angiotensin-converting enzyme 2 gene: lack of association with SARS in the Vietnamese population. Am J Med Genet A 136(1):52–57. https://doi.org/10.1002/ajmg.a.30779

Iversen L (2006) Neurotransmitter transporters and their impact on the development of psychopharmacology. Br J Pharmacol 147(Suppl 1):S82–S88. https://doi.org/10.1038/sj.bjp.0706428

Iwanaga T, Kishimoto A (2015) Cellular distributions of monocarboxylate transporters: a review. Biomed Res 36(5):279–301. https://doi.org/10.2220/biomedres.36.279

Jajosky RP, Jajosky AN, Jajosky PG (2017) Can exchange transfusions using red blood cells from donors with Southeast Asian ovalocytosis prevent or ameliorate cerebral malaria in patients with multi-drug resistant Plasmodium falciparum? Transfus Apher Sci 56 (6):865–866. https://doi.org/10.1016/j.transci.2017.10.003

Jamali S, Klier M, Ames S, Barros LF, McKenna R, Deitmer JW, Becker HM (2015) Hypoxia-induced carbonic anhydrase IX facilitates lactate flux in human breast cancer cells by non-catalytic function. Sci Rep 5:13605. https://doi.org/10.1038/srep13605

Janecek S (1997) alpha-Amylase family: molecular biology and evolution. Prog Biophys Mol Biol 67 (1):67–97

Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP (1994) Amelioration of negative symptoms in schizophrenia by glycine. Am J Psychiatry 151(8):1234–1236. https://doi.org/10.1176/ajp.151.8.1234

Jayaraman K, Morley AN, Szöllősi D, Wassenaar TA, Sitte HH, Stockner T (2018) Dopamine transporter oligomerization involves the scaffold domain, but spares the bundle domain. PLoS Comput Biol 14(6):e1006229. https://doi.org/10.1371/journal.pcbi.1006229

Jeannotte AM, Sidhu A (2007) Regulation of the norepinephrine transporter by alpha-synuclein-mediated interactions with microtubules. Eur J Neurosci 26 (6):1509–1520. https://doi.org/10.1111/j.1460-9568.2007.05757.x

Jeckelmann JM, Fotiadis D (2019) Volta phase plate cryo-EM structure of the human heterodimeric amino acid transporter 4F2hc-LAT2. Int J Mol Sci 20(4). https://doi.org/10.3390/ijms20040931

Jedlitschky G, Greinacher A, Kroemer HK (2012) Transporters in human platelets: physiologic function and impact for pharmacotherapy. Blood 119 (15):3394–3402. https://doi.org/10.1182/blood-2011-09-336933

Jeffers SA, Tusell SM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, Thomas WD Jr, Thackray LB, Young MD, Mason RJ, Ambrosino DM, Wentworth DE, Demartini JC, Holmes KV (2004) CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A 101(44):15748–15753. https://doi.org/10.1073/pnas.0403812101

Jennings ML (1984) Oligomeric structure and the anion transport function of human erythrocyte band 3 protein. J Membr Biol 80(2):105–117

Jensen H, Potempa M, Gotthardt D, Lanier LL (2017) Cutting edge: IL-2-induced expression of the amino acid transporters SLC1A5 and CD98 is a prerequisite for NKG2D-mediated activation of human NK cells. J Immunol 199(6):1967–1972. https://doi.org/10.4049/jimmunol.1700497

Jeong HJ, Vandenberg RJ, Vaughan CW (2010) N-arachidonyl-glycine modulates synaptic transmission in superficial dorsal horn. Br J Pharmacol 161 (4):925–935. https://doi.org/10.1111/j.1476-5381.2010.00935.x

Ji TH (1974) Cross-linking of glycolipids in erythrocyte ghost membrane. J Biol Chem 249(24):7841–7847

Jiang JL, Zhou Q, Yu MK, Ho LS, Chen ZN, Chan HC (2001) The involvement of HAb18G/CD147 in regulation of store-operated calcium entry and metastasis of human hepatoma cells. J Biol Chem 276 (50):46870–46877. https://doi.org/10.1074/jbc.M108291200

Jiang F, Yang J, Zhang Y, Dong M, Wang S, Zhang Q, Liu FF, Zhang K, Zhang C (2014) Angiotensin-converting enzyme 2 and angiotensin 1-7: novel therapeutic targets. Nat Rev Cardiol 11(7):413–426. https://doi.org/10.1038/nrcardio.2014.59

Jiang Y, Rose AJ, Sijmonsma TP, Broer A, Pfenninger A, Herzig S, Schmoll D, Broer S (2015) Mice lacking neutral amino acid transporter B(0)AT1 (Slc6a19) have elevated levels of FGF21 and GLP-1 and improved glycaemic control. Mol Metab 4 (5):406–417. https://doi.org/10.1016/j.molmet.2015.02.003

Jiang Y, Cao Y, Wang Y, Li W, Liu X, Lv Y, Li X, Mi J (2017) Cysteine transporter SLC3A1 promotes breast cancer tumorigenesis. Theranostics 7(4):1036–1046. https://doi.org/10.7150/thno.18005

Jokinen M, Gahmberg CG, Andersson LC (1979) Biosynthesis of the major human red cell sialoglycoprotein, glycophorin A, in a continuous cell line. Nature 279 (5714):604–607

Jokinen M, Ulmanen I, Andersson LC, Kaariainen L, Gahmberg CG (1981) Cell-free synthesis and glycosylation of the major human-red-cell sialoglycoprotein, glycophorin A. Eur J Biochem 114(2):393–397

Jones RS, Parker MD, Morris ME (2017) Quercetin, morin, luteolin, and phloretin are dietary flavonoid inhibitors of monocarboxylate transporter 6. Mol Pharm 14(9):2930–2936. https://doi.org/10.1021/acs.molpharmaceut.7b00264

Joseph D, Pidathala S, Mallela AK, Penmatsa A (2019) Structure and gating dynamics of Na(+)/Cl(-) coupled neurotransmitter transporters. Front Mol Biosci 6:80–80. https://doi.org/10.3389/fmolb.2019.00080

Jungnickel KEJ, Parker JL, Newstead S (2018) Structural basis for amino acid transport by the CAT family of SLC7 transporters. Nat Commun 9(1):550. https://doi.org/10.1038/s41467-018-03066-6

Kager L, Bruce LJ, Zeitlhofer P, Flatt JF, Maia TM, Ribeiro ML, Fahrner B, Fritsch G, Boztug K, Haas OA (2017) Band 3 null(VIENNA), a novel homozygous SLC4A1 p.Ser477X variant causing severe hemolytic anemia, dyserythropoiesis and complete distal renal tubular acidosis. Pediatr Blood Cancer 64(3). https://doi.org/10.1002/pbc.26227

Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Kawashima O, Kamide Y, Ishizuka T, Kanai Y, Nakajima T, Mori M (2009) CD98 expression is associated with poor prognosis in resected non-small-cell lung cancer with lymph node metastases. Ann Surg Oncol 16(12):3473–3481. https://doi.org/10.1245/s10434-009-0685-0

Kaira K, Kawashima O, Endoh H, Imaizumi K, Goto Y, Kamiyoshihara M, Sugano M, Yamamoto R, Osaki T, Tanaka S, Fujita A, Imai H, Kogure Y, Seki Y, Shimizu K, Mogi A, Shitara Y, Oyama T, Kanai Y, Asao T (2018) Expression of amino acid transporter (LAT1 and 4F2hc) in pulmonary pleomorphic carcinoma. Hum Pathol. https://doi.org/10.1016/j.humpath.2018.09.020

Kalea AZ, Batlle D (2010) Apelin and ACE2 in cardiovascular disease. Curr Opin Investig Drugs 11 (3):273–282

Kaleeba JA, Berger EA (2006) Kaposi's sarcoma-associated herpesvirus fusion-entry receptor: cystine

transporter xCT. Science 311(5769):1921–1924. https://doi.org/10.1126/science.1120878

Kalli AC, Reithmeier RAF (2018) Interaction of the human erythrocyte Band 3 anion exchanger 1 (AE1, SLC4A1) with lipids and glycophorin A: molecular organization of the Wright (Wr) blood group antigen. PLoS Comput Biol 14(7):e1006284. https://doi.org/10.1371/journal.pcbi.1006284

Kampen KR (2011) Membrane proteins: the key players of a cancer cell. J Membr Biol 242(2):69–74. https://doi.org/10.1007/s00232-011-9381-7

Kanai Y, Endou H (2001) Heterodimeric amino acid transporters: molecular biology and pathological and pharmacological relevance. Curr Drug Metab 2 (4):339–354. https://doi.org/10.2174/1389200013338324

Kanai Y, Stelzner MG, Lee WS, Wells RG, Brown D, Hediger MA (1992) Expression of mRNA (D2) encoding a protein involved in amino acid transport in S3 proximal tubule. Am J Physiol 263(6 Pt 2): F1087–F1092. https://doi.org/10.1152/ajprenal.1992.263.6.F1087

Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J Biol Chem 273(37):23629–23632

Kanai Y, Fukasawa Y, Cha SH, Segawa H, Chairoungdua A, Kim DK, Matsuo H, Kim JY, Miyamoto K, Takeda E, Endou H (2000a) Transport properties of a system y+L neutral and basic amino acid transporter. Insights into the mechanisms of substrate recognition. J Biol Chem 275(27):20787–20793. https://doi.org/10.1074/jbc.M000634200

Kanai Y, Segawa H, Chairoungdua A, Kim JY, Kim DK, Matsuo H, Cha SH, Endou H (2000b) Amino acid transporters: molecular structure and physiological roles. Nephrol Dial Transplant 15(Suppl 6):9–10

Kandasamy P, Gyimesi G, Kanai Y, Hediger MA (2018) Amino acid transporters revisited: new views in health and disease. Trends Biochem Sci 43(10):752–789. https://doi.org/10.1016/j.tibs.2018.05.003

Kanki T, Young MT, Sakaguchi M, Hamasaki N, Tanner MJ (2003) The N-terminal region of the transmembrane domain of human erythrocyte band 3. Residues critical for membrane insertion and transport activity. J Biol Chem 278(8):5564–5573. https://doi.org/10.1074/jbc.M211662200

Kantcheva AK, Quick M, Shi L, Winther AM, Stolzenberg S, Weinstein H, Javitch JA, Nissen P (2013) Chloride binding site of neurotransmitter sodium symporters. Proc Natl Acad Sci U S A 110 (21):8489–8494. https://doi.org/10.1073/pnas.1221279110

Karet FE, Gainza FJ, Gyory AZ, Unwin RJ, Wrong O, Tanner MJ, Nayir A, Alpay H, Santos F, Hulton SA, Bakkaloglu A, Ozen S, Cunningham MJ, di Pietro A, Walker WG, Lifton RP (1998) Mutations in the chloride-bicarbonate exchanger gene AE1 cause autosomal dominant but not autosomal recessive distal renal tubular acidosis. Proc Natl Acad Sci U S A 95 (11):6337–6342

Katada T, Sakurai H (2019) Xenopus slc7a5 is essential for notochord function and eye development. Mech Dev. https://doi.org/10.1016/j.mod.2019.01.001

Kau KS, Madayag A, Mantsch JR, Grier MD, Abdulhameed O, Baker DA (2008) Blunted cystine-glutamate antiporter function in the nucleus accumbens promotes cocaine-induced drug seeking. Neuroscience 155(2):530–537. https://doi.org/10.1016/j.neuroscience.2008.06.010

Kazmier K, Sharma S, Islam SM, Roux B, McHaourab HS (2014a) Conformational cycle and ion-coupling mechanism of the Na+/hydantoin transporter Mhp1. Proc Natl Acad Sci U S A 111(41):14752–14757. https://doi.org/10.1073/pnas.1410431111

Kazmier K, Sharma S, Quick M, Islam SM, Roux B, Weinstein H, Javitch JA, McHaourab HS (2014b) Conformational dynamics of ligand-dependent alternating access in LeuT. Nat Struct Mol Biol 21 (5):472–479. https://doi.org/10.1038/nsmb.2816

Kazmier K, Claxton DP, McHaourab HS (2017) Alternating access mechanisms of LeuT-fold transporters: trailblazing towards the promised energy landscapes. Curr Opin Struct Biol 45:100–108. https://doi.org/10.1016/j.sbi.2016.12.006

Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ (2015) The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 10 (6):845–858. https://doi.org/10.1038/nprot.2015.053

Kendrick AA, Schafer J, Dzieciatkowska M, Nemkov T, D'Alessandro A, Neelakantan D, Ford HL, Pearson CG, Weekes CD, Hansen KC, Eisenmesser EZ (2017) CD147: a small molecule transporter ancillary protein at the crossroad of multiple hallmarks of cancer and metabolic reprogramming. Oncotarget 8 (4):6742–6762. https://doi.org/10.18632/oncotarget.14272

Kern C, Erdem FA, El-Kasaby A, Sandtner W, Freissmuth M, Sucic S (2017) The N terminus specifies the switch between transport modes of the human serotonin transporter. J Biol Chem 292 (9):3603–3613. https://doi.org/10.1074/jbc.M116.771360

Khafizov K, Staritzbichler R, Stamm M, Forrest LR (2010) A study of the evolution of inverted-topology repeats from LeuT-fold transporters using AlignMe. Biochemistry 49(50):10702–10713. https://doi.org/10.1021/bi101256x

Khelashvili G, Weinstein H (2015) Functional mechanisms of neurotransmitter transporters regulated by lipid-protein interactions of their terminal loops. Biochim Biophys Acta 1848(9):1765–1774. https://doi.org/10.1016/j.bbamem.2015.03.025

Khelashvili G, Galli A, Weinstein H (2012) Phosphatidylinositol 4,5-biphosphate (PIP(2)) lipids regulate the phosphorylation of syntaxin N-terminus by modulating both its position and local structure. Biochemistry 51 (39):7685–7698. https://doi.org/10.1021/bi300833z

Khelashvili G, Doktorova M, Sahai MA, Johner N, Shi L, Weinstein H (2015a) Computational modeling of the

N-terminus of the human dopamine transporter and its interaction with PIP2 -containing membranes. Proteins 83(5):952–969. https://doi.org/10.1002/prot.24792

Khelashvili G, Stanley N, Sahai MA, Medina J, LeVine MV, Shi L, De Fabritiis G, Weinstein H (2015b) Spontaneous inward opening of the dopamine transporter is triggered by PIP2-regulated dynamics of the N-terminus. ACS Chem Neurosci 6(11):1825–1837. https://doi.org/10.1021/acschemneuro.5b00179

Khunweeraphong N, Nagamori S, Wiriyasermkul P, Nishinaka Y, Wongthai P, Ohgaki R, Tanaka H, Tominaga H, Sakurai H, Kanai Y (2012) Establishment of stable cell lines with high expression of heterodimers of human 4F2hc and human amino acid transporter LAT1 or LAT2 and delineation of their differential interaction with alpha-alkyl moieties. J Pharmacol Sci 119(4):368–380

Kilisch M, Lytovchenko O, Schwappach B, Renigunta V, Daut J (2015) The role of protein-protein interactions in the intracellular traffic of the potassium channels TASK-1 and TASK-3. Pflug Arch 467(5):1105–1120. https://doi.org/10.1007/s00424-014-1672-2

Kilty JE, Lorang D, Amara SG (1991) Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254(5031):578–579

Kim DK, Kanai Y, Choi HW, Tangtrongsup S, Chairoungdua A, Babu E, Tachampa K, Anzai N, Iribe Y, Endou H (2002a) Characterization of the system L amino acid transporter in T24 human bladder carcinoma cells. Biochim Biophys Acta 1565 (1):112–121

Kim DK, Kanai Y, Matsuo H, Kim JY, Chairoungdua A, Kobayashi Y, Enomoto A, Cha SH, Goya T, Endou H (2002b) The human T-type amino acid transporter-1: characterization, gene organization, and chromosomal location. Genomics 79(1):95–103. https://doi.org/10.1006/geno.2001.6678

Kim DK, Ahn SG, Park JC, Kanai Y, Endou H, Yoon JH (2004) Expression of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (4F2hc) in oral squamous cell carcinoma and its precusor lesions. Anticancer Res 24(3a):1671–1675

Kim CH, Park KJ, Park JR, Kanai Y, Endou H, Park JC, Kim DK (2006) The RNA interference of amino acid transporter LAT1 inhibits the growth of KB human oral cancer cells. Anticancer Res 26(4B):2943–2948

Kinne A, Schulein R, Krause G (2011) Primary and secondary thyroid hormone transporters. Thyroid Res 4 (Suppl 1):S7. https://doi.org/10.1186/1756-6614-4-s1-s7

Kirchdoerfer RN, Wang N, Pallesen J, Wrapp D, Turner HL, Cottrell CA, Corbett KS, Graham BS, McLellan JS, Ward AB (2018a) Publisher Correction: Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. Sci Rep 8(1):17823. https://doi.org/10.1038/s41598-018-36918-8

Kirchdoerfer RN, Wang N, Pallesen J, Wrapp D, Turner HL, Cottrell CA, Corbett KS, Graham BS, McLellan JS, Ward AB (2018b) Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. Sci Rep 8(1):15701. https://doi.org/10.1038/s41598-018-34171-7

Kirk P, Wilson MC, Heddle C, Brown MH, Barclay AN, Halestrap AP (2000) CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO J 19 (15):3896–3904. https://doi.org/10.1093/emboj/19.15.3896

Kittana N (2018) Angiotensin-converting enzyme 2-Angiotensin 1-7/1-9 system: novel promising targets for heart failure treatment. Fundam Clin Pharmacol 32 (1):14–25. https://doi.org/10.1111/fcp.12318

Kittanakom S, Cordat E, Akkarapatumwong V, Yenchitsomanus PT, Reithmeier RA (2004) Trafficking defects of a novel autosomal recessive distal renal tubular acidosis mutant (S773P) of the human kidney anion exchanger (kAE1). J Biol Chem 279 (39):40960–40971. https://doi.org/10.1074/jbc.M405356200

Klaassen CD, Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacol Rev 62(1):1–96. https://doi.org/10.1124/pr.109.002014

Kleta R, Romeo E, Ristic Z, Ohura T, Stuart C, Arcos-Burgos M, Dave MH, Wagner CA, Camargo SR, Inoue S, Matsuura N, Helip-Wooley A, Bockenhauer D, Warth R, Bernardini I, Visser G, Eggermann T, Lee P, Chairoungdua A, Jutabha P, Babu E, Nilwarangkoon S, Anzai N, Kanai Y, Verrey F, Gahl WA, Koizumi A (2004) Mutations in SLC6A19, encoding B0AT1, cause Hartnup disorder. Nat Genet 36(9):999–1002. https://doi.org/10.1038/ng1405

Klier M, Schuler C, Halestrap AP, Sly WS, Deitmer JW, Becker HM (2011) Transport activity of the high-affinity monocarboxylate transporter MCT2 is enhanced by extracellular carbonic anhydrase IV but not by intracellular carbonic anhydrase II. J Biol Chem 286(31):27781–27791. https://doi.org/10.1074/jbc.M111.255331

Klier M, Andes FT, Deitmer JW, Becker HM (2014) Intracellular and extracellular carbonic anhydrases cooperate non-enzymatically to enhance activity of monocarboxylate transporters. J Biol Chem 289 (5):2765–2775. https://doi.org/10.1074/jbc.M113.537043

Kloeckener-Gruissem B, Vandekerckhove K, Nurnberg G, Neidhardt J, Zeitz C, Nurnberg P, Schipper I, Berger W (2008) Mutation of solute carrier SLC16A12 associates with a syndrome combining juvenile cataract with microcornea and renal glucosuria. Am J Hum Genet 82(3):772–779. https://doi.org/10.1016/j.ajhg.2007.12.013

Knopfel EB, Vilches C, Camargo SMR, Errasti-Murugarren E, Staubli A, Mayayo C, Munier FL, Miroshnikova N, Poncet N, Junza A, Bhattacharya SS, Prat E, Berry V, Berger W, Heon E, Moore AT,

Yanes O, Nunes V, Palacin M, Verrey F, Kloeckener-Gruissem B (2019) Dysfunctional LAT2 amino acid transporter is associated with cataract in mouse and humans. Front Physiol 10:688. https://doi.org/10.3389/fphys.2019.00688

Knowles DW, Chasis JA, Evans EA, Mohandas N (1994) Cooperative action between band 3 and glycophorin A in human erythrocytes: immobilization of band 3 induced by antibodies to glycophorin A. Biophys J 66(5):1726–1732. https://doi.org/10.1016/s0006-3495(94)80965-8

Ko JM, Shin CH, Yang SW, Seong MW, Park SS, Song J (2012) The first Korean case of lysinuric protein intolerance: presented with short stature and increased somnolence. J Korean Med Sci 27(8):961–964. https://doi.org/10.3346/jkms.2012.27.8.961

Kobayashi K, Ohnishi A, Promsuk J, Shimizu S, Kanai Y, Shiokawa Y, Nagane M (2008) Enhanced tumor growth elicited by L-type amino acid transporter 1 in human malignant glioma cells. Neurosurgery 62(2):493–503; discussion 503–494. https://doi.org/10.1227/01.neu.0000316018.51292.19

Kodippili GC, Spector J, Hale J, Giger K, Hughes MR, McNagny KM, Birkenmeier C, Peters L, Ritchie K, Low PS (2012) Analysis of the mobilities of band 3 populations associated with ankyrin protein and junctional complexes in intact murine erythrocytes. J Biol Chem 287(6):4129–4138. https://doi.org/10.1074/jbc.M111.294439

Koehnke J, Jin X, Budreck EC, Posy S, Scheiffele P, Honig B, Shapiro L (2008) Crystal structure of the extracellular cholinesterase-like domain from neuroligin-2. Proc Natl Acad Sci U S A 105(6):1873–1878. https://doi.org/10.1073/pnas.0711701105

Kohyama N, Shiokawa H, Ohbayashi M, Kobayashi Y, Yamamoto T (2013) Characterization of monocarboxylate transporter 6: expression in human intestine and transport of the antidiabetic drug nateglinide. Drug Metab Dispos 41(11):1883–1887. https://doi.org/10.1124/dmd.113.051854

Kokkonen JO, Saarinen J, Kovanen PT (1997) Regulation of local angiotensin II formation in the human heart in the presence of interstitial fluid. Inhibition of chymase by protease inhibitors of interstitial fluid and of angiotensin-converting enzyme by Ang-(1-9) formed by heart carboxypeptidase A-like activity. Circulation 95(6):1455–1463. https://doi.org/10.1161/01.cir.95.6.1455

Kolesnikova TV, Mannion BA, Berditchevski F, Hemler ME (2001) Beta1 integrins show specific association with CD98 protein in low density membranes. BMC Biochem 2:10

Kollert-Jons A, Wagner S, Hubner S, Appelhans H, Drenckhahn D (1993) Anion exchanger 1 in human kidney and oncocytoma differs from erythroid AE1 in its NH2 terminus. Am J Physiol 265(6 Pt 2):F813–F821. https://doi.org/10.1152/ajprenal.1993.265.6.F813

Konstantinopoulos PA, Spentzos D, Fountzilas E, Francoeur N, Sanisetty S, Grammatikos AP, Hecht JL, Cannistra SA (2011) Keap1 mutations and Nrf2 pathway activation in epithelial ovarian cancer. Cancer Res 71(15):5081–5089. https://doi.org/10.1158/0008-5472.CAN-10-4668

Kopito RR, Lodish HF (1985a) Primary structure and transmembrane orientation of the murine anion exchange protein. Nature 316(6025):234–238

Kopito RR, Lodish HF (1985b) Structure of the murine anion exchange protein. J Cell Biochem 29(1):1–17. https://doi.org/10.1002/jcb.240290102

Koshy C, Ziegler C (2015) Structural insights into functional lipid-protein interactions in secondary transporters. Biochim Biophys Acta 1850(3):476–487. https://doi.org/10.1016/j.bbagen.2014.05.010

Koshy C, Schweikhard ES, Gartner RM, Perez C, Yildiz O, Ziegler C (2013) Structural evidence for functional lipid interactions in the betaine transporter BetP. EMBO J 32(23):3096–3105. https://doi.org/10.1038/emboj.2013.226

Kowalczuk S, Broer A, Munzinger M, Tietze N, Klingel K, Broer S (2005) Molecular cloning of the mouse IMINO system: an Na+- and Cl--dependent proline transporter. Biochem J 386(Pt 3):417–422. https://doi.org/10.1042/bj20050100

Kowalczuk S, Broer A, Tietze N, Vanslambrouck JM, Rasko JE, Broer S (2008) A protein complex in the brush-border membrane explains a Hartnup disorder allele. FASEB J 22(8):2880–2887. https://doi.org/10.1096/fj.08-107300

Kowalczyk L, Ratera M, Paladino A, Bartoccioni P, Errasti-Murugarren E, Valencia E, Portella G, Bial S, Zorzano A, Fita I, Orozco M, Carpena X, Vazquez-Ibar JL, Palacin M (2011) Molecular basis of substrate-induced permeation by an amino acid antiporter. Proc Natl Acad Sci U S A 108(10):3935–3940. https://doi.org/10.1073/pnas.1018081108

Kramkowski K, Mogielnicki A, Leszczynska A, Buczko W (2010) Angiotensin-(1-9), the product of angiotensin I conversion in platelets, enhances arterial thrombosis in rats. J Physiol Pharmacol 61(3):317–324

Krause G, Hinz KM (2017) Thyroid hormone transport across L-type amino acid transporters: what can molecular modelling tell us? Mol Cell Endocrinol 458:68–75. https://doi.org/10.1016/j.mce.2017.03.018

Kreutz MR, Langnaese K, Dieterich DC, Seidenbecher CI, Zuschratter W, Beesley PW, Gundelfinger ED (2001) Distribution of transcript and protein isoforms of the synaptic glycoprotein neuroplastin in rat retina. Invest Ophthalmol Vis Sci 42(8):1907–1914

Krishnamurthy H, Gouaux E (2012) X-ray structures of LeuT in substrate-free outward-open and apo inward-open states. Nature 481(7382):469–474. https://doi.org/10.1038/nature10737

Kristensen AS, Andersen J, Jorgensen TN, Sorensen L, Eriksen J, Loland CJ, Stromgaard K, Gether U (2011) SLC6 neurotransmitter transporters: structure,

function, and regulation. Pharmacol Rev 63 (3):585–640. https://doi.org/10.1124/pr.108.000869

Kristiansen TZ, Bunkenborg J, Gronborg M, Molina H, Thuluvath PJ, Argani P, Goggins MG, Maitra A, Pandey A (2004) A proteomic analysis of human bile. Mol Cell Proteomics 3(7):715–728. https://doi.org/10.1074/mcp.M400015-MCP200

Krogsgaard-Larsen P, Frolund B, Frydenvang K (2000) GABA uptake inhibitors. Design, molecular pharmacology and therapeutic aspects. Curr Pharm Des 6 (12):1193–1209

Kropski JA, Blackwell TS (2018) Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. J Clin Invest 128(1):64–73. https://doi.org/10.1172/jci93560

Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 11(8):875–879. https://doi.org/10.1038/nm1267

Kuba K, Imai Y, Penninger JM (2013) Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. Circ J 77(2):301–308

Kurayama R, Ito N, Nishibori Y, Fukuhara D, Akimoto Y, Higashihara E, Ishigaki Y, Sai Y, Miyamoto K, Endou H, Kanai Y, Yan K (2011) Role of amino acid transporter LAT2 in the activation of mTORC1 pathway and the pathogenesis of crescentic glomerulonephritis. Lab Invest 91(7):992–1006. https://doi.org/10.1038/labinvest.2011.43

Kurko J, Vaha-Makila M, Tringham M, Tanner L, Paavanen-Huhtala S, Saarinen M, Nanto-Salonen K, Simell O, Niinikoski H, Mykkanen J (2015) Dysfunction in macrophage toll-like receptor signaling caused by an inborn error of cationic amino acid transport. Mol Immunol 67(2 Pt B):416–425. https://doi.org/10.1016/j.molimm.2015.07.006

Lafreniere RG, Carrel L, Willard HF (1994) A novel transmembrane transporter encoded by the XPCT gene in Xq13.2. Hum Mol Genet 3(7):1133–1139

Lahoutte T, Caveliers V, Camargo SM, Franca R, Ramadan T, Veljkovic E, Mertens J, Bossuyt A, Verrey F (2004) SPECT and PET amino acid tracer influx via system L (h4F2hc-hLAT1) and its transstimulation. J Nucl Med 45(9):1591–1596

Lambert DW, Hooper NM, Turner AJ (2008) Angiotensin-converting enzyme 2 and new insights into the renin-angiotensin system. Biochem Pharmacol 75(4):781–786

Lan T, Haywood J, Rao A, Dawson PA (2011) Molecular mechanisms of altered bile acid homeostasis in organic solute transporter-alpha knockout mice. Dig Dis 29 (1):18–22. https://doi.org/10.1159/000324124

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X (2020a) Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor. bioRxiv:2020.2002.2019.956235. https://doi.org/10.1101/2020.02.19.956235

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X (2020b) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581(7807):215–220. https://doi.org/10.1038/s41586-020-2180-5

Lang T, Jahn R (2008) Core proteins of the secretory machinery. Handb Exp Pharmacol 184:107–127. https://doi.org/10.1007/978-3-540-74805-2_5

Langley JW, Issitt PD, Anstee DJ, McMahan M, Smith N, Pavone BG, Tessel JA, Carlin MA (1981) Another individual (J.R.) whose red blood cells appear to carry a hybrid MNSs sialoglycoprotein. Transfusion 21(1):15–24

Langnaese K, Beesley PW, Gundelfinger ED (1997) Synaptic membrane glycoproteins gp65 and gp55 are new members of the immunoglobulin superfamily. J Biol Chem 272(2):821–827

Laursen L, Severinsen K, Kristensen KB, Periole X, Overby M, Muller HK, Schiott B, Sinning S (2018) Cholesterol binding to a conserved site modulates the conformation, pharmacology, and transport kinetics of the human serotonin transporter. J Biol Chem 293 (10):3510–3523. https://doi.org/10.1074/jbc.M117.809046

Le Floch R, Chiche J, Marchiq I, Naiken T, Ilc K, Murray CM, Critchlow SE, Roux D, Simon MP, Pouyssegur J (2011) CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. Proc Natl Acad Sci U S A 108(40):16663–16668. https://doi.org/10.1073/pnas.1106123108

Lebowitz JJ, Pino JA, Mackie PM, Lin M, Hurst C, Divita K, Collins AT, Koutzoumis DN, Torres GE, Khoshbouei H (2019) Clustered Kv2.1 decreases dopamine transporter activity and internalization. J Biol Chem 294(17):6957–6971. https://doi.org/10.1074/jbc.RA119.007441

Lee WS, Wells RG, Sabbag RV, Mohandas TK, Hediger MA (1993) Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic, and neutral amino acid transport. J Clin Invest 91 (5):1959–1963. https://doi.org/10.1172/jci116415

Lee FJ, Liu F, Pristupa ZB, Niznik HB (2001) Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. FASEB J 15(6):916–926. https://doi.org/10.1096/fj.00-0334com

Lee KH, Kim MY, Kim DH, Lee YS (2004) Syntaxin 1A and receptor for activated C kinase interact with the N-terminal region of human dopamine transporter. Neurochem Res 29(7):1405–1409

Lee Y, Wiriyasermkul P, Jin C, Quan L, Ohgaki R, Okuda S, Kusakizako T, Nishizawa T, Oda K, Ishitani R, Yokoyama T, Nakane T, Shirouzu M, Endou H, Nagamori S, Kanai Y, Nureki O (2019) Cryo-EM structure of the human L-type amino acid transporter 1 in complex with glycoprotein CD98hc.

Nat Struct Mol Biol 26(6):510–517. https://doi.org/10.1038/s41594-019-0237-7

Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B (2009) Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 89(1):147–191. https://doi.org/10.1152/physrev.00010.2008

Leung YM, Kwan EP, Ng B, Kang Y, Gaisano HY (2007) SNAREing voltage-gated K+ and ATP-sensitive K+ channels: tuning beta-cell excitability with syntaxin-1A and other exocytotic proteins. Endocr Rev 28 (6):653–663. https://doi.org/10.1210/er.2007-0010

LeVine MV, Weinstein H (2014) NbIT – a new information theory-based analysis of allosteric mechanisms reveals residues that underlie function in the leucine transporter LeuT. PLoS Comput Biol 10(5):e1003603–e1003603. https://doi.org/10.1371/journal.pcbi.1003603

LeVine MV, Cuendet MA, Razavi AM, Khelashvili G, Weinstein H (2018) Thermodynamic coupling function analysis of allosteric mechanisms in the human dopamine transporter. Biophys J 114(1):10–14. https://doi.org/10.1016/j.bpj.2017.10.030

LeVine MV, Terry DS, Khelashvili G, Siegel ZS, Quick M, Javitch JA, Blanchard SC, Weinstein H (2019) The allosteric mechanism of substrate-specific transport in SLC6 is mediated by a volumetric sensor. Proc Natl Acad Sci U S A 116(32):15947–15956. https://doi.org/10.1073/pnas.1903020116

Lewerenz J, Maher P (2009) Basal levels of eIF2alpha phosphorylation determine cellular antioxidant status by regulating ATF4 and xCT expression. J Biol Chem 284(2):1106–1115. https://doi.org/10.1074/jbc.M807325200

Lewerenz J, Albrecht P, Tien ML, Henke N, Karumbayaram S, Kornblum HI, Wiedau-Pazos M, Schubert D, Maher P, Methner A (2009) Induction of Nrf2 and xCT are involved in the action of the neuroprotective antibiotic ceftriaxone in vitro. J Neurochem 111(2):332–343. https://doi.org/10.1111/j.1471-4159.2009.06347.x

Lewerenz J, Maher P, Methner A (2012a) Regulation of xCT expression and system x (c) (-) function in neuronal cells. Amino Acids 42(1):171–179. https://doi.org/10.1007/s00726-011-0862-x

Lewerenz J, Sato H, Albrecht P, Henke N, Noack R, Methner A, Maher P (2012b) Mutation of ATF4 mediates resistance of neuronal cell lines against oxidative stress by inducing xCT expression. Cell Death Differ 19(5):847–858. https://doi.org/10.1038/cdd.2011.165

Lewerenz J, Hewett SJ, Huang Y, Lambros M, Gout PW, Kalivas PW, Massie A, Smolders I, Methner A, Pergande M, Smith SB, Ganapathy V, Maher P (2013) The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities. Antioxid Redox Signal 18(5):522–555. https://doi.org/10.1089/ars.2011.4391

Li H, Zhang H, Wang HY (2003a) Collectrin, a kidney-specific novel gene, is over-expressed in the hypertrophic phase in 5/6 nephrectomized rat kidney. Zhonghua Yi Xue Za Zhi 83(8):684–687

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M (2003b) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426 (6965):450–454. https://doi.org/10.1038/nature02145

Li N, Cui Z, Fang F, Lee JY, Ballatori N (2007) Heterodimerization, trafficking and membrane topology of the two proteins, Ost alpha and Ost beta, that constitute the organic solute and steroid transporter. Biochem J 407 (3):363–372. https://doi.org/10.1042/bj20070716

Li W, Hu M, Wang C, Lu H, Chen F, Xu J, Shang Y, Wang F, Qin J, Yan Q, Krueger BJ, Renne R, Gao SJ, Lu C (2017) A viral microRNA downregulates metastasis suppressor CD82 and induces cell invasion and angiogenesis by activating the c-Met signaling. Oncogene 36(38):5407–5420. https://doi.org/10.1038/onc.2017.139

Li J, Zhao Z, Tajkhorshid E (2019) Locking two rigid-body bundles in an outward-facing conformation: the ion-coupling mechanism in a LeuT-fold transporter. Sci Rep 9(1):19479. https://doi.org/10.1038/s41598-019-55722-6

Lin PJ, Williams WP, Luu Y, Molday RS, Orlowski J, Numata M (2005) Secretory carrier membrane proteins interact and regulate trafficking of the organellar (Na+, K+)/H+ exchanger NHE7. J Cell Sci 118 (Pt 9):1885–1897. https://doi.org/10.1242/jcs.02315

Lin L, Yee SW, Kim RB, Giacomini KM (2015) SLC transporters as therapeutic targets: emerging opportunities. Nat Rev Drug Discov 14(8):543–560. https://doi.org/10.1038/nrd4626

Liu QR, Lopez-Corcuera B, Nelson H, Mandiyan S, Nelson N (1992a) Cloning and expression of a cDNA encoding the transporter of taurine and beta-alanine in mouse brain. Proc Natl Acad Sci U S A 89 (24):12145–12149

Liu QR, Nelson H, Mandiyan S, Lopez-Corcuera B, Nelson N (1992b) Cloning and expression of a glycine transporter from mouse brain. FEBS Lett 305 (2):110–114

Liu QR, Lopez-Corcuera B, Mandiyan S, Nelson H, Nelson N (1993) Cloning and expression of a spinal cord- and brain-specific glycine transporter with novel structural features. J Biol Chem 268(30):22802–22808

Liu SC, Jarolim P, Rubin HL, Palek J, Amato D, Hassan K, Zaik M, Sapak P (1994) The homozygous state for the band 3 protein mutation in Southeast Asian ovalocytosis may be lethal. Blood 84(10):3590–3591

Liu X, Charrier L, Gewirtz A, Sitaraman S, Merlin D (2003) CD98 and intracellular adhesion molecule I regulate the activity of amino acid transporter LAT-2 in polarized intestinal epithelia. J Biol Chem 278 (26):23672–23677. https://doi.org/10.1074/jbc.M302777200

Liu Q, Dou S, Wang G, Li Z, Feng Y (2008) Evolution and functional divergence of monocarboxylate transporter genes in vertebrates. Gene 423(1):14–22. https://doi.org/10.1016/j.gene.2008.07.003

Liu CL, Zhong W, He YY, Li X, Li S, He KL (2016) Genome-wide analysis of tunicamycin-induced endoplasmic reticulum stress response and the protective effect of endoplasmic reticulum inhibitors in neonatal rat cardiomyocytes. Mol Cell Biochem 413 (1–2):57–67. https://doi.org/10.1007/s11010-015-2639-0

Liu C, Li X, Li C, Zhang Z, Gao X, Jia Z, Chen H, Jia Q, Zhao X, Liu J, Liu B, Xu Z, Tian Y, He K (2018) SLC3A2 is a novel endoplasmic reticulum stress-related signaling protein that regulates the unfolded protein response and apoptosis. PLoS One 13(12): e0208993. https://doi.org/10.1371/journal.pone.0208993

Lo M, Wang YZ, Gout PW (2008) The x(c)- cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases. J Cell Physiol 215 (3):593–602. https://doi.org/10.1002/jcp.21366

Longhena F, Faustini G, Missale C, Pizzi M, Bellucci A (2018) Dopamine transporter/α-synuclein complexes are altered in the post mortem caudate putamen of Parkinson's disease: an in situ proximity ligation assay study. Int J Mol Sci 19(6):1611. https://doi.org/10.3390/ijms19061611

Lopez-Corcuera B, Liu QR, Mandiyan S, Nelson H, Nelson N (1992) Expression of a mouse brain cDNA encoding novel gamma-aminobutyric acid transporter. J Biol Chem 267(25):17491–17493

Lopez-Corcuera B, Aragon C, Geerlings A (2001) Regulation of glycine transporters. Biochem Soc Trans 29 (Pt 6):742–745

Low PS (1986) Structure and function of the cytoplasmic domain of band 3: center of erythrocyte membrane-peripheral protein interactions. Biochim Biophys Acta 864(2):145–167

Lu F, Li S, Jiang Y, Jiang J, Fan H, Lu G, Deng D, Dang S, Zhang X, Wang J, Yan N (2011) Structure and mechanism of the uracil transporter UraA. Nature 472 (7342):243–246. https://doi.org/10.1038/nature09885

Lumadue JA, Glick AB, Ruddle FH (1987) Cloning, sequence analysis, and expression of the large subunit of the human lymphocyte activation antigen 4F2. Proc Natl Acad Sci U S A 84(24):9204–9208

Luo J, Teplyakov A, Obmolova G, Malia T, Wu SJ, Beil E, Baker A, Swencki-Underwood B, Zhao Y, Sprenkle J, Dixon K, Sweet R, Gilliland GL (2009) Structure of the EMMPRIN N-terminal domain 1: dimerization via beta-strand swapping. Proteins 77 (4):1009–1014. https://doi.org/10.1002/prot.22577

Lyons SA, Chung WJ, Weaver AK, Ogunrinu T, Sontheimer H (2007) Autocrine glutamate signaling promotes glioma cell invasion. Cancer Res 67 (19):9463–9471. https://doi.org/10.1158/0008-5472.can-07-2034

Machnicka B, Czogalla A, Hryniewicz-Jankowska A, Boguslawska DM, Grochowalska R, Heger E, Sikorski AF (2014) Spectrins: a structural platform for stabilization and activation of membrane channels, receptors and transporters. Biochim Biophys Acta 1838 (2):620–634. https://doi.org/10.1016/j.bbamem.2013.05.002

MacKenzie KR, Prestegard JH, Engelman DM (1997) A transmembrane helix dimer: structure and implications. Science 276(5309):131–133

Magagnin S, Bertran J, Werner A, Markovich D, Biber J, Palacin M, Murer H (1992) Poly(A)+ RNA from rabbit intestinal mucosa induces b0,+ and y+ amino acid transport activities in Xenopus laevis oocytes. J Biol Chem 267(22):15384–15390

Mahdavi M, Koulivand L, Khorrami M, Mirsafaie M, Kheirollahi M (2018) In silico analysis of SLC3A1 and SLC7A9 mutations in Iranian patients with Cystinuria. Mol Biol Rep 45(5):1165–1173. https://doi.org/10.1007/s11033-018-4269-6

Maiya R, Ponomarev I, Linse KD, Harris RA, Mayfield RD (2007) Defining the dopamine transporter proteome by convergent biochemical and in silico analyses. Genes Brain Behav 6(1):97–106. https://doi.org/10.1111/j.1601-183X.2006.00236.x

Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B (1999) Identification of a nuclear receptor for bile acids. Science 284(5418):1362–1365

Makrides V, Camargo SM, Verrey F (2014) Transport of amino acids in the kidney. Compr Physiol 4 (1):367–403. https://doi.org/10.1002/cphy.c130028

Malakauskas SM, Quan H, Fields TA, McCall SJ, Yu MJ, Kourany WM, Frey CW, Le TH (2007) Aminoaciduria and altered renal expression of luminal amino acid transporters in mice lacking novel gene collectrin. Am J Physiol Renal Physiol 292(2):F533–F544. https://doi.org/10.1152/ajprenal.00325.2006

Malakauskas SM, Kourany WM, Zhang XY, Lu D, Stevens RD, Koves TR, Hohmeier HE, Muoio DM, Newgard CB, Le TH (2009) Increased insulin sensitivity in mice lacking collectrin, a downstream target of HNF-1alpha. Mol Endocrinol 23(6):881–892. https://doi.org/10.1210/me.2008-0274

Malinauskaite L, Quick M, Reinhard L, Lyons JA, Yano H, Javitch JA, Nissen P (2014) A mechanism for intracellular release of Na+ by neurotransmitter/sodium symporters. Nat Struct Mol Biol 21 (11):1006–1012. https://doi.org/10.1038/nsmb.2894

Malinauskaite L, Said S, Sahin C, Grouleff J, Shahsavar A, Bjerregaard H, Noer P, Severinsen K, Boesen T, Schiott B, Sinning S, Nissen P (2016) A conserved leucine occupies the empty substrate site of LeuT in the Na(+)-free return state. Nat Commun 7:11673. https://doi.org/10.1038/ncomms11673

Mandal PK, Seiler A, Perisic T, Kolle P, Banjac Canak A, Forster H, Weiss N, Kremmer E, Lieberman MW, Bannai S, Kuhlencordt P, Sato H, Bornkamm GW,

Conrad M (2010) System x(c)- and thioredoxin reductase 1 cooperatively rescue glutathione deficiency. J Biol Chem 285(29):22244–22253. https://doi.org/10.1074/jbc.M110.121327

Manning Fox JE, Meredith D, Halestrap AP (2000) Characterisation of human monocarboxylate transporter 4 substantiates its role in lactic acid efflux from skeletal muscle. J Physiol 529(Pt 2):285–293

Mannion BA, Kolesnikova TV, Lin SH, Wang S, Thompson NL, Hemler ME (1998) The light chain of CD98 is identified as E16/TA1 protein. J Biol Chem 273(50):33127–33129

Mannowetz N, Wandernoth P, Wennemuth G (2012) Basigin interacts with both MCT1 and MCT2 in murine spermatozoa. J Cell Physiol 227 (5):2154–2162. https://doi.org/10.1002/jcp.22949

Manoharan C, Wilson MC, Sessions RB, Halestrap AP (2006) The role of charged residues in the transmembrane helices of monocarboxylate transporter 1 and its ancillary protein basigin in determining plasma membrane expression and catalytic activity. Mol Membr Biol 23(6):486–498. https://doi.org/10.1080/09687860600841967

Marchesi VT, Furthmayr H, Tomita M (1976) The red cell membrane. Annu Rev Biochem 45:667–698. https://doi.org/10.1146/annurev.bi.45.070176.003315

Margheritis E, Imperiali FG, Cinquetti R, Vollero A, Terova G, Rimoldi S, Girardello R, Bossi E (2016) Amino acid transporter B(0)AT1 (slc6a19) and ancillary protein: impact on function. Pflug Arch 468 (8):1363–1374. https://doi.org/10.1007/s00424-016-1842-5

Marieb EA, Zoltan-Jones A, Li R, Misra S, Ghatak S, Cao J, Zucker S, Toole BP (2004) Emmprin promotes anchorage-independent growth in human mammary carcinoma cells by stimulating hyaluronan production. Cancer Res 64(4):1229–1232

Markazi S, Kheirollahi M, Doosti A, Mohammadi M, Koulivand L (2016) A novel mutation in SLC3A1 gene in patients with cystinuria. Iran J Kidney Dis 10 (1):44–47

Markovich D, Stange G, Bertran J, Palacin M, Werner A, Biber J, Murer H (1993) Two mRNA transcripts (rBAT-1 and rBAT-2) are involved in system b0,(+)-related amino acid transport. J Biol Chem 268 (2):1362–1367

Marzban H, Khanzada U, Shabir S, Hawkes R, Langnaese K, Smalla KH, Bockers TM, Gundelfinger ED, Gordon-Weeks PR, Beesley PW (2003) Expression of the immunoglobulin superfamily neuroplastin adhesion molecules in adult and developing mouse cerebellum and their localisation to parasagittal stripes. J Comp Neurol 462(3):286–301. https://doi.org/10.1002/cne.10719

Massie A, Schallier A, Kim SW, Fernando R, Kobayashi S, Beck H, De Bundel D, Vermoesen K, Bannai S, Smolders I, Conrad M, Plesnila N, Sato H, Michotte Y (2011) Dopaminergic neurons of system x (c)(-)-deficient mice are highly protected against 6-hydroxydopamine-induced toxicity. FASEB J 25 (4):1359–1369. https://doi.org/10.1096/fj.10-177212

Massie A, Boillee S, Hewett S, Knackstedt L, Lewerenz J (2015) Main path and byways: non-vesicular glutamate release by system xc(-) as an important modifier of glutamatergic neurotransmission. J Neurochem 135 (6):1062–1079. https://doi.org/10.1111/jnc.13348

Mastroberardino L, Spindler B, Pfeiffer R, Skelly PJ, Loffing J, Shoemaker CB, Verrey F (1998) Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. Nature 395 (6699):288–291. https://doi.org/10.1038/26246

Matskevitch I, Wagner CA, Stegen C, Broer S, Noll B, Risler T, Kwon HM, Handler JS, Waldegger S, Busch AE, Lang F (1999) Functional characterization of the Betaine/gamma-aminobutyric acid transporter BGT-1 expressed in Xenopus oocytes. J Biol Chem 274 (24):16709–16716

Matsuo H, Kanai Y, Kim JY, Chairoungdua A, Kim DK, Inatomi J, Shigeta Y, Ishimine H, Chaekuntode S, Tachampa K, Choi HW, Babu E, Fukuda J, Endou H (2002) Identification of a novel Na+-independent acidic amino acid transporter with structural similarity to the member of a heterodimeric amino acid transporter family associated with unknown heavy chains. J Biol Chem 277(23):21017–21026. https://doi.org/10.1074/jbc.M200019200

Matthies HJ, Han Q, Shields A, Wright J, Moore JL, Winder DG, Galli A, Blakely RD (2009) Subcellular localization of the antidepressant-sensitive norepinephrine transporter. BMC Neurosci 10:65. https://doi.org/10.1186/1471-2202-10-65

Mauhin W, Habarou F, Gobin S, Servais A, Brassier A, Grisel C, Roda C, Pinto G, Moshous D, Ghalim F, Krug P, Deltour N, Pontoizeau C, Dubois S, Assoun M, Galmiche L, Bonnefont JP, Ottolenghi C, de Blic J, Arnoux JB, de Lonlay P (2017) Update on Lysinuric Protein Intolerance, a Multi-faceted Disease Retrospective cohort analysis from birth to adulthood. Orphanet J Rare Dis 12(1):3. https://doi.org/10.1186/s13023-016-0550-8

Mauna JC, Harris SS, Pino JA, Edwards CM, DeChellis-Marks MR, Bassi CD, Garcia-Olivares J, Amara SG, Guajardo FG, Sotomayor-Zarate R, Terminel M, Castañeda E, Vergara M, Baust T, Thiels E, Torres GE (2019) G protein βγ subunits play a critical role in the actions of amphetamine. Transl Psychiatry 9 (1):81–81. https://doi.org/10.1038/s41398-019-0387-8

Maurer-Spurej E, Pittendreigh C, Solomons K (2004) The influence of selective serotonin reuptake inhibitors on human platelet serotonin. Thromb Haemost 91 (1):119–128. https://doi.org/10.1160/th03-05-0330

Mayya V, Lundgren DH, Hwang SI, Rezaul K, Wu L, Eng JK, Rodionov V, Han DK (2009) Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions. Sci Signal 2(84):ra46. https://doi.org/10.1126/scisignal.2000007

Mboge MY, Chen Z, Khokhar D, Wolff A, Ai L, Heldermon CD, Bozdag M, Carta F, Supuran CT, Brown KD, McKenna R, Frost CJ, Frost SC (2019) A non-catalytic function of carbonic anhydrase IX contributes to the glycolytic phenotype and pH

regulation in human breast cancer cells. Biochem J 476 (10):1497–1513. https://doi.org/10.1042/bcj20190177
McConnell RE, Benesh AE, Mao S, Tabb DL, Tyska MJ (2011) Proteomic analysis of the enterocyte brush border. Am J Physiol Gastrointest Liver Physiol 300(5): G914–G926. https://doi.org/10.1152/ajpgi.00005.2011
McCoy KE, Zhou X, Vize PD (2008) Collectrin/tmem27 is expressed at high levels in all segments of the developing Xenopus pronephric nephron and in the Wolffian duct. Gene Expr Patterns 8(4):271–274. https://doi.org/10.1016/j.gep.2007.12.002
McQueen G, Lally J, Collier T, Zelaya F, Lythgoe DJ, Barker GJ, Stone JM, McGuire P, MacCabe JH, Egerton A (2018) Effects of N-acetylcysteine on brain glutamate levels and resting perfusion in schizophrenia. Psychopharmacology (Berl) 235 (10):3045–3054. https://doi.org/10.1007/s00213-018-4997-2
Medin T, Medin H, Hefte MB, Storm-Mathisen J, Bergersen LH (2019) Upregulation of the lactate transporter monocarboxylate transporter 1 at the blood-brain barrier in a rat model of attention-deficit/hyperactivity disorder suggests hyperactivity could be a form of self-treatment. Behav Brain Res 360:279–285. https://doi.org/10.1016/j.bbr.2018.12.023
Meier C, Ristic Z, Klauser S, Verrey F (2002) Activation of system L heterodimeric amino acid exchangers by intracellular substrates. EMBO J 21(4):580–589
Meier A, Erler H, Beitz E (2018) Targeting channels and transporters in protozoan parasite infections. Front Chem 6:88. https://doi.org/10.3389/fchem.2018.00088
Melendez RI, Vuthiganon J, Kalivas PW (2005) Regulation of extracellular glutamate in the prefrontal cortex: focus on the cystine glutamate exchanger and group I metabotropic glutamate receptors. J Pharmacol Exp Ther 314(1):139–147. https://doi.org/10.1124/jpet.104.081521
Mentlein R (2004) Cell-surface peptidases. Int Rev Cytol 235:165–213. https://doi.org/10.1016/S0074-7696(04)35004-7
Mercado CP, Kilic F (2010) Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. Mol Interv 10(4):231–241. https://doi.org/10.1124/mi.10.4.6
Merezhinskaya N, Fishbein WN, Davis JI, Foellmer JW (2000) Mutations in MCT1 cDNA in patients with symptomatic deficiency in lactate transport. Muscle Nerve 23(1):90–97
Mesuret G, Khabbazzadeh S, Bischoff AM, Safory H, Wolosker H, Hulsmann S (2018) A neuronal role of the Alanine-Serine-Cysteine-1 transporter (SLC7A10, Asc-1) for glycine inhibitory transmission and respiratory pattern. Sci Rep 8(1):8536. https://doi.org/10.1038/s41598-018-26868-6
Meury M, Costa M, Harder D, Stauffer M, Jeckelmann JM, Bruhlmann B, Rosell A, Ilgu H, Kovar K, Palacin M, Fotiadis D (2014) Detergent-induced stabilization and improved 3D map of the human heteromeric amino acid transporter 4F2hc-LAT2. PLoS One 9(10):e109882. https://doi.org/10.1371/journal.pone.0109882
Milewski K, Bogacinska-Karas M, Fresko I, Hilgier W, Jazwiec R, Albrecht J, Zielinska M (2017) Ammonia reduces intracellular asymmetric dimethylarginine in cultured astrocytes stimulating its y(+)LAT2 carrier-mediated loss. Int J Mol Sci 18(11). https://doi.org/10.3390/ijms18112308
Milkereit R, Persaud A, Vanoaica L, Guetg A, Verrey F, Rotin D (2015) LAPTM4b recruits the LAT1-4F2hc Leu transporter to lysosomes and promotes mTORC1 activation. Nat Commun 6:7250. https://doi.org/10.1038/ncomms8250
Miller LH, Haynes JD, McAuliffe FM, Shiroishi T, Durocher JR, McGinniss MH (1977) Evidence for differences in erythrocyte surface receptors for the malarial parasites, Plasmodium falciparum and Plasmodium knowlesi. J Exp Med 146(1):277–281
Mina-Osorio P (2008) The moonlighting enzyme CD13: old and new functions to target. Trends Mol Med 14 (8):361–371
Mineev KS, Bocharov EV, Volynsky PE, Goncharuk MV, Tkach EN, Ermolyuk YS, Schulga AA, Chupin VV, Maslennikov IV, Efremov RG, Arseniev AS (2011) Dimeric structure of the transmembrane domain of glycophorin a in lipidic and detergent environments. Acta Naturae 3(2):90–98
Miyamoto K, Katai K, Tatsumi S, Sone K, Segawa H, Yamamoto H, Taketani Y, Takada K, Morita K, Kanayama H et al (1995) Mutations of the basic amino acid transporter gene associated with cystinuria. Biochem J 310(Pt 3):951–955
Miyamoto K, Segawa H, Tatsumi S, Katai K, Yamamoto H, Taketani Y, Haga H, Morita K, Takeda E (1996) Effects of truncation of the COOH-terminal region of a Na+-independent neutral and basic amino acid transporter on amino acid transport in Xenopus oocytes. J Biol Chem 271(28):16758–16763
Mizoguchi K, Cha SH, Chairoungdua A, Kim DK, Shigeta Y, Matsuo H, Fukushima J, Awa Y, Akakura K, Goya T, Ito H, Endou H, Kanai Y (2001) Human cystinuria-related transporter: localization and functional characterization. Kidney Int 59 (5):1821–1833. https://doi.org/10.1046/j.1523-1755.2001.0590051821.x
Mogielnicki A, Kramkowski K, Hermanowicz JM, Leszczynska A, Przyborowski K, Buczko W (2014) Angiotensin-(1-9) enhances stasis-induced venous thrombosis in the rat because of the impairment of fibrinolysis. J Renin Angiotensin Aldosterone Syst 15 (1):13–21. https://doi.org/10.1177/1470320313498631
Mondal S, Khelashvili G, Weinstein H (2014) Not just an oil slick: how the energetics of protein-membrane interactions impacts the function and organization of transmembrane proteins. Biophys J 106 (11):2305–2316. https://doi.org/10.1016/j.bpj.2014.04.032
Moran MM, McFarland K, Melendez RI, Kalivas PW, Seamans JK (2005) Cystine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability

to cocaine seeking. J Neurosci 25(27):6389–6393. https://doi.org/10.1523/jneurosci.1007-05.2005

Morita K, Motoyama N, Kitayama T, Morioka N, Kifune K, Dohi T (2008) Spinal antiallodynia action of glycine transporter inhibitors in neuropathic pain models in mice. J Pharmacol Exp Ther 326 (2):633–645. https://doi.org/10.1124/jpet.108.136267

Moschen I, Setiawan I, Palacin M, Broer S, Albers A, Lang F (2002) Influence of rBAT-mediated amino acid transport on cytosolic pH. Nephron 91 (4):631–636

Mostyn SN, Wilson KA, Schumann-Gillett A, Frangos ZJ, Shimmon S, Rawling T, Ryan RM, O'Mara ML, Vandenberg RJ (2019) Identification of an allosteric binding site on the human glycine transporter, GlyT2, for bioactive lipid analgesics. Elife 8:e47150. https://doi.org/10.7554/eLife.47150

Moszczynska A, Saleh J, Zhang H, Vukusic B, Lee FJS, Liu F (2007) Parkin disrupts the alpha-synuclein/dopamine transporter interaction: consequences toward dopamine-induced toxicity. J Mol Neurosci 32(3):217–227. https://doi.org/10.1007/s12031-007-0037-0

Moulin PA, Nivaggioni V, Saut N, Grosdidier C, Bernot D, Baccini V (2017) Southeast Asian ovalocytosis: the need for a carefull observation of red cell indices and blood smear. Ann Biol Clin (Paris) 75(6):699–702. https://doi.org/10.1684/abc.2017.1291

Mount DB (2007) Collectrin and the kidney. Curr Opin Nephrol Hypertens 16(5):427–429

Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A, Kalivas PW (2009) N-Acetylcysteine reverses cocaine-induced metaplasticity. Nat Neurosci 12(2):182–189. https://doi.org/10.1038/nn.2250

Muller HK, Wiborg O, Haase J (2006) Subcellular redistribution of the serotonin transporter by secretory carrier membrane protein 2. J Biol Chem 281(39):28901–28909. https://doi.org/10.1074/jbc.M602848200

Muller HK, Kragballe M, Fjorback AW, Wiborg O (2014) Differential regulation of the serotonin transporter by vesicle-associated membrane protein 2 in cells of neuronal versus non-neuronal origin. PLoS One 9(5): e97540. https://doi.org/10.1371/journal.pone.0097540

Murakami Y, Kohyama N, Kobayashi Y, Ohbayashi M, Ohtani H, Sawada Y, Yamamoto T (2005) Functional characterization of human monocarboxylate transporter 6 (SLC16A5). Drug Metab Dispos 33 (12):1845–1851. https://doi.org/10.1124/dmd.105.005264

Muramatsu T (2012) Basigin: a multifunctional membrane protein with an emerging role in infections by malaria parasites. Expert Opin Ther Targets 16(10):999–1011. https://doi.org/10.1517/14728222.2012.711818

Muramatsu T (2016) Basigin (CD147), a multifunctional transmembrane glycoprotein with various binding partners. J Biochem 159(5):481–490. https://doi.org/10.1093/jb/mvv127

Muramatsu T, Miyauchi T (2003) Basigin (CD147): a multifunctional transmembrane protein involved in reproduction, neural function, inflammation and tumor invasion. Histol Histopathol 18(3):981–987. https://doi.org/10.14670/hh-18.981

Mykkanen J, Torrents D, Pineda M, Camps M, Yoldi ME, Horelli-Kuitunen N, Huoponen K, Heinonen M, Oksanen J, Simell O, Savontaus ML, Zorzano A, Palacin M, Aula P (2000) Functional analysis of novel mutations in y(+)LAT-1 amino acid transporter gene causing lysinuric protein intolerance (LPI). Hum Mol Genet 9(3):431–438

Nabeyama A, Kurita A, Asano K, Miyake Y, Yasuda T, Miura I, Nishitai G, Arakawa S, Shimizu S, Wakana S, Yoshida H, Tanaka M (2010) xCT deficiency accelerates chemically induced tumorigenesis. Proc Natl Acad Sci U S A 107(14):6436–6441. https://doi.org/10.1073/pnas.0912827107

Nagamori S, Wiriyasermkul P, Guarch ME, Okuyama H, Nakagomi S, Tadagaki K, Nishinaka Y, Bodoy S, Takafuji K, Okuda S, Kurokawa J, Ohgaki R, Nunes V, Palacin M, Kanai Y (2016) Novel cystine transporter in renal proximal tubule identified as a missing partner of cystinuria-related plasma membrane protein rBAT/SLC3A1. Proc Natl Acad Sci U S A 113 (3):775–780. https://doi.org/10.1073/pnas.1519959113

Nakada N, Mikami T, Hana K, Ichinoe M, Yanagisawa N, Yoshida T, Endou H, Okayasu I (2014) Unique and selective expression of L-amino acid transporter 1 in human tissue as well as being an aspect of oncofetal protein. Histol Histopathol 29(2):217–227. https://doi.org/10.14670/hh-29.217

Nakamura E, Sato M, Yang H, Miyagawa F, Harasaki M, Tomita K, Matsuoka S, Noma A, Iwai K, Minato N (1999) 4F2 (CD98) heavy chain is associated covalently with an amino acid transporter and controls intracellular trafficking and membrane topology of 4F2 heterodimer. J Biol Chem 274(5):3009–3016

Nakauchi J, Matsuo H, Kim DK, Goto A, Chairoungdua A, Cha SH, Inatomi J, Shiokawa Y, Yamaguchi K, Saito I, Endou H, Kanai Y (2000) Cloning and characterization of a human brain Na(+)-independent transporter for small neutral amino acids that transports D-serine with high affinity. Neurosci Lett 287(3):231–235

Napolitano L, Scalise M, Galluccio M, Pochini L, Albanese LM, Indiveri C (2015) LAT1 is the transport competent unit of the LAT1/CD98 heterodimeric amino acid transporter. Int J Biochem Cell Biol 67:25–33. https://doi.org/10.1016/j.biocel.2015.08.004

Napolitano L, Galluccio M, Scalise M, Parravicini C, Palazzolo L, Eberini I, Indiveri C (2017) Novel insights into the transport mechanism of the human amino acid transporter LAT1 (SLC7A5). Probing critical residues for substrate translocation. Biochim Biophys Acta Gen Subj 1861(4):727–736. https://doi.org/10.1016/j.bbagen.2017.01.013

Nash SR, Giros B, Kingsmore SF, Rochelle JM, Suter ST, Gregor P, Seldin MF, Caron MG (1994) Cloning, pharmacological characterization, and genomic localization of the human creatine transporter. Recept Channels 2(2):165–174

Navratna V, Gouaux E (2019) Insights into the mechanism and pharmacology of neurotransmitter sodium symporters. Curr Opin Struct Biol 54:161–170. https://doi.org/10.1016/j.sbi.2019.03.011

Nawashiro H, Otani N, Shinomiya N, Fukui S, Ooigawa H, Shima K, Matsuo H, Kanai Y, Endou H (2006) L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. Int J Cancer 119(3):484–492. https://doi.org/10.1002/ijc.21866

Nazir S, Kumar A, Chatterjee I, Anbazhagan AN, Gujral T, Priyamvada S, Saksena S, Alrefai WA, Dudeja PK, Gill RK (2015) Mechanisms of intestinal serotonin transporter (SERT) upregulation by TGF-beta1 induced non-smad pathways. PLoS One 10(5):e0120447. https://doi.org/10.1371/journal.pone.0120447

Nemeroff CB, Owens MJ (2002) Treatment of mood disorders. Nat Neurosci 5(Suppl):1068–1070. https://doi.org/10.1038/nn943

Newstead S (2019) Insights into L-type heteromeric amino acid transporters. Nat Struct Mol Biol 26(6):395–396. https://doi.org/10.1038/s41594-019-0240-z

Nguyen HT, Dalmasso G, Yan Y, Obertone TS, Sitaraman SV, Merlin D (2008) Ecto-phosphorylation of CD98 regulates cell-cell interactions. PLoS One 3(12):e3895. https://doi.org/10.1371/journal.pone.0003895

Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, Yang H, Hild M, Kung C, Wilson C, Myer VE, MacKeigan JP, Porter JA, Wang YK, Cantley LC, Finan PM, Murphy LO (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. Cell 136(3):521–534. https://doi.org/10.1016/j.cell.2008.11.044

