What Do We Really Know and What Do We Need to Know: Some Controversies, Perspectives, and Surprises

Bernd Nilius and Veit Flockerzi

Contents

B. Nilius (✉)
Department Cell Mol Medicine, Laboratory Ion Channel Research, KU Leuven, Campus Gasthuisberg, O&N 1, Herestraat 49-Bus 802, 3000 Leuven, Belgium
e-mail: bernd.nilius@med.kuleuven.be

V. Flockerzi
Universität des Saarlandes, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Gebäude 46, 66421 Homburg, Germany
e-mail: veit.flockerzi@uks.eu

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Abstract

TRP channels comprise one of the most rapid growing research topics in ion channel research, in fields related to ion channels including channelopathies and translational medicine. We provide here a critical survey on our current knowledge of TRP channels and highlight some of the still open or controversial questions. This comprises questions related to evolution of TRP channels; biophysics, i.e., permeation; pore properties and gating; modulation; the still-elusive 3D structure; and channel subunits but also their role as general sensory channels and in human diseases. We will conclude that our knowledge on TRP channels is still at the very beginning of an exciting research journey.

Keywords

TRP channels • Evolution • Pores • Gating • Associated proteins • Subunits • Modulation by calcium • Sensory physiology • Phosphoinositides • Channelopathy • TRPs in networks

1 Introduction

There is no doubt: The discovery of the *trp* gene superfamily which encodes TRP (transient receptor potential) cation channels has opened a highly exciting field to understand physiological and pathophysiological basic mechanisms of cell functions which were until this time inaccessible. This book reviews many examples reaching from sensory physiology, such as vision, hearing, and touch, to homeostatic functions such as Ca^{2+} and Mg^{2+} (re)absorption and thermoregulation and to hereditary diseases ranging from stationary night blindness to neurodegenerative disease, kidney diseases, chronic pain, and skeletal abnormalities (reviewed in Nilius and Owsianik 2010b, 2011; Nilius et al. 2007). If we date the advent of "TRP research" with the isolation of the Drosophila "trp" (transient receptor potential) mutant (Cosens and Manning 1969), approximately 20 years passed until the Drosophila *trp* gene was cloned (Montell and Rubin 1989) and a role of TRP ion channels was explicitly mentioned (Hardie and Minke 1992, 1993). Now, again 20 years later, the progress in this field is overwhelming, but there are still many open questions, controversies, and surprises. Only some of them can be addressed in such a review, and we do not claim any complete view but will provide a few snapshots.

2 The Basics: Nomenclature

As always in the beginning of a rapidly extending research field, some Babylonian language disasters happened. Some TRP clones received up to five different names, e.g., OTRPC4, VR-OAC, TRP12, VRL-2, and presently TRPV4 (Everaerts

et al. 2010). Fortunately, this Babylonian disaster was at least partially solved by agreeing on a unifying nomenclature based on homology (Montell et al. 2002). All 8 subfamilies were given the name TRP (for transient receptor potential, maybe not the best decision) followed by the subfamily indicators, C, V, M, A, P, ML, and N.

However, one problem recently appeared: The polycystin family comprises 5 TRPPs (TRPP1–5). However, only three are really channel proteins. Therefore, it was suggested by a recent review from the Clapham group that this family only contains the three channel members, TRPP1 (previously known as TRPP2 or PKD2, PC2 (polycystin 2) derived from the second gene which causes autosomal dominant polycystic kidney disease (ADPKD)), TRPP2 (previously TRPP3, polycystin-like (PCL) or PKD2-like 1 (PKD2L1)), and TRPP3 (also known as TRPP5 or PKD2L2) (Wu et al. 2010). The former TRPP1 (PKD1, PC1) and TRPP4 (PKD1L1) have been eliminated because they are indeed glycoproteins which contain a large N-terminal extracellular region and multiple transmembrane domains and have no homology with ion channels and also do not function as channels. This seems to be more logical but confuses again the ADPKD community. The HUGO nomenclature still considers the old description as TRPP2, TRPP3, and TRPP5 as the TRP family polycystin members. So we stick to the HUGO nomenclature throughout this volume.

3 Some Evolutionary Puzzles

TRPs appear relatively late in the evolution. No TRPs are expressed in bacteria in contrast to most of the well-described voltage-dependent ion channels (Fig. 1). They are expressed in some algae, yeast, and other fungi. Expression occurs in the unicellular choanoflagellate *Monosiga brevicollis* and importantly in many mammalian parasites. Five metazoan TRP subfamily members (TRPA, TRPC, TRPM, TRPML, and TRPV) were identified in choanoflagellates, demonstrating that they evolved before the emergence of multicellular animals (Fig. 1). One puzzle is the complete absence in plants (see for details Cai 2008; Prole and Taylor 2011, 2012). But why make these plants drugs for TRPs, like capsaicin, tetrahydrocannabinol, nicotine, and all the other spices being effective TRP modulators (Nilius and Appendino 2011, 2013)? They make them as "secondary metabolites" which are not essential for normal growth and development of the plant but appear to function in the defense of plants against herbivores and pathogens. TRPs are widely expressed in insects which may be targeted by these plant compounds (Matsuura et al. 2009). For example, a plant can produce substances that result in adverse physiological effects in the insect, such as a bitter taste or even poisoning. Ironically, the medicinal applications and pharmacological effects of secondary metabolites in animals (insects) and humans are better understood than their functions in the plants that produce them (Liscombe and Facchini 2008).

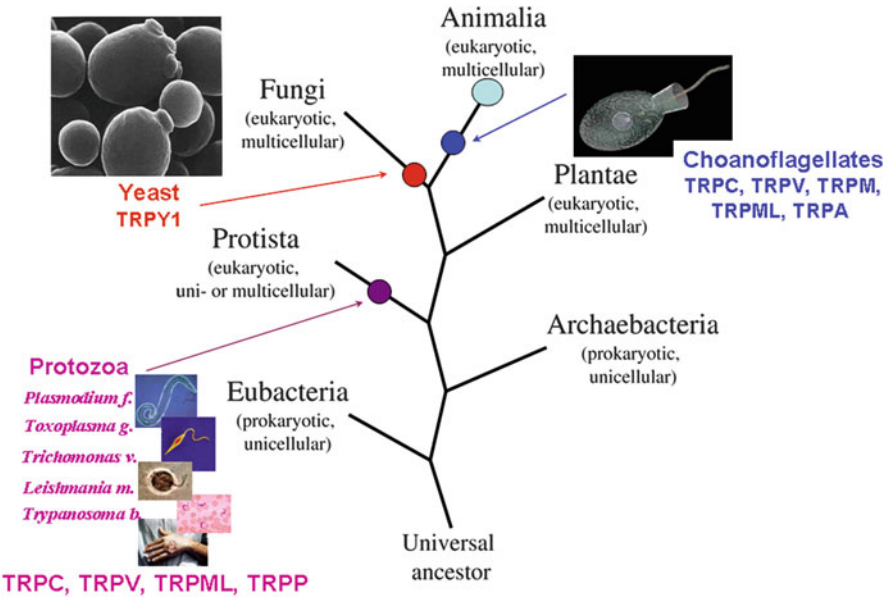

Fig. 1 TRPs in the kingdom of life and the appearance of TRPs (*circles*). See for detailed information (Cai 2008; Ihara et al. 2013; Prole and Taylor 2011, 2012; Wheeler and Brownlee 2008)

4 Do We Have the Correct Clones? Contribution of Endogenous TRPs?

There is a certain discrepancy between annotated TRP entries in protein and gene databases (e.g., UniProtKB and Ensembl Genome Browser), TRP cDNAs used for functional expression studies in vitro, and, apparently, TRP proteins expressed in vivo. For example, for human TRPC3, most in vitro studies have been performed with a cDNA encoding an 848-amino acid (aa) protein which is different to the 836-aa TRPC3 UniProtKB entry and the predicted 921-aa TRPC3 entry in the Ensembl Genome Browser. For human TRPC1, most in vitro studies have been done with the TRPC1beta variant (759 aa, Zitt et al. 1996) or with the TRPC1alfa variant (793 aa, Zhu et al. 1995), although the endogenous human TRPC1 protein may well cover an additional ~100 aa extension at its N-terminus (Ong et al. 2013). In fact, the identity of the endogenous TRP proteins is only known to a certain level of approximation: There is little direct experimental evidence to what extent the DNA sequences are translated to real proteins. This limitation refers not only to TRP channel genes but to most protein-coding genes in humans and many other organisms. In 2013, more than 95 % of protein sequences provided by the UniProt resource came from translations of coding sequences generated by gene prediction programs. Only less than 5 % of the protein entries rely on sequence data obtained by direct protein sequencing, by Edman degradation or MS/MS experiments (http://www.uniprot.org). New developments in protein analytical methods including

mass spectrometry-based targeted proteomics will improve this situation in the future; but current approaches heavily rely on antibodies to pull out the protein of interest from complex biological samples. Using three independent antibodies, the endogenous protein sequence of TRPV6 could be obtained by this approach (Fecher-Trost et al. 2013). Compared to the 725-amino acid (aa) annotated sequence, the "real" TRPV6 protein contained a 40-aa extension within its N-terminus. However, adequate antibodies for most TRP proteins are not generally available (Wu et al. 2010), and therefore protein sequence information on most TRPs using mass spectrometry based has still to be shown.

Because of their convenience, many laboratories use HEK293 cells for functional expression of TRP cDNAs. *Trpc1* (Zhu et al. 1995) and the *Trpc3* cDNAs (Zhu et al. 1996) were originally cloned from HEK293 mRNA. It cannot be avoided but is hardly considered in the interpretation of data that the endogenous TRP proteins of HEK cells (TRPC1,TRPC3, and, maybe, TRPC7 and TRPM4) associate with the TRP proteins of expressed cDNAs to form functional channel complexes (Zagranichnaya et al. 2005). Considering the assumed broad expression of *Trp* genes, a similar situation will exist in most cell lines used for heterologous expression studies.

5 The Still-Elusive 3D Structure

It is agreed that all TRP consists of intracellular N- and C-termini and has six transmembrane spanning helices (TM) in which TM5 and TM6 probably form a classical channel pore. So far, only parts of TRP proteins have been crystallized, and the whole protein structure for some TRPs can be approached in electron cryomicroscopy pictures in a not very high resolution, e.g., for TRPV1 in a 19 Å resolution (Moiseenkova-Bell et al. 2008). Only 30 % of the channel protein is located in the plasma membrane (transmembrane domain TM); the other part forms a large cytosolic bulk. Both parts are connected by a fenestrated domain which might be important as an intracellular access to the TM region. Typical for the TRPV family and for TRPA1 is the N-terminal ankyrin repeat domain (ARD) (Fig. 3). TRPVs have an ARD formed by 6 ankyrin repeats (ARs) consisting of an inner and outer helix connected by unusual long "fingers" at the ARD's "concave" side (Gaudet 2008a, b) which are considered to be quite flexible. TRPA1 is proposed to have an ARD consisting of 14–19 ARs (Wang et al. 2012). It is highly speculative whether this domain is involved in gating (probably for TRPA1 via covalent cys/lys modification) or whether it is mainly required for channel trafficking and insertion in the plasma membrane (Nilius et al. 2011) (Fig. 2).

