Sodium Channel Trafficking

A. Mercier, P. Bois, and A. Chatelier

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Abstract

Voltage-gated sodium channels (VGSC) are critical determinants of cellular electrical activity through the control of initiation and propagation of action potential. To ensure this role, these proteins are not consistently delivered to the plasma membrane but undergo drastic quality controls throughout various adaptive processes such as biosynthesis, anterograde and retrograde trafficking, and membrane targeting. In pathological conditions, this quality control could lead to the retention of functional VGSC and is therefore the target of different pharmacological approaches. The present chapter gives an overview of the current understanding of the facets of VGSC life cycle in the context of both cardiac and neuronal cell types.

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Handbook of Experimental Pharmacology, DOI 10.1007/164_2017_47

Keywords
Forward trafficking • Membrane targeting • Retrograde transport • Trafficking modulators • Voltage-gated sodium channel

1 Introduction

Voltage-gated sodium channels (VGSC) play a critical role in electrical signaling of excitable tissues through the control of initiation and propagation of action potentials. This function is strongly correlated to the nature of VGSC isoforms associated with their functional membrane expression and localization. To date, nine mammalian pore forming α-subunits ($Na_v1.1$–$Na_v1.9$) have been identified and divided into two categories based on their sensitivity to the puffer fish toxin tetrodotoxin (TTX). $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, $Na_v1.6$, and $Na_v1.7$ are sensitive to TTX, while $Na_v1.5$, $Na_v1.8$, and $Na_v1.9$ are TTX-resistant. At least seven VGSC are mainly expressed in the nervous system. $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$ are predominantly expressed in the central nervous system (CNS), while $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are principally found in the peripheral nervous system (PNS). $Na_v1.5$ and $Na_v1.4$ are muscular sodium channels and are mainly expressed in cardiac tissue and skeletal muscle, respectively (Chahine et al. 2008; Savio-Galimberti et al. 2012). Each VGSC exists as multimeric complex at the plasma membrane presumably composed of one or several auxiliary β-subunits associated with the α-subunit. To date, four β-subunits ($\beta_{1–4}$) have been identified and are divided into two groups: β_1 and β_3 associate non-covalently with the VGSC α-subunit, whereas β_2 and β_4 bind covalently to the α-subunit through a cysteine residue on the extracellular loop (reviewed in Patino and Isom 2010).

Like most integral proteins, the membrane expression of functional Na_v channels is drastically controlled by both cell-type and common components of the trafficking machinery. These were identified as molecular chaperones, enzymes, motor, anchoring and scaffold proteins whose key functions are to ensure the effective transport of VGSC α- and β-subunits through intracellular compartments to their final functional locations. Instant membrane expression of VGSC directly reflects the cellular proteostasis environment. This balance involves various adaptive processes such as biogenesis, trafficking, and degradation of functional channels and is known to be highly cell-type- and stimulus-dependent.

In the present chapter, we provide a selective review of research focusing on the biosynthetic transport of VGSC and more specifically the general and cell-type mechanisms involved in their anterograde/retrograde trafficking, subcellular targeting and pharmacological behavior in two types of polarized cells, neurons and cardiomyocytes (see Fig. 1).