Nielsen EB, Suzdak PD, Andersen KE, Knutsen LJ, Sonnewald U, Braestrup C (1991) Characterization of tiagabine (NO-328), a new potent and selective GABA uptake inhibitor. Eur J Pharmacol 196(3):257–266

Nigg EA, Bron C, Girardet M, Cherry RJ (1980) Band 3-glycophorin A association in erythrocyte membrane demonstrated by combining protein diffusion measurements with antibody-induced cross-linking. Biochemistry 19(9):1887–1893

Nikkuni O, Kaira K, Toyoda M, Shino M, Sakakura K, Takahashi K, Tominaga H, Oriuchi N, Suzuki M, Iijima M, Asao T, Nishiyama M, Nagamori S, Kanai Y, Oyama T, Chikamatsu K (2015) Expression of amino acid transporters (LAT1 and ASCT2) in patients with stage III/IV laryngeal squamous cell carcinoma. Pathol Oncol Res 21(4):1175–1181. https://doi.org/10.1007/s12253-015-9954-3

Noble RA, Bell N, Blair H, Sikka A, Thomas H, Phillips N, Nakjang S, Miwa S, Crossland R, Rand V, Televantou D, Long A, Keun HC, Bacon CM, Bomken S, Critchlow SE, Wedge SR (2017) Inhibition of monocarboxyate transporter 1 by AZD3965 as a novel therapeutic approach for diffuse large B-cell lymphoma and Burkitt lymphoma. Haematologica 102(7):1247–1257. https://doi.org/10.3324/haematol.2016.163030

Noguchi A, Nakamura K, Murayama K, Yamamoto S, Komatsu H, Kizu R, Takayanagi M, Okuyama T, Endo F, Takasago Y, Shoji Y, Takahashi T (2016) Clinical and genetic features of lysinuric protein intolerance in Japan. Pediatr Int 58(10):979–983. https://doi.org/10.1111/ped.12946

Noor SI, Dietz S, Heidtmann H, Boone CD, McKenna R, Deitmer JW, Becker HM (2015) Analysis of the binding moiety mediating the interaction between monocarboxylate transporters and carbonic anhydrase II. J Biol Chem 290(7):4476–4486. https://doi.org/10.1074/jbc.M114.624577

Noor SI, Jamali S, Ames S, Langer S, Deitmer JW, Becker HM (2018) A surface proton antenna in carbonic anhydrase II supports lactate transport in cancer cells. Elife 7. https://doi.org/10.7554/eLife.35176

Nuiplot NO, Junking M, Duangtum N, Khunchai S, Sawasdee N, Yenchitsomanus PT, Akkarapatumwong V (2015) Transmembrane protein 139 (TMEM139) interacts with human kidney isoform of anion exchanger 1 (kAE1). Biochem Biophys Res Commun 463(4):706–711. https://doi.org/10.1016/j.bbrc.2015.05.128

O'Mara M, Oakley A, Broer S (2006) Mechanism and putative structure of B(0)-like neutral amino acid transporters. J Membr Biol 213(2):111–118. https://doi.org/10.1007/s00232-006-0879-3

Ocaranza MP, Godoy I, Jalil JE, Varas M, Collantes P, Pinto M, Roman M, Ramirez C, Copaja M, Diaz-Araya G, Castro P, Lavandero S (2006) Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. Hypertension 48(4):572–578. https://doi.org/10.1161/01.HYP.0000237862.94083.45

Ochrietor JD, Linser PJ (2004) 5A11/Basigin gene products are necessary for proper maturation and function of the retina. Dev Neurosci 26(5–6):380–387. https://doi.org/10.1159/000082280

Ochrietor JD, Moroz TP, van Ekeris L, Clamp MF, Jefferson SC, deCarvalho AC, Fadool JM, Wistow G, Muramatsu T, Linser PJ (2003) Retina-specific expression of 5A11/Basigin-2, a member of the immunoglobulin gene superfamily. Invest Ophthalmol Vis Sci 44(9):4086–4096

Oda K, Hosoda N, Endo H, Saito K, Tsujihara K, Yamamura M, Sakata T, Anzai N, Wempe MF, Kanai Y, Endou H (2010) L-type amino acid transporter 1 inhibitors inhibit tumor cell growth. Cancer Sci 101(1):173–179. https://doi.org/10.1111/j.1349-7006.2009.01386.x

Ohgimoto S, Tabata N, Suga S, Nishio M, Ohta H, Tsurudome M, Komada H, Kawano M, Watanabe N,

Ito Y (1995) Molecular characterization of fusion regulatory protein-1 (FRP-1) that induces multinucleated giant cell formation of monocytes and HIV gp160-mediated cell fusion. FRP-1 and 4F2/CD98 are identical molecules. J Immunol 155(7):3585–3592

Ohkame H, Masuda H, Ishii Y, Kanai Y (2001) Expression of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (4F2hc) in liver tumor lesions of rat models. J Surg Oncol 78(4):265–271; discussion 271-262

Ohno K, Koroll M, El Far O, Scholze P, Gomeza J, Betz H (2004) The neuronal glycine transporter 2 interacts with the PDZ domain protein syntenin-1. Mol Cell Neurosci 26(4):518–529. https://doi.org/10.1016/j.mcn.2004.04.007

Ohno Y, Suda K, Masuko K, Yagi H, Hashimoto Y, Masuko T (2008) Production and characterization of highly tumor-specific rat monoclonal antibodies recognizing the extracellular domain of human L-type amino-acid transporter 1. Cancer Sci 99 (5):1000–1007. https://doi.org/10.1111/j.1349-7006.2008.00770.x

Ohno C, Nakanishi Y, Honma T, Henmi A, Sugitani M, Kanai Y, Nemoto N (2009) Significance of system L amino acid transporter 1 (LAT-1) and 4F2 heavy chain (4F2hc) expression in human developing intestines. Acta Histochem Cytochem 42(3):73–81. https://doi.org/10.1267/ahc09010

Okamoto Y, Sakata M, Ogura K, Yamamoto T, Yamaguchi M, Tasaka K, Kurachi H, Tsurudome M, Murata Y (2002) Expression and regulation of 4F2hc and hLAT1 in human trophoblasts. Am J Physiol Cell Physiol 282(1):C196–C204. https://doi.org/10.1152/ajpcell.2002.282.1.C196

Okano N, Kawai K, Yamauchi Y, Kobayashi T, Naruge D, Nagashima F, Endou H, Furuse J (2018) First-in-human phaseIstudy of JPH203 in patients with advanced solid tumors. J Clin Oncol 36 (4 Suppl):419–419. https://doi.org/10.1200/JCO.2018.36.4_suppl.419

Okubo K, Hamasaki N, Hara K, Kageura M (1991) Palmitoylation of cysteine 69 from the COOH-terminal of band 3 protein in the human erythrocyte membrane. Acylation occurs in the middle of the consensus sequence of F--I-IICLAVL found in band 3 protein and G2 protein of Rift Valley fever virus. J Biol Chem 266(25):16420–16424

Olschok K, Vester U, Lahme S, Kurth I, Eggermann T (2018) No evidence for point mutations in the novel renal cystine transporter AGT1/SLC7A13 contributing to the etiology of cystinuria. BMC Nephrol 19(1):278. https://doi.org/10.1186/s12882-018-1080-5

Olsen J, Kokholm K, Noren O, Sjostrom H (1997) Structure and expression of aminopeptidase N. Adv Exp Med Biol 421:47–57

Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M (2006) Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 127(3):635–648. https://doi.org/10.1016/j.cell.2006.09.026

Olsen JV, Vermeulen M, Santamaria A, Kumar C, Miller ML, Jensen LJ, Gnad F, Cox J, Jensen TS, Nigg EA, Brunak S, Mann M (2010) Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. Sci Signal 3 (104):ra3. https://doi.org/10.1126/scisignal.2000475

Onda M, Fukuda M (1995) Detailed physical mapping of the genes encoding glycophorins A, B and E, as revealed by P1 plasmids containing human genomic DNA. Gene 159(2):225–230

Onda M, Kudo S, Rearden A, Mattei MG, Fukuda M (1993) Identification of a precursor genomic segment that provided a sequence unique to glycophorin B and E genes. Proc Natl Acad Sci U S A 90(15):7220–7224

Oppedisano F, Indiveri C (2008) Reconstitution into liposomes of the B degrees -like glutamine-neutral amino acid transporter from renal cell plasma membrane. Biochim Biophys Acta 1778(10):2258–2265. https://doi.org/10.1016/j.bbamem.2008.05.011

Oppedisano F, Pochini L, Broer S, Indiveri C (2011) The B degrees AT1 amino acid transporter from rat kidney reconstituted in liposomes: kinetics and inactivation by methylmercury. Biochim Biophys Acta 1808 (10):2551–2558. https://doi.org/10.1016/j.bbamem.2011.05.011

Ortiz-Melo DI, Gurley SB (2016) Angiotensin converting enzyme 2 and the kidney. Curr Opin Nephrol Hypertens 25(1):59–66. https://doi.org/10.1097/mnh.0000000000000182

Otonkoski T, Jiao H, Kaminen-Ahola N, Tapia-Paez I, Ullah MS, Parton LE, Schuit F, Quintens R, Sipila I, Mayatepek E, Meissner T, Halestrap AP, Rutter GA, Kere J (2007) Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. Am J Hum Genet 81 (3):467–474. https://doi.org/10.1086/520960

Ovens MJ, Davies AJ, Wilson MC, Murray CM, Halestrap AP (2010a) AR-C155858 is a potent inhibitor of monocarboxylate transporters MCT1 and MCT2 that binds to an intracellular site involving transmembrane helices 7-10. Biochem J 425(3):523–530. https://doi.org/10.1042/bj20091515

Ovens MJ, Manoharan C, Wilson MC, Murray CM, Halestrap AP (2010b) The inhibition of Monocarboxylate Transporter 2 (MCT2) by AR-C155858 is modulated by the associated ancillary protein. Biochem J. https://doi.org/10.1042/bj20100890

Owczarek S, Berezin V (2012) Neuroplastin: cell adhesion molecule and signaling receptor. Int J Biochem Cell Biol 44(1):1–5. https://doi.org/10.1016/j.biocel.2011.10.006

Owczarek S, Soroka V, Kiryushko D, Larsen MH, Yuan Q, Sandi C, Berezin V, Bock E (2011) Neuroplastin-65 and a mimetic peptide derived from its homophilic binding site modulate neuritogenesis and neuronal plasticity. J Neurochem 117

(6):984–994. https://doi.org/10.1111/j.1471-4159.2011.07269.x
Owens MJ, Nemeroff CB (1994) Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 40(2):288–295
Ozaslan D, Wang S, Ahmed BA, Kocabas AM, McCastlain JC, Bene A, Kilic F (2003) Glycosyl modification facilitates homo- and hetero-oligomerization of the serotonin transporter. A specific role for sialic acid residues. J Biol Chem 278(45):43991–44000. https://doi.org/10.1074/jbc.M306360200
Ozawa M, Huang RP, Furukawa T, Muramatsu T (1988) A teratocarcinoma glycoprotein carrying a developmentally regulated carbohydrate marker is a member of the immunoglobulin gene superfamily. J Biol Chem 263(7):3059–3062
Pacholczyk T, Blakely RD, Amara SG (1991) Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature 350 (6316):350–354. https://doi.org/10.1038/350350a0
Paizis G, Tikellis C, Cooper ME, Schembri JM, Lew RA, Smith AI, Shaw T, Warner FJ, Zuilli A, Burrell LM, Angus PW (2005) Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut 54(12):1790–1796. https://doi.org/10.1136/gut.2004.062398
Palacajornsuk P (2006) Review: molecular basis of MNS blood group variants. Immunohematology 22 (4):171–182
Palacin M (1994) A new family of proteins (rBAT and 4F2hc) involved in cationic and zwitterionic amino acid transport: a tale of two proteins in search of a transport function. J Exp Biol 196:123–137
Palacin M, Kanai Y (2004) The ancillary proteins of HATs: SLC3 family of amino acid transporters. Pflug Arch 447(5):490–494. https://doi.org/10.1007/s00424-003-1062-7
Palacin M, Estevez R, Bertran J, Zorzano A (1998a) Molecular biology of mammalian plasma membrane amino acid transporters. Physiol Rev 78(4):969–1054. https://doi.org/10.1152/physrev.1998.78.4.969
Palacin M, Estevez R, Zorzano A (1998b) Cystinuria calls for heteromultimeric amino acid transporters. Curr Opin Cell Biol 10(4):455–461
Palacin M, Bertran J, Zorzano A (2000) Heteromeric amino acid transporters explain inherited aminoacidurias. Curr Opin Nephrol Hypertens 9 (5):547–553
Palacin M, Bertran J, Chillaron J, Estevez R, Zorzano A (2004) Lysinuric protein intolerance: mechanisms of pathophysiology. Mol Genet Metab 81(Suppl 1):S27–S37. https://doi.org/10.1016/j.ymgme.2003.11.015
Palacin M, Nunes V, Font-Llitjos M, Jimenez-Vidal M, Fort J, Gasol E, Pineda M, Feliubadalo L, Chillaron J, Zorzano A (2005) The genetics of heteromeric amino acid transporters. Physiology (Bethesda) 20:112–124. https://doi.org/10.1152/physiol.00051.2004
Palacin M, Errasti-Murugarren E, Rosell A (2016) Heteromeric amino acid transporters. In search of the molecular bases of transport cycle mechanisms. Biochem Soc Trans 44(3):745–752. https://doi.org/10.1042/bst20150294
Pang AJ, Reithmeier RA (2009) Interaction of anion exchanger 1 and glycophorin A in human erythroleukaemic K562 cells. Biochem J 421 (3):345–356. https://doi.org/10.1042/bj20090345
Paquette AM, Harahap A, Laosombat V, Patnode JM, Satyagraha A, Sudoyo H, Thompson MK, Yusoff NM, Wilder JA (2015) The evolutionary origins of Southeast Asian ovalocytosis. Infect Genet Evol 34:153–159. https://doi.org/10.1016/j.meegid.2015.06.002
Parajuli N, Ramprasath T, Patel VB, Wang W, Putko B, Mori J, Oudit GY (2014) Targeting angiotensin-converting enzyme 2 as a new therapeutic target for cardiovascular diseases. Can J Physiol Pharmacol 92 (7):558–565. https://doi.org/10.1139/cjpp-2013-0488
Parker MD, Boron WF (2013) The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. Physiol Rev 93(2):803–959. https://doi.org/10.1152/physrev.00023.2012
Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM (1999) Bile acids: natural ligands for an orphan nuclear receptor. Science 284(5418):1365–1368
Parks SK, Cormerais Y, Marchiq I, Pouyssegur J (2016) Hypoxia optimises tumour growth by controlling nutrient import and acidic metabolite export. Mol Aspects Med 47-48:3–14. https://doi.org/10.1016/j.mam.2015.12.001
Parmacek MS, Karpinski BA, Gottesdiener KM, Thompson CB, Leiden JM (1989) Structure, expression and regulation of the murine 4F2 heavy chain. Nucleic Acids Res 17(5):1915–1931
Parra LA, Baust T, El Mestikawy S, Quiroz M, Hoffman B, Haflett JM, Yao JK, Torres GE (2008) The orphan transporter Rxt1/NTT4 (SLC6A17) functions as a synaptic vesicle amino acid transporter selective for proline, glycine, leucine, and alanine. Mol Pharmacol 74(6):1521–1532. https://doi.org/10.1124/mol.108.050005
Parvari R, Brodyansky I, Elpeleg O, Moses S, Landau D, Hershkovitz E (2001) A recessive contiguous gene deletion of chromosome 2p16 associated with cystinuria and a mitochondrial disease. Am J Hum Genet 69 (4):869–875. https://doi.org/10.1086/323624
Parvari R, Gonen Y, Alshafee I, Buriakovsky S, Regev K, Hershkovitz E (2005) The 2p21 deletion syndrome: characterization of the transcription content. Genomics 86(2):195–211. https://doi.org/10.1016/j.ygeno.2005.04.001
Pasquali L, d'Annunzio G, Gastaldi R, Di Battista E, Calcaterra V, Larizza D, Lorini R, D'Amato E (2009) Collectrin gene screening in Turner syndrome patients with kidney malformation. J Genet 88(1):105–108
Passow H (1986) Molecular aspects of band 3 protein-mediated anion transport across the red blood cell

membrane. Rev Physiol Biochem Pharmacol 103:61–203
Patel SA, Warren BA, Rhoderick JF, Bridges RJ (2004) Differentiation of substrate and non-substrate inhibitors of transport system xc(-): an obligate exchanger of L-glutamate and L-cystine. Neuropharmacology 46(2):273–284
Patel VB, Parajuli N, Oudit GY (2014) Role of angiotensin-converting enzyme 2 (ACE2) in diabetic cardiovascular complications. Clin Sci (Lond) 126 (7):471–482. https://doi.org/10.1042/cs20130344
Patterson ST, Li J, Kang JA, Wickrema A, Williams DB, Reithmeier RA (2009) Loss of specific chaperones involved in membrane glycoprotein biosynthesis during the maturation of human erythroid progenitor cells. J Biol Chem 284(21):14547–14557. https://doi.org/10.1074/jbc.M809076200
Paulitschke M, Nash GB, Anstee DJ, Tanner MJ, Gratzer WB (1995) Perturbation of red blood cell membrane rigidity by extracellular ligands. Blood 86(1):342–348
Pavone BG, Issitt PD, Wagstaff W (1977) Independence of Wright from many other blood group systems. Transfusion 17(1):47–49
Payen VL, Hsu MY, Radecke KS, Wyart E, Vazeille T, Bouzin C, Porporato PE, Sonveaux P (2017) Monocarboxylate transporter MCT1 promotes tumor metastasis independently of its activity as a lactate transporter. Cancer Res 77(20):5591–5601. https://doi.org/10.1158/0008-5472.Can-17-0764
Paz A, Claxton DP, Kumar JP, Kazmier K, Bisignano P, Sharma S, Nolte SA, Liwag TM, Nayak V, Wright EM, Grabe M, McHaourab HS, Abramson J (2018) Conformational transitions of the sodium-dependent sugar transporter, vSGLT. Proc Natl Acad Sci U S A 115(12):E2742–E2751. https://doi.org/10.1073/pnas.1718451115
Pedersen SF, Counillon L (2019) The SLC9A-C mammalian Na(+)/H(+) exchanger family: molecules, mechanisms, and physiology. Physiol Rev 99 (4):2015–2113. https://doi.org/10.1152/physrev.00028.2018
Penmatsa A, Gouaux E (2014) How LeuT shapes our understanding of the mechanisms of sodium-coupled neurotransmitter transporters. J Physiol 592 (Pt 5):863–869. https://doi.org/10.1113/jphysiol.2013.259051
Penmatsa A, Wang KH, Gouaux E (2013) X-ray structure of dopamine transporter elucidates antidepressant mechanism. Nature 503(7474):85–90. https://doi.org/10.1038/nature12533
Penmatsa A, Wang KH, Gouaux E (2015) X-ray structures of Drosophila dopamine transporter in complex with nisoxetine and reboxetine. Nat Struct Mol Biol 22 (6):506–508. https://doi.org/10.1038/nsmb.3029
Pepaj M, Gjerlaugsen N, Julien K, Thorsby PM (2014) Tmem27 is upregulated by vitamin D in INS-1 cells and its serum concentrations are low in patients with autoimmune diabetes. Scand J Clin Lab Invest 74 (4):358–365. https://doi.org/10.3109/00365513.2014.898322
Pereira CA, Saye M, Wrenger C, Miranda MR (2014) Metabolite transporters in trypanosomatid parasites: promising therapeutic targets but... How to deal with them? Curr Med Chem 21(15):1707–1712
Perez C, Koshy C, Yildiz O, Ziegler C (2012) Alternating-access mechanism in conformationally asymmetric trimers of the betaine transporter BetP. Nature 490 (7418):126–130. https://doi.org/10.1038/nature11403
Perez C, Faust B, Mehdipour AR, Francesconi KA, Forrest LR, Ziegler C (2014) Substrate-bound outward-open state of the betaine transporter BetP provides insights into Na+ coupling. Nat Commun 5:4231. https://doi.org/10.1038/ncomms5231
Perheentupa J, Visakorpi JK (1965) Protein intolerance with deficient transport of basic aminoacids. Another inborn error of metabolism. Lancet 2(7417):813–816
Perland E, Fredriksson R (2017) Classification systems of secondary active transporters. Trends Pharmacol Sci 38(3):305–315. https://doi.org/10.1016/j.tips.2016.11.008
Perlot T, Penninger JM (2013) ACE2 – from the renin-angiotensin system to gut microbiota and malnutrition. Microbes Infect 15(13):866–873. https://doi.org/10.1016/j.micinf.2013.08.003
Peter GJ, Panova TB, Christie GR, Taylor PM (2000) Cysteine residues in the C-terminus of the neutral- and basic-amino-acid transporter heavy-chain subunit contribute to functional properties of the system b(0,+)-type amino acid transporter. Biochem J 351 (Pt 3):677–682
Peura L, Malmioja K, Laine K, Leppanen J, Gynther M, Isotalo A, Rautio J (2011) Large amino acid transporter 1 (LAT1) prodrugs of valproic acid: new prodrug design ideas for central nervous system delivery. Mol Pharm 8(5):1857–1866. https://doi.org/10.1021/mp2001878
Peura L, Malmioja K, Huttunen K, Leppanen J, Hamalainen M, Forsberg MM, Gynther M, Rautio J, Laine K (2013) Design, synthesis and brain uptake of LAT1-targeted amino acid prodrugs of dopamine. Pharm Res 30(10):2523–2537
Pfeiffer R, Spindler B, Loffing J, Skelly PJ, Shoemaker CB, Verrey F (1998) Functional heterodimeric amino acid transporters lacking cysteine residues involved in disulfide bond. FEBS Lett 439(1–2):157–162
Pfeiffer R, Loffing J, Rossier G, Bauch C, Meier C, Eggermann T, Loffing-Cueni D, Kuhn LC, Verrey F (1999a) Luminal heterodimeric amino acid transporter defective in cystinuria. Mol Biol Cell 10 (12):4135–4147
Pfeiffer R, Rossier G, Spindler B, Meier C, Kuhn L, Verrey F (1999b) Amino acid transport of y+L-type by heterodimers of 4F2hc/CD98 and members of the glycoprotein-associated amino acid transporter family. EMBO J 18(1):49–57. https://doi.org/10.1093/emboj/18.1.49

Philp NJ, Yoon H, Grollman EF (1998) Monocarboxylate transporter MCT1 is located in the apical membrane and MCT3 in the basal membrane of rat RPE. Am J Physiol 274(6 Pt 2):R1824–R1828

Philp NJ, Ochrietor JD, Rudoy C, Muramatsu T, Linser PJ (2003a) Loss of MCT1, MCT3, and MCT4 expression in the retinal pigment epithelium and neural retina of the 5A11/basigin-null mouse. Invest Ophthalmol Vis Sci 44(3):1305–1311

Philp NJ, Wang D, Yoon H, Hjelmeland LM (2003b) Polarized expression of monocarboxylate transporters in human retinal pigment epithelium and ARPE-19 cells. Invest Ophthalmol Vis Sci 44(4):1716–1721

Picard V, Proust A, Eveillard M, Flatt JF, Couec ML, Caillaux G, Feneant-Thibault M, Finkelstein A, Raphael M, Delaunay J, Bruce LJ, Pissard S, Thomas C (2014) Homozygous Southeast Asian ovalocytosis is a severe dyserythropoietic anemia associated with distal renal tubular acidosis. Blood 123(12):1963–1965. https://doi.org/10.1182/blood-2014-01-548149

Pillai NR, Yubero D, Shayota BJ, Oyarzabal A, Ghosh R, Sun Q, Azamian MS, Arjona C, Brandi N, Palau F, Lalani SR, Artuch R, Garcia-Cazorla A, Scott DA (2019) Loss of CLTRN function produces a neuropsychiatric disorder and a biochemical phenotype that mimics Hartnup disease. Am J Med Genet A 179 (12):2459–2468. https://doi.org/10.1002/ajmg.a.61357

Pinard E, Alanine A, Alberati D, Bender M, Borroni E, Bourdeaux P, Brom V, Burner S, Fischer H, Hainzl D, Halm R, Hauser N, Jolidon S, Lengyel J, Marty HP, Meyer T, Moreau JL, Mory R, Narquizian R, Nettekoven M, Norcross RD, Puellmann B, Schmid P, Schmitt S, Stalder H, Wermuth R, Wettstein JG, Zimmerli D (2010) Selective GlyT1 inhibitors: discovery of [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a promising novel medicine to treat schizophrenia. J Med Chem 53(12):4603–4614. https://doi.org/10.1021/jm100210p

Pineda M, Fernandez E, Torrents D, Estevez R, Lopez C, Camps M, Lloberas J, Zorzano A, Palacin M (1999) Identification of a membrane protein, LAT-2, that Co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. J Biol Chem 274 (28):19738–19744

Pineda M, Wagner CA, Broer A, Stehberger PA, Kaltenbach S, Gelpi JL, Martin Del Rio R, Zorzano A, Palacin M, Lang F, Broer S (2004) Cystinuria-specific rBAT(R365W) mutation reveals two translocation pathways in the amino acid transporter rBAT-b0,+AT. Biochem J 377(Pt 3):665–674. https://doi.org/10.1042/bj20030956

Pinheiro C, Longatto-Filho A, Scapulatempo C, Ferreira L, Martins S, Pellerin L, Rodrigues M, Alves VA, Schmitt F, Baltazar F (2008) Increased expression of monocarboxylate transporters 1, 2, and 4 in colorectal carcinomas. Virchows Arch 452(2):139–146. https://doi.org/10.1007/s00428-007-0558-5

Pinheiro C, Longatto-Filho A, Pereira SM, Etlinger D, Moreira MA, Jube LF, Queiroz GS, Schmitt F, Baltazar F (2009a) Monocarboxylate transporters 1 and 4 are associated with CD147 in cervical carcinoma. Dis Markers 26(3):97–103. https://doi.org/10.3233/dma-2009-0596

Pinheiro C, Longatto-Filho A, Simoes K, Jacob CE, Bresciani CJ, Zilberstein B, Cecconello I, Alves VA, Schmitt F, Baltazar F (2009b) The prognostic value of CD147/EMMPRIN is associated with monocarboxylate transporter 1 co-expression in gastric cancer. Eur J Cancer 45(13):2418–2424. https://doi.org/10.1016/j.ejca.2009.06.018

Pinheiro C, Albergaria A, Paredes J, Sousa B, Dufloth R, Vieira D, Schmitt F, Baltazar F (2010a) Monocarboxylate transporter 1 is up-regulated in basal-like breast carcinoma. Histopathology 56 (7):860–867. https://doi.org/10.1111/j.1365-2559.2010.03560.x

Pinheiro C, Reis RM, Ricardo S, Longatto-Filho A, Schmitt F, Baltazar F (2010b) Expression of monocarboxylate transporters 1, 2, and 4 in human tumours and their association with CD147 and CD44. J Biomed Biotechnol 2010:427694. https://doi.org/10.1155/2010/427694

Pinheiro C, Sousa B, Albergaria A, Paredes J, Dufloth R, Vieira D, Schmitt F, Baltazar F (2011) GLUT1 and CAIX expression profiles in breast cancer correlate with adverse prognostic factors and MCT1 overexpression. Histol Histopathol 26 (10):1279–1286. https://doi.org/10.14670/hh-26.1279

Pisano A, Redmond JW, Williams KL, Gooley AA (1993) Glycosylation sites identified by solid-phase Edman degradation: O-linked glycosylation motifs on human glycophorin A. Glycobiology 3(5):429–435

Piscitelli CL, Gouaux E (2012) Insights into transport mechanism from LeuT engineered to transport tryptophan. EMBO J 31(1):228–235. https://doi.org/10.1038/emboj.2011.353

Piscitelli CL, Krishnamurthy H, Gouaux E (2010) Neurotransmitter/sodium symporter orthologue LeuT has a single high-affinity substrate site. Nature 468 (7327):1129–1132. https://doi.org/10.1038/nature09581

Pitt BR, Weng W, Steve AR, Blakely RD, Reynolds I, Davies P (1994) Serotonin increases DNA synthesis in rat proximal and distal pulmonary vascular smooth muscle cells in culture. Am J Physiol 266(2 Pt 1): L178–L186

Plakidou-Dymock S, McGivan JD (1993) The oligomeric structure of renal aminopeptidase N from bovine brush-border membrane vesicles. Biochim Biophys Acta 1145(1):105–112. https://doi.org/10.1016/0005-2736(93)90386-e

Plakidou-Dymock S, Tanner MJ, McGivan JD (1993) A role for aminopeptidase N in Na(+)-dependent amino acid transport in bovine renal brush-border membranes. Biochem J 290(Pt 1):59–65

Planells-Cases R, Ferrer-Montiel A (2007) Frontiers in neuroscience TRP channel trafficking. In: Liedtke WB, Heller S (eds) TRP ion channel function in

sensory transduction and cellular signaling cascades. CRC Press/Taylor & Francis, Boca Raton

Pochini L, Seidita A, Sensi C, Scalise M, Eberini I, Indiveri C (2014) Nimesulide binding site in the B0AT1 (SLC6A19) amino acid transporter. Mechanism of inhibition revealed by proteoliposome transport assay and molecular modelling. Biochem Pharmacol 89(3):422–430. https://doi.org/10.1016/j.bcp.2014.03.014

Polanski R, Hodgkinson CL, Fusi A, Nonaka D, Priest L, Kelly P, Trapani F, Bishop PW, White A, Critchlow SE, Smith PD, Blackhall F, Dive C, Morrow CJ (2014) Activity of the monocarboxylate transporter 1 inhibitor AZD3965 in small cell lung cancer. Clin Cancer Res 20(4):926–937. https://doi.org/10.1158/1078-0432.Ccr-13-2270

Ponzoni L, Zhang S, Cheng MH, Bahar I (2018) Shared dynamics of LeuT superfamily members and allosteric differentiation by structural irregularities and multimerization. Philos Trans R Soc Lond B Biol Sci 373(1749). https://doi.org/10.1098/rstb.2017.0177

Poole J (2000) Red cell antigens on band 3 and glycophorin A. Blood Rev 14(1):31–43. https://doi.org/10.1054/blre.1999.0124

Poole RC, Halestrap AP (1992) Identification and partial purification of the erythrocyte L-lactate transporter. Biochem J 283(Pt 3):855–862

Poole RC, Halestrap AP (1994) N-terminal protein sequence analysis of the rabbit erythrocyte lactate transporter suggests identity with the cloned monocarboxylate transport protein MCT1. Biochem J 303(Pt 3):755–759

Poole RC, Halestrap AP (1997) Interaction of the erythrocyte lactate transporter (monocarboxylate transporter 1) with an integral 70-kDa membrane glycoprotein of the immunoglobulin superfamily. J Biol Chem 272 (23):14624–14628

Poole J, Banks J, Bruce LJ, Ring SM, Levene C, Stern H, Overbeeke MA, Tanner MJ (1999) Glycophorin A mutation Ala65 --> Pro gives rise to a novel pair of MNS alleles ENEP (MNS39) and HAG (MNS41) and altered Wrb expression: direct evidence for GPA/band 3 interaction necessary for normal Wrb expression. Transfus Med 9(2):167–174

Poulopoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, Jedlicka P, Schwarzacher SW, Betz H, Harvey RJ, Brose N, Zhang W, Varoqueaux F (2009) Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. Neuron 63(5):628–642. https://doi.org/10.1016/j.neuron.2009.08.023

Prager GW, Feral CC, Kim C, Han J, Ginsberg MH (2007) CD98hc (SLC3A2) interaction with the integrin beta subunit cytoplasmic domain mediates adhesive signaling. J Biol Chem 282(33):24477–24484. https://doi.org/10.1074/jbc.M702877200

Pramod AB, Foster J, Carvelli L, Henry LK (2013) SLC6 transporters: structure, function, regulation, disease association and therapeutics. Mol Aspects Med 34 (2–3):197–219. https://doi.org/10.1016/j.mam.2012.07.002

Pras E, Raben N, Golomb E, Arber N, Aksentijevich I, Schapiro JM, Harel D, Katz G, Liberman U, Pras M et al (1995) Mutations in the SLC3A1 transporter gene in cystinuria. Am J Hum Genet 56(6):1297–1303

Prasad PD, Wang H, Huang W, Kekuda R, Rajan DP, Leibach FH, Ganapathy V (1999) Human LAT1, a subunit of system L amino acid transporter: molecular cloning and transport function. Biochem Biophys Res Commun 255(2):283–288. https://doi.org/10.1006/bbrc.1999.0206

Price NT, Jackson VN, Halestrap AP (1998) Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochem J 329(Pt 2):321–328

Puris E, Gynther M, Huttunen J, Petsalo A, Huttunen KM (2017) L-type amino acid transporter 1 utilizing prodrugs: how to achieve effective brain delivery and low systemic exposure of drugs. J Control Release 261:93–104. https://doi.org/10.1016/j.jconrel.2017.06.023

Qaradakhi T, Gadanec LK, McSweeney KR, Tacey A, Apostolopoulos V, Levinger I, Rimarova K, Egom EE, Rodrigo L, Kruzliak P, Kubatka P, Zulli A (2020) The potential actions of angiotensin converting enzyme II (ACE2) activator Diminazene aceturate (DIZE) in various diseases. Clin Exp Pharmacol Physiol. https://doi.org/10.1111/1440-1681.13251

Qian Y, Galli A, Ramamoorthy S, Risso S, DeFelice LJ, Blakely RD (1997) Protein kinase C activation regulates human serotonin transporters in HEK-293 cells via altered cell surface expression. J Neurosci 17 (1):45–57

Qin Z, Freitas E, Sullivan R, Mohan S, Bacelieri R, Branch D, Romano M, Kearney P, Oates J, Plaisance K, Renne R, Kaleeba J, Parsons C (2010) Upregulation of xCT by KSHV-encoded microRNAs facilitates KSHV dissemination and persistence in an environment of oxidative stress. PLoS Pathog 6(1): e1000742. https://doi.org/10.1371/journal.ppat.1000742

Quackenbush E, Clabby M, Gottesdiener KM, Barbosa J, Jones NH, Strominger JL, Speck S, Leiden JM (1987) Molecular cloning of complementary DNAs encoding the heavy chain of the human 4F2 cell-surface antigen: a type II membrane glycoprotein involved in normal and neoplastic cell growth. Proc Natl Acad Sci U S A 84(18):6526–6530

Quick MW (2002) Role of syntaxin 1A on serotonin transporter expression in developing thalamocortical neurons. Int J Dev Neurosci 20(3–5):219–224

Quick MW (2003) Regulating the conducting states of a mammalian serotonin transporter. Neuron 40 (3):537–549

Quick MW (2006) The role of SNARE proteins in trafficking and function of neurotransmitter transporters. Handb Exp Pharmacol 175:181–196

Quick M, Javitch JA (2007) Monitoring the function of membrane transport proteins in detergent-solubilized form. Proc Natl Acad Sci U S A 104(9):3603–3608. https://doi.org/10.1073/pnas.0609573104

Quick MW, Corey JL, Davidson N, Lester HA (1997) Second messengers, trafficking-related proteins, and amino acid residues that contribute to the functional regulation of the rat brain GABA transporter GAT1. J Neurosci 17(9):2967–2979

Quick M, Winther AM, Shi L, Nissen P, Weinstein H, Javitch JA (2009) Binding of an octylglucoside detergent molecule in the second substrate (S2) site of LeuT establishes an inhibitor-bound conformation. Proc Natl Acad Sci U S A 106(14):5563–5568. https://doi.org/10.1073/pnas.0811322106

Quick M, Abramyan AM, Wiriyasermkul P, Weinstein H, Shi L, Javitch JA (2018) The LeuT-fold neurotransmitter: sodium symporter MhsT has two substrate sites. Proc Natl Acad Sci U S A 115(34):E7924–e7931. https://doi.org/10.1073/pnas.1717444115

Quistgaard EM, Low C, Guettou F, Nordlund P (2016) Understanding transport by the major facilitator superfamily (MFS): structures pave the way. Nat Rev Mol Cell Biol 17(2):123–132. https://doi.org/10.1038/nrm.2015.25

Radestock S, Forrest LR (2011) The alternating-access mechanism of MFS transporters arises from inverted-topology repeats. J Mol Biol 407(5):698–715. https://doi.org/10.1016/j.jmb.2011.02.008

Rahbek-Clemmensen T, Lycas MD, Erlendsson S, Eriksen J, Apuschkin M, Vilhardt F, Jorgensen TN, Hansen FH, Gether U (2017) Super-resolution microscopy reveals functional organization of dopamine transporters into cholesterol and neuronal activity-dependent nanodomains. Nat Commun 8(1):740. https://doi.org/10.1038/s41467-017-00790-3

Rainero E, Howe JD, Caswell PT, Jamieson NB, Anderson K, Critchley DR, Machesky L, Norman JC (2015) Ligand-occupied integrin internalization links nutrient signaling to invasive migration. Cell Rep. https://doi.org/10.1016/j.celrep.2014.12.037

Rajan DP, Kekuda R, Huang W, Wang H, Devoe LD, Leibach FH, Prasad PD, Ganapathy V (1999) Cloning and expression of a b(0,+)-like amino acid transporter functioning as a heterodimer with 4F2hc instead of rBAT. A new candidate gene for cystinuria. J Biol Chem 274(41):29005–29010

Rajan DP, Kekuda R, Huang W, Devoe LD, Leibach FH, Prasad PD, Ganapathy V (2000) Cloning and functional characterization of a Na(+)-independent, broad-specific neutral amino acid transporter from mammalian intestine. Biochim Biophys Acta 1463(1):6–14

Ramamoorthy S, Prasad PD, Kulanthaivel P, Leibach FH, Blakely RD, Ganapathy V (1993) Expression of a cocaine-sensitive norepinephrine transporter in the human placental syncytiotrophoblast. Biochemistry 32(5):1346–1353

Ramamoorthy S, Leibach FH, Mahesh VB, Han H, Yang-Feng T, Blakely RD, Ganapathy V (1994) Functional characterization and chromosomal localization of a cloned taurine transporter from human placenta. Biochem J 300(Pt 3):893–900

Ramamoorthy S, Giovanetti E, Qian Y, Blakely RD (1998) Phosphorylation and regulation of antidepressant-sensitive serotonin transporters. J Biol Chem 273(4):2458–2466

Ramos HE (2014) Thyroid hormone cell membrane transport defect. Endocr Dev 26:108–117. https://doi.org/10.1159/000363158

Rao A, Haywood J, Craddock AL, Belinsky MG, Kruh GD, Dawson PA (2008) The organic solute transporter alpha-beta, Ostalpha-Ostbeta, is essential for intestinal bile acid transport and homeostasis. Proc Natl Acad Sci U S A 105(10):3891–3896. https://doi.org/10.1073/pnas.0712328105

Rasola A, Galietta LJ, Barone V, Romeo G, Bagnasco S (1995) Molecular cloning and functional characterization of a GABA/betaine transporter from human kidney. FEBS Lett 373(3):229–233

Razavi AM, Khelashvili G, Weinstein H (2017) A Markov state-based quantitative kinetic model of sodium release from the dopamine transporter. Sci Rep 7:40076. https://doi.org/10.1038/srep40076

Razavi AM, Khelashvili G, Weinstein H (2018) How structural elements evolving from bacterial to human SLC6 transporters enabled new functional properties. BMC Biol 16(1):31. https://doi.org/10.1186/s12915-018-0495-6

Rearden A (1985) Phospholipid dependence of Wrb antigen expression in human erythrocyte membranes. Vox Sang 49(5):346–353

Rearden A, Taetle R, Elmajian DA, Majda JA, Baird SM (1985) Glycophorin A on normal and leukemia cells detected by monoclonal antibodies, including a new monoclonal antibody reactive with glycophorins A and B. Mol Immunol 22(4):369–378

Redzic JS, Armstrong GS, Isern NG, Jones DN, Kieft JS, Eisenmesser EZ (2011) The retinal specific CD147 Ig0 domain: from molecular structure to biological activity. J Mol Biol 411(1):68–82. https://doi.org/10.1016/j.jmb.2011.04.060

Rees DC, Johnson E, Lewinson O (2009) ABC transporters: the power to change. Nat Rev Mol Cell Biol 10(3):218–227. https://doi.org/10.1038/nrm2646

Reid ME (2009) MNS blood group system: a review. Immunohematology 25(3):95–101

Reig N, Chillaron J, Bartoccioni P, Fernandez E, Bendahan A, Zorzano A, Kanner B, Palacin M, Bertran J (2002) The light subunit of system b(o,+) is fully functional in the absence of the heavy subunit. EMBO J 21(18):4906–4914

Reithmeier RA (1979) Fragmentation of the band 3 polypeptide from human erythrocyte membranes. Size and

detergent binding of the membrane-associated domain. J Biol Chem 254(8):3054–3060
Reithmeier RA, Casey JR, Kalli AC, Sansom MS, Alguel Y, Iwata S (2016) Band 3, the human red cell chloride/bicarbonate anion exchanger (AE1, SLC4A1), in a structural context. Biochim Biophys Acta 1858(7 Pt A):1507–1532. https://doi.org/10.1016/j.bbamem.2016.03.030
Rella M, Rushworth CA, Guy JL, Turner AJ, Langer T, Jackson RM (2006) Structure-based pharmacophore design and virtual screening for novel angiotensin converting enzyme 2 inhibitors. J Chem Inf Model 46 (2):708–716. https://doi.org/10.1021/ci0503614
Ressl S, Terwisscha van Scheltinga AC, Vonrhein C, Ott V, Ziegler C (2009) Molecular basis of transport and regulation in the Na(+)/betaine symporter BetP. Nature 458(7234):47–52. https://doi.org/10.1038/nature07819
Ridgwell K, Tanner MJ, Anstee DJ (1983) The Wrb antigen, a receptor for Plasmodium falciparum malaria, is located on a helical region of the major membrane sialoglycoprotein of human red blood cells. Biochem J 209(1):273–276
Ridgwell K, Tanner MJ, Anstee DJ (1984) The Wrb antigen in Sta-positive and Dantu-positive human erythrocytes. J Immunogenet 11(5–6):365–370
Riemann D, Kehlen A, Langner J (1999) CD13 – not just a marker in leukemia typing. Immunol Today 20 (2):83–88
Riethdorf S, Reimers N, Assmann V, Kornfeld JW, Terracciano L, Sauter G, Pantel K (2006) High incidence of EMMPRIN expression in human tumors. Int J Cancer 119(8):1800–1810. https://doi.org/10.1002/ijc.22062
Rigbolt KT, Prokhorova TA, Akimov V, Henningsen J, Johansen PT, Kratchmarova I, Kassem M, Mann M, Olsen JV, Blagoev B (2011) System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. Sci Signal 4(164):rs3. https://doi.org/10.1126/scisignal.2001570
Ring SM, Tippett P, Swallow DM (1994) Comparative immunochemical analysis of Wra and Wrb red cell antigens. Vox Sang 67(2):226–230
Risinger MA, Dotimas EM, Cohen CM (1992) Human erythrocyte protein 4.2, a high copy number membrane protein, is N-myristylated. J Biol Chem 267 (8):5680–5685
Ritchie JW, Taylor PM (2001) Role of the System L permease LAT1 in amino acid and iodothyronine transport in placenta. Biochem J 356(Pt 3):719–725
Rius M, Chillaron J (2012) Carrier subunit of plasma membrane transporter is required for oxidative folding of its helper subunit. J Biol Chem 287 (22):18190–18200. https://doi.org/10.1074/jbc.M111.321943
Rius M, Sala L, Chillaron J (2016) The role of N-glycans and the C-terminal loop of the subunit rBAT in the biogenesis of the cystinuria-associated transporter. Biochem J 473(3):233–244. https://doi.org/10.1042/bj20150846
Rives ML, Javitch JA, Wickenden AD (2017) Potentiating SLC transporter activity: emerging drug discovery opportunities. Biochem Pharmacol 135:1–11. https://doi.org/10.1016/j.bcp.2017.02.010
Rizo J, Xu J (2015) The synaptic vesicle release machinery. Annu Rev Biophys 44:339–367. https://doi.org/10.1146/annurev-biophys-060414-034057
Rogers ME, Williams DT, Niththyananthan R, Rampling MW, Heslop KE, Johnston DG (1992) Decrease in erythrocyte glycophorin sialic acid content is associated with increased erythrocyte aggregation in human diabetes. Clin Sci (Lond) 82(3):309–313
Romei C, Raiteri L (2016) Advances in understanding the functions of native GlyT1 and GlyT2 neuronal glycine transporters. Neurochem Int 99:169–177. https://doi.org/10.1016/j.neuint.2016.07.001
Romeo E, Dave MH, Bacic D, Ristic Z, Camargo SM, Loffing J, Wagner CA, Verrey F (2006) Luminal kidney and intestine SLC6 amino acid transporters of B0AT-cluster and their tissue distribution in Mus musculus. Am J Physiol Renal Physiol 290(2):F376–F383. https://doi.org/10.1152/ajprenal.00286.2005
Romero MF, Chen AP, Parker MD, Boron WF (2013) The SLC4 family of bicarbonate (HCO(3)(-)) transporters. Mol Aspects Med 34(2–3):159–182. https://doi.org/10.1016/j.mam.2012.10.008
Rosario FJ, Kanai Y, Powell TL, Jansson T (2013) Mammalian target of rapamycin signalling modulates amino acid uptake by regulating transporter cell surface abundance in primary human trophoblast cells. J Physiol 591(3):609–625. https://doi.org/10.1113/jphysiol.2012.238014
Rosell A, Meury M, Alvarez-Marimon E, Costa M, Perez-Cano L, Zorzano A, Fernandez-Recio J, Palacin M, Fotiadis D (2014) Structural bases for the interaction and stabilization of the human amino acid transporter LAT2 with its ancillary protein 4F2hc. Proc Natl Acad Sci U S A 111(8):2966–2971. https://doi.org/10.1073/pnas.1323779111
Rosenberg LE, Downing S, Durant JL, Segal S (1966) Cystinuria: biochemical evidence for three genetically distinct diseases. J Clin Invest 45(3):365–371. https://doi.org/10.1172/jci105351
Rosenberg D, Artoul S, Segal AC, Kolodney G, Radzishevsky I, Dikopoltsev E, Foltyn VN, Inoue R, Mori H, Billard JM, Wolosker H (2013) Neuronal D-serine and glycine release via the Asc-1 transporter regulates NMDA receptor-dependent synaptic activity. J Neurosci 33(8):3533–3544. https://doi.org/10.1523/jneurosci.3836-12.2013
Rossier G, Meier C, Bauch C, Summa V, Sordat B, Verrey F, Kuhn LC (1999) LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. J Biol Chem 274(49):34948–34954
Rotmann A, Simon A, Martine U, Habermeier A, Closs EI (2007) Activation of classical protein kinase C decreases transport via systems y+ and y+L. Am J

Physiol Cell Physiol 292(6):C2259–C2268. https://doi.org/10.1152/ajpcell.00323.2006

Rotoli BM, Bussolati O, Sala R, Barilli A, Talarico E, Gazzola GC, Dall'Asta V (2004) INFgamma stimulates arginine transport through system y+L in human monocytes. FEBS Lett 571(1–3):177–181. https://doi.org/10.1016/j.febslet.2004.06.086

Rotoli BM, Bussolati O, Sala R, Gazzola GC, Dall'Asta V (2005) The transport of cationic amino acids in human airway cells: expression of system y+L activity and transepithelial delivery of NOS inhibitors. FASEB J 19(7):810–812. https://doi.org/10.1096/fj.04-2924fje

Rotoli BM, Barilli A, Visigalli R, Ingoglia F, Milioli M, Di Lascia M, Riccardi B, Puccini P, Dall'Asta V (2018) Downregulation of SLC7A7 triggers an inflammatory phenotype in human macrophages and airway epithelial cells. Front Immunol 9:508. https://doi.org/10.3389/fimmu.2018.00508

Rudnick G (1977) Active transport of 5-hydroxytryptamine by plasma membrane vesicles isolated from human blood platelets. J Biol Chem 252(7):2170–2174

Rudnick G, Kramer R, Blakely RD, Murphy DL, Verrey F (2014) The SLC6 transporters: perspectives on structure, functions, regulation, and models for transporter dysfunction. Pflug Arch 466(1):25–42. https://doi.org/10.1007/s00424-013-1410-1

Rungroj N, Devonald MA, Cuthbert AW, Reimann F, Akkarapatumwong V, Yenchitsomanus PT, Bennett WM, Karet FE (2004) A novel missense mutation in AE1 causing autosomal dominant distal renal tubular acidosis retains normal transport function but is mistargeted in polarized epithelial cells. J Biol Chem 279(14):13833–13838. https://doi.org/10.1074/jbc.M400188200

Rusu V, Hoch E, Mercader JM, Tenen DE, Gymrek M, Hartigan CR, DeRan M, von Grotthuss M, Fontanillas P, Spooner A, Guzman G, Deik AA, Pierce KA, Dennis C, Clish CB, Carr SA, Wagner BK, Schenone M, Ng MCY, Chen BH, Centeno-Cruz F, Zerrweck C, Orozco L, Altshuler DM, Schreiber SL, Florez JC, Jacobs SBR, Lander ES (2017) Type 2 diabetes variants disrupt function of SLC16A11 through two distinct mechanisms. Cell 170(1):199–212.e120. https://doi.org/10.1016/j.cell.2017.06.011

Sacher A, Nelson N, Ogi JT, Wright EM, Loo DD, Eskandari S (2002) Presteady-state and steady-state kinetics and turnover rate of the mouse gamma-aminobutyric acid transporter (mGAT3). J Membr Biol 190(1):57–73. https://doi.org/10.1007/s00232-002-1024-6

Sahota A, Tischfield JA, Goldfarb DS, Ward MD, Hu L (2019) Cystinuria: genetic aspects, mouse models, and a new approach to therapy. Urolithiasis 47(1):57–66. https://doi.org/10.1007/s00240-018-1101-7

Sahr KE, Taylor WM, Daniels BP, Rubin HL, Jarolim P (1994) The structure and organization of the human erythroid anion exchanger (AE1) gene. Genomics 24 (3):491–501. https://doi.org/10.1006/geno.1994.1658

Saier MH Jr (2016) Transport protein evolution deduced from analysis of sequence, topology and structure. Curr Opin Struct Biol 38:9–17. https://doi.org/10.1016/j.sbi.2016.05.001

Saier MH Jr, Reddy VS, Tamang DG, Vastermark A (2014) The transporter classification database. Nucleic Acids Res 42(Database issue):D251–D258. https://doi.org/10.1093/nar/gkt1097

Saier MH Jr, Reddy VS, Tsu BV, Ahmed MS, Li C, Moreno-Hagelsieb G (2016) The Transporter Classification Database (TCDB): recent advances. Nucleic Acids Res 44(D1):D372–D379. https://doi.org/10.1093/nar/gkv1103

Saisho K, Fukuhara A, Yasuda T, Sato Y, Fukui K, Iwahashi H, Imagawa A, Hatta M, Shimomura I, Yamagata K (2009) Glucose enhances collectrin protein expression in insulin-producing MIN6 beta cells. Biochem Biophys Res Commun 389(1):133–137. https://doi.org/10.1016/j.bbrc.2009.08.111

Saito M, Kondo M, Ohshima M, Deguchi K, Hayashi H, Inoue K, Tsuji D, Masuko T, Itoh K (2014) Identification of anti-CD98 antibody mimotopes for inducing antibodies with antitumor activity by mimotope immunization. Cancer Sci 105(4):396–401. https://doi.org/10.1111/cas.12365

Sakamoto S, Chairoungdua A, Nagamori S, Wiriyasermkul P, Promchan K, Tanaka H, Kimura T, Ueda T, Fujimura M, Shigeta Y, Naya Y, Akakura K, Ito H, Endou H, Ichikawa T, Kanai Y (2009) A novel role of the C-terminus of b 0,+ AT in the ER-Golgi trafficking of the rBAT-b 0,+ AT heterodimeric amino acid transporter. Biochem J 417(2):441–448. https://doi.org/10.1042/bj20081798

Sakimura K, Nakao K, Yoshikawa M, Suzuki M, Kimura H (2016) A novel Na(+) -Independent alanine-serine-cysteine transporter 1 inhibitor inhibits both influx and efflux of D-Serine. J Neurosci Res 94(10):888–895. https://doi.org/10.1002/jnr.23772

Santiago C, Mudgal G, Reguera J, Recacha R, Albrecht S, Enjuanes L, Casasnovas JM (2017) Allosteric inhibition of aminopeptidase N functions related to tumor growth and virus infection. Sci Rep 7:46045. https://doi.org/10.1038/srep46045

Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, Campagnole-Santos MJ (2018) The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). Physiol Rev 98(1):505–553. https://doi.org/10.1152/physrev.00023.2016

Sason H, Billard JM, Smith GP, Safory H, Neame S, Kaplan E, Rosenberg D, Zubedat S, Foltyn VN, Christoffersen CT, Bundgaard C, Thomsen C, Avital A, Christensen KV, Wolosker H (2017) Asc-1 transporter regulation of synaptic activity via the tonic release of d-Serine in the forebrain. Cereb Cortex 27 (2):1573–1587. https://doi.org/10.1093/cercor/bhv350

Satchwell TJ, Bell AJ, Pellegrin S, Kupzig S, Ridgwell K, Daniels G, Anstee DJ, van den Akker E, Toye AM (2011) Critical band 3 multiprotein complex interactions establish early during human erythropoiesis. Blood 118(1):182–191. https://doi.org/10.1182/blood-2010-10-314187

Sato H, Tamba M, Ishii T, Bannai S (1999) Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. J Biol Chem 274(17):11455–11458

Sato H, Tamba M, Kuriyama-Matsumura K, Okuno S, Bannai S (2000) Molecular cloning and expression of human xCT, the light chain of amino acid transport system xc. Antioxid Redox Signal 2(4):665–671. https://doi.org/10.1089/ars.2000.2.4-665

Savaskan NE, Heckel A, Hahnen E, Engelhorn T, Doerfler A, Ganslandt O, Nimsky C, Buchfelder M, Eyupoglu IY (2008) Small interfering RNA-mediated xCT silencing in gliomas inhibits neurodegeneration and alleviates brain edema. Nat Med 14(6):629–632. https://doi.org/10.1038/nm1772

Saxena S, Quick MW, Warnock DG (2000) Interaction of syntaxins with epithelial ion channels. Curr Opin Nephrol Hypertens 9(5):523–527

Scalise M, Pochini L, Giangregorio N, Tonazzi A, Indiveri C (2013) Proteoliposomes as tool for assaying membrane transporter functions and interactions with xenobiotics. Pharmaceutics 5(3):472–497. https://doi.org/10.3390/pharmaceutics5030472

Scalise M, Galluccio M, Console L, Pochini L, Indiveri C (2018) The human SLC7A5 (LAT1): the intriguing histidine/large neutral amino acid transporter and its relevance to human health. Front Chem 6:243. https://doi.org/10.3389/fchem.2018.00243

Schmidt RS, Macedo JP, Steinmann ME, Salgado AG, Butikofer P, Sigel E, Rentsch D, Maser P (2018) Transporters of Trypanosoma brucei-phylogeny, physiology, pharmacology. FEBS J 285(6):1012–1023. https://doi.org/10.1111/febs.14302

Schneiderhan W, Scheler M, Holzmann KH, Marx M, Gschwend JE, Bucholz M, Gress TM, Seufferlein T, Adler G, Oswald F (2009) CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in in vivo and in vitro models. Gut 58(10):1391–1398. https://doi.org/10.1136/gut.2009.181412

Schofield AE, Martin PG, Spillett D, Tanner MJ (1994) The structure of the human red blood cell anion exchanger (EPB3, AE1, band 3) gene. Blood 84 (6):2000–2012

Scholze P, Freissmuth M, Sitte HH (2002) Mutations within an intramembrane leucine heptad repeat disrupt oligomer formation of the rat GABA transporter 1. J Biol Chem 277(46):43682–43690. https://doi.org/10.1074/jbc.M205602200

Schulze U, Brast S, Grabner A, Albiker C, Snieder B, Holle S, Schlatter E, Schröter R, Pavenstädt H, Herrmann E, Lambert C, Spoden GA, Florin L, Saftig P, Ciarimboli G (2017) Tetraspanin CD63 controls basolateral sorting of organic cation transporter 2 in renal proximal tubules. FASEB J 31 (4):1421–1433. https://doi.org/10.1096/fj.201600901R

Schumann T, König J, Henke C, Willmes DM, Bornstein SR, Jordan J, Fromm MF, Birkenfeld AL (2020) Solute carrier transporters as potential targets for the treatment of metabolic disease. Pharmacol Rev 72 (1):343–379. https://doi.org/10.1124/pr.118.015735

Schumann-Gillett A, O'Mara ML (2019) Lipid-based inhibitors act directly on GlyT2. ACS Chem Neurosci 10(3):1668–1678. https://doi.org/10.1021/acschemneuro.8b00586

Schumann-Gillett A, Blyth MT, O'Mara ML (2018) Is protein structure enough? A review of the role of lipids in SLC6 transporter function. Neurosci Lett. https://doi.org/10.1016/j.neulet.2018.05.020

Schwartz CE, Stevenson RE (2007) The MCT8 thyroid hormone transporter and Allan-Herndon-Dudley syndrome. Best Pract Res Clin Endocrinol Metab 21 (2):307–321. https://doi.org/10.1016/j.beem.2007.03.009

Segawa H, Fukasawa Y, Miyamoto K, Takeda E, Endou H, Kanai Y (1999) Identification and functional characterization of a Na+-independent neutral amino acid transporter with broad substrate selectivity. J Biol Chem 274(28):19745–19751

Seib TM, Patel SA, Bridges RJ (2011) Regulation of the system x(C)- cystine/glutamate exchanger by intracellular glutathione levels in rat astrocyte primary cultures. Glia 59(10):1387–1401. https://doi.org/10.1002/glia.21176

Seow HF, Broer S, Broer A, Bailey CG, Potter SJ, Cavanaugh JA, Rasko JE (2004) Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. Nat Genet 36 (9):1003–1007. https://doi.org/10.1038/ng1406

Sery O, Paclt I, Drtilkova I, Theiner P, Kopeckova M, Zvolsky P, Balcar VJ (2015) A 40-bp VNTR polymorphism in the 3′-untranslated region of DAT1/SLC6A3 is associated with ADHD but not with alcoholism. Behav Brain Funct 11:21. https://doi.org/10.1186/s12993-015-0066-8

Seward DJ, Koh AS, Boyer JL, Ballatori N (2003) Functional complementation between a novel mammalian polygenic transport complex and an evolutionarily ancient organic solute transporter, OSTalpha-OSTbeta. J Biol Chem 278(30):27473–27482. https://doi.org/10.1074/jbc.M301106200

Shaffer PL, Goehring A, Shankaranarayanan A, Gouaux E (2009) Structure and mechanism of a Na+-independent amino acid transporter. Science 325 (5943):1010–1014. https://doi.org/10.1126/science.1176088

Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F (2020) Structural basis of receptor recognition by SARS-CoV-2. Nature 581 (7807):221–224. https://doi.org/10.1038/s41586-020-2179-y

Shayakul C, Alper SL (2004) Defects in processing and trafficking of the AE1 Cl-/HCO3- exchanger associated with inherited distal renal tubular acidosis. Clin Exp Nephrol 8(1):1–11. https://doi.org/10.1007/s10157-003-0271-x

Shen L, Qian C, Cao H, Wang Z, Luo T, Liang C (2018) Upregulation of the solute carrier family 7 genes is indicative of poor prognosis in papillary thyroid carcinoma. World J Surg Oncol 16(1):235. https://doi.org/10.1186/s12957-018-1535-y

Shi Y (2013) Common folds and transport mechanisms of secondary active transporters. Annu Rev Biophys 42:51–72. https://doi.org/10.1146/annurev-biophys-083012-130429

Shigeta Y, Kanai Y, Chairoungdua A, Ahmed N, Sakamoto S, Matsuo H, Kim DK, Fujimura M, Anzai N, Mizoguchi K, Ueda T, Akakura K, Ichikawa T, Ito H, Endou H (2006) A novel missense mutation of SLC7A9 frequent in Japanese cystinuria cases affecting the C-terminus of the transporter. Kidney Int 69(7):1198–1206. https://doi.org/10.1038/sj.ki.5000241

Shimamura T, Weyand S, Beckstein O, Rutherford NG, Hadden JM, Sharples D, Sansom MS, Iwata S, Henderson PJ, Cameron AD (2010) Molecular basis of alternating access membrane transport by the sodium-hydantoin transporter Mhp1. Science 328 (5977):470–473. https://doi.org/10.1126/science.1186303

Shin SS, Jeong BS, Wall BA, Li J, Shan NL, Wen Y, Goydos JS, Chen S (2018) Participation of xCT in melanoma cell proliferation in vitro and tumorigenesis in vivo. Oncogenesis 7(11):86. https://doi.org/10.1038/s41389-018-0098-7

Shoji Y, Noguchi A, Shoji Y, Matsumori M, Takasago Y, Takayanagi M, Yoshida Y, Ihara K, Hara T, Yamaguchi S, Yoshino M, Kaji M, Yamamoto S, Nakai A, Koizumi A, Hokezu Y, Nagamatsu K, Mikami H, Kitajima I, Takada G (2002) Five novel SLC7A7 variants and y+L gene-expression pattern in cultured lymphoblasts from Japanese patients with lysinuric protein intolerance. Hum Mutat 20 (5):375–381. https://doi.org/10.1002/humu.10140

Showe LC, Ballantine M, Huebner K (1987) Localization of the gene for the erythroid anion exchange protein, band 3 (EMPB3), to human chromosome 17. Genomics 1(1):71–76

Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S, Gallagher T (2011) A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. J Virol 85(2):873–882. https://doi.org/10.1128/jvi.02062-10

Simmons KJ, Jackson SM, Brueckner F, Patching SG, Beckstein O, Ivanova E, Geng T, Weyand S, Drew D, Lanigan J, Sharples DJ, Sansom MS, Iwata S, Fishwick CW, Johnson AP, Cameron AD, Henderson PJ (2014) Molecular mechanism of ligand recognition by membrane transport protein, Mhp1. EMBO J 33(16):1831–1844. https://doi.org/10.15252/embj.201387557

Singer D, Camargo SM, Huggel K, Romeo E, Danilczyk U, Kuba K, Chesnov S, Caron MG, Penninger JM, Verrey F (2009) Orphan transporter SLC6A18 is renal neutral amino acid transporter B0AT3. J Biol Chem 284(30):19953–19960. https://doi.org/10.1074/jbc.M109.011171

Singer D, Camargo SM, Ramadan T, Schafer M, Mariotta L, Herzog B, Huggel K, Wolfer D, Werner S, Penninger JM, Verrey F (2012) Defective intestinal amino acid absorption in Ace2 null mice. Am J Physiol Gastrointest Liver Physiol 303(6):G686–G695. https://doi.org/10.1152/ajpgi.00140.2012

Singh SK, Yamashita A, Gouaux E (2007) Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. Nature 448(7156):952–956. https://doi.org/10.1038/nature06038

Singh SK, Piscitelli CL, Yamashita A, Gouaux E (2008) A competitive inhibitor traps LeuT in an open-to-out conformation. Science 322(5908):1655–1661. https://doi.org/10.1126/science.1166777

Sitte HH, Huck S, Reither H, Boehm S, Singer EA, Pifl C (1998) Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells transfected with the human dopamine transporter. J Neurochem 71 (3):1289–1297. https://doi.org/10.1046/j.1471-4159.1998.71031289.x

Sitte HH, Farhan H, Javitch JA (2004) Sodium-dependent neurotransmitter transporters: oligomerization as a determinant of transporter function and trafficking. Mol Interv 4(1):38–47

Sjostrom H, Noren O, Olsen J (2000) Structure and function of aminopeptidase N. Adv Exp Med Biol 477:25–34

Skelton LA, Boron WF, Zhou Y (2010) Acid-base transport by the renal proximal tubule. J Nephrol 23(Suppl 16):S4–18

Skopkova Z, Hrabincova E, Stastna S, Kozak L, Adam T (2005) Molecular genetic analysis of SLC3A1 and SLC7A9 genes in Czech and Slovak cystinuric patients. Ann Hum Genet 69(Pt 5):501–507. https://doi.org/10.1111/j.1529-8817.2005.00185.x

Sloan JL, Mager S (1999) Cloning and functional expression of a human Na(+) and Cl(-)-dependent neutral and cationic amino acid transporter B(0+). J Biol Chem 274(34):23740–23745

Sloan JL, Grubb BR, Mager S (2003) Expression of the amino acid transporter ATB 0+ in lung: possible role in luminal protein removal. Am J Physiol Lung Cell Mol Physiol 284(1):L39–L49. https://doi.org/10.1152/ajplung.00164.2002

Slomiany MG, Grass GD, Robertson AD, Yang XY, Maria BL, Beeson C, Toole BP (2009) Hyaluronan, CD44, and emmprin regulate lactate efflux and membrane localization of monocarboxylate transporters in human breast carcinoma cells. Cancer Res 69 (4):1293–1301. https://doi.org/10.1158/0008-5472.can-08-2491