Thus, the functional role of the ARD is still unknown. It provides ATP and calmodulin binding which interferes in the TRPV family with channel activation and desensitization (inactivation) (Lishko et al. 2007; Phelps et al. 2007, 2008, 2010). Characteristically, ankyrin repeats are connected by large "fingers" which might be important for channel–protein interactions. The ARD is involved in TRPV4 dysfunction in several channelopathies (Inada et al. 2012). Interestingly,

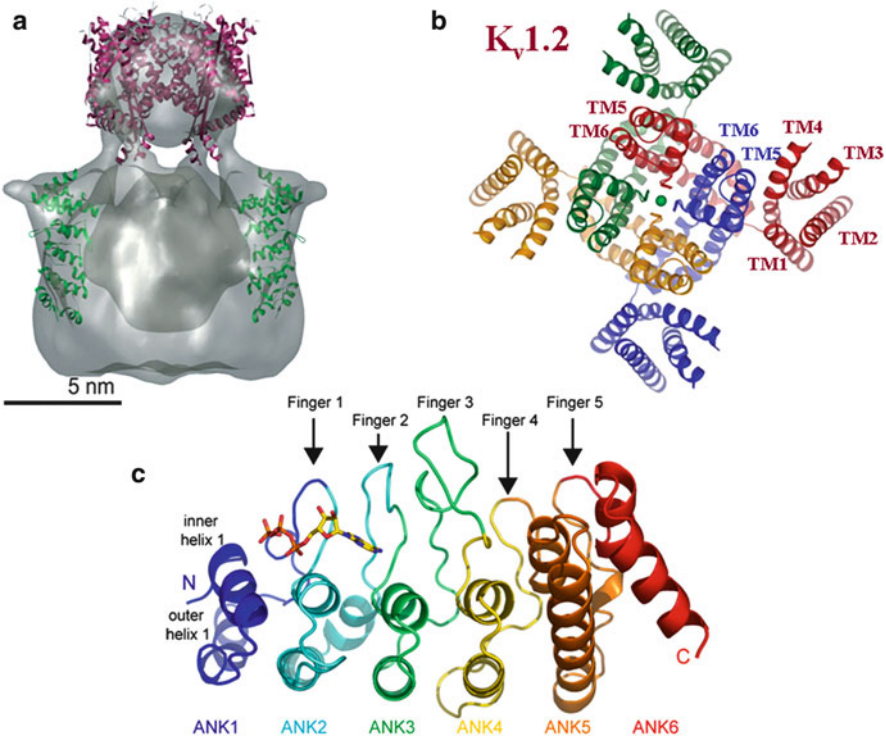

Fig. 2 Structure of TRPV1. (**a**) 3D reconstruction of TRPV1 obtained from cryo-electron microscopy. Inserted are in the plasma membrane part the atomic structures of Kv1.2 potassium channel (TM1–TM4, *pink*) and in the cytosolic part the TRPV1 ankyrin repeats (*green*). See the nice match of these structures. (**b**) High-resolution structure of Kv1.2 transmembrane domains. (**c**) TRPV1 ankyrin domains. Indicated is the ATP-binding site in TRPV1 [adapted from Lishko et al. (2007); Long et al. (2005); Moiseenkova-Bell et al. (2008) with permission]

the TM1-4 domains in TRPV1, TRPM4, and TRPM8 have some structural similarities with the TM domain of Kv1.2 including its voltage sensor (Nilius et al. 2005b). These TMs also nicely fit into the 3D structure of the transmembrane part of TRPV1 (Moiseenkova-Bell et al. 2008). It is extremely important to decipher a role of a potential voltage sensor in the TM region and the functional role of ARD in channel gating and trafficking. We refer to new and exciting data obtained from a 3D analysis of TRPV1 with a 3.4 A resolution to Chap. 11 in this book. *"High-resolution views of TRPV1 and their implications for the TRP superfamily" by Ute. A. Hellmicu and Rachelle Gaudet*.

All TRPM channels have coiled-coil domains, i.e., α-helices which form helical bundles of a preferred stoichiometry. This domain was crystallized in the C-terminus of TRPM7 where it is proposed to trigger tetramerization (Fujiwara and Minor 2008; Tsuruda et al. 2006). A similar domain in TRPP2 (PKD2) has been crystallographically analyzed, and the structure provides insight into TRPP2–PKD1

heteromerization (Yu et al. 2009; Zhu et al. 2011). Also the 3D structure of the TRPM7 kinase domain was analyzed by crystallography (Yamaguchi et al. 2001). However, these are only some snapshots into the TRP structure. Thus, the high-resolution 3D structure is highly expected to solve one of the mysteries of TRP channel gating.

6 α-Subunits and Elusive β-Subunits

Most of the TRP channels are most probably tetramers as shown first for TRPV1 (Kedei et al. 2001) and later for TRPV5 and TRPV6 (Hoenderop et al. 2003). Probably, nearly all TRPs form tetrahomomers. Several heteromeric TRP channels have been described. TRPP2 forms a trimer with PKD1 (for a review see Zheng 2013). Heteromeric channels are still a matter of debate, and their functional role must be evaluated. In the classical view, these tetramers are formed from the pore-carrying α-subunit. The mechanism of co-assembly is still not solved, although several mechanisms have been proposed. N-terminal ARD domains are protein–protein interaction sites (Sedgwick and Smerdon 1999). However, its role for α-subunit assembly is very much uncertain because isolated ARDs from TRPVs do not interact (Lishko et al. 2007; Phelps et al. 2007, 2008, 2010; Zhang et al. 2011) and TRPV1 and TRPV2 crystals do not multimerize (Jin et al. 2006; Lishko et al. 2007). Also for TRPA1, evidence has been reported that the ARD probably controls membrane insertion of the channels rather than tetramerization (Nilius et al. 2011). The role of coiled-coil domains for multimerization of TRPM channels has been discussed above. It is also reported that for TRPVs a region proximal of the TRP box might be required for subunit assembly (Zhang et al. 2011; Garcia-Sanz et al. 2004). Obviously, we do not yet understand the assembly of TRP subunits to form functional channel.

Another pertinent urgent problem to solve is the existence and possible function of β-subunits, i.e., proteins which bind to the α-subunit and modulate channel function by direct interference with channel gating or regulation of membrane insertion as studied extensively for voltage-operated Ca^{2+} channels (Richards et al. 2004; Hofmann et al. 2014). Such subunits are not yet discovered for TRP channels. A putative β-subunit, although not yet confirmed in detail, might be PACSIN 3, a member of a protein family that has been implicated in synaptic vesicular membrane trafficking and regulation of dynamin-mediated endocytotic processes. PACSIN 3 binds via its SH3 domain to a site in the proline-rich domain (PRD) proximal to the ARD in TRPV4 and not only increases channel insertion in the plasma membrane but also selects the activation mode of this channel, i.e., it suppressed activation by cell swelling which is thought to be an EET-dependent process (Cuajungco et al. 2006; D'hoedt et al. 2007).

A pertinent question is not yet answered: Is TRPC1 α-subunit? Originally described as a cation channel activated by store depletion (Mori et al. 2002; Zitt et al. 1996), it was later shown that TRPC1 proteins do not form functional channels by themselves (Storch et al. 2012; Strubing et al. 2001) but contribute to

heterotetrameric TRPC channels, preferably with TRPC4 and TRPC5 (Hofmann et al. 2002; Strubing et al. 2001). Apparently, TRPC4 and TRPC5 but also TRPP2 (Tsiokas et al. 1999) and TRPV4 (Ma et al. 2010, 2011) pick up TRPC1 from the ER to the plasma membrane and the primary cilium (Bai et al. 2008), whereas in their absence TRPC1 may act as a leak channel of the ER (Berbey et al. 2009). Recently, it was shown that the full-length endogenous TRPC1 amplifies Ca^{2+} release-activated Ca^{2+} currents mediated by Orai channels (Ong et al. 2013) by direct interactions with components of the store-operated Ca^{2+} entry machinery (Liao et al. 2008; Singh et al. 2002; Yuan et al. 2007). The *Trpc1* gene is supposed to be ubiquitously expressed, but how can one tell considering that there are no TRPC1 ion currents nor appropriate antibodies?

Another surprising example concerns the *Xenopus* TRPV6 homologue. xTRPC1 interacts with xTRPV6, thereby inhibiting the channel. TRPC1 is probably not part of the channel but regulates the activity of TRPV6 under physiological conditions (Schindl et al. 2012; Courjaret et al. 2013). Obviously, the search for TRP β-subunits is another high priority in TRP channel research.

Recently, it has been shown that an alternative splice variant of the mouse *Trpa1* gene (TRPA1b) can physically interact with the main variant *Trpa1* and increases the expression of TRPA1a in the plasma membrane. TRPA1a and TRPA1b co-expression significantly increases current density in response to different agonists without affecting their single-channel conductance. TRPA1 is obviously regulated through alternative splicing under physiological and also pathological conditions (Zhou et al. 2013). Thus, can such a variant in fact be considered as a β-subunit?

Another example came as a surprise. TRPM4 interacts with the sulfonylurea receptor 1 SUR1 and may confer the sensitivity of glibenclamide to this channel (Sala-Rabanal et al. 2012; Woo et al. 2013).

If a β-subunit is mainly defined as non-pore forming but with a positive effect on plasma membrane insertion of the α-subunit, then a β-subunit of voltage-dependent K^+ channels, Kvβ2, must be considered as a novel TRP β-subunit. Kvβ2 interacts with TRPV1, increases the cell surface expression levels of this channel, and results in a significant increase of TRPV1 sensitivity to capsaicin, as functionally measured with patch clamp. Thus, Kvβ2 plays a role in TRPV1 channel trafficking to the plasma membrane (Bavassano et al. 2013). It is now intriguing to ask whether β-subunits among several channel families have a certain degree of promiscuity.