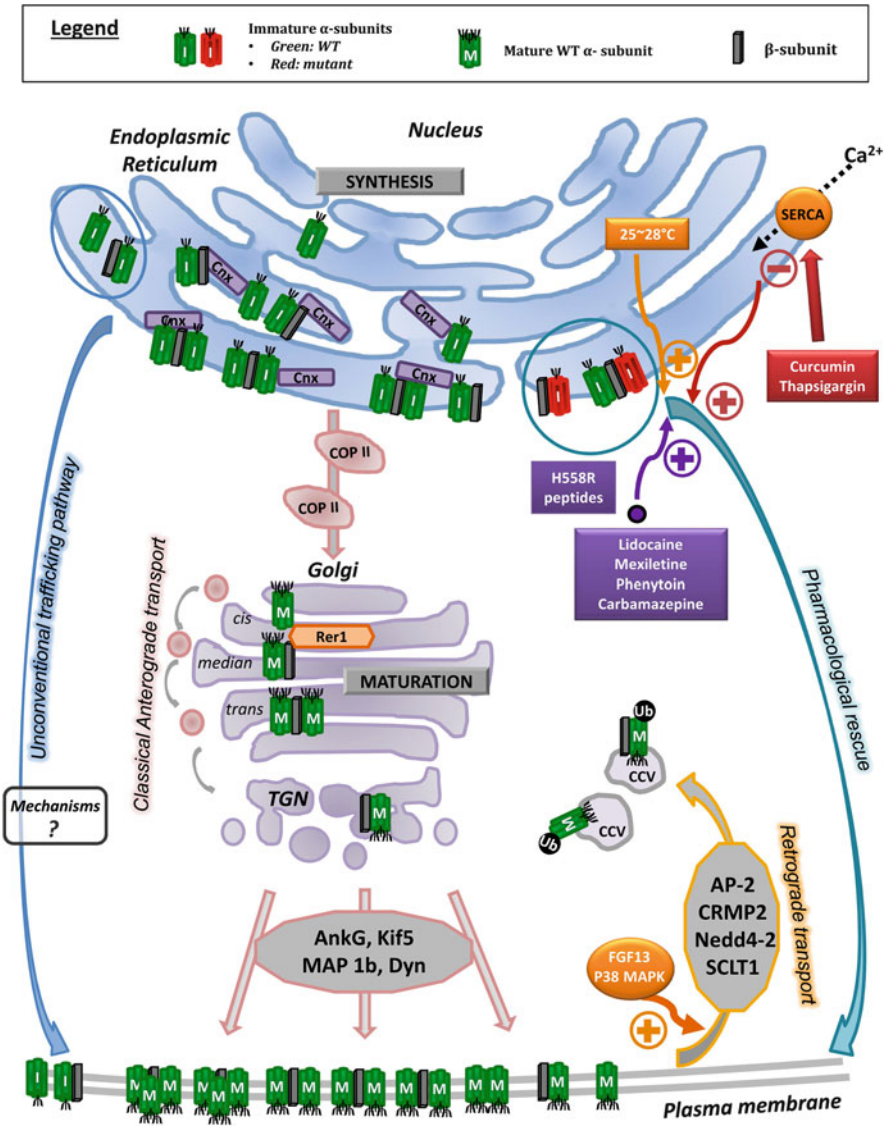

Fig. 1 Illustration of VGSC transport pathways and pharmacological trafficking modulations. This scheme summarizes the main steps and modulations reported for VGSC trafficking without any distinction between cardiac or neuronal α and β-subunit subtypes. *AnkG* Ankyrin G, *AP-2* adaptor protein complex 2, *CCV* clathrin-coated vesicle, *Cnx* calnexin, *COP II* coat protein II-coated vesicles, *CRMP2* SUMOylated collapsin response mediator protein 2, *Dyn* dynamitin, *FGF13* Fibroblast Growth Factor 13, *Kif5* kinesin 5 protein, *MAP 1b* microtubule-associated protein 1b, *Nedd4-2* precursor cell-expressed developmentally downregulated gene 4-2, *P38 MAPK* P38 mitogen-activated protein kinase, *SCLT1* sodium channel and clathrin linker 1, *TGN* trans-Golgi network, *Ub* ubiquitin, *WT* wild type

2 Biosynthesis and Anterograde Transport

2.1 VGSC Processing and ER Quality Control

Like all voltage-gated channels, VGSC's life begins in the endoplasmic reticulum (ER). Once synthesized and inserted into this organelle membrane, the fate of Na_v subunits largely depends on their glycosylation status and the folding assistance of molecular chaperones.

N-glycosylation is a co- and post-translational process initiated by the transfer of high-mannose carbohydrate chains to specific asparagine residues on nascent Na_v polypeptides (Schmidt and Catterall 1986). VGSC core-glycosylation appears to be a prerequisite for proper subunit folding and subsequent surface expression as well as assembly with $Na_v\beta$ subunits (Laedermann et al. 2013; Schmidt and Catterall 1986). As reliable markers of forward transport, different glycosylation patterns were observed for $Na_v1.5$ proteins from distinct cardiac chambers. This suggests their ability to exhibit differential trafficking behaviors, probably mediated by multiple interacting protein partners (Arakel et al. 2014).

Like many glycoproteins, they are not consistently delivered to the cell surface and undergo drastic quality controls throughout the early biosynthetic pathway. The first checkpoint for the quality control of nascent Na_v proteins occurs in the ER and implies the involvement of ER-resident chaperones including calnexin (CNX). There is substantial evidence that wild type (WT) and defective-trafficking $Na_v1.5$ proteins colocalize intracellularly with CNX in rat neonatal cardiomyocytes and Human Embryonic Kidney (HEK) cells (Clatot et al. 2012; Dulsat et al. 2017). However, a physical interaction was only demonstrated between this lectin and the $Na_v1.8$ subtype and appeared to positively influence VGSC degradation through the ER-associated degradation pathway (Li et al. 2010). It was recently reported that the Drosophila Para neuronal sodium channel also happened to be a substrate for CNX binding. These data confirm that the ER-resident lectin contributes as a molecular chaperone to ER quality control of Na_v glycoprotein folding and to their efficient exit towards the Golgi (Xiao et al. 2017). Under the action of CNX, misfolded or misassembled channels can be retained in the ER to eventually reach their correct conformation. Only after multiple unsuccessful attempts, they can be sorted to the ERAD pathway by being escorted to the proteasome for subsequent degradation (McArdle et al. 2008; Ogino et al. 2015).