Smalla KH, Matthies H, Langnase K, Shabir S, Bockers TM, Wyneken U, Staak S, Krug M, Beesley PW, Gundelfinger ED (2000) The synaptic glycoprotein neuroplastin is involved in long-term potentiation at

hippocampal CA1 synapses. Proc Natl Acad Sci U S A 97(8):4327–4332. https://doi.org/10.1073/pnas.080389297

Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL (1992a) Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron 8(5):927–935

Smith KE, Borden LA, Wang CH, Hartig PR, Branchek TA, Weinshank RL (1992b) Cloning and expression of a high affinity taurine transporter from rat brain. Mol Pharmacol 42(4):563–569

Snieder B, Brast S, Grabner A, Buchholz S, Schröter R, Spoden GA, Florin L, Salomon J, Albrecht T, Barz V, Sparreboom A, Ciarimboli G (2019) Identification of the tetraspanin CD9 as an interaction partner of organic cation transporters 1 and 2. SLAS Discov 24 (9):904–914. https://doi.org/10.1177/2472555219859837

Sorensen JB (2005) SNARE complexes prepare for membrane fusion. Trends Neurosci 28(9):453–455. https://doi.org/10.1016/j.tins.2005.06.007

Soroka CJ, Ballatori N, Boyer JL (2010) Organic solute transporter, OSTalpha-OSTbeta: its role in bile acid transport and cholestasis. Semin Liver Dis 30 (2):178–185. https://doi.org/10.1055/s-0030-1253226

Soroka CJ, Velazquez H, Mennone A, Ballatori N, Boyer JL (2011) Ostalpha depletion protects liver from oral bile acid load. Am J Physiol Gastrointest Liver Physiol 301(3):G574–G579. https://doi.org/10.1152/ajpgi.00141.2011

Sperandeo MP, Andria G, Sebastio G (2008) Lysinuric protein intolerance: update and extended mutation analysis of the SLC7A7 gene. Hum Mutat 29(1):14–21

Staubli A, Capatina N, Fuhrer Y, Munier FL, Labs S, Schorderet DF, Tiwari A, Verrey F, Heon E, Cheng CY, Wong TY, Berger W, Camargo SMR, Kloeckener-Gruissem B (2017) Abnormal creatine transport of mutations in monocarboxylate transporter 12 (MCT12) found in patients with age-related cataract can be partially rescued by exogenous chaperone CD147. Hum Mol Genet 26(21):4203–4214. https://doi.org/10.1093/hmg/ddx310

Steeves CL, Baltz JM (2005) Regulation of intracellular glycine as an organic osmolyte in early preimplantation mouse embryos. J Cell Physiol 204(1):273–279. https://doi.org/10.1002/jcp.20284

Steeves CL, Hammer MA, Walker GB, Rae D, Stewart NA, Baltz JM (2003) The glycine neurotransmitter transporter GLYT1 is an organic osmolyte transporter regulating cell volume in cleavage-stage embryos. Proc Natl Acad Sci U S A 100(24):13982–13987. https://doi.org/10.1073/pnas.2334537100

Stewart AK, Chebib FT, Akbar SW, Salas MJ, Sonik RA, Shmukler BE, Alper SL (2011) Interactions of mouse glycophorin A with the dRTA-related mutant G719D of the mouse Cl-/HCO3- exchanger Ae1. Biochem Cell Biol 89(2):224–235. https://doi.org/10.1139/o10-147

Storry JR, Castilho L, Daniels G, Flegel WA, Garratty G, de Haas M, Hyland C, Lomas-Francis C, Moulds JM, Nogues N, Olsson ML, Poole J, Reid ME, Rouger P, van der Schoot E, Scott M, Tani Y, Yu LC, Wendel S, Westhoff C, Yahalom V, Zelinski T (2014) International Society of Blood Transfusion Working Party on red cell immunogenetics and blood group terminology: Cancun report (2012). Vox Sang 107(1):90–96. https://doi.org/10.1111/vox.12127

Stridh MH, Alt MD, Wittmann S, Heidtmann H, Aggarwal M, Riederer B, Seidler U, Wennemuth G, McKenna R, Deitmer JW, Becker HM (2012) Lactate flux in astrocytes is enhanced by a non-catalytic action of carbonic anhydrase II. J Physiol 590 (10):2333–2351. https://doi.org/10.1113/jphysiol.2011.220152

Su J, Chen X, Kanekura T (2009) A CD147-targeting siRNA inhibits the proliferation, invasiveness, and VEGF production of human malignant melanoma cells by down-regulating glycolysis. Cancer Lett 273 (1):140–147. https://doi.org/10.1016/j.canlet.2008.07.034

Su J, Gao T, Jiang M, Wu L, Zeng W, Zhao S, Peng C, Chen X (2016) CD147 silencing inhibits tumor growth by suppressing glucose transport in melanoma. Oncotarget 7(40):64778–64784. https://doi.org/10.18632/oncotarget.11415

Subramanian N, Scopelitti AJ, Carland JE, Ryan RM, O'Mara ML, Vandenberg RJ (2016a) Identification of a 3rd Na+ binding site of the glycine transporter, GlyT2. PLoS One 11(6):e0157583. https://doi.org/10.1371/journal.pone.0157583

Subramanian N, Scopelliti AJ, Carland JE, Ryan RM, O'Mara ML, Vandenberg RJ (2016b) Correction: identification of a 3rd Na+ binding site of the glycine transporter, GlyT2. PLoS One 11(7):e0159896. https://doi.org/10.1371/journal.pone.0159896

Sucic S, Dallinger S, Zdrazil B, Weissensteiner R, Jorgensen TN, Holy M, Kudlacek O, Seidel S, Cha JH, Gether U, Newman AH, Ecker GF, Freissmuth M, Sitte HH (2010) The N terminus of monoamine transporters is a lever required for the action of amphetamines. J Biol Chem 285(14):10924–10938. https://doi.org/10.1074/jbc.M109.083154

Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, Altmaier E, Deloukas P, Erdmann J, Grundberg E, Hammond CJ, de Angelis MH, Kastenmuller G, Kottgen A, Kronenberg F, Mangino M, Meisinger C, Meitinger T, Mewes HW, Milburn MV, Prehn C, Raffler J, Ried JS, Romisch-Margl W, Samani NJ, Small KS, Wichmann HE, Zhai G, Illig T, Spector TD, Adamski J, Soranzo N, Gieger C (2011) Human metabolic individuality in biomedical and pharmaceutical research. Nature 477 (7362):54–60. https://doi.org/10.1038/nature10354

Suina K, Tsuchihashi K, Yamasaki J, Kamenori S, Shintani S, Hirata Y, Okazaki S, Sampetrean O, Baba E, Akashi K, Mitsuishi Y, Takahashi F, Takahashi K, Saya H, Nagano O (2018) Epidermal

growth factor receptor promotes glioma progression by regulating xCT and GluN2B-containing N-methyl-d-aspartate-sensitive glutamate receptor signaling. Cancer Sci 109(12):3874–3882. https://doi.org/10.1111/cas.13826

Sumorok N, Goldfarb DS (2013) Update on cystinuria. Curr Opin Nephrol Hypertens 22(4):427–431. https://doi.org/10.1097/MNH.0b013e3283621c5d

Sun AQ, Balasubramaniyan N, Xu K, Liu CJ, Ponamgi VM, Liu H, Suchy FJ (2007) Protein-protein interactions and membrane localization of the human organic solute transporter. Am J Physiol Gastrointest Liver Physiol 292(6):G1586–G1593. https://doi.org/10.1152/ajpgi.00457.2006

Sung U, Apparsundaram S, Galli A, Kahlig KM, Savchenko V, Schroeter S, Quick MW, Blakely RD (2003) A regulated interaction of syntaxin 1A with the antidepressant-sensitive norepinephrine transporter establishes catecholamine clearance capacity. J Neurosci 23(5):1697–1709

Supplisson S, Roux MJ (2002) Why glycine transporters have different stoichiometries. FEBS Lett 529 (1):93–101

Tachikui H, Kurosawa N, Kadomatsu K, Muramatsu T (1999) Genomic organization and promoter activity of embigin, a member of the immunoglobulin superfamily. Gene 240(2):325–332

Takanaga H, Mackenzie B, Suzuki Y, Hediger MA (2005) Identification of mammalian proline transporter SIT1 (SLC6A20) with characteristics of classical system imino. J Biol Chem 280(10):8974–8984. https://doi.org/10.1074/jbc.M413027200

Takesono A, Moger J, Farooq S, Cartwright E, Dawid IB, Wilson SW, Kudoh T (2012) Solute carrier family 3 member 2 (Slc3a2) controls yolk syncytial layer (YSL) formation by regulating microtubule networks in the zebrafish embryo. Proc Natl Acad Sci U S A 109 (9):3371–3376. https://doi.org/10.1073/pnas.1200642109

Tamai S, Masuda H, Ishii Y, Suzuki S, Kanai Y, Endou H (2001) Expression of L-type amino acid transporter 1 in a rat model of liver metastasis: positive correlation with tumor size. Cancer Detect Prev 25(5):439–445

Tang BL, Gee HY, Lee MG (2011) The cystic fibrosis transmembrane conductance regulator's expanding SNARE interactome. Traffic 12(4):364–371. https://doi.org/10.1111/j.1600-0854.2011.01161.x

Tanner MJ (1993) The major integral proteins of the human red cell. Baillieres Clin Haematol 6(2):333–356

Tanner MJ, Anstee DJ (1976a) The membrane change in En(a-) human erythrocytes. Absence of the major erythrocyte sialoglycoprotein. Biochem J 153 (2):271–277

Tanner MJ, Anstee DJ (1976b) A method for the direct demonstration of the lectin-binding components of the human erythrocyte membrane. Biochem J 153 (2):265–270

Tanner MJ, Jenkins RE, Anstee DJ, Clamp JR (1976) Abnormal carbohydrate composition of the major penetrating membrane protein of En(a-) human erythrocytes. Biochem J 155(3):701–703

Tanphaichitr VS, Sumboonnanonda A, Ideguchi H, Shayakul C, Brugnara C, Takao M, Veerakul G, Alper SL (1998) Novel AE1 mutations in recessive distal renal tubular acidosis. Loss-of-function is rescued by glycophorin A. J Clin Invest 102 (12):2173–2179. https://doi.org/10.1172/jci4836

Tarlungeanu DC, Deliu E, Dotter CP, Kara M, Janiesch PC, Scalise M, Galluccio M, Tesulov M, Morelli E, Sonmez FM, Bilguvar K, Ohgaki R, Kanai Y, Johansen A, Esharif S, Ben-Omran T, Topcu M, Schlessinger A, Indiveri C, Duncan KE, Caglayan AO, Gunel M, Gleeson JG, Novarino G (2016) Impaired amino acid transport at the blood brain barrier is a cause of autism spectrum disorder. Cell 167 (6):1481–1494.e1418. https://doi.org/10.1016/j.cell.2016.11.013

Tartia AP, Rudraraju N, Richards T, Hammer MA, Talbot P, Baltz JM (2009) Cell volume regulation is initiated in mouse oocytes after ovulation. Development 136(13):2247–2254. https://doi.org/10.1242/dev.036756

Tate SS, Yan N, Udenfriend S (1992) Expression cloning of a Na(+)-independent neutral amino acid transporter from rat kidney. Proc Natl Acad Sci U S A 89(1):1–5

Tatsumi M, Groshan K, Blakely RD, Richelson E (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol 340(2–3):249–258

Tavoulari S, Forrest LR, Rudnick G (2009) Fluoxetine (Prozac) binding to serotonin transporter is modulated by chloride and conformational changes. J Neurosci 29 (30):9635–9643. https://doi.org/10.1523/jneurosci.0440-09.2009

Teixeira S, Di Grandi S, Kuhn LC (1987) Primary structure of the human 4F2 antigen heavy chain predicts a transmembrane protein with a cytoplasmic NH2 terminus. J Biol Chem 262(20):9574–9580

Telen MJ, Chasis JA (1990) Relationship of the human erythrocyte Wrb antigen to an interaction between glycophorin A and band 3. Blood 76(4):842–848

ter Beek J, Guskov A, Slotboom DJ (2014) Structural diversity of ABC transporters. J Gen Physiol 143 (4):419–435. https://doi.org/10.1085/jgp.201411164

Terry DS, Kolster RA, Quick M, LeVine MV, Khelashvili G, Zhou Z, Weinstein H, Javitch JA, Blanchard SC (2018) A partially-open inward-facing intermediate conformation of LeuT is associated with Na(+) release and substrate transport. Nat Commun 9(1):230–230. https://doi.org/10.1038/s41467-017-02202-y

Thornell IM, Bevensee MO (2015) Regulators of Slc4 bicarbonate transporter activity. Front Physiol 6:166. https://doi.org/10.3389/fphys.2015.00166

Tian JH, Das S, Sheng ZH (2003) Ca2+-dependent phosphorylation of syntaxin-1A by the death-associated protein (DAP) kinase regulates its interaction with Munc18. J Biol Chem 278(28):26265–26274. https://doi.org/10.1074/jbc.M300492200

Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol

Chem 275(43):33238–33243. https://doi.org/10.1074/jbc.M002615200

To KF, Lo AW (2004) Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2). J Pathol 203(3):740–743. https://doi.org/10.1002/path.1597

Tokunaga E, Sasakawa S, Tamaka K, Kawamata H, Giles CM, Ikin EW, Poole J, Anstee DJ, Mawby W, Tanner MJ (1979) Two apparently healthy Japanese individuals of type MkMk have erythrocytes which lack both the blood group MN and Ss-active sialoglycoproteins. J Immunogenet 6(6):383–390

Tomi M, Mori M, Tachikawa M, Katayama K, Terasaki T, Hosoya K (2005) L-type amino acid transporter 1-mediated L-leucine transport at the inner blood-retinal barrier. Invest Ophthalmol Vis Sci 46 (7):2522–2530. https://doi.org/10.1167/iovs.04-1175

Tomita M, Marchesi VT (1975) Amino-acid sequence and oligosaccharide attachment sites of human erythrocyte glycophorin. Proc Natl Acad Sci U S A 72 (8):2964–2968

Tomita M, Furthmayr H, Marchesi VT (1978) Primary structure of human erythrocyte glycophorin A. Isolation and characterization of peptides and complete amino acid sequence. Biochemistry 17 (22):4756–4770

Torrents D, Estevez R, Pineda M, Fernandez E, Lloberas J, Shi YB, Zorzano A, Palacin M (1998) Identification and characterization of a membrane protein (y+L amino acid transporter-1) that associates with 4F2hc to encode the amino acid transport activity y+L. A candidate gene for lysinuric protein intolerance. J Biol Chem 273(49):32437–32445

Torrents D, Mykkanen J, Pineda M, Feliubadalo L, Estevez R, de Cid R, Sanjurjo P, Zorzano A, Nunes V, Huoponen K, Reinikainen A, Simell O, Savontaus ML, Aula P, Palacin M (1999) Identification of SLC7A7, encoding y+LAT-1, as the lysinuric protein intolerance gene. Nat Genet 21(3):293–296. https://doi.org/10.1038/6809

Torres GE (2006) The dopamine transporter proteome. J Neurochem 97(Suppl 1):3–10. https://doi.org/10.1111/j.1471-4159.2006.03719.x

Torres GE, Carneiro A, Seamans K, Fiorentini C, Sweeney A, Yao W-D, Caron MG (2003) Oligomerization and trafficking of the human dopamine transporter. Mutational analysis identifies critical domains important for the functional expression of the transporter. J Biol Chem 278(4):2731–2739. https://doi.org/10.1074/jbc.M201926200

Towler P, Staker B, Prasad SG, Menon S, Tang J, Parsons T, Ryan D, Fisher M, Williams D, Dales NA, Patane MA, Pantoliano MW (2004) ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J Biol Chem 279(17):17996–18007. https://doi.org/10.1074/jbc.M311191200

Toye AM (2005) Defective kidney anion-exchanger 1 (AE1, Band 3) trafficking in dominant distal renal tubular acidosis (dRTA). Biochem Soc Symp 72:47–63

Toye AM, Ghosh S, Young MT, Jones GK, Sessions RB, Ramauge M, Leclerc P, Basu J, Delaunay J, Tanner MJ (2005) Protein-4.2 association with band 3 (AE1, SLCA4) in Xenopus oocytes: effects of three natural protein-4.2 mutations associated with hemolytic anemia. Blood 105(10):4088–4095. https://doi.org/10.1182/blood-2004-05-1895

Toye AM, Williamson RC, Khanfar M, Bader-Meunier B, Cynober T, Thibault M, Tchernia G, Dechaux M, Delaunay J, Bruce LJ (2008) Band 3 Courcouronnes (Ser667Phe): a trafficking mutant differentially rescued by wild-type band 3 and glycophorin A. Blood 111 (11):5380–5389. https://doi.org/10.1182/blood-2007-07-099473

Toyoda M, Kaira K, Ohshima Y, Ishioka NS, Shino M, Sakakura K, Takayasu Y, Takahashi K, Tominaga H, Oriuchi N, Nagamori S, Kanai Y, Oyama T, Chikamatsu K (2014) Prognostic significance of amino-acid transporter expression (LAT1, ASCT2, and xCT) in surgically resected tongue cancer. Br J Cancer 110(10):2506–2513. https://doi.org/10.1038/bjc.2014.178

Trenker R, Call ME, Call MJ (2015) Crystal Structure of the Glycophorin A Transmembrane Dimer in Lipidic Cubic Phase. J Am Chem Soc 137(50):15676–15679. https://doi.org/10.1021/jacs.5b11354

Treutlein HR, Lemmon MA, Engelman DM, Brunger AT (1992) The glycophorin A transmembrane domain dimer: sequence-specific propensity for a right-handed supercoil of helices. Biochemistry 31 (51):12726–12732

Tsai CJ, Khafizov K, Hakulinen J, Forrest LR, Forrest LR, Kramer R, Kuhlbrandt W, Ziegler C (2011) Structural asymmetry in a trimeric Na+/betaine symporter, BetP, from Corynebacterium glutamicum. J Mol Biol 407 (3):368–381. https://doi.org/10.1016/j.jmb.2011.01.028

Tsuchihashi K, Okazaki S, Ohmura M, Ishikawa M, Sampetrean O, Onishi N, Wakimoto H, Yoshikawa M, Seishima R, Iwasaki Y, Morikawa T, Abe S, Takao A, Shimizu M, Masuko T, Nagane M, Furnari FB, Akiyama T, Suematsu M, Baba E, Akashi K, Saya H, Nagano O (2016) The EGF receptor promotes the malignant potential of glioma by regulating amino acid transport system xc(-). Cancer Res 76(10):2954–2963. https://doi.org/10.1158/0008-5472.Can-15-2121

Tsumura H, Suzuki N, Saito H, Kawano M, Otake S, Kozuka Y, Komada H, Tsurudome M, Ito Y (2003) The targeted disruption of the CD98 gene results in embryonic lethality. Biochem Biophys Res Commun 308(4):847–851

Turner AJ, Hooper NM (2002) The angiotensin-converting enzyme gene family: genomics and pharmacology. Trends Pharmacol Sci 23(4):177–183

Turner AJ, Tipnis SR, Guy JL, Rice G, Hooper NM (2002) ACEH/ACE2 is a novel mammalian metallocarboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ACE inhibitors. Can J Physiol Pharmacol 80(4):346–353

Uchigashima M, Ohtsuka T, Kobayashi K, Watanabe M (2016) Dopamine synapse is a neuroligin-2-mediated contact between dopaminergic presynaptic and GABAergic postsynaptic structures. Proc Natl Acad Sci U S A 113(15):4206–4211. https://doi.org/10.1073/pnas.1514074113

Ueda S, Hayashi H, Miyamoto T, Abe S, Hirai K, Matsukura K, Yagi H, Hara Y, Yoshida K, Okazaki S, Tamura M, Abe Y, Agatsuma T, Niwa SI, Masuko K, Masuko T (2018) Anti-tumor effects of mAb against l-type amino acid transporter 1 (LAT1) bound to human and monkey LAT1 with dual avidity modes. Cancer Sci. https://doi.org/10.1111/cas.13908

Ueda S, Hayashi H, Miyamoto T, Abe S, Hirai K, Matsukura K, Yagi H, Hara Y, Yoshida K, Okazaki S, Tamura M, Abe Y, Agatsuma T, Niwa SI, Masuko K, Masuko T (2019) Anti-tumor effects of mAb against L-type amino acid transporter 1 (LAT1) bound to human and monkey LAT1 with dual avidity modes. Cancer Sci 110(2):674–685. https://doi.org/10.1111/cas.13908

Uosaki H, Cahan P, Lee DI, Wang S, Miyamoto M, Fernandez L, Kass DA, Kwon C (2015) Transcriptional landscape of cardiomyocyte maturation. Cell Rep 13(8):1705–1716. https://doi.org/10.1016/j.celrep.2015.10.032

Usdin TB, Mezey E, Chen C, Brownstein MJ, Hoffman BJ (1991) Cloning of the cocaine-sensitive bovine dopamine transporter. Proc Natl Acad Sci U S A 88 (24):11168–11171

van den Akker E, Satchwell TJ, Williamson RC, Toye AM (2010) Band 3 multiprotein complexes in the red cell membrane; of mice and men. Blood Cells Mol Dis 45 (1):1–8. https://doi.org/10.1016/j.bcmd.2010.02.019

van der Deure WM, Peeters RP, Visser TJ (2010) Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. J Mol Endocrinol 44 (1):1–11. https://doi.org/10.1677/jme-09-0042

Van Dort HM, Moriyama R, Low PS (1998) Effect of band 3 subunit equilibrium on the kinetics and affinity of ankyrin binding to erythrocyte membrane vesicles. J Biol Chem 273(24):14819–14826

van Hasselt PM, Ferdinandusse S, Monroe GR, Ruiter JP, Turkenburg M, Geerlings MJ, Duran K, Harakalova M, van der Zwaag B, Monavari AA, Okur I, Sharrard MJ, Cleary M, O'Connell N, Walker V, Rubio-Gozalbo ME, de Vries MC, Visser G, Houwen RH, van der Smagt JJ, Verhoeven-Duif NM, Wanders RJ, van Haaften G (2014) Monocarboxylate transporter 1 deficiency and ketone utilization. N Engl J Med 371(20):1900–1907. https://doi.org/10.1056/NEJMoa1407778

Van Winkle LJ (1988) Amino acid transport in developing animal oocytes and early conceptuses. Biochim Biophys Acta 947(1):173–208

Vandenberg RJ, Ryan RM, Carland JE, Imlach WL, Christie MJ (2014) Glycine transport inhibitors for the treatment of pain. Trends Pharmacol Sci 35(8):423–430. https://doi.org/10.1016/j.tips.2014.05.006

Vanslambrouck JM, Broer A, Thavyogarajah T, Holst J, Bailey CG, Broer S, Rasko JE (2010) Renal imino acid and glycine transport system ontogeny and involvement in developmental iminoglycinuria. Biochem J 428(3):397–407. https://doi.org/10.1042/BJ20091667

Vaquero J, Monte MJ, Dominguez M, Muntane J, Marin JJ (2013) Differential activation of the human farnesoid X receptor depends on the pattern of expressed isoforms and the bile acid pool composition. Biochem Pharmacol 86(7):926–939. https://doi.org/10.1016/j.bcp.2013.07.022

Vastermark A, Saier MH Jr (2014) Evolutionary relationship between 5+5 and 7+7 inverted repeat folds within the amino acid-polyamine-organocation superfamily. Proteins 82(2):336–346. https://doi.org/10.1002/prot.24401

Vastermark A, Wollwage S, Houle ME, Rio R, Saier MH Jr (2014) Expansion of the APC superfamily of secondary carriers. Proteins 82(10):2797–2811. https://doi.org/10.1002/prot.24643

Vats D, Wang H, Esterhazy D, Dikaiou K, Danzer C, Honer M, Stuker F, Matile H, Migliorini C, Fischer E, Ripoll J, Keist R, Krek W, Schibli R, Stoffel M, Rudin M (2012) Multimodal imaging of pancreatic beta cells in vivo by targeting transmembrane protein 27 (TMEM27). Diabetologia 55(9):2407–2416. https://doi.org/10.1007/s00125-012-2605-2

Veettil MV, Sadagopan S, Sharma-Walia N, Wang FZ, Raghu H, Varga L, Chandran B (2008) Kaposi's sarcoma-associated herpesvirus forms a multimolecular complex of integrins (alphaVbeta5, alphaVbeta3, and alpha3beta1) and CD98-xCT during infection of human dermal microvascular endothelial cells, and CD98-xCT is essential for the postentry stage of infection. J Virol 82(24):12126–12144. https://doi.org/10.1128/jvi.01146-08

Vengelen-Tyler V, Anstee DJ, Issitt PD, Pavone BG, Ferguson SJ, Mawby WJ, Tanner MJ, Blajchman MA, Lorque P (1981) Studies on the blood of an MiV homozygote. Transfusion 21(1):1–14

Vergara-Jaque A, Fenollar-Ferrer C, Kaufmann D, Forrest LR (2015) Repeat-swap homology modeling of secondary active transporters: updated protocol and prediction of elevator-type mechanisms. Front Pharmacol 6:183. https://doi.org/10.3389/fphar.2015.00183

Verrey F (2003) System L: heteromeric exchangers of large, neutral amino acids involved in directional transport. Pflugers Arch 445(5):529–533

Verrey F, Jack DL, Paulsen IT, Saier MH Jr, Pfeiffer R (1999) New glycoprotein-associated amino acid transporters. J Membr Biol 172(3):181–192

Verrey F, Meier C, Rossier G, Kuhn LC (2000) Glycoprotein-associated amino acid exchangers: broadening the range of transport specificity. Pflug Arch 440(4):503–512

Verrey F, Closs EI, Wagner CA, Palacin M, Endou H, Kanai Y (2004) CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch 447 (5):532–542. https://doi.org/10.1007/s00424-003-1086-z

Verrey F, Ristic Z, Romeo E, Ramadan T, Makrides V, Dave MH, Wagner CA, Camargo SM (2005) Novel renal amino acid transporters. Annu Rev Physiol 67:557–572. https://doi.org/10.1146/annurev.physiol.67.031103.153949

Verrey F, Singer D, Ramadan T, Vuille-dit-Bille RN, Mariotta L, Camargo SM (2009) Kidney amino acid transport. Pflug Arch 458(1):53–60. https://doi.org/10.1007/s00424-009-0638-2

Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 277(17):14838–14843. https://doi.org/10.1074/jbc.M200581200

Vilches C, Boiadjieva-Knopfel E, Bodoy S, Camargo S, Lopez de Heredia M, Prat E, Ormazabal A, Artuch R, Zorzano A, Verrey F, Nunes V, Palacin M (2018) Cooperation of antiporter LAT2/CD98hc with uniporter TAT1 for renal reabsorption of neutral amino acids. J Am Soc Nephrol 29(6):1624–1635. https://doi.org/10.1681/asn.2017111205

Visser TJ (2013) Thyroid hormone transporters and resistance. Endocr Dev 24:1–10. https://doi.org/10.1159/000343695

Visser WE, Philp NJ, van Dijk TB, Klootwijk W, Friesema EC, Jansen J, Beesley PW, Ianculescu AG, Visser TJ (2009) Evidence for a homodimeric structure of human monocarboxylate transporter 8. Endocrinology 150(11):5163–5170. https://doi.org/10.1210/en.2009-0699

Visser WE, Friesema EC, Visser TJ (2011) Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol Endocrinol 25(1):1–14. https://doi.org/10.1210/me.2010-0095

Voss DM, Spina R, Carter DL, Lim KS, Jeffery CJ, Bar EE (2017) Disruption of the monocarboxylate transporter-4-basigin interaction inhibits the hypoxic response, proliferation, and tumor progression. Sci Rep 7 (1):4292. https://doi.org/10.1038/s41598-017-04612-w

Vuille-dit-Bille RN, Camargo SM, Emmenegger L, Sasse T, Kummer E, Jando J, Hamie QM, Meier CF, Hunziker S, Forras-Kaufmann Z, Kuyumcu S, Fox M, Schwizer W, Fried M, Lindenmeyer M, Gotze O, Verrey F (2015) Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. Amino Acids 47(4):693–705. https://doi.org/10.1007/s00726-014-1889-6

Vuong LA, Mitchell VA, Vaughan CW (2008) Actions of N-arachidonyl-glycine in a rat neuropathic pain model. Neuropharmacology 54(1):189–193. https://doi.org/10.1016/j.neuropharm.2007.05.004

Wagner CA, Lang F, Broer S (2001) Function and structure of heterodimeric amino acid transporters. Am J Physiol Cell Physiol 281(4):C1077–C1093. https://doi.org/10.1152/ajpcell.2001.281.4.C1077

Wagner CA, Imenez Silva PH, Bourgeois S (2019) Molecular pathophysiology of acid-base disorders. Semin Nephrol 39(4):340–352. https://doi.org/10.1016/j.semnephrol.2019.04.004

Wahlgren WY, Dunevall E, North RA, Paz A, Scalise M, Bisignano P, Bengtsson-Palme J, Goyal P, Claesson E, Caing-Carlsson R, Andersson R, Beis K, Nilsson UJ, Farewell A, Pochini L, Indiveri C, Grabe M, Dobson RCJ, Abramson J, Ramaswamy S, Friemann R (2018) Substrate-bound outward-open structure of a Na(+)-coupled sialic acid symporter reveals a new Na(+) site. Nat Commun 9(1):1753. https://doi.org/10.1038/s41467-018-04045-7

Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S (2002) Guidelines for human gene nomenclature. Genomics 79(4):464–470. https://doi.org/10.1006/geno.2002.6748

Wakabayashi S, Hisamitsu T, Nakamura TY (2013) Regulation of the cardiac Na(+)/H(+) exchanger in health and disease. J Mol Cell Cardiol 61:68–76. https://doi.org/10.1016/j.yjmcc.2013.02.007

Wallimann T, Tokarska-Schlattner M, Schlattner U (2011) The creatine kinase system and pleiotropic effects of creatine. Amino Acids 40(5):1271–1296. https://doi.org/10.1007/s00726-011-0877-3

Walters DK, Arendt BK, Jelinek DF (2013) CD147 regulates the expression of MCT1 and lactate export in multiple myeloma cells. Cell Cycle 12 (19):3175–3183. https://doi.org/10.4161/cc.26193

Wang H, Gouaux E (2012) Substrate binds in the S1 site of the F253A mutant of LeuT, a neurotransmitter sodium symporter homologue. EMBO Rep 13(9):861–866. https://doi.org/10.1038/embor.2012.110

Wang Y, Tate SS (1995) Oligomeric structure of a renal cystine transporter: implications in cystinuria. FEBS Lett 368(2):389–392

Wang DN, Kühlbrandt W, Sarabia VE, Reithmeier RA (1993) Two-dimensional structure of the membrane domain of human band 3, the anion transport protein of the erythrocyte membrane. EMBO J 12 (6):2233–2239

Wang DN, Sarabia VE, Reithmeier RA, Kühlbrandt W (1994) Three-dimensional map of the dimeric membrane domain of the human erythrocyte anion exchanger, Band 3. EMBO J 13(14):3230–3235

Wang W, Seward DJ, Li L, Boyer JL, Ballatori N (2001) Expression cloning of two genes that together mediate organic solute and steroid transport in the liver of a marine vertebrate. Proc Natl Acad Sci U S A 98 (16):9431–9436. https://doi.org/10.1073/pnas.161099898

Wang D, Deken SL, Whitworth TL, Quick MW (2003a) Syntaxin 1A inhibits GABA flux, efflux, and exchange mediated by the rat brain GABA transporter GAT1. Mol Pharmacol 64(4):905–913. https://doi.org/10.1124/mol.64.4.905

Wang Y, Tonouchi M, Miskovic D, Hatta H, Bonen A (2003b) T3 increases lactate transport and the expression of MCT4, but not MCT1, in rat skeletal muscle. Am J Physiol Endocrinol Metab 285(3):E622–E628. https://doi.org/10.1152/ajpendo.00069.2003

Wang H, Goehring A, Wang KH, Penmatsa A, Ressler R, Gouaux E (2013a) Structural basis for action by diverse antidepressants on biogenic amine transporters. Nature 503(7474):141–145. https://doi.org/10.1038/nature12648

Wang Y, Tikellis C, Thomas MC, Golledge J (2013b) Angiotensin converting enzyme 2 and atherosclerosis. Atherosclerosis 226(1):3–8. https://doi.org/10.1016/j.atherosclerosis.2012.08.018

Wang KH, Penmatsa A, Gouaux E (2015) Neurotransmitter and psychostimulant recognition by the dopamine transporter. Nature 521(7552):322–327. https://doi.org/10.1038/nature14431

Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY, Wang Q, Zhou H, Yan J, Qi J (2020) Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell 181(4):894–904. e899. https://doi.org/10.1016/j.cell.2020.03.045

Ward PE, Benter IF, Dick L, Wilk S (1990) Metabolism of vasoactive peptides by plasma and purified renal aminopeptidase M. Biochem Pharmacol 40(8):1725–1732

Warskulat U, Borsch E, Reinehr R, Heller-Stilb B, Roth C, Witt M, Haussinger D (2007) Taurine deficiency and apoptosis: findings from the taurine transporter knockout mouse. Arch Biochem Biophys 462(2):202–209. https://doi.org/10.1016/j.abb.2007.03.022

Watanabe A, Choe S, Chaptal V, Rosenberg JM, Wright EM, Grabe M, Abramson J (2010) The mechanism of sodium and substrate release from the binding pocket of vSGLT. Nature 468(7326):988–991. https://doi.org/10.1038/nature09580

Watts SW, Morrison SF, Davis RP, Barman SM (2012) Serotonin and blood pressure regulation. Pharmacol Rev 64(2):359–388. https://doi.org/10.1124/pr.111.004697

Weidle UH, Scheuer W, Eggle D, Klostermann S, Stockinger H (2010) Cancer-related issues of CD147. Cancer Genomics Proteomics 7(3):157–169

Weiner DB, Kokai Y, Wada T, Cohen JA, Williams WV, Greene MI (1989) Linkage of tyrosine kinase activity with transforming ability of the p185neu oncoprotein. Oncogene 4(10):1175–1183

Weiss N, Zamponi GW (2012) Regulation of voltage-gated calcium channels by synaptic proteins. Adv Exp Med Biol 740:759–775. https://doi.org/10.1007/978-94-007-2888-2_33

Wells RG, Hediger MA (1992) Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. Proc Natl Acad Sci U S A 89(12):5596–5600

Wells RG, Lee WS, Kanai Y, Leiden JM, Hediger MA (1992) The 4F2 antigen heavy chain induces uptake of neutral and dibasic amino acids in Xenopus oocytes. J Biol Chem 267(22):15285–15288

Weng L, Han YP, Enomoto A, Kitaura Y, Nagamori S, Kanai Y, Asai N, An J, Takagishi M, Asai M, Mii S, Masuko T, Shimomura Y, Takahashi M (2018) Negative regulation of amino acid signaling by MAPK-regulated 4F2hc/Girdin complex. PLoS Biol 16(3): e2005090. https://doi.org/10.1371/journal.pbio.2005090

Wersinger C, Sidhu A (2003) Differential cytotoxicity of dopamine and H2O2 in a human neuroblastoma divided cell line transfected with alpha-synuclein and its familial Parkinson's disease-linked mutants. Neurosci Lett 342(1–2):124–128. https://doi.org/10.1016/s0304-3940(03)00212-x

Wersinger C, Sidhu A (2009) Partial regulation of serotonin transporter function by gamma-synuclein. Neurosci Lett 453(3):157–161. https://doi.org/10.1016/j.neulet.2009.02.033

Wersinger C, Rusnak M, Sidhu A (2006) Modulation of the trafficking of the human serotonin transporter by human alpha-synuclein. Eur J Neurosci 24(1):55–64. https://doi.org/10.1111/j.1460-9568.2006.04900.x

Weyand S, Shimamura T, Yajima S, Suzuki S, Mirza O, Krusong K, Carpenter EP, Rutherford NG, Hadden JM, O'Reilly J, Ma P, Saidijam M, Patching SG, Hope RJ, Norbertczak HT, Roach PC, Iwata S, Henderson PJ, Cameron AD (2008) Structure and molecular mechanism of a nucleobase-cation-symport-1 family transporter. Science 322 (5902):709–713. https://doi.org/10.1126/science.1164440

Weyand S, Shimamura T, Beckstein O, Sansom MS, Iwata S, Henderson PJ, Cameron AD (2011) The alternating access mechanism of transport as observed in the sodium-hydantoin transporter Mhp1. J Synchrotron Radiat 18(1):20–23. https://doi.org/10.1107/s0909049510032449

Wheeler SG, Hammond CL, Jornayvaz FR, Samuel VT, Shulman GI, Soroka CJ, Boyer JL, Hinkle PM, Ballatori N (2014) Ostalpha-/- mice exhibit altered expression of intestinal lipid absorption genes, resistance to age-related weight gain, and modestly improved insulin sensitivity. Am J Physiol Gastrointest Liver Physiol 306(5):G425–G438. https://doi.org/10.1152/ajpgi.00368.2013

Willford SL, Anderson CM, Spencer SR, Eskandari S (2015) Evidence for a revised ion/substrate coupling stoichiometry of GABA transporters. J Membr Biol 248(4):795–810. https://doi.org/10.1007/s00232-015-9797-6

Williams VR, Scholey JW (2018) Angiotensin-converting enzyme 2 and renal disease. Curr Opin Nephrol Hypertens 27(1):35–41. https://doi.org/10.1097/MNH.0000000000000378

Williams AL, Jacobs SB, Moreno-Macias H, Huerta-Chagoya A, Churchhouse C, Marquez-Luna C, Garcia-Ortiz H, Gomez-Vazquez MJ, Burtt NP, Aguilar-Salinas CA, Gonzalez-Villalpando C, Florez

JC, Orozco L, Haiman CA, Tusie-Luna T, Altshuler D (2014) Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature 506 (7486):97–101. https://doi.org/10.1038/nature12828

Williamson RC, Toye AM (2008) Glycophorin A: band 3 aid. Blood Cells Mol Dis 41(1):35–43. https://doi.org/10.1016/j.bcmd.2008.01.001

Wilson MC, Meredith D, Halestrap AP (2002) Fluorescence resonance energy transfer studies on the interaction between the lactate transporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex in situ. J Biol Chem 277(5):3666–3672. https://doi.org/10.1074/jbc.M109658200

Wilson MC, Meredith D, Fox JE, Manoharan C, Davies AJ, Halestrap AP (2005) Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: the ancillary protein for the insensitive MCT2 is EMBIGIN (gp70). J Biol Chem 280(29):27213–27221. https://doi.org/10.1074/jbc.M411950200

Wilson MC, Meredith D, Bunnun C, Sessions RB, Halestrap AP (2009) Studies on the DIDS-binding site of monocarboxylate transporter 1 suggest a homology model of the open conformation and a plausible translocation cycle. J Biol Chem 284 (30):20011–20021. https://doi.org/10.1074/jbc.M109.014217

Wilson MC, Kraus M, Marzban H, Sarna JR, Wang Y, Hawkes R, Halestrap AP, Beesley PW (2013) The neuroplastin adhesion molecules are accessory proteins that chaperone the monocarboxylate transporter MCT2 to the neuronal cell surface. PLoS One 8(11):e78654. https://doi.org/10.1371/journal.pone.0078654

Winter M, Funk J, Korner A, Alberati D, Christen F, Schmitt G, Altmann B, Pospischil A, Singer T (2016) Effects of GlyT1 inhibition on erythropoiesis and iron homeostasis in rats. Exp Hematol 44(10):964–974. e964. https://doi.org/10.1016/j.exphem.2016.07.003

Wollscheid B, Bausch-Fluck D, Henderson C, O'Brien R, Bibel M, Schiess R, Aebersold R, Watts JD (2009) Mass-spectrometric identification and relative quantification of N-linked cell surface glycoproteins. Nat Biotechnol 27(4):378–386. https://doi.org/10.1038/nbt.1532

Wong AH, Zhou D, Rini JM (2012a) The X-ray crystal structure of human aminopeptidase N reveals a novel dimer and the basis for peptide processing. J Biol Chem 287(44):36804–36813. https://doi.org/10.1074/jbc.M112.398842

Wong FH, Chen JS, Reddy V, Day JL, Shlykov MA, Wakabayashi ST, Saier MH Jr (2012b) The amino acid-polyamine-organocation superfamily. J Mol Microbiol Biotechnol 22(2):105–113. https://doi.org/10.1159/000338542

Wong KA, Mein R, Wass M, Flinter F, Pardy C, Bultitude M, Thomas K (2015) The genetic diversity of cystinuria in a UK population of patients. BJU Int 116(1):109–116. https://doi.org/10.1111/bju.12894

Wong AHM, Tomlinson ACA, Zhou D, Satkunarajah M, Chen K, Sharon C, Desforges M, Talbot PJ, Rini JM (2017) Receptor-binding loops in alphacoronavirus adaptation and evolution. Nat Commun 8(1):1735. https://doi.org/10.1038/s41467-017-01706-x

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367(6483):1260–1263. https://doi.org/10.1126/science.abb2507

Wright KE, Hjerrild KA, Bartlett J, Douglas AD, Jin J, Brown RE, Illingworth JJ, Ashfield R, Clemmensen SB, de Jongh WA, Draper SJ, Higgins MK (2014) Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies. Nature 515 (7527):427–430. https://doi.org/10.1038/nature13715

Wu F, Saleem MA, Kampik NB, Satchwell TJ, Williamson RC, Blattner SM, Ni L, Toth T, White G, Young MT, Parker MD, Alper SL, Wagner CA, Toye AM (2010) Anion exchanger 1 interacts with nephrin in podocytes. J Am Soc Nephrol 21(9):1456–1467. https://doi.org/10.1681/asn.2009090921

Wu D, Grund TN, Welsch S, Mills DJ, Michel M, Safarian S, Michel H (2020) Structural basis for amino acid exchange by a human heteromeric amino acid transporter. Proc Natl Acad Sci U S A 117(35):21281–21287. https://doi.org/10.1073/pnas.2008111117

Xie X, Dumas T, Tang L, Brennan T, Reeder T, Thomas W, Klein RD, Flores J, O'Hara BF, Heller HC, Franken P (2005) Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice. Brain Res 1052(2):212–221. https://doi.org/10.1016/j.brainres.2005.06.039

Xu D, Hemler ME (2005) Metabolic activation-related CD147-CD98 complex. Mol Cell Proteomics 4 (8):1061–1071. https://doi.org/10.1074/mcp.M400207-MCP200

Xue Y, Ma C, Hanna I, Pan G (2019) Intestinal Transporter-Associated Drug Absorption and Toxicity. Adv Exp Med Biol 1141:361–405. https://doi.org/10.1007/978-981-13-7647-4_8

Yadav A, Shah N, Tiwari PK, Javed K, Cheng Q, Aidhen IS, Bröer S (2020) Novel chemical scaffolds to inhibit the neutral amino acid transporter B0AT1 (SLC6A19), a potential target to treat metabolic diseases. Front Pharmacol 11:140. https://doi.org/10.3389/fphar.2020.00140

Yagita H, Masuko T, Hashimoto Y (1986) Inhibition of tumor cell growth in vitro by murine monoclonal antibodies that recognize a proliferation-associated cell surface antigen system in rats and humans. Cancer Res 46(3):1478–1484

Yahyaoui R, Pérez-Frías J (2019) Amino acid transport defects in human inherited metabolic disorders. Int J Mol Sci 21(1):E119. https://doi.org/10.3390/ijms21010119

Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E (2005) Crystal structure of a bacterial homologue of Na+/Cl--dependent neurotransmitter transporters. Nature 437(7056):215–223. https://doi.org/10.1038/nature03978

Yan N (2015) Structural biology of the major facilitator superfamily transporters. Annu Rev Biophys

44:257–283. https://doi.org/10.1146/annurev-biophys-060414-033901

Yan Y, Vasudevan S, Nguyen HT, Merlin D (2008) Intestinal epithelial CD98: an oligomeric and multifunctional protein. Biochim Biophys Acta 1780 (10):1087–1092

Yan R, Zhao X, Lei J, Zhou Q (2019) Structure of the human LAT1-4F2hc heteromeric amino acid transporter complex. Nature 568(7750):127–130. https://doi.org/10.1038/s41586-019-1011-z

Yan R, Li Y, Shi Y, Zhou J, Lei J, Huang J, Zhou Q (2020a) Cryo-EM structure of the human heteromeric amino acid transporter $b^{0,+}$AT-rBAT. Sci Adv 6(16): eaay6379. https://doi.org/10.1126/sciadv.aay6379

Yan R, Zhang Y, Guo Y, Xia L, Zhou Q (2020b) Structural basis for the recognition of the 2019-nCoV by human ACE2. bioRxiv:2020.2002.2019.956946. https://doi.org/10.1101/2020.02.19.956946

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q (2020c) Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 367 (6485):1444–1448. https://doi.org/10.1126/science.abb2762

Yan R, Zhang Y, Li Y, Xia L, Zhou Q (2020d) Structure of dimeric full-length human ACE2 in complex with B^0AT1. bioRxiv:2020.2002.2017.951848. https://doi.org/10.1101/2020.02.17.951848

Yanagida O, Kanai Y, Chairoungdua A, Kim DK, Segawa H, Nii T, Cha SH, Matsuo H, Fukushima J, Fukasawa Y, Tani Y, Taketani Y, Uchino H, Kim JY, Inatomi J, Okayasu I, Miyamoto K, Takeda E, Goya T, Endou H (2001) Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim Biophys Acta 1514 (2):291–302

Yanagisawa N, Hana K, Nakada N, Ichinoe M, Koizumi W, Endou H, Okayasu I, Murakumo Y (2014) High expression of L-type amino acid transporter 1 as a prognostic marker in bile duct adenocarcinomas. Cancer Med 3(5):1246–1255. https://doi.org/10.1002/cam4.272

Yang E, Seo-Mayer P, Lezon-Geyda K, Badior KE, Li J, Casey JR, Reithmeier RAF, Gallagher PG (2018) A Ser725Arg mutation in Band 3 abolishes transport function and leads to anemia and renal tubular acidosis. Blood 131(15):1759–1763. https://doi.org/10.1182/blood-2018-01-827725

Yannoukakos D, Vasseur C, Blouquit Y, Bursaux E, Wajcman H (1989) Primary structure of the cytoplasmic domain of human erythrocyte protein band 3. Comparison with its sequence in the mouse. Biochim Biophys Acta 998(1):43–49

Yannoukakos D, Vasseur C, Piau JP, Wajcman H, Bursaux E (1991) Phosphorylation sites in human erythrocyte band 3 protein. Biochim Biophys Acta 1061(2):253–266

Yasuhara A, Wada J, Malakauskas SM, Zhang Y, Eguchi J, Nakatsuka A, Murakami K, Kanzaki M, Teshigawara S, Yamagata K, Le TH, Makino H (2008) Collectrin is involved in the development of salt-sensitive hypertension by facilitating the membrane trafficking of apical membrane proteins via interaction with soluble N-ethylmaleiamide-sensitive factor attachment protein receptor complex. Circulation 118 (21):2146–2155. https://doi.org/10.1161/circulationaha.108.787259

Ye R, Carneiro AM, Airey D, Sanders-Bush E, Williams RW, Lu L, Wang J, Zhang B, Blakely RD (2014a) Evaluation of heritable determinants of blood and brain serotonin homeostasis using recombinant inbred mice. Genes Brain Behav 13(3):247–260. https://doi.org/10.1111/gbb.12092

Ye R, Carneiro AM, Han Q, Airey D, Sanders-Bush E, Zhang B, Lu L, Williams R, Blakely RD (2014b) Quantitative trait loci mapping and gene network analysis implicate protocadherin-15 as a determinant of brain serotonin transporter expression. Genes Brain Behav 13(3):261–275. https://doi.org/10.1111/gbb.12119

Ye R, Quinlan MA, Iwamoto H, Wu HH, Green NH, Jetter CS, McMahon DG, Veestra-VanderWeele J, Levitt P, Blakely RD (2015) Physical interactions and functional relationships of neuroligin 2 and midbrain serotonin transporters. Front Synaptic Neurosci 7:20. https://doi.org/10.3389/fnsyn.2015.00020

Ylikangas H, Peura L, Malmioja K, Leppanen J, Laine K, Poso A, Lahtela-Kakkonen M, Rautio J (2013) Structure-activity relationship study of compounds binding to large amino acid transporter 1 (LAT1) based on pharmacophore modeling and in situ rat brain perfusion. Eur J Pharm Sci 48(3):523–531. https://doi.org/10.1016/j.ejps.2012.11.014

Ylikangas H, Malmioja K, Peura L, Gynther M, Nwachukwu EO, Leppanen J, Laine K, Rautio J, Lahtela-Kakkonen M, Huttunen KM, Poso A (2014) Quantitative insight into the design of compounds recognized by the L-type amino acid transporter 1 (LAT1). ChemMedChem 9(12):2699–2707. https://doi.org/10.1002/cmdc.201402281

Yoon H, Fanelli A, Grollman EF, Philp NJ (1997) Identification of a unique monocarboxylate transporter (MCT3) in retinal pigment epithelium. Biochem Biophys Res Commun 234(1):90–94. https://doi.org/10.1006/bbrc.1997.6588

Young MT, Tanner MJ (2003) Distinct regions of human glycophorin A enhance human red cell anion exchanger (band 3; AE1) transport function and surface trafficking. J Biol Chem 278(35):32954–32961. https://doi.org/10.1074/jbc.M302527200

Young MT, Beckmann R, Toye AM, Tanner MJ (2000) Red-cell glycophorin A-band 3 interactions associated with the movement of band 3 to the cell surface. Biochem J 350(Pt 1):53–60

Yu XL, Hu T, Du JM, Ding JP, Yang XM, Zhang J, Yang B, Shen X, Zhang Z, Zhong WD, Wen N, Jiang H, Zhu P, Chen ZN (2008) Crystal structure of HAb18G/CD147: implications for immunoglobulin superfamily homophilic adhesion. J Biol Chem 283

(26):18056–18065. https://doi.org/10.1074/jbc.M802694200

Yu X, Yang G, Yan C, Baylon JL, Jiang J, Fan H, Lu G, Hasegawa K, Okumura H, Wang T, Tajkhorshid E, Li S, Yan N (2017) Dimeric structure of the uracil: proton symporter UraA provides mechanistic insights into the SLC4/23/26 transporters. Cell Res 27 (8):1020–1033. https://doi.org/10.1038/cr.2017.83

Yu Z, Lin W, Rui Z, Jihong P (2018) Fibroblast-like synoviocyte migration is enhanced by IL-17-mediated overexpression of L-type amino acid transporter 1 (LAT1) via the mTOR/4E-BP1 pathway. Amino Acids 50(2):331–340. https://doi.org/10.1007/s00726-017-2520-4

Zaarour N, Defontaine N, Demaretz S, Azroyan A, Cheval L, Laghmani K (2011) Secretory carrier membrane protein 2 regulates exocytic insertion of NKCC2 into the cell membrane. J Biol Chem 286 (11):9489–9502. https://doi.org/10.1074/jbc.M110.166546

Zaia KA, Reimer RJ (2009) Synaptic vesicle protein NTT4/XT1 (SLC6A17) catalyzes Na+-coupled neutral amino acid transport. J Biol Chem 284 (13):8439–8448. https://doi.org/10.1074/jbc.M806407200

Zeilhofer HU, Acuna MA, Gingras J, Yevenes GE (2018) Glycine receptors and glycine transporters: targets for novel analgesics? Cell Mol Life Sci 75(3):447–465. https://doi.org/10.1007/s00018-017-2622-x

Zhang G, Cao L (2017) New mutations in the SLC7A7 gene of two chinese sisters with lysinuric protein intolerance. Pediatr Pulmonol 52(11):E94–e96. https://doi.org/10.1002/ppul.23760

Zhang H, Wada J, Kanwar YS, Tsuchiyama Y, Hiragushi K, Hida K, Shikata K, Makino H (1999) Screening for genes up-regulated in 5/6 nephrectomized mouse kidney. Kidney Int 56 (2):549–558. https://doi.org/10.1046/j.1523-1755.1999.00561.x

Zhang D, Kiyatkin A, Bolin JT, Low PS (2000) Crystallographic structure and functional interpretation of the cytoplasmic domain of erythrocyte membrane band 3. Blood 96(9):2925–2933

Zhang H, Wada J, Hida K, Tsuchiyama Y, Hiragushi K, Shikata K, Wang H, Lin S, Kanwar YS, Makino H (2001) Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. J Biol Chem 276(20):17132–17139. https://doi.org/10.1074/jbc.M006723200

Zhang H, Li XJ, Martin DB, Aebersold R (2003) Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. Nat Biotechnol 21(6):660–666. https://doi.org/10.1038/nbt827

Zhang H, Wang XL, Zhang YL, Hou P, Li H, Wang HY (2004) Construction of sense and antisense eukaryotic expression vector of novel gene Collectrin and its function in cell growth. Beijing Da Xue Xue Bao Yi Xue Ban 36(2):181–184

Zhang Y, Wada J, Yasuhara A, Iseda I, Eguchi J, Fukui K, Yang Q, Yamagata K, Hiesberger T, Igarashi P, Zhang H, Wang H, Akagi S, Kanwar YS, Makino H (2007) The role for HNF-1beta-targeted collectrin in maintenance of primary cilia and cell polarity in collecting duct cells. PLoS One 2(5):e414. https://doi.org/10.1371/journal.pone.0000414

Zhang M, Wang C, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Bronner IF, Casandra D, Mayho M, Brown J, Li S, Swanson J, Rayner JC, Jiang RHY, Adams JH (2018) Uncovering the essential genes of the human malaria parasite Plasmodium falciparum by saturation mutagenesis. Science 360(6388). https://doi.org/10.1126/science.aap7847

Zhao C, Wilson MC, Schuit F, Halestrap AP, Rutter GA (2001) Expression and distribution of lactate/monocarboxylate transporter isoforms in pancreatic islets and the exocrine pancreas. Diabetes 50 (2):361–366

Zhao Y, Terry DS, Shi L, Quick M, Weinstein H, Blanchard SC, Javitch JA (2011) Substrate-modulated gating dynamics in a Na+-coupled neurotransmitter transporter homologue. Nature 474(7349):109–113. https://doi.org/10.1038/nature09971

Zhao C, Stolzenberg S, Gracia L, Weinstein H, Noskov S, Shi L (2012) Ion-controlled conformational dynamics in the outward-open transition from an occluded state of LeuT. Biophys J 103(5):878–888. https://doi.org/10.1016/j.bpj.2012.07.044

Zheng JC, Tham CT, Keatings K, Fan S, Liou AY, Numata Y, Allan D, Numata M (2014) Secretory Carrier Membrane Protein (SCAMP) deficiency influences behavior of adult flies. Front Cell Dev Biol 2:64. https://doi.org/10.3389/fcell.2014.00064

Zhong H, Sanchez C, Caron MG (2012) Consideration of allosterism and interacting proteins in the physiological functions of the serotonin transporter. Biochem Pharmacol 83(4):435–442. https://doi.org/10.1016/j.bcp.2011.09.020

Zhou H, Di Palma S, Preisinger C, Peng M, Polat AN, Heck AJ, Mohammed S (2013) Toward a comprehensive characterization of a human cancer cell phosphoproteome. J Proteome Res 12(1):260–271. https://doi.org/10.1021/pr300630k

Zhou K, Dichlberger A, Martinez-Seara H, Nyholm TKM, Li S, Kim YA, Vattulainen I, Ikonen E, Blom T (2018) A ceramide-regulated element in the late endosomal protein LAPTM4B controls amino acid transporter interaction. ACS Cent Sci 4(5):548–558. https://doi.org/10.1021/acscentsci.7b00582

Zielinska M, Ruszkiewicz J, Hilgier W, Fresko I, Albrecht J (2011) Hyperammonemia increases the expression and activity of the glutamine/arginine transporter y+ LAT2 in rat cerebral cortex: implications for the nitric oxide/cGMP pathway. Neurochem Int 58(2):190–195. https://doi.org/10.1016/j.neuint.2010.11.015

Zielinska M, Skowronska M, Fresko I, Albrecht J (2012) Upregulation of the heteromeric y(+)LAT2 transporter contributes to ammonia-induced increase of arginine uptake in rat cerebral cortical astrocytes. Neurochem

Int 61(4):531–535. https://doi.org/10.1016/j.neuint.2012.02.021

Zielinska M, Milewski K, Skowronska M, Gajos A, Zieminska E, Beresewicz A, Albrecht J (2015) Induction of inducible nitric oxide synthase expression in ammonia-exposed cultured astrocytes is coupled to increased arginine transport by upregulated y(+) LAT2 transporter. J Neurochem 135(6):1272–1281. https://doi.org/10.1111/jnc.13387

Ziu E, Mercado CP, Li Y, Singh P, Ahmed BA, Freyaldenhoven S, Lensing S, Ware J, Kilic F (2012) Down-regulation of the serotonin transporter in hyperreactive platelets counteracts the pro-thrombotic effect of serotonin. J Mol Cell Cardiol 52(5):1112–1121. https://doi.org/10.1016/j.yjmcc.2012.02.004

Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V, Prisinzano TE, Baumann MH (2009) Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. J Pharmacol Exp Ther 329(2):738–746. https://doi.org/10.1124/jpet.108.146142

Zur AA, Chien HC, Augustyn E, Flint A, Heeren N, Finke K, Hernandez C, Hansen L, Miller S, Lin L, Giacomini KM, Colas C, Schlessinger A, Thomas AA (2016) LAT1 activity of carboxylic acid bioisosteres: evaluation of hydroxamic acids as substrates. Bioorg Med Chem Lett 26(20):5000–5006. https://doi.org/10.1016/j.bmcl.2016.09.001

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Collagen IV Exploits a Cl^- Step Gradient for Scaffold Assembly

Sergey V. Ivanov, Ryan Bauer, Elena N. Pokidysheva, and Sergei P. Boudko

Abstract

Collagen molecules are crucial extracellular players in animal tissue development and in functions ranging from ultrafiltration to organism locomotion. Among the 28 types of collagen found in human, type IV collagen stands out as a primordial type found in all species of the animal kingdom. Collagen IV forms smart scaffolds for basement membranes, sheet-like acellular structures that isolate, coordinate, and direct cells during morphogenesis. Collagen IV is also involved in multiple functions in developed tissues. As part of the basement membrane, collagen IV scaffolds provide mechanical strength, spatially tether extracellular macromolecules and directly signal to cells via receptor binding sites. Proper assembly and structure of the scaffolds are critical for development and function of multiple types of basement membranes. Within last 5 years it was established that Cl^- concentration is a key factor for initiating collagen IV scaffold assembly. The biological role of Cl^- in multiple physiological processes and detailed mechanisms for its signaling and structural impacts are well established. Cl^- gradients are generated across the plasma and intracellular organelle membranes. As collagen IV molecules are secreted outside the cell, they experience a switch from low to high Cl^- concentration. This transition works as a trigger for collagen IV scaffold assembly. Within the scaffold, collagen IV remains to be a Cl^- sensor as its structural integrity continues to depend on Cl^- concentration. Here, we review recent findings and set future directions for studies on the role of Cl^- in type IV collagen assembly, function, and disease.

Keywords

Basement membrane · Cl^- · Collagen IV · Extracellular matrix · Kinetics · NC1 domain · Protein assembly

Abbreviations

NC1	non-collagenous domain 1 of collagen IV
7S	7 Svedberg, the dodecameric region of collagen IV

S. V. Ivanov, R. Bauer, and E. N. Pokidysheva
Department of Medicine, Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN, USA

Vanderbilt Center for Matrix Biology, Vanderbilt University Medical Center, Nashville, TN, USA

S. P. Boudko (✉)
Department of Medicine, Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN, USA

Vanderbilt Center for Matrix Biology, Vanderbilt University Medical Center, Nashville, TN, USA

Department of Biochemistry, Vanderbilt University, Nashville, TN, USA
e-mail: sergey.budko@vumc.org

ECM	extracellular matrix
BM	basement membrane
GBM	glomerular BM
LBM	lens capsule BM
$NC1^{sc}$	single polypeptide chain NC1 trimer
PEG	polyethylene glycol

1 Introduction

Basement membranes (BMs) are evolutionary ancient and highly conserved sheet-like structures of extracellular matrix (ECM) that coordinate cells, direct their polarization, and play a formative role in shaping tissues (Morrissey and Sherwood 2015). In adult as all animals are multicellular, BMs serve structural and protective functions by providing signaling stimuli for cell behaviors and tissue regeneration (Jayadev and Sherwood 2017; Morrissey and Sherwood 2015). Prominent cases of highly specialized BMs are glomerular basement membrane (GBM), which provides ultrafiltration in the kidney (Gunwar et al. 1998), lens capsule basement membrane (LBM), which acts as anchor points for lens cells during migration and proliferation (Lu et al. 2008), and seminiferous tubule basement membrane, which plays a role in spermatogenesis (Kahsai et al. 1997). Type IV collagen is a structural scaffold in all BMs that integrates other ECM components, such as laminins, nidogens and proteoglycans (Fidler et al. 2017).

Genetic variants in collagen IV genes, COL4A1-6, cause BM pathologies in various tissues. Central nervous system and cerebral vasculature are primary targets of pathogenic variants in COL4A1 and COL4A2 genes, while pathogenic variants in COL4A3-5 genes almost exclusively lead to kidney diseases, hearing defects and ocular abnormalities (Cosgrove and Liu 2017; Meuwissen et al. 2015; Stokman et al. 2016; Wang et al. 2018). Whereas connection between gene variant and resulting abnormalities is firmly established in most cases, the mechanism of pathogenesis remains unclear. To provide better insight into why and how these diseases develop, it is essential to understand molecular mechanisms of collagen IV scaffold assembly.

2 Structural Organization of Collagen IV

Collagen IV was discovered in kidney GBM over 50 years ago in the seminal works of Spiro and Kefalides groups (Spiro 1967; Kefalides 1968) as a novel crosslinked molecule with unusual properties. Structural organization of the collagen IV scaffold was later analyzed using acid extraction and limited proteolysis of tumor BM, which led to a discovery of hexamer and dodecamer oligomers (Fig. 1a) (Timpl et al. 1981). Subsequently, two key structural elements responsible for forming each of these complexes, the 7S dodecamer and the NC1 hexamer (Fig. 1a), were isolated using proteolytic enzymes (Weber et al. 1984; Risteli et al. 1980; Timpl et al. 1979). These and other functional domains of collagen IV can be extracted and purified to homogeneity from different tissues, tumors, and matrix deposited by cultured cells (Boudko et al. 2018). The type IV collagen family has six genetically distinct α-chains designated α1 to α6 (Fig. 1b). Each α-chain contains an N-terminal 7S region, collagenous domain, and C-terminal NC1 domain. Initially, three α chains form collagen IV protomer that further assembles into the scaffolds composed of the hexameric and dodecameric assemblies. Studies of the NC1 hexamers extracted from tissues by treatment with collagenase (Fig. 1c) led to discoveries of three compositions of NC1 hexamers: α121, α345, and α121/α556 as well as a new type of covalent cross-link, the sulfilimine bond, stabilizing these hexamers. (Fig. 1d) (Hudson et al. 2003; Hudson et al. 1994; Borza et al. 2001; Boutaud et al. 2000; Vanacore et al. 2009).