7 The Correct Expression Pattern

This is one of the main but still not completely solved problems. To solve this problem, specific TRP antibodies must be available, and transgenic models and reporter strategies must be employed to identify the correct cellular localization of TRP. The poor quality of available antibodies for TRPs has caused considerable frustration and may have led to publication and perpetuation of erroneous research results. It is true that to generate an antibody for a low abundant membrane protein

like a TRP is a painstaking effort (Meissner et al. 2011) requiring months of laborious benchwork and money for synthesizing, expressing, and purifying the antigen, for hosting the suitable animals to be immunized, for generating and selecting hybridoma cells, and for purifying and characterizing the final antibodies. Especially the analysis of the antibody specificity is crucial. Only in very few cases, this major ordeal is accomplished by the many commercial suppliers that have proliferated and sell antibodies directed at a wide range of proteins including TRPs (Flockerzi et al. 2005; Ong et al. 2002, 2013; Wu et al. 2010).

In addition, we need to know the subcellular, e.g., intra-organelle, localization because intracellular TRPs are important functional players in cell organelles such as the endosomes, lysosomes, SR and ER, and Golgi apparatus (for a reviews see Cheng et al. 2010; Gees et al. 2010). Probably, also mitochondria may have TRP channels. TRPC3 is probably localized in mitochondria and carries a significant fraction of mitochondrial Ca^{2+} uptake that relies on extramitochondrial Ca^{2+} concentration. Up- and downregulation of TRPC3 expression influences also the mitochondrial membrane potential (Feng et al. 2013). It has to be noted that substantial discrepancies exist in the literature concerning TRP channel distribution. The use of mRNA detection is an indicator but has several drawbacks (Anderson and Seilhamer 1997; Anderson and Anderson 1998; Gygi et al. 1999; Pradet-Balade et al. 2001; Tew et al. 1996).

Immunohistochemistry relies on selective antibodies, which might be different from the ones used for protein detection in Western blotting. Radioactive ligands might be a complementary tool for detection of TRP channels. Now, promising is the genetic modification of a TRP channel locus which allows introduction of reporter genes such as *lacZ* or *fluorescent proteins* into living animals. Expression of these genes reflects the TRP distribution. Just as an example, many functions have been attributed to TRPV1 in the central nervous system, e.g., the development of cerebellar Purkinje cells and hippocampal pyramidal neurons and its contribution to glutamatergic synapse stabilization as important player in the neonatal cerebellar cortex and in the refinement of synaptic plasticity (Vennekens et al. 2012). It came as a big surprise that TRPV1 is probably even not expressed in these brain areas (Cavanaugh et al. 2011a, b). This causes another problem. Do we really know where the TRP channels that we wish to target are really located? A crucial test is always the detection of ion current through identified TRP channels (see below).

8 News About the Pore

As required for a channel protein, we do not understand yet the regulation of permeation. Is the pore of many TRPs in fact a dynamic structure contributing to gating much more than the known α-pore of classical channels (between TM5 and TM6)?

One of the most important functional features of many TRP pores is their permeability for Mg^{2+} and other heavy metals. The Mg^{2+} permeation is still a biophysical puzzle. So far, all ion channels refuse Mg^{2+} permeation because of its

very high dehydration energy. TRP channels therefore provide a unique Mg^{2+} entry pathway. However, the biophysics behind is not yet unraveled (for a review see Owsianik et al. 2006).

There is now growing evidence that upon agonist stimulation some TRP channels such as TRPV1 (Chung et al. 2008) and TRPA1 (Karashima et al. 2010) show a dynamic pore behavior, i.e., a dilatation of the pore upon agonist binding. This dilatation is in the range of several Å. Mechanistically important seems to be the fact that this dilation of the TRPV1 pore decreases after cholesterol depletion of the plasma membrane (Jansson et al. 2013). In TRPA1, it causes an increase in divalent cation selectivity and fractional Ca^{2+} current and is therefore functionally very important. How does an agonist dilate the pore? Pore dilation has been also described in the TRPC family. Activation of TRPC4β with the G protein $G\alpha_{i2}$ as compared with activation by GTPγS causes a small but significant dilation of the channel's pore and increases Ca^{2+} permeability like the dilation of the TRPA1 pore (Jeon et al. 2013). This dynamic pore behavior needs to be explained soon because it will shed some light in the gating behavior of TRP channels. Of note, a pore dilation from 3 to 12 Å occurs by binding of G proteins to open the G-loop gate in GIRK2 potassium channels, a mechanism that has been explained by changes of the pore structure (Whorton and MacKinnon 2011).

Another route to follow is even more spectacular. TRP channels might posses and exploit under certain circumstances other pathways for ion conduction as shown for voltage-activated potassium and sodium channels. This pathways comprise a permeation gate through the voltage-sensing domain (TM1–TM4) of the channel, the ω-pore (Starace and Bezanilla 2004; Tombola et al. 2007; Gamal El-Din et al. 2011; Jurkat-Rott et al. 2010; Prütting and Grissmer 2011). It might be possible that TRP channels have a similar pathway which is active during distinct modes of activation (Vriens et al. 2014).

9 Fractional Ca^{2+} Currents in TRPs

Importantly, most of the TRP channels have in fact a small Ca^{2+} selectivity, and the "fractional Ca^{2+} current" is often less than 5 %. However, for understanding the functional impact of TRP channels, we need to know which partition of the current under physiological condition is carried by Ca^{2+} compared with monovalent cation. We need to measure this "fraction" to check which amount of Ca^{2+} is really contributing to the current (Karashima et al. 2010; Samways and Egan 2011). Unfortunately, such measurements are rare (done for TRPV1, TRPA1, TRPM3, and TRPM8). TRPV5 and TRPV6 are the only "real" Ca^{2+} channels with fractional Ca^{2+} currents of ~100 %. TRPA1 and TRPM3, with a fractional Ca^{2+} current of ~20 %, can be considered as relatively efficient Ca^{2+} channels. Probably, all other TRP channels have fractional Ca^{2+} currents of less than 10 % (Gees et al. 2010). Importantly, TRPV1 is known as a TRP channel with a relatively high Ca^{2+} permeability ($P_{Ca}/P_{Na} \sim 10$) (Owsianik et al. 2006). However, its fractional Ca^{2+} current is only in the range of 5 % of the total current. High fractional Ca^{2+} current

so far is only measured for TRPV5, TRPV6, TRPM3, and TRPA1 (reviewed in Gees et al. 2010). Even for the Drosophila TRP and TRPL channel, which are regarded as highly Ca^{2+}-selective channels, the fractional Ca^{2+} current is only in the range of 29 % and 17 %, respectively (Chu et al. 2013).

Knowing the fractional Ca^{2+} current will also at least partially answer the questions: which increases in $[Ca^{2+}]_i$ can be expected under physiological conditions and whether it is likely that the TRP-induced depolarization is essential rather than the Ca^{2+} influx. Obviously, TRP channels are functionally coupled with Ca^{2+}-sensing effector proteins and may therefore achieve a high signal gain. It is therefore probably an important feature of TRP channels to provide only a low fractional Ca^{2+} current or in the case of high fractional Ca^{2+} current have only a low-density expression.

Obviously, these issues have to be urgently addressed for TRPC channels.

10 Gating

TRP channels have been widely described as receptor-activated channels, ligand-activated channels, and "directly" gated channels. However, some TRP channels are constitutively open, at least in several heterologous expression systems (e.g., TRPV5, TRPV6, TRPC3, TRPA1, and others) raising the question whether a crucial gating partner is missing in the expression system. This question of constitutive open TRP channels seems to be especially intriguing in the brain: TRPV4 is active at physiological brain temperature in hippocampal neurons and thereby controls their excitability in vitro. Local changes in temperature, which occur in the brain, cause modulation of brain activity, e.g., local cooling might reduce neuronal electric excitability via a decreased TRPV4 activity and thereby contribute to brain functions (Shibasaki et al. 2007; Tominaga 2013, personal communication).

The question whether TRP channels like some K_2P channels are responsible for background currents is unanswered.

Receptor activation comprises G protein-coupled receptors (GPCRs) and receptor tyrosine kinases that activate phospholipases C (PLCs) which gate/modulate TRP channel activity by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), production of diacylglycerol (DAG) or inositol 1,4,5-trisphosphate (IP3), and subsequent liberation of Ca^{2+} from intracellular stores. Ligand activation is due to exogenous small organic molecules, e.g., capsaicin, endogenous lipids, or products of lipid metabolism (diacylglycerols, phosphoinositides, eicosanoids, anandamide), and purine nucleotides and their metabolites (adenosine diphosphoribose (ADP-ribose), βNAD) but also by proteins (like G proteins for TRPCs).

Direct activation can occur via changes of temperature, changes in the membrane potential (voltage activation), and maybe also mechanical forces (Nilius and Owsianik 2011; Ramsey et al. 2006; Wu et al. 2010).

Thus, TRP channels are polymodal channels, opposite to voltage-gated channels, which only sense changes in the transmembrane electric field. TRP channel might be considered as integrators of multiple cellular signals and might be preferred coincidence detectors (Ramsey et al. 2006). However, this makes the identification of a basic gating mechanism difficult if not impossible, and probably each channel exploits multiple mechanisms to open the gate. So far, we do not have any structural insight how the gating process is initiated (Nieto-Posadas et al. 2011).

Let's first consider possibilities of direct gating. Maybe the most intriguing mechanism is TRP gating by depolarization. In this view, gating would share some similarities with the activation mechanism of classical voltage-dependent channels. However, half maximal activation occurs at very positive membrane potentials, which are physiologically irrelevant. However, several signals, like temperature changes and agonist binding, cause a shift in the activation curve toward more negative potentials, thereby increasing the fraction of open channels under steady state conditions (Nilius et al. 2005b; Voets et al. 2004). This paradigm "gating by shift" has been described for several channel types (TRPV1, TRPA1, TRPM4, TRPM8, etc.) but is mechanistically not yet understood. The shift is supported by a small apparent gating charge of TRP channels which is in the range of 0.5–0.8e (Nilius et al. 2005b; Voets et al. 2007; Zheng 2013). A proposed voltage-sensing role of basic residues in TM4 and the TM4–TM5 linker of TRPM8 might be considered as a first hint (Voets et al. 2007). Although the architectural and pharmacological similarities exist between TRP channels and Shaker K^+ channels, replacing previously identified domains and critical structural motifs of the membrane-spanning portions of Kv2.1 with corresponding regions of two TRP channels, TRPM8 and TRPV1, did not allow the formation of hybrid channels, i.e., the TM3b–TM4 paddle motif of Kv2.1 can be replaced by the analogous regions of both TRP channels without abolishing voltage activation, but swapping putative voltage-sensing TRP channel regions with the Kv2.1 voltage sensor yields non-functional channels (Kalia and Swartz 2013). Obviously, such studies should be extended. Obviously, the true voltage sensors need to be pinned down. Probably, the measurement of gating currents should be envisaged. Because of the small gating charge of TRP channels [0.4–0.9e (Nilius et al. 2005b)], better methods must be applied, e.g., the *limited slope method* (Almers 1978; Sigg and Bezanilla 1997), to measure in a low open probability range the effective gating charge or changes in gating charge caused by mutations in a putative voltage sensor (see Voets et al. 2007). From the *voltage dependence* point of view, temperature and agonists are modulators of the voltage-dependent opening of the channel gate. The true nature of this shift is still elusive. However, the existence of the open probability shift is a clear fact that needs to be explained (for a critical discussion and challenges of this simplified view see, e.g., Nieto-Posadas et al. 2011; Zheng 2013).