2.2 VGSC ER-to-Golgi Transport

One noteworthy feature of VGSC is their predominant ER localization consistent with the existence of an intracellular reservoir of Na_v α-subunits, readily mobilizable to mediate cell adaptation to varying environmental changes (Schmidt et al. 1985; Zimmer et al. 2002). ER subpopulations of recombinant $Na_v1.5$ were shown

to be redistributed to the plasma membrane upon PKA stimulation, corroborating the increase in native Na^+ current amplitude observed following β-adrenergic receptor stimulation in rat cardiomyocytes (Hallaq et al. 2006; Lu et al. 1999). As for neuronal VGSC, large somatic cytoplasmic pools of Na^+ channels have long been described such as in invertebrate Aplysia sensory neurons or in developing rat brain (Johnston et al. 1996; Schmidt et al. 1985). More recently, intracellular pools of $Na_v1.6$ proteins were identified as a ready reserve that was thought to be potentially recruited to dendritic synapses in the rat cerebral cortex (Caldwell et al. 2000).

The control of Na_v α-subunit ER exit was proposed to be partially mediated through association with $Na_vβ$ proteins, suggesting a putative role for these regulatory subunits as chaperone-like proteins (Laedermann et al. 2013). Zhang and colleagues identified that the DI-DII linker of rat $Na_v1.8$ channels contained a functional ER-retention motif (RRR) that was shown to regulate their surface expression and be antagonized by $Na_vβ_3$ assembly (Zhang et al. 2008). Interestingly, surface expression of human $Na_v1.8$ channels that do not harbor this rodent-conserved motif remains unaffected following PKA activation, reflecting species-dependent differences in the ER exit rate (Schirmeyer et al. 2014). Numerous putative ER retention/retrieval signals were also found in other Na_v subtypes such as $Na_v1.2$ and $Na_v1.5$ (Lee and Goldin 2009; Zhou et al. 2002). Since Na^+ current potentiation mediated by PKA activation requires the masking of $Na_v1.5$ retention motifs, the role of these sequences might contribute to the rate-limiting step in Na_v export from the ER, thus maintaining a reserve pool of channels with exposed retention signals.

Once correctly folded Na_v channels have undergone adequate posttranslational maturation, they travel from ER to the Golgi complex by being packaged in coat protein II (COPII)-coated vesicles. Although the Golgi apparatus and the trans-Golgi network exhibit pivotal roles in the maturation and polarized intracellular sorting of membrane proteins (Zhang and Wang 2016), there are currently few studies that address the VGSC fate in these compartments. To date, the vast majority of defective-trafficking Na_v mutants were found to accumulate in the ER (Baroudi et al. 2001; Clatot et al. 2012; Ruan et al. 2010). However, the functional characterization of the ataxia3 $Na_v1.6$/S21P mutants revealed evidence of their sequestration in the *cis*-Golgi compartment as a result of their defective Golgi export (Sharkey et al. 2009). Lastly, in a recent report, Valkova and colleagues have examined the impact of Rer1 deficiency on cerebellar Purkinje cells in mice (Valkova et al. 2017). Loss of this *cis*-Golgian sorting receptor was shown to disrupt $Na_v1.1$ and $Na_v1.6$ expression levels and their terminal maturation. Thus, Rer1 was proposed to act as a member of the glycoprotein quality control system in the assembly and transport of mature VGSC in a subtype-dependent manner (Valkova et al. 2017).

2.3 VGSC Microtubule-Based Delivery

From their ER exit to their plasma membrane insertion, ion channels are actively transported through the cell along microtubule tracks (see for review Steele and Fedida 2014). The post-Golgi anterograde vesicular transport of Na_v channels is driven by motor proteins including members of the kinesin superfamily proteins (KIFs). While limited data exists on the final delivery of cardiac VGSC into sarcolemma, so far most research studies have focused on neuronal Na_v channels. It has been reported that the ubiquitous KIF5B isoform was associated in a subtype-specific manner with the two neuronal $Na_v1.8$ and $Na_v1.9$ subunits in rat Dorsal Root Ganglia (DRG) (Su et al. 2013). More specifically, the functional interaction between $Na_v1.8$ and KIF5B resulted in both promoting $Na_v1.8$ forward transport and preventing its degradation. Another study provided strong evidence that the scaffolding protein Ankyrin G (AnkG) could act as a cargo adaptor protein to bind KIF5 to $Na_v1.2$ during their axonal transport (Barry et al. 2014). Interestingly, $Na_v1.6$ microtubular trafficking would be mediated by its own adaptor protein, the neuron-specific microtubule-associated protein Map 1b (O'Brien et al. 2012). As for $Na_v1.5$, Casini et al. have highlighted the importance of a functional microtubule network which is required for normal $Na_v1.5$ sarcolemmal expression (Casini et al. 2010). In fact, pre-treatment with the microtubule-stabilizing agent Taxol reduced sodium current density in primary neonatal rat cardiomyocytes. Another protein from the dynactin complex, dynamitin was found to be involved in the vectorial delivery of $Na_v1.5$-containing vesicles to the cardiomyocyte surface (Chatin et al. 2014).