3 Approaches to Overcome Hurdles to Study NC1 Hexamer Assembly

Collagen IV is notoriously insoluble in tissue due to extensive cross-linking, though this hurdle can be partially resolved by applying β-aminoproprionitrile, a lathrytic agent that prevents the formation of cross-links, during animal development or tumor growth. Although full-

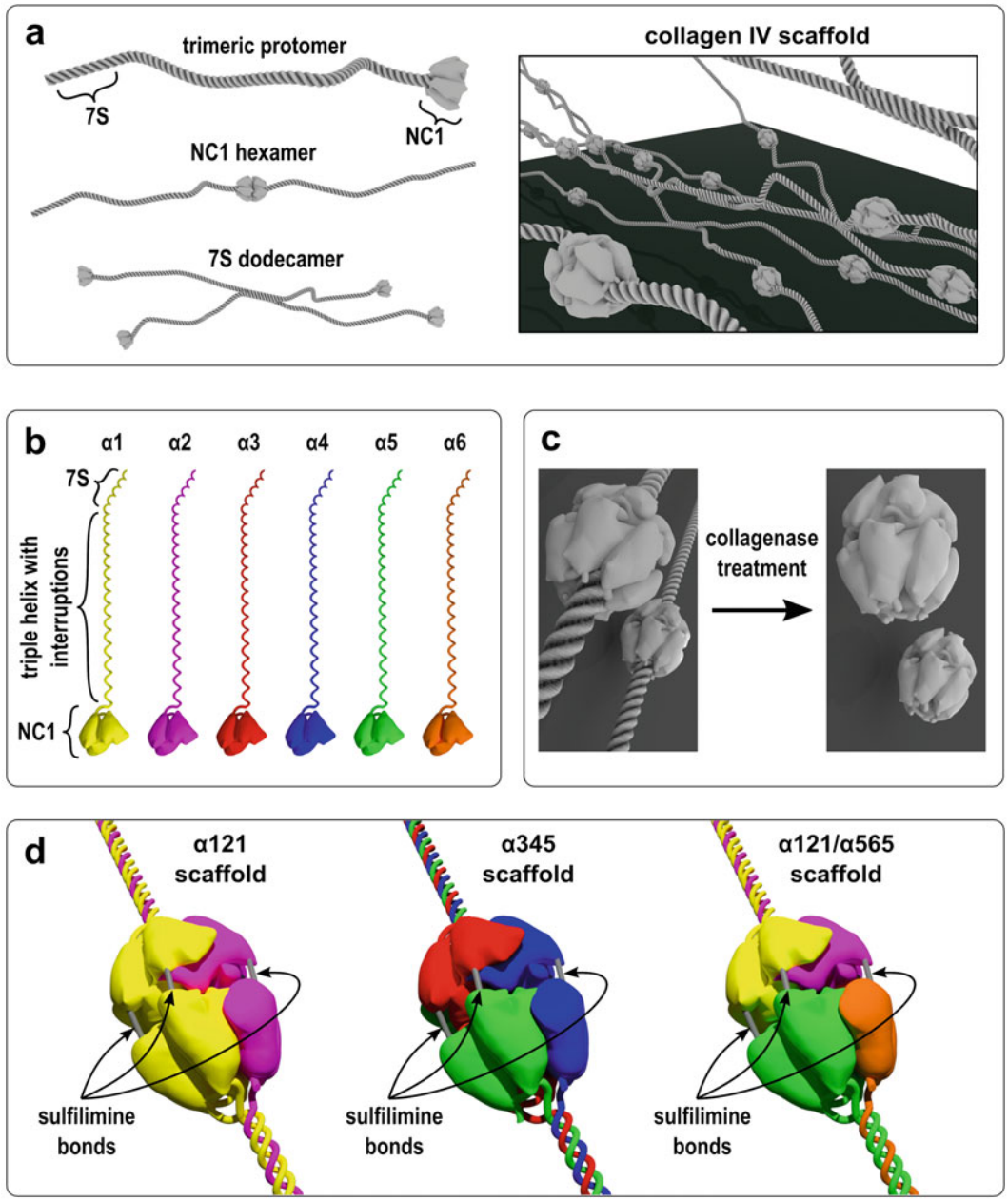

Fig. 1 **Collagen IV**. (**a**) Two key assemblies, NC1-to-NC1 hexamer formation and 7S dodecameric assembly drive the formation of collagen IV scaffolds that are further re-enforced by lateral interactions. (**b**) The six α-chains, α1-α6, of collagen IV found in humans. (**c**) The non-collagenous (NC1) domain can be solubilized from tissues and matrix deposited by cultured cells after collagenase treatment. (**d**) The three reported types of collagen IV scaffolds based on analysis of solubilized NC1 hexamers. The NC1 hexamers are often re-enforced by sulfilimine bonds between two protomers

length collagen IV protomers can be extracted from animal tissues or cell cultures, they are quite challenging to study under physiological conditions as they naturally tend to aggregate (Bachinger et al. 1982). These challenges make full-length protomers prohibitively difficult to use

to study NC1 hexamer assembly. Currently, there are three approaches available to bypass full-length protomers when studying hexamer formation (Fig. 2). The first approach (Fig. 2a) utilizes either recombinantly produced NC1 monomers (Casino et al. 2018) or collagenase liberated native NC1 from bovine LBM or PFHR9 cell culture (Bhave et al. 2012). These hexamers dissociate into monomers upon removal of Cl^- ions using dialysis or desalting columns. These monomers can then be analyzed in hexamer assembly assays by addition of Cl^-. This approach though does not reflect natural hexamer formation as it combines two steps, trimerization and hexamerization, into one process. The second approach (Fig. 2b) utilizes recombinant constructs consisting of the NC1 domain and an N-terminal stretch of 28 GXY repeats essential for forming a stable triple helix (Cummings et al. 2016). These constructs form trimeric molecules in the absence of Cl^- ions and thus, can be used for direct hexamer assembly experiments. The third approach (Fig. 2c) utilizes single-chain recombinant technology in which all three chains of the NC1 domain are tied together in a desired domain composition and registry to form a stable, single polypeptide chain NC1 trimer ($NC1^{sc}$) (Pedchenko et al. 2019). All three approaches bear unique advantages and have certain limitations, but collectively provide tools for studying assembly of the NC1 hexamer.

4 Role of Cl^- in NC1 Hexamer Assembly

The initial clue for how NC1 domains assemble into hexamers was provided by crystal structures of human and bovine $NC1^{\alpha 121}$ hexamers that presented acetate, Br^-, Cl^-, Ca^{2+}, and K^+ coordinated by residues from two opposite trimers (Sundaramoorthy et al. 2002; Than et al. 2002; Vanacore et al. 2004). These and chemically similar ions were extensively studied for their abilities to trigger or facilitate the hexamer assembly (Cummings et al. 2016). Neither Ca^{2+} or K^+

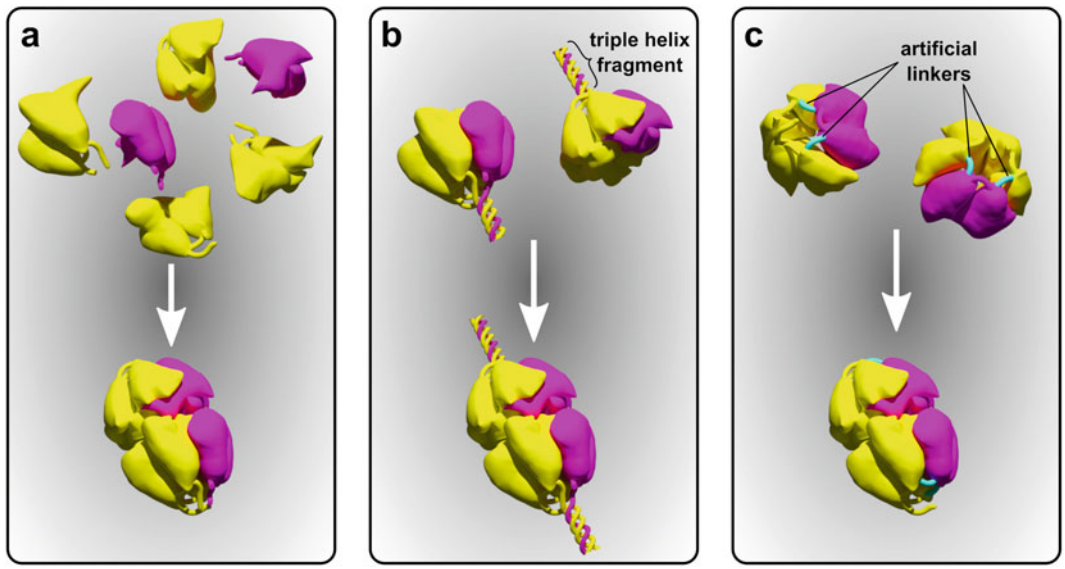

Fig. 2 Experimental models of collagen IV hexamer assembly. (**a**) NC1 monomers from either recombinant expression or from collagenase liberated NC1 extracted from bovine lens capsules or murine PFHR9 cell culture matrix. (**b**) Recombinant truncated protomers containing 28 GXY repeats necessary to form the triple helix and the whole NC1 domain. (**c**) Recombinant single-chain NC1 trimers. Three NC1 chains are linked by artificial 3-residue linkers (shown in cyan)

were able to induce the hexamer formation without presence of negatively charged ions. In contrast, halides were able to induce the hexamer assembly with the following relative efficiency: $Br^- > Cl^- >> I^- >> F^-$, where F^- had negligible effect. Whereas Br^- was the most efficient in initiating hexamerization, Cl^- was the only ion that could efficiently assemble NC1 hexamers at its physiologically relevant extracellular concentration (Cummings et al. 2016). Interestingly, addition of 1 mM Ca^{2+} in the presence of physiologically relevant (100 mM) Cl^- further increased efficiency of hexamerization suggesting complementary effect of divalent cations in this process (Cummings et al. 2016).

The role of Cl^- in assembly and stability of the hexamer can be illustrated in several ways. Size-exclusion chromatography demonstrates assembly (Fig. 3a, left) and disassembly (Fig. 3a, right) of the hexamer by the presence of hexamer or trimer peaks in elution profiles upon addition or removal of Cl^- (Pedchenko et al. 2019). Atomic force microscopy was found to be a very illustrative technique to assess the presence of hexamers in a sample (Fig. 3b) (Pedchenko et al. 2019). When a sample with NC1 trimers was imaged, individual randomly dispersed particles were seen (Fig. 3b, left). Surprisingly, when a sample with NC1 hexamers was imaged, pairs of individual particles were seen (Fig. 3b, right). Explanation of this phenomenon can be found in the method of sample preparation. After absorbing the NC1 hexamers onto the mica surface using the working solution (with

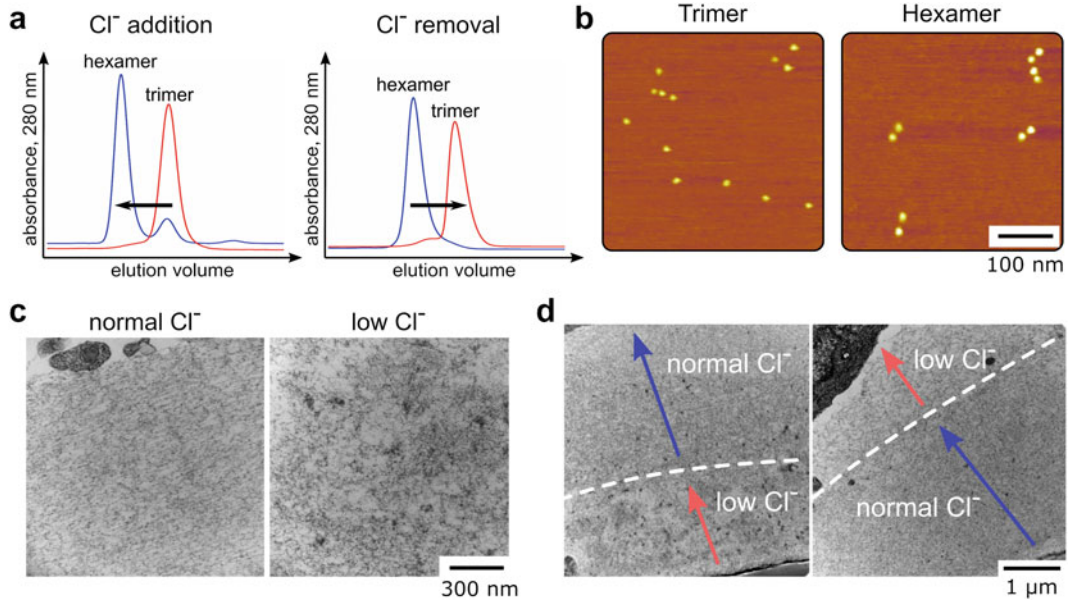

Fig. 3 Cl^- concentration affects collagen IV. (**a**) Elution profiles from size-exclusion chromatography demonstrate that single-chain NC1 trimers (red line) associate into hexamers (blue line) at high Cl^- concentration (left) but dissociate back to trimers when Cl^- are replaced with acetate (right). (**b**) Atomic force microscopy of single-chain NC1 trimers (left) or hexamers (right). Hexamers dissociate into pairs of trimers after washing the sample with water, which initiates Cl^- removal from the NC1. (**c**) Effect of Cl^- on the quality of deposited basement membrane-like matrix by cultured PFHR9 cells as visualized by transmission electron microscopy. Left, cells were grown using normal medium with physiological Cl^- concentration for 10 days. Right, cells were grown in the medium with low Cl^- concentration for 10 days. (**d**) Effects of medium changes on the matrix. Left, initially cells were grown in the medium with low Cl^- concentration for 5 days and then in the normal medium for additional 5 days. Right, initially cells were grown in the normal medium for 5 days and then switched to low Cl^- concentration for additional 5 days. Overall, the matrix deposited under low Cl^- concentration is less dense and a boarder is noticeable between two types of matrix. When cells were initially grown in the low Cl^- concentration the matrix was partially restored (patches of dense matrix) after switching to the normal medium. Arrows indicate direction of basement membrane-like matrix deposition over time

enough Cl^- concentration), the surface is briefly washed with water and immediately air dried for subsequent imaging. This short washing step removes Cl^- and thus, initiates dissociation into trimers, which remain non-covalently absorbed to the surface.

Depletion of Cl^- had drastic effect on quantity and quality of the growing BM as visualized in cell culture experiments with varying Cl^- concentration in the medium (Cummings et al. 2016). At low Cl^- concentration (~5 mM) cells deposit disorganized matrix with lesser density compared to normal conditions (Fig. 3c). Switching from low to normal Cl^- concentration partially repairs the abnormal BM (Fig. 3d, left panel). Meanwhile, switching from normal to low Cl^- concentration seems to not significantly disrupt BM deposited under normal conditions (Fig. 3d, right panel), suggesting stabilization of the NC1 hexamer through sulfilimine cross-linking (Bhave et al. 2012; Vanacore et al. 2009) and possibly complexation with other macromolecules in the BM. Nevertheless, Cl^- depletion can disrupt NC1 hexamer assembly, and may disrupt yet unknown macromolecular complexes within BM.

The development of a single-chain NC1 trimer as a tool to study the assembly of the NC1 hexamer (Fig. 2c) allowed quantitative analysis of this process and establishment of a kinetics model (Pedchenko et al. 2019). Plotting hexamer formation versus Cl^- concentration results in a sigmoidal curve (Fig. 4a). Cl^- concentrations below 10 mM (intracellularly relevant) appear to prohibit hexamer formation, whereas at 100 mM (extracellular concentration), the fraction of hexamer formed approaches a plateau. This titration curve surprisingly fits the two extremes of the Cl^- step gradient across the cell membrane (e.g. 7 mM inside the cell and 100 mM outside the cell (Andersen 2013; Armstrong 2003)) and suggests a major role for Cl^- in collagen IV scaffold assembly outside the cell. Experimentally measured kinetics of the hexamer assembly at high Cl^- concentration and protein concentration dependence revealed that this Cl^- ion driven process fits a simple bimolecular reaction model with the rate constant $k_a = 3.45 \pm 0.12\ M^{-1}\ s^{-1}$ (Pedchenko et al. 2019). Projections of the hexamer assembly demonstrate slow kinetics with half-times ranging from 20 min to 7 h at 25 to 1 mg/ml protein concentration (Fig. 4b). The correlation between this *in vitro* data and the *in vivo* processes remains unknown.

5 Cl^- Ions Are Structural Components of the NC1 Hexamer

Recently reported crystal structures of Cl^- bound hexamers $NC1^{sc\text{-}\alpha121}$ (Fig. 2c), $NC1^{\alpha111}$, $NC1^{\alpha333}$, and $NC1^{\alpha555}$ finally revealed the

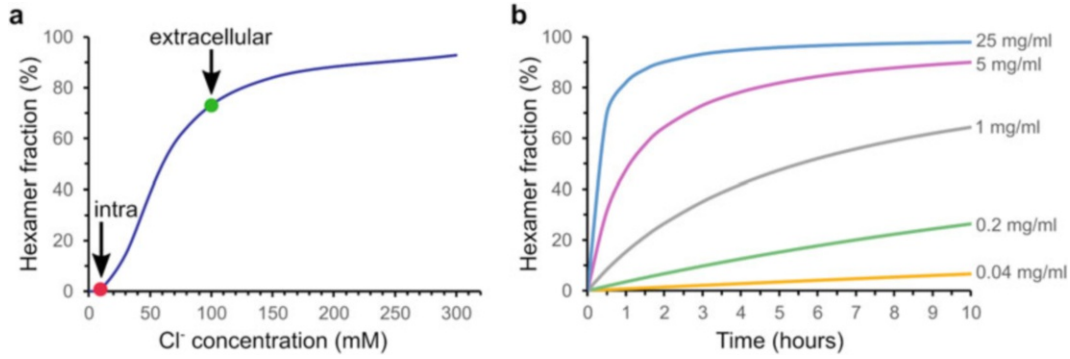

Fig. 4 Cl^- titration and kinetics of the hexamer assembly. (**a**) Cl^- titration curve, adapted from (Pedchenko et al. 2019). The amount of the hexamer formed depends on Cl^- concentration. The red dot indicates approximate intracellular Cl^- concentration, while the green dot indicates approximate extracellular Cl^- concentration under normal physiological conditions. (**b**) Calculated kinetics of hexamer formation at various protein concentrations, based on experimentally defined mechanism (Pedchenko et al. 2019)

number and positions of Cl^- within each hexamer (Casino et al. 2018; Pedchenko et al. 2019). The hexamers were specifically held at high Cl^- concentration prior and during crystallization to assure complete saturation of the structure with the Cl^- ions (Pedchenko et al. 2019). Each of these hexamer structures revealed 12 Cl^- ions at the trimer-trimer interface as shown for the α121 composition (Fig. 5). These 12 ions form a Cl^- ring, composed of two groups of 6, based on their structural environment.

In the following detailed descriptions of Cl^- environment, $NC1^{sc\text{-}\alpha121}$ and $NC1^{\alpha555}$ structures will be used as they were solved at the highest resolution. Group 1 Cl^- intercalate into the base of the NC1 trimers, where they are centered in loops formed by amino acids 74–78 and coordinated by these residues' backbone atoms (Fig. 6, top panel). In addition, an arginine residue from the opposite trimer forms a salt bridge to each of these ions. Molecular dynamics simulations also suggest that binding Group 1 ions causes the re-arrangement of the R76 side chain to break intra-trimeric salt bridges in order to form inter-trimeric salt bridges that contribute to hexamer formation (Cummings et al. 2016).

Group 2 Cl^- are located closer to the equatorial plane of the hexamer (Fig. 5). Instead of intercalating into the base of the NC1 trimers, these ions sit directly at the interface and act like a bridge that connects two protomers by accepting hydrogen bonds from each NC1 trimer (Fig. 6, bottom panel). A unique aspect of the Group 2 Cl^- that will be discussed further in the subsequent section is the fragility of this coordination. Aliphatic C-H groups from alanine and aromatic residue side chains serve as donors for the Cl^- interaction. In addition, the solution outside the hexamer can donate hydrogen bonds not only from water, but also from polyethylene glycol

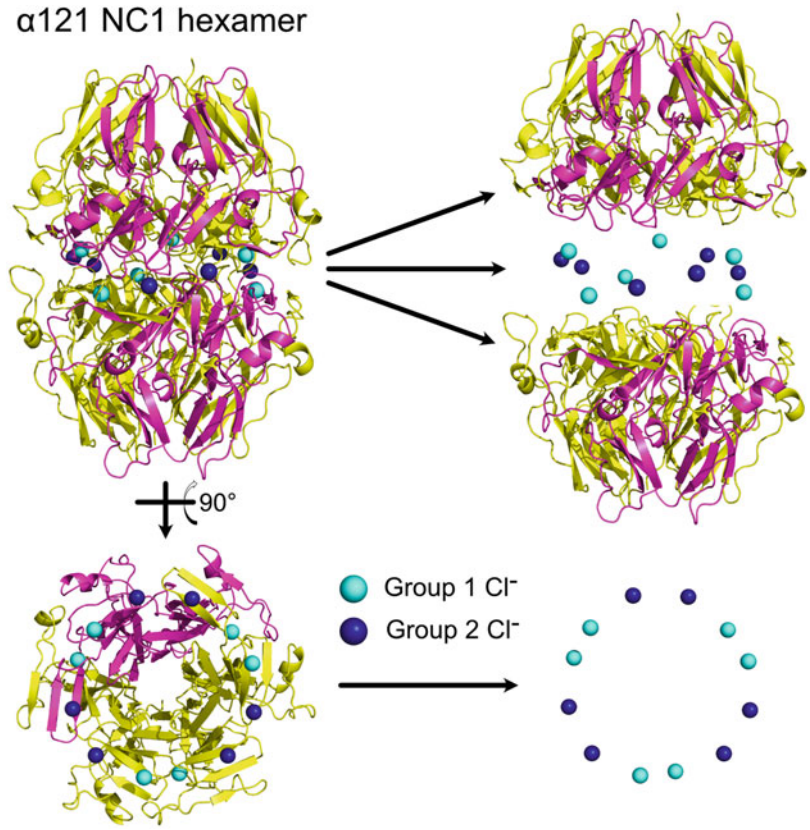

Fig. 5 Cl^- at the hexamer interface of $NC1^{\alpha121}$. Cartoon representation of the crystal structure of the $NC1^{\alpha121}$ hexamer. Coloring for α1 and α2 chains are yellow and magenta, respectively. Spaced-out image reveals 12 Cl^- (shown as spheres) sandwiched between NC1 trimers. Rotation of the hexamer presents an interfacial view that reveals the distribution of Cl^- resembles a ring around the interface. The Cl^- can be further split into Group 1 (colored cyan) and Group 2 (colored blue) depending on the nature of their coordination. The figure was generated using coordinates of the crystal structure of $NC1^{sc\text{-}\alpha121}$ (PBD code: 6MPX)

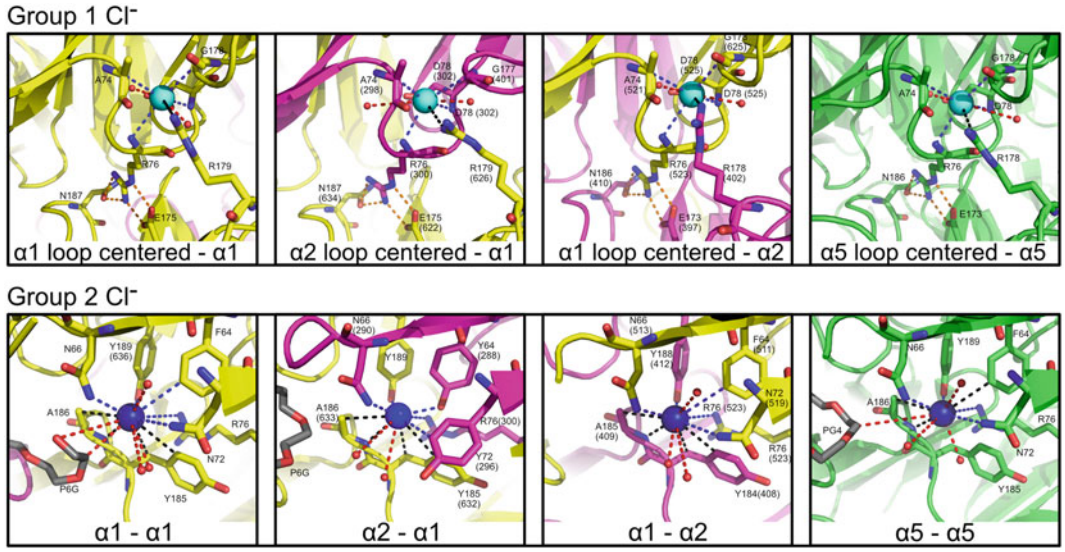

Fig. 6 Cl^- coordination at the interface between NC1 trimers. The top panel shows coordination of Group 1 Cl^- (shown as cyan spheres), while the bottom panel depicts coordination of Group 2 Cl^- (shown as blue spheres). The α1, α2, and α5 NC1 domains are colored yellow, magenta, and green, respectively. Water molecules are shown as red spheres. PEG molecules found near Group 2 ions are shown as gray carbon chains with red oxygens. Interactions between Cl^- and either water or PEG are denoted by red dashes. Blue dashes denote Cl^- interactions with one NC1 trimer, while black dashes denote Cl^- interactions with the opposite NC1 trimer. Residue numbering follows the convention used for non-single chain structures with single-chain structure numbering in parenthesis. The figure was generated using coordinates of the crystal structures of $NC1^{sc\text{-}\alpha121}$ and $NC1^{\alpha555}$ hexamers (PBD codes: 6MPX and 5NAZ)

(PEG) molecules included in the crystallization solution. Combined, these interactions represent a weak interaction network that could allow for relatively easy exchange with solvent Cl^- ions suggesting highly dynamic nature of Group 2 Cl^- coordination within the NC1 domain.

6 Surface Environment Accessibility of Cl^- Ions

Surface analysis of ions at the center of the NC1 hexamer presents a putative binding order and differentiation of roles between Group 1 and Group 2 Cl^-. In both the $NC1^{\alpha121}$ and $NC1^{\alpha555}$ hexamers, Group 1 Cl^- are visible only when one of the trimers is removed (Fig. 7). Hence, it appears the Group 1 Cl^- must bind to the NC1 before Group 2 Cl^- and thus, play a signaling role in assembly. It appears that Group 1 Cl^- ions in the $NC1^{\alpha555}$ trimer are embedded deeper into the NC1 surface compared to equivalent Cl^- in the $NC1^{\alpha121}$ trimer. Whether this indicates $NC1^{\alpha555}$ bind tighter to this Cl^- group or is also relevant to $NC1^{\alpha345}$ or $NC1^{\alpha121/\alpha565}$ hexamers is unknown currently.

Whereas the Group 1 Cl^- are sequestered inside the core of the NC1 hexamers, Group 2 Cl^- are readily accessible to solvent through portals in the hexamer (Fig. 8). Both structures contain polyethylene glycol (PEG) fragments, though their location in the structure leads to a curious observation. While both PEG and water molecules are found near the portals in odd-numbered NC1 alignments, only water was identified near the portals for $NC1^{\alpha2}$ (Fig. 8). Conclusions cannot be drawn from the $NC1^{\alpha2,\ \alpha3,\ \alpha4}$ homo-oligomer structures (PDB IDs: 5NB2, 5NB0, and 5NB1) as none were solved at sufficient resolution to observe PEG molecules included in the crystallization drop and $NC1^{\ \alpha2,\ \alpha4}$ structures form unusual non-hexameric assemblies. Meanwhile, the $NC1^{\alpha1}$ homo-hexamer

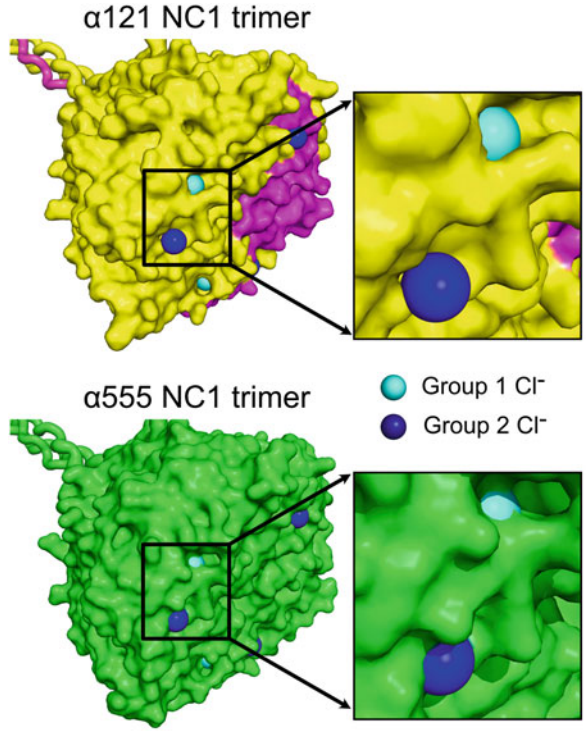

Fig. 7 Interfacial view of the surface of NC1$^{\alpha 121}$ and NC1$^{\alpha 555}$ trimers as part of a hexameric assembly. As Group 1 Cl^- (cyan) are embedded into the NC1 trimer, they are accessed only through channels leading into the interface. Group 2 Cl^-, meanwhile, lie in the interface itself, appearing as though they "sit" on the NC1 surface. The triple helical collagen segments in this figure are added to orient the reader to the interface surface and are not a part of the crystal structures. Arrows extending from each box point to an expand out of the interfacial region within the box. The figure was generated using coordinates of the crystal structures of NC1$^{sc\text{-}\alpha 121}$ and NC1$^{\alpha 555}$ hexamers (PBD codes: 6MPX and 5NAZ)

was not crystallized in the presence of PEG molecules and no structures including NC1$^{\alpha 6}$ are available. Nevertheless, observation of the position of PEG molecules raises the possibility that the surface topography of odd-numbered NC1 pairings is distinguished from the surface topography of even-numbered NC1 pairings in that it could accommodate environmental perturbations near Cl^- that are necessary for NC1 assembly.

Whereas Group 1 is solvent-accessible only in the trimer configuration (Fig. 7), Group 2 remains exposed to solvent upon hexamer assembly (Fig. 8). Thus, each Group 2 Cl^-, positioned in a pocket communicating to the outside through a portal, can therefore be in a dynamic equilibrium with free ions in the solution. Remarkably, the nature and geometry of this arrangement seems to be also conserved for all reported types of NC1 hexamers (Fig. 1d) suggesting a common sensing mechanism to Cl^- concentration.

7 Conclusions

Assembling of collagen IV structures has distinct stages both inside and outside of the cell (Fig. 9). The NC1 domain plays dual intra- and extracellular roles. Inside the cell, the domain is responsible for selecting three α chains, bringing them together and nucleating folding of triple helix in a zipper-like fashion (Soder and Poschl 2004). Outside the cell, trimeric NC1 domains from two protomers are connected by Cl^- to form hexamers, which can be then covalently

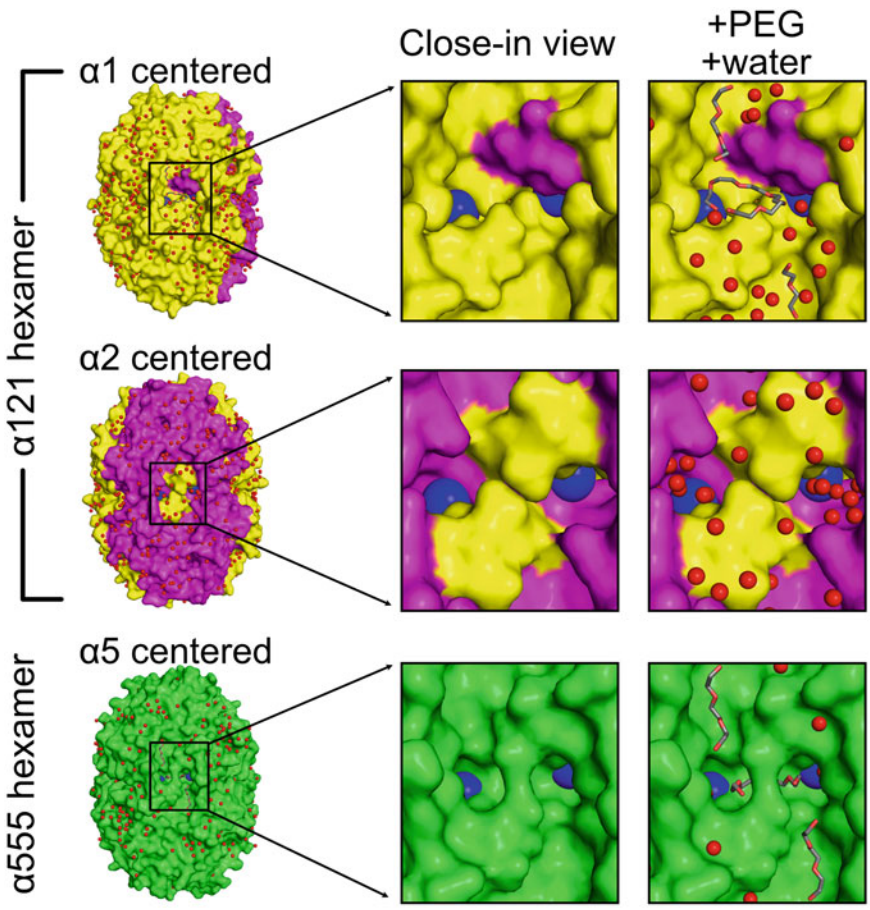

Fig. 8 Group 2 Cl^- are solvent accessible in the NC1 hexamers. Solvent accessible portals on the surface of the NC1 hexamers reveal Group 2 Cl^- (blue spheres) and unique portal environments. Group 1 Cl^- are not directly solvent accessible. Structured water molecules are shown as smaller red spheres, while structured PEG molecules are shown with gray carbon chains. Coloring for each NC1 domain is yellow for α1, magenta for α2, and green for α5. Only one $NC1^{\alpha121}$ trimer is found in the asymmetric unit of the crystal structure and hence, the two α1 centered faces of the hexamer are similar due to the twofold crystallographic symmetry rotation necessary to complete the hexamer. Meanwhile, only one $NC1^{\alpha5}$ domain is found in the asymmetric unit of the corresponding structure. Thus, each α5 centered face is identical due to the threefold and twofold crystallographic symmetry rotations necessary to complete the hexamer. The figure was generated using coordinates of the crystal structures of $NC1^{sc\text{-}\alpha121}$ and $NC1^{\alpha555}$ hexamers (PBD codes: 6MPX and 5NAZ)

connected by sulfilimine cross-links with the help of the peroxide reductase, peroxidasin (Fig. 1d). (Brown et al. 2017; Bhave et al. 2012; Vanacore et al. 2009). Until recently, the exact molecular mechanism of the NC1-driven collagen scaffold assembly remained unclear. Biochemical and structural data have now demonstrated that NC1 domains of collagen IV are equipped with a Cl^- sensing mechanism, which is capable of triggering conformational switches essential for hexamer assembly. It also appears that this mechanism has emerged early in evolution (Cummings et al. 2016; Pedchenko et al. 2019). Structural organization resulting in two groups of Cl^- ions suggests distinct steps take place during hexamer assembly. Group 1 ions bind to the trimer and re-organize the surface for trimer-trimer docking. Group 2 Cl^- bind to the formed hexamer and ultimately stabilize it (Fig. 9) (Pedchenko et al. 2019). The hexamer structure is sufficiently

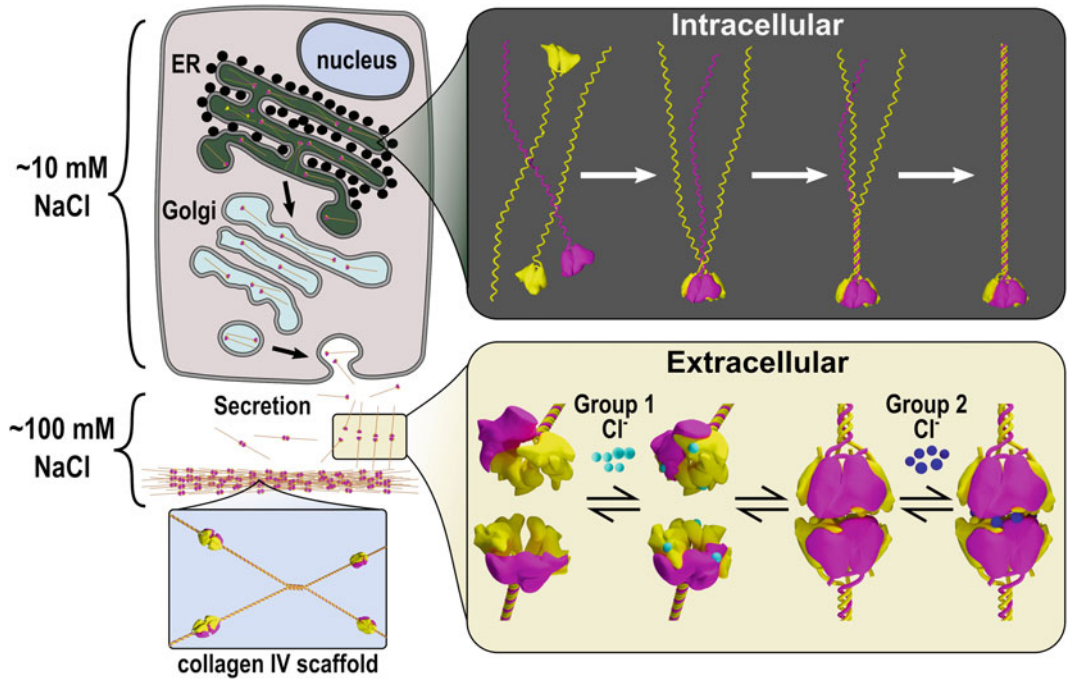

Fig. 9 Collagen IV assembly steps driven by the NC1 domain. Once collagen IV chains are translated into the ER lumen the NC1 domain drives chain selection and trimerization event. Trimerization of the NC1 domain subsequently nucleates the formation of the triple helix which propagates along the molecule from the NC1 domain to the N-termini of the chains in a zipper-like fashion. After multiple post-translational modifications and quality control events, the collagen IV protomers are secreted outside the cell into a high Cl^- concentration environment. In the current model, once in the extracellular space the NC1 trimers undergo conformational transitions upon binding Cl^- (Group 1 (shown as cyan spheres) first, followed by Group 2 (shown as blue spheres)). Subsequently, physiologically high extracellular Cl^- concentration is critical for maintaining the hexamer structure, as Cl^- depletion results in dissociation of the hexamer. This hexamer is a critical step towards collagen IV scaffold assembly

dynamic to require high Cl^- concentration in the surrounding environment as depletion of free Cl^- causes dissociation of the hexamer.

Thus far there is limited structural information on the nature of the extracellular Cl^- binding sites, and therefore, no consensus motifs or even essential residues for Cl^- binding have emerged to allow searching for similar sites in other proteins (Luscher et al. 2020). Establishing Cl^- binding sites within the NC1 hexamer of collagen IV provides a new brick for building our understanding of role of Cl^- in structural organization, functional aspects, and signaling events happening in the extracellular milieu. The step gradient of Cl^- concentration between intracellular and extracellular space can also be exploited by other ECM proteins, yet to be discovered, as a common mechanism. Involvement of Cl^- concentration in control of assembly, function and signaling can also be considered for development of new types of therapies.

There are several interesting questions to be addressed in the future studies of collagen IV scaffold assembly and function related to the NC1 hexamer and Cl^-: (1) Does the sequence similarity between α1-α6 chains lead to the same Cl^--dependent mechanism for NC1 hexamer formation for other known compositions, *i.e.* α345 and α121/α565? (2) Will the Cl^- titration curve and kinetics observed for α121 apply to other assemblies? (3) What is the mechanism of discrimination between α121 and α121/α565 hexamers? (4) What cofactors or helper proteins could accelerate the remarkably

slow rate-limiting step of the hexamer assembly (Sect. 4) and/or reduce the high protein concentration necessary for hexamer formation? (5) What mechanisms, besides sulfilimine cross-linking (Bhave et al. 2012; Vanacore et al. 2009), could protect the NC1 hexamer in the event of a Cl^- concentration drop? (6) How much variability in BM Cl^- concentration exists in health and disease? (7) Is there a cell signaling role for the NC1 domain that utilizes BM Cl^- concentration sensing?

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Conflicts of Interests Authors declare no conflicts of interest.

References

Andersen O (2013) Cellular electrolyte metabolism. Encyclopedia of metalloproteins. Springer, New York

Armstrong CM (2003) The Na/K pump, Cl ion, and osmotic stabilization of cells. Proc Natl Acad Sci U S A 100(10):6257–6262. https://doi.org/10.1073/pnas.0931278100

Bachinger HP, Fessler LI, Fessler JH (1982) Mouse procollagen IV. Characterization and supramolecular association. J Biol Chem 257(16):9796–9803

Bhave G, Cummings CF, Vanacore RM, Kumagai-Cresse-C, Ero-Tolliver IA, Rafi M, Kang JS, Pedchenko V, Fessler LI, Fessler JH, Hudson BG (2012) Peroxidasin forms sulfilimine chemical bonds using hypohalous acids in tissue genesis. Nat Chem Biol 8(9):784–790. https://doi.org/10.1038/nchembio.1038

Borza DB, Bondar O, Ninomiya Y, Sado Y, Naito I, Todd P, Hudson BG (2001) The NC1 domain of collagen IV encodes a novel network composed of the alpha 1, alpha 2, alpha 5, and alpha 6 chains in smooth muscle basement membranes. J Biol Chem 276 (30):28532–28540. https://doi.org/10.1074/jbc.M103690200

Boudko SP, Danylevych N, Hudson BG, Pedchenko VK (2018) Basement membrane collagen IV: isolation of functional domains. Methods Cell Biol 143:171–185. https://doi.org/10.1016/bs.mcb.2017.08.010

Boutaud A, Borza DB, Bondar O, Gunwar S, Netzer KO, Singh N, Ninomiya Y, Sado Y, Noelken ME, Hudson BG (2000) Type IV collagen of the glomerular basement membrane. Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. J Biol Chem 275(39):30716–30724. https://doi.org/10.1074/jbc.M004569200

Brown KL, Cummings CF, Vanacore RM, Hudson BG (2017) Building collagen IV smart scaffolds on the outside of cells. Protein Sci 26(11):2151–2161. https://doi.org/10.1002/pro.3283

Casino P, Gozalbo-Rovira R, Rodriguez-Diaz J, Banerjee S, Boutaud A, Rubio V, Hudson BG, Saus J, Cervera J, Marina A (2018) Structures of collagen IV globular domains: insight into associated pathologies, folding and network assembly. IUCr J 5 (Pt 6):765–779. https://doi.org/10.1107/S2052252518012459

Cosgrove D, Liu S (2017) Collagen IV diseases: a focus on the glomerular basement membrane in Alport syndrome. Matrix Biol 57-58:45–54. https://doi.org/10.1016/j.matbio.2016.08.005

Cummings CF, Pedchenko V, Brown KL, Colon S, Rafi M, Jones-Paris C, Pokydeshava E, Liu M, Pastor-Pareja JC, Stothers C, Ero-Tolliver IA, McCall AS, Vanacore R, Bhave G, Santoro S, Blackwell TS, Zent R, Pozzi A, Hudson BG (2016) Extracellular chloride signals collagen IV network assembly during basement membrane formation. J Cell Biol 213(4):479–494. https://doi.org/10.1083/jcb.201510065

Fidler AL, Darris CE, Chetyrkin SV, Pedchenko VK, Boudko SP, Brown KL, Gray Jerome W, Hudson JK, Rokas A, Hudson BG (2017) Collagen IV and basement membrane at the evolutionary dawn of metazoan tissues. elife 6. https://doi.org/10.7554/eLife.24176

Gunwar S, Ballester F, Noelken ME, Sado Y, Ninomiya Y, Hudson BG (1998) Glomerular basement membrane. Identification of a novel disulfide-cross-linked network of alpha3, alpha4, and alpha5 chains of type IV collagen and its implications for the pathogenesis of Alport syndrome. J Biol Chem 273 (15):8767–8775. https://doi.org/10.1074/jbc.273.15.8767

Hudson BG, Kalluri R, Gunwar S, Noelken ME (1994) Structure and organization of type IV collagen of renal glomerular basement membrane. Contrib Nephrol 107:163–167. https://doi.org/10.1159/000422975

Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG (2003) Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med 348 (25):2543–2556. https://doi.org/10.1056/NEJMra022296

Jayadev R, Sherwood DR (2017) Basement membranes. Curr Biol 27(6):R207–R211. https://doi.org/10.1016/j.cub.2017.02.006

Kahsai TZ, Enders GC, Gunwar S, Brunmark C, Wieslander J, Kalluri R, Zhou J, Noelken ME, Hudson BG (1997) Seminiferous tubule basement membrane. Composition and organization of type IV collagen chains, and the linkage of alpha3(IV) and alpha5

(IV) chains. J Biol Chem 272(27):17023–17032. https://doi.org/10.1074/jbc.272.27.17023

Kefalides NA (1968) Isolation and characterization of the collagen from glomerular basement membrane. Biochemistry 7(9):3103–3112. https://doi.org/10.1021/bi00849a012

Lu JY, Mohammed TA, Donohue ST, Al-Ghoul KJ (2008) Distribution of basal membrane complex components in elongating lens fibers. Mol Vis 14:1187–1203

Luscher BP, Vachel L, Ohana E, Muallem S (2020) Cl(−) as a bona fide signaling ion. Am J Physiol Cell Physiol 318(1):C125–C136. https://doi.org/10.1152/ajpcell.00354.2019

Meuwissen ME, Halley DJ, Smit LS, Lequin MH, Cobben JM, de Coo R, van Harssel J, Sallevelt S, Woldringh G, van der Knaap MS, de Vries LS, Mancini GM (2015) The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. Genet Med 17 (11):843–853. https://doi.org/10.1038/gim.2014.210

Morrissey MA, Sherwood DR (2015) An active role for basement membrane assembly and modification in tissue sculpting. J Cell Sci 128(9):1661–1668. https://doi.org/10.1242/jcs.168021

Pedchenko V, Bauer R, Pokidysheva EN, Al-Shaer A, Forde NR, Fidler AL, Hudson BG, Boudko SP (2019) A chloride ring is an ancient evolutionary innovation mediating the assembly of the collagen IV scaffold of basement membranes. J Biol Chem 294 (20):7968–7981. https://doi.org/10.1074/jbc.RA119.007426

Risteli J, Bachinger HP, Engel J, Furthmayr H, Timpl R (1980) 7-S collagen: characterization of an unusual basement membrane structure. Eur J Biochem 108 (1):239–250. https://doi.org/10.1111/j.1432-1033.1980.tb04717.x

Soder S, Poschl E (2004) The NC1 domain of human collagen IV is necessary to initiate triple helix formation. Biochem Biophys Res Commun 325(1):276–280. https://doi.org/10.1016/j.bbrc.2004.10.034

Spiro RG (1967) Studies on the renal glomerular basement membrane. Preparation and chemical composition. J Biol Chem 242(8):1915–1922

Stokman MF, Renkema KY, Giles RH, Schaefer F, Knoers NV, van Eerde AM (2016) The expanding phenotypic spectra of kidney diseases: insights from genetic studies. Nat Rev Nephrol 12(8):472–483. https://doi.org/10.1038/nrneph.2016.87

Sundaramoorthy M, Meiyappan M, Todd P, Hudson BG (2002) Crystal structure of NC1 domains. Structural basis for type IV collagen assembly in basement membranes. J Biol Chem 277(34):31142–31153. https://doi.org/10.1074/jbc.M201740200

Than ME, Henrich S, Huber R, Ries A, Mann K, Kuhn K, Timpl R, Bourenkov GP, Bartunik HD, Bode W (2002) The 1.9-A crystal structure of the noncollagenous (NC1) domain of human placenta collagen IV shows stabilization via a novel type of covalent Met-Lys cross-link. Proc Natl Acad Sci U S A 99 (10):6607–6612. https://doi.org/10.1073/pnas.062183499

Timpl R, Risteli J, Bachinger HP (1979) Identification of a new basement membrane collagen by the aid of a large fragment resistant to bacterial collagenase. FEBS Lett 101(2):265–268. https://doi.org/10.1016/0014-5793(79)81022-4

Timpl R, Wiedemann H, van Delden V, Furthmayr H, Kuhn K (1981) A network model for the organization of type IV collagen molecules in basement membranes. Eur J Biochem 120(2):203–211. https://doi.org/10.1111/j.1432-1033.1981.tb05690.x

Vanacore RM, Shanmugasundararaj S, Friedman DB, Bondar O, Hudson BG, Sundaramoorthy M (2004) The alpha1.alpha2 network of collagen IV. Reinforced stabilization of the noncollagenous domain-1 by noncovalent forces and the absence of met-Lys cross-links. J Biol Chem 279 (43):44723–44730. https://doi.org/10.1074/jbc.M406344200

Vanacore R, Ham AJ, Voehler M, Sanders CR, Conrads TP, Veenstra TD, Sharpless KB, Dawson PE, Hudson BG (2009) A sulfilimine bond identified in collagen IV. Science 325(5945):1230–1234. https://doi.org/10.1126/science.1176811

Wang X, Li W, Wei K, Xiao R, Wang J, Ma H, Qin L, Shao W, Li C (2018) Missense mutations in COL4A5 or COL4A6 genes may cause cerebrovascular fibromuscular dysplasia: case report and literature review. Medicine (Baltimore) 97(30):e11538. https://doi.org/10.1097/MD.0000000000011538

Weber S, Engel J, Wiedemann H, Glanville RW, Timpl R (1984) Subunit structure and assembly of the globular domain of basement-membrane collagen type IV. Eur J Biochem 139(2):401–410. https://doi.org/10.1111/j.1432-1033.1984.tb08019.x

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Roles of Slit Ligands and Their Roundabout (Robo) Family of Receptors in Bone Remodeling

Tomoaki Niimi

Abstract

Slit guidance ligands (Slits) and their roundabout (Robo) family of receptors are well-known axon guidance molecules that were originally identified in *Drosophila* mutants with commissural axon pathfinding defects. However, Slit-Robo signaling has been shown to be involved in not only neurogenesis, but also the development of other organs such as the kidney and heart. Recently, it was also revealed that Slit-Robo signaling plays an important role in bone metabolism. For example, osteoclast-derived Slit3 plays an osteoprotective role by synchronously stimulating bone formation by osteoblasts and suppressing bone resorption by osteoclasts through Robo receptors expressed on osteoblastic and osteoclastic cell lineages, making it a potential therapeutic target for metabolic bone disorders. Furthermore, osteoblast-derived Slit3 promotes bone formation indirectly as a proangiogenic factor. This review summarizes the recent progress on defining the roles of the Slit-Robo signaling in bone metabolism, and discusses the possible roles of the interaction between Robo and neural epidermal growth factor-like (NEL)-like (NELL) proteins that are novel ligands for Robo receptors.

Keywords

Clastokine · Coupling factor · NELL1 · Osteoblast · Osteoclast

Abbreviations

BMP	bone morphogenetic protein
EGFL	epidermal growth factor-like
FNIII	fibronectin type III
GTPase	guanosine triphosphatase
Ig	immunoglobulin
NELL	neural EGFL (NEL)-like
RANK	receptor activator of nuclear factor-κB
RANKL	RANK ligand
Robo	roundabout
Sema	semaphorin
Slit	Slit guidance ligand

1 Introduction

Bone locally repeats ossification and bone resorption for a lifetime to build skeletal structures without changing its morphology and serves as a calcium reservoir to maintain calcium homeostasis. These turnover processes are called bone remodeling (Zaidi 2007). Bone remodeling is

T. Niimi (✉)
Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan
e-mail: tniimi@agr.nagoya-u.ac.jp

carried out by temporary structures known as the basic multicellular units (BMUs) that are composed of two main cell types: osteoclasts and osteoblasts (Sims and Martin 2015, 2020; Kenkre and Bassett 2018). The bone remodeling cycle begins with the activation of osteoclastic bone resorption. After the resorption phase is completed, osteoclasts disappear and osteoblast precursors are recruited to the bone surface, and this phase is called the reversal phase. The formation phase follows with osteoblasts laying down bone matrix until the resorbed bone is completely replaced by new bone. The same amount of bone that was resorbed by osteoclasts must be newly formed to maintain the balance between osteoclastic bone resorption and osteoblastic bone formation. A local coupling factor linking bone resorption to subsequent bone formation has long been proposed to explain the coordination of bone resorption and formation in BMUs (Sims and Martin 2020; Martin and Sims 2005; Kim and Koh 2019). Several coupling factors have been identified, including those derived from the bone matrix, such as transforming growth factor-β1 (TGF-β1) (Tang et al. 2009) and insulin-like growth factor 1 (IGF-1) (Xian et al. 2012), and those secreted by osteoclasts, which act on osteoblasts, such as cardiotrophin-1 (Walker et al. 2008), sphingosine-1-phosphate (S1P) (Ryu et al. 2006), collagen triple helix repeat containing 1 (CTHRC1) (Takeshita et al. 2013) and complement factor 3a (Matsuoka et al. 2014).

In contrast to these coupling factors that act on osteoblasts, semaphorin (Sema) 3A, which belongs to the semaphorin family of axon guidance molecules, is known as a dual-acting factor that acts on both osteoblasts and osteoclasts to control their differentiation (Hayashi et al. 2012). Sema3A is a secreted protein that binds to plexin A/neuropilin-1 receptor complexes to suppress osteoclast differentiation and promote osteoblast differentiation synchronously. Although Sema3A plays an osteoprotective role, it is not produced by osteoclasts, but by osteoblasts and nerves. Interestingly, axon guidance molecules other than Sema3A have also been shown to be involved in bone remodeling. Sema4D, which is a transmembrane-type semaphorin family member, is produced by osteoclasts to suppress osteoblast maturation by binding to the plexin B1 receptor on osteoblasts (i.e., a "negative coupling factor") (Negishi-Koga et al. 2011). Netrin-1 has been reported to both promote and inhibit osteoclastogenesis through binding to the UNC5B receptor on osteoclasts (Mediero et al. 2015; Maruyama et al. 2016). These findings raise the question of whether Slit-Robo signaling is also involved in bone remodeling.

Slit ligands and their Robo family of receptors were originally identified as axon guidance molecules in *Drosophila* mutants that show abnormal projections of commissure neurons in the central nervous system (Rothberg et al. 1988; Seeger et al. 1993). However, Slit-Robo-mediated signaling is not limited to neural development, but is also involved in the development of various organs such as the kidney and heart (Lu et al. 2007; Wainwright et al. 2015; Zhao and Mommersteeg 2018). In addition, Slit-Robo signaling is involved in the regulation of angiogenesis, cancer development, cell migration, and cell proliferation (Dai et al. 2019; Gara et al. 2015; Blockus and Chedotal 2016). Recently, it was revealed that Slit-Robo signaling plays an important role in bone metabolism (Kim et al. 2018a; Xu et al. 2018). In this review, I discuss the roles of Slit-Robo signaling in bone remodeling and future research directions.

2 Slit Ligands and Robo Receptors: Structure and Interactions

In mammals, three *Slit* genes (*Slit1–3*) have been identified (Fig. 1) (Li et al. 1999). All *Slit* genes encode large (~200 kDa) secreted glycoproteins that comprise, from their N- to C-terminus, four leucine-rich repeats (LRRs), six EGFL repeats, a laminin G (LG) domain, a further three EGFL repeats, and a C-terminal cysteine knot-like domain. Slit proteins can be cleaved into large N-terminal (~140 kDa; Slit-N) and short C-terminal (~60 kDa; Slit-C) fragments at the putative proteolytic site between the fifth and sixth EGFL repeats (Brose et al. 1999). Both the

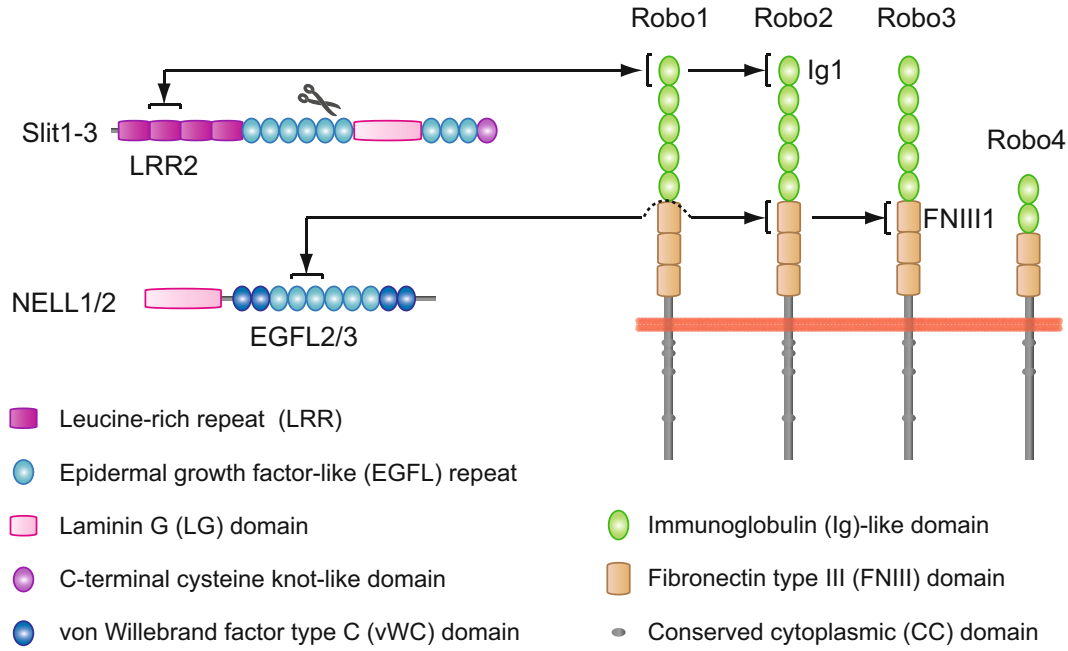

Fig. 1 Structures of the Slit, Robo, and NELL protein families. Slit ligands bind to the Robo1/2 Ig1 domain through their LRR2 domain, whereas NELL1/2 bind to the Robo2/3 FNIII1 domain through their EGFL2/3 domains. Slit proteins are proteolytically cleaved between EGFL5 and EGFL6 domains to create N- (Slit-N) and C-terminal (Slit-C) fragments. Both fragments bind to different receptors. A direct interaction between Slit/NELL ligands and Robo4 has not been demonstrated. The intracellular domains of Robo have several short conserved motifs that are important for Robo signaling

uncleaved and cleaved Slit proteins induce chemorepulsion by binding to Robo receptors through their second LRR within Slit-N or by binding to plexin A1 receptor for Slit-C (Delloye-Bourgeois et al. 2015).

The first *Robo* gene was named after the phenotype of the *Drosophila* mutant in which commissural axons inappropriately cross the midline many times in the manner of a roundabout (Seeger et al. 1993). *Robo* was found to encode a protein that is a single-pass transmembrane receptor for Slit ligands (Brose et al. 1999). Four *Robo* genes (*Robo1–4*) have been identified in mammals (Fig. 1) (Kidd et al. 1998; Huminiecki et al. 2002). The extracellular domains of Robo1–3 have five immunoglobulin (Ig)-like domains and three fibronectin type III (FNIII) domains, whereas Robo4, an endothelial-specific member of the Robo family, has only two Ig domains and two FNIII domains in its extracellular domain. The intracellular domain of Robo is poorly conserved, but there are four short conserved cytoplasmic (CC) motifs (CC0–3). Because the intracellular domain has no catalytic activity, various intracellular signaling molecules that bind to these CC motifs mediate the signal. Both Robo1 and Robo2 contain the binding site for Slit proteins in their first Ig domain (Morlot et al. 2007). However, Robo3 has lost the ability to bind Slit proteins because of a few amino acid changes in its first Ig domain (Zelina et al. 2014). Instead, Robo3 interacts with neural EGFL (NEL)-like protein 2 (NELL2) to induce chemorepulsion (Jaworski et al. 2015). Robo4 has been suggested to transduce Slit2/3 signaling in endothelial cells, but a direct interaction between Robo4 and Slit2/3 has not been demonstrated so far (Dai et al. 2019). Transmembrane heparan sulfate proteoglycans (HSPGs), such as syndecan and glypican, bind to both Slit and Robo via the heparan sulfate moiety, forming a ternary complex and stabilizing their interaction

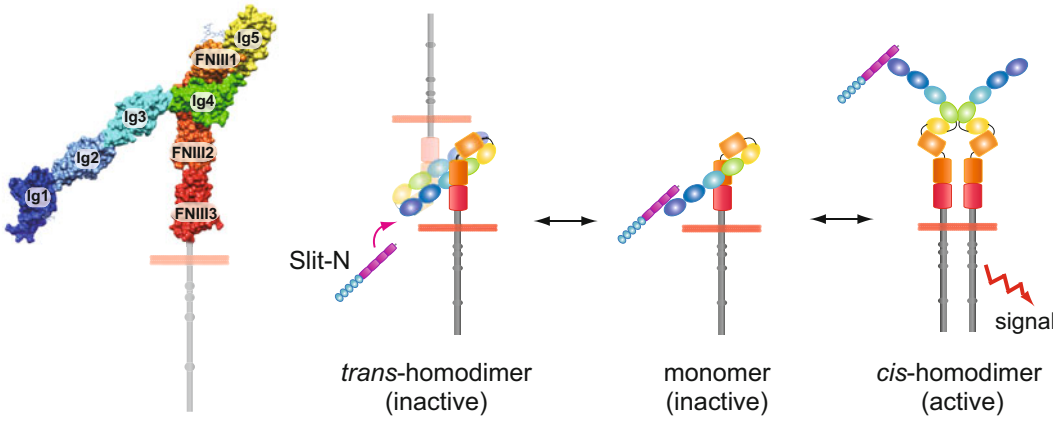

Fig. 2 Hairpin-like architecture of the Robo2 ectodomain and a model of Robo2 activation. (*Left*) Crystal structure of the human Robo2 ectodomain in surface representation (PDB ID: 6IAA). The nomenclature of Robo extracellular Ig and FNIII domains is shown on each domain structure. (*Right*) Model of Slit-induced Robo2 activation. *Trans*-interacting Robo2 homodimers are tightly auto-inhibited. Both *trans*-interacting Robo2 homodimers and monomers are inactive. Slit-N binding to the Robo2 Ig1 domain induces dissociation from *trans*-homodimers to monomers and facilitates formation of Ig4-mediated *cis*-homodimers following activation of the intracellular signals

at cell surfaces (Hussain et al. 2006). Robo receptors interact with other membrane receptors, such as deleted in colorectal carcinoma (DCC) and C-X-C motif chemokine receptor 4 (Cxcr4), to elicit their functions via shared downstream partners, including small guanosine triphosphatases (GTPases), cytoplasmic kinases, and adaptor molecules (Stein and Tessier-Lavigne 2001; Wu et al. 2001).

Robo1/2 form homo- and heterodimers in *cis*- and *trans*-positions (Fig. 2) (Hivert et al. 2002; Zakrys et al. 2014). Crystal structure and biochemical analyses have revealed that *cis*-homodimers form via the fourth Ig domain (Ig4) of Robo (Seiradake et al. 2009; Yom-Tov et al. 2017), whereas *trans*-homodimers are formed by head-to-head interactions between Ig1 and Ig5 of Robo on adjacent cell surfaces (Barak et al. 2019). Barak et al. recently demonstrated that the extracellular domain of Robo2 has a hairpin-like architecture as a monomer, which is unable to form *cis*-homodimers, but *trans*-homodimers because the Ig4-mediated dimerization interface is intramolecularly blocked by the FNIII domains (Barak et al. 2019). Because *cis*-dimerization is essential for Robo receptor activation, a hairpin-like architecture enables an auto-inhibited conformation of Robo receptors. Furthermore, *trans*-homodimer formation reinforces the auto-inhibition state strongly. It has been suggested that Slit binding to Robo reverses Robo auto-inhibition following by *cis*-dimerization and activation.

3 Roles of Slit-Robo Signaling in Bone Remodeling

Slit1 is minimally expressed in bone cells, whereas *Slit2* is expressed in osteoblastic cell lineages (Sun et al. 2009). *Slit3* is expressed in vascular endothelial cells other than the nervous system and has angiogenic functions (Zhang et al. 2009). In bone, *Slit3* is expressed in both osteoclastic and osteoblastic cell lineages (Kim et al. 2018a; Xu et al. 2018), although the expression levels may vary depending on the status of the cell (discussed later). *Robo1* and *Robo3* are expressed in osteoclastic cell lineages, while *Robo1* and *Robo2* are expressed in osteoblastic cell lineages (Kim et al. 2018a). *Robo4* is hardly expressed in bone cells (Xu et al. 2018). In a previous study, Slit2 was shown to inhibit osteoblast differentiation *in vitro* (Sun et al. 2009).

However, a recent study could not detect the inhibitory effect of Slit2 on osteoblast differentiation, probably because of the difference in methodology using recombinant Slit2 protein instead of conditioned medium containing Slit2 (Park et al. 2019). Accordingly, Slit2 may have no activity in osteoblasts but suppresses osteoclast differentiation through the Robo1 receptor by reducing the migration and fusion of preosteoclasts (Fig. 3a) (Park et al. 2019). Slit2 has been shown to interact with Gremlin, an antagonist of bone morphogenetic proteins (BMPs), to constitute a negative feedback loop where Slit2 blocks Gremlin inhibition of BMP2 and canonical BMP2 signaling downregulates Slit2 expression, although the role in bone remodeling is not presently known (Tumelty et al. 2018).