Direct channel gating is caused by the covalent modification of cysteine and lysine residues. Such covalent modifications of Cys and Lys residues in the N-terminus of TRPA1 by electrophiles have been extensively described (for a comprehensive review, see Nilius et al. 2012). However, there are many issues to consider. First, covalent modification by electrophilic compounds may result in the

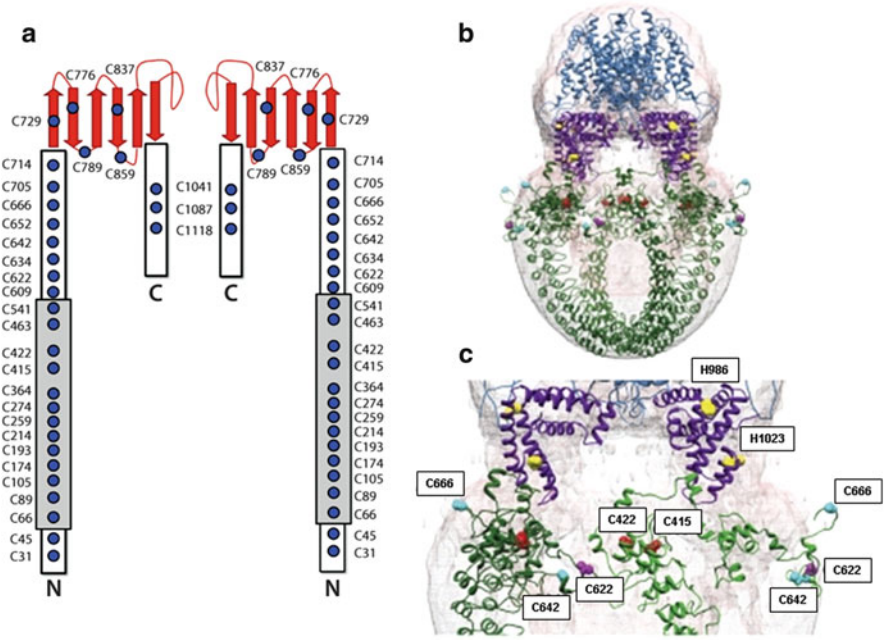

Fig. 3 (**a**) Two-dimensional representation of a TRPA1 dimer indicating the reactive cysteine residues. The cytoplasmic N- and C-termini are separated by six transmembrane helices. Each monomer contains 31 cysteine residues (*blue circles*), and the N-terminus has an extended ankyrin repeat domain (*box*). (**b**) TRPA1 is shown in the cryo-electron microscopic 3D structure. The modeled TRPV1 TM domain fits into the TRPA1 structure (*blue ribbon*). The TRPA1 N-terminus (*green*) and the C-terminus are shown. (**c**) Conserved cysteines involved in electrophilic activation in the mouse (Cys-415, Cys-422, Cys-622), human (Cys-622, Cys-642, Cys-666), and *Drosophila* (Cys-622, Cys-642) homologues are highlighted in the zoomed-in view (ball-and-stick structures and labeled, *right panel*). Histidines involved in zinc activation are also indicated in *yellow* (adapted from Cvetkov et al. (2011) with permission)

formation of Michael adducts, thiocarbamates, alkylation products of cysteine, and disulfides. The chemistry of these modified residues is not known, i.e., the subsequent intracellular modification to make the channel again available. Second, it is not yet completely clear which cysteines are really required for gating. Critical in mouse TRPA1 are C415, C422 (in the ARD), and C622 (between ARD and TM1) and in human C622, C642, and C666 (between ARD and TM1); in addition, modification of K710 is involved. However, 30 from the 31 cysteine are reactive 174, 193, 415, 422, 463, 609, 622, 634, 642, 666, and 859, and in addition four sulfide intra-chain bridges are formed between cys666-662, cys666-463, cys622-609, and cys666-193. Eleven cysteines have been identified to be possibly involved in gating (Fig. 3).

This disulfide bonding might be also involved in channel gating via an unknown mechanism. Probably, critical N-terminal cysteine residues involved in

electrophilic activation are located at the interface between neighboring subunits, and conformational changes at this region might lead to unknown conformational changes resulting in channel activation (Cvetkov et al. 2011; Wang et al. 2012). How the covalent modification of the intracellular core signals to the channel gate is completely unknown. Third, surprisingly, that activation of the channel in inside-out patches is difficult and requires the presence of MgATP or polyphosphates (Kim and Cavanaugh 2007; Karashima et al. 2008). However, channel gating by non-electrophilic agonists such as Δ9-THC and menthol is still possible (Cavanaugh et al. 2008). It seems, yet not at all understood, that some intracellular factors, such as polyphosphates, may act as a scaffold to stabilize the channel in an available conformation.

Another direct form of gating remains mechanistically also enigmatic and came as a surprise. Heavy metals such as Zn^{2+}, Cd^{2+}, and Cu^{2+} can directly gate TRPA1. Activating Zn^{2+} binds to the C-terminal His983 and Cys1021 (Andersson et al. 2009; Banke and Wickenden 2009; Gu and Lin 2010; Hu et al. 2009b).

Another gating puzzle emerged with the identification of an obvious role of the pore region as part of the gating machinery. Activation of TRPV1 occurs by low pH and requires the pore residues E648 (E600 for potentiation of TRPV1 activity by low pH). Surprisingly, the non-charged T633 is also required for proton activation but not for activation by agonists (Ryu et al. 2007). In addition, TRPV1 is also activated by the double-knot toxin DkTx from the Earth tiger tarantula. This activation requires binding of the toxin to residues in the outer pore (Bohlen et al. 2010). This binding probably causes conformational changes in the pore region, thereby opening the channel gate (Bright and Sansom 2004). Also the inner pore of some TRP channels, TRPV1, TRPV3, TRPV4, and TRPA1, seems to be involved in direct channel gating as evidenced from mutagenesis experiments. TM6 in TRPA1 may possess two points of flexibility which could function as a gating hinge similar to Kv channels (Benedikt et al. 2009; Bright and Sansom 2004). Specific point mutations within the cytosolic TM4–TM5 linker of TRPA1 (N885S) (Kremeyer et al. 2010) or TRPV3 (G573C or G573S) (Asakawa et al. 2006; Xiao et al. 2008c) or the introduced G503S (TRPC4) and G504S (TRPC5) mutations in the corresponding sequences of TRPC4 and TRPC5 (Beck et al. 2013) lead to constitutively active channels indicating that the non-mutated amino acid residues are essential to keep the channels in a gateable configuration.

Obviously, many TRP channels are activated by binding of agonists. In this view, they can be considered as "ligand"-activated channels. Such ligands function endogenously or are externally applied. The most studied ligand is certainly capsaicin, a TRPV1 agonist. Capsaicin activates TRPV1 with $K_{1/2}$ values varying with temperature and voltage. Capsaicin binding occurs to the closed channel state probably at several binding sites mapped in the TM2–TM4 region (Jordt and Julius 2002), but other sites have been identified at the TRP box, the pore domain, and the N- and C-termini TRPV1. The docking of the capsaicin molecule to the interface between two channel monomers in the TM3/TM4 region, i.e., the probably voltage-sensing domain, has been analyzed in detail. The vanillyl moiety forms a $\pi-\pi$ stacking and hydrophobic interactions with Tyr511 and H-bonding with Ser512. In

addition, the carbonyl group (B region) made H-bonding interactions with Tyr511 and Lys571. This binding favors an open-channel configuration (Lee et al. 2011). Although this structural modeling is intriguing, we still have no clue, even for this most advanced case, how the channel gates by ligand binding. The existence of probably more binding sites with positive cooperativity makes the underlying mechanism of channel opening still more uncertain not to speak of the still-elusive docking sites for endogenous agonists like the endocannabinoid anandamide and 2-arachidonoylglycerol (2-AG) and the lipid 12-HPETE. The situation for other TRP channels is still much less clear. Certainly, we have to fill the gap how ligand binding gates a TRP channel and binding studies have to be connected with functional data.

The situation of pinning down clear-cut gating mechanisms for TRPCs seems especially difficult. What do we really understand of TRPC gating? Obviously, Gi,o and Gq,11 are required at the same time to activate TRPC4/TRPC5. Simultaneously, an elevation of $[Ca^{2+}]_i$ is facilitating. Activation is also voltage dependent, and PI(4,5)P2 is a unique negative gating modulator (Otsuguro et al. 2008; Tsvilovskyy et al. 2009; Zholos et al. 2004). The complexity of these gating mechanisms is striking and puzzling. In addition, it has also been shown that TRPC4/TRPC5 are activated by Gαi rather than Gq/11. But what about the endogenous environment of the cell systems used? Gi,o and Gq,11 appear to be present in most cell lines used for *Trp* cDNA expression, but what about the receptors activating these G proteins (http://www.uni-leipzig.de/~strotm/molbio/endogenous_gpcr.htm)? In another approach, interaction of C-terminal SEC14-like and spectrin-type domain SESTD with Gαi was required for gating. What is the mechanism (Jeon et al. 2008, 2012, 2013)?