2.4 VGSC Oligomerization

Until recently, it was assumed that VGSC were expressed as functional monomers associated with one or more β-subunits (Catterall 2014). However, the validity of this assumption has been challenged by experiments involving co-expressions of WT with mutant $Na_v1.5$ channels. In fact, a subset of defective-trafficking mutants was shown to exert a dominant negative effect upon Na_v channel surface expression (see for review Sottas and Abriel 2016). It now appears that $Na_v1.5$ α-subunits can interact with each other physically, as shown by immunoprecipitation experiments in HEK cells (Clatot et al. 2012; Mercier et al. 2012). Interestingly, depending on the experimental conditions, this α-α assembly was found to occur through a β_1-subunit-dependent process (Mercier et al. 2012). AFM imaging has recently provided substantial evidence that heterogeneous populations of $Na_v1.5$ oligomers could coexist at the cell surface and that relative proportions of α-subunit monomers, dimers, and trimers were largely conditioned by the presence of the β_3-subunit (Namadurai et al. 2014). As $Na_v\beta_1$ and β_3 share a high degree of amino acid sequence, it is not unreasonable to suggest that they both play a key role in facilitating $Na_v\alpha$ oligomerization, possibly through the stabilization of these macromolecular complexes. However, it remains to be clarified in which compartment(s) this

interaction might occur and whether stable $Na_v\alpha$ complexes could still persist after reaching the plasma membrane.

2.5 VGSC Local Translation and Alternative Transports

Neurons, by their very nature, need to precisely control the segregation and the subsequent asymmetric distribution of plasma membrane VGSC. Over the past decade, substantial evidence has accumulated demonstrating that mature axons have the capacity for local membrane protein translation independently from the soma (see for review Cornejo et al. 2017). This biosynthetic process could be considered to overcome spatial limitations reflecting the slow axonal protein transport velocity along axons, and might allow neurons to either maintain or adjust their local proteome in distal domains (see for review Jung et al. 2012).

$Na_v1.8$ mRNA level was found to be locally up-regulated in a subtype-specific manner in sciatic nerve axons in a rat model of neuropathic pain (Ruangsri et al. 2011; Thakor et al. 2009). The authors postulated that the observed hyperexcitability of sensory neurons might be caused by the local translation of axonal accumulated $Na_v1.8$ transcripts, this hypothesis remaining speculative until recently. González and colleagues have demonstrated that mammalian peripheral axons harbor all the necessary secretory machinery required for local membrane protein synthesis (González et al. 2016). Their findings provide strong evidence that newly synthesized VGSC channels can result from both somatic and axonal biosynthetic processes. In fact, $Na_v\alpha$ subunits were shown to transit through the axonal secretory organelles before being efficiently delivered to the plasma membrane locally.

Non-conventional protein transport is not exclusive to the axonal compartment. In a recent extensive study, Hanus and colleagues revealed that a multitude of transmembrane proteins including $Na_v1.2$–1.4 and $Na_v1.7$ could reach the dendritic plasma membrane in their core-glycosylated ER forms. The authors concluded that these neuronal proteins with immature glycosylation profiles might be trafficked to the cell surface through a Golgi bypass route (Hanus et al. 2016). These findings are consistent with our previous observations showing that a pool of $Na_v1.5$ subunits that have not completed their terminal maturation could be efficiently delivered to the plasma membrane via an alternative anterograde route in HEK cells (Mercier et al. 2015). Thus, this "improper" surface expression might concern all VGSC subtypes and its functional impact should be investigated further in both cardiac and neuronal contexts. Also, in human macrophages, functional $Na_v1.5$ α-subunits were shown to be localized in late endosomal compartments, where they mediate endosomal acidification and phagocytosis (Carrithers et al. 2007). However, how this VGSC subtype is specifically targeted to these organelles and not to the plasma membrane remains to be determined.