Recently, two different groups identified Slit3 as a bone anabolic factor. Kim et al. identified Slit3 in conditioned medium of osteoclasts as a coupling factor that coordinates bone resorption and formation (Kim et al. 2018a). They demonstrated that osteoclast-derived Slit3 acts on Robo1/2 receptors expressed by osteoblasts to promote osteoblast migration and proliferation, and also acts on Robo1 expressed by osteoclasts in an autocrine manner to suppress osteoclast differentiation (Fig. 3a). Mice lacking Slit3 specifically in osteoclasts have a low bone mass due to decreased bone formation and increased bone resorption, whereas mice with either neuron-specific Slit3 deletion or osteoblast-specific Slit3 deletion have a normal bone mass. These results indicate that Slit3 is a "clastokine", an osteoclast-derived osteoblast differentiation factor. They

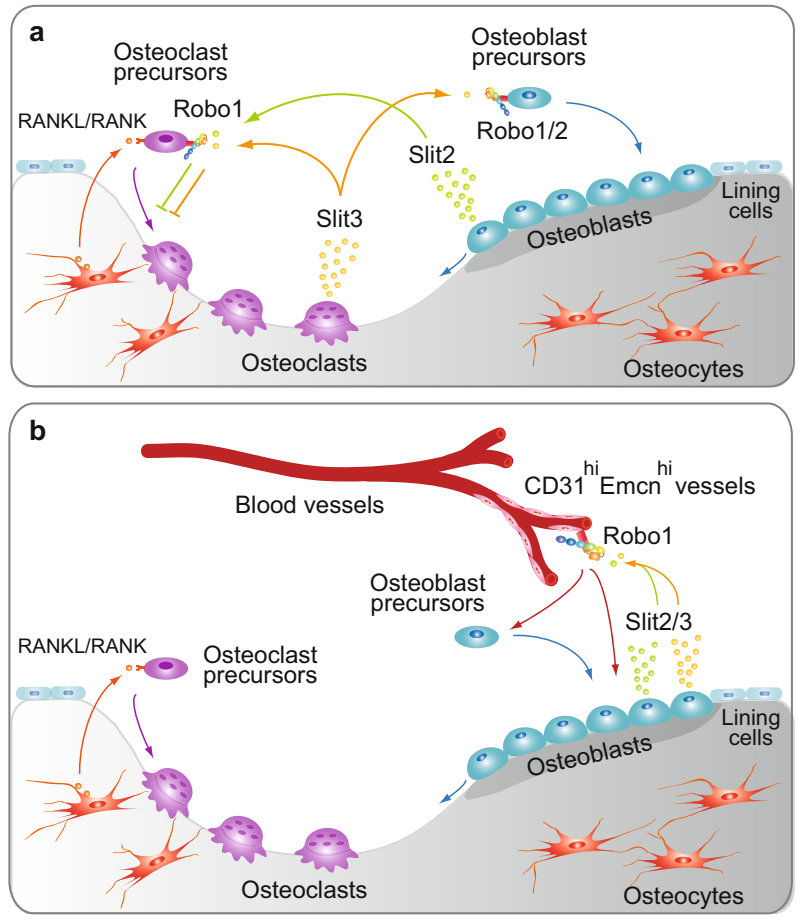

Fig. 3 Roles of Slit-Robo signaling in bone remodeling. (**a**) Slit3 secreted by osteoclasts and Slit2 secreted by osteoblasts suppress osteoclastogenesis through Robo1 binding. In addition, Slit3 promotes osteoblast migration and proliferation through Robo1/2 binding. (**b**) Slit3 secreted by osteoblasts induces $CD31^{hi}Emcn^{hi}$ blood vessels through Robo1 binding to promote osteoblast differentiation indirectly. Slit2 has a similar effect. The signaling protein RANKL secreted by osteocytes activates the RANK receptor on osteoclast precursors to stimulate osteoclastogenesis

also demonstrated that a genetic variant of the *SLIT3* gene was associated with bone parameters in postmenopausal women, and that high circulating SLIT3 levels in plasma are associated with high bone mineral density values. These observations suggest that SLIT3 is a potential biomarker to predict bone health in humans. Finally, administration of a truncated recombinant Slit3 to ovariectomized (OVX) mice significantly rescues bone loss by promoting osteoblastic bone formation and inhibiting osteoclastic bone resorption, indicating that Slit3 can be used as a therapeutic target for osteoporosis.

Shortly after this work, Xu et al. identified Slit3 as a proangiogenic factor that increases $CD31^{hi}endomucin^{hi}$ ($CD31^{hi}Emcn^{hi}$) vascular endothelium (type H vessel) levels, which positively regulate bone formation (Fig. 3b) (Xu et al. 2018). Mice lacking adaptor protein Schnurri-3 (SHN3) specifically in osteoblasts have markedly elevated bone formation with increased levels of Slit3 and $CD31^{hi}Emcn^{hi}$ endothelium, suggesting that Slit3 is an SHN3-controlled and osteoblast-derived regulator of bone formation by coupling between osteoblasts and $CD31^{hi}Emcn^{hi}$ endothelium. However, there are some differences in the results between the two groups, probably due in part to the differences in experimental methods and design. For example, Xu et al. observed robust *Slit3* expression in osteoblasts, but not in osteoclasts (Xu et al. 2018). They did not observe a direct effect of Slit3 on osteoblasts, but observed osteopenic phenotypes in osteoblast-specific *Slit3*-deficient mice with three different cre-deleter strains (Xu et al. 2018). They also demonstrated that Slit3 treatment had only a modest effect on osteoclastogenesis *in vitro* and osteoclast-specific *Slit3*-deficient mice showed normal bone mass (Li et al. 2020). These results indicate that osteoblasts are the major source of skeletal Slit3. However, another group has shown recently that osteoclast-derived Slit3 promotes osteoblast differentiation (Shin et al. 2020). The discrepancy between the two groups might be due in part to differences in tissue-specific cre-deleter strains utilized for generating conditional knockout mice. Although the primary source of skeletal Slit3 and the direct effect on bone cells continue to be debated, it seems certain that Slit3 acts as a potent angiogenic factor through Robo2 binding on endothelial cells to evoke osteoanabolic responses because Kim et al. also observed a decrease in type H vessels in global *Slit3*-deficient mice (Kim et al. 2018a; Peng et al. 2020). Further studies are required to better understand the role of Slit3-Robo signaling in bone remodeling.

4 Downstream of Slit-Robo Signaling in Bone Remodeling

The major signaling molecules downstream of Robo receptors are cytoplasmic kinases. Abelson (Abl) tyrosine kinase associates with the intracellular domain of the Robo receptor (Rhee et al. 2007). Upon binding to Slit ligands, Robo forms a multimolecular complex containing Abl kinase, adaptor protein Cables (Cdk5 and Abl enzyme substrate), N-cadherin, and β-catenin. Abl kinase subsequently phosphorylates β-catenin on tyrosine 489, resulting in the release of cadherin-associated β-catenin and the loss of N-cadherin-mediated cell adhesion. Slit3 has been shown to promote chondrocyte differentiation through the Robo2 receptor by suppression of β-catenin activity, thereby regulating endochondral ossification (Kim et al. 2018b). Interestingly, this is the opposite of the effect on osteoblasts in which Slit3 activates β-catenin to stimulate migration and proliferation (Kim et al. 2018a), indicating that the effect of Slit3 on β-catenin activity depends on the cell type.

The Rho family of small GTPases (Rac, Cdc42, and RhoA) also plays an important role in regulating the downstream responses of Slit-Robo signaling. They are key regulators of cytoskeletal reorganization critical for cell motility. Activation of Slit-Robo signaling recruits Slit-Robo GTPase-activating proteins (srGAPs) to the intracellular domain of the Robo receptor and inactivates Rho GTPases, thereby inhibiting actin polymerization and stress fiber formation (Wong et al. 2001). In Slit2-suppressed osteoclastogenesis, inhibition of Cdc42 GTPase by Slit2 is critical (Park et al. 2019). In contrast, Slit3-suppressed osteoclastogenesis is mediated by

inhibition of Rac1 GTPase (Kim et al. 2018a). A recent study showed that female mice deficient for srGAP2 specifically in myeloid lineages have high levels of Rac1 activation and increased bone formation with enhanced Slit3 production (Shin et al. 2020). A feedback loop in which Rac1 activity stimulates expression of Slit3 has been suggested to exist, which in turn acts to limit Rac1 activation through Robo1 and srGAP2 in osteoclastic cells.

5 NELL Family Proteins: Novel Ligands of Robo2/3 Receptors

Slit ligands have been shown to interact with several receptor proteins other than Robo, such as plexin A1 and dystroglycan (Delloye-Bourgeois et al. 2015; Wright et al. 2012). However, Slit proteins were the only known ligands of Robo receptors until NELL2 was recently identified as a ligand for Robo3 to function as a repulsive axon guidance cue that contributes to commissural axon guidance to the midline (Jaworski et al. 2015). Although NELL1 also binds to Robo3, NELL1 has a weak effect on commissural axon guidance repulsion (Pak et al. 2020). NELL1 and NELL2 constitute a NELL family of large secretory glycoproteins that contain an LG domain, four von Willebrand factor type C (vWC) domains, and six EGFL repeats (Fig. 1) (Zhang et al. 2010). Both *NELL1* and *NELL2* genes are expressed primarily in the brain and have high similarity in their amino acid sequences. However, the biological functions of the proteins they encode are greatly different (Kuroda et al. 1999). NELL2 plays multiple roles in neuronal development, survival, and activity (Aihara et al. 2003), whereas NELL1 mainly functions as a regulator of craniofacial skeletal morphogenesis (Zhang et al. 2010; Li et al. 2019). Most recently, NELL2 was shown to function as a regulator of sperm maturation by binding to an orphan receptor tyrosine kinase, c-ros oncogene 1 (ROS1) (Kiyozumi et al. 2020). The *NELL1* gene was originally identified as a gene overexpressed in human sporadic coronal craniosynostosis characterized by premature cranial suture closure with bone over growth (Ting et al. 1999). Transgenic mice overexpressing NELL1 display a phenotype similar to human craniosynostosis patients (Zhang et al. 2002), whereas *NELL1*-deficient mice have cranial and vertebral skeletal defects (Desai et al. 2006; Zhang et al. 2012). Currently, NELL2 targets Robo3 and ROS1 receptors (Jaworski et al. 2015; Kiyozumi et al. 2020), whereas the receptors targeted by NELL1 include integrin β1, HSPGs and contactin-associated protein-like 4 (Cntnap4) (Shen et al. 2012; Takahashi et al. 2015; Li et al. 2018).

To gain more insight into NELL-Robo interactions, our group examined the binding activity between NELL1/2 and the Robo family of receptors, and found that Robo2 is a potential receptor for NELL1/2 (Yamamoto et al. 2019). When using a fragment of Robo FNIII domains, NELL1/2 bind to the first FNIII domain of Robo2 and Robo3 through their EGFL domains, but not to those of Robo1 and Robo4 (Fig. 1). However, NELL1/2 bind to the whole ectodomain of Robo3, but not to that of Robo2. We hypothesized that this is because the conformation of the ectodomain of Robo2 might mask the binding site for NELL1/2, which is supported by a recent study showing that the Robo2 ectodomain has a hairpin-like architecture (Barak et al. 2019). Importantly, we observed that mild acidic conditions (pH 5.5–6.5) facilitate the interaction between NELL1/2 and intact Robo2, probably because of the conformational change to the Robo2 ectodomain (Fig. 4). Local extracellular acidification has been observed under many pathological conditions, including ischemia, inflammation, and tumor progression (Okajima 2013). Tissue acidosis causes a decrease in the bone mineral density by inducing cell death of osteoblasts and activation of osteoclasts to resorb bone (Arnett 2008; Arnett 2010). However, a recent study indicated that short-term acidic stimulation enhances the stem cell phenotype of mesenchymal stem cells, which facilitates differentiation to osteoblasts during bone tissue healing (Hazehara-Kunitomo et al. 2019). Although we have no direct evidence that NELL1 interacts with intact Robo2 *in vivo*, the

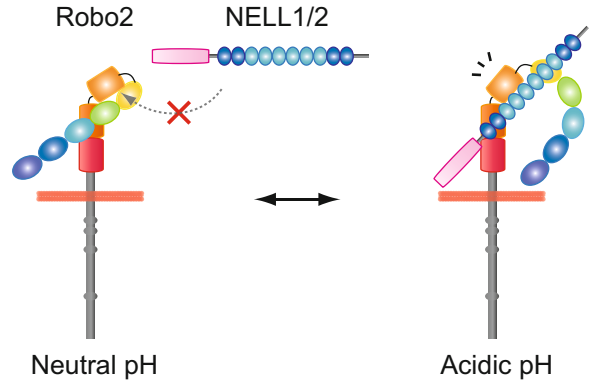

Fig. 4 Model showing the possible interaction between NELL proteins and Robo2 under acidic conditions. NELL1/2 only bind to Robo2 under acidic pH conditions. Conformational changes to the Robo2 ectodomain may unmask the binding site for NELL proteins under acidic conditions

temporal interaction between NELL1 and Robo2 in osteoblast precursors under acidic conditions may enhance bone formation to recover bone mass (Fig. 5).

The identification of the NELL proteins as novel ligands for the Robo receptors has raised questions concerning how NELL proteins activate Robo receptors and which signals are downstream of NELL-Robo interactions. Although NELL2 acts as repulsive axon guidance cue by binding to Robo3 and antagonizes Slits-Robo1/2-mediated repulsion, the molecular mechanisms of NELL2-Robo3 signaling remain unclear (Jaworski et al. 2015; Pak et al. 2020). In the case of the NELL1/2-Robo2 interaction, it is possible that Slit proteins and NELL1/2 act on Robo2 in cooperation because both ligands bind to Robo2 simultaneously. Thus, the signaling pathways downstream of the two classes of interaction, NELL-Robo2 and NELL-Robo3, may have different components. The identification of the NELL-Robo interaction has also stimulated further research. As described above, both NELL1 and NELL2 bind to Robo3, but NELL1 is not an effective ligand for Robo3 in commissural axons (Pak et al. 2020). It remains possible that NELL1 functions as a ligand for Robo3 at another spatiotemporal location. Because Robo3 is expressed in osteoclastic cell lineages (Kim et al. 2018a), NELL1 may have some effect on osteoclasts through binding to Robo3 (Fig. 5). In addition, conformational changes in the ectodomain of Robo2 allow both NELL1 and NELL2 to interact with Robo2, suggesting that NELL1/2-Robo2 interaction under acidic conditions functions in neuronal development.

6 Concluding Remarks

Because Slit ligands and their Robo receptors were originally identified in *Drosophila* mutants, many studies of *Drosophila* have been performed and the findings have contributed to the research progress on mammalian Slit-Robo signaling. However, *Drosophila* is not a suitable model to study bone metabolism because insects have an exoskeleton rather than a bony endoskeleton, which may be one of the reasons why studies of Slit-Robo signaling in bone remodeling have been recent. The identification of Slit3 as a coupling factor has revealed that Slit-Robo signaling acts with other axon guidance molecules in bone remodeling and the nervous system. As described above, Sema3A is a dual-acting factor that inhibits osteoclast differentiation and promotes osteoblast differentiation synchronously (Hayashi et al. 2012). Interestingly, neuron-specific, but not osteoblast-specific, *Sema3A*-deficient mice show a markedly low bone mass, suggesting that Sema3A affects bone remodeling through the nervous system. In comparison, osteoblast-specific, but not neuron-specific, *Slit3*-deficient mice show a significantly lower bone mass (Kim et al. 2018a; Xu et al. 2018). Thus, these two factors may act in a non-redundant and cooperative manner to fine-tune bone homeostasis. Recently, Ikebuchi et al. identified a unique

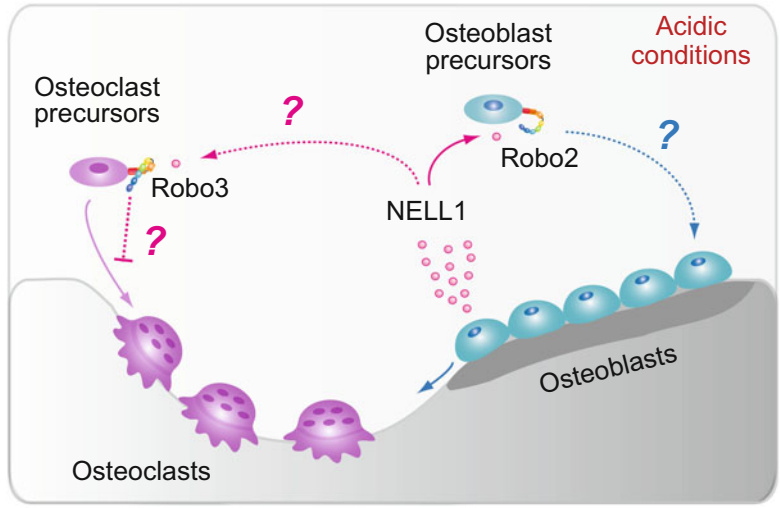

Fig. 5 Proposed model of the roles of NELL1-Robo signaling in bone remodeling. Robo2 is expressed in osteoblast precursors, whereas Robo3 is expressed in osteoclast precursors. NELL1 is possibly secreted by osteoblasts and may regulate osteoblast differentiation through Robo2 binding under acidic conditions. NELL1 may have some effects on osteoclasts through Robo3 binding

coupling factor, namely small extracellular vesicles (SEVs) containing receptor activator of nuclear factor-κB (RANK) secreted by mature osteoclasts (Ikebuchi et al. 2018). The RANK ligand (RANKL) produced by osteoblastic cell lineages acts on RANK expressed on the cell membrane of osteoclast precursor cells to differentiate osteoclasts. Ikebuchi et al. demonstrated that RANK-loaded SEVs secreted from mature osteoclasts act on RANKL on osteoblasts to promote osteoblast differentiation by activating the mTOR (mammalian target of rapamycin) pathway (RANKL reverse signaling). The interplay of these signaling systems, including RANKL-RANK, Sema3A-Plexin A1, Slit3-Robo, and other coupling factors, between osteoclasts and osteoblasts may contribute to the tight regulation of bone homeostasis. Future studies are required to elucidate how these factors coordinate bone-coupling events.

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Conflict of Interest The author declares that they have no conflicts of interest with the contents of this article.

References

Aihara K, Kuroda S, Kanayama N, Matsuyama S, Tanizawa K, Horie M (2003) A neuron-specific EGF family protein, NELL2, promotes survival of neurons through mitogen-activated protein kinases. Brain Res Mol Brain Res 116:86–93

Arnett TR (2008) Extracellular pH regulates bone cell function. J Nutr 138:415S–418S

Arnett TR (2010) Acidosis, hypoxia and bone. Arch Biochem Biophys 503:103–109

Barak R, Yom-Tov G, Guez-Haddad J, Gasri-Plotnitsky L, Maimon R, Cohen-Berkman M, McCarthy AA, Perlson E, Henis-Korenblit S, Isupov MN, Opatowsky Y (2019) Structural principles in Robo activation and auto-inhibition. Cell 177:272–285

Blockus H, Chedotal A (2016) Slit-Robo signaling. Development 143:3037–3044

Brose K, Bland KS, Wang KH, Arnott D, Henzel W, Goodman CS, Tessier-Lavigne M, Kidd T (1999) Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell 96:795–806

Dai C, Gong Q, Cheng Y, Su G (2019) Regulatory mechanisms of Robo4 and their effects on angiogenesis. Biosci Rep 39:BSR20190513

Delloye-Bourgeois C, Jacquier A, Charoy C, Reynaud F, Nawabi H, Thoinet K, Kindbeiter K, Yoshida Y, Zagar Y, Kong Y, Jones YE, Falk J, Chedotal A, Castellani V (2015) PlexinA1 is a new Slit receptor and mediates axon guidance function of Slit C-terminal fragments. Nat Neurosci 18:36–45

Desai J, Shannon ME, Johnson MD, Ruff DW, Hughes LA, Kerley MK, Carpenter DA, Johnson DK, Rinchik EM, Culiat CT (2006) Nell1-deficient mice have

reduced expression of extracellular matrix proteins causing cranial and vertebral defects. Hum Mol Genet 15:1329–1341

Gara RK, Kumari S, Ganju A, Yallapu MM, Jaggi M, Chauhan SC (2015) Slit/Robo pathway: a promising therapeutic target for cancer. Drug Discov Today 20:156–164

Hayashi M, Nakashima T, Taniguchi M, Kodama T, Kumanogoh A, Takayanagi H (2012) Osteoprotection by semaphorin 3A. Nature 485:69–74

Hazehara-Kunitomo Y, Hara ES, Ono M, Aung KT, Komi K, Pham HT, Akiyama K, Okada M, Oohashi T, Matsumoto T, Kuboki T (2019) Acidic pre-conditioning enhances the stem cell phenotype of human bone marrow stem/progenitor cells. Int J Mol Sci 20:1097

Hivert B, Liu Z, Chuang C-Y, Doherty P, Sundaresan V (2002) Robo1 and Robo2 are homophilic binding molecules that promote axonal growth. Mol Cell Neurosci 21:534–545

Huminiecki L, Gorn M, Suchting S, Poulsom R, Bicknell R (2002) Magic roundabout is a new member of the roundabout receptor family that is endothelial specific and expressed at sites of active angiogenesis. Genomics 79:547–552

Hussain SA, Piper M, Fukuhara N, Strochlic L, Cho G, Howitt JA, Ahmed Y, Powell AK, Turnbull JE, Holt CE, Hohenester E (2006) A molecular mechanism for the heparan sulfate dependence of slit-robo signaling. J Biol Chem 281:39693–39698

Ikebuchi Y, Aoki S, Honma M, Hayashi M, Sugamori Y, Khan M, Kariya Y, Kato G, Tabata Y, Penninger JM, Udagawa N, Aoki K, Suzuki H (2018) Coupling of bone resorption and formation by RANKL reverse signalling. Nature 561:195–200

Jaworski A, Tom I, Tong RK, Gildea HK, Koch AW, Gonzalez LC, Trssier-Lavigne M (2015) Operational redundancy in axon guidance through the multifunctional receptor Robo3 and its ligand NELL2. Science 350:961–965

Kenkre JS, Bassett J (2018) The bone remodelling cycle. Ann Clin Biochem 55:308–327

Kidd T, Brose K, Mitchell KJ, Fetter RD, Tessier-Lavigne-M, Goodman CS, Tear G (1998) Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. Cell 92:205–215

Kim BJ, Koh JM (2019) Coupling factors involved in preserving bone balance. Cell Mol Life Sci 76:1243–1253

Kim BJ, Lee YS, Lee SY, Baek WY, Choi YJ, Moon SA, Lee SH, Kim JE, Chang EJ, Kim EY, Yoon J, Kim SW, Ryu SH, Lee SK, Lorenzo JA, Ahn SH, Kim H, Lee KU, Kim GS, Koh JM (2018a) Osteoclast-secreted SLIT3 coordinates bone resorption and formation. J Clin Invest 128:1429–1441

Kim H, Choi YJ, Lee YS, Park SY, Baek JE, Kim HK, Kim BJ, Lee SH, Koh JM (2018b) SLIT3 regulates endochondral ossification by beta-catenin suppression in chondrocytes. Biochem Biophys Res Commun 506:847–853

Kiyozumi D, Noda T, Yamaguchi R, Tobita T, Matsumura T, Shimada K, Kodani M, Kohda T, Fujihara Y, Ozawa M, Yu Z, Miklossy G, Bohren KM, Horie M, Okabe M, Matzuk MM, Ikawa M (2020) NELL2-mediated lumicrine signaling through OVCH2 is required for male fertility. Science 368:1132–1135

Kuroda S, Oyasu M, Kawakami M, Kanayama N, Tanizawa K, Saito N, Abe T, Matsuhashi S, Ting K (1999) Biochemical characterization and expression analysis of neural thrombospondin-1-like proteins NELL1 and NELL2. Biochem Biophys Res Commun 265:79–86

Li HS, Chen JH, Wu W, Fagaly T, Zhou L, Yuan W, Dupuis S, Jiang ZH, Nash W, Gick C, Ornitz DM, Wu JY, Rao Y (1999) Vertebrate slit, a secreted ligand for the transmembrane protein roundabout, is a repellent for olfactory bulb axons. Cell 96:807–818

Li C, Zheng Z, Ha P, Chen X, Jiang W, Sun S, Chen F, Asatrian G, Berthiaume EA, Kim JK, Chen EC, Pang S, Zhang X, Ting K, Soo C (2018) Neurexin superfamily cell membrane receptor contactin-associated protein like-4 (Cntnap4) is involved in neural EGFL-like 1 (Nell-1)-responsive osteogenesis. J Bone Miner Res 33:1813–1825

Li C, Zhang X, Zheng Z, Nguyen A, Ting K, Soo C (2019) Nell-1 is a key functional modulator in osteochondrogenesis and beyond. J Dent Res 98:1458–1468

Li N, Inoue K, Sun J, Niu Y, Lalani S, Yallowitz A, Yang X, Zhang C, Shen R, Zhao B, Xu R, Greenblatt MB (2020) Osteoclasts are not a source of SLIT3. Bone Res 8:11

Lu W, van Eerde AM, Fan X, Quintero-Rivera F, Kulkarni S, Ferguson H, Kim HG, Fan Y, Xi Q, Li QG, Sanlaville D, Andrews W, Sundaresan V, Bi W, Yan J, Giltay JC, Wijmenga C, de Jong TP, Feather SA, Woolf AS, Rao Y, Lupski JR, Eccles MR, Quade BJ, Gusella JF, Morton CC, Maas RL (2007) Disruption of ROBO2 is associated with urinary tract anomalies and confers risk of vesicoureteral reflux. Am J Hum Genet 80:616–632

Martin TJ, Sims NA (2005) Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol Med 11:76–81

Maruyama K, Kawasaki T, Hamaguchi M, Hashimoto M, Furu M, Ito H, Fujii T, Takemura N, Karuppuchamy T, Kondo T, Kawasaki T, Fukasaka M, Misawa T, Saitoh T, Suzuki Y, Martino MM, Kumagai Y, Akira S (2016) Bone-protective functions of Netrin 1 protein. J Biol Chem 291:23854–23868

Matsuoka K, Park KA, Ito M, Ikeda K, Takeshita S (2014) Osteoclast-derived complement component 3a stimulates osteoblast differentiation. J Bone Miner Res 29:1522–1530

Mediero A, Ramkhelawon B, Perez-Aso M, Moore KJ, Cronstein BN (2015) Netrin-1 is a critical autocrine/

paracrine factor for osteoclast differentiation. J Bone Miner Res 30:837–854
Morlot C, Thielens NM, Ravelli RB, Hemrika W, Romijn RA, Gros P, Cusack S, McCarthy AA (2007) Structural insights into the Slit-Robo complex. Proc Natl Acad Sci U S A 104:14923–14928
Negishi-Koga T, Shinohara M, Komatsu N, Bito H, Kodama T, Friedel RH, Takayanagi H (2011) Suppression of bone formation by osteoclastic expression of semaphorin 4D. Nat Med 17:1473–1480
Okajima F (2013) Regulation of inflammation by extracellular acidification and proton-sensing GPCRs. Cell Signal 25:2263–2271
Pak JS, DeLoughery ZJ, Wang J, Acharya N, Park Y, Jaworski A, Ozkan E (2020) NELL2-Robo3 complex structure reveals mechanisms of receptor activation for axon guidance. Nat Commun 11:1489
Park SJ, Lee JY, Lee SH, Koh JM, Kim BJ (2019) SLIT2 inhibits osteoclastogenesis and bone resorption by suppression of Cdc42 activity. Biochem Biophys Res Commun 514:868–874
Peng Y, Wu S, Li Y, Crane JL (2020) Type H blood vessels in bone modeling and remodeling. Theranostics 10:426–436
Rhee J, Buchan T, Zukerberg L, Lilien J, Balsamo J (2007) Cables links Robo-bound Abl kinase to N-cadherin-bound beta-catenin to mediate Slit-induced modulation of adhesion and transcription. Nat Cell Biol 9:883–892
Rothberg JM, Hartley DA, Walther Z, Artavanis-Tsakonas S (1988) Slit: an EGF-homologous locus of D. melanogaster involved in the development of the embryonic central nervous system. Cell 55:1047–1059
Ryu J, Kim HJ, Chang EJ, Huang H, Banno Y, Kim HH (2006) Sphingosine 1-phosphate as a regulator of osteoclast differentiation and osteoclast-osteoblast coupling. EMBO J 25:5840–5851
Seeger M, Tear G, Ferres-Marco D, Goodman CS (1993) Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline. Neuron 10:409–426
Seiradake E, von Philipsborn AC, Henry M, Fritz M, Lortat-Jacob H, Jamin M, Hemrika W, Bastmeyer M, Cusack S, McCarthy AA (2009) Structure and functional relevance of the Slit2 homodimerization domain. EMBO Rep 10:736–741
Shen J, James AW, Chung J, Lee K, Zhang JB, Ho S, Lee KS, Kim TM, Niimi T, Kuroda S, Ting K, Soo C (2012) NELL-1 promotes cell adhesion and differentiation via integrin β1. J Cell Biochem 113:3620–3628
Shin B, Kupferman J, Schmidt E, Polleux F, Delany AM, Lee SK (2020) Rac1 inhibition via Srgap2 restrains inflammatory osteoclastogenesis and limits the clastokine, SLIT3. J Bone Miner Res 35:789–800
Sims NA, Martin TJ (2015) Coupling signals between the osteoclast and osteoblast: how are messages transmitted between these temporary visitors to the bone surface? Front Endocrinol (Lausanne) 6:41
Sims NA, Martin TJ (2020) Osteoclasts provide coupling signals to osteoblast lineage cells through multiple mechanisms. Annu Rev Physiol 82:507–529
Stein E, Tessier-Lavigne M (2001) Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science 291:1928–1938
Sun H, Dai K, Tang T, Zhang X (2009) Regulation of osteoblast differentiation by slit2 in osteoblastic cells. Cells Tissues Organs 190:69–80
Takahashi K, Imai A, Iijima M, Yoshimoto N, Maturana AD, Kuroda S, Niimi T (2015) Mapping the heparin-binding site of the osteoinductive protein NELL1 by site-directed mutagenesis. FEBS Lett 589:4026–4032
Takeshita S, Fumoto T, Matsuoka K, Park KA, Aburatani H, Kato S, Ito M, Ikeda K (2013) Osteoclast-secreted CTHRC1 in the coupling of bone resorption to formation. J Clin Invest 123:3914–3924
Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, Zhao L, Nagy TR, Peng X, Hu J, Feng X, Van Hul W, Wan M, Cao X (2009) TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med 15:757–765
Ting K, Vastardis H, Mulliken JB, Soo C, Tieu A, Do H, Kwong E, Bertolami CN, Kawamoto H, Kuroda S, Longaker MT (1999) Human NELL-1 expressed in unilateral coronal synostosis. J Bone Miner Res 14:80–89
Tumelty KE, Higginson-Scott N, Fan X, Bajaj P, Knowlton KM, Shamashkin M, Coyle AJ, Lu W, Berasi SP (2018) Identification of direct negative cross-talk between the SLIT2 and bone morphogenetic protein-Gremlin signaling pathways. J Biol Chem 293:3039–3055
Wainwright EN, Wilhelm D, Combes AN, Little MH, Koopman P (2015) ROBO2 restricts the nephrogenic field and regulates Wolffian duct-nephrogenic cord separation. Dev Biol 404:88–102
Walker EC, McGregor NE, Poulton IJ, Pompolo S, Allan EH, Quinn JM, Gillespie MT, Martin TJ, Sims NA (2008) Cardiotrophin-1 is an osteoclast-derived stimulus of bone formation required for normal bone remodeling. J Bone Miner Res 23:2025–2032
Wong K, Ren XR, Huang YZ, Xie Y, Liu G, Saito H, Tang H, Wen L, Brady-Kalnay SM, Mei L, Wu JY, Xiong WC, Rao Y (2001) Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. Cell 107:209–221
Wright KM, Lyon KA, Leung H, Leahy DJ, Ma L, Ginty DD (2012) Dystroglycan organizes axon guidance cue localization and axonal pathfinding. Neuron 76:931–944
Wu JY, Feng L, Park HT, Havlioglu N, Wen L, Tang H, Bacon KB, Jiang Z, Zhang X, Rao Y (2001) The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. Nature 410:948–952

Xian L, Wu X, Pang L, Lou M, Rosen CJ, Qiu T, Crane J, Frassica F, Zhang L, Rodriguez JP, Jia X, Yakar S, Xuan S, Efstratiadis A, Wan M, Cao X (2012) Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. Nat Med 18:1095–1101

Xu R, Yallowitz A, Qin A, Wu Z, Shin DY, Kim JM, Debnath S, Ji G, Bostrom MP, Yang X, Zhang C, Dong H, Kermani P, Lalani S, Li N, Liu Y, Poulos MG, Wach A, Zhang Y, Inoue K, Di Lorenzo A, Zhao B, Butler JM, Shim JH, Glimcher LH, Greenblatt MB (2018) Targeting skeletal endothelium to ameliorate bone loss. Nat Med 24:823–833

Yamamoto N, Kashiwagi M, Ishihara M, Kojima T, Maturana AD, Kuroda S, Niimi T (2019) Robo2 contains a cryptic binding site for neural EGFL-like (NELL) protein 1/2. J Biol Chem 294:4693–4703

Yom-Tov G, Barak R, Matalon O, Barda-Saad M, Guez-Haddad J, Opatowsky Y (2017) Robo Ig4 is a dimerization domain. J Mol Biol 429:3606–3616

Zaidi M (2007) Skeletal remodeling in health and disease. Nat Med 13:791–801

Zakrys L, Ward RJ, Pediani JD, Godin AG, Graham GJ, Milligan G (2014) Roundabout 1 exists predominantly as a basal dimeric complex and this is unaffected by binding of the ligand Slit2. Biochem J 461:61–73

Zelina P, Blockus H, Zagar Y, Peres A, Friocourt F, Wu Z, Rama N, Fouquet C, Hohenester E, Tessier-Lavigne M, Schweitzer J, Roest Crollius H, Chedotal A (2014) Signaling switch of the axon guidance receptor Robo3 during vertebrate evolution. Neuron 84:1258–1272

Zhang X, Kuroda S, Carpenter D, Nishimura I, Soo C, Moats R, Iida K, Wisner E, Hu FY, Miao S, Beanes S, Dang C, Vastardis H, Longaker M, Tanizawa K, Kanayama N, Saito N, Ting K (2002) Craniosynostosis in transgenic mice overexpressing Nell-1. J Clin Invest 110:861–870

Zhang B, Dietrich UM, Geng JG, Bicknell R, Esko JD, Wang L (2009) Repulsive axon guidance molecule Slit3 is a novel angiogenic factor. Blood 114:4300–4309

Zhang X, Zara J, Siu RK, Ting K, Soo C (2010) The role of NELL-1, a growth factor associated with craniosynostosis, in promoting bone regeneration. J Dent Res 89:865–878

Zhang X, Ting K, Pathmanathan D, Ko T, Chen W, Chen F, Lee H, James AW, Siu RK, Shen J, Culiat CT, Soo C (2012) Calvarial cleidocraniodysplasia-like defects with ENU-induced Nell-1 deficiency. J Craniofac Surg 23:61–66

Zhao J, Mommersteeg MTM (2018) Slit-Robo signalling in heart development. Cardiovasc Res 114:794–804

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Characterization of *C. elegans* Chondroitin Proteoglycans and Their Large Functional and Structural Heterogeneity; Evolutionary Aspects on Structural Differences Between Humans and the Nematode

Fredrik Noborn and Göran Larson

Abstract

Proteoglycans regulate important cellular pathways in essentially all metazoan organisms. While considerable effort has been devoted to study structural and functional aspects of proteoglycans in vertebrates, the knowledge of the core proteins and proteoglycan-related functions in invertebrates is relatively scarce, even for *C.elegans*. This nematode produces a large amount of non-sulfated chondroitin in addition to small amount of low-sulfated chondroitin chains (Chn and CS chains, respectively). Until recently, 9 chondroitin core proteins (CPGs) had been identified in *C.elegans*, none of which showed any homology to vertebrate counterparts or to other invertebrate core proteins. By using a glycoproteomic approach, we recently characterized the chondroitin glycoproteome of *C.elegans,* resulting in the identification of 15 novel CPG core proteins in addition to the 9 previously established. Three of the novel core proteins displayed homology to human proteins, indicating that CPG and CSPG core proteins may be more conserved throughout evolution than previously perceived. Bioinformatic analysis of the primary amino acid sequences revealed that the core proteins contained a broad range of functional domains, indicating that specialization of proteoglycan-mediated functions may have evolved early in metazoan evolution. This review specifically discusses our recent data in relation to previous knowledge of core proteins and GAG-attachment sites in Chn and CS proteoglycans of *C.elegans* and humans, and point out both converging and diverging aspects of proteoglycan evolution.

Keywords

Proteoglycan · Caenorhabditis elegans · Glycosaminoglycan · Chondroitin · Chondroitin sulfate · Core protein · Protein functional domains · Attachment site · Metazoan evolution

F. Noborn
Department of Laboratory Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

G. Larson (✉)
Department of Laboratory Medicine, Sahlgrenska University Hospital, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
e-mail: goran.larson@clinchem.gu.se

1 Introduction

The notion of "proteoglycans" as discrete entities first became apparent during the 1950s (Yanagishita 1993). By isolating and analyzing material from bovine cartilage, it was found that glycosaminoglycan (GAG) chains were associated with a protein component (Yanagishita 1993; Schatton and Schubert 1954). These compounds were referred to as "mucoproteins" although it was unclear at that time whether the GAG-protein association involved covalent bonds or not. In the following years, a covalent association was indeed demonstrated between chondroitin sulfate and serine residues (Muir 1958; Lindahl 2014). Furthermore, a tetrasaccharide "linkage region" [Glucuronic acid (GlcA) – Galactose (Gal) – Galactose (Gal) – Xylose (Xyl)] was identified that covalently linked the GAG chain to specific serine residues of the corresponding core proteins (Roden and Smith 1966). Since then, core proteins have gradually become recognized as distinct molecular entities, each with differences in their protein structures and cellular functions, as well as with differences in the number and types of GAG chains attached (Lindahl 2014; Murdoch and Iozzo 1993; Kjellen et al. 1989; Lindahl et al. 2015).

The identification of proteoglycans is often difficult from a methodological perspective, since proteoglycan identification requires the combined sequencing of a given core protein, together with the characterization of which type, and where along the amino acid sequence the GAG chain(s) are attached. Biochemical and immunological techniques are often hampered by the size and heterogeneity of the GAG side chains, which preclude effective core protein sequencing and characterization. Molecular cloning techniques offer a solution to these difficulties by allowing the identification and sequencing of mRNAs and protein coding genes. However, these methods do not provide information on any post-translational modifications, which creates ambiguity as to the identification of a proteoglycan (Kjellen et al. 1989; Bourdon et al. 1985). Therefore, studies on identifying proteoglycans have earlier been focused mostly on isolation and characterization of a single core protein in specific model systems, whereas unbiased and global characterizations of all proteoglycans of a specific tissue or organism have not been systematically attempted.

The number of core proteins identified in vertebrates is limited. Less than 20 heparan sulfate proteoglycans (HSPGs) and about 60 chondroitin sulfate proteoglycans (CSPGs) have so far been identified in humans (Lindahl 2014; Zhang et al. 2018; Noborn et al. 2015; Nasir et al. 2016). This is a very limited number in relation to other types of glycoproteins, such as N- and O-glycosylated proteins, which are counted in their thousands (Nilsson et al. 2013; Joshi et al. 2018). We have recently developed a glycoproteomic approach that may assist in identifying how many and which type of proteoglycans are indeed expressed in different animal tissues and species. The aim was to characterize linkage regions, attachment sites and identities of CS core proteins (Noborn et al. 2015). In this approach, trypsin-treated proteoglycans were enriched from various sample matrices by strong-anion-exchange chromatography, and then digested with chondroitinase ABC to specifically reduce the CS chain lengths. The preparations were thereafter analyzed by nLC-MS/MS and the data from remaining linkage regions, linked to tryptic or semi-tryptic peptides, was processed by a novel glycopeptide search algorithm. Analysis of human urine and CSF resulted in the identification of 13 novel CSPGs, many of which were previously defined as peptide prohormones (Noborn et al. 2015). This suggested that many novel proteoglycans and proteoglycan-related functions are yet to be discovered, and that new methodological approaches may assist in such an endeavor.

While proteoglycans in vertebrates has been the focus of several structural and functional studies, the knowledge of proteoglycans in invertebrates is still relatively scarce, even for the otherwise well-studied nematode *C.elegans*. Until recently, 5 HSPGs and 9 CPG core proteins had been identified in the nematode (Wilson et al. 2015; Olson et al. 2006). Using our

glycoproteomic strategy, we mapped the chondroitin glycoproteome of *C.elegans*, confirming the identities of the 9 previously established core proteins, but also identifying an additional 15 chondroitin core proteins (Noborn et al. 2018). Three of the novel core proteins displayed homologies to human proteins, which was surprising since no chondroitin core proteins have previously been found to display homology to human proteins, and were therefore not considered to be well-conserved throughout evolution (Olson et al. 2006). Bioinformatic analysis of the primary amino acid sequences was performed to provide insights of the structural domain organization of each core protein. This analysis revealed a previously unknown structural complexity of CPGs in *C.elegans*, indicating that complex proteoglycan-related functions may have evolved early in metazoan evolution.

Additional glycoproteomic analyses of proteoglycans of various animals, vertebrates as well as invertebrates, are likely to expand our understanding of the structural heterogeneity of Chn and CS core proteins during metazoan evolution. However, at present it is difficult to fully appreciate the evolutionary aspects on core protein alterations in large, simply because the number of studies on core proteins in invertebrates is too limited (and that available studies typically focus only on a single core protein). Thus, this review will concentrate on the recent findings of CPGs and CSPGs in *C.elegans* and humans and points to similarities and differences between core proteins between these two evolutionary distant species. Although core proteins are the primary focus of this review, the initiating GAG-biosynthetic machinery in *C.elegans* and humans will also be discussed to highlight both converging and diverging aspects of proteoglycan evolution. References to relevant reviews relating to structural diversity of GAGs and proteoglycans in other organisms are given in their conceptual contexts in the following paragraphs. Our general and specific conclusions are summarized in Fig. 1, exemplifying our conclusions on some evolutionary principles of proteoglycan development from *C.elegans* to Homo sapiens.

2 Proteoglycan Diversity from *C. elegans* to Humans

Glycoconjugates constitute the structurally most diverse group of organic molecules in nature. This diversity poses a great challenge in analyzing glycan structures and also in assigning glycan-specific functions (Joshi et al. 2018; Gagneux et al. 2015; Mulloy et al. 2009). Although GAGs constitute a small subgroup of all glycans, their structural complexity is still considerable. GAGs are divided into four subclasses depending on the repeating disaccharides of the polysaccharide chains: heparan sulfate (HS)/heparin (GlcA/IdoA-GlcN), chondroitin sulfate (CS) /dermatan sulfate (DS) (GlcA/IdoA-GalNAc), keratan sulfate (Gal-GlcNAc) and hyaluronan (GlcA-GlcNAc). The molecular heterogeneity is influenced by large differences in polysaccharide chain length, domain organization and unique monosaccharide modifications, e.g. O- and N-sulfations, phosphorylations, sialylations etc. All GAGs, except for hyaluronan, are invariably attached to core proteins to form unique proteoglycans (Hascall et al. 2014; Weigel et al. 1997; Saied-Santiago and Bulow 2018). This provides additional complexity since different core proteins, have unique gene coded primary, secondary and tertiary sequences, with major consequences for where, which type and which number of GAG chains that are initiated, extended and modified.

Proteoglycans have a long evolutionary history and are expressed in all bilateral animals investigated to date (Couchman and Pataki 2012). HS appeared early in metazoan organisms and essentially all cells produce complex sulfated HS structures (Esko and Lindahl 2001). In contrast, CS/DS from lower organisms have a limited structural complexity, a complexity which increases with evolutionary higher organisms (Yamada et al. 2011). For extensive information on proteoglycan diversity and GAG-specific functions we highly recommend the following reviews (Couchman and Pataki 2012; Iozzo and Schaefer 2015; Kjellen and Lindahl 2018; Weiss et al. 2017). Here, examples are selected to focus

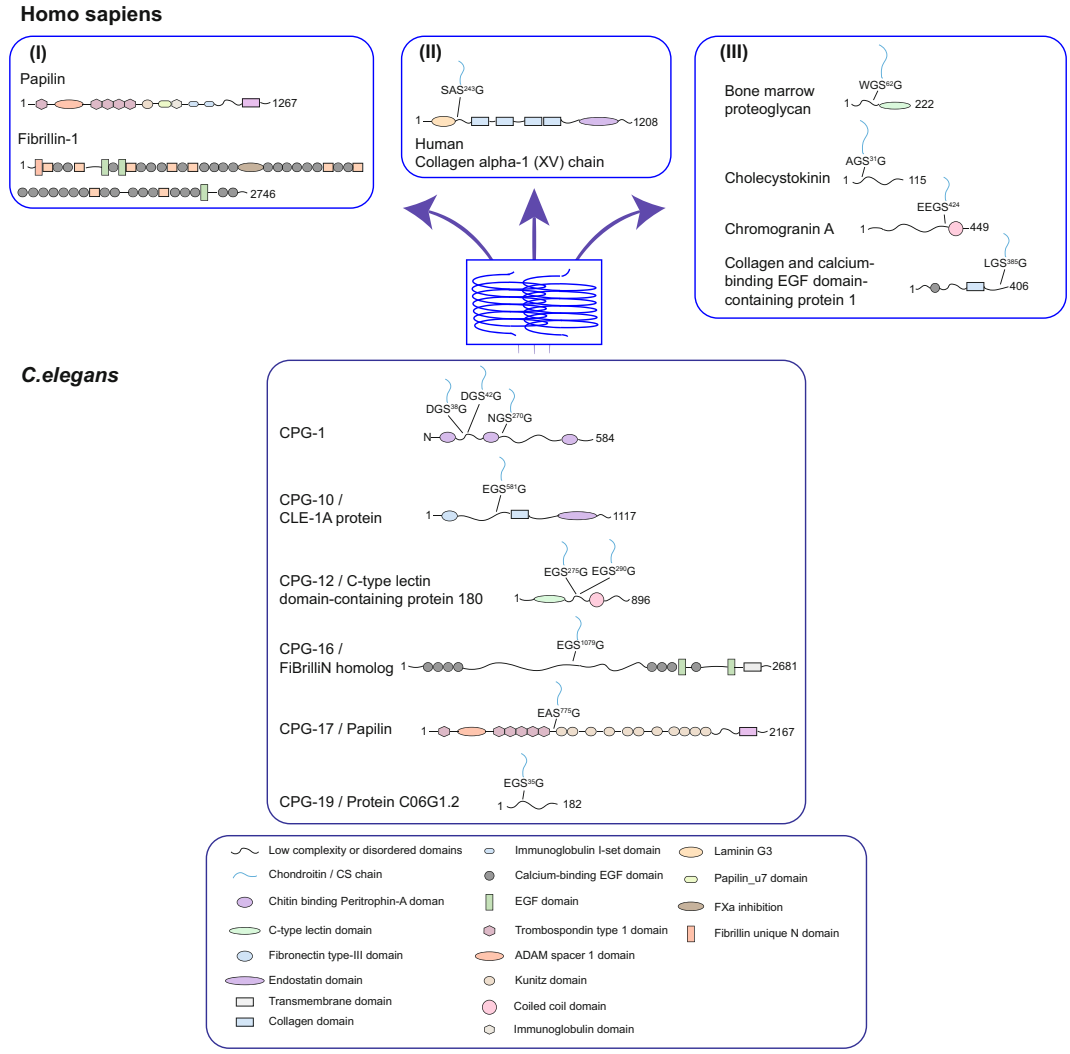

Fig. 1 Schematic illustration of evolutionary principles bridging millions of years of proteoglycan development from *C.elegans* to Homo sapiens. (I) Divergent evolution where the GAG chain is lost but the protein is conserved, (II) Parallel evolution conserving both the GAG chain and the protein, (III) Convergent evolution where conserved GAG chains are added to novel core proteins. Note that some functional core protein domains are conserved throughout evolution whereas other core proteins lack such domains. The spirals/arrows are representing the evolutionary multi-interaction steps for the two primary constituents of proteoglycans, i.e. GAGs and core proteins

primarily on different aspects of CPGs in *C. elegans* and are not meant to provide a comprehensive review on proteoglycan structure and evolution in general. Hopefully, this review will provide some new aspects in proteoglycan structure and perhaps inspire to novel concepts that can be experimentally tested.

3 Structural Diversity of Chondroitin and Heparan Sulfate Proteoglycans in Invertebrates

The number of CSPGs identified in humans is around 60 (Noborn et al. 2015; Nasir et al.

2016) but in invertebrates, the number of CSPGs is even lower and the reports are restricted to only a few species. A proteomic-based study identified tryptic peptides from versican, neurocan (CSPG3) and neuroglycan (CSPG4-NG2) in the gastropoda *Achatina fulica (*Gesteira et al. 2011*)*. Since these proteins are well-establish CSPGs in vertebrates, assumptions of their CS substitutions were also made in *A. fulica.* Moreover, two populations of CSPGs with different molecular weights were isolated from squid skin (*Ilex illecebrosus*) using a combination of ion-exchange chromatography and ultracentrifugation (Karamanos et al. 1990). Biochemical analysis showed different amino acid composition of these core proteins, although the exact peptide sequences could not be resolved (Karamanos et al. 1990). Surprisingly, information on CSPGs is lacking in *Drosophila melanogaster*, regarded as one of the most studied invertebrates in glycobiology (Zhu et al. 2019), which supports our perception that there is a general gap of knowledge of these structures in invertebrates.

However, earlier studies identified various HSPGs in *D. melanogaster* and *C.elegans* which display homologies to vertebrate core proteins. There are 5 known HSPGs in *D. melanogaster* that are all homologues of mammalian counterparts: syndecan, 2 glypicans (*dally* and *dlp*), perlecan (*trol*) and testican (*cow*) (Bernfield et al. 1999; Nakato et al. 1995; Baeg et al. 2001; Park et al. 2003; Chang and Sun 2014; Nakato and Li 2016). Similar to these findings, five homologues to vertebrate genes encoding for HSPG core proteins have been identified in *C. elegans*: syndecan (*sdn-1*), *2* glypicans *(lon-2* and *gpn-1*), perlecan (*unc-52*) and agrin (*agr-1)* (Blanchette et al. 2015*;* Consortium CeS 1998*;* Rogalski et al. 1993*;* Hrus et al. 2007*;* Hutter et al. 2000*;* Blanchette et al. 2017). The *unc-52* gene encodes the homologue of the vertebrate gene perlecan, a major component of the extracellular matrix, which in vertebrates is substituted with both HS and CS (Rogalski et al. 1993; Yamada et al. 2002; Noborn et al. 2016). There is of course a possibility that additional HSPGs, yet unidentified and maybe not conserved, may exist in both *Drosophila* and *C.elegans*. Nevertheless, the findings so far indicate a high degree of conservation of genes encoding for HSPG core proteins throughout evolution.

Nine CPGs have previously been identified in *C.elegans*, which were designated CPG-1 to CPG-9 (Olson et al. 2006). In contrast to the HSPGs, none of these core proteins showed homology to vertebrate proteins or to proteins in other invertebrates such as *Drosophila melanogaster (*Olson et al. 2006*)*. Two of the CPGs (CPG-1 and CPG-2) in *C.elegans* contain chitin-binding domains and were therefore assumed to interact with the chitin layer in the cuticle (Wilson et al. 2015). Detailed functional analysis showed that CPG-1 and CPG-2 are indeed important for the hierarchical assembly of the egg shell layer during embryogenesis, resulting in an outer vitelline layer, a middle chitin layer and an inner CPG-1 and CPG-2 layer (Olson et al. 2012). This specific function confers to the classical notion of CSPGs as structural components in cartilage and other connective tissues. Since vertebrate CSPGs display a wide range of functional diversity, we argued that additional CPG core proteins are likely present in *C. elegans*, which not only relate to extracellular matrix formation, but may also accommodate more specialized functions.

Indeed, in our investigation of the chondroitin glycoproteome of *C.elegans*, we found 15 novel core proteins that were designated CPG-10 through −24, in accordance with previous introduced terminology (Olson et al. 2006). Six of the 15 novel core proteins were previously uncharacterized proteins, and were only annotated in UniProt based on the open reading frame (ORF) names (e.g. Protein C45E5.4/CPG-18) (Noborn et al. 2018). The other novel CPGs have previously been assigned names based on phenotypes in mutation studies (e.g. High incidence of males, isoform b/CPG-14), or based on sequence similarities to vertebrate proteins (e.g. FiBrilliN homolog/CPG-16). The identified core proteins displayed a wide range in their molecular weights, from 7.1 kDa (CPG-9) to 568 kDa (high incidence of males, isoform b/CPG-14). The number of chondroitin attachment

sites also varied depending on the core protein, from one (e.g. CPG-3, CPG-5) to four sites (CPG-4). Moreover, bioinformatics analysis of the primary amino acid sequences revealed that the core proteins contained a broad range of functional domains, assuming their involvement in a wide-range of physiological functions. In total, 19 unique domains were retrieved form the 24 core protein sequences. Apart from the expected chitin-binding domains on CPG-1 and CPG-2, additional domains were identified that indicate their involvement in extracellular matrix formation, such as fibronectin type-III domain (CLE-1A protein/CPG-10) and collagen domain (COLlagen/CPG-11). Other identified domains indicate a role in more specialized functions, such as thrombospondin type-1 domain (Papilin/CPG-17) and endostatin domain (CLE-1A protein/CPG-10), both of which are known to be involved in axon guidance and neuronal development (Adams and Tucker 2000; Ackley et al. 2001). Notably, 9 core protein sequences did not retrieve any hits and displayed only low complexity/disordered domains.

Bioinformatic analysis was also conducted on human CSPGs, previously identified in human samples with the same approach. The analysis retrieved 40 unique domains for 28 core proteins sequences (Noborn et al. 2015; Nasir et al. 2016). Certain domains were found in both species, such as collagen domain and the Kunitz domain. Of the 50 unique domain structures identified in the two species, 31 were uniquely found in human CSPGs, 10 uniquely found in *C. elegans* CPGs, and 9 found in both species. Moreover, sequences that only display disordered domains were also found in humans, although to a lesser degree than in *C.elegans*. Three of 28 human core proteins (10.7%) displayed this characteristic, compared to 9 out of 24 (37.5%) in *C.elegans*. This may indicate a selection process where core proteins with functional domains are conserved throughout evolution. A certain amount of research bias regarding detection of functional domains in the data base (e.g. more information of human proteins), may however also explain the higher incidence of known domains in human. Nevertheless, this analysis suggests a great structural and also functional diversity of CPGs in *C. elegans* and indicates that some, but not all, functions overlap with those of human CSPGs. Furthermore, this indicates that also specialized CS proteoglycan-mediated functions may have evolved early in metazoan evolution.

4 Evolutionary Aspects of CS Biosynthesis in *C.elegans* and Humans

Although *C.elegans* is a well-studied model organism with regard to genomics, proteomics and certain aspects of glycosylation (Consortium CeS 1998; Antoshechkin and Sternberg 2007; Shim and Paik 2010; Schachter 2004), information on CS proteoglycans and proteoglycan-mediated functions is limited. This is unfortunate since *C.elegans* is often used to study the influence of genes and proteins in evolutionary conserved processes (Maduro 2017; Vuong-Brender et al. 2016). Such processes, e.g. morphogen distribution in embryogenesis of e.g. *D. melanogaster*, have been shown to involve HS proteoglycans which fine tunes the cellular response (Nakato and Li 2016; Bishop et al. 2007). Structural information of CS proteoglycans in *C.elegans* would therefore probably assist to our functional understanding of these processes in the worm.

The CS (and GAG) biosynthesis is always initiated by the transfer of a Xyl to a serine residue in the core protein. The xylosylation typically occurs at certain serine residues with a glycine residue at the carboxyl-terminal side (-SG-), and with a cluster of acidic residues in close proximity (Esko and Zhang 1996). This motif was initially observed for vertebrate core proteins and a similar motif has also been suggested for *C.elegans* (Olson et al. 2006). The chondroitin sulfate biosynthesis continues with the addition of two galactose (Gal) and one glucuronic acid (GlcA) residue, completing the formation of the consensus tetrasaccharide linkage region. The biosynthesis then continues with polymerization of the chain through the addition of alternating units of N-acetylgalactosamine (GalNAc) and GlcA

residues. The individual enzymes for each step in the chondroitin biosynthesis in *C.elegans* have been well established. In a mutagenesis experiment, eight mutations that perturb vulval development in the growing embryo were identified (designated *sqv* or squashed vulva). All eight mutations (*sqv-1* to *8*) produced similar phenotypes, such as a defective vulval epithelial invagination and for some mutations oocyte development was also affected (Wilson et al. 2015; Herman et al. 1999). Moreover, all *sqv* genes showed homology to vertebrate enzymes and were found to be involved in different aspects of the GAG-biosynthesis. Biochemical analysis showed that *sqv-6*, *sqv-3*, *sqv-2*, and *sqv-8* encode for vertebrate homologues of glycosyltransferases required for the formation of the tetrasaccharide linkage regions, whereas *sqv-1, sqv-4,* and *sqv-7* encode proteins that have roles in nucleotide sugar metabolism and transport (Wilson et al. 2015; Herman and Horvitz 1999; Bulik et al. 2000; Berninsone et al. 2001; Hwang and Horvitz 2002; Hwang et al. 2003; Izumikawa et al. 2004). Taken together, all components required for the initial part of the biosynthesis is highly conserved between *C.elegans* and humans, including nucleotide sugar precursors and their transport into the Golgi, as well as enzymes required for linkage formation and chain polymerization (Olson et al. 2006).

In vertebrates, the chondroitin polysaccharide undergoes extensive modifications of sulfotransferases and chondroitin-specific epimerases (Mizumoto et al. 2013; Ly et al. 2011). This results in complex yet defined CS/DS structures that may interact with various protein ligands with different degree of specificities (Le Jan et al. 2012; Mizumoto et al. 2015; Sugiura et al. 2016). In contrast, chondroitin in *C.elegans* is considerably less complex and the general view was, until recently, that the nematode only produces non-sulfated chondroitin (Yamada et al. 1999; Toyoda et al. 2000). This was puzzling since *C.elegans*, which belongs to Ecdysozoa clade, appeared to be an exception to other animals within the same clade, such as *D. melanogaster*, which was known to produce sulfated structures (Toyoda et al. 2000). Moreover, even animals in the evolutionary older phylum of Cniderians, containing simple organisms such as hydrozoans, produce CS (Yamada et al. 2011). This paradox was recently settled when two separate groups demonstrated that Chn may indeed be sulfated in *C.elegans*, although to a smaller extent (Dierker et al. 2016; Izumikawa et al. 2016). So far only one single sulfotransferase, which catalyzes the GalNAc 4-*O* sulfation has been identified in *C.elegans*. However, the presence of both 4-*O* and 6-*O* sulfated GalNAc residues was shown by MS/MS analysis of CS disaccharides, and indicated that at least one additional sulfotransferase should be expressed in the nematode (Dierker et al. 2016). In contrast, *C.elegans* seems to lack the chondroitin-specific epimerases present in vertebrates and no DS structures have yet been detected in this nematode. Taken together, apart from the epimerase, all the components required for CS biosynthesis are highly conserved between *C.elegans* and humans, demonstrating an essential role for CS throughout metazoan evolution (Olson et al. 2006; Yamada et al. 2002; Berninsone et al. 2001).

5 Glycosaminoglycan Diversity from *C.elegans* to Human

However, not all aspects of GAG-evolution seems well conserved. Hyaluronan (HA), a non-sulfated GAG composed of long repeating units of GlcNAc and GlcA disaccharides, seems to have appeared quite late in evolution (Csoka and Stern 2013). The genome in *C.elegans* does not contain the necessary synthases for HA and there is no structural evidence of HA in the nematode (Yamada et al. 1999; Stern 2003). HA has various biological roles and is a prominent component of hydrated matrices in the extracellular matrix. Since Chn/CS only contains a few percent of sulfated disaccharides in *C.elegans* (Dierker et al. 2016), the large majority of the chains are therefore likely non-sulfated structures. Apart from their sizes, chondroitin and HA are relatively similar in structure with differences only in the isomeric identities of the HexNAc residues

(GalNAc vs GlcNAc). It has been suggested that chondroitin in *C.elegans* is a possible HA ancestor, carrying out functions in *C.elegans* that are assigned to HA in vertebrates (Stern 2003). While vertebrates have evolved two different GAG structures, CS and HA, to accommodate separate cellular functions, one may speculate that this structural-functional specialization also occurs in *C.elegans* to a certain extent. It is thus possible that non-sulfated chondroitin accommodate HA-like functions (e.g. provide hydrated matrices); whereas sulfated chondroitin structures accommodate more specialized functions (e.g. provide binding motifs to specific ligands).

Typically, a "GAG-perspective" or a "core protein perspective" is applied when studying the role of proteoglycans in various pathophysiological settings. This structural and conceptual separation is natural, given their vast structural heterogeneity and the limited number of analytical methods that provides integrated GAG-protein characterization. However, integrating structural information on the GAG chains, their attachment sites and the potential functional domains of the corresponding core protein, will likely provide new perspective when studying proteoglycan-related functions. For instance, the effect of chondroitin on neuronal migration in *C.elegans* has been studied by targeting two proteins in the chondroitin biosynthetic pathway: the chondroitin synthase (SQV-5) and a UDP-sugar transporter (SQV-7) (Pedersen et al. 2013). Worms with hypomorphic alleles in these proteins showed aberrant migration of hermaphrodite-specific neurons (HSN) (Pedersen et al. 2013). Although a functional relationship between reduced Chn synthesis and impaired neuronal migration was established, the molecular involvement of the corresponding core protein (s) remains unclear. Different scenarios are possible: the migration requires free Chn, or the migration requires Chn attached to a specific core protein, or even the active involvement of both Chn and a specific core protein. In fact, it was recently shown that neurexin, an essential component in synapse organization, was modified with HS (Zhang et al. 2018). The binding of neurexin to its post-synaptic partner, neuroligin, involved an intrinsic mode of interaction, which required both the HS chain and the protein domain of neurexin. This underlines the importance of site-specific characterization to further delineate GAG-mediated functions in all organisms.

6 Chondroitin Sulfate and Core Proteins

The selective binding of specific protein ligands to structural variants of GAG chains regulates a diverse set of biological- and pathological processes (Kjellen and Lindahl 2018; Salanti et al. 2015; Kreuger et al. 2006; Sarrazin et al. 2011). Determining the fine-structure of binding domains or, when possible, intact GAG chains is therefore essential for understanding GAG-protein interactions and their down-stream cellular events. As *C.elegans* was recently found to have CS structures (Dierker et al. 2016; Izumikawa et al. 2016), characterization of the sulfate distribution on the polysaccharides will likely improve our understanding of CSPG- and CPG-related functions.

In our glycoproteomic approach, the Chn and CS chains are depolymerized with chondroitinase ABC, generating free disaccharides and a residual hexameric structure composed of the linkage region and a GlcA-GalNAc disaccharide, dehydrated on the terminal GlcA residue (Noborn et al. 2015; Noborn et al. 2018). This strategy reduces the complexity of the analysis significantly, but at the same time structural information towards the non-reducing end is omitted. Our analysis of CPGs in *C.elegans* did not however reveal any sulfate groups on the residual hexasacharide structure, although the method is fully capable of detecting such modifications (Noborn et al. 2015). This may suggest that the sulfate groups are located further out on the chains, or in quantities below the present limit of detection. Regardless of their position, one may speculate whether the sulfate modifications are evenly distributed between the 24 different CPGs, or if only a subset of CPGs carries sulfated structures. Regulation of GAG-biosynthesis is

believed to be largely cell-specific as for instance GAG-structures from one mouse tissue differ from those of other mouse tissues (Kjellen and Lindahl 2018; Ledin et al. 2004). Cell-specific co-expression of GalNAc 4-*O* sulfotransferase and certain CPGs may thus result in CS chains on only a subset of core proteins, in a cell specific manner. Moreover, the modification pattern may also involve type of core proteins, although such reports are relatively scarce (Li et al. 2011). Apart from these two principles of regulation, the sulfation pattern may also be lineage specific, in that the sulfation varies in response to developmental stages and possibly disease states (Shao et al. 2013).

If sulfate groups are limited to a subset of CPGs, one may speculate which CPGs that carries sulfated structures. Three homologues to human proteins were found in *C.elegans*; CLE-1A protein/CPG-10, FiBrilliN/CPG-16 and Papilin/CPG-17. The CLE-1A protein/CPG-10 is encoded by the *cle-1* gene which produces three developmentally regulated protein isoforms (CLE-1A-C), which are expressed predominantly in neurons (Ackley et al. 2003). The CLE-1A protein is the homologue to human collagen alpha-1 XV/XVIII (Ackley et al. 2003). Interestingly, we recently found that the human collagen XV alpha-1 chain is substituted with CS in human tissue fluids (Noborn et al. 2015) and this is to our knowledge the first example of an invertebrate chondroitin core protein that shows homology to a vertebrate counterpart. Since all vertebrate core proteins carry CS chains, one may thus speculate that the three vertebrate homologues are likely candidates to be substituted with CS. Moreover, each of the CPG-vertebrate homologues contains functional domains that assume involvement in specialized proteoglycan-mediated functions. As mentioned previously, CLE-1A/CPG-10 contains an endostatin domain and deletion of this domain resulted in worms with defects in cell migration and axon guidance (Ackley et al. 2001, 2003). In vertebrates, CS inhibits nerve regeneration upon binding to the receptor protein tyrosine phosphatase sigma (RPTPσ), an interaction that requires uniform distribution of sulfate groups along the CS chain (Shen et al. 2009; Coles et al. 2011; Katagiri et al. 2018; Sakamoto et al. 2019). Given that advanced functions, such as neurogenesis, require CS with certain sulfate distribution in vertebrates, it is plausible that this is also a requirement in *C.elegans*. Moreover, Papilin/CPG-17 is also claimed to be involved in neurogenesis in *C.elegans*, regulating and forming specific nerve tracts, although this potential role of the Chn or CS chain is unclear (Ramirez-Suarez et al. 2019). Regardless of the *in vivo* situation, future structural studies, using site-specific sequencing of longer GAG chains, will likely determine which core proteins (all or a subset) that indeed carry sulfated structures. We recently showed site-specific sequencing of longer chains in perlecan (8-mer and 10-mers), indicating that a similar approach is feasible also for CSPGs/CPGs (Noborn et al. 2016).