11 Positive and Negative Ca^{2+} Feedback

Probably all TRP channels are modulated by Ca^{2+}. Most TRPs show an activity-dependent inactivation which is mediated by Ca^{2+} and involves as possible mechanisms Ca^{2+}-dependent kinases, Ca^{2+}-dependent phosphatases, Ca^{2+}-regulated PLCs, and especially calmodulin. The mechanisms of this modulation is not clear (Gordon-Shaag et al. 2008); often Ca^{2+}-dependent $PI(4,5)P_2$ depletion is involved (Rohacs 2013; Rohacs and Nilius 2007). In many cases, this modulation is bimodal, e.g., comprises activation and negative feedback inhibition. As for all Ca^{2+}-permeable channels, negative feedback inhibition is extremely useful to avoid cellular Ca^{2+} overload. But do we really understand this mechanism? Some TRP channels such as TRPM4 and TRPM5 are directly activated by Ca^{2+}. Indeed, an increase in $[Ca^{2+}]_i$ is required to activate these channels in a voltage-dependent manner. Without Ca^{2+}, even very large depolarizations cannot activate the channel. Thus, this situation is different from, e.g., BK_{Ca} channels. Unfortunately, an activating binding site for Ca^{2+} has not been reliably determined. However, it has been shown that the presence of calmodulin which binds to probably several C-terminal sites shifts the voltage-dependent activation toward much more negative

potentials (Nilius et al. 2005a). Again, the gating mechanism remains unclear. Both Ca^{2+}-activated channels desensitize/inactivate upon prolonged exposure to elevated $[Ca^{2+}]_i$. This effect can be nicely explained by a Ca^{2+}-dependent $PI(4,5)P_2$ depletion via activation of a Ca^{2+}-activated PLCβ4 (Nilius et al. 2006, 2008; Rohacs and Nilius 2007). A pleckstrin-like homology domain is required for $PI(4,5)P_2$ binding. Another intriguing example for the dual regulation by Ca^{2+} is TRPA1. Channel activity, if it is activated, is strongly potentiated by extracellular Ca^{2+} followed by channel desensitization. Thus, Ca^{2+} is one of the most important endogenous modulators of TRPA1. Both mechanisms are not understood. TRPA1 is affected by $[Ca^{2+}]_e$ due to entry through TRPA1 and subsequent elevation of intracellular calcium. The mutation of Asp918 in the putative TRPA1 pore greatly reduces Ca^{2+} permeability. Extracellular Ca^{2+} alone produced neither potentiation nor inactivation, e.g., channel activation is required. Application of Ca^{2+} to the cytosolic face of excised patches is sufficient to produce both potentiation and inactivation of TRPA1 channels. Moreover, in whole-cell recordings, elevation of intracellular Ca^{2+} potentiated, but did not inactivate TRPA1. Thus, potentiation and inactivation are two independent mechanistically not understood processes (Nilius et al. 2011, 2012; Wang et al. 2008). In this case, desensitization is probably much less attributed to an increased $PI(4,5)P_2$ breakdown as for other channels such as TRPM4, TRPM8, TRPV5, and TRPV6 (Karashima et al. 2008; Rohacs and Nilius 2007) or is even opposite (Kim et al. 2009). These are only examples for the probably universal modulatory activity of Ca^{2+} on TRP channels. Because of the bimodal effect of even extracellular Ca^{2+} on TRPA1, the use of a TRP-current readout in a Ca^{2+}-free solutions screening of channel modulators can easily generate false-negative (missing potentiation) or false-positive (lacking inactivation) results.

Because of the extremely important regulation of many TRP channels by Ca^{2+}, it is indicated to search downstream of Ca^{2+} for effects of Ca^{2+}-binding signaling proteins, e.g., calmodulin (CaM). CaM in most cases exerts an inhibitory effect on the channel gating. This negative feedback loop works locally and rapidly to regulate the level of channel activity suitable for cellular physiology. Activation of CaM by Ca^{2+} influx following TRP channel activation can also affect channel function indirectly, e.g., by binding of CaM to other proteins such as CaM-sensitive protein kinases. Given the diversity of TRP channels, it is perhaps not surprising that the sites of CaM binding are also quite diverse and multiple sites have been detected on many TRP channels at the N- and C-terminus (Zhu 2005). Because of this diversity, clear-cut functional data related to CaM should always substantiate such binding studies. As one striking example, the crystal form of the ARD of TRPV1 exists as a complex with CaM (Lishko et al. 2007). Thus, the ARD of TRPV1 (TRPV3, TRPV4) serves as a CaM-binding site. ATP was also found to bind to the CaM-binding site of TRPV1 and competes. CaM binding occurs in the absence of Ca^{2+}. The functional impact of the ATP–CaM competition is accelerated channel desensitization in the presence of CaM and a delay in the presence of ATP and absence of CaM. The diversity of CaM action is also shown in the surprising findings that Ca^{2+} binding to CaM may underlie the Ca^{2+}-dependent activation of

TRPM4 (Nilius et al. 2005a) and the Ca^{2+}-dependent potentiation of TRPV3 (Xiao et al. 2008b) and TRPV4 (Strotmann et al. 2003, 2010). Again, this diversity of CaM action needs to be understood and underpinned by more functional and structural analysis.

12 The $PI(4,5)P_2$ Puzzle

One exciting aspect of TRP channel regulation is the interaction with and modulation by plasma membrane phosphatidylinositol phosphates (PIPs) and in particular by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). Probably all TRPs are regulated by PIPs. PI(4,5)P2-binding sites in nearly all PIP-modulated TRPs have been proposed and functionally tested via mutagenesis (Nilius et al. 2008). $PI(4,5)P_2$ positively modulates the activity of TRPM4, TRPM5, TRPM7, TRPM8, TRPV5, and TRPV6 but inhibits TRPC4α. Effects on TRPA1 are still somewhat controversial (reviewed in Rohacs 2013; Rohacs and Nilius 2007). Probably the most intriguing yet important problem to solve refers to a complex regulation of TRPV1 by PI(4,5)P2. It has been first shown that nerve growth factor (NGF) and bradykinin activate PLC, which hydrolyzes $PI(4,5)P_2$ and subsequently sensitizes the channel through interactions with a region in the C-terminus (Chuang et al. 2001; Prescott and Julius 2003). The opposite effect has been later described. Indeed, depletion of PI(4,5)P2 in inside-out patches leads to channel inhibition, and direct application of PI(4,5)P2 activated the channel (Klein et al. 2008). Again somewhat later, a new transmembrane protein has been found, Pirt (phosphoinositide interacting regulator of TRP), that interacts with both $PI(4,5)P_2$ and TRPV1, thereby inducing an activating effect on TRPV1. Thus, PI(4,5)P2 only activated TRPV1 in the presence of Pirt (Kim et al. 2008). However, this mechanism has been recently challenged. Pirt is probably not required for PI(4,5)P2 modulation of TRPV1. Pirt does not alter the phosphoinositide sensitivity of TRPV1 in HEK293 cells. Dorsal root ganglion neurons from Pirt knockout mice have an apparent affinity for $PI(4,5)P_2$ indistinguishable from that of their wild-type littermates. A proximal C-terminal region of TRPV1 is sufficient for $PI(4,5)P_2$ binding. This proximal C-terminal region of TRPV1 can interact directly with $PI(4,5)P_2$ and may play a key role in PIP2 regulation of the channel. Importantly, Pirt does probably not bind to TRPV1 (Ufret-Vincenty et al. 2011). However, Pirt might be an accessory protein for other channels, e.g., TRPM8, and may act as a positive modulator under conditions of channel activation (Tang et al. 2013). In another approach, PLC activation by bradykinin leads to a moderate decrease in PI(4,5)P2), but no sustained change in the levels of its precursor PI(4)P. Preventing this selective decrease in PI(4,5)P2 inhibited TRPV1 sensitization, while selectively decreasing PI(4,5)P2 independently of PLC potentiated the sensitizing effect of protein kinase C (PKC) on the channel, thereby inducing increased TRPV1 responsiveness. Maximal pharmacological TRPV1 stimulation leads to a large decrease of both PI(4,5)P2 and its precursor PI(4)P. Attenuating the decrease of either lipid significantly reduced desensitization, and simultaneous reduction of PI

(4,5)P2 and PI(4)P independently of PLC inhibited TRPV1. In this view, differential changes in phosphoinositide levels are mediated by probably distinct PLC isoforms which can result in opposite effects on TRPV1 (Lukacs et al. 2013). Recently, TRPV1 was inserted into artificial liposomes. It can be activated by capsaicin, protons, and heat. Under these conditions, TRPV1 is fully functional in the absence of phosphoinositides, i.e., does not require PI(4,5)P2 for activation. PI (4,5)P2, PI(4)P, and phosphatidylinositol inhibit TRPV1 (Cao et al. 2013). Obviously, there is still no unifying view how PIPs act on the probably best studied TRP channel, TRPV1. However, this modulation is critical for the understanding of TRPV1 function under conditions of inflammation and other disease-causing influences. In addition, we are still at the beginning of the beginning to understand specific actions of different PIPs on TRP channels. For the TRPML family, a completely different modulation pattern as for plasma membrane TRPs has been described. In endolysosome, PI(3)P and specifically PI(3,5)P2 are positive regulators for TRPML1–3. These lipids are also required for the correct channel trafficking. In late endosomes, PI(3,5)P2 is the positive regulator, whereas $PI(4,5)P_2$ is a negative regulator, and its depletion activated TRPML1 (Cheng et al. 2010; Zhang et al. 2012; Dong et al. 2010) indicating a probably highly diverse modulation pattern of TRPs by PIPs.

13 Understanding Networks

The last example on modulation of TRPV1 by $PI(4,5)P_2$ points to an integrated action of Ca^{2+} influx via the channel and activation of different isoforms of PLC (PLCδ, PLCβ4) and PKCε. Obviously, this is an example for a functional network which causes extremely variable channel reaction. Submaximal activation via bradykinin receptors (coupled to PLCβ4) causes a restricted $PI(4,5)P_2$ depletion and no change in PI(4)P; activation of TRPV1 by PKCε activation via the Ca^{2+} influx overwrites the negative effect of $PI(4,5)P_2$ depletion. On the other hand, maximal TRPV1 activation, e.g., by high concentrations of capsaicin, causes a large $PI(4,5)P_2$ and PI(4)P depletion via PLCδ activation which overwrites activating effects of PKCε (Lukacs et al. 2013).