3 Targeting and Subcellular Distribution of VGSC

The localization of VGSC at the plasma membrane is a controlled phenomenon that constitutes the final step of the trafficking process. According to their tissue function, excitable cells display various morphologies associated with specialized electrical membrane domains. VGSC subcellular targeting is therefore essential to ensure the electrophysiological function of the cell and is strongly dependent on associated proteins often involved in the intracellular trafficking of VGSC.

Neurons are polarized cells with specialized electrical microdomains such as axon initial segments (AIS), axons or somatodendritic compartment. AIS plays a key role in the action potential initiation and is therefore a site of high VGSC concentration. Axons are more specialized in action potential conduction and in myelinated axons, particular structures such as nodes of Ranvier are well known to arbor high concentrations of VGSC for saltatory conduction (for review see Nelson and Jenkins 2017). Electrophysiological function of these subcellular compartments is related to the specific functional expression of VGSC isoforms (review in Boiko et al. 2001). $Na_v1.1$ and $Na_v1.3$ have a somatodendritic localization (reviewed in Vacher et al. 2008) and could possibly control the neuronal excitability threshold through post-synaptic potential integration. On the other hand, $Na_v1.2$ and $Na_v1.6$ seem to specialize in axonal conduction. $Na_v1.2$ is present in unmyelinated axons, including the AIS and at immature nodes of Ranvier (Westenbroek et al. 1989). A recent study revealed that $Na_v1.6$ channels are preferentially inserted into the AIS membrane during hippocampal neurons development via direct vesicular trafficking and this localization is dependent on the binding with AnkG (Akin et al. 2015). In mature nodes of Ranvier of myelinated axons, $Na_v1.2$ is replaced by $Na_v1.6$, which is also present in AIS and dendrites (Boiko et al. 2001; Caldwell et al. 2000; Kaplan et al. 2001). These localizations, together with their specific biophysical properties (Rush et al. 2005), indicate that $Na_v1.2$ may be important for the conduction capabilities of unmyelinated axons, whereas $Na_v1.6$ may be more adapted to saltatory conduction. In sensory neurons, $Na_v1.7$ and $Na_v1.8$ are widely expressed from peripheral terminals in the skin to central branches and terminals in the dorsal horn of the spinal cord (Black et al. 2012).

The macromolecular complexes that underly these VGSC targeting and sequestration involve anchoring proteins such as AnkG and βIV spectrin or cell adhesion molecules and β-subunits (Jenkins and Bennett 2001; Nelson and Jenkins 2017; Zhou et al. 1998). In this macromolecular complex, contactin, a cell adhesion molecule, is an important modulator of sodium channel expression and localization. In optic nerve, $Na_v1.2$ subunits define the nodes and the axon initial segment during early postnatal development and a progressive switch from $Na_v1.2$ to $Na_v1.6$ channel subtypes occurs with maturation (Boiko et al. 2001; Kaplan et al. 2001). Experiments that knocked out the expression of contactin in optic nerve revealed a decrease in $Na_v1.6$ nodal sodium channels (Çolakoğlu et al. 2014). An interaction of contactin with $Na_v1.2$ and $Na_v1.3$ has already been described suggesting a similar mechanism (Kazarinova-Noyes et al. 2001; McEwen et al. 2004; Shah et al. 2004). AnkG is a key modulator of VGSC membrane clustering at the AIS

and node of Ranvier (Kordeli et al. 1995; Leterrier et al. 2011). As described previously in this chapter, this anchoring protein could be a cargo adaptor in the trafficking pathway but it is also necessary for the stability of VGSC clusters at the plasma membranes (Barry et al. 2014; Hedstrom et al. 2008). The first interaction between AnkG and VGSC was observed in 2003 with $Na_v1.2$ and a highly conserved nine-amino acid motif in intracellular loop 2 of VGSC was identified for binding AnkG (Garrido et al. 2003; Lemaillet et al. 2003). AnkG interaction has then been characterized for other VGSC such as $Na_v1.6$ and $Na_v1.8$ (Gasser et al. 2012; Montersino et al. 2014). Proteins that interact with AnkG such as Neural cell adhesion molecule L1 (also known as L1CAM) could also contribute to functional expression and localization of Na^+ channels to the neuronal plasma membrane, ensuring correct initiation of action potential and normal firing activity (Valente et al. 2016).

Interestingly, these mechanisms of VGSC targeting seem scalable. This is the case for the interaction between VGSC and AnkG that was shown to be enhanced through the phosphorylation of the Ankyrin-binding motif of VGSC by the protein casein kinase 2 (CK2) (Bréchet et al. 2008; Hien et al. 2014). These data strongly suggest a regulatory mechanism for changes in VGSC subcellular domains such as AIS. Similarly, a recent study has shown that Fibroblast Growth Factor (FGF) 13 binds directly to VGSC in hippocampal neurons to limit their somatodendritic surface expression, although exerting little effect on VGSC within the AIS. In contrast, homologous FGF14, which is highly concentrated in the proximal axon, binds directly to VGSC to promote their axonal localization (Pablo et al. 2016). At the node of Ranvier, the role of AnkG in clustering VGSC was recently shown to be dispensable. Indeed, using a conditional knock-out model, a study revealed that in the absence of AnkG, Ankyrin R-βI spectrin protein complexes function as secondary reserve for Na^+ channel clustering machinery (Ho et al. 2014).