7 Attachment Motifs in *C.elegans* and Humans

The composition and sequence of certain amino acid in defined motifs influence whether a given serine residue is selected for GAG-biosynthesis. This attachment motif was originally observed for vertebrates core proteins and may assist in the prediction of potential GAG-sites (Esko and Zhang 1996; Zhang and Esko 1994). Large scale analysis of attachment motifs in invertebrates is still lacking and it is unknown to which degree invertebrate motifs conform to the vertebrate counterpart. We prepared a frequency plot of the neighboring amino acids in the region from −9 to +9 of the glycosylated serine residue in *C. elegans*. As a comparison we aligned 20 human CS-sites that we previously identified in human urine and CSF, identified with the same analytical procedure (Noborn et al. 2015; Noborn et al. 2018). The analysis showed that the *C.elegans*-attachment motif was similar to the vertebrate counterpart, although with certain exceptions. In both species, the glycosylated serine residue was characteristically flanked by a glycine residue in the C-terminal direction and acidic residues were present in proximity to the attachment site. However, a more stringent motif was seen in *C.*

elegans in the immediate N-terminal direction. A large portion of the sequences (80%) had "Glu" or "Asp" at the −2 position and "Gly" or "Ala" at the −1 position ([ED] − [GA] − S – G). Two vertebrate xylosyltransferases (XT-I and XT-II) have been identified, whereas only a single xylosyltransferase has been found in the nematode (Wilson 2004). One may speculate that the less stringent motif in humans reflect the activities of two different xylosyltransferases, each with slightly different specificities with regard to the amino acid motifs that are required for the enzymes to bind and initiate the first step in the GAG-linkage region. The mouse XT-1 and XT-II display different tissue-specific expression pattern: XT-I is highly expressed in mouse testis, kidney, and brain, while XT-II is highly expressed in mouse liver (Ponighaus et al. 2007). Our frequency plot of the human motif was based on CS-sites found in both urine and CSF thereby representing a mixture of CSPGs from different tissues. Preparing separate plots based on which tissue the CSPGs derives from, different CS-attachment motifs may emerge, which would probably represent differences in XT-I and XT-II specificities.

The attachment motif ([ED] − [GA] − S − G) defined *in C.elegans* was further used to investigate if additional potential CPGs may be present in the nematode. A search against the Swiss-prot data base for sequences containing this motif resulted in the identification of 19 additional potential CPGs, indicating that the chondroitin glycoproteome in *C.elegans* may probably expand even further with future studies (Noborn et al. 2018). Notably, since Swiss-prot is a curated data base, additional hits may be retrieved when searches are made against a more general data base, such the NCBI protein database. Nevertheless, additional CPGs are likely to be identified and this bioinformatic strategy may be useful for identifying potential CPGs/CSPGs also in other model organisms, such as *Danio rerio* and *Drosophila melanogaster*.

Inspection of the attachment motifs in relation to the functional domains, demonstrated that all motifs were present in disordered regions of the core proteins. A similar observation was made for mucin-type O-glycans (King et al. 2017), suggesting that glycosylation in disordered regions is a general phenomenon in metazoan organisms. Further, some of the attachment motifs in our study of *C.elegans* were found in close proximity to a functional domain (e.g. on Papilin/ CPG-17), while others were found in disordered regions distant, in the primary sequence, from any functional domains (e.g on FiBrilliN homologue/CPG-16). It is unclear how the distance to a functional domain affects the specificity of the xylosyltransferase at a given attachment motif. In vertebrates, several proteoglycans have been identified that have a time-dependent presence of GAGs, so-called part-time proteoglycans which vary their degree of occupancy at specific sites (Iozzo and Schaefer 2015; Nadanaka et al. 1998; Aono et al. 2004; Oohira et al. 2004). Sometimes, this is regulated by the synthesis of splice variants lacking or presenting a GAG-attachment motif (Wight 2002; Pangalos et al. 1995). However, one may speculate that positioning of the attachment motif and its distance to a functional domain, may influence the efficiency of the biosynthesis and thereby contribute to the glycosylation heterogeneity seen in proteoglycans.

8 Evolutionary Aspects when Comparing Chondroitin Sulfate Proteoglycans (CSPGs) and Heparan Sulfate Proteoglycans (HSPGs)

Several HSPGs in *C.elegans* display homology to vertebrate core proteins. In line with these findings, neurexin, which was recently defined as a HSPG in mouse brain tissue, also displays a high degree of similarity between distant species (Zhang et al. 2018). The HS site is conserved in all vertebrate neurexin genes from zebrafish to human. *C.elegans* also contains a homologue to the vertebrate neurexin gene, corroborating the notion that HSPGs are highly conserved throughout evolution. Although the primary sequence of neurexin is more divergent in *C.elegans*, the nematode has a consensus HS site approximately in

the same region as that of the mouse protein (Zhang et al. 2018). Other HSPG core proteins in *C.elegans* also display this degree of similarity to vertebrate HSPGs. For instance, mouse perlecan has three SG repeats in close proximity to the N-terminal domain (62-DDASGDGLGSGDVGSGDFQMVYFR-85), all of which are modified with HS (Noborn et al. 2016). The nematode-homologue (*unc-52*) has also several potential HS attachment sites in the primary sequence, but none of these is located in the N-terminal domain. As mentioned previously, the large majority of CPG core protein in *C.elegans* do not display homology to vertebrate counterparts. However, we found a chondroitin modification on CLE-1A protein/ CPG-10 (Q9U9K7) which display homology to the human CSPG collagen α-1 (XV) chain (P39059). These proteins display a high degree of sequence similarity regarding functional domains and their order of organization. However, the Chn or CS attachment site is different in the nematode compared to the human protein, as well as the sequence and composition of amino acids surrounding the attachment site, thus principally displaying a similar degree of conservation as found for HSPGs. Furthermore, we recently identified several novel human CSPGs in tissue samples, that had previously been defined as prohormones (Noborn et al. 2015). Cholecystokinin, a peptide hormone of the gut and central nervous system, was found to be modified with CS in its propeptide region. Alignment of mammalian cholecystokinins shows a relatively low degree of sequence homology for the CS-site. For instance, the sequences of mouse and cat contain a proline instead of a serine residue at the attachment site, thereby excluding the possibility of CS-modification. Taken together, this indicates that certain CPGs/CSPGs are conserved throughout evolution to the same extent as HSPGs, whereas others display a very short evolutionary history.

One might question why HSPGs are generally more conserved throughout evolution compared with CSPGs. The difference in conservation may reflect differences in physiological functions, as HSPGs and CSPGs often induce opposite effects on similar cellular events. In neurogenesis, CS and HS have a dual mode of action for regulating neuronal outgrowth, where both GAGs compete for the same binding sites on RPTPσ-receptors. CS chains inhibit nerve regeneration upon binding to RPTPσ-receptors, whereas HS promotes nerve regeneration upon binding to the same receptors (Shen et al. 2009; Coles et al. 2011; Katagiri et al. 2018; Sakamoto et al. 2019). Given this proteoglycan-switch, it is conceivable that HSPGs work in strict regulation with other promoting factors to navigate the growing axon along a precisely defined path. In contrast, CSPGs, which have a negative regulatory role, may be less specific in its action, providing foremost an outer perimeter for the process. A clinical example is the potential use of chondroitinase ABC in the treatment of spinal cord injury. At the injured site, axons fail to regenerate due to the formation of a glial scar, which is composed of extracellular matrix components including CSPGs (Bradbury et al. 2002). Intrathecal administration of chondroitinase ABC degrades the CS chains and thereby increases neuronal plasticity (Hu et al. 2018). Therefore, CSPG-mediated functions may display less stringent spatiotemporal requirements compared to HSPGs. To exert a particular CSPG-mediated function a CS chain is likely necessary, but its exact attachment site along the complete amino acid sequence, or even the exact identity of the core protein may have less importance, as long as the CS chain is presented in its functional context. This would impose an evolutionary selection pressure to conserve the mechanisms for CS biosynthesis and attachment motifs, but not to the same extent to a particular core protein.

A wide range of microbial pathogens uses GAG-specific interactions for their adhesion to host tissues and invasion of target cells (Bartlett and Park 2010). In nature, *C.elegans* is found in microbe-rich environments, such as rotting plant matter, containing a multitude of microbial antagonists to the nematode (Schulenburg and Felix 2017). As parasites and pathogens reduce host fitness they often impose high selective pressure on their hosts. The nematode's natural biotic environment has therefore been suggested to have

strong impact on *C.elegans* evolution and of great importance for understanding its biology (Schulenburg and Felix 2017). Given that GAGs serve as an entry point for different pathogens, changes in the underlying genomic characteristics to introduce additional chondroitin-attachment motifs on different core proteins, may have served as a strategy to evade infections for *C. elegans* throughout evolution. A more divergent chondroitin glycoproteome may present more 'decoy sites' for chondroitin-binding pathogens, thereby reducing pathogen attachment and entry to specific target cells. Indeed, the complexity of glycans has been suggested to be driven by an evolutionary arms race due to the exploitation of host glycans by parasites and pathogens (Gagneux et al. 2015). One may speculate that other invertebrates, whose natural habitats present lower microbe-induced selective pressure, would have less CSPGs. Regardless of the *in vivo* situation, full appreciation of the functional roles and evolutionary perspectives of CPGs/CSPGs warrants further studies in *C.elegans* and in other invertebrates. Taken together, our findings suggest that several aspects regarding chondroitin and chondroitin sulfate proteoglycan biosynthesis are conserved throughout evolution. This includes the glycosylation motif, the mechanisms for saccharide initiation and polymerization and in some cases also the splicing and the presentation of core protein domains. However, since the majority of core proteins seems not to be conserved between the species, our findings point to both converging and diverging selective forces during the proteoglycan evolution.

9 Conclusions

Our use of a novel glycoproteomic method for identifying CS-glycopeptides enabled the identification of several novel core proteins in *C. elegans* and in humans. Bioinformatic analysis of the primary amino acid sequence revealed great structural and also functional diversity of CPGs in the nematode and indicates that some, but not all, functions overlap with those of human CSPGs. Moreover, three of the novel core proteins display homology to vertebrate counterparts, indicating that CPG / CSPGs may be more conserved throughout evolution than previously perceived. The future use of similar glycoproteomic strategies may thus be helpful in identifying CPG / CSPGs also in other important model organisms, such as *Drosophila melanogaster* and *Danio rerio*. This will likely expand the number of identified core proteins and may also provide new perspectives on proteoglycan-mediated functions and how these have persisted or developed throughout evolution. Further, obtaining global information on attachment sites and core protein identities will likely assist in assigning CPG/CSPG specific functions, both in vertebrates and in invertebrates. In addition, novel methods to site-specifically analyze the structures of extended CS chains may also be important to better understand the structure-function relationship of CPG/CSPG-mediated functions.

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References

Ackley BD, Crew JR, Elamaa H, Pihlajaniemi T, Kuo CJ, Kramer JM (2001) The NC1/endostatin domain of Caenorhabditis elegans type XVIII collagen affects cell migration and axon guidance. J Cell Biol 152 (6):1219–1232

Ackley BD, Kang SH, Crew JR, Suh C, Jin Y, Kramer JM (2003) The basement membrane components nidogen and type XVIII collagen regulate organization of neuromuscular junctions in Caenorhabditis elegans. J Neurosci 23(9):3577–3587

Adams JC, Tucker RP (2000) The thrombospondin type 1 repeat (TSR) superfamily: diverse proteins with related roles in neuronal development. Dev Dyn 218 (2):280–299. https://doi.org/10.1002/(SICI)1097-0177(200006)218:2<280::AID-DVDY4>3.0.CO;2-0

Antoshechkin I, Sternberg PW (2007) The versatile worm: genetic and genomic resources for Caenorhabditis elegans research. Nat Rev Genet 8(7):518–532. https://doi.org/10.1038/nrg2105

Aono S, Tokita Y, Shuo T, Yamauchi S, Matsui F, Nakanishi K, Hirano K, Sano M, Oohira A (2004) Glycosylation site for chondroitin sulfate on the neural part-time proteoglycan, neuroglycan C. J Biol Chem 279(45):46536–46541. https://doi.org/10.1074/jbc.M403263200

Baeg GH, Lin X, Khare N, Baumgartner S, Perrimon N (2001) Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. Development 128(1):87–94

Bartlett AH, Park PW (2010) Proteoglycans in host-pathogen interactions: molecular mechanisms and therapeutic implications. Expert Rev Mol Med 12:e5. https://doi.org/10.1017/S1462399409001367

Bernfield M, Gotte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M (1999) Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem 68:729–777. https://doi.org/10.1146/annurev.biochem.68.1.729

Berninsone P, Hwang HY, Zemtseva I, Horvitz HR, Hirschberg CB (2001) SQV-7, a protein involved in Caenorhabditis elegans epithelial invagination and early embryogenesis, transports UDP-glucuronic acid, UDP-N- acetylgalactosamine, and UDP-galactose. Proc Natl Acad Sci U S A 98(7):3738–3743. https://doi.org/10.1073/pnas.061593098

Bishop JR, Schuksz M, Esko JD (2007) Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 446(7139):1030–1037. https://doi.org/10.1038/nature05817

Blanchette CR, Perrat PN, Thackeray A, Benard CY (2015) Glypican is a modulator of Netrin-mediated axon guidance. PLoS Biol 13(7):e1002183. https://doi.org/10.1371/journal.pbio.1002183

Blanchette CR, Thackeray A, Perrat PN, Hekimi S, Benard CY (2017) Functional requirements for Heparan sulfate biosynthesis in morphogenesis and nervous system development in C. elegans. PLoS Genet 13(1):e1006525. https://doi.org/10.1371/journal.pgen.1006525

Bourdon MA, Oldberg A, Pierschbacher M, Ruoslahti E (1985) Molecular cloning and sequence analysis of a chondroitin sulfate proteoglycan cDNA. Proc Natl Acad Sci U S A 82(5):1321–1325. https://doi.org/10.1073/pnas.82.5.1321

Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, SB MM (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416(6881):636–640. https://doi.org/10.1038/416636a

Bulik DA, Wei G, Toyoda H, Kinoshita-Toyoda A, Waldrip WR, Esko JD, Robbins PW, Selleck SB (2000) sqv-3, -7, and -8, a set of genes affecting morphogenesis in Caenorhabditis elegans, encode enzymes required for glycosaminoglycan biosynthesis. Proc Natl Acad Sci U S A 97(20):10838–10843. https://doi.org/10.1073/pnas.97.20.10838

Chang YH, Sun YH (2014) Carrier of Wingless (Cow), a secreted heparan sulfate proteoglycan, promotes extracellular transport of Wingless. PLoS One 9(10):e111573. https://doi.org/10.1371/journal.pone.0111573

Coles CH, Shen Y, Tenney AP, Siebold C, Sutton GC, Lu W, Gallagher JT, Jones EY, Flanagan JG, Aricescu AR (2011) Proteoglycan-specific molecular switch for RPTPsigma clustering and neuronal extension. Science 332(6028):484–488. https://doi.org/10.1126/science.1200840

Consortium CeS (1998) Genome sequence of the nematode C. elegans: a platform for investigating biology. Science 282(5396):2012–2018

Couchman JR, Pataki CA (2012) An introduction to proteoglycans and their localization. J Histochem Cytochem 60(12):885–897. https://doi.org/10.1369/0022155412464638

Csoka AB, Stern R (2013) Hypotheses on the evolution of hyaluronan: a highly ironic acid. Glycobiology 23(4):398–411. https://doi.org/10.1093/glycob/cws218

Dierker T, Shao C, Haitina T, Zaia J, Hinas A, Kjellen L (2016) Nematodes join the family of chondroitin sulfate-synthesizing organisms: identification of an active chondroitin sulfotransferase in Caenorhabditis elegans. Sci Rep 6:34662. https://doi.org/10.1038/srep34662

Esko JD, Lindahl U (2001) Molecular diversity of heparan sulfate. J Clin Invest 108(2):169–173. https://doi.org/10.1172/JCI13530

Esko JD, Zhang L (1996) Influence of core protein sequence on glycosaminoglycan assembly. Curr Opin Struct Biol 6(5):663–670

Gagneux P, Aebi M, Varki A (2015) Evolution of glycan diversity. In: Varki A, Cummings RD et al (eds) Essentials of Glycobiology, Cold Spring Harbor, pp 253–264. https://doi.org/10.1101/glycobiology.3e.020

Gesteira TF, Coulson-Thomas VJ, Ogata FT, Farias EH, Cavalheiro RP, de Lima MA, Cunha GL, Nakayasu ES, Almeida IC, Toma L, Nader HB (2011) A novel approach for the characterisation of proteoglycans and biosynthetic enzymes in a snail model. Biochim Biophys Acta 1814(12):1862–1869. https://doi.org/10.1016/j.bbapap.2011.07.024

Hascall VC, Wang A, Tammi M, Oikari S, Tammi R, Passi A, Vigetti D, Hanson RW, Hart GW (2014) The dynamic metabolism of hyaluronan regulates the cytosolic concentration of UDP-GlcNAc. Matrix Biol 35:14–17. https://doi.org/10.1016/j.matbio.2014.01.014

Herman T, Horvitz HR (1999) Three proteins involved in Caenorhabditis elegans vulval invagination are similar to components of a glycosylation pathway. Proc Natl Acad Sci U S A 96(3):974–979. https://doi.org/10.1073/pnas.96.3.974

Herman T, Hartwieg E, Horvitz HR (1999) sqv mutants of Caenorhabditis elegans are defective in vulval epithelial invagination. Proc Natl Acad Sci U S A 96(3):968–973. https://doi.org/10.1073/pnas.96.3.968

Hrus A, Lau G, Hutter H, Schenk S, Ferralli J, Brown-Luedi M, Chiquet-Ehrismann R, Canevascini S (2007) C. elegans agrin is expressed in pharynx, IL1 neurons and distal tip cells and does not genetically interact

with genes involved in synaptogenesis or muscle function. PLoS One 2(8):e731. https://doi.org/10.1371/journal.pone.0000731

Hu HZ, Granger N, Pai SB, Bellamkonda RV, Jeffery ND (2018) Therapeutic efficacy of microtube-embedded chondroitinase ABC in a canine clinical model of spinal cord injury. Brain 141(4):1017–1027. https://doi.org/10.1093/brain/awy007

Hutter H, Vogel BE, Plenefisch JD, Norris CR, Proenca RB, Spieth J, Guo C, Mastwal S, Zhu X, Scheel J, Hedgecock EM (2000) Conservation and novelty in the evolution of cell adhesion and extracellular matrix genes. Science 287(5455):989–994. https://doi.org/10.1126/science.287.5455.989

Hwang HY, Horvitz HR (2002) The Caenorhabditis elegans vulval morphogenesis gene sqv-4 encodes a UDP-glucose dehydrogenase that is temporally and spatially regulated. Proc Natl Acad Sci U S A 99 (22):14224–14229. https://doi.org/10.1073/pnas.172522499

Hwang HY, Olson SK, Brown JR, Esko JD, Horvitz HR (2003) The Caenorhabditis elegans genes sqv-2 and sqv-6, which are required for vulval morphogenesis, encode glycosaminoglycan galactosyltransferase II and xylosyltransferase. J Biol Chem 278 (14):11735–11738. https://doi.org/10.1074/jbc.C200518200

Iozzo RV, Schaefer L (2015) Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. Matrix Biol 42:11–55. https://doi.org/10.1016/j.matbio.2015.02.003

Izumikawa T, Kitagawa H, Mizuguchi S, Nomura KH, Nomura K, Tamura J, Gengyo-Ando K, Mitani S, Sugahara K (2004) Nematode chondroitin polymerizing factor showing cell−/organ-specific expression is indispensable for chondroitin synthesis and embryonic cell division. J Biol Chem 279 (51):53755–53761. https://doi.org/10.1074/jbc.M409615200

Izumikawa T, Dejima K, Watamoto Y, Nomura KH, Kanaki N, Rikitake M, Tou M, Murata D, Yanagita E, Kano A, Mitani S, Nomura K, Kitagawa H (2016) Chondroitin 4-O-Sulfotransferase is indispensable for Sulfation of chondroitin and plays an important role in maintaining Normal life span and oxidative stress responses in nematodes. J Biol Chem 291(44):23294–23304. https://doi.org/10.1074/jbc.M116.757328

Joshi HJ, Narimatsu Y, Schjoldager KT, Tytgat HLP, Aebi M, Clausen H, Halim A (2018) SnapShot: O-Glycosylation pathways across kingdoms. Cell 172 (3):632–632 e632. https://doi.org/10.1016/j.cell.2018.01.016

Karamanos KK, Aletras AJ, Antonopoulos CA, Hjerpe A, Tsiganos CP (1990) Chondroitin proteoglycans from squid skin. Isolation, characterization and immunological studies. Eur J Biochem 192(1):33–38. https://doi.org/10.1111/j.1432-1033.1990.tb19191.x

Katagiri Y, Morgan AA, Yu P, Bangayan NJ, Junka R, Geller HM (2018) Identification of novel binding sites for heparin in receptor protein-tyrosine phosphatase (RPTPsigma): implications for proteoglycan signaling. J Biol Chem 293(29):11639–11647. https://doi.org/10.1074/jbc.RA118.003081

King SLJH, Schjoldager KT, Halim A, Madsen TD, Dzielgiel MH, Woetmann A, Vakhrushev SY, Wandall H (2017) Characterizing the O-glycosylation landscape of human plasma, platelets, and endothelial cells. Blood Adv 1(7):429–442

Kjellen L, Lindahl U (2018) Specificity of glycosaminoglycan-protein interactions. Curr Opin Struct Biol 50:101–108. https://doi.org/10.1016/j.sbi.2017.12.011

Kjellen L, Pettersson I, Lillhager P, Steen ML, Pettersson U, Lehtonen P, Karlsson T, Ruoslahti E, Hellman L (1989) Primary structure of a mouse mastocytoma proteoglycan core protein. Biochem J 263(1):105–113. https://doi.org/10.1042/bj2630105

Kreuger J, Spillmann D, Li JP, Lindahl U (2006) Interactions between heparan sulfate and proteins: the concept of specificity. J Cell Biol 174(3):323–327. https://doi.org/10.1083/jcb.200604035

Le Jan S, Hayashi M, Kasza Z, Eriksson I, Bishop JR, Weibrecht I, Heldin J, Holmborn K, Jakobsson L, Soderberg O, Spillmann D, Esko JD, Claesson-Welsh-L, Kjellen L, Kreuger J (2012) Functional overlap between chondroitin and heparan sulfate proteoglycans during VEGF-induced sprouting angiogenesis. Arterioscler Thromb Vasc Biol 32(5):1255–1263. https://doi.org/10.1161/ATVBAHA.111.240622

Ledin J, Staatz W, Li JP, Gotte M, Selleck S, Kjellen L, Spillmann D (2004) Heparan sulfate structure in mice with genetically modified heparan sulfate production. J Biol Chem 279(41):42732–42741. https://doi.org/10.1074/jbc.M405382200

Li F, Shi W, Capurro M, Filmus J (2011) Glypican-5 stimulates rhabdomyosarcoma cell proliferation by activating Hedgehog signaling. J Cell Biol 192 (4):691–704. https://doi.org/10.1083/jcb.201008087

Lindahl U (2014) A personal voyage through the proteoglycan field. Matrix Biol 35:3–7. https://doi.org/10.1016/j.matbio.2014.01.001

Lindahl U, Couchman J, Kimata K, Esko JD (2015) Proteoglycans and Sulfated Glycosaminoglycans. In: Varki A, Cummings RD et al (eds) Essentials of glycobiology, Cold Spring Harbor, pp 207–221. https://doi.org/10.1101/glycobiology.3e.017

Ly M, Leach FE 3rd, Laremore TN, Toida T, Amster IJ, Linhardt RJ (2011) The proteoglycan bikunin has a defined sequence. Nat Chem Biol 7(11):827–833. https://doi.org/10.1038/nchembio.673

Maduro MF (2017) Gut development in C. elegans. Semin Cell Dev Biol 66:3–11. https://doi.org/10.1016/j.semcdb.2017.01.001

Mizumoto S, Ikegawa S, Sugahara K (2013) Human genetic disorders caused by mutations in genes encoding biosynthetic enzymes for sulfated glycosaminoglycans. J Biol Chem 288(16):10953–10961. https://doi.org/10.1074/jbc.R112.437038

Mizumoto S, Yamada S, Sugahara K (2015) Molecular interactions between chondroitin-dermatan sulfate and growth factors/receptors/matrix proteins. Curr Opin

Struct Biol 34:35–42. https://doi.org/10.1016/j.sbi.2015.06.004

Muir H (1958) The nature of the link between protein and carbohydrate of a chondroitin sulphate complex from hyaline cartilage. Biochem J 69(2):195–204. https://doi.org/10.1042/bj0690195

Mulloy B, Hart GW, Stanley P (2009) Structural analysis of Glycans. In: Varki A, Cummings RD et al (eds) Essentials of Glycobiology, Cold Spring Harbor

Murdoch AD, Iozzo RV (1993) Perlecan: the multidomain heparan sulphate proteoglycan of basement membrane and extracellular matrix. Virchows Arch A Pathol Anat Histopathol 423(4):237–242. https://doi.org/10.1007/bf01606885

Nadanaka S, Kitagawa H, Sugahara K (1998) Demonstration of the immature glycosaminoglycan tetrasaccharide sequence GlcAbeta1-3Galbeta1-3Galbeta1-4Xyl on recombinant soluble human alpha-thrombomodulin. An oligosaccharide structure on a "part-time" proteoglycan. J Biol Chem 273 (50):33728–33734. https://doi.org/10.1074/jbc.273.50.33728

Nakato H, Li JP (2016) Functions of Heparan sulfate proteoglycans in development: insights from Drosophila models. Int Rev Cell Mol Biol 325:275–293. https://doi.org/10.1016/bs.ircmb.2016.02.008

Nakato H, Futch TA, Selleck SB (1995) The division abnormally delayed (dally) gene: a putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in Drosophila. Development 121 (11):3687–3702

Nasir W, Toledo AG, Noborn F, Nilsson J, Wang M, Bandeira N, Larson G (2016) SweetNET: a bioinformatics workflow for Glycopeptide MS/MS spectral analysis. J Proteome Res 15(8):2826–2840. https://doi.org/10.1021/acs.jproteome.6b00417

Nilsson J, Halim A, Grahn A, Larson G (2013) Targeting the glycoproteome. Glycoconj J 30(2):119–136. https://doi.org/10.1007/s10719-012-9438-6

Noborn F, Gomez Toledo A, Sihlbom C, Lengqvist J, Fries E, Kjellen L, Nilsson J, Larson G (2015) Identification of chondroitin sulfate linkage region glycopeptides reveals prohormones as a novel class of proteoglycans. Mol Cell Proteomics 14(1):41–49. https://doi.org/10.1074/mcp.M114.043703

Noborn F, Gomez Toledo A, Green A, Nasir W, Sihlbom C, Nilsson J, Larson G (2016) Site-specific identification of heparan and chondroitin sulfate glycosaminoglycans in hybrid proteoglycans. Sci Rep 6:34537. https://doi.org/10.1038/srep34537

Noborn F, Gomez Toledo A, Nasir W, Nilsson J, Dierker T, Kjellen L, Larson G (2018) Expanding the chondroitin glycoproteome of Caenorhabditis elegans. J Biol Chem 293(1):379–389. https://doi.org/10.1074/jbc.M117.807800

Olson SK, Bishop JR, Yates JR, Oegema K, Esko JD (2006) Identification of novel chondroitin proteoglycans in Caenorhabditis elegans: embryonic cell division depends on CPG-1 and CPG-2. J Cell Biol 173(6):985–994. https://doi.org/10.1083/jcb.200603003

Olson SK, Greenan G, Desai A, Muller-Reichert T, Oegema K (2012) Hierarchical assembly of the eggshell and permeability barrier in C. elegans. J Cell Biol 198(4):731–748. https://doi.org/10.1083/jcb.201206008

Oohira A, Shuo T, Tokita Y, Nakanishi K, Aono S (2004) Neuroglycan C, a brain-specific part-time proteoglycan, with a particular multidomain structure. Glycoconj J 21(1–2):53–57. https://doi.org/10.1023/B:GLYC.0000043748.90896.83

Pangalos MN, Efthimiopoulos S, Shioi J, Robakis NK (1995) The chondroitin sulfate attachment site of appican is formed by splicing out exon 15 of the amyloid precursor gene. J Biol Chem 270 (18):10388–10391. https://doi.org/10.1074/jbc.270.18.10388

Park Y, Rangel C, Reynolds MM, Caldwell MC, Johns M, Nayak M, Welsh CJ, McDermott S, Datta S (2003) Drosophila perlecan modulates FGF and hedgehog signals to activate neural stem cell division. Dev Biol 253(2):247–257. https://doi.org/10.1016/s0012-1606(02)00019-2

Pedersen ME, Snieckute G, Kagias K, Nehammer C, Multhaupt HA, Couchman JR, Pocock R (2013) An epidermal microRNA regulates neuronal migration through control of the cellular glycosylation state. Science 341(6152):1404–1408. https://doi.org/10.1126/science.1242528

Ponighaus C, Ambrosius M, Casanova JC, Prante C, Kuhn J, Esko JD, Kleesiek K, Gotting C (2007) Human xylosyltransferase II is involved in the biosynthesis of the uniform tetrasaccharide linkage region in chondroitin sulfate and heparan sulfate proteoglycans. J Biol Chem 282(8):5201–5206. https://doi.org/10.1074/jbc.M611665200

Ramirez-Suarez NJ, Belalcazar HM, Salazar CJ, Beyaz B, Raja B, Nguyen KCQ, Celestrin K, Fredens J, Faergeman NJ, Hall DH, Bulow HE (2019) Axon-dependent patterning and maintenance of somatosensory dendritic Arbors. Dev Cell 48(2):229–244 e224. https://doi.org/10.1016/j.devcel.2018.12.015

Roden L, Smith R (1966) Structure of the neutral trisaccharide of the chondroitin 4-sulfate-protein linkage region. J Biol Chem 241(24):5949–5954

Rogalski TM, Williams BD, Mullen GP, Moerman DG (1993) Products of the unc-52 gene in Caenorhabditis elegans are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. Genes Dev 7(8):1471–1484. https://doi.org/10.1101/gad.7.8.1471

Saied-Santiago K, Bulow HE (2018) Diverse roles for glycosaminoglycans in neural patterning. Dev Dyn 247(1):54–74. https://doi.org/10.1002/dvdy.24555

Sakamoto K, Ozaki T, Ko YC, Tsai CF, Gong Y, Morozumi M, Ishikawa Y, Uchimura K, Nadanaka S, Kitagawa H, Zulueta MML, Bandaru A, Tamura JI, Hung SC, Kadomatsu K (2019) Glycan sulfation patterns define autophagy flux at axon tip via

PTPRsigma-cortactin axis. Nat Chem Biol 15 (7):699–709. https://doi.org/10.1038/s41589-019-0274-x

Salanti A, Clausen TM, Agerbaek MO, Al Nakouzi N, Dahlback M, Oo HZ, Lee S, Gustavsson T, Rich JR, Hedberg BJ, Mao Y, Barington L, Pereira MA, LoBello J, Endo M, Fazli L, Soden J, Wang CK, Sander AF, Dagil R, Thrane S, Holst PJ, Meng L, Favero F, Weiss GJ, Nielsen MA, Freeth J, Nielsen TO, Zaia J, Tran NL, Trent J, Babcook JS, Theander TG, Sorensen PH, Daugaard M (2015) Targeting human Cancer by a glycosaminoglycan binding malaria protein. Cancer Cell 28(4):500–514. https://doi.org/10.1016/j.ccell.2015.09.003

Sarrazin S, Lamanna WC, Esko JD (2011) Heparan sulfate proteoglycans. Cold Spring Harb Perspect Biol 3(7). https://doi.org/10.1101/cshperspect.a004952

Schachter H (2004) Protein glycosylation lessons from Caenorhabditis elegans. Curr Opin Struct Biol 14 (5):607–616. https://doi.org/10.1016/j.sbi.2004.09.005

Schatton J, Schubert M (1954) Isolation of a mucoprotein from cartilage. J Biol Chem 211:565–573

Schulenburg H, Felix MA (2017) The natural biotic environment of Caenorhabditis elegans. Genetics 206 (1):55–86. https://doi.org/10.1534/genetics.116.195511

Shao C, Shi X, White M, Huang Y, Hartshorn K, Zaia J (2013) Comparative glycomics of leukocyte glycosaminoglycans. FEBS J 280(10):2447–2461. https://doi.org/10.1111/febs.12231

Shen Y, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, He Z, Silver J, Flanagan JG (2009) PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. Science 326 (5952):592–596. https://doi.org/10.1126/science.1178310

Shim YH, Paik YK (2010) Caenorhabditis elegans proteomics comes of age. Proteomics 10(4):846–857. https://doi.org/10.1002/pmic.200900542

Stern R (2003) Devising a pathway for hyaluronan catabolism: are we there yet? Glycobiology 13(12):105R–115R. https://doi.org/10.1093/glycob/cwg112

Sugiura N, Clausen TM, Shioiri T, Gustavsson T, Watanabe H, Salanti A (2016) Molecular dissection of placental malaria protein VAR2CSA interaction with a chemo-enzymatically synthesized chondroitin sulfate library. Glycoconj J 33(6):985–994. https://doi.org/10.1007/s10719-016-9685-z

Toyoda H, Kinoshita-Toyoda A, Selleck SB (2000) Structural analysis of glycosaminoglycans in Drosophila and Caenorhabditis elegans and demonstration that tout-velu, a Drosophila gene related to EXT tumor suppressors, affects heparan sulfate in vivo. J Biol Chem 275(4):2269–2275

Vuong-Brender TT, Yang X, Labouesse M (2016) C. elegans embryonic morphogenesis. Curr Top Dev Biol 116:597–616. https://doi.org/10.1016/bs.ctdb.2015.11.012

Weigel PH, Hascall VC, Tammi M (1997) Hyaluronan synthases. J Biol Chem 272(22):13997–14000. https://doi.org/10.1074/jbc.272.22.13997

Weiss RJ, Esko JD, Tor Y (2017) Targeting heparin and heparan sulfate protein interactions. Org Biomol Chem 15(27):5656–5668. https://doi.org/10.1039/c7ob01058c

Wight TN (2002) Versican: a versatile extracellular matrix proteoglycan in cell biology. Curr Opin Cell Biol 14 (5):617–623

Wilson IB (2004) The never-ending story of peptide O-xylosyltransferase. Cell Mol Life Sci 61 (7–8):794–809. https://doi.org/10.1007/s00018-003-3278-2

Wilson IBH, Cummings RD, Aebi M (2015) Nematoda. In: Varki A, Cummings RD et al (eds) Essentials of Glycobiology, Cold Spring Harbor, pp 321–333. https://doi.org/10.1101/glycobiology.3e.025

Yamada S, Van Die I, Van den Eijnden DH, Yokota A, Kitagawa H, Sugahara K (1999) Demonstration of glycosaminoglycans in Caenorhabditis elegans. FEBS Lett 459(3):327–331. https://doi.org/10.1016/s0014-5793(99)01286-7

Yamada S, Okada Y, Ueno M, Iwata S, Deepa SS, Nishimura S, Fujita M, Van Die I, Hirabayashi Y, Sugahara K (2002) Determination of the glycosaminoglycan-protein linkage region oligosaccharide structures of proteoglycans from Drosophila melanogaster and Caenorhabditis elegans. J Biol Chem 277(35):31877–31886. https://doi.org/10.1074/jbc.M205078200

Yamada S, Sugahara K, Ozbek S (2011) Evolution of glycosaminoglycans: comparative biochemical study. Commun Integr Biol 4(2):150–158. https://doi.org/10.4161/cib.4.2.14547

Yanagishita M (1993) A brief history of proteoglycans. Experientia 49(5):366–368. https://doi.org/10.1007/bf01923581

Zhang L, Esko JD (1994) Amino acid determinants that drive heparan sulfate assembly in a proteoglycan. J Biol Chem 269(30):19295–19299

Zhang P, Lu H, Peixoto RT, Pines MK, Ge Y, Oku S, Siddiqui TJ, Xie Y, Wu W, Archer-Hartmann S, Yoshida K, Tanaka KF, Aricescu AR, Azadi P, Gordon MD, Sabatini BL, Wong ROL, Craig AM (2018) Heparan sulfate organizes neuronal synapses through Neurexin partnerships. Cell 174(6):1450–1464 e1423. https://doi.org/10.1016/j.cell.2018.07.002

Zhu F, Li D, Chen K (2019) Structures and functions of invertebrate glycosylation. Open Biol 9(1):180232. https://doi.org/10.1098/rsob.180232

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Structural Basis of a Conventional Recognition Mode of IGHV1-69 Rheumatoid Factors

Mitsunori Shiroishi

Abstract

Rheumatoid factors (RFs) are autoantibodies that recognize the fragment crystallizable (Fc) region of immunoglobulin G (IgG). Genetically diverse RFs are produced in rheumatoid arthritis patients; however, in hematologic diseases, such as cryoglobulinemia and B cell lymphoma, RFs from a limited combination of heavy chain V-region genes and J-region genes are produced in large quantities and forms immune complexes with IgG. These genetically limited RFs have historically been used for the immunochemical characterization of RFs. Among them, RFs derived from the heavy-chain germline gene *IGHV1-69* are the most common. Recently, the crystal structure of an *IGHV1-69*-derived RF named YES8c was elucidated in complex with human IgG1-Fc. Based on the structure and mutant analyses, a recognition mechanism for the autoantigen (IgG-Fc) common to *IGHV1-69*-derived RFs was proposed. This review summarizes the immunochemical character of the *IGHV1-69*-derived RFs, and then focuses on the recognition mechanism of the *IGHV1-69*-derived RFs, referring the structural features of the *IGHV1-69*-derived neutralizing antibodies.

M. Shiroishi (✉)
Department of Biological Science and Technology, Tokyo University of Science, Tokyo, Japan
e-mail: shiroishi@rs.tus.ac.jp

Keywords

Autoantibody · B cell lymphoma · Cryoglobulinemia · Crystal structure · Immunoglobulin-G (IgG) · Molecular recognition · Rheumatoid factor

1 Rheumatoid Factors (RF)

RFs are autoantibodies that recognize the fragment crystallizable (Fc) region of immunoglobulin G (IgG). RFs have been used as important diagnostic markers of rheumatoid arthritis (RA); an autoimmune disease characterized by the chronic joint inflammation leading to systemic inflammation (van Delft and Huizinga 2020). RFs are detected not only in RA but also in other inflammatory diseases or in the serum of some healthy individuals. In particular, a large amount of monoclonal RF (mainly IgM) is observed in the patients with some B-cell malignancies, such as mixed cryoglobulinemia (MC) and Waldenströms macroglobulinemia (WM) (Randen et al. 1992a). In these diseases, precipitation of a mixture of IgG and monoclonal IgM with RF activity, named cryoglobulin, is observed in the serum of the patient below body temperature. The cryoglobulin deposits on tissues such as small blood vessel and kidneys, causing inflammation and tissue injury (Muchtar et al. 2017). The abundant abnormal immunoglobulins secreted from malignant B cells are called "paraprotein". Immunochemical studies of RFs,

such as those to elucidate their molecular-recognition mechanisms, have evolved from studies using monoclonal IgM paraprotein with RF activity derived from B cell malignancies (Randen et al. 1992a).

There is a difference in genetic diversity and Fc-binding patterns between RA-derived RF and B cell lymphoma-derived RF. RFs derived from B cells isolated from RA patients are more diverse in terms of germline gene types than RFs derived from B cell malignancies (Pascual et al. 1990, 1992; Victor et al. 1991; Ermel et al. 1993; Mantovani et al. 1993; Youngblood et al. 1994). Additionally, RA-derived RFs exhibit various binding patterns for different IgG subclasses (IgG1, IgG2, IgG3, and IgG4) (Artandi et al. 1991, 1992), and its epitopes are thought to be widely distributed in the Fc region (Bonagura et al. 1993). By contrast, in B cell malignancies, such as MC and WM, monoclonal IgM-type RFs with specific idiotype combinations are frequently observed, and the binding pattern of the RFs is limited (Silverman et al. 1988; Kunkel et al. 1973).

2 RFs from a Common VH and VL Gene

In the serum of healthy individuals immunized with foreign antigen (mismatched red blood cells) (Thompson et al. 1994) and in patients who developed MC due to hepatitis C virus (HCV) infection (Charles et al. 2008), mucosal-associated lymphoid tissue-type lymphoma (Bende et al. 2005), HCV-associated lymphoma (De Re et al. 2002), or B cell chronic lymphocytic leukemia (Stamatopoulos et al. 2007), RFs with a combination of heavy and light chains derived from a specific germline gene are frequently observed (Bende et al. 2015). In such cases, the combination of a heavy chain with a cross-reactive idiotype (CRI) called Wa and a light chain with a CRI called 17.109 are the most frequent. Each of these heavy and light chains is derived from *IGHV1-69* and *IGKV3-20*, respectively, which are frequently used V-region genes for RFs. RFs derived from a specific germline gene (especially H chain) having a specific length in the third complementarity determining region of the H chain (CDR-H3), and highly homologous to the RF are specifically referred to as "stereotypic RFs". *IGHV1-69* is the most major germline gene for stereotypic RFs; however, recently, stereotypic RFs derived from germline genes such as *IGHV3-7* and *IGHV4-59*, have also been reported (Hoogeboom et al. 2013; Bende et al. 2016).

The heavy chain germline gene *IGHV1-69* is used in the heavy chain of neutralizing antibodies against the following viruses and virulence factors: HCV (Kong et al. 2013; Chan et al. 2001), human immunodeficiency virus (HIV) (Luftig et al. 2006), influenza virus (Ekiert et al. 2009), Middle East respiratory syndrome coronavirus (MERS-CoV) (Ying et al. 2015), and the *Staphylococcus aureus* virulence factor NEAT (Yeung et al. 2016). The germline gene for the light chain is the kappa chain IGKV3-20, which normally pairs with the heavy chain IGHV1-69. Additionally, *IGHV1-69*-derived RF is reportedly highly associated with HCV-related lymphoma (Ivanovski et al. 1998), and notably, >80% of patients with MC are carriers of HCV (Agnello et al. 1992). Moreover, IgM antibody derived from *IGHV1-69*, which occurs in patients with HCV-related MC, reportedly exhibits RF activity with or without somatic mutation (Charles et al. 2013). These findings suggest that the antibody derived from *IGHV1-69* harbors characteristics of molecular recognition related to RF activity.

Therefore, although *IGHV1-69*-derived RF is physiologically and pathologically important, details of the antigen-recognition mechanism have not been clarified. Previous reports suggest that *IGHV1-69*-derived RFs isolated from immunized healthy individuals, RA patients, or MC patients have a CDR-H3 with a length restriction of 12–15 residues but with diverse amino acid composition (Bende et al. 2005; Charles et al. 2013; Borretzen et al. 1995). On the other hand, most *IGHV1-69*-derived RFs recognize the CH2-CH3 elbow region of Fc as their epitope (Artandi et al. 1992). The question of whether *IGHV1-69*-derived RFs recognize this epitope in a common recognition mode, and whether the

antibodies from this germline gene have structural features likely to exhibit RF activity, are interesting from a structural biology perspective.

3 Structure of the *IGHV1-69*-Derived Antibodies

As mentioned above, the *IGHV1-69* gene-encoded antibodies are frequently observed as neutralizing antibodies against viruses and bacterial pathogens. Understanding the recognition mechanism of neutralizing antibodies provide important insights into rational vaccine design. So, what are the structural features of molecular recognition of this germline antibodies? This section summarizes the structure of IGHV1-69 antibodies other than RF. A systematic analysis of antigen-free Fab structures reveals that the structures of CDR-H1 of IGHV1-69 antibodies are substantially flexible, whereas the canonical structures of CDR-H2 have consistent conformations (Teplyakov et al. 2016). This study also reveals that heavy chains derived from *IGHV1-69* gene form thermally stable Fab fragments.

What is common to neutralizing antibodies is that the germline sequence of *IGHV1-69* originally has the properties of a neutralizing antibody, and becomes a neutralizing antibody without introducing many mutations. The structure of D5, a broadly neutralizing antibody for HIV-1, in complex with the glycoprotein gp41 shows that the hydrophobic CDR-H2 protrudes into the conserved gp41 hydrophobic pocket, which determines neutralizing ability (Luftig et al. 2006). The structure of m336, a neutralizing antibody for the MERS-CoV receptor-binding domain, reveals that the heavy chain is a major contributor to antigen binding, and that the V(D)J junctional and *IGHV1-69* allele-specific residues play an important role in the neutralization of MERS-CoV (Ying et al. 2015). The structure of the neutralizing antibodies against *Staphylococcus aureus* virulence factor NEAT2 also reveals that the interaction by the germline-encoded CDR-H2 is critical for neutralization (Yeung et al. 2016). HCV is closely related to IGHV1-69 RFs. The structure of AR3C, a broadly neutralizing antibody for HCV, in complex with the envelope protein E2 reveals that the heavy chain is responsible for 86% of the antigen-antibody binding (Kong et al. 2013). The CDR-H2 of AR3C interacts with the hydrophobic cluster on E2 important for binding to the cell-surface CD81. The structures of the four independent neutralizing antibodies against HCV suggest the mechanism of the broad neutralization of the *IGHV1-69*-derived antibodies (Tzarum et al. 2019).

Among the *IGHV1-69*-derived neutralization antibody, the antibodies for influenzae virus are most reported. The structure of F10 and CR6261, broadly neutralizing antibodies against H5 hemagglutinin (HA), reveals that these antibodies block infection by inserting its heavy chain, which is the only contributor to the interaction, into a conserved pocket in the stem region (Ekiert et al. 2009; Sui et al. 2009). The germline-encoded CDR-H1 and CDR-H2 of the antibody CR6261 interact with the highly conserved stem region in HAs, demonstrating the broad neutralization and high affinity (Ekiert et al. 2009). Furthermore, only a small number of somatic mutations on germline residues enables strong neutralization, suggesting that selected immunoglobulin genes such as *IGHV1-69* are adopted for neutralizing antibody (Lingwood et al. 2012). The *IGHV1-69*-derived neutralizing antibodies, 8M2 and 2G1, recognize the receptor binding region of H2 HA. The structure of 8M2 and 2G1 reveals that the Phe54 in CDR-H2 plugs a conserved cavity in the HA receptor binding site (Xu et al. 2013).

4 Structure of Determined RFs

The following sections focus on the molecular-recognition mechanisms of the RF derived from the germline gene *IGHV1-69* based on the crystal structure of RF YES8c previously described (Fig. 1a) (Shiroishi et al. 2018). Before reporting the YES8c structure, the crystal structures of the two RFs had been revealed in complex with Fc

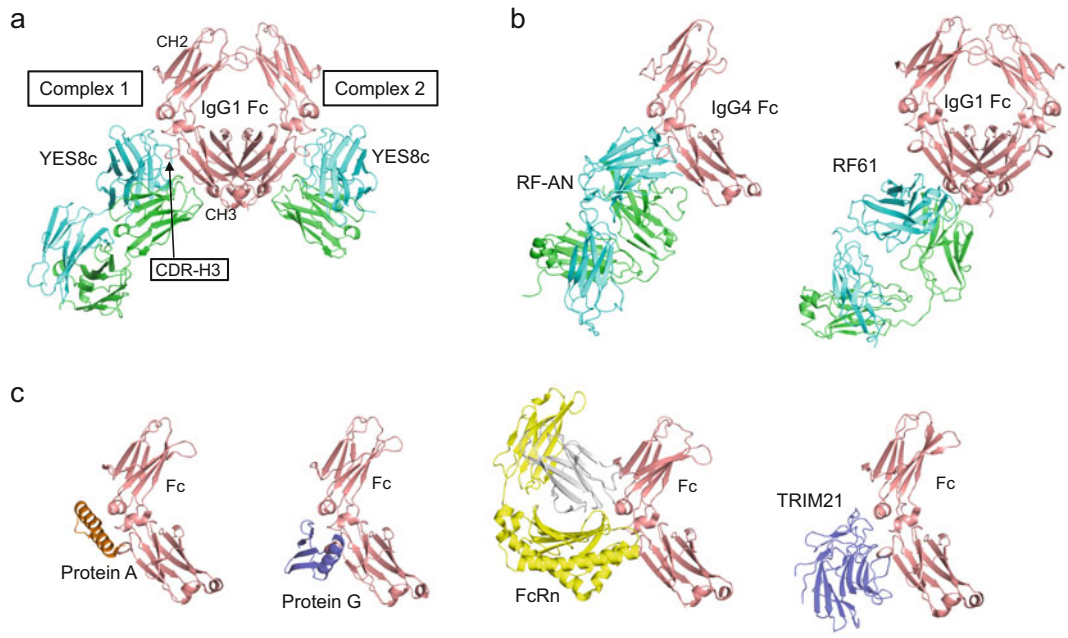

Fig. 1 Crystal structure of YES8c in complex with IgG1-Fc and other Fc-binding proteins (**a**) Overall structure of the YES8c-Fab–Fc complex in an asymmetric unit. Fc and heavy and light chains are shown in pink, cyan, and green, respectively. In complex 2, atomic models of the constant region of YES8c could not be built due to weak electron density. (**b**) Overall structure of the RF-AN–Fc complex (PDB ID: 1ADQ) and the RF61–Fc complex (PDB ID: 2J6E). (**c**) Overall structure of other Fc-binding proteins in complex with Fc: minimized B domain of *S. aureus* protein A (PDB ID: 1L6X), Streptococcal protein G (PDB ID: 1FCC), neonatal Fc receptor (FcRn) (PDB ID: 1I1A), and TRIM21 (PDB ID: 2IWG)

(Corper et al. 1997; Duquerroy et al. 2007), providing interesting insights into the molecular-recognition mechanism and the origin of RFs. One is an IgM-type RF named RF-AN derived from an RA patient and that recognizes the CH2-CH3 cleft region of Fc, a major epitope recognized by many RFs (Fig. 1b). Unlike general antibodies, this RF has the unusual feature of binding Fc at the edge of the conventional paratope (antigen-recognition site) (Corper et al. 1997). The authors considered that due to its unique binding mode, an antibody originally against another antigen acquired binding ability to IgG-Fc at sites other than the original antigen-binding site by somatic mutation (Sutton et al. 2000). The other is a high-affinity IgM-type RF called RF61, which does not recognize the CH2-CH3 cleft but recognizes the CH3 domain near the C-terminus of Fc across two CH3 domains (Fig. 1b) (Duquerroy et al. 2007). The crystal structure of RF61 revealed a new RF epitope, as well as highlighted the diversity of RF epitopes. However, these two RFs were not stereotypic RFs and did not answer the questions presented in this review.

5 Structural Basis for Molecular Recognition of the RF YES8c

5.1 RF YES8c

YES8c is a mono-reactive and IgM-type RF isolated from bone marrow B cells from RA patients and does not exhibit cross-reactivity with autoantigens other than IgG-Fc (Ezaki et al. 1991). This particular patient also had macroglobulinemia due to macroglobulin (IgM) produced by B cell lymphoma. The germline genes in the V, D, and J regions that constitute the heavy chain variable region of YES8c are *IGHV1-69*, *IGHD2-2*, and *IGHJ4*, respectively, and the

germline genes in the V and J regions of the light chain are *IGKV3-20* and *IGKJ2*, respectively. Therefore, YES8c is a stereotype RF derived from the *IGHV1-69/IGKV3-20* germline gene combination common in B cell lymphomas.

5.2 Overall Structure of the YES8c−Fc Complex

We elucidated the crystal structure of the complex of the YES8c Fab fragment and human IgG1-Fc with a resolution of 2.8 Å (Shiroishi et al. 2018). Two YES8c−IgG1-Fc complexes were observed in the asymmetric unit of the crystal, with these two complexes nearly identical structures, except for the constant region (Fig. 1a), hence complex 1 is discussed below. YES8c bound to the CH2-CH3 elbow region of Fc similar to RF-AN (Fig. 1b) (Corper et al. 1997). Additionally, the crystal structure revealed that several proteins, such as protein A, protein G, FcRn, and tripartite motif-containing protein 21 (TRIM21) recognize the CH2-CH3 elbow (Fig. 1c) (James et al. 2007; Martin et al. 2001; Sauer-Eriksson et al. 1995; Deisenhofer 1981). Therefore, this region is thought to have an intrinsic physiochemical property that is easily recognized by other proteins (DeLano et al. 2000). Because the complex structure of protein A and Fc was elucidated long ago, protein A has been used to characterize the interaction of many RFs by the competitive-inhibition assay for Fc (Deisenhofer 1981; Sasso et al. 1988). The heavy chain of YES8c recognizes mainly the CH2−CH3 cleft, and its binding site overlaps with the site where protein A binds. Therefore, YES8c interaction was expected to be inhibited by protein A. Most regions of the light chain of YES8c were utilized for interaction with the CH3 domain.

One characteristic of the YES8c−Fc complex is the presence of a wider interaction interface than that of RF-AN and RF61 (Fig. 1b). The buried surface area involved in forming the YES8c−Fc complex is 1996–2101 $Å^2$, which is larger than RF-AN (1458 $Å^2$) and RF61 (1689 $Å^2$). Additionally, the YES8c heavy chain has a larger interaction interface involved with the Fc interaction than the light chain, and the shape complementarity is also high, suggesting that the heavy chain plays a more central role in Fc recognition. Included in the large interaction interface are 22 amino acid residues on the YES8c side involved in Fc binding, which is a higher number of involved residues than RF-AN (9) and RF61 (14).

Here, we discuss structural differences with RF-AN, an RF that recognizes the CH2-CH3 elbow, as well as YES8c. The binding mode of RF-AN and YES8c differ, as the RF-AN antigen-recognition site deviates from the pseudo two-fold axis formed between VH and VL, and this RF also has the unusual feature of recognizing Fc at the edge of the general paratope (Fig. 2) (Corper et al. 1997). On the other hand, in YES8c, Fc is bound near the center of the pseudo two-fold axis of VH and VL, which is the normal binding mode of antibody (Fig. 2). Additionally, the binding mode of CDR-H3 located at the center of the antigen-binding site is also greatly different between RF-AN and YES8c. In RF-AN, the interaction area of CDR-H3 occupies 44% of the entire antibody binding site, and side chain residues of CDR-H3 (H-Arg96, H-Tyr98, and H-Val99), which is the key to the interaction, form a salt bridge, hydrogen bonds, and hydrophobic interactions with Fc, thereby greatly contributing to antigen recognition. This suggests that CDR-H3 plays a central role in the interaction between RF-AN and Fc. By contrast, the YES8c CDR-H3 occupies only 21% of the interaction area, and the interaction formed between CDR-H3 and Fc is not as strong, suggesting that regions other than YES8c CDR-H3 play a central role in Fc binding.

5.3 YES8c Subclass Specificity and Affinity

The binding specificity of RFs to the IgG subclass is largely divided into two groups: Ga (binding to IgG1, 2, and 4) and Pan (binding to all subclasses) (Bonagura et al. 1993). Binding experiments show that YES8c binds to both IgG1 and IgG4 subclasses (Shiroishi et al. 2018); therefore,

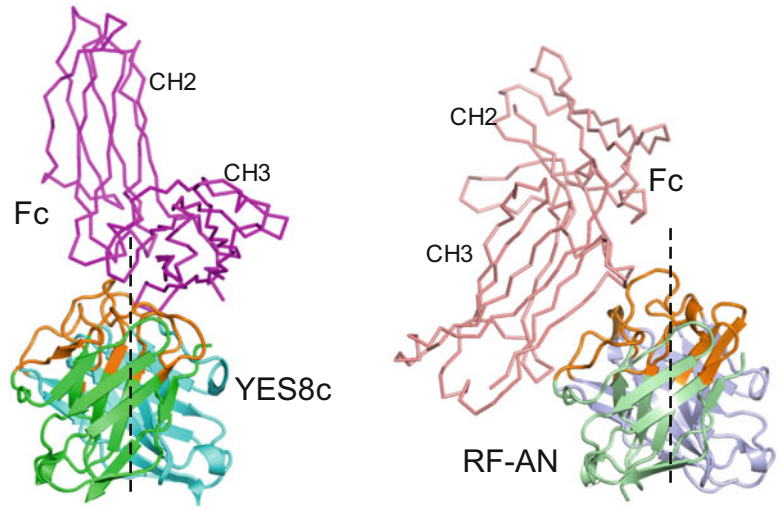

Fig. 2 Comparison of the YES8c and RF-AN binding modes In YES8c, Fc and heavy and light chains are shown in magenta, cyan, and green, respectively, and in light pink, light blue, and light green, respectively, in RF-AN. CDRs are shown in orange in the both structures. The pseudo two-fold axis between VH and VL domains is shown as a dashed line

YES8c belongs to a group with subclass specificity different from Ga and Pan. Here we discuss the binding specificity observed in YES8c according to the three-dimensional structure. Comparison of residues of the IgG3 epitope with other IgG subclasses reveals amino acid residue differences at positions 384, 422, 435, and 436. In particular, position 435 in Fc, which is the key to Ga specificity (Artandi et al. 1992), is a histidine in IgG1, IgG2, and IgG4 but an arginine in IgG3. According to a previous report, although RF-AN exhibits Ga specificity, the reason for its weak affinity with IgG3 cannot be clarified from the RF-AN crystal structure (Corper et al. 1997). The YES8c-Fc complex structure shows that arginine at position 435 would collide with H-Phe54 of YES8c, thereby generating subclass specificity. However, the reason for the reduced affinity with IgG2 remains unknown. Given the possibility that the three-dimensional structure of Fc is slightly different for each IgG subclass due to the influence of amino acid residues other than those at the interaction site, it is necessary to clarify the detailed structure of the subclass.

Similar to RF-AN, YES8c displays a low affinity for Fc. The affinity of YES8c Fab for IgG1 determined by surface plasmon resonance analysis is 160 ± 30 μM (K_d) (Shiroishi et al. 2018). Although this represents low affinity, these RFs are present in the form of IgM with 10 Fab "arms", suggesting that they exhibit strong binding affinity to antigens along with high avidity effect. As noted, the YES8c−Fc complex has a large interface area, with many residues involved in the interaction; however, the distance of the hydrogen bonds between YES8c and Fc are relatively long, implying low bond strength. Moreover, the complex included no salt bridge, which is an interaction that generally promotes affinity, although the number of van der Waals interactions was similar to that in other RF (RF-AN and RF61) complexes. Therefore, the structure suggests that YES8c is loosely bound to Fc, which might also explain its low affinity for IgG1-Fc, despite the large interaction interface. Such a "loose" binding mode might be an important feature for *IGHV1-69*-derived RF recognition of common epitopes in Fc, regardless of the diversity of the CDR-H3 or the introduction of somatic hyper mutations (SMHs).

5.4 Length Limit and Amino Acid Diversity of the CDR-H3 in Fc Recognition

As previously reported, in *IGHV1-69*-derived RFs, the length of the CDR-H3 is limited to 12–15 residues, although some variation in

amino acid composition is observed (Fig. 3a) (Bende et al. 2005, 2015; Borretzen et al. 1995). Based on the crystal structure of the YES8c–Fc complex, we proposed a structural model for allowing amino acid diversity in the CDR-H3 loop associated with Fc recognition by *IGHV1-69*-derived RFs (Shiroishi et al. 2018). In the structure of the YES8c–Fc complex the Leu432-His435 region protruding at the Fc elbow penetrates into the pocket formed between the RF VL and VH regions, and Asn434 interacts with the CDR-H3 from the inside of the loop (Fig. 3b), representing a characteristic feature of YES8c recognition. In particular, the area where the Leu432–His435 region interacts with Fc (~ 320 $Å^2$) is large, occupying one third of the total interaction area. The Asn434 side chain is located near the center of the antigen-binding site and interacts with the CDR-H3 loop to primarily forms hydrogen bonds with CDR-H3 main-chain atoms (the N of H-Gly100 and the O of H-Thr100A). The CDR-H3 residues (especially from H-Ala99 to H-Pro100B) are subsequently located in the space formed between CH2-CH3 and without insertion into the binding interface (Fig. 3c). This binding mode allows accommodation of CDR-H3 in the space formed between CH2 and CH3 by only slight changes to the loop structure, regardless of amino acid composition. If the loop length is <12 residues, this will likely preclude formation of numerous interactions between CDR-H3 and Fc. On the other hand, a length > 15 residues would prevent CDR-H3 from fitting in the space between CH2 and CH3 or result in CDR-H3 covering the pocket between VL and VH, thereby interfering with interactions with the Leu432–His435 region and preventing RF activity.

5.5 The Hydrophobic Region in CDR-H2 Common to *IGHV1-69*-Derived Antibodies

The neutralizing antibodies anti-influenza hemagglutinin antibody CR6261 (Lingwood et al. 2012), the anti-HCV E2 envelope antibody AR3C (Kong et al. 2013), and the anti-HIV-1 gp41 antibody D5A (Luftig et al. 2006) are all derived from the *IGHV1-69* germline gene. Crystal structures of these antibody−antigen complexes revealed that the hydrophobic residues in the CDR-H2 commonly recognize the hydrophobic patch of the antigen. Therefore, the naive antibody derived from *IGHV1-69* is considered to be a "pattern-recognizing" antibody capable of hydrophobic interactions with CDR-H2 residues. CDR-H2 hydrophobicity is highly conserved in *IGHV1-69*-derived RFs (Fig. 3d). In YES8c, the hydrophobic residues (H-Leu53 and H-Phe54) at the tip of the CDR-H2 loop are located at the CH2-CH3 cleft and form hydrophobic interactions with Ile253 and Leu314 across a large interfacial area (Fig. 3e). Because the H-L53A and H-F54A mutants show significantly decreased affinity, recognition by H-Leu53 and H-Phe54 might significantly contribute to RF activity (Shiroishi et al. 2018).

5.6 The Importance of the Light Chain Paired with IGHV1-69 Heavy Chain

In *IGHV1-69*-derived RFs, the paired light chain is derived from *IGKV3-20* in most cases, and the amino acid sequence of the light chain is highly conserved throughout. In the YES8c−Fc complex, 10–11 residues of the light chain are involved in Fc interaction, and the interfacial area between the light chain and Fc is 430–450 $Å^2$, which is considerably larger than the light chains of RF-AN (2 residues and 196 $Å^2$) and RF61 (5 residues and 255 $Å^2$). CDR-L1 and CDR-L3 in YES8c form a flat binding surface and recognize the flat β-sheet region of the CH3 domain (Fig. 3f). Although the area of the interaction interface between the light chain and Fc is large, the calculated shape complementarity between the light chain and Fc is low, indicating that the light chain is loosely bound with the flat binding interface. Surprisingly, however, substitution of various residues in the binding site in the light-chain region with an alanine (L-S29A, L-S31A, L-Y32A, and L-S94A) resulted in a significant

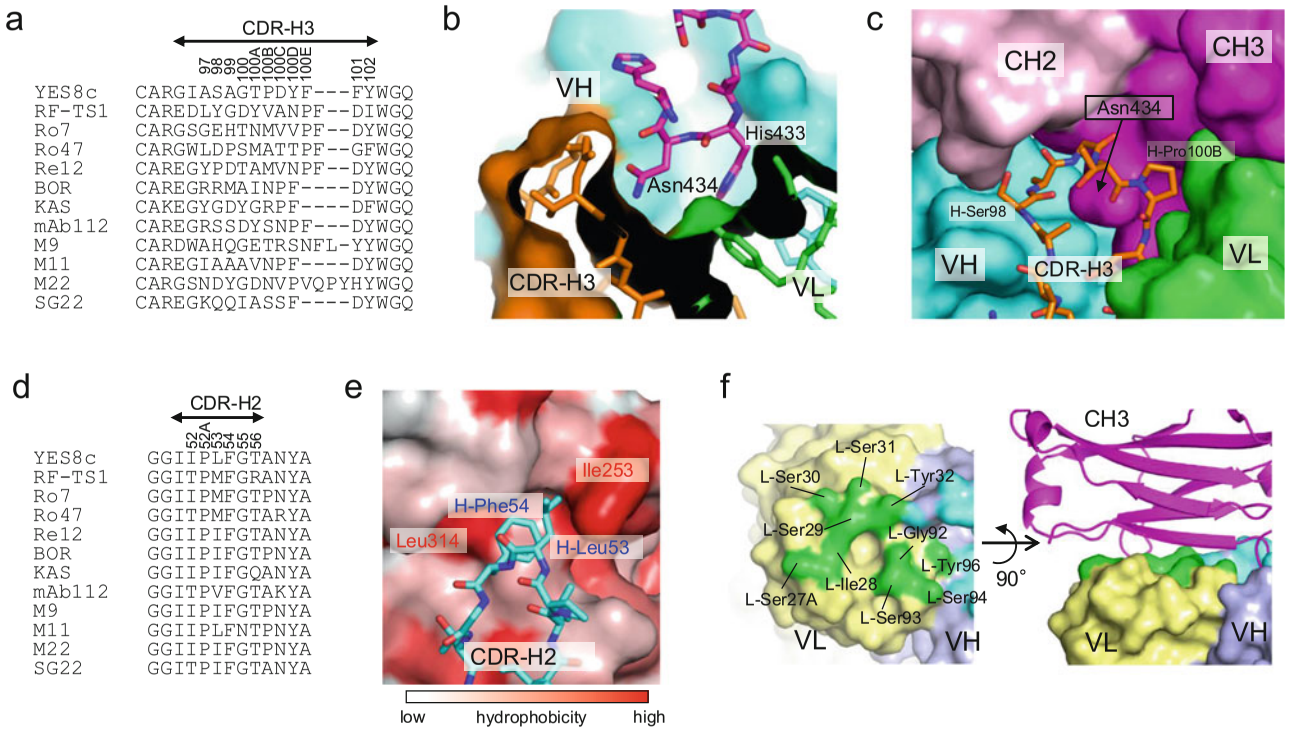

Fig. 3 Structural features of Fc recognition by YES8c (**a**) Alignment of the amino acid residues in the CDR-H3 of YES8c and the other *IGHV1–69/IGKV3–20*-derived RFs. The residues are numbered based on the Kabat numbering scheme. (**b**) The protruding region in Leu432-His435 region of Fc bound to YES8c. The heavy and light chains of YES8c are shown as cyan and green surfaces, respectively, and Fc is shown as magenta sticks. The CDR-H3 is shown in orange. (**c**) The interaction between the CDR-H3 and the CH2–CH3 cleft of Fc. The surfaces of CH2 and CH3 are shown in light pink and magenta, respectively. VH (excluding the CDR-H3) and VL domains are shown as cyan and green surfaces, respectively. The CDR-H3 is shown as orange sticks. (**d**) Alignment of the amino acid residues of CDR-H2 in YES8c and the *IGHV1–69/IGKV3–20*-derived RFs. (**e**) Interactions between CDR-H2 of YES8c and Fc. The Fc surface is shown with the hydrophobicity scale (Eisenberg et al. 1984). (**f**) Interaction footprints in the light chain of YES8c with Fc (left), and the 90° rotated view (right). The contact area of the light chain with Fc is shown in green. The other surface of the light chain is shown in pale yellow. Fc is shown as a magenta cartoon model

decrease in Fc binding, suggesting that highly conserved light-chain residues might contribute important interactions with Fc via hydrophilic residues (Shiroishi et al. 2018).