Another striking example for a network modulating function of TRPC1 has been recently published and is discussed here as another example. Protein kinase C (PKC) and $PI(4,5)P_2$ are obligatory for the activation of TRPC1 in vascular smooth muscle cells (VSMCs). This activation is coordinated by myristoylated alanine-rich C kinase substrate (MARCKS) which is required for the TRPC1 channel's activation by PKC and $PI(4,5)P_2$. TRPC1 channels and MARCKS form signaling complexes. If $PI(4,5)P_2$ is bound to MARCKS, TRPC1 is closed. Activators of TRPC1 channels induce PKC phosphorylation of TRPC1 proteins, which causes dissociation of TRPC1 subunits from MARCKS and a release of the bound $PI(4,5)P_2$. The released $PI(4,5)P_2$ may now bind to TRPC1 and causes channel opening. Thus, MARCKS regulates the native TRPC1 in VSMCs by acting as a reversible $PI(4,5)P_2$ buffer, which in turn is regulated by PKC-mediated TRPC1

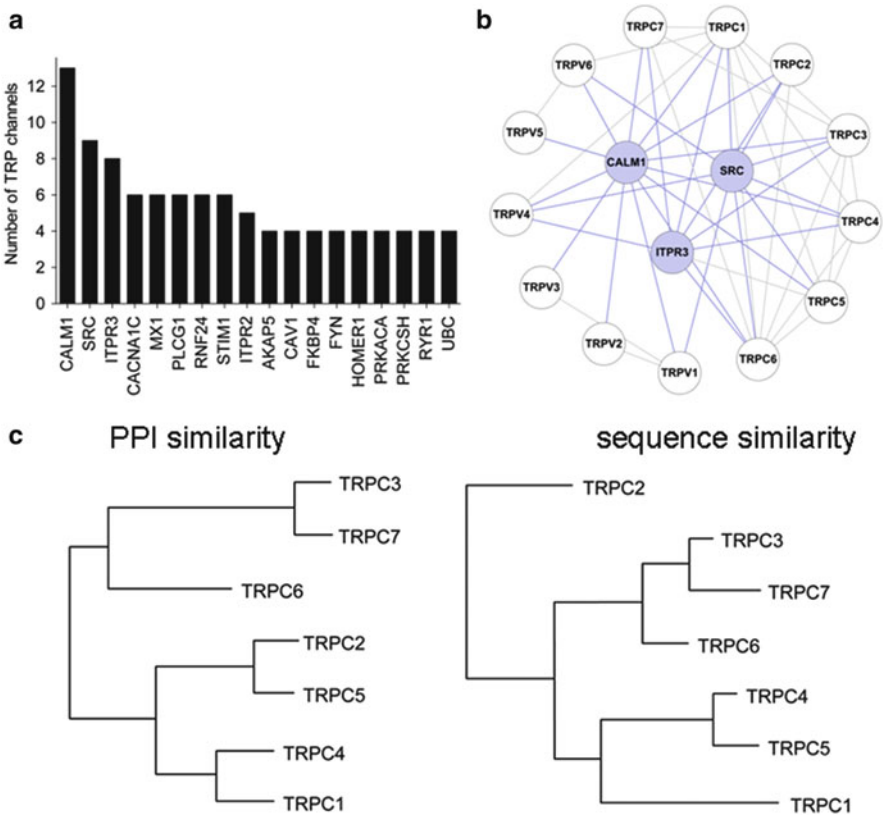

Fig. 4 (**a**) TRP channel-interacting proteins published to bind to multiple TRP channels. (**b**) CALM1, SRC, and ITPR3 interact with at least eight TRP channel isotypes within or across the subfamilies (*light blue* edges). *Gray line* marks the interactions among TRP channels. (**c**) Discordance between protein–protein interactions (PPI) and sequence similarities. PPI similarity denotes here how many interacting proteins are shared among TRPC channels. Sequence similarity is calculated using a standard protein BLAST tool (from Chun et al. (2013) with permission)

phosphorylation, and PI(4,5)P_2 acts finally as gating ligand of TRPC1 (Shi et al. 2013a). Because of the complexity of such a mechanism in native cells, it is important to verify similar mechanisms in other TRPC1 expressing cell types!

These are only a very simple example that TRPs function in complex networks. Probably, network-based approaches may facilitate the understanding of TRP channel biology and their function in disease. Using databases of protein–protein interaction (PPI) (Chun et al. 2013), the multifunctionality of TRP channels might be better understandable. An example is given in Fig. 4a showing the number of interacting TRP channels with several proteins and in Fig. 4b representing the network of the interfering interaction of TRP channels with 3 proteins (ITPR3, inositol 1,4,5-trisphosphate receptor, type 3; CALM1, calmodulin 1; and the SRC tyrosine-protein kinase). Interestingly, comparing the similarity of TRP subfamilies

based on PPI or sequence data may give additional information (Fig. 4c). We refer to a review on network-based approaches in biomedical science, which describes the current state of TRP channel network biology and which discusses a promising future direction of TRP channel research (Chun et al. 2013). However, as always with conclusions drawn from databases, they heavily rely on the data present in the databases. So far, no rigorous and comprehensive targeted high-resolution proteomic study is available on the nano-environment of any endogenous TRP channel. Such studies are available for other ion channels such as voltage-activated Cav2 channels (Muller et al. 2010) or AMPA receptors (Schwenk et al. 2012). The very few published studies on TRP proteomes even failed to identify the "targeted" TRP. As long as we do not know details of the specific makeup of endogenous TRP channel complexes, conclusions on networks may be premature.

14 The Quest for Selective Tools

The pharmaceutical industry focuses on generation of small molecules, which are synthesized in vitro and screened with high-throughput methods. Although there is now considerable progress in the synthesis of highly selective and also reversible TRP channel modulators (like for TRPV1, TRPV4, TRPA1), this is not yet the case for all TRP channels. It is highly desirable to ask the pharmaceutical industry for distributing such selective tools especially if they are not golden bullets for the therapeutically useful TRP targets. Obviously, the progress in the research on TRPV4 was mainly triggered by the early release of the relatively selective agonist 4αPDD from Glaxo (Watanabe et al. 2002).

15 The Use of Natural Compounds

Natural compounds teach us a lot about TRP channels. The problem seems to be that they are difficult to synthesize, but is there not a huge intellectual quest for natural compounds? Since probably more than 5,000 years, mankind fights for spices added to our food. Many of them are just efficient TRP channel modulators (Nilius and Appendino 2011, 2013). Interestingly, some natural compounds have been used in traditional medicine, e.g., as antidepressants have TRP channels as targets. One of these compounds is incensole acetate which is released by the burning of resin from the *Boswellia* plant and has been used for religious and cultural ceremonies for millennia. It activates TRPV3, which is expressed in the brain and causes anxiolytic-like and antidepressant-like behavioral effects (Moussaieff and Mechoulam 2009; Moussaieff et al. 2008). St. John's wort has been used medicinally for over 5,000 years. Relatively recently, one of its phloroglucinol derivatives, hyperforin, an antidepressant compound, has been identified as effective activator of TRPC6 (Leuner et al. 2007, 2010). Hyperforin has been shown to have cognitive-enhancing, memory-facilitating properties and has probably neuroprotective effects (Griffith et al. 2010). This example is only

mentioned to open the interest of TRPists for natural compounds. Estimations are done that less than 1 % of all blooming plants have never been tested for natural compounds which might be useful as medical compounds. One has to remember that these molecules were evaluated during more than 3.5 billions of years in evolution! A striking example how optimized screening natural compounds, i.e., of flavanone derivatives from citrus plants, has been successfully used to identify highly potent blockers of TRPM3, a channel with a rather elusive and intractable pharmacology (Straub et al. 2013a, b).

16 Why Is It So Difficult to Measure TRP Currents in Native Cells?

TRP proteins form channels. Obviously, the most direct evidence that such a channel generates physiological signals is the detection of a current via TRP channels in native cells. It turned out that this is a difficult task. First, for reasons explained above, TRP channel expression might be low, and especially only few channels are located in the plasma membrane where they form available ion channels. Second, although the single-channel conductance of several TRPs might be large under conditions of permeation of monovalent ions, under physiological conditions in the presence of Ca^{2+}, this conductance might be low. However, because of all difficulties with an exact localization of TRP channels in the plasma membrane, such a functional readout is unavoidable and necessary. As a striking example, when TRPV5 and TRPV6 were first described as Ca^{2+}-selective ion channels (Nilius et al. 2000), in heterologous expression systems, whole-cell currents and channel current (in the absence of Ca^{2+}) could be measured but not in the physiological most relevant native cell system, the Ca^{2+}-reabsorbing cells in the rabbit kidney connecting tubule and cortical collecting duct epithelial cells from which the *trpv5* gene was cloned (Vennekens et al. 2000); also TRPV5-dependent $^{45}Ca^{2+}$ fluxes could be measured. This is probably due to the low single-channel conductance if Ca^{2+} is the charge carrier (in contrast to a high conductance of 77pS if monovalent cations are the charge carrier). Currents are probably too small to be detected indicating the need of using more refined methods such as single-channel measurements and fluctuation analysis. On the other hand, the amount of eGFP-tagged TRPV5 (den Dekker et al. 2005) or TRPV6 protein (Fecher-Trost et al. 2013) routed to the plasma membrane after cDNA expression was too small to be detectable by confocal laser scanning microscopy. That means that also after cDNA expression the amount of functional channels in the plasma membrane is very low, like in native cells, but sufficient for current recording in one system but not in the other. May measuring native currents under divalent-free conditions improve this situation?

The measurement of currents through TRP channel is also very much complicated because of the lack of selective and reversible inhibitory tools. Also for Ca^{2+}-activated TRP channels, TRM4 and TRPM5, identification of those currents is possible by selective activation of those channel by Ca^{2+} uncaging (Ullrich

et al. 2005). However, in native cells, there are always the problems to dissect these currents through TRP channels from other Ca^{2+}-activated currents. In addition, using "selective" tools like siRNA or comparison of currents in native cells from wild-type and knockout animals is difficult and often based on unreliable readouts. Thus, any functional mechanism involving TRP channels should address this problem of measuring TRP-mediated currents in native cells by the use of identified pharmacological agonist and antagonists which act reversible and selective. The problem of tiny current should be tried to overcome by fluctuation analysis and single-channel measurements, e.g., TRPM4 identification in inside-out patches from sinoatrial nodes (Demion et al. 2007).

17 Species Dependence

There are plenty of examples that activation and modulation of TRP channels are highly species dependent, also within mammalian species. Only in some examples, in the presence of saturating capsaicin, rat TRPV1 reaches full activation, with no further stimulation by protons. In contrast, human TRPV1 (hTRPV1) is potentiated by extracellular protons and magnesium, even at saturating capsaicin (probably due to differences in negatively charged residues in the TM3–TM4 linker) (Wang et al. 2010). Also in the search for effective TRPV1 antagonists, the significant lack of consistency of the pharmacology of many TRPV1 antagonists across different species has created a substantial obstacle (probably due to differences in the pore) (Papakosta et al. 2011). In the search for TRPA1 antagonists, trichloro (sulfanyl)ethyl benzamides were discovered to be active in humans but had no or even activating activity in rodents (Klionsky et al. 2007). Both rodent and rhesus monkey TRPA1 do not respond to extracellular acidosis, and protons even inhibited rodent TRPA1, but human TRPA1 is activated by protons (an effect probably caused by differences in TM5, TM6) (de la Roche et al. 2013). Electrophilic thiamines block human TRPA1 but activate rat TRPA1 (Chen et al. 2008). Non-electrophilic menthol is an activator of mouse TRPA1 at low concentrations and a blocker at high concentrations; but it only activates human TRPA1 (differences in the TM5–TM6 pore region) (Xiao et al. 2008a). Caffeine activates mouse TRPA1 but blocks human TRPA1 (Met268Pro point mutation in the mouse TRPA1 N-terminus changes activation into inhibition) (Nagatomo et al. 2010). Many more of such examples could be mentioned. Even more important, in invertebrates and ancestral vertebrates, TRPA1 serves as a heat receptor. In mammals, TRPA1 is probably a receptor for noxious cold. This is, however, controversial. Cold activates rat and mouse TRPA1 but not human or rhesus monkey TRPA1. At the molecular level, a single residue within the S5 transmembrane domain (G878 in rodent but V875 in primate) seems to account for the observed difference in cold sensitivity (Chen et al. 2013).