As for neuronal cells, cardiac myocytes display various subcellular electrophysiological needs to ensure cell–cell conduction and excitation/contraction coupling. Targeting cardiac VGSC to specific membrane areas is therefore important for the cell function. There are several lines of evidence demonstrating that the most prominent cardiac Na^+ channel is $Na_v1.5$. However, whereas their functional role is not clearly determined, numerous studies revealed that neuronal sodium channels are also present in cardiac tissues (Kaufmann et al. 2013; for review see Zimmer et al. 2014). In cardiac myocytes, VGSC membrane expression can be divided into two pools: the lateral membrane and the intercalated disc pools (for review see Abriel et al. 2015). Interestingly, the macromolecular complexes that govern these pools depend on the cardiomyocyte location. Briefly, like for neuronal AIS and node of Ranvier, targeting $Na_v1.5$ to the intercalated discs involved the AnkG anchoring protein (Makara et al. 2014) and interacting proteins such as SAP97 (Petitprez et al. 2011), N-cadherin and connexin 43 (Malhotra et al. 2004; Rhett et al. 2012) as well as plakophilin-2 and desmoglein-2 (Rizzo et al. 2012; Sato et al. 2009). At the lateral membrane of cardiomyocytes, $Na_v1.5$ targeting depends on different macromolecular complexes related to syntrophin/dystrophin expression

(Gavillet et al. 2006; Petitprez et al. 2011). This occurs through a PDZ domain binding motif on $Na_v1.5$ (Shy et al. 2014).

4 VGSC Retrograde Transport

The regulation of Na_v channel late trafficking occurs even after VGSC subunits have reached their ultimate site of function, by undergoing constitutive and dynamic turnover. Their steady-state surface expression is governed by a balance between channel anterograde trafficking, endocytic recycling, and degradative pathways. More specifically, it has been reported that sarcolemmal $Na_v1.5$ subunits have apparent long half-lives of about ~35 h whereas other "neuronal" VGSC were estimated to be turned over with half-lives ranging from 17 to 50 h, depending on the cell-type or culture conditions, but reflecting their relative stability within their macromolecular complex (Maltsev et al. 2008; Monjaraz et al. 2000; Schmidt and Catterall 1986; Sherman et al. 1985).

Whether it concerns muscle, cardiomyocyte or neuronal cells, ion channels are subject to endocytic events. These processes allow cells to tune their response to environmental stimuli by modulating their electrical excitability. To date, clathrin-mediated endocytosis (CME) is identified as the major mechanism of VGSC internalization (Dustrude et al. 2013; Garrido 2001; Liu et al. 2005). This critical process by which specific mono-ubiquitinated Na_v channels are recruited and packaged into clathrin-coated vesicles is controlled by the selective binding of different accessory proteins (see for review McMahon and Boucrot 2011).

The initial CME step related to cargo recognition requires the recruitment of adaptor protein complexes (APs) (Popova et al. 2013). Among these, the clathrin-adaptor complex AP-2 might play dual roles in vesicular VGSC transport. The first one involves a di-leucine-based motif identified in the C-terminal region of $Na_v1.2$ that can be recognized by AP-2 as an endocytic machinery component (Garrido 2001). Indeed, it appears that this internalization signal is required for the somatodendritic selective endocytosis of $Na_v1.2$ subtype, indirectly conditioning its axonal compartmentalization. It is worth adding that the $Na_v1.2$ DII-DIII loop contains an additional endocytosis signal that also promotes selective elimination of untrapped $Na_v1.2$ from the somatodendritic membrane (Fache et al. 2004).

Besides, AP-2 seems to modulate correct VGSC targeting and clustering to the AIS in early stages of neurodevelopment by preventing $Na_v1.6$ enrichment at this specialized signaling domain (Kyung et al. 2017).

Another adaptor protein, sodium channel and clathrin linker 1 (SCLT1), was identified as a subtype-specific binding partner mediating indirect $Na_v1.8$ and clathrin association (Liu et al. 2005). The formation of this tri-molecular protein complex is thought to facilitate VGSC internalization, given that SCLT1 overexpression leads to reduced current Na^+ density in murine DRG neurons.