5.7 Effects of SHMs in RFs

Many identified RFs harbor SHMs; however, some antibodies exhibit RF activity in the absence of SHMs (Charles et al. 2013; Randen et al. 1992b; Carayannopoulos et al. 2000). A naive antibody of RF-TS1, an RF derived from *IGHV1-69*, displays higher affinity for Fc than RF-TS1 (Carayannopoulos et al. 2000), whereas in mixed macroglobulinemia related to HCV, IgM derived from *IGHV1-69* reportedly acquires RF activity through SHMs (Charles et al. 2013). However, the degree to which SHMs affect the acquisition of RF activity and by what mechanism it imparts higher RF activity remain unknown. The structure of RF-AN and RF61 suggested that SHMs might be important for RF activity, but there previously had been no mutants created or analyzed to investigate the effects of SHMs. Therefore, we generated mutants reverting to the germline amino acid residues and analyzed their interactions with Fc (Shiroishi et al. 2018). Three SHMs (L-V28I, L-K39R, and L-S53T) exist in the VL domain, and seven SHMs (H-A33P, H-M48V, H-I53L, H-K62R, H-S76R, H-L82V, H-S82BV, and H-D100EF) exist in the VH domain. Among these, we selected three sites (L28, H33, and H53) close to the Fc-binding site and prepared mutants (L-I28V, H-P33A, and H-L53I) by reverting to the respective germline amino acid. Because the affinity of the H-P33A mutant for Fc was greatly reduced, SHM at this site might be important for acquisition of RF activity. On the other hand, the affinity for Fc was increased in the H-L53I mutant, whereas that in the L-I28V mutant was unchanged. These findings suggest that during the process of B cell proliferation, mutations are introduced that result in a spectrum of contributions to Fc affinity, thereby dictating changes in RF activity. In the case of YES8c, it is considered that higher RF activity is gradually acquired as a result of SHMs.

Poly-reactive RFs with low affinity are found in healthy individuals as naturally occurring IgM. On the other hand, mono-reactive RFs with high affinity are observed in various autoimmune diseases, implying that increased affinity might be related to disease progression. Based on the structure of the YES8c−Fc complex, we designed mutants potentially capable of forming interactions resulting in improved affinity. Our analyses using Fc showed K_d values for H-T56K, H-N58K, L-Q27E, and L-S27AN of 42 μM, 94 μM, 78 μM, and 69 μM, respectively, revealing increased affinities relative to wild type (160 μM). Similar to results observed with the H-L53I mutant, these are amino acid mutations derived from a single-base substitution, suggesting that the antibody derived from *IGHV1-69* can easily increase its affinity via various SHMs to become a disease-aggravation factor.

6 Concluding Remarks

RFs derived from the germline gene combination of *IGHV1-69* have been studied for many years as a paraprotein IgM-RF in MC and WM. Additionally, *IGHV1-69*-derived RFs are found at high levels as stereotype RFs in patients with B cell lymphoma caused by HCV infection (Charles et al. 2008; Bende et al. 2005; De Re et al. 2002). The crystal structure and mutant analyses of YES8c revealed the following possible antigen-recognition mechanisms common to *IGHV1-69*-derived stereotypic RFs: (1) interaction with the Leu432−His435 region of Fc allows orientation of the CDR-H3 within the space formed by the CH2-CH3 elbow, which allows amino acid diversity but limits the number of residues in the CDR-H3; (2) hydrophobic residues in the CDR-H2 typical of *IGHV1-69*-derived antibodies interact with a hydrophobic region in Fc; and (3) paired light chains are highly conserved and form a loose but significant interaction with Fc. Confirmation of these models require clarification of the complex structures of

other *IGHV1-69*-derived RFs in complex with Fc. Furthermore, the structures of other germline-derived RFs will allow the elucidation of the whole picture of autoreactivity of RFs.

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References

Agnello V, Chung RT, Kaplan LM (1992) A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 327(21):1490–1495. https://doi.org/10.1056/NEJM199211193272104

Artandi SE, Canfield SM, Tao MH, Calame KL, Morrison SL, Bonagura VR (1991) Molecular analysis of IgM rheumatoid factor binding to chimeric IgG. J Immunol 146(2):603–610

Artandi SE, Calame KL, Morrison SL, Bonagura VR (1992) Monoclonal IgM rheumatoid factors bind IgG at a discontinuous epitope comprised of amino acid loops from heavy-chain constant-region domains 2 and 3. Proc Natl Acad Sci U S A 89(1):94–98

Bende RJ, Aarts WM, Riedl RG, de Jong D, Pals ST, van Noesel CJ (2005) Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. J Exp Med 201(8):1229–1241. https://doi.org/10.1084/jem.20050068

Bende RJ, Slot LM, Hoogeboom R, Wormhoudt TA, Adeoye AO, Guikema JE, van Noesel CJ (2015) Stereotypic rheumatoid factors that are frequently expressed in mucosa-associated lymphoid tissue-type lymphomas are rare in the labial salivary glands of patients with Sjogren's syndrome. Arthritis Rheumatol 67(4):1074–1083. https://doi.org/10.1002/art.39002

Bende RJ, Janssen J, Wormhoudt TA, Wagner K, Guikema JE, van Noesel CJ (2016) Identification of a novel stereotypic IGHV4-59/IGHJ5-encoded B-cell receptor subset expressed by various B-cell lymphomas with high affinity rheumatoid factor activity. Haematologica 101(5):e200–e203. https://doi.org/10.3324/haematol.2015.139626

Bonagura VR, Artandi SE, Davidson A, Randen I, Agostino N, Thompson K, Natvig JB, Morrison SL (1993) Mapping studies reveal unique epitopes on IgG recognized by rheumatoid arthritis-derived monoclonal rheumatoid factors. J Immunol 151 (7):3840–3852

Borretzen M, Randen I, Natvig JB, Thompson KM (1995) Structural restriction in the heavy chain CDR3 of human rheumatoid factors. J Immunol 155 (7):3630–3637

Carayannopoulos MO, Potter KN, Li Y, Natvig JB, Capra JD (2000) Evidence that human immunoglobulin M rheumatoid factors can Be derived from the natural autoantibody pool and undergo an antigen driven immune response in which somatically mutated rheumatoid factors have lower affinities for immunoglobulin G Fc than their germline counterparts. Scand J Immunol 51(4):327–336

Chan CH, Hadlock KG, Foung SK, Levy S (2001) V(H)1-69 gene is preferentially used by hepatitis C virus-associated B cell lymphomas and by normal B cells responding to the E2 viral antigen. Blood 97 (4):1023–1026

Charles ED, Green RM, Marukian S, Talal AH, Lake-Bakaar GV, Jacobson IM, Rice CM, Dustin LB (2008) Clonal expansion of immunoglobulin M +CD27+ B cells in HCV-associated mixed cryoglobulinemia. Blood 111(3):1344–1356. https://doi.org/10.1182/blood-2007-07-101717

Charles ED, Orloff MI, Nishiuchi E, Marukian S, Rice CM, Dustin LB (2013) Somatic hypermutations confer rheumatoid factor activity in hepatitis C virus-associated mixed cryoglobulinemia. Arthritis Rheum 65(9):2430–2440. https://doi.org/10.1002/art.38041

Corper AL, Sohi MK, Bonagura VR, Steinitz M, Jefferis R, Feinstein A, Beale D, Taussig MJ, Sutton BJ (1997) Structure of human IgM rheumatoid factor Fab bound to its autoantigen IgG Fc reveals a novel topology of antibody-antigen interaction. Nat Struct Biol 4(5):374–381

De Re V, De Vita S, Gasparotto D, Marzotto A, Carbone A, Ferraccioli G, Boiocchi M (2002) Salivary gland B cell lymphoproliferative disorders in Sjogren's syndrome present a restricted use of antigen receptor gene segments similar to those used by hepatitis C virus-associated non-Hodgkins's lymphomas. Eur J Immunol 32(3):903–910. https://doi.org/10.1002/1521-4141(200203)32:3<903::AID-IMMU903>3.0.CO;2-D

Deisenhofer J (1981) Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8-A resolution. Biochemistry 20 (9):2361–2370

DeLano WL, Ultsch MH, de Vos AM, Wells JA (2000) Convergent solutions to binding at a protein-protein interface. Science 287(5456):1279–1283. https://doi.org/10.1126/science.287.5456.1279

Duquerroy S, Stura EA, Bressanelli S, Fabiane SM, Vaney MC, Beale D, Hamon M, Casali P, Rey FA, Sutton BJ, Taussig MJ (2007) Crystal structure of a human autoimmune complex between IgM rheumatoid factor RF61 and IgG1 Fc reveals a novel epitope and evidence for affinity maturation. J Mol Biol 368(5):1321–1331. https://doi.org/10.1016/j.jmb.2007.02.085

Eisenberg D, Schwarz E, Komaromy M, Wall R (1984) Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J Mol Biol 179 (1):125–142

Ekiert DC, Bhabha G, Elsliger MA, Friesen RH, Jongeneelen M, Throsby M, Goudsmit J, Wilson IA (2009) Antibody recognition of a highly conserved influenza virus epitope. Science 324(5924):246–251. https://doi.org/10.1126/science.1171491

Ermel RW, Kenny TP, Chen PP, Robbins DL (1993) Molecular analysis of rheumatoid factors derived from rheumatoid synovium suggests an antigen-driven response in inflamed joints. Arthritis Rheum 36 (3):380–388

Ezaki I, Kanda H, Sakai K, Fukui N, Shingu M, Nobunaga M, Watanabe T (1991) Restricted diversity of the variable region nucleotide sequences of the heavy and light chains of a human rheumatoid factor. Arthritis Rheum 34(3):343–350

Hoogeboom R, Wormhoudt TA, Schipperus MR, Langerak AW, Dunn-Walters DK, Guikema JE, Bende RJ, van Noesel CJ (2013) A novel chronic lymphocytic leukemia subset expressing mutated IGHV3-7-encoded rheumatoid factor B-cell receptors that are functionally proficient. Leukemia 27 (3):738–740. https://doi.org/10.1038/leu.2012.238

Ivanovski M, Silvestri F, Pozzato G, Anand S, Mazzaro C, Burrone OR, Efremov DG (1998) Somatic hypermutation, clonal diversity, and preferential expression of the VH 51p1/VL kv325 immunoglobulin gene combination in hepatitis C virus-associated immunocytomas. Blood 91(7):2433–2442

James LC, Keeble AH, Khan Z, Rhodes DA, Trowsdale J (2007) Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. Proc Natl Acad Sci U S A 104(15):6200–6205. https://doi.org/10.1073/pnas.0609174104

Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M (2013) Hepatitis C virus E2 envelope glycoprotein core structure. Science 342 (6162):1090–1094. https://doi.org/10.1126/science.1243876

Kunkel HG, Agnello V, Joslin FG, Winchester RJ, Capra JD (1973) Cross-idiotypic specificity among monoclonal IgM proteins with anti- -globulin activity. J Exp Med 137(2):331–342. https://doi.org/10.1084/jem.137.2.331

Lingwood D, McTamney PM, Yassine HM, Whittle JR, Guo X, Boyington JC, Wei CJ, Nabel GJ (2012) Structural and genetic basis for development of broadly neutralizing influenza antibodies. Nature 489 (7417):566–570. https://doi.org/10.1038/nature11371

Luftig MA, Mattu M, Di Giovine P, Geleziunas R, Hrin R, Barbato G, Bianchi E, Miller MD, Pessi A, Carfi A (2006) Structural basis for HIV-1 neutralization by a gp41 fusion intermediate-directed antibody. Nat Struct Mol Biol 13(8):740–747. https://doi.org/10.1038/nsmb1127

Mantovani L, Wilder RL, Casali P (1993) Human rheumatoid B-1a (CD5+ B) cells make somatically hypermutated high affinity IgM rheumatoid factors. J Immunol 151(1):473–488

Martin WL, West AP Jr, Gan L, Bjorkman PJ (2001) Crystal structure at 2.8 A of an FcRn/heterodimeric Fc complex: mechanism of pH-dependent binding. Mol Cell 7(4):867–877

Muchtar E, Magen H, Gertz MA (2017) How I treat cryoglobulinemia. Blood 129(3):289–298. https://doi.org/10.1182/blood-2016-09-719773

Pascual V, Randen I, Thompson K, Sioud M, Forre O, Natvig J, Capra JD (1990) The complete nucleotide sequences of the heavy chain variable regions of six monospecific rheumatoid factors derived from Epstein-Barr virus-transformed B cells isolated from the synovial tissue of patients with rheumatoid arthritis. Further evidence that some autoantibodies are unmutated copies of germ line genes. J Clin Invest 86(4):1320–1328. https://doi.org/10.1172/JCI114841

Pascual V, Victor K, Randen I, Thompson K, Steinitz M, Forre O, Fu SM, Natvig JB, Capra JD (1992) Nucleotide sequence analysis of rheumatoid factors and polyreactive antibodies derived from patients with rheumatoid arthritis reveals diverse use of VH and VL gene segments and extensive variability in CDR-3. Scand J Immunol 36(2):349–362

Randen I, Thompson KM, Pascual V, Victor K, Beale D, Coadwell J, Forre O, Capra JD, Natvig JB (1992a) Rheumatoid factor V genes from patients with rheumatoid arthritis are diverse and show evidence of an antigen-driven response. Immunol Rev 128:49–71

Randen I, Brown D, Thompson KM, Hughes-Jones N, Pascual V, Victor K, Capra JD, Forre O, Natvig JB (1992b) Clonally related IgM rheumatoid factors undergo affinity maturation in the rheumatoid synovial tissue. J Immunol 148(10):3296–3301

Sasso EH, Barber CV, Nardella FA, Yount WJ, Mannik M (1988) Antigenic specificities of human monoclonal and polyclonal IgM rheumatoid factors. The C gamma 2-C gamma 3 interface region contains the major determinants. J Immunol 140(9):3098–3107

Sauer-Eriksson AE, Kleywegt GJ, Uhlen M, Jones TA (1995) Crystal structure of the C2 fragment of streptococcal protein G in complex with the Fc domain of human IgG. Structure 3(3):265–278

Shiroishi M, Ito Y, Shimokawa K, Lee JM, Kusakabe T, Ueda T (2018) Structure-function analyses of a stereotypic rheumatoid factor unravel the structural basis for germline-encoded antibody autoreactivity. J Biol Chem 293(18):7008–7016. https://doi.org/10.1074/jbc.M117.814475

Silverman GJ, Goldfien RD, Chen P, Mageed RA, Jefferis R, Goni F, Frangione B, Fong S, Carson DA (1988) Idiotypic and subgroup analysis of human monoclonal rheumatoid factors. Implications for structural and genetic basis of autoantibodies in humans. J Clin Invest 82(2):469–475. https://doi.org/10.1172/JCI113620

Stamatopoulos K, Belessi C, Moreno C, Boudjograh M, Guida G, Smilevska T, Belhoul L, Stella S, Stavroyianni N, Crespo M, Hadzidimitriou A, Sutton L, Bosch F, Laoutaris N, Anagnostopoulos A, Montserrat E, Fassas A, Dighiero G, Caligaris-Cappio-F, Merle-Beral H, Ghia P, Davi F (2007) Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood 109(1):259–270. https://doi.org/10.1182/blood-2006-03-012948

Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, Santelli E, Stec B, Cadwell G, Ali M, Wan H, Murakami A, Yammanuru A, Han T, Cox NJ, Bankston LA, Donis RO, Liddington RC, Marasco WA (2009) Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nat Struct Mol Biol 16(3):265–273. https://doi.org/10.1038/nsmb.1566

Sutton B, Corper A, Bonagura V, Taussig M (2000) The structure and origin of rheumatoid factors. Immunol Today 21(4):177–183

Teplyakov A, Obmolova G, Malia TJ, Luo J, Muzammil S, Sweet R, Almagro JC, Gilliland GL (2016) Structural diversity in a human antibody germline library. MAbs 8(6):1045–1063. https://doi.org/10.1080/19420862.2016.1190060

Thompson KM, Randen I, Borretzen M, Forre O, Natvig JB (1994) Variable region gene usage of human monoclonal rheumatoid factors derived from healthy donors following immunization. Eur J Immunol 24 (8):1771–1778. https://doi.org/10.1002/eji.1830240808

Tzarum N, Giang E, Kong L, He L, Prentoe J, Augestad E, Hua Y, Castillo S, Lauer GM, Bukh J, Zhu J, Wilson IA, Law M (2019) Genetic and structural insights into broad neutralization of hepatitis C virus by human VH1-69 antibodies. Sci Adv 5(1):eaav1882. https://doi.org/10.1126/sciadv.aav1882

van Delft MAM, Huizinga TWJ (2020) An overview of autoantibodies in rheumatoid arthritis. J Autoimmun 102392. https://doi.org/10.1016/j.jaut.2019.102392

Victor KD, Randen I, Thompson K, Forre O, Natvig JB, Fu SM, Capra JD (1991) Rheumatoid factors isolated from patients with autoimmune disorders are derived from germline genes distinct from those encoding the Wa, Po, and Bla cross-reactive idiotypes. J Clin Invest 87(5):1603–1613. https://doi.org/10.1172/JCI115174

Xu R, Krause JC, McBride R, Paulson JC, Crowe JE Jr, Wilson IA (2013) A recurring motif for antibody recognition of the receptor-binding site of influenza hemagglutinin. Nat Struct Mol Biol 20(3):363–370. https://doi.org/10.1038/nsmb.2500

Yeung YA, Foletti D, Deng X, Abdiche Y, Strop P, Glanville J, Pitts S, Lindquist K, Sundar PD, Sirota M, Hasa-Moreno A, Pham A, Melton Witt J, Ni I, Pons J, Shelton D, Rajpal A, Chaparro-Riggers J (2016) Germline-encoded neutralization of a Staphylococcus aureus virulence factor by the human antibody repertoire. Nat Commun 7:13376. https://doi.org/10.1038/ncomms13376

Ying T, Prabakaran P, Du L, Shi W, Feng Y, Wang Y, Wang L, Li W, Jiang S, Dimitrov DS, Zhou T (2015) Junctional and allele-specific residues are critical for MERS-CoV neutralization by an exceptionally potent germline-like antibody. Nat Commun 6:8223. https://doi.org/10.1038/ncomms9223

Youngblood K, Fruchter L, Ding G, Lopez J, Bonagura V, Davidson A (1994) Rheumatoid factors from the peripheral blood of two patients with rheumatoid arthritis are genetically heterogeneous and somatically mutated. J Clin Invest 93(2):852–861. https://doi.org/10.1172/JCI117040

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Regulated Alternative Translocation: A Mechanism Regulating Transmembrane Proteins Through Topological Inversion

Jin Ye

Abstract

Transmembrane proteins must adopt a proper topology to execute their functions. In mammalian cells, a transmembrane protein is believed to adopt a fixed topology. This assumption has been challenged by recent reports that ceramide or related sphingolipids regulate some transmembrane proteins by inverting their topology. Ceramide inverts the topology of certain newly synthesized polytopic transmembrane proteins by altering the direction through which their first transmembrane helices are translocated across membranes. Thus, this regulatory mechanism has been designated as Regulated Alternative Translocation (RAT). The physiological importance of this topological regulation has been demonstrated by the finding that ceramide-induced RAT of TM4SF20 (Transmembrane 4 L6 family member 20) is crucial for the effectiveness of doxorubicin-based chemotherapy, and that dihydroceramide-induced RAT of CCR5 (C-C chemokine receptor type 5), a G protein-coupled receptor, is required for lipopolysaccharide (LPS) to inhibit chemotaxis of macrophages. These observations suggest that topological inversion through RAT could be an emerging mechanism to regulate transmembrane proteins.

Keywords

CCR5 · Ceramide · Protein translocation · TM4SF20 · Transmembrane proteins

Abbreviations

CCR5	C-C chemokine receptor 5
CREB3L1	cAMP responsive element binding protein 3-like 1
ER	endoplasmic reticulum
GPCR	G protein-coupled receptor
LPS	lipopolysaccharide
RAT	regulated alternative translocation
RIP	regulated intramembrane proteolysis
TM4SF20	Transmembrane 4 L6 family member 20
TRAM	Translocating chain-associated membrane protein

1 Introduction

Cellular membranes are physical barriers that are impermeable to many molecules. Thus, unlike cytosolic and nuclear proteins that are surrounded by homogenous environment, transmembrane proteins are all polarized, as segments of

J. Ye (✉)
Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX, USA
e-mail: Jin.ye@utsouthwestern.edu

transmembrane proteins localized at different sides of membranes are exposed to distinct environment. As a result, the topology of transmembrane proteins, which depicts their orientation across membranes, is critical for their functions.

The topology of transmembrane proteins is primarily determined during their synthesis on endoplasmic reticulum (ER) membranes (Zimmermann et al. 2011). For transmembrane proteins that contain a single transmembrane helix, the translocation process has been categorized into three classes (Fig. 1) (Lodish et al. 2007). Type I insertion refers to proteins that contain a cleavable ER-targeting signal peptide N-terminal to the first transmembrane helix. The signal recognition particle binds to the hydrophobic sequence within a nascent signal peptide, directing the ribosome/nascent polypeptide complex to the ER membranes. The signal peptide is then inserted into membranes adjacent to the Sec61 ER translocon through a direction where its N-terminus faces cytosol. This insertion opens the lateral gate of the translocon, enabling hydrophilic sequence C-terminal to the signal peptide to be transported into ER lumen (Rapoport et al. 2017). Following the translocation, the signal peptide is removed from the mature protein by the signal peptidase, causing the N-terminus of the mature protein to be embedded into ER lumen (Fig. 1a). The other two insertions do not use a signal peptide. Instead, their insertions are initiated by recognition of the hydrophobic sequence present in the transmembrane helix of nascent peptides by the signal recognition particle, which directs the nascent peptide/ribosome complex to the Sec61 ER translocon. In Type II insertion, the direction through which the transmembrane helix is inserted into membranes is the same as that of the signal peptide in Type I insertion, allowing sequence C-terminal to the transmembrane helix to be imported into ER lumen (Rapoport et al. 2017). As a result, the N-terminus of the Type II transmembrane proteins is located in cytosol (Fig. 1b). In Type III insertion, the transmembrane helix is inserted through an opposite

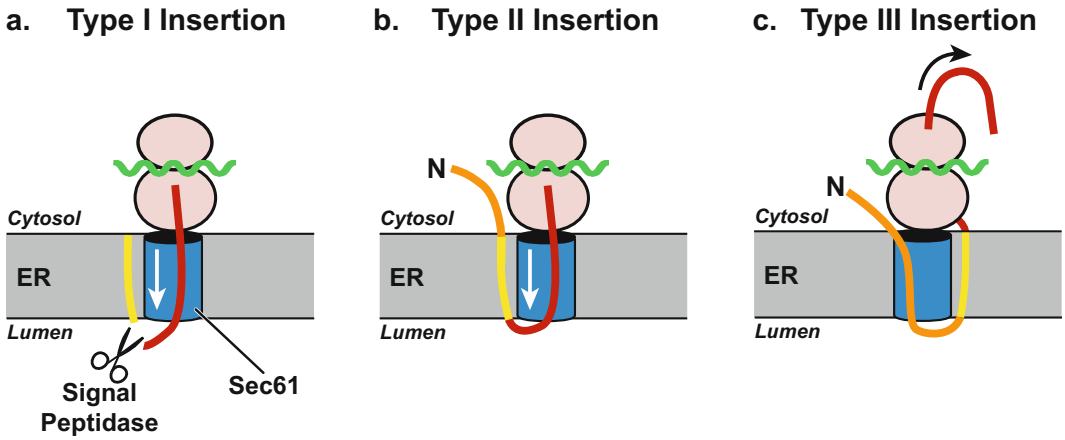

Fig. 1 Three classes of membrane translocation process
(**a**) During Type I insertion, the signal peptide is inserted into membranes with its N-terminus facing cytosol before it is removed from the mature protein by the signal peptidase. This insertion allows nascent peptides C-terminal to the signal peptide to be pushed through the Sec61 translocon by ribosomes. (**b**) Type II insertion is similar to the Type I insertion except that the translocation process is initiated by the transmembrane helix that cannot be cleaved by the signal peptidase. (**c**) During Type III insertion, the transmembrane helix is inserted into membranes with the N-terminus embedded into the ER lumen. This insertion enables peptides N-terminal to the transmembrane helix to be pulled through the translocation channel. (**a**–**c**) The sequence N-terminal to the transmembrane helix, the signal peptide (**a**) or the transmembrane helix (**b**, **c**), and the sequence C-terminal to the transmembrane helix are highlighted in orange, yellow, and red, respectively. The arrow indicates the direction of nascent peptide elongation during translation

direction so that its N-terminus faces the ER lumen. This insertion enables hydrophilic sequence N-terminal to the transmembrane helix to be transported into the ER lumen (Rapoport et al. 2017). Following this type of insertion, the N-terminus of the proteins is located in the ER lumen (Fig. 1c).

For polytopic transmembrane proteins, their topology is determined by the direction through which the first transmembrane helix is inserted into membranes, which follows one of the three types of the insertion illustrated above (Rapoport et al. 2017). In bacteria, the transmembrane helices in polytopic membrane proteins can be reoriented co-translationally or post-translationally upon alterations in phospholipid contents in membranes (Bogdanov and Dowhan 1998; Bogdanov et al. 2002, 2014; Dowhan et al. 2019). However, this reposition of transmembrane helices has not been extensively reported for mammalian transmembrane proteins except for a few proteins such as aquaporin 1 and CD38 (Bogdanov et al. 2018; Lee and Zhao 2019; Lu et al. 2000). Recently, there are several reports demonstrating that topology of some mammalian transmembrane proteins can be reversed under certain physiological conditions, and this topological inversion is critical to regulate functions of these transmembrane proteins.

2 Topological Inversion of TM4SF20: A Critical Step in Doxorubicin-Based Chemotherapy

Doxorubicin, a chemotherapeutic reagent extensively used to treat various cancers, is believed to inhibit cancer cell proliferation through DNA damage by inhibiting DNA topoisomerase II (Gewirtz 1999; Yang et al. 2014). However, recent studies suggest that activation of a transcription factor called cAMP responsive element binding protein 3-like 1 (CREB3L1) is also critical for the cytostatic activity of doxorubicin (Denard et al. 2012; Patel and Kaufmann 2012). Unlike typical transcription factors, CREB3L1 is synthesized as a transmembrane precursor that contains a cytosolic N-terminal domain capable of functioning as a transcription factor (Denard et al. 2011; Murakami et al. 2006) (Fig. 2). In resting cells, CREB3L1 remains as the inactive transmembrane precursor. Doxorubicin enhances production of ceramide, which in turn triggers proteolytic activation of CREB3L1 through a pathway called Regulated Intramembrane Proteolysis (RIP) (Brown et al. 2000; Ye 2013). This pathway activates CREB3L1 through two proteolytic events: The first cleavage in the luminal domain catalyzed by Site-1 protease followed by

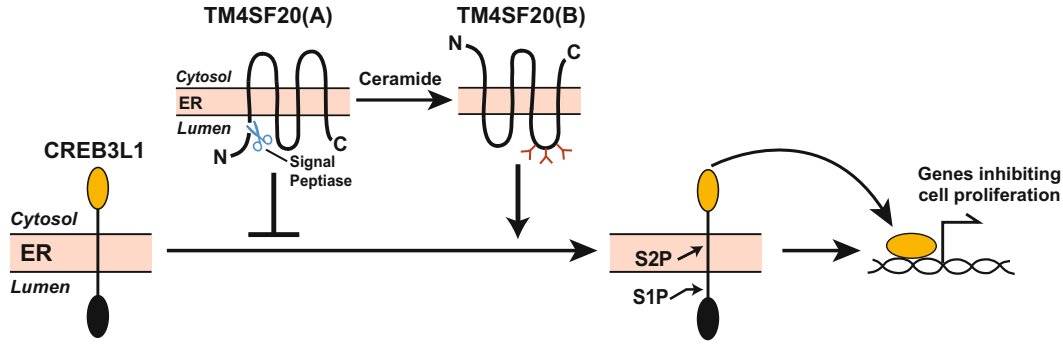

Fig. 2 RIP of CREB3L1 activated by RAT of TM4SF20
In the absence of ceramide, TM4SF20 adopts a topology (TM4SF20(A)) that inhibits proteolytic activation CREB3L1. Ceramide inverts the topology of newly synthesized TM4SF20 through RAT. The protein with the inverted topology (TM4SF20(B)) activates RIP of CREB3L1 catalyzed by Site-1 protease (S1P) and Site-2 protease (S2P). These cleavages release the N-terminal domain of CREB3L1 from membranes, allowing it to activate genes inhibiting cell proliferation

the second cleavage at the interface between the transmembrane helix and the cytosolic domain (Fig. 2). The final cleavage catalyzed by Site-2-protease releases the N-terminal domain of CREB3L1 from membranes, allowing it to activate transcription of genes such as p21 that inhibit cell proliferation (Cui et al. 2016; Denard et al. 2011, 2012) (Fig. 2). The importance of this CREB3L1-mediated pathway was illustrated by observations that at clinically relevant doses, doxorubicin was much more effective in cancer cells that expressed high levels of *CREB3L1* than in those expressing low levels of the gene. This correlation was documented in cancer cells cultured in vitro, in xenograft tumors established in mice, and in human tumor samples (Denard et al. 2012, 2015, 2018; Xiao et al. 2019). Thus, ceramide-induced RIP of CREB3L1 plays a critical role in doxorubicin-based cancer chemotherapy.

Later studies identified Transmembrane 4 L6 family member 20 (TM4SF20) as a polytopic transmembrane protein crucial for ceramide to induce RIP of CREB3L1. In the absence of ceramide, the N-terminus of TM4SF20 is inserted into the ER lumen, and the sequence N-terminal to the first transmembrane helix is removed co-translationally by the signal peptidase (Fig. 2). Under this configuration, the loop between the third and fourth transmembrane helix that contains three potential sites for N-linked glycosylation is located in the cytosol so that glycosylation cannot occur, and the C-terminus of the protein is located within the lumen as demonstrated by the protease protection assay (Fig. 2) (Chen et al. 2016). This form of the protein, which is designated as TM4SF20(A), inhibits RIP of CREB3L1 (Fig. 2) (Chen et al. 2014). Treatments of cells with exogenous ceramide or bacterial sphingomyelinase and doxorubicin that stimulates endogenous production of ceramide inverted the topology of TM4SF20 (Chen et al. 2016). In these cells, the N and C-termini of newly synthesized TM4SF20 are all located in the cytosol as demonstrated by the protease protection assay, and the loop containing the potential glycosylation sites is located in the ER lumen where it is glycosylated (Fig. 2) (Chen et al. 2016). This form of the protein, which is designated as TM4SF20(B), activates RIP of CREB3L1 (Chen et al. 2016). Thus, the ceramide-induced topological inversion turns TM4SF20 from an inhibitor to an activator for proteolytic activation of CREB3L1 (Fig. 2). Importantly, ceramide does not flip the topology of pre-existing TM4SF20 but inverts the topology of the newly synthesized protein, as treatment of cycloheximide, an inhibitor of protein synthesis, blocked ceramide-induced production of TM4SF20(B) (Chen et al. 2016). Therefore, ceramide appears to invert the topology of TM4SF20 by altering the direction through which it is translocated across membranes during its synthesis. This regulatory mechanism was thus designated as Regulated Alternative Translocation (RAT).

Since the N-terminal sequence of TM4SF20 (A) produced in the absence of ceramide is cleaved off from the mature protein by the signal peptidase, it is tempting to conclude that the first transmembrane helix of TM4SF20(A) is translocated through the Type I insertion, the mechanism responsible for insertion of proteins with an N-terminal signal peptide. However, several lines of evidence argue against this conclusion: First, the N-terminal sequence of TM4SF20 does not contain any hydrophobic residues characteristic for a signal peptide (Petersen et al. 2011); Second, the N-terminal sequence of TM4SF20 did not function as a signal peptide when it was fused to another protein (Chen et al. 2016); Third, the N-terminal sequence of TM4SF20 could be replaced by other peptides without altering the topology of TM4SF20 (A) and ceramide-induced RAT of TM4SF20 (Chen et al. 2016). Thus, even though the N-terminal sequence of TM4SF20(A) is cleaved off from the mature protein by signal peptidase, there is no evidence to suggest that the sequence actually functions as a signal peptide. It is more likely that this cleavage is caused by the accessibility of the peptide to signal peptidase in the ER lumen. This type of proteolysis by signal peptidase has been reported for processing of hepatitis C virus polyprotein in which the protease cleaves the protein at multiple sites in the ER lumen distal to the N-terminal sequence (Hijikata et al. 1991).

Since TM4SF20(A) does not contain a functional signal peptide, the first transmembrane helix of the protein is translocated across ER membranes through the Type III insertion. In contrast, the first transmembrane helix of TM4SF20(B) produced in the presence of ceramide is translocated through the Type II insertion. Thus, ceramide-induced RAT of TM4SF20 shifts the translocation of the first transmembrane helix of TM4SF20 from the Type III to Type II insertion. Since both of these insertions are initiated by interaction of the first transmembrane helix with the ER translocon, the first transmembrane helix of TM4SF20 may be critical for the topological regulation through RAT. Indeed, replacing the signal peptide of alkaline phosphatase with the N-terminal domain of TM4SF20 that contains the first transmembrane helix of the protein led to ceramide-induced RAT of the fusion protein (Chen et al. 2016). Further analysis revealed that a GXXXN motif present in the first transmembrane helix of TM4SF20 is critical for RAT of the protein, as mutating the Gly or Asn residue in the motif to Leu locked TM4SF20 in the reversed orientation (TM4SF20(B)) regardless of the presence of ceramide (Chen et al. 2016; Wang et al. 2019). These results suggest that in the absence of ceramide, the GXXXN motif present in the nascent peptide may interact with a component of the translocation machinery, allowing the first transmembrane helix to be translocated through the Type III insertion to produce TM4SF20(A).

Translocating chain-associated membrane protein 2 (TRAM2) may be such a translocation component involved in RAT of TM4SF20. TRAM2, a poorly characterized protein, is highly homologous to TRAM1, an accessory protein for the Sec61 translocation channel (Do et al. 1996; Görlich et al. 1992; Voigt et al. 1996). Knockdown of TRAM2 but not TRAM1 through RNAi facilitated production of TM4SF20(B) even in the absence of ceramide (Chen et al. 2016). This observation suggests that in the absence of ceramide, TRAM2 may interact with the GXXXN motif present in the first transmembrane helix of nascent TM4SF20 to enable the type III insertion of the transmembrane helix. Interestingly, TRAM2, and all TRAM protein, contains a TLC domain that is postulated to bind ceramide or related sphingolipids (Winter and Ponting 2002). It will be interesting to determine whether ceramide regulates translocation of TM4SF20 through its interaction with TRAM2.

3 Topological Inversion of CCR5: A Critical Step for LPS to Inhibit Chemotaxis

A bioinformatics analysis revealed that ~100 proteins contain in their first transmembrane helix a GXXXN motif that is critical for RAT of TM4SF20. Remarkably, more than ~90% of these proteins are G protein-coupled receptors (GPCRs) (Denard et al. 2019). Interestingly, the first transmembrane helix of the majority of GPCRs is inserted into membranes through the Type III orientation (Guan et al. 1992; Von Heijne 2006). Since RAT alters the translocation of the first transmembrane helix of TM4SF20 from the Type III to Type II insertion, these observations raise the possibility that these GPCRs may also subject to ceramide-induced topological inversion through RAT.

One of these GPCRs, namely C-C chemokine receptor 5 (CCR5), indeed subjects to ceramide-induced topological inversion. In unstimulated macrophages, CCR5 adopts a configuration consistent with that of GPCRs. The N- and C-terminus of the protein with this topology, which was designated as CCR5(A), is localized at extracellular space and cytosol, respectively (Fig. 3). Under this configuration, CCR5 functions as a chemokine receptor, directing macrophages migrating toward its ligand, CCL5 (Denard et al. 2019; Duma et al. 2007; Oppermann 2004) (Fig. 3). Upon stimulation by lipopolysaccharide (LPS), macrophages markedly increased production of dihydroceramide, which in turn inverted the topology of newly synthesized CCR5. While still reaching the cell surface, the N- and C-terminus of CCR5 with the inversed topology, which was designated as CCR5(B), is localized at cytosol and extracellular space, respectively (Denard et al. 2019) (Fig. 3).

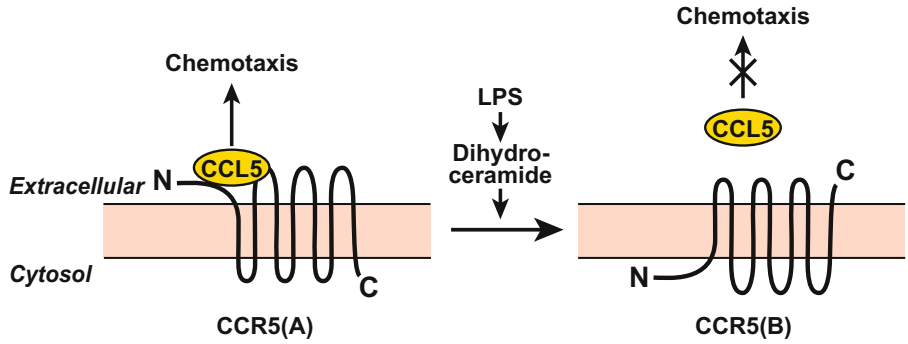

Fig. 3 LPS inhibits chemotaxis through RAT of CCR5 In the absence of LPS, CCR5 adopts a topology consistent with that of GPCR (CCR5(A)) to guide macrophages migrating towards the gradient of its ligand, CCL5. LPS induces production of dihydroceramide, which in turn triggers topological inversion of CCR5 through RAT. CCR5 with the inverted topology (CCR5(B)) no longer binds CCL5, leading to inhibition of the chemotaxis reaction

CCR5(B) no longer functioned as a chemokine receptor, leading to inhibition of chemotaxis (Denard et al. 2019) (Fig. 3). The dihydroceramide-induced topological inversion of CCR5 was critical for LPS to prevent macrophages from migrating toward CCL5, as treatments inhibiting production of dihydroceramide relieved this inhibition of chemotaxis (Denard et al. 2019). These findings may explain the well-known observation that LPS-activated macrophages are insensitive to chemotaxis (Biswas and Lopez-Collazo 2009).

4 Concluding Remarks

The concept of translocation regulation has been proposed more than a decade ago (Hegde and Kang 2008). Ceramide-induced RAT of TM4SF20 and CCR5 discovered recently are the first two examples that this regulation indeed takes place in mammalian cells. Both TM4SF20 and CCR5 are polytopic transmembrane proteins, and their first transmembrane helix is translocated across membranes through the Type III insertion in the absence of excess ceramide. In contrast to the well-studied Type I and II insertions, the Type III insertion is much less characterized (Rapoport et al. 2017). Unlike the Type I and II insertions during which the newly synthesized peptide C-terminal to the transmembrane helix is pushed through the translocation channel by ribosomes, sequence N-terminal to the transmembrane helix, which may have already been folded before initiation of the membrane translocation process, has to be pulled through the translocation channel during the Type III insertion. The driving force and unfolding mechanism that allows this sequence to be translocated through the Sec61 translocon remains obscure. Understanding the mechanism behind the Type III insertion may provide mechanistic insights into RAT of transmembrane proteins.

Perhaps the most important question raised by the discovery of RAT is the breadth of this regulatory mechanism. How many transmembrane proteins are regulated by RAT? Can RAT be triggered by other stimulations in addition to ceramide? The major obstacle to answer these questions is our limited knowledge on topology of transmembrane proteins in mammalian cells. According to UniProt, only less than 10% of transmembrane proteins expressed in mammalian cells have their topology experimentally defined. The topology of the rest of the transmembrane proteins is either unknown or predicted by sequence analysis, which may be plagued by errors. This problem is difficult to address by the currently available techniques, as they can only measure topology of one protein at a time, and often require overexpression of the protein tagged with an epitope, which may alter the topology of

the transmembrane protein. Thus, a proteome-wide approach capable of measuring topology of endogenous untagged transmembrane proteins expressed in mammalian cells globally is needed to systematically identify transmembrane proteins subjected to topological regulation.

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Conflicts of Interest None.

References

Biswas SK, Lopez-Collazo E (2009) Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol 30:475–487

Bogdanov M, Dowhan W (1998) Phospholipid-assisted protein folding: phosphatidylethanolamine is required at a late step of the conformational maturation of the polytopic membrane protein lactose permease. EMBO J 17:5255–5264

Bogdanov M, Dowhan W, Vitrac H (2014) Lipids and topological rules governing membrane protein assembly. Biochem Biophys Acta 1843:1475–1488

Bogdanov M, Heacock PN, Dowhan W (2002) A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition. EMBO J 21:2107–2116

Bogdanov M, Vitrac H, Dowhan W (2018) Flip-fopping membrane proteins: how the charge balance rule governs dynamic membrane protein topology. In: Geiger O (ed) Biogenesis of fatty acids, lipids and membranes. Cham, Springer International Publishing, pp 1–28

Brown M, Ye J, Rawson R, Goldstein J (2000) Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell 100:391–398

Chen Q, Denard B, Lee C-E, Han S, Ye JS, Ye J (2016) Inverting the topology of a transmembrane protein by regulating the translocation of the first transmembrane helix. Mol Cell 63:567–578

Chen Q, Lee C-E, Denard B, Ye J (2014) Sustained induction of collagen synthesis by TGF-β requires regulated intramembrane proteolysis of CREB3L1. PLoS One 9:e108528

Cui X, Cui M, Asada R, Kanemoto S, Saito A, Matsuhisa K, Kaneko M, Imaizumi K (2016) The androgen-induced protein AIbZIP facilitates proliferation of prostate cancer cells through downregulation of p21 expression. Sci Rep 6:37310

Denard B, Han S, Kim J, Ross EM, Ye J (2019) Regulating G protein-coupled receptors by topological inversion. elife 8:e40234

Denard B, Jiang S, Peng Y, Ye J (2018) CREB3L1 as a potential biomarker predicting response of triple negative breast cancer to doxorubicin-based chemotherapy. BMC Cancer 18:813

Denard B, Lee C, Ye J (2012) Doxorubicin blocks proliferation of cancer cells through proteolytic activation of CREB3L1. eLife 1. https://doi.org/10.7554/eLife.00090

Denard B, Pavia-Jimenez A, Chen W, Williams NS, Naina H, Collins R, Brugarolas J, Ye J (2015) Identification of CREB3L1 as a biomarker predicting doxorubicin treatment outcome. PLoS One 10:e0129233

Denard B, Seemann J, Chen Q, Gay A, Huang H, Chen Y, Ye J (2011) The membrane-bound transcription factor CREB3L1 is activated in response to virus infection to inhibit proliferation of virus-infected cells. Cell Host Microbe 10:65–74

Do H, Falcone D, Lin J, Andrews DW, Johnson AE (1996) The cotranslational integration of membrane proteins into the phospholipid bilayer is a multistep process. Cell 85:369–378

Dowhan W, Vitrac H, Bogdanov M (2019) Lipid-assisted membrane protein folding and topogenesis. Protein J 38:274–288

Duma L, Häussinger D, Rogowski M, Lusso P, Grzesiek S (2007) Recognition of RANTES by extracellular parts of the CCR5 receptor. J Mol Biol 365:1063–1075

Gewirtz DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem Pharmacol 57:727–741

Görlich D, Hartmann E, Prehn S, Rapoport TA (1992) A protein of the endoplasmic reticulum involved early in polypeptide translocation. Nature 357:47–52

Guan XM, Kobilka TS, Kobilka BK (1992) Enhancement of membrane insertion and function in a type IIIb membrane protein following introduction of a cleavable signal peptide. J Biol Chem 267:21995–21998

Hegde RS, Kang S-W (2008) The concept of translocational regulation. J Cell Biol 182:225–232

Hijikata M, Kato N, Ootsuyama Y, Nakagawa M, Shimotohno K (1991) Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. Proc Natl Acad Sci U S A 88:5547–5551

Lee HC, Zhao YJ (2019) Resolving the topological enigma in Ca(2+) signaling by cyclic ADP-ribose and NAADP. J Biol Chem 294:19831–19843

Lodish H, Berk A, Kaiser C, Krieger M, Scott M, Bretscher A, Ploegh H, Matsudaira P (2007) Molecular cell biology, 6th edn. W. H. Freeman, New York

Lu Y, Turnbull IR, Bragin A, Carveth K, Verkman AS, Skach WR (2000) Reorientation of aquaporin-1 topology during maturation in the endoplasmic reticulum. Mol Biol Cell 11:2973–2985

Murakami T, Kondo S, Ogata M, Kanemoto S, Saito A, Wanaka A, Imaizumi K (2006) Cleavage of the membrane-bound transcription factor OASIS in response to endoplasmic reticulum stress. J Neurochem 96:1090–1100

Oppermann M (2004) Chemokine receptor CCR5: insights into structure, function, and regulation. Cell Signal 16:1201–1210

Patel AG, Kaufmann SH (2012) How does doxorubicin work? eLife 1. https://doi.org/10.7554/eLife.00387

Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8:785–786

Rapoport TA, Li L, Park E (2017) Structural and mechanistic insights into protein translocation. Annu Rev Cell Dev Biol 33:369–390

Voigt S, Jungnickel B, Hartmann E, Rapoport TA (1996) Signal sequence-dependent function of the TRAM protein during early phases of protein transport across the endoplasmic reticulum membrane. J Cell Biol 134:25–35

Von Heijne G (2006) Membrane-protein topology. Nat Rev Mol Cell Biol 7:909–918

Wang J, Kinch LN, Denard B, Lee C-E, Esmaeilzadeh Gharehdaghi E, Grishin N, Ye J (2019) Identification of residues critical for topology inversion of the transmembrane protein TM4SF20 through regulated alternative translocation. J Biol Chem 294:6054–6061

Winter E, Ponting CP (2002) TRAM, LAG1 and CLN8: members of a novel family of lipid-sensing domains? Trends Biochem Sci 27:381–383

Xiao W, Liang Y, Que Y, Li J, Peng R, Xu B, Wen X, Zhao J, Guan Y, Zhang X (2019) Comparison of the MAID (AI) and CAV/IE regimens with the predictive value of cyclic AMP-responsive element-binding protein 3 like protein 1 (CREB3L1) in palliative chemotherapy for advanced soft-tissue sarcoma patients. J Cancer 10:3517–3525

Yang F, Teves SS, Kemp CJ, Henikoff S (2014) Doxorubicin, DNA torsion, and chromatin dynamics. Biochim Biophys Acta 1845:84–89

Ye J (2013) Regulated intramembrane proteolysis. In: Lennarz W, Lane D (eds) Encyclopedia of biological chemistry. Waltham, Academic, pp 50–55

Zimmermann R, Eyrisch S, Ahmad M, Helms V (2011) Protein translocation across the ER membrane. Biochim Biophys Acta 1808:912–924

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Interleukin-36: Structure, Signaling and Function

Li Zhou and Viktor Todorovic

Abstract

The IL-36 family belongs to a larger IL-1 superfamily and consists of three agonists (IL-36α/β/γ), one antagonist (IL-36Ra), one cognate receptor (IL-36R) and one accessory protein (IL-1RAcP). The receptor activation follows a two-step mechanism in that the agonist first binds to IL-36R and the resulting binary complex recruits IL-1RAcP. Assembled ternary complex brings together intracellular TIR domains of receptors which activate downstream NF-κB and MAPK signaling. Antagonist IL-36Ra inhibits the signaling by binding to IL-36R and preventing recruitment of IL-1RAcP. Members of IL-36 are normally expressed at low levels. Upon stimulation, they are inducted and act on a variety of cells including epithelial and immune cells. Protease mediated N-terminal processing is needed for cytokine activation. In the skin, the functional role of IL-36 is to contribute to host defense through inflammatory response. However, when dysregulated, IL-36 stimulates keratinocyte and immune cells to enhance the Th17/Th23 axis and induces psoriatic-like skin disorder. Genetic mutations of the antagonist IL-36Ra are associated with occurrence of generalized pustular psoriasis, a rare but life-threatening skin disease. Anti-IL-36 antibodies attenuate IMQ or IL-23 induced skin inflammation in mice, illustrating IL-36's involvement in mouse model of psoriasis. Other organs such as the lungs, the intestine, the joints and the brain also express IL-36 family members upon stimulation. The physiological and pathological roles of IL-36 are less well defined in these organs than in the skin. In this chapter, current progress on IL-36 protein and biology is reviewed with a discussion on investigative tools for this novel target.

Keywords

Generalized pustular psoriasis · IL-1 superfamily · IL-36 · Inflammation · Psoriasis · Therapeutics

1 Introduction

Interleukin-36 (IL-36) cytokines were first described by multiple groups as homologs of Interleukin-1 (IL-1) cytokines around 2000 (Mulero et al. 1999; Smith et al. 2000; Kumar et al. 2000; Busfield et al. 2000; Barton et al. 2000; Debets et al. 2001), when the analysis of DNA database was proceeding at a rapid pace. Based on their similarity in conserved sequences

Authors Li Zhou and Viktor Todorovic have been equally contributed for this chapter.

L. Zhou (✉)
AbbVie Bioresearch Center, Worcester, MA, USA
e-mail: li.zhou@abbvie.com

V. Todorovic
AbbVie Inc., North Chicago, IL, USA

and the predicted structures, IL-36 cytokines were predicted to hold similar functions as the IL-1 cytokines (Smith et al. 2000); for example, IL-1 has been shown to modulate innate immune responses and inflammation. Two decades later, accumulated knowledge of expression, activation, structure and function on IL-36 is helping us better define its biological roles. Cytokines from IL-36 family are involved in innate and inflammatory response in multiple organs, especially in the skin. In this chapter, current progress on the structure and function of IL-36 is reviewed with a discussion on the therapeutic implications.

2 IL-36 Cytokines Are Members of the IL-1 Superfamily

There are 11 cytokines in the IL-1 superfamily, which are grouped into four families (IL-1, IL-18, IL-33 and IL-36) based on their conserved sequence and recognition of cognate receptors. The sequence phylogenetic tree shows that the three human IL-36 agonists (IL-36α, IL-36β and IL-36γ) are grouped together due to their sequence similarity (Fig. 1) (Sievers et al. 2011). IL-36α and IL-36γ are even more similar in sequence with each other than with IL-36β, suggesting the former two share more related functions than with IL-36β. The two IL-1 agonists (IL-1α and IL-β) are grouped together, consistent with their family origin and their similar functions. Interestingly, the three antagonists from different families (IL-1Ra, IL-36Ra and IL-38) share more sequence similarity among each other than with the agonists from their own families, which suggests a conserved sequence and structural requirement exists for cytokine antagonistic activity. The two remaining agonists IL-18 and IL-33 are in different groups since they are from different families. It is interesting to note that IL-37, which is an antagonist of IL-18 family, is separated from the other antagonists in the IL-1 superfamily, suggesting IL-37 may rely on a different binding mode for antagonistic activity.

The genomic location of IL-36 cytokines are mapped to human chromosome 2q14.1. The other cytokines of the IL-1 superfamily, except for IL-18 and IL-33 which are on chromosomes 11q23.1–23.2 and 9p23–24 respectively, are clustered at the same locus as IL-36 (Fig. 1) (Nicklin et al. 2002; Taylor et al. 2002). Because of the conserved sequence and the proximity in genomic location, genes of IL-36 cytokines and the other cytokines in the IL-1 superfamily are probably duplicated from a common ancestor gene during evolution (Rivers-Auty et al. 2018).

The naming of members in the IL-1 superfamily have been through several rounds of change for reconciling confusion. Multiple names have been proposed for the same molecules due to simultaneous discovery by different groups. For ease of reading, uniform naming system is adopted throughout the chapter instead of taking the names from literature verbatim (Tables 1 and 2).

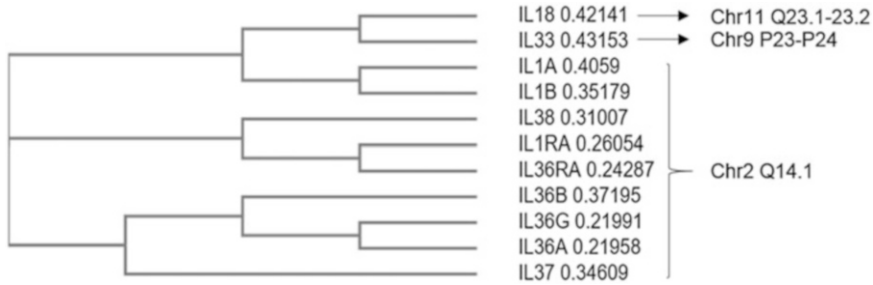

Fig. 1 Sequence phylogenetic tree shows the agonists are grouped according to the family origin while the antagonists from different families are grouped together. IL-36 agonists (IL-36α/β/γ), IL-1 agonists (IL- 1 α/β) and antagonists (IL-1Ra/IL-36Ra/IL-38) are grouped into separate bins. IL-18, IL-33 and IL-37 are in their own bins. Except for IL-18 and IL-33, all other cytokines in the IL-1 superfamily are located at Chr2 Q14.1 region on human chromosome. The dendrogram was generated by using Clustal Omega from EMBL-EBI

Table 1 Cytokines in the IL-1 superfamily

Entry	UniProt	Gene names	Protein names	Length
IL-1α	P01583	IL1A IL1F1	Interleukin-1 alpha (IL-1 alpha) (Hematopoietin-1)	271
IL-1β	P01584	IL1B IL1F2	Interleukin-1 beta (IL-1 beta) (Catabolin)	269
IL-1Ra	P18510	IL1RN IL1F3 IL1RA	Interleukin-1 receptor antagonist protein (IL-1RN) (IL-1ra) (IRAP) (ICIL-1RA) (IL1 inhibitor) (Anakinra)	177
IL-36α	Q9UHA7	IL36A FIL1E IL1E IL1F6	Interleukin-36 alpha (FIL1 epsilon) (Interleukin-1 epsilon) (IL-1 epsilon) (Interleukin-1 family member 6) (IL-1F6)	158
IL-36β	Q9NZH7	IL36B IL1F8 IL1H2	Interleukin-36 beta (FIL1 eta) (Interleukin-1 eta) (IL-1 eta) (Interleukin-1 family member 8) (IL-1F8) (Interleukin-1 homolog 2) (IL-1H2)	164
IL-36γ	Q9NZH8	IL36G IL1E IL1F9 IL1H1 IL1RP2 UNQ2456/PRO5737	Interleukin-36 gamma (IL-1-related protein 2) (IL-1RP2) (Interleukin-1 epsilon) (IL-1 epsilon) (Interleukin-1 family member 9) (IL-1F9) (Interleukin-1 homolog 1) (IL-1H1)	169
IL-36Ra	Q9UBH0	IL36RN FIL1D IL1F5 IL1HY1 IL1L1 IL1RP3 UNQ1896/PRO4342	Interleukin-36 receptor antagonist protein (IL-36Ra) (FIL1 delta) (IL-1-related protein 3) (IL-1RP3) (Interleukin-1 HY1) (IL-1HY1) (Interleukin-1 delta) (IL-1 delta) (Interleukin-1 family member 5) (IL-1F5) (Interleukin-1 receptor antagonist homolog 1) (IL-1ra homolog 1) (Interleukin-1-like protein 1) (IL-1 L1)	155
IL-38	Q8WWZ1	IL1F10 FIL1T IL1HY2 IL38 FKSG75 UNQ6119/PRO20041	Interleukin-1 family member 10 (IL-1F10) (Family of interleukin 1-theta) (FIL1 theta) (Interleukin-1 HY2) (IL-1HY2) (Interleukin-1 theta) (IL-1 theta) (Interleukin-38) (IL-38)	152
IL-18	Q14116	IL18 IGIF IL1F4	Interleukin-18 (IL-18) (Iboctadekin) (Interferon gamma-inducing factor) (IFN-gamma-inducing factor) (Interleukin-1 gamma) (IL-1 gamma)	193
IL-33	O95760	IL33 C9orf26 IL1F11 NFHEV	Interleukin-33 (IL-33) (Interleukin-1 family member 11) (IL-1F11) (Nuclear factor from high endothelial venules) (NF-HEV) [Cleaved into: Interleukin-33 (95–270); Interleukin-33 (99–270); Interleukin-33 (109–270)]	270
IL-37	Q9NZH6	IL37 FIL1Z IL1F7 IL1H4 IL1RP1	Interleukin-37 (FIL1 zeta) (IL-1X) (Interleukin-1 family member 7) (IL-1F7) (Interleukin-1 homolog 4) (IL-1H) (IL-1H4) (Interleukin-1 zeta) (IL-1 zeta) (Interleukin-1-related protein) (IL-1RP1) (Interleukin-23) (IL-37)	218

2.1 The Secretion Mechanism of IL-36 Cytokines Is Not Completely Understood

Except for IL-1Ra, the other IL-1 superfamily cytokines including those in the IL-36 family do not encode a signal peptide, which suggests a canonical endoplasmic reticulum and Golgi dependent secretion mechanism is unlikely for them. IL-36 cytokines are unlikely to be secreted by a caspase-1 dependent mechanism which is adopted by IL-1β and IL-18. In the inflammasome, IL-1β and IL-18 are processed by caspase-1 (Martinon et al. 2002; Ghayur et al. 1997) and subsequently released through membrane pores induced by gasdermin D (He et al. 2015; Shi et al. 2015; Kayagaki et al. 2015). Although there are several reports showing that the secretion of IL-36α requires caspase 3/7 in alveolar epithelial cells (Aoyagi

Table 2 Receptors and accessory proteins in the IL-1 superfamily

Entry	UniProt	Gene names	Protein names	Length	Ligand
IL-1R-1	P14778	IL1R1 IL1R IL1RA IL1RT1	Interleukin-1 receptor type 1 (IL-1R-1) (IL-1RT-1) (IL-1RT1) (CD121 antigen-like family member A) (Interleukin-1 receptor alpha) (IL-1R-alpha) (Interleukin-1 receptor type I) (p80) (CD antigen CD121a) [Cleaved into: Interleukin-1 receptor type 1, membrane form (mIL-1R1) (mIL-1RI); Interleukin-1 receptor type 1, soluble form (sIL-1R1) (sIL-1RI)]	569	IL-1
IL-1R-2	P27930	IL1R2 IL1RB	Interleukin-1 receptor type 2 (IL-1R-2) (IL-1RT-2) (IL-1RT2) (CD121 antigen-like family member B) (CDw121b) (IL-1 type II receptor) (Interleukin-1 receptor beta) (IL-1R-beta) (Interleukin-1 receptor type II) (CD antigen CD121b) [Cleaved into: Interleukin-1 receptor type 2, membrane form (mIL-1R2) (mIL-1RII); Interleukin-1 receptor type 2, soluble form (sIL-1R2) (sIL-1RII)]	398	IL-1
IL-36R	Q9HB29	IL1RL2 IL1RRP2	Interleukin-1 receptor-like 2 (IL-36 receptor) (IL-36R) (Interleukin-1 receptor-related protein 2) (IL-1Rrp2) (IL1R-rp2)	575	IL-36
IL-18R-1	Q13478	IL18R1 IL1RRP	Interleukin-18 receptor 1 (IL-18R-1) (IL-18R1) (CD218 antigen-like family member A) (CDw218a) (IL1 receptor-related protein) (IL-1Rrp) (IL1R-rp) (Interleukin-18 receptor alpha) (IL-18R-alpha) (IL-18Ralpha) (CD antigen CD218a)	541	IL-18, IL-37
IL-33R	Q01638	IL1RL1 DER4 ST2 T1	Interleukin-1 receptor-like 1 (Protein ST2)	556	IL-33
IL-1RAcP	Q9NPH3	IL1RAP C3orf13 IL1R3	Interleukin-1 receptor accessory protein (IL-1 receptor accessory protein) (IL-1RAcP) (Interleukin-1 receptor 3) (IL-1R-3) (IL-1R3)	570	IL-1, IL-33, IL-36,
IL-18RAcP	O95256	IL18RAP IL1R7	Interleukin-18 receptor accessory protein (IL-18 receptor accessory protein) (IL-18RAcP) (Accessory protein-like) (AcPL) (CD218 antigen-like family member B) (CDw218b) (IL-1R accessory protein-like) (IL-1RAcPL) (Interleukin-1 receptor 7) (IL-1R-7) (IL-1R7) (Interleukin-18 receptor accessory protein-like) (Interleukin-18 receptor beta) (IL-18R-beta) (IL-18Rbeta) (CD antigen CD218b)	599	IL-18
SIGIRR	Q6IA17	SIGIRR UNQ301/ PRO342	Single Ig IL-1-related receptor (Single Ig IL-1R-related molecule) (Single immunoglobulin domain-containing IL1R-related protein) (Toll/interleukin-1 receptor 8) (TIR8)	410	IL-1R-1
TIGIRR-1	Q9NP60	IL1RAPL2 IL1R9	X-linked interleukin-1 receptor accessory protein-like 2 (IL-1 receptor accessory protein-like 2) (IL-1-RAPL-2) (IL-1RAPL-2) (IL1RAPL-2) (IL1RAPL-2-related protein) (Interleukin-1 receptor 9) (IL-1R-9) (IL-1R9) (Three immunoglobulin domain-containing IL-1 receptor-related 1) (TIGIRR-1)	686	Orphan
TIGIRR-2	Q9NZN1	IL1RAPL1 OPHN4	Interleukin-1 receptor accessory protein-like 1 (IL-1-RAPL-1) (IL-1RAPL-1) (IL1RAPL-1) (Oligophrenin-4) (Three immunoglobulin domain-containing IL-1 receptor-related 2) (TIGIRR-2) (X-linked interleukin-1 receptor accessory protein-like 1)	696	Orphan

et al. 2017a) and that polyinosinic-polycytidylic acid (poly(I:C)) induces both IL-36γ expression and caspase-dependent keratinocyte death and release of IL-36γ (Lian et al. 2012), none of the cytokines in the IL-36 family contains a caspase cleavage site. Therefore, it is unlikely that the cytokine secretion is directly dependent on caspase. Several studies report evidence suggesting IL-36 cytokines adopt an alternative secretion mechanism. Lipopolysaccharide (LPS) and adenosine triphosphate (ATP) treated bone marrow-derived macrophages (BMDMs) release IL-36α (Martin et al. 2009), suggesting IL-36α is secreted after external stimulation. Upon bacterial stimulation, IL-36γ is induced and secreted from lung macrophages and the secretion is detected in microparticles and exosome fractions, suggesting a non-Golgi dependent secretion mechanism (Kovach et al. 2016). Although remaining elusive, current studies suggest IL-36 cytokines adopt a secretion mechanism that is distinct from IL-1β and IL-18.