It might be important to consider that such species-dependent effects may give some insight into structural requirements of channel activation and modulation. It should also be mentioned that of course basic functional differences in organ

function, e.g., the beat frequency in rodents as compared to human hearts, might substantially alter effects of TRP activation raising, e.g., the problem whether rodent models are in general appropriate for an extrapolation to humans (e.g., the TRPM4 effect in mice might be underestimated as compared to humans).

18 Are TRPs Really the Aristotle's Main Sensory Players? Surprises?

Since their discovery, TRP channels have been always considered as the main players in the sensory system and were even considered as the main signal transducers providing via Aristotle's classical five-sense recognition of the outer world (Damann et al. 2008). Vision is in Aristotle's view the noblest of all five senses. So far, TRPs were only considered as photoactive channel in invertebrates. In fact, TRP and TRPL in Drosophila are main players in vision as many times excellently reviewed (Hardie and Postma 2008; Montell 2012). A more recently evolving topic in mammals concerns intrinsically photosensitive retinal ganglion cells (ipRGCs) which are discovered photoreceptors in the mammalian eye which mediate primarily nonimage visual functions, such as pupillary light reflex and circadian photo-entrainment, and are expected to respond to the absolute light intensity (for a recent reviews see Lucas 2013; Pickard and Sollars 2012). It is intriguing to unravel the functioning of this photo-sensing including possible TRP channels and its modulation by different chromophores (Schmidt et al. 2011). A first surprise, melanopsin-expressing photosensitive retinal ganglion cells (pRGCs) express TRPM1 and TRPM3 which are involved in the nonimage-forming responses to light (Hughes et al. 2012). Melanopsin signaling depends also on TRPC6 and TRPC7. Iris muscles isolated from nocturnal mammals such as mice contract when exposed to light through the action of a melanopsin-based signaling pathway. Ablating in mouse the expression of both TRPC6 and TRPC7 also eliminated the light response in the M1 subtype of melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (M1 ipRGCs) (Xue et al. 2011).

At note, very surprisingly, it was recently shown that gating of Drosophila TRP involved not only a photosensitive step coupled to light sensing by rhodopsin but also a mechano-sensitive step (Hardie and Franze 2012). In general, we have to expect that polymodal stimuli, such as light, heat, and force, converge on chromophores which in turn modulate various TRP channels, as shown now in Drosophila (Kirkwood and Albert 2013).

Highly hypothetical but intriguing to consider is the possible existence of "deep brain photoreception" by extraocular light detectors also in mammals, e.g., in dopaminergic amacrine neurons, which may again settled by a melanopsin–TRP interaction. Such sensing may still form a connection with neuroendocrine cells for the control of mood (Fernandes et al. 2012, 2013).

TRPM1 has now been also described as a direct player in image-visual function. It is TRPM1 expressed on ON bipolar cells in the retina which invert the sign of light responses from hyperpolarizing to depolarizing before passing them on to

ganglion cells (ON response). TRPM1 colocalizes with metabotropic glutamate receptor mGluR6 (GRM6) coupled to $G\alpha_o$ and Gβ3 proteins. Light responses are generated when TRPM1 is activated. The gating mode of this channel is still obscure: Unbinding of glutamate, which is released from adjacent rod photoreceptor cells, from mGluR6 activates TRPM1. Binding of glutamate to mGluR6 closes the channel. The signaling cascade is still not known. After activation, TRPM1 desensitizes and the light response decays (Koike et al. 2010; Morgans et al. 2009). Underlining the important role of this novel TRP-mediated vision pathway, TRPM1 is linked to the autosomal-recessive congenital stationary night blindness type 1C (CSNB1C), a retinal disorder characterized by nonprogressive impaired night vision and decreased visual activity due to ON bipolar cell dysfunction (Audo et al. 2009; Nakamura et al. 2010).

The question remains: Are other TRP channels involved in vision (Gilliam and Wense 2011)? TRPC1 has been detected in cones and rods which may hint to still unknown function beyond light sensing but regulation of Ca^{2+} homeostasis in photoreceptors (Molnar et al. 2012).

TRPs have now been more and more involved in light sensing in cells outside the visual system. Melanocytes sense UV light (mainly UVB light) via TRPM1-mediating melanogenesis (Devi et al. 2009). TRPA1 is also involved in UVB sensing and early melanogenesis; a retinal-dependent G protein-coupled signaling pathway is involved in TRPA1 activation (Bellono et al. 2013; Bellono and Oancea 2013). UVB light is also supposed to activate TRPV4 (Moore et al. 2013). Obviously, the mechanism underlying activation of TRP channels by light is of high interest.

It came also as a surprise that at least in rattlesnake and in bats TRPA1 can be activated by infrared light (Gracheva et al. 2010, 2011; Panzano et al. 2010; Geng et al. 2012; Yokoyama et al. 2011). Activation is reportedly due to heat response of TRPA1 above a temperature threshold for activation at ~28 °C. TRPA1 is a heat-activated channel in these species. Again, the underlying activation mechanism of TRPA1 is highly interesting. If snakes would detect temperature differences of 0.01 °C, which is probably even an underestimation (Ebert and Westhoff 2006), currents through TRPA1 would exhibit a Q^{10} of $>10^{15}$. Obviously, this temperature sensing must involve a nonlinear signal amplification process upstream of channel opening. Vice versa, considering a Q_{10} of ~10, as typical for thermoTRPs, the increase in open probability induced by a warming of 0.003 °C would be only ~0.2 %! It is very unlikely that this would be enough to generate sufficient depolarization to trigger action potentials in the snake trigeminal neurons. One has also to consider that the radiation energy decays with the fourth power of the distance (Stefan–Boltzmann law), which would predict very small activation energies for TRPA1 in the snake's pit organ. Of note, it has never been shown that TRPA1 antagonists impair the infrared sensing of snake or bats! However, infrared radiation sensing by TRP channels is a highly exciting, yet unsolved, topic.

In Aristotle's view, touch, or now more generally described as "somato-sensitivity," was the most primitive sense. However, somato-sensitivity is the most essential and necessary sense for survival, far beyond the other four senses.

This has now become one of the most exciting topics in the TRP field. We will only refer to two examples: mechano- and temperature sensing.

How are TRP channels involved in mechano-sensation? There are several examples which are all reviewed in detail (described in the issue of HEP Plant and in Eijkelkamp et al. 2013). However, with the advent of completely unexpected mechano-sensing channels, Piezo1 and Piezo2, some of the earlier mechano-TRP concepts have certainly to be revised (for a critical discussion, see Nilius and Honore 2013). One recent finding might be mentioned to open our interest novel forms of mechano-sensing. Probably TRPV4 in glia cells can mechanically sense infrasound (16 Hz) which can even be involved in infrasound-caused neurodegeneration (Shi et al. 2013b).

Another important part of somato-sensing is temperature (less correct "thermo-") sensing. It has been one of the most accomplishments in the TRP field to identify the so-called thermoTRPs which are supposed as the main temperature sensors. Without any doubt, some TRPs have a very high temperature sensitivity as expressed in Q_{10} values >10. Mechanistic aspects of thermosensing by TRPs have been addressed in many excellent reviews and will not be further mentioned (Dhaka et al. 2006; Ferrer-Montiel et al. 2012; Patapoutian et al. 2003; Vay et al. 2011; Voets 2012; Wetsel 2011) (also described in this volume by Voets, T. "TRP channels and thermosensation"). The list of thermoTRPs is constantly changing, e.g., TRPV2, TRPV3, and TRPV4 (reviewed by Voets 2012; Nilius et al. 2014) are not anymore considered as functionally involved in temperature sensing, but TRPM3 must be added (Vriens et al. 2011). Several "thermo"TRPs are located in thermosensitive regions such as in skin keratinocytes, in thermosensory nerve endings, and in blood vessels. However, many other cell types are of course also thermosensitive and may not exploit TRP channels. As an important surprise for the coupling of temperature and metabolism, fat cells sense temperature TRP independent (Ye et al. 2013). Temperature sensing has probably many other faces such as metabolic control of many cell functions and homeostasis. At note, TRP channels must be considered as only one player in temperature sensing, as many other channels have been recognized to play an important role in determining the action potential frequency and pattern upon heat or cold stimulation in sensory nerve fibers, in mediating thermoregulatory response, or in triggering temperature-mediated response from the cellular to the behavioral level. Many other proteins, not only ion channel, are involved in orchestrating temperature sensing in temperature-mediated responses. Rhodopsin can function as a temperature sensor and might be involved as an amplifier for thermoTRP activation (Shen et al. 2011). The melatonin-related orphan receptor GPR50 is involved in determining the basal metabolic rate and is a temperature sensor (Swoap 2013). The Ca^{2+} sensor STIM1 is a thermosensor (Xiao et al. 2011). Nav1.7, Nav1.8, IK_D, Kv1, Kv7 (M-current), K_2P channels, the proton channel Hv1 and TMEM16A (Ano1, a Ca^{2+}-activated Cl^- channel), HCN channels, and probably several more function as temperature sensors (see recent reviews Cho et al. 2012; Fujiwara et al. 2012; Madrid et al. 2009; Orio et al. 2013; Voets 2012). This all indicates that TRP will not be the only clue for understanding temperature sensing. Interestingly and illustrating

the still mediocre understanding of temperature sensing as a whole physiopsychological event, we refer to the fact that classical phenomena related to thermosensation, including "Weber's silver *Thaler* illusion," the "thermal grill illusion," and "Weber's three-bowl experiment," are not yet understood and cannot be explained at the level of TRPs. It is even difficult to find a quantitative model involving several classes of ion channels to describe the fast and slow adaptation in thermosensory fibers and the mostly unimodal (bell-shaped) steady state discharge.

We will not refer to many unsolved problems in taste, olfaction, and hearing. Many open questions have been addressed in this volume of HEP, e.g., the controversial role of PKD2L1 (TRPP3), the existence of a salt receptor in the TRP family (TRPML3, filed as Senomyx Patent, 2009[1]), the role of TRPV1 in taste rather than chemesthesis, the real function of the many TRP channels in the inner ear [e.g., TRPC3, TRPC5; TRPV1, TRPV4, TRPV5; TRPA1; TRPML3; TRPP3; etc. (Asai et al. 2010)] beyond hearing after identification of the probably main mechano-transducer channels TMC1 and TMC2 (Pan et al. 2013), and a still somewhat elusive role of TRPs in olfaction (Dong et al. 2012).