As is the case during their forward trafficking, surface VGSC appear to interact constantly with multiple protein partners which can be involved in their endocytic internalization. αB-crystallin, a member of the small heat shock protein family, is

known to be a stress-responsive protein displaying chaperone-like activities (Gangalum et al. 2012; Minami et al. 2003). Although its protective role in preventing aggregation of unstable proteins has been demonstrated in cardiac and lens cells, little is known about its potential role in the folding of VGSC channels (Horwitz 1992; Wang 2003). However, it eventually appeared that αB-crystallin can affect the stability of cell surface $Na_v1.5$ channels by reducing their Nedd4-2-dependent ubiquitylation and internalization for degradation (Huang et al. 2016).

A recent study postulates that the subcellular distribution of two members of the FGF homologous factors, FGF13 and FGF14, could be relevant for the differential modulation of VGSC membrane expression in rat hippocampal neurons (Pablo et al. 2016). While FGF14B is axonally restricted and promotes VGSC surface expression, the broadly distributed FGF13 protein has been shown to regulate VGSC selective internalization from the somatodendritic compartment via a dynamin-dependent endocytotic pathway. It is worthy to mention that FGF13 knockdown produced a cellular context-specific disruption in Na^+ current density in mouse cardiomyocytes, this being in contrast to the increase observed in hippocampal neurons (Wang et al. 2011).

The stress-activated p38 mitogen-activated protein kinase was shown to reduce $Na_v1.6$ peak currents in both native neurons and neuronal cell lines (Gasser et al. 2012; Wittmack 2005). It appeared that this effect could be attributed to the phosphorylation of a single serine residue allowing Nedd4-like (neural precursor cell-expressed developmentally downregulated gene 4) protein to bind and ubiquitylate $Na_v1.6$ channels, which in turn promotes their internalization into neurons. Subsequent diminished neuronal excitability might constitute an adaptive and protective mechanism after cellular stress or injury (Gasser et al. 2012). As for surface ubiquitylated VGSC fate, evidence suggests that the ubiquitin E3 protein ligase Nedd4-2 would induce their internalization rather than their degradation, though the occurrence of these processes seems to be subtype-and tissue-dependent (as reviewed in Laedermann et al. 2013).

Apart from classical interacting protein partners, as those described above, VGSC proteostasis was found to be directly controlled by collapsin response mediator protein 2 (CRMP2) SUMOylation (Dustrude et al. 2013). Loss of this post-translational modification results in reduced Na^+ current density in rat DRG neurons, by promoting $Na_v1.7$ endocytosis through a clathrin-mediated mechanism (Dustrude et al. 2016). The axonal CRMP2 in its SUMOylated state was demonstrated as playing a central role in recruiting endocytosis-related proteins such as Numb and Eps15, and indirectly Nedd4-2, all of these affecting $Na_v1.7$ internalization in a subtype-specific manner.

5 Trafficking Modulation in Physiopathology

The identification and characterization of VGSC mutations have considerably increased our understanding of ion channel function. Various genetic mutations lead to misfolded proteins and subsequent trafficking defects. These mutations could cover either the α-subunit or a protein involved in VGSC macromolecular

complex such as β-subunits (Adsit et al. 2013; Amin et al. 2010; Vacher and Trimmer 2012). As a consequence, the reduction of the membrane functional proteins leads to electrophysiological defects and subsequent pathological manifestations. To date, most of these mutants have been associated with $Na_v1.1$ and $Na_v1.5$ sodium channels. $Na_v1.1$ is predominately expressed in the CNS and particularly in γ-aminobutyric acid (GABA)ergic neurons (Duflocq et al. 2008). Characterized mutations that affect the trafficking of $Na_v1.1$ are related to different types of epilepsia such as generalized epilepsy with febrile seizure plus (GEFS+) or severe myoclonic epilepsy of infancy (SMEI) (Rusconi et al. 2007, 2009; Thompson et al. 2012). Numerous *SCN5A* mutations leading to trafficking defective $Na_v1.5$ have been extensively studied in cardiac disease. These mutations are associated with cardiac conduction defects that could lead to sudden death as in Brugada Syndrome or Sudden Infant Death Syndrome (Amin et al. 2010).

Most of the trafficking defective proteins can be functional when they are correctly inserted into the plasma membrane. Developing strategies to restore the trafficking of these proteins is therefore of interest. Impaired trafficking of mutant ion channels is a well-established pathological mechanism of channelopathies such as Cystic Fibrosis or cardiac Long QT syndrome and many strategies used to rescue VGSC were adapted from studies on rescue of the cystic fibrosis transmembrane conductance regulator (CFTR) channel or Human ether-a-go-go-related gene (hERG) potassium channel (Farinha and Canato 2017; Ficker et al. 2002; Lukacs and Verkman 2012; Zhou et al. 1999). One method commonly used consists in the modulation of the sarcoendoplasmic reticulum calcium transport ATPase pump by treating cells with thapsigargin or curcumin (Keller et al. 2005; Moreau et al. 2012; Rusconi et al. 2007, 2009). These pharmacological treatments would act on Ca^{2+}-dependent chaperones that retain the misfolded protein in the ER (Egan et al. 2002, 2004).