2.2 Full-Length IL-36 Cytokines Require N-Terminal Processing Before Activating NF-κB and MAPK Signaling Pathways

In a biochemical deletion study, N-terminal deletion of IL-36 cytokines significantly increased cytokine activity (Towne et al. 2011). The consensus site for deletion is localized to 9 amino acids in the N-terminus of a conserved A-X-D motif (A is an aliphatic amino acid, X is any amino acid and D is aspartic acid) (Towne et al. 2011). Unlike IL-1β and IL-18 that utilize intracellular caspase-1 and inflammasome for activation (Martinon et al. 2002; Ghayur et al. 1997), IL-36 cytokines do not contain any caspase cleavage site. Thus, IL-36 cytokines require different enzymes for N-terminal processing and activation. It has been shown that neutrophil-derived proteases cleave and activate IL-36 cytokines, which resembles the activation of IL-1α (Henry et al. 2016; Macleod et al. 2016; Clancy et al. 2017; Clancy et al. 2018; Guo et al. 2019; Afonina et al. 2011). IL-36α is cleaved at Lys3 by cathepsin G or at Ala4 by elastase. IL-36β is activated by cathepsin G after being processed at Arg5. IL-36γ is mainly cleaved at Val15 by elastase under a protease concentration that is normally released during neutrophil degranulation (Henry et al. 2016). Both cathepsin G and elastase are present in the neutrophil extracellular traps where IL-36 cytokines can be activated (Clancy et al. 2017). Recombinant neutrophil elastase, a serine protease expressed in polymorphonuclear cells, activates IL-36Ra by cleaving the N-terminal methionine (Macleod et al. 2016). In the psoriatic skin, IL-36β is activated by elevated expression of cathepsin G while IL-36α or IL-36γ is not activated, likely due to the absence of elevated elastase or proteinase-3 (Henry et al. 2016). Interestingly, cathepsin S, which is also upregulated in psoriatic skin lesions, has been shown to activate IL-36γ after cleaving the cytokine at Ser-18. Since cathepsin S is readily expressed by a range of resident cells in the barrier tissues, it is reported as the major activator of IL-36γ in the skin and the activation is independent, contrary to previous reports, of neutrophil recruitment (Bal et al. 2017). Nevertheless, the activity of IL-36 cytokines is regulated by enzymes other than caspase-1 and more studies are needed to confirm the presence of neutrophil derived proteases or barrier resident cells derived cathepsin S in patients with upregulated IL-36 activity.

2.3 IL-36 Cytokines Adopt a Similar Structural Fold as the Other Cytokines in the IL-1 Superfamily

Crystal structures are available for human IL-36γ (PDB: 4IZE, 2 Å), mouse IL-36Ra (PDB: 1MD6, 1.6 Å) and several chimeric IL-36γ constructs with the β4/5 or the β11/12 loop swapped with IL-36Ra (PDB: 4P0J, 2.298 Å, 4P0K, 1.7 Å and 4P0L, 1.55 Å). Human IL-36γ maintains a β-trefoil fold (Gunther and Sundberg 2014),

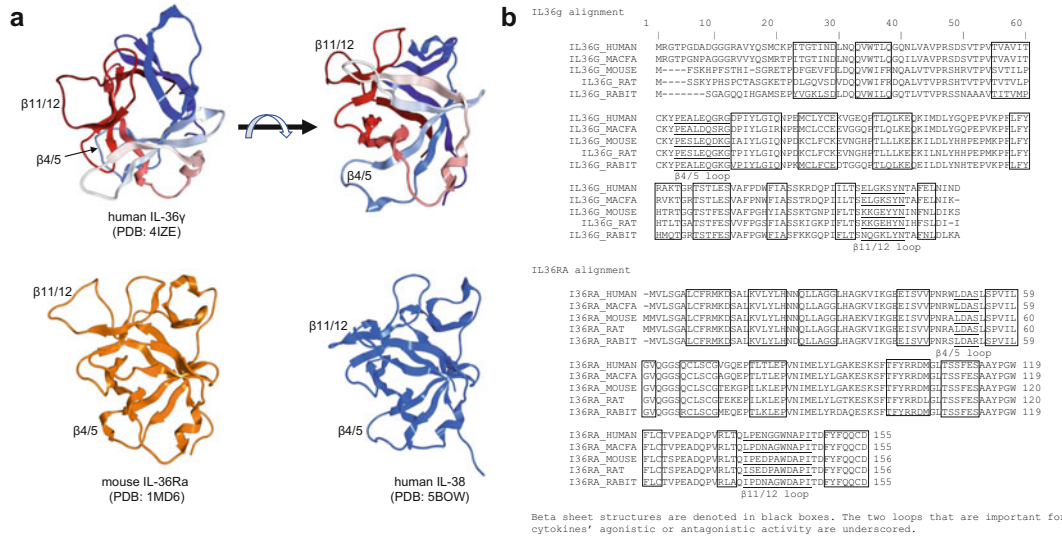

Fig. 2 (**a**) Human IL-36γ adopts a β-trefoil fold, which is typical for cytokines in the IL-1 superfamily. This structural fold contains 12 β-strands connected by 11 loops. The structure consists of six beta hairpins in a threefold symmetry. The blue (N-terminus) and red (C-terminus) color gradient highlights the symmetry of the molecule. Mouse IL-36Ra (orange) and Human IL-38 (blue) also adopts the same fold. (**b**) Sequence alignment of IL-36γ and IL-36Ra among different species. The β11/12 and β4/5 loops are conserved across species. Compared to the agonist IL-36γ, the β11/12 loop of IL-36Ra is several amino acids longer

which is typical for the cytokines in the IL-1 superfamily (Fields et al. 2019). This structural fold contains 12 β-strands connected by 11 loops (Fig. 2). The structure consists of six β-hairpins in a threefold symmetry. The blue (N-terminus) and red (C-terminus) color gradient highlights the symmetry of the molecule. Although currently there is no human IL-36Ra structure, the mouse IL-36Ra structure shows it adopts a β-trefoil fold as well (Dunn et al. 2003). Considering human and mouse IL-36Ra share more than 90% sequence similarity, it is reasonable to consider that the human antagonist structure resembles the mouse IL-36Ra. Notably, even though the human IL-36γ and the mouse IL-36Ra have similar structures (RMSD 0.746 Å), their total sequence similarity is relatively low. β4/5 and β11/12 loop are distinctly different between the agonist and antagonist. Structure alignment without these loops results in a higher RMSD at 0.619 Å. It has been reported that by exchanging these two loops, one can tune the agonistic and antagonistic activity of IL-36 cytokines (Gunther and Sundberg 2014).

3 IL-36 Receptors Are Multi-domain Transmembrane Receptors That Require an Agonist for Activation

There are a total of 10 receptors and accessory proteins in the IL-1 superfamily (Table 2). Each family has at least one unique cognate receptor and one accessory protein. For example, IL-1R-1 and IL-1R-2 belong to IL-1 family; IL-18R-1 is from IL-18; IL-33R and IL-36R are from IL-33 and IL-36 families respectively. Every family in the IL-1 superfamily shares the same accessory protein IL-1RAcP except for IL-18 family which has a different accessory protein IL-18RAcP. There are three receptors SIGIRR, TIGIRR-1 and TIGIRR-2 in the IL-1 superfamily with less known family origin and functions. SIGIRR is reported to be a negative regulator of IL-1 signaling, possibly by interfering the IL-1R and IL-1RAcP heterodimerization (Qin et al. 2005).

The cognate receptor IL-36R, together with the other receptors in the IL-1 superfamily such

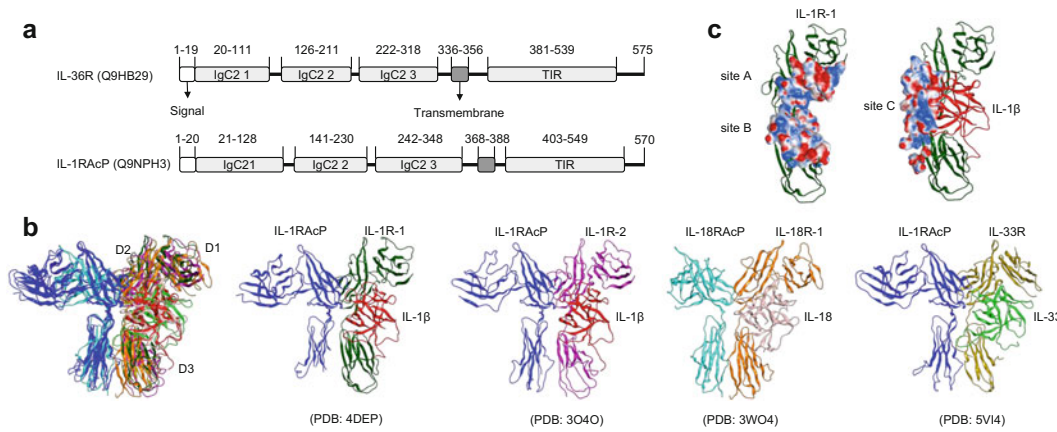

Fig. 3 (**a**) IL-36 receptors are multidomain transmembrane receptors. Each receptor consists of three Ig-like C-2 type domains, one transmembrane domain and one Toll-Interleukin receptor domain. Illustration is rendered with information from UniProt last accessed on 8/8/2019. (**b**) Existing structures show that the cytokines and the ECDs of receptors from different families form similar ternary complexes. Overlay of these complexes reveal the similarity in cytokine and receptor binding mode. The cognate receptor ECDs consist of D1, D2 and D3 domains. (**c**) The IL-1R-1 structure shows D1 and D2 domains form the binding site A for IL-1β while D3 contains the binding site B for the cytokine. The binary IL-1R-1/IL-1β complex form a new interface, the binding site C, that interacts with IL-1RAcP. Electrostatics surface is shown in red patches as negative and blue patches as positive

as IL-1R-2, IL-1R-1, IL-33R and IL-18R-1, are mapped to IL-1 receptor cluster on human chromosome 2q12 (Dale and Nicklin 1999). IL-36R is a 575 amino acid-long, multi-domain, single-pass transmembrane protein (Fig. 3a). The first 19 amino acid signal peptide is cleaved in the mature protein form. Sequence from 20 to 335 is the extracellular region containing three Immunoglobulin (Ig) like C2-type domains. Sequence from 336 to 356 is the helical transmembrane region. Sequence from 357 to 575 is the intracellular region containing Toll-interleukin receptor (TIR) domain (381–539). There are multiple glycosylation sites and disulfide bonds in the sequence, which help maintain the stability of the receptor (Yi et al. 2016). Either alanine mutation of the conserved cysteine residues or deglycosylation by PNGase F treatment was shown to lead to a lower accumulation of IL-36R in HEK293 reporter cells (Yi et al. 2016). Three human single nucleotide polymorphisms (SNPs) (V352I, A471T and L550P) were examined in HEK293 reporter cells. A471T, which accounted for 2.3% frequency in population, reduced NF-κB activation by about 60% (Yi et al. 2016).

Accessory protein IL-1RAcP is a 580 amino acid-long, multi-domain, transmembrane protein which contains a signal peptide of 20 amino acid-long at the N-terminus (Fig. 3a). Like IL-36R, mature IL-1RAcP consists of three Ig-like C2-type extracellular domains and one intracellular TIR domain. There are multiple glycosylation sites and disulfide bonds in the sequence as well. This co-receptor is also shared among IL-1 and IL-33 families for signaling activation.

3.1 IL-36R Is Likely to Adopt a Similar Structural Fold as the Other Receptors in the IL-1 Superfamily

Even though the structure of IL-36R has not been determined yet, the extracellular domains (ECDs) of the cognate receptors from IL-1, IL-18 and IL-33 families have been separately published in the form of ternary complexes (Wang et al. 2010; Thomas et al. 2012; Tsutsumi et al. 2014; Gunther et al. 2017). The ECDs of IL-1R-1, IL-1R-2, IL-18R-1 and IL-33R contain three Ig-like domains (D1, D2 and D3) (Fig. 3b). Overlay of

these structures reveals the high similarity among these ternary complexes from the IL-1 superfamily (Fig. 3b). Given the similarity, it is reasonable to consider that IL-36R also adopts a similar structure as the other cognate receptors such as IL-1R-1. The IL-1R-1 structure shows domains D1 and D2 are tightly packed with each other to form a binding site A interacting with the cytokine IL-1β (Fig. 3c). Domain D3, which contains a binding site B, is linked with D2 by a linker with no secondary structure. About 50 amino acids in total from both sites contribute to the entire cytokine binding interface, covering more than 1500 $Å^2$ surface (Fields et al. 2019). IL-1RAcP binds to an interface that is newly formed by the cytokine and the cognate receptor, binding site C, in all three ternary complex structures (Fig. 3c).

3.2 Receptors from the IL-1 Superfamily Require Corresponding Cytokines for Activation and Inhibition

Agonists IL-1α, IL-1β and antagonist IL-1Ra bind IL-1Rs. Agonist IL-18 and antagonist IL-37 bind IL-18R-1 and agonist IL-33 binds IL-33R. IL-1RAcP is shared among all families except for IL-18, which requires its own accessory protein (IL-18RAcP) for signal activation. IL-36R was first identified in 1996 by cDNA cloning as an orphan receptor from the rat brain (Lovenberg et al. 1996). Its sequence homology with IL-1R-1 or IL-1R-2 is 42% or 26%, respectively. IL-36R was first believed to be related to IL-1 family receptors in that it would bind IL-1 ligands. However, IL-36R did not show binding to the IL-1 cytokines (Lovenberg et al. 1996). Instead, IL-36R requires its own ligand (IL-36α, IL-36β and IL-36γ) for activation. IL-36 cytokines activate NF-κB and MAPK through IL-36R and a shared accessory protein IL-1RAcP in a MYD88 (myeloid differentiation factor 88) dependent manner. IL-36α, IL-36β, IL-36γ and IL-36Ra were shown to activate or deactivate NF-κB signaling through IL-36R in transfected Jurkat cells (Debets et al. 2001). Additionally, IL-36α and IL-36β activated NF-κB signaling in NCI/ADR-RES cells, which naturally expressed IL-36R (Towne et al. 2004). Inhibition of either IL-36R or IL-1RAcP blocked the signaling, suggesting both IL-36R and IL-1RAcP were needed for signaling (Towne et al. 2004). *In vivo*, IL-36α transgenic mice displayed skin abnormalities in mice with genetic background containing both receptors and accessory protein ($IL\text{-}36R^{+/+}$, $IL\text{-}1RAcP^{+/+}$, $IL\text{-}36R^{+/-}$ and $IL\text{-}1RAcP^{+/-}$) but not in mice with either component knocked out ($IL\text{-}36R^{-/-}$ or $IL\text{-}1RAcP^{-/-}$). Additionally, antibody targeting mouse IL-36R blocked the skin phenotype *in vivo* (Blumberg et al. 2007). Collectively, these studies further suggest IL-36α induces skin phenotype that is dependent on both IL-36R and IL-1RAcP (Blumberg et al. 2007).

Although the activation requirement for both IL-36R and IL-1RAcP has been established, the temporal order of receptor activation is only recently proven. Activation of IL-36 was suggested to be like IL-1 in that the agonistic cytokine bound to cognate receptor and recruited the accessory protein following a two-step mechanism (Wang et al. 2010). However, both IL-36R and IL-1RAcP co-immunoprecipitated in one biochemical study, suggesting IL-36R and IL-1RAcP might form a pre-associated receptor heterodimer in the absence of an agonist cytokine (Yi et al. 2016). This observation implied the signaling activation was perhaps due to cytokine mediated heterodimeric receptor conformational change, a mechanism that was different from the two-step mechanism. In a quantitative binding study, interactions between the ECDs of IL-36R and IL-1RAcP were undetectable in the absence of IL-36α, which suggested a pre-associated heterodimeric receptor was unlikely to be stable under physiological conditions (Zhou et al. 2018). The dominant path for receptor activation is indeed like IL-1, which is through the intermediate binary complex formed by agonist and cognate receptor rather than through the pre-associated heterodimeric receptor path. The newly formed binary complex recruits accessory protein to form IL-36α/IL-36R/IL-1RAcP ternary complex (Zhou et al. 2018).

In this two-step receptor activation process, the specificity of signaling is achieved by the first step-cytokine recognition by cognate receptor. The secondary receptor IL-1RAcP is shared among IL-1, IL-33 and IL-36 families. The promiscuous use of a shared receptor among the IL-1 superfamily represents a common theme in cytokine signaling. In fact, the vast majority of heterodimeric receptor systems employ a shared receptor (e.g. βc, γc, gp130, IL-12Rβ1) (Spangler et al. 2015; Ozaki and Leonard 2002). Cells utilize this economical approach to achieve signaling specificity.

The antagonist IL-36Ra binds IL-36R with a higher affinity than the agonists (IL-36α or IL-36γ) (Fig. 4). It suggests one efficient mechanism for IL-36 negative regulation is through the preferable binding of antagonist over agonists. When both types of cytokines were present at similar concentrations, the ones with higher affinity occupy more receptors based on the law of mass action (Zhou et al. 2018). The structures of human IL-36γ and murine IL-36Ra reveal the biggest differences reside in two loops (β4/5 and the β11/12) (Fig. 5a) (Gunther and Sundberg 2014). The extended β11/12 loop in IL-36Ra causes steric clash with IL-1RAcP and therefore prevents recruitment of IL-1RAcP (Fig. 5b). Swapping these loops between agonists and antagonist reverse activity of the cytokine (Gunther and Sundberg 2014). Similarly, the largest structural difference between the IL-1β and IL-1Ra is in β4/5 and β11/12 loops. β11/12 loop is several amino acids longer in IL-1Ra and causes steric clash with the IL-1RAcP (Wang et al. 2010). The agonistic and antagonistic functions of cytokines are therefore determined by their structures.

Once a ternary complex forms, IL-36 utilizes MYD88 and TOLLIP to transduce signaling intracellularly like IL-1 does. The intracellular TIR domains of IL-1 receptors and IL-1RAcP are brought together through extracellular domain interactions mediated by IL-1β. The resulting ternary complex recruit intracellular adaptor protein such as MYD88, Toll-interacting protein kinase

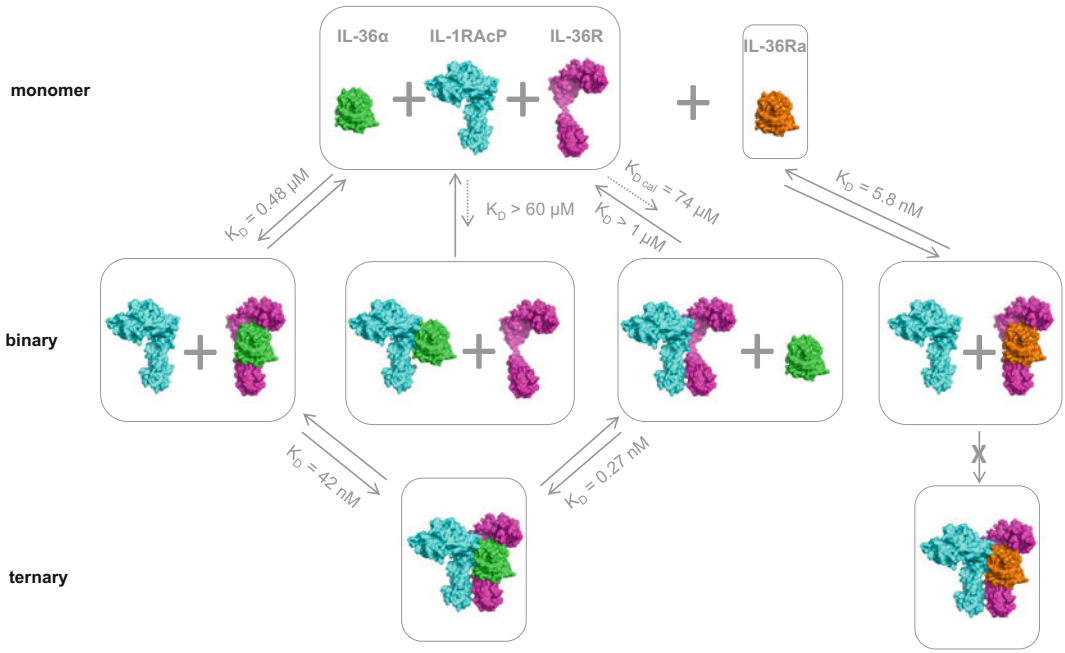

Fig. 4 IL-36 receptors activation and inhibition mechanism. The main activation path for IL-36 is through agonist IL-36α binding to IL-36R, which then recruits IL-1RAcP to form a signaling ternary complex. IL-36Ra shows stronger binding than IL-36α, indicating an efficient negative regulation by the antagonist. Figure is based on reference (Thomas et al. 2012)

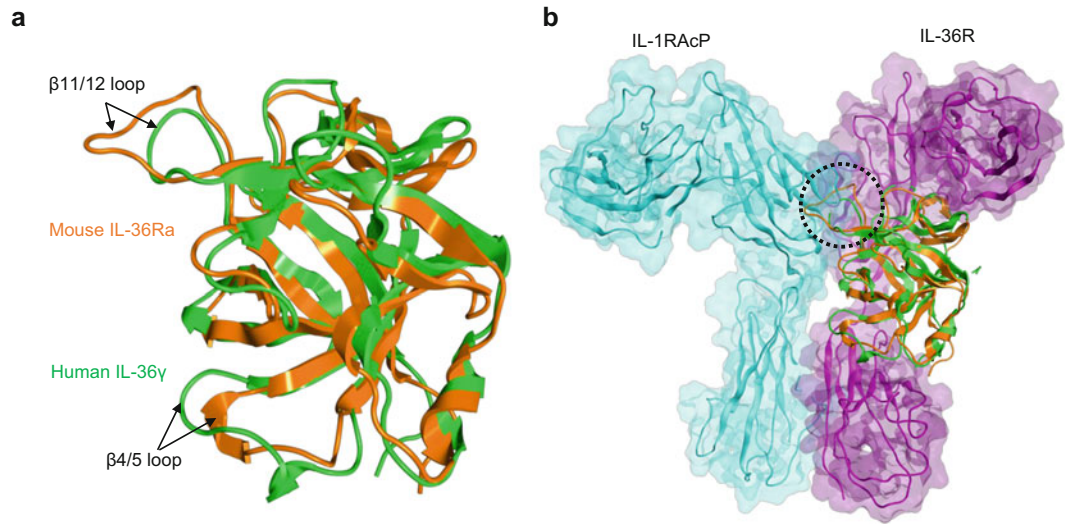

Fig. 5 Structural difference reveal the basis of functional difference for IL-36 agonist and antagonist. (**a**) overlay of human IL-36γ and mouse IL-36Ra shows the differences in β11/12 and β4/5 loops. β11/12 in IL-36Ra are several amino acids longer than that of IL-36γ. (**b**) The ternary complex model shows β11/12 loop of IL-36Ra causes significant steric clash with IL-1RAcP. IL-36R is a homology model based on IL-1R-2 (PDB: 3O4O). Human IL-36γ (PDB: 4IZE), mouse IL-36Ra (PDB: 1MD6) and the IL-36R model are docked with a template (IL-1R-2/IL-1RAcP/IL-1β, PDB: 3O4O). Only extracellular domains of receptors are shown

1 (IRAK-1) and the Toll-interacting protein (TOLLIP), which then activate transcription factors such as NF-κB (Medzhitov et al. 1998; Dunne and O'Neill 2003; Tiwari et al. 2011). In the IL-36 stimulated signaling, IL-6 secretion was inhibited when either MYD88 or TOLLIP was knocked down by siRNA in NCI/ADR-RES cells (Saha et al. 2015). Activated IL-36R entered LAMP1 lysosomes via a clathrin-mediated pathway and the lysosomal accumulation of IL-36R did not change the receptor turnover (Saha et al. 2015). In a RNA-seq based transcriptome study, IL-1β or IL-36α/β/γ treated primary epidermal keratinocytes transcribed a shared set of genes (Swindell et al. 2018), suggesting IL-1 and IL-36 employed a similar intracellular signaling transduction route. Although there were some differences at an earlier time point, 225 genes were similarly induced for both IL-1β and IL-36 treatment groups. Furthermore, these differently expressed genes were significantly overlapping with genes altered in psoriasis and generalized pustular psoriasis, suggesting IL-1β and IL-36 were involved in these inflammatory diseases (Swindell et al. 2018). After MYD88 being inactivated through CRISPR/Cas9, these differentially expressed genes lose their response (Swindell et al. 2018). Given the similarity in the use of adaptor protein cascade and the transcriptomic regulation, the biological specificity of IL-1 and IL-36 is therefore unlikely to be achieved by intracellular factors but more likely due to other factors such as recognition of cognate receptors, temporal and spatial co-localization of cytokines and receptors, and selective activation through elevated proteases.

3.3 IL-38, Also Identified Through DNA Database Analysis, Is a 152 Amino Acid-Long Antagonistic Cytokine and Shows about 40% Sequence Similarity with IL-1Ra and IL-36Ra (Lin et al. 2001; Bensen et al. 2001)

IL-38 was mapped to the human chromosome 2q13-14.1, a region near IL1RN (Bensen et al. 2001). The similarity in both conserved sequences and the genomic location suggest

IL-38 may be another antagonist in the IL-1 family (Lin et al. 2001; Bensen et al. 2001). However, it bound to IL-1R with low affinity (Lin et al. 2001). Instead, IL-38 was shown to bind recombinant IL-36R and had similar biological effects as IL-36Ra on immune cells. For example, it inhibited the secretion of LPS-induced inflammatory cytokines (van de Veerdonk et al. 2012). IL-38 also adopts a similar fold as IL-36Ra (PDB: 5BOW) (Fig. 2). It remains to be seen whether IL-38 exerts its antagonistic activities by blocking IL-1RAcP or by recruiting an inhibitory receptor from the IL-1 superfamily such as SIGIRR, TIGIRR1 or TIGIRR2 (van de Veerdonk et al. 2018).

4 IL-36 Tissue Expression, Function and Regulation

IL-36 family members are endogenously expressed in the tissues and the organs at very low levels, if at all (Buhl and Wenzel 2019). Barrier tissues including skin, lung and intestine express the highest levels of IL-36, especially after stimulation. The emerging role for IL-36 is to act as a bridge between innate and inflammatory responses. As such, it is responsible for coordinating inflammatory and anti-microbial responses in the barrier tissues. IL-36 agonists induce epithelial cells to produce anti-microbial peptides, cytokines and chemokines (Carrier et al. 2011; Blumberg et al. 2010; Johnston et al. 2011); drive the differentiation and maturation of tissue resident immune cells (Vigne et al. 2011; Mutamba et al. 2012); and recruit and activate circulating immune cells to the tissues (Vigne et al. 2011; Gresnigt et al. 2013). The strong pro-inflammatory effects of IL-36 agonistic cytokines are opposed by the negative regulation of antagonist IL-36Ra (Debets et al. 2001). Disruption of this delicate balance creates pathological changes. In chronic inflammatory conditions, sustained upregulation of IL-36 agonists or downregulation of IL-36Ra is associated with inflammatory changes in the affected tissues and organs.

4.1 Skin

In the skin, the normal function of IL-36 appears to be host defense against pathogenic microbes. Current findings implicate IL-36α in responses to bacterial infections while IL-36γ is associated with responses to fungi and virus infection. Research on *Staphylococcus aureus* (*S. aureus*) skin colonization shows both IL-1 and IL-36 are associated with skin inflammation (Liu et al. 2017; Nakagawa et al. 2017; Miller et al. 2006). Considering that *S. aureus* colonization is one of the main drivers for atopic dermatitis and that IL-36 is upregulated in this inflammatory skin disease, it will be interesting to see whether a deeper mechanistic connection exists (Brunner et al. 2018). Multiple lines of evidence from *in vitro*, *in vivo* and patient studies suggest that IL-36 plays an important role in response to herpes simplex virus infection by inhibiting viral replication and by establishing anti-viral defense in the skin (Kumar et al. 2000; Milora et al. 2017; Gardner and Herbst-Kralovetz 2018).

IL-36 agonists act as amplifiers of inflammatory responses in the skin. Family members are induced by some early skin injury and infection markers such as alarmin LL37. Once secreted and processed, IL-36 cytokines activate keratinocytes and resident immune cells to produce several chemokines (e.g. IL-8, CXCL1, CCL20) for recruiting more immune cells to the skin (Li et al. 2014; Gabay and Towne 2015). IL-36 itself is upregulated by cytokines produced by infiltrating Th1 and Th17 lymphocytes and synergizes with the other inflammatory cytokines to stimulate additional production of pro-inflammatory cytokines and chemokines, further enhancing the inflammation (Carrier et al. 2011). IL-36R and IL-36Ra knockout mice showed differential responses to IMQ induced dermatitis, suggesting IL-36 pathway was involved in induction and propagation of skin inflammation (Tortola et al. 2012). Moreover, anti-IL-36R antibodies attenuated skin inflammation induced by IL-23 in a mouse model. This suggested IL-36 signaling was involved in Th17/

Th23 signaling, a clinically validated axis for psoriasis (Su et al. 2019; Ganesan et al. 2017).

Given its involvement in both innate and inflammatory responses, it should come as no surprise that dysregulation of IL-36 signaling leads to various skin disorders. Mutations of IL36RN have been implicated in the pathogenesis of general pustular psoriasis (GPP) and related palmoplantar pustulosis (PPP) (Marrakchi et al. 2011; Farooq et al. 2013). Mutations of this natural antagonist generate a phenotype resembling severe psoriasis with abundant neutrophil infiltrate and creating life-threatening pustular skin lesions (Onoufriadis et al. 2011). In addition to their association with this rare form of psoriasis, IL-36 family members are substantially upregulated in both the skin and the serum from patients with psoriasis vulgaris (PV). The expression level of IL-36 agonists correlates with disease severity (Blumberg et al. 2007, 2010; Johnston et al. 2011; Sehat et al. 2018; Mercurio et al. 2018). Recent studies show that IL-36 cytokine expression is highly correlated with the severity of skin ichthyoses, which has substantial involvement of Th17 (Malik et al. 2019). IL-36 family members are also highly upregulated in allergic contact dermatitis and administration of IL-36Ra into patient's skin biopsies significantly reduces the secretion of IL-36 and IL-8 (Mattii et al. 2013).

4.2 Intestine

The normal function for IL-36 in the human intestine is under investigation. IL-36α, IL-36γ and IL-36R are upregulated in patients with Crohn's disease (CD), ulcerative colitis (UC), inflammatory bowel disease (IBD) and Hirschsprung's disease (Nishida et al. 2016; Russell et al. 2016; Scheibe et al. 2017; Tomuschat et al. 2017; Fonseca-Camarillo et al. 2018; Scheibe et al. 2019). The expression is driven by T-cells, monocytes, plasma cells and myofibroblasts. IL-36R knockout mice exhibited reduced innate response in *Citrobacter rodentium* colonization (Russell et al. 2016). IL-36 alone, or in combination with IL-17 and TNFα, drove the expression of pro-inflammatory chemokines in the intestinal cells (Kanda et al. 2015). In an acute mouse model of intestinal injury (DSS), IL-36α, IL-36γ and IL-17 could be induced (Boutet et al. 2016). Notably, inhibition by an anti-IL-36R antibody or IL-36R knockout mice developed less severe fibrosis and colitis (Scheibe et al. 2019). In the T-cell transfer model of colitis, both IL-36R and IL-36γ knockout mice were protected from T-cell driven inflammation (Harusato et al. 2017). However, IL-36 has been reported to also play a role in gut healing after injury, likely through the induction of IL-22 (Scheibe et al. 2017; Medina-Contreras et al. 2016; Ngo et al. 2018). Collectively, IL-36 has been shown to be involved in reducing *Citrobacter rodentium* colonization, participating in intestine inflammation, and interestingly, inducing gut healing. The complex roles of IL-36 in the gut require additional elucidation given its association with multiple intestinal diseases.

4.3 Lungs

IL-36 mediates inflammatory response in the lungs upon infection. IL-36R was found in human lung fibroblasts and bronchial epithelial cells (Zhang et al. 2017). In the lung, IL-36 cytokines can be induced by house dust mites, bacteria and virus. In the lung homogenates of A/J mice challenged with house dust mite, IL-36γ was significantly increased in the airway epithelial cells (Ramadas et al. 2011). Similarly, IL-36α, IL-36γ but not IL-36β were increased in the mouse lung after bacterial infection such as *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis (M. tuberculosis)* and *M. bovis* BCG (a less virulent mycobacteria) (Segueni et al. 2015; Aoyagi et al. 2017b; Nanjo et al. 2019). Influenza virus upregulated IL-36α in the mouse lung (Aoyagi et al. 2017a). In children with *Mycoplasma pneumoniae* pneumonia, IL-36 was elevated in the bronchoalveolar lavage fluid (BALF) (Chen et al. 2016). *In vitro*, both murine pulmonary macrophages and human alveolar macrophages

expressed higher levels of IL-36γ after stimulation with recombinant pathogen associated molecular patterns from *Klebsiella pneumoniae* and *Streptococcus pneumoniae (*Kovach et al. 2016*)*. The activated IL-36 signaling stimulates production of pro-inflammatory cytokines, chemokines and recruits neutrophils and monocytes into the lungs (Aoyagi et al. 2017a, b; Kovach et al. 2016, 2017; Zhang et al. 2017; Ramadas et al. 2011, 2012; Nanjo et al. 2019). This inflammatory response is consistent with IL-36 signaling and its interplay with immune cells.

The host response in the lungs for IL-36 is less definitive. Although IL-36 members were expressed in the airway epithelial cells and the lung-resident macrophages (Kovach et al. 2016; Ramadas et al. 2011), IL-36R deficient mice showed no worse mortality than the wild type after bacterial infection in the lungs (Aoyagi et al. 2017a, b; Segueni et al. 2015). The IL-36 mediated inflammatory response, after infection, increased lung injury and mouse mortality (Aoyagi et al. 2017a; b). However, in recent reports, IL-36γ was shown to play an important role in clearing *Streptococcus pneumoniae* in the lungs and to increase mortality in mice (Kovach et al. 2017). IL-36R$^{-/-}$ mice were less capable in bacterial clearance with a significantly reduced inflammatory response (Nanjo et al. 2019). Interestingly, inactivation of either IL-36α or IL-36γ alone did not exacerbate the bacterial clearance compared to wild type, suggesting there might be a functional overlap between IL-36 agonists in the protection against infection (Nanjo et al. 2019).

4.4 Joints

It is now well established that IL-36 family members play important roles in inflammatory responses in barrier tissues and that their deregulation can be paramount in diseases such as psoriasis. In inflammatory joint disorders such as rheumatoid arthritis (RA), psoriatic arthritis (PsA) and osteoarthirtis (OA), there is upregulation of IL-36 family members. A subset of RA patients (~20%) had a substantial elevation of the IL-36 agonist and antagonist ratio in synovial fluids (Boutet et al. 2016). Similar findings showing upregulation of IL-36α in PsA and OA patients have also been reported (Frey et al. 2013; Li et al. 2019). In addition, it has been demonstrated that synovial fibroblasts, as well as a variety of immune cells, produce IL-36 cytokines, which induce the expression of pro-inflammatory chemokines in the synovial tissues (Boutet et al. 2016; Frey et al. 2013).

Although IL-36 family members are expressed in synovial tissues, several models of arthritis show no correlation between IL-36 (IL-36γ, IL-36Ra and IL-36R) and arthritis severity. Moreover, IL-36R neutralizing antibodies and IL-36R genetic ablation did not affect arthritis severity in either collagen induced or TNF-induced arthritis models (Lamacchia et al. 2013; Derer et al. 2014; Dietrich and Gabay 2014). The lack of efficacy in mouse models could potentially be explained by IL-36's functional redundancy with IL-1, which might play a dominant role in rodent arthritis models. The stimulatory effect of IL-36 in synovial fibroblasts and articular chondrocytes was significantly lower than that of IL-1 (Magne et al. 2006), consistent with the suggestion that IL-36 might not play a prominent role in driving arthritis. Nonetheless, since the RA, PsA and OA patients overexpress IL-36 cytokines and the clinical and histopathological features have not been clearly defined, further research is warranted.

4.5 Brain

In the brain, the role for IL-1 has been well established. Infections lead to IL-1 mediated fever and appetite suppression. It is therefore no surprise that both IL-36R and IL-36Ra were first cloned from brain tissue (Mulero et al. 1999; Lovenberg et al. 1996). However, limited studies have not shed much light on the functional roles of IL-36 in the brain and other nervous tissues. High mRNA expression of IL-36R was detected in mouse glia cells including astrocytes and microglia, which was counterintuitively downregulated by exposure to bacterial

lipopolysaccharide (Berglof et al. 2003). Furthermore, IL-36γ failed to induce any classical IL-1β response such as fever, reduced body weight and food intake (Berglof et al. 2003). Similarly, mRNA of IL-36β was detected in both mouse primary neuronal and mixed glial cells but stimulation with IL-36β showed no classical IL-1β responses (Wang et al. 2005). In the experimental allergic encephalomyelitis (EAE) model of inflammatory demyelinating disease, both IL-36γ and IL-36R were upregulated in the nervous and hematopoietic tissues (Bozoyan et al. 2015). Microglial cells in culture were able to respond to IL-36γ by robustly expressing pro-inflammatory mediators of neutrophil recruitment. However, IL-36γ deficient and IL-36R$^{-/-}$ mouse still developed all of the histopathological signs of EAE (Bozoyan et al. 2015), suggesting IL-36γ might be a biomarker and not a driver for EAE. Recent studies show increased levels of IL-36γ in the serum from MS patients (Alsahebfosoul et al. 2018) and elevated IL-36α in the serum and the CSF from neuromyelitis optica spectrum disorder (NMOSD) patients (Song et al. 2019). It will be interesting to see whether IL-36 drives pro-inflammatory responses in the human nervous system.

5 Tools and Investigative Therapeutics

Considering its pro-inflammatory role in the skin and the other organs, IL-36 appears to be an attractive therapeutic target. It has recently been shown that individuals carrying loss of function mutations in the IL36R gene do not have compromised host defense or abnormal immune function (Mahil et al. 2017), suggesting IL-36 may be a safe target. To inhibit the pathogenic inflammatory processes, either IL-36 cytokines or the receptors can be targeted directly or indirectly with a therapeutic agent.

As mentioned above, IL-36 cytokines require proteolytic cleavage to achieve their full signaling activity. IL-36α, IL-36β and IL-36γ are activated at the N-terminus by the neutrophil granule-derived proteases cathepsin G, elastase and proteinase-3; or by the keratinocyte and fibroblast-derived cathepsin S. Therefore, one targeting strategy is to inhibit these tissue specific proteases (Henry et al. 2016; Sullivan et al. 2018; Ainscough et al. 2017). Recently, small molecule inhibitors of elastase were identified by *in silico* screening (Sullivan et al. 2018). These inhibitors attenuated the activation of full-length IL-36γ and the downstream pro-inflammatory signaling (Sullivan et al. 2018). However, safety concerns regarding systemic use of these protease inhibitors exist as the elastase, for instance, is important for normal neutrophil mediated antibacterial responses (Belaaouaj et al. 2000; Weinrauch et al. 2002).

Dosing a natural antagonist has been an effective approach in dampening the inflammatory response mediated by IL-1 signaling. The recombinant IL-1 receptor antagonist (IL-1Ra), anakinra, has been approved for the use in rheumatoid arthritis and neonatal-onset multisystem inflammatory disease (NOMID). It has also shown efficacy in the treatment of Deficiency of Il-1 Receptor Antagonist (DIRA), a rare immune disorder caused by function blocking mutations of IL1RN (Aksentijevich et al. 2009). IL-36Ra has the same inhibitory activity in IL-36 signaling as does IL-1Ra in IL-1 signaling. However, there is currently no report on the clinical use or development of IL-36Ra as therapeutics.

On the other hand, direct inhibition of cytokine and signaling receptors by monoclonal antibodies has been a successful means of intervention for numerous targets. In psoriasis for instance, antibodies targeting TNFα, IL-23p40, IL-17 or IL-23p19 have shown transformative efficacy with a favorable safety profile. Although there are many therapeutics antibodies approved for treating psoriasis, opportunities exist for IL-36 antibodies in other rare but life-threatening diseases such as GPP. Since there are three IL-36 agonists that share low overall sequence similarity, inhibiting the receptors instead may be an efficient way to antagonize the entire IL-36 pathway. Two anti-IL-36R antibodies are being developed for several clinical indications. Boehringer Ingelheim's antibody against IL-36R (BI 655130) rapidly improved symptoms in

patients with GPP in the 20-week Phase 1 clinical trial. The average improvement in patients' skin symptoms was close to 80% at week four and was maintained until the end of the study (Bachelez et al. 2019). BI 655130 is currently in Phase 2 studies for GPP, PPP, Crohn's Disease, IBD and Ulcerative Colitis. AnaptysBio reported Phase 1 study results of their anti-IL-36R antibody (ANB019) in healthy volunteers, demonstrating favorable safety and PK/PD. The on-going Phase 2 studies are being conducted in GPP and PPP (Irina Khanskaya et al. 2018). In addition to antibodies targeting human IL-36R in clinic, there are several reported anti-mouse IL-36R antibody tools (Su et al. 2019; Ganesan et al. 2017) which should enable further drug discovery efforts.

Antibodies against IL-1RAcP have been evaluated in multiple pre-clinical disease models (Zheng et al. 2018). This targeting approach is proposed to have a broader therapeutic impact on inflammatory diseases driven by multiple cytokines from the IL-1 superfamily (Højen et al. 2019). Additionally, IL-1RAcP is highly expressed on candidate chronic myeloid leukemia stem cells. Antibodies against IL-1RAcP are reported to hold promise for treating chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) (Agerstam et al. 2015, 2016; Askmyr et al. 2013; Jaras et al. 2010; Yoon and Dinarello 1998). However, more studies are needed to evaluate the translational value of these antibodies due to the promiscuous nature of IL-1RAcP and its common expression in various cells and tissues.

Small molecules are attractive modalities for their convenient administration to treat dermatological diseases involving IL-36 dysregulation. However, targeting protein-protein interactions with small molecules has proven to be challenging (Arkin et al. 2003, 2014; Sundberg et al. 2014). The interface of protein-protein interaction (PPI) is relatively flat, extended and adaptable. To accommodate the topological features of PPI, small molecules need to be of a certain size to generate enough binding free energy so that they can compete with the target's natural ligand. However, as the molecule increases in size, other properties such as bioavailability can be compromised. Recent advances in the expanding chemical space (Whitty and Zhou 2015) and the increasing availability of structural information for the IL-1 superfamily members (Gunther and Sundberg 2014; Yi et al. 2016; Thomas et al. 2012; Tsutsumi et al. 2014; Liu et al. 2013) have opened up the possibility for in-depth *in silico* analysis of potential druggable sites for small molecules (Gunther and Sundberg 2014; Dunn et al. 2003; Yang 2015). Short peptide inhibitors for IL-1R have been reported, demonstrating the feasibility of such an approach (Klementiev et al. 2014; Quiniou et al. 2008). Recently, we have published a first-in-class small molecule tool compound binding to IL-36γ which was identified though high throughput screening. This IL-36γ inhibitor blocked interactions with IL-36R and inhibited *in vitro* and *in vivo* inflammation (Todorovic et al. 2019). The report highlights the possibility of targeting the IL-1 superfamily cytokines with small molecules.

6 Conclusions

Almost 20 years have passed since the discovery of IL-36. Since then, our understanding of protein expression, processing, structure and signaling activation of IL-36 has significantly improved. However, there are gaps remaining. For example, the structures of IL-36R/IL-36/IL-1RAcP ternary complex and human IL-36Ra have not been determined yet. The proteases involved in the N-terminal processing of cytokines need to be confirmed in relevant disease populations. The secretion mechanism of IL-36 is far from clear. The individual roles for IL-36α, IL-36β and IL-36γ are unknown. Transcriptome analysis of keratinocytes stimulated with IL-1β and IL-36 agonists only show some small differences in gene expression. However, the differences disappear at later time points (Swindell et al. 2018). Given the similarities between IL-1 and IL-36 in the protein structure, receptor activation mechanism, intracellular signaling transduction and gene regulation; it will be interesting to know

whether IL-36 performs a unique function that is not overlapping with IL-1. IL-36 is reported to hold an important role in the skin's host defense and is also involved in the inflammatory response. Emerging data suggest that it may be an attractive target for skin disorders such as generalized pustular psoriasis. IL-36 should be studied and compared with related signaling pathways such as IL-1, IL-17 and IL-23. Additionally, it remains to be seen whether IL-36 has any translational value in other organs such as the lung and the gut.

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References

Afonina IS et al (2011) Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. Mol Cell 44(2):265–278

Agerstam H et al (2015) Antibodies targeting human IL1RAP (IL1R3) show therapeutic effects in xenograft models of acute myeloid leukemia. Proc Natl Acad Sci U S A 112(34):10786–10791

Agerstam H et al (2016) IL1RAP antibodies block IL-1-induced expansion of candidate CML stem cells and mediate cell killing in xenograft models. Blood 128 (23):2683–2693

Ainscough JS et al (2017) Cathepsin S is the major activator of the psoriasis-associated proinflammatory cytokine IL-36gamma. Proc Natl Acad Sci U S A 114(13): E2748–E2757

Aksentijevich I et al (2009) An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. N Engl J Med 360(23):2426–2437

Alsahebfosoul F et al (2018) Serum level of interleukin 36 in patients with multiple sclerosis. J Immunoassay Immunochem 39(5):558–564

Aoyagi T et al (2017a) IL-36 receptor deletion attenuates lung injury and decreases mortality in murine influenza pneumonia. Mucosal Immunol 10(4):1043–1055

Aoyagi T et al (2017b) Interleukin-36gamma and IL-36 receptor signaling mediate impaired host immunity and lung injury in cytotoxic Pseudomonas aeruginosa pulmonary infection: role of prostaglandin E2. PLoS Pathog 13(11):e1006737

Arkin MR et al (2003) Binding of small molecules to an adaptive protein-protein interface. Proc Natl Acad Sci U S A 100(4):1603–1608

Arkin MR, Tang Y, Wells JA (2014) Small-molecule inhibitors of protein-protein interactions: progressing toward the reality. Chem Biol 21(9):1102–1114

Askmyr M et al (2013) Selective killing of candidate AML stem cells by antibody targeting of IL1RAP. Blood 121 (18):3709–3713

Bachelez H et al (2019) Inhibition of the interleukin-36 pathway for the treatment of generalized pustular psoriasis. N Engl J Med 380(10):981–983

Bal E et al (2017) Mutation in IL36RN impairs the processing and regulatory function of the interleukin-36-receptor antagonist and is associated with DITRA syndrome. Exp Dermatol

Barton JL et al (2000) A tissue specific IL-1 receptor antagonist homolog from the IL-1 cluster lacks IL-1, IL-1ra, IL-18 and IL-18 antagonist activities. Eur J Immunol 30(11):3299–3308

Belaaouaj A, Kim KS, Shapiro SD (2000) Degradation of outer membrane protein a in Escherichia coli killing by neutrophil elastase. Science 289(5482):1185–1188

Bensen JT et al (2001) Identification of a novel human cytokine gene in the interleukin gene cluster on chromosome 2q12–14. J Interf Cytokine Res 21 (11):899–904

Berglof E et al (2003) IL-1Rrp2 expression and IL-1F9 (IL-1H1) actions in brain cells. J Neuroimmunol 139 (1–2):36–43

Blumberg H et al (2007) Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. J Exp Med 204(11):2603–2614

Blumberg H et al (2010) IL-1RL2 and its ligands contribute to the cytokine network in psoriasis. J Immunol 185 (7):4354–4362

Boutet MA et al (2016) Distinct expression of interleukin (IL)-36alpha, beta and gamma, their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis and Crohn's disease. Clin Exp Immunol 184 (2):159–173

Bozoyan L et al (2015) Interleukin-36gamma is expressed by neutrophils and can activate microglia, but has no role in experimental autoimmune encephalomyelitis. J Neuroinflammation 12:173

Brunner PM et al (2018) Early-onset pediatric atopic dermatitis is characterized by TH2/TH17/TH22-centered inflammation and lipid alterations. J Allergy Clin Immunol 141(6):2094–2106

Buhl AL, Wenzel J (2019) Interleukin-36 in infectious and inflammatory skin diseases. Front Immunol 10:1162

Busfield SJ et al (2000) Identification and gene organization of three novel members of the IL-1 family on human chromosome 2. Genomics 66(2):213–216

Carrier Y et al (2011) Inter-regulation of Th17 cytokines and the IL-36 cytokines in vitro and in vivo:

implications in psoriasis pathogenesis. J Invest Dermatol 131(12):2428–2437
Chen Z et al (2016) Increased concentrations of soluble B7-H3 and interleukin 36 in bronchoalveolar lavage fluid of children with mycoplasma pneumoniae pneumonia. BMC Infect Dis 16:212
Clancy DM et al (2017) Neutrophil extracellular traps can serve as platforms for processing and activation of IL-1 family cytokines. FEBS J 284(11):1712–1725
Clancy DM et al (2018) Extracellular neutrophil proteases are efficient regulators of IL-1, IL-33, and IL-36 cytokine activity but poor effectors of microbial killing. Cell Rep 22(11):2937–2950
Dale M, Nicklin MJ (1999) Interleukin-1 receptor cluster: gene organization of IL1R2, IL1R1, IL1RL2 (IL-1Rrp2), IL1RL1 (T1/ST2), and IL18R1 (IL-1Rrp) on human chromosome 2q. Genomics 57(1):177–179
Debets R et al (2001) Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. J Immunol 167(3):1440–1446
Derer A et al (2014) Blockade of IL-36 receptor signaling does not prevent from TNF-induced arthritis. PLoS One 9(8):e101954
Dietrich D, Gabay C (2014) Inflammation: IL-36 has proinflammatory effects in skin but not in joints. Nat Rev Rheumatol 10(11):639–640
Dunn EF et al (2003) High-resolution structure of murine interleukin 1 homologue IL-1F5 reveals unique loop conformations for receptor binding specificity. Biochemistry 42(37):10938–10944
Dunne A, O'Neill LA (2003) The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. Sci STKE 2003 (171):re3
Farooq M et al (2013) Mutation analysis of the IL36RN gene in 14 Japanese patients with generalized pustular psoriasis. Hum Mutat 34(1):176–183
Fields JK, Gunther S, Sundberg EJ (2019) Structural basis of IL-1 family cytokine Signaling. Front Immunol 10:1412
Fonseca-Camarillo G et al (2018) Differential expression of IL-36 family members and IL-38 by immune and nonimmune cells in patients with active inflammatory bowel disease. Biomed Res Int 2018:5140691
Frey S et al (2013) The novel cytokine interleukin-36alpha is expressed in psoriatic and rheumatoid arthritis synovium. Ann Rheum Dis 72(9):1569–1574
Gabay C, Towne JE (2015) Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions. J Leukoc Biol 97(4):645–652
Ganesan R et al (2017) Generation and functional characterization of anti-human and anti-mouse IL-36R antagonist monoclonal antibodies. MAbs 9(7):1143–1154
Gardner JK, Herbst-Kralovetz MM (2018) IL-36gamma induces a transient HSV-2 resistant environment that protects against genital disease and pathogenesis. Cytokine 111:63–71
Ghayur T et al (1997) Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. Nature 386(6625):619–623
Gresnigt MS et al (2013) The IL-36 receptor pathway regulates Aspergillus fumigatus-induced Th1 and Th17 responses. Eur J Immunol 43(2):416–426
Gunther S, Sundberg EJ (2014) Molecular determinants of agonist and antagonist signaling through the IL-36 receptor. J Immunol 193(2):921–930
Gunther S et al (2017) IL-1 family cytokines use distinct molecular mechanisms to signal through their shared co-receptor. Immunity 47(3):510–523.e4
Guo J et al (2019) Cathepsin G cleaves and activates IL-36gamma and promotes the inflammation of psoriasis. Drug Des Devel Ther 13:581–588
Harusato A et al (2017) IL-36gamma signaling controls the induced regulatory T cell-Th9 cell balance via NFkappaB activation and STAT transcription factors. Mucosal Immunol 10(6):1455–1467
He WT et al (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1beta secretion. Cell Res 25(12):1285–1298
Henry CM et al (2016) Neutrophil-derived proteases escalate inflammation through activation of IL-36 family cytokines. Cell Rep 14(4):708–722
Højen JF et al (2019) IL-1R3 blockade broadly attenuates the functions of six members of the IL-1 family, revealing their contribution to models of disease. Nat Immunol
Irina Khanskaya, J.P., Margaret H Marino, Traci Savall, Jie Li, Marco Londei (2018) A phase 1 study of ANB019, an anti-IL-36 receptor monoclonal antibody, in healthy volunteers. Available from https://www2.anaptysbio.com/wp-content/uploads/ANB019-Phase-1-Study-Poster-EAACI-2018.pdf
Jaras M et al (2010) Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. Proc Natl Acad Sci U S A 107(37):16280–16285
Johnston A et al (2011) IL-1F5, -F6, -F8, and -F9: a novel IL-1 family signaling system that is active in psoriasis and promotes keratinocyte antimicrobial peptide expression. J Immunol 186(4):2613–2622
Kanda T et al (2015) Interleukin(IL)-36alpha and IL-36gamma induce proinflammatory mediators from human colonic subepithelial myofibroblasts. Front Med (Lausanne) 2:69
Kayagaki N et al (2015) Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature 526(7575):666–671
Klementiev B et al (2014) Anti-inflammatory properties of a novel peptide interleukin 1 receptor antagonist. J Neuroinflammation 11:27
Kovach MA et al (2016) IL-36gamma is secreted in microparticles and exosomes by lung macrophages in response to bacteria and bacterial components. J Leukoc Biol 100(2):413–421
Kovach MA et al (2017) IL-36gamma is a crucial proximal component of protective type-1-mediated lung

mucosal immunity in gram-positive and -negative bacterial pneumonia. Mucosal Immunol 10(5):1320–1334
Kumar S et al (2000) Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem 275(14):10308–10314
Lamacchia C et al (2013) The severity of experimental arthritis is independent of IL-36 receptor signaling. Arthritis Res Ther 15(2):R38
Li N et al (2014) Alarmin function of cathelicidin antimicrobial peptide LL37 through IL-36gamma induction in human epidermal keratinocytes. J Immunol 193 (10):5140–5148
Li T et al (2019) TGF-beta type 2 receptor-mediated modulation of the IL-36 family can be therapeutically targeted in osteoarthritis. Sci Transl Med 11(491)
Lian LH et al (2012) The double-stranded RNA analogue polyinosinic-polycytidylic acid induces keratinocyte pyroptosis and release of IL-36gamma. J Invest Dermatol 132(5):1346–1353
Lin H et al (2001) Cloning and characterization of IL-1HY2, a novel interleukin-1 family member. J Biol Chem 276(23):20597–20602
Liu X et al (2013) Structural insights into the interaction of IL-33 with its receptors. Proc Natl Acad Sci U S A 110 (37):14918–14923
Liu H et al (2017) Staphylococcus aureus Epicutaneous exposure drives skin inflammation via IL-36-mediated T cell responses. Cell Host Microbe 22(5):653–666. e5
Lovenberg TW et al (1996) Cloning of a cDNA encoding a novel interleukin-1 receptor related protein (IL 1R-rp2). J Neuroimmunol 70(2):113–122
Macleod T et al (2016) Neutrophil elastase-mediated proteolysis activates the anti-inflammatory cytokine IL-36 receptor antagonist. Sci Rep 6:24880
Magne D et al (2006) The new IL-1 family member IL-1F8 stimulates production of inflammatory mediators by synovial fibroblasts and articular chondrocytes. Arthritis Res Ther 8(3):R80
Mahil SK et al (2017) An analysis of IL-36 signature genes and individuals with IL1RL2 knockout mutations validates IL-36 as a psoriasis therapeutic target. Sci Transl Med 9(411)
Malik K et al (2019) Ichthyosis molecular fingerprinting shows profound TH17 skewing and a unique barrier genomic signature. J Allergy Clin Immunol 143 (2):604–618
Marrakchi S et al (2011) Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. N Engl J Med 365(7):620–628
Martin U et al (2009) Externalization of the leaderless cytokine IL-1F6 occurs in response to lipopolysaccharide/ATP activation of transduced bone marrow macrophages. J Immunol 183(6):4021–4030
Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 10(2):417–426
Mattii M et al (2013) The balance between pro- and anti-inflammatory cytokines is crucial in human allergic contact dermatitis pathogenesis: the role of IL-1 family members. Exp Dermatol 22(12):813–819
Medina-Contreras O et al (2016) Cutting edge: IL-36 receptor promotes resolution of intestinal damage. J Immunol 196(1):34–38
Medzhitov R et al (1998) MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell 2(2):253–258
Mercurio L et al (2018) IL-38 has an anti-inflammatory action in psoriasis and its expression correlates with disease severity and therapeutic response to anti-IL-17A treatment. Cell Death Dis 9(11):1104
Miller LS et al (2006) MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity 24(1):79–91
Milora KA et al (2017) Interleukin-36beta provides protection against HSV-1 infection, but does not modulate initiation of adaptive immune responses. Sci Rep 7 (1):5799
Mulero JJ et al (1999) IL1HY1: a novel interleukin-1 receptor antagonist gene. Biochem Biophys Res Commun 263(3):702–706
Mutamba S et al (2012) Expression of IL-1Rrp2 by human myelomonocytic cells is unique to DCs and facilitates DC maturation by IL-1F8 and IL-1F9. Eur J Immunol 42(3):607–617
Nakagawa S et al (2017) Staphylococcus aureus virulent PSMalpha peptides induce keratinocyte Alarmin release to orchestrate IL-17-dependent skin inflammation. Cell Host Microbe 22(5):667–677. e5
Nanjo Y et al (2019) Overlapping roles for interleukin-36 cytokines in protective host defense against murine Legionella pneumophila pneumonia. Infect Immun 87 (1)
Ngo VL et al (2018) A cytokine network involving IL-36gamma, IL-23, and IL-22 promotes antimicrobial defense and recovery from intestinal barrier damage. Proc Natl Acad Sci U S A 115(22):E5076–E5085
Nicklin MJ et al (2002) A sequence-based map of the nine genes of the human interleukin-1 cluster. Genomics 79 (5):718–725
Nishida A et al (2016) Increased expression of Interleukin-36, a member of the interleukin-1 cytokine family, in inflammatory bowel disease. Inflamm Bowel Dis 22 (2):303–314
Onoufriadis A et al (2011) Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet 89(3):432–437
Ozaki K, Leonard WJ (2002) Cytokine and cytokine receptor pleiotropy and redundancy. J Biol Chem 277 (33):29355–29358
Qin J et al (2005) SIGIRR inhibits interleukin-1 receptor- and toll-like receptor 4-mediated signaling through

different mechanisms. J Biol Chem 280 (26):25233–25241
Quiniou C et al (2008) Development of a novel noncompetitive antagonist of IL-1 receptor. J Immunol 180 (10):6977–6987
Ramadas RA et al (2011) Interleukin-1 family member 9 stimulates chemokine production and neutrophil influx in mouse lungs. Am J Respir Cell Mol Biol 44 (2):134–145
Ramadas RA et al (2012) IL-36alpha exerts pro-inflammatory effects in the lungs of mice. PLoS One 7(9):e45784
Rivers-Auty J et al (2018) Redefining the ancestral origins of the interleukin-1 superfamily. Nat Commun 9 (1):1156
Russell SE et al (2016) IL-36alpha expression is elevated in ulcerative colitis and promotes colonic inflammation. Mucosal Immunol 9(5):1193–1204
Saha SS et al (2015) Signal transduction and intracellular trafficking by the interleukin 36 receptor. J Biol Chem 290(39):23997–24006
Scheibe K et al (2017) IL-36R signalling activates intestinal epithelial cells and fibroblasts and promotes mucosal healing in vivo. Gut 66(5):823–838
Scheibe K et al (2019) Inhibiting interleukin 36 receptor signaling reduces fibrosis in mice with chronic intestinal inflammation. Gastroenterology 156 (4):1082–1097. e11
Segueni N et al (2015) Limited contribution of IL-36 versus IL-1 and TNF pathways in host response to mycobacterial infection. PLoS One 10(5):e0126058
Sehat M et al (2018) Evaluating serum levels of IL-33, IL-36, IL-37 and gene expression of IL-37 in patients with psoriasis vulgaris. Iran J Allergy Asthma Immunol 17(2):179–187
Shi J et al (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526 (7575):660–665
Sievers F et al (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal omega. Mol Syst Biol 7:539. https://doi.org/10.1038/msb.2011.75
Smith DE et al (2000) Four new members expand the interleukin-1 superfamily. J Biol Chem 275 (2):1169–1175
Song Y et al (2019) Interleukin-36 alpha levels are elevated in the serum and cerebrospinal fluid of patients with neuromyelitis optica spectrum disorder and correlate with disease activity. Immunobiology
Spangler JB et al (2015) Insights into cytokine-receptor interactions from cytokine engineering. Annu Rev Immunol 33:139–167
Su Z et al (2019) IL-36 receptor antagonistic antibodies inhibit inflammatory responses in preclinical models of psoriasiform dermatitis. Exp Dermatol 28(2):113–120
Sullivan GP et al (2018) Identification of small-molecule elastase inhibitors as antagonists of IL-36 cytokine activation. FEBS Open Bio 8(5):751–763
Sundberg TB et al (2014) Small-molecule control of cytokine function: new opportunities for treating immune disorders. Curr Opin Chem Biol 23:23–30
Swindell WR et al (2018) RNA-Seq analysis of IL-1B and IL-36 responses in epidermal keratinocytes identifies a shared MyD88-dependent gene signature. Front Immunol 9:80
Taylor SL et al (2002) Genomic organization of the interleukin-1 locus. Genomics 79(5):726–733
Thomas C, Bazan JF, Garcia KC (2012) Structure of the activating IL-1 receptor signaling complex. Nat Struct Mol Biol 19(4):455–457
Tiwari RL et al (2011) IL-1R-associated kinase-1 mediates protein kinase Cdelta-induced IL-1beta production in monocytes. J Immunol 187(5):2632–2645
Todorovic V et al (2019) Small molecule IL-36gamma antagonist as a novel therapeutic approach for plaque psoriasis. Sci Rep 9(1):9089
Tomuschat C et al (2017) Altered expression of IL36gamma and IL36 receptor (IL1RL2) in the colon of patients with Hirschsprung's disease. Pediatr Surg Int 33(2):181–186
Tortola L et al (2012) Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. J Clin Invest 122(11):3965–3976
Towne JE et al (2004) Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. J Biol Chem 279(14):13677–13688
Towne JE et al (2011) Interleukin-36 (IL-36) ligands require processing for full agonist (IL-36alpha, IL-36beta, and IL-36gamma) or antagonist (IL-36Ra) activity. J Biol Chem 286(49):42594–42602
Tsutsumi N et al (2014) The structural basis for receptor recognition of human interleukin-18. Nat Commun 5:5340
van de Veerdonk FL et al (2012) IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. Proc Natl Acad Sci U S A 109(8):3001–3005
van de Veerdonk FL et al (2018) Biology of IL-38 and its role in disease. Immunol Rev 281(1):191–196
Vigne S et al (2011) IL-36R ligands are potent regulators of dendritic and T cells. Blood 118(22):5813–5823
Wang P et al (2005) The interleukin-1-related cytokine IL-1F8 is expressed in glial cells, but fails to induce IL-1beta signalling responses. Cytokine 29 (6):245–250
Wang D et al (2010) Structural insights into the assembly and activation of IL-1beta with its receptors. Nat Immunol 11(10):905–911
Weinrauch Y et al (2002) Neutrophil elastase targets virulence factors of enterobacteria. Nature 417 (6884):91–94
Whitty A, Zhou L (2015) Horses for courses: reaching outside drug-like chemical space for inhibitors of challenging drug targets. Future Med Chem 7 (9):1093–1095

Yang CY (2015) Identification of potential small molecule allosteric modulator sites on IL-1R1 ectodomain using accelerated conformational sampling method. PLoS One 10(2):e0118671

Yi G et al (2016) Structural and functional attributes of the Interleukin-36 receptor. J Biol Chem 291 (32):16597–16609

Yoon DY, Dinarello CA (1998) Antibodies to domains II and III of the IL-1 receptor accessory protein inhibit IL-1 beta activity but not binding: regulation of IL-1 responses is via type I receptor, not the accessory protein. J Immunol 160(7):3170–3179

Zhang J et al (2017) IL-36 induces cytokine IL-6 and chemokine CXCL8 expression in human lung tissue cells: implications for pulmonary inflammatory responses. Cytokine 99:114–123

Zheng P et al (2018) Synthetic human monoclonal antibody targets hIL1 receptor accessory protein chain with therapeutic potential in triple-negative breast cancer. Biomed Pharmacother 107:1064–1073

Zhou L et al (2018) Quantitative ligand and receptor binding studies reveal the mechanism of interleukin-36 (IL-36) pathway activation. J Biol Chem 293 (2):403–411

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