19 TRPs Go Metabolic

We all agree that TRPs have a huge impact on many sensory functions and behave like unique cellular sensors which are activated by polymodal stimuli. However, more and more evidence defines a role of TRP channels in the regulation of metabolic networks and metabolic diseases, e.g., type 2 diabetes, obesity, dyslipidemia, metabolic syndrome, atherosclerosis, metabolic bone diseases, and electrolyte disturbances. This is certainly a line which deserves a high consideration. Intriguing examples are as follows: (1) The weight of *Trpv1*$^{-/-}$mice heavily exceeds that of controls, which is coupled with a predisposition to age-associated overweight (Garami et al. 2010, 2011); (2) dietary TRPV1 activation improves energy metabolism, muscle growth, and exercise endurance by upregulating PGC-1α and nNOS/mTOR in skeletal muscles (Ito et al. 2012; Luo et al. 2012); (3) TRPV4 antagonists elevate thermogenesis in adipose tissues and protect from diet-induced obesity, adipose inflammation, and insulin resistance (Ye et al. 2012); (4) TRPC1, TRPC5, and TRPC6 expression is altered in a more humanlike metabolic syndrome model, the *Ossabaw miniature pigs* (Hu et al. 2009a); (5) brown fat activity is, besides β3-adrenergic stimulation, modulated by peroxisome proliferator-activated receptor γ (PPARγ) and the connected stimulation of TRPV1 by dietary capsaicin and monoacylglycerols (Birerdinc et al. 2012); (6) TRPV1 activation prevents nonalcoholic fatty liver disease (Li et al. 2012,

[1] Moyer B, Zlotnik A, Hevezi P, Soto H, Lu M, Gao N, Servant G, Brust P, Williams M, Kalabat D, et al. (2009) Identification of Trpml3 (Mcoln3) as a salty taste receptor and use in assays for identifying taste (salty) modulators and/or therapeutics that modulate sodium transport, absorption or excretion and/or aldosterone and/or vasopressin production or release. SENOMYX patent.

2013); and much more published evidence (for detailed reviews see Nilius and Appendino 2011, 2013; Zhu et al. 2010). Concerning these obviously exciting novel approaches to metabolic diseases, some pertinent questions have to be answered: What are the precise signaling pathways up- and downstream of TRPs in adipocytes and in the hypothalamus?

How do TRPs such as TRPV1 exert tissue-specific, positive, and negative effects on metabolism? Do TRPs participate in metabolic diseases, and do known genetic variants (SNPs) in TRP genes influence disease prevalence? Are TRPs useful pharmacological targets for treating obesity and insulin resistance (see for reviews Liu et al. 2008; Nilius and Appendino 2011, 2013; Zhu et al. 2010)?

20 TRPs and Disease

We have to accept a general scheme: TRP channels are not highly expressed on most native cells, and the current density will be low. Therefore, a pertinent question is whether these obviously more "modulatory" ion channels will have such a striking functional impact to cause diseases. Importantly, although many knockout models do not show per se very striking phenotypes, the number of channelopathies connected to dysfunctional TRP channels is amazingly high (for reviews see Cornell et al. 2008; Kiselyov et al. 2007; Nilius 2007; Nilius and Owsianik 2010b; Nilius et al. 2007). One of the probably most exciting puzzles that we have to solve in understanding TRP channelopathies is related to TRPV4 (Nilius and Owsianik 2010a). The Trpv4 gene is prone to many mutations a surprising number of different diseases. Familial digital arthropathy–brachydactyly only affects fingers and toes. Mutations in Trpv4 have also been identified in motor/sensory neuropathies, such as the congenital distal spinal muscle atrophy, scapuloperoneal spinal muscle atrophy, and Charcot–Marie–Tooth disease type 2C,with highly variable phenotypes, i.e., skeletal dysplasias (*brachyolmia, spondylometaphyseal dysplasia Kozlowski, spondyloepimetaphyseal dysplasia Maroteaux pseudo-Morquio type 2, metatropic dysplasia,* and *parastremmatic dysplasia* with a unifying feature short trunk with vertebral platyspondyly and scoliosis), skeletal diseases (*familial digital arthropathy–brachydactyly* only affecting fingers and toes), and motor/sensory neuropathies (*congenital distal spinal muscle atrophy, scapuloperoneal spinal muscle atrophy*, and *Charcot–Marie–Tooth disease type 2C* characterized by degeneration of motor and sensory neurons and peripheral nerves) (for a review, see Nilius and Voets 2013). More than 50 disease-causing mutants have been so far identified. They are distributed over the whole channel protein, with only two hot spots, the ADR (mainly neuropathies) and a region around the MAP-binding site in the C-terminus (skeletal dysplasias) (Nilius and Voets 2013). Another puzzle appeared concerning TRPV4 channelopathies: It is widely discussed that TRPV4 plays an important role in endothelium, contributes to the EDHF mechanism, and subsequently is an endothelial player for the regulation of blood pressure (Earley et al. 2009; Filosa et al. 2013; Kassmann et al. 2013; Kohler et al. 2006; Loot et al. 2008; Rath et al. 2009; Saliez

et al. 2008; Vriens et al. 2005; Zhang and Gutterman 2010). However, any vascular phenotype in the TRPV4 patients is missing. The striking challenge is that mutations which are often located in the same domains of the channel proteins and even three identical mutations cause different diseases.

Most TRP-caused diseases have a dominant inheritance pattern, which implies that the patients carry both a mutant and a wild-type allele, which creates a general often overlooked problem: TRPs function probably as tetramers; thus, patients are expected to express TRP channels with variable stoichiometry of WT and mutant subunits. Assuming that wild-type and mutant subunits are equally expressed and assemble into tetrameric channels in a random fashion, one would expect that the TRP channels in a patient's cell consist of 1/16 (6.25 %) pure wild-type channels and 1/16 pure mutant channels and that the 14/16 (87.5 %) of functional channels are heteromultimeric channels consisting of a mixture of wild-type and mutant subunits. This obviously requires the understanding of the properties of such heteromultimeric channels, which have often even not been studied. Understanding a disease needs the functional analysis of such heteromers! It is very likely that they exhibit properties that differ strongly from either pure wild-type or pure mutant homotetramers.

Understanding of the mechanistic background of these channel malfunctions not only is essential for a treatment of these diseases but may also help to understand the functioning of these exciting proteins and their involvement in different signaling cascades.

Obviously, TRP channels are connected to acquired diseases. In these cases, a critical mechanistic understanding is often missing, and far-reaching extrapolations and hopes are focused on TRP channels. We will also refer to a puzzle, seen the huge social impact: TRP causes diseases in the brain. Many discussions have been started about *age as a drug target*, and TRPs are considered as main actors. TRPM7 and TRPM2 have been put forward as potential factors in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, stroke, and diseases associated with oxidant-mediated neuronal damage (McNulty and Fonfria 2005). Especially TRPM2, which is highly expressed in the striatum (caudate nucleus and putamen), is supposed to play a key role in bipolar disorders (Uemura et al. 2005; Xu et al. 2006). Recent case–control studies implicate TRPM2 conferring risk for bipolar disorder (BD), and genetic variants of TRPM2 have been identified to be coupled with BD supporting a role for this channel in the pathogenesis of this disorder (see for a review Chahl 2007; Xu et al. 2009). Depletion of intracellular Mg^{2+}, a symptom of ischemic brain injury, and a reduction of extracellular Ca^{2+} are both associated with poor neurological outcome and are both conditions which activate TRPM7 and possibly increase the Ca^{2+} load of neuronal cells. This leads to a secondary injury processes and to cell death (Cook et al. 2009). Downregulation of TRPM2 and TRPM7 causes probably neuroprotective effect in the cerebral cortex and hippocampus after brain injury, oxidative stress, inflammation, and neuronal death (Cook et al. 2010). Therefore, TRPM7 might be a potential target for neuroprotection after brain injury. Suppressing the expression of TRPM7 in hippocampal CA1, neurons conferred resistance to ischemic cell death, preserved

cell function, and prevented ischemia-induced deficits in memory (Rempe et al. 2009; Sun et al. 2009). Is this an antiaging strategy? On the other hand, mutations in TRPM2 have been discovered which result in rapidly inactivated channels which are unable to maintain sustained Mg^{2+} influx (Hermosura et al. 2002, 2005, 2008; Hermosura and Garruto 2007). Unfortunately, although quite a lot of evidence has been published, we face now clinical studies which completely refuse this even mechanistically underpinned view (Hara et al. 2010). However, still, TRPM7 might be involved in the *familial Alzheimer's disease (FAD)* but mechanistically linked to aberrant $PI(4,5)P_2$ causing TRPM7 dysfunction (Landman et al. 2006). In another view as the abovementioned role for "switching on" of light responses or as tumor suppressor, TRPM1 could be linked to a complex neurodevelopmental disorder characterized by severe visual impairment, intellectual disability, and refractory epilepsy. This disease is caused by a microdeletion in chromosome 15q13.3 carrying the *trpm1* gene (Lepichon et al. 2010). Aging increases also a risk for ischemic brain injury by oxidative stress, edema, inflammation, and excitotoxicity. TRPM4 is reportedly involved in the damaging secondary events that accompany brain injury. Inhibition of TRPM4 channels provides beneficial effects in ischemia-associated cerebral edema and secondary hemorrhage (Simard et al. 2010) and has been considered as a neuroprotective target in the brain or spinal cord lesion (Gerzanich et al. 2009). However, the pharmacological evidence that TRPM4 is really blocked by K_{ATP} (SUR1 bound) inhibitors is still controversial. Also the question arises whether SUR1 is indeed a TRPM4 subunit as suggested (Woo et al. 2013).

This is just to say that although brain injury confers a major burden to Western society and effective treatments are urgently required, the enthusiasm that TRP channel may solve many of such problems should be qualified by a more rigorous investigation of TRP channel functions in more refined and suitable models! But which mechanisms are really involved? How can we really understand that TRP channels are causative in all the sketched diseases?

Obviously, we have to admit that in many diseases the role of TRPs might be somewhat overinterpreted/overstressed. Better pathophysiological models which consider the difficulties to handle TRP must be developed. We are still in the very beginning of understanding disease-causing dysfunctions of TRP channels.

Because of all the controversies and gaps in our understanding on TRP, we have to confess that we have opened an exciting field, but the current state of TRP channel research, as described in this book, is still in its infancy!

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