Direct interaction of a molecule with VGSC α-subunit can also modulate its trafficking. These small molecules, also called pharmacological chaperones, are generally blockers of cardiac and neuronal VGSC. For example, cell incubation with class I antiarrhythmic drugs such as mexiletine or antiepileptic drugs like phenytoin and carbamazepine can functionally restore a fraction of $Na_v1.5$ trafficking defective mutants to the plasma membrane (Keller et al. 2005; Moreau et al. 2012; Rusconi et al. 2007, 2009; Thompson et al. 2012; Valdivia et al. 2004). Interestingly, whereas these strategies are almost exclusively tested on trafficking defective mutants, they could also impact wild type protein trafficking as it was observed for $Na_v1.8$ after incubation with the local anesthetic lidocaine (Zhao et al. 2007).

Cell culture at low temperature (~25–28°C) for few hours could also trigger the rescue of misfolded VGSC mutants (Keller et al. 2005; Moreau et al. 2012; Rusconi et al. 2007, 2009; Valdivia et al. 2004). While little is known concerning this effect for VGSC, it seems likely to be the consequence of a slowed folding process which acts in preventing protein misfolding and aggregation (Ulloa-Aguirre et al. 2004). Moreover, thermosensitive proteins such as Heat Shock Proteins (HSP) have been involved in protein folding processes and in the rescue of delta508 CFTR protein

(Lopes-Pacheco et al. 2015; Lukacs and Verkman 2012). These proteins could therefore be involved in VGSC correct folding processes.

Interestingly, whereas the positive influence of low temperature and calcium modulators on surface membrane channel expression could be generalized to different misfolded ion channels, the pharmacological chaperones seem to act in a substrate-specific manner. Indeed, specific molecules published to restore the trafficking of the delta F508 CTFR mutant were tested and remained without effect on rescuable folding defects of $Na_v1.1$ and $Na_v1.5$ (Norez et al. 2014; Thompson et al. 2012). Such a specificity of action is interesting since it could limit potential side effects of pharmacological rescue strategies.

The recent advances in VGSC conformational structures (Payandeh et al. 2012; Shen et al. 2017; Sula et al. 2017) will bring fundamental information to understand VGSC folding as well as misfolding consequences of mutations. This should release new pharmacological approaches for rescuing VGSC trafficking defects. An exciting strategy in the future would be to design new molecules based on VGSC structural misfolding knowledge to restore the correct structural folding of mutant VGSC and its membrane localization. Pharmacological assays could not be the only way to recover proper trafficking of VGSC. For example, a polymorphism H558R observed in $Na_v1.5$ has been shown to have a corrective effect on the trafficking defective mutation R282H associated with Brugada Syndrome (Poelzing et al. 2006). Interestingly, the expression of a 20–40 amino acid peptide that contains H558R was shown to be sufficient to restore the trafficking of R282H $Na_v1.5$ mutant (Shinlapawittayatorn et al. 2011). This peptide could be sufficient by itself to limit the misfolding of the mutant through a physical interaction. However, if trafficking rescue is interesting for therapeutic approaches, it is probably not sufficient. Indeed, in addition to their trafficking defects, VGSC mutations can display gating defects once restored to the plasma membrane (Thompson et al. 2012). Rescuing such mutants to the plasma membrane in a therapeutic perspective can therefore uncover deleterious manifestations for patients.

6 Conclusion

This chapter reviews the current understanding of the facets of VGSC trafficking (See Fig. 1). The needs of highly specialized neuronal and cardiac cells are partially fulfilled by the differential expression of key protein partners. These actors appear to determine Na_v subtypes' fate at different stages of trafficking including their anchoring to specialized targeting regions. The most interesting question is now to decipher how these protein partners dynamically and sequentially interact with VGSC. Whereas numerous studies have investigated VGSC trafficking in heterologous expression systems, this dynamic of VGSC trafficking could only be studied from native cells which represents the next challenge for research laboratories. This knowledge should result in new pharmaceutical strategies. Indeed, most of the existing approaches are focused on the modulation of biophysical properties of VGSC localized at the plasma membrane. However, understanding the different

steps of VGSC life cycle and quality controls should open new avenues to modulate VGSC expression in time and space such as their plasma localization, their membrane half-life or the rescue of trafficking defective mutants.

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