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Trafficking Mechanisms Underlying Neuronal Voltage-gated Ion Channel Localization at the Axon Initial Segment

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Summary

Voltage-gated ion channels are diverse and fundamental determinants of neuronal intrinsic excitability. Voltage-gated K⁺ (Kv) and Na⁺ (Nav) channels play complex yet fundamentally important roles in determining intrinsic excitability. The Kv and Nav channels located at the axon initial segment (AIS) play a unique and especially important role in generating neuronal output in the form of anterograde axonal and backpropagating action potentials. Aberrant intrinsic excitability in individual neurons within networks contributes to synchronous neuronal activity leading to seizures. Mutations in ion channel genes gives rise to a variety of seizure-related “Channelopathies”, and many of the ion channel subunits associated with epilepsy mutations are localized at the AIS, making this a hotspot for epileptogenesis. Here we review the cellular mechanisms that underlie the trafficking of Kv and Nav channels found at the AIS, and how Kv and Nav channel mutations associated with epilepsy can alter these processes.

Keywords

Potassium; channel-Sodium; channel- Neuron-Subcellular; localization-Seizures

Introduction

Voltage-gated ion channels are fundamental determinants of intrinsic excitability in neurons (Hille, 2001). Changes in expression, localization and function of ion channels underlie changes in neuronal excitability (Nusser, 2011). Epilepsy is characterized by the occurrence of spontaneous seizures, which consist of large populations of brain neurons exhibiting bursts of synchronous firing. Aberrant intrinsic excitability in individual neurons within networks contributes to synchronous neuronal activity leading to seizures. A variety of mechanisms working to alter expression, localization and function of voltage-gated ion channels lead to aberrant intrinsic excitability. Among the intrinsic ionic conductances that

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govern neuronal excitability, voltage-gated K⁺ (Kv) and voltage-gated Na⁺ (Nav) currents play complex and fundamentally important roles in fine-tuning cellular and network activity. While Kv and Nav channels are found throughout neurons, those located at the axon initial segment (AIS) play a unique and especially important role in generating neuronal output in the form of axonal action potentials (Bender & Trussell, 2012), and backpropagating action potentials that invade the soma and dendritic tree to influence computational and integrative events (Hu et al., 2009). Moreover, many of the ion channel subunits associated with epilepsy mutations are localized at the AIS, making this a hotspot for epileptogenesis (Wimmer et al., 2010b). We note that recent reviews have focused on both general aspects of ion channel trafficking (Jensen et al., 2011, Leterrier et al., 2011, Vacher & Trimmer, 2011) and on the generation and maintenance of the AIS (Grubb & Burrone, 2010b, Rasband, 2010, 2011). Here we review the cellular mechanisms that underlie the trafficking of Kv and Nav channels found at the AIS, and how Kv and Nav channel mutations associated with epilepsy can alter these processes. Mutations involved in numerous human diseases can cause protein trafficking defects *via* diverse mechanisms, in some cases due to mutations in bona fide trafficking motifs, and in others due to a more general misfolding of the mutant protein that cannot pass the rough endoplasmic reticulum (ER) quality control systems (Braakman & Bulleid, 2011) leading to degradation (Cobbold et al., 2003, Sitia & Braakman, 2003). There is increased interest in identifying molecules can rescue trafficking defective mutants and restore expression and function (Bernier et al., 2004). Note that a series of recent papers have highlighted cell-specific differences in the composition of the AIS [*e.g.*, (Duflocq et al., 2008, Lorincz & Nusser, 2008), etc.], here we focus primarily on the AIS in glutamatergic neurons.

I- Voltage-gated potassium (Kv) channels

I.1- Structure and expression of principal and auxiliary subunits of native Kv channels in mammalian brain

Kv channels are the most diverse subfamily of voltage-gated ion channel α subunits, with over 40 human genes in 12 subfamilies, termed Kv1-Kv12 (Yu & Catterall, 2004). Kv channel α subunits have six transmembrane segments, the first four (S1-S4) form the voltage-sensing module and the region between transmembrane segments S5-S6 the pore module (Figure 1). Four independent α subunits from the same subfamily assemble to form functional homo- or hetero-meric Kv channels, and coassembly with auxiliary subunits further enhances Kv channel diversity. Subunit composition determines the trafficking, localization, biophysical, pharmacological and modulation characteristics of a given Kv channel.

In general, Kv channels exhibit subfamily-specific patterns of subcellular localization (Vacher et al., 2008), with Kv1, Kv2 and Kv7 family members unique in that they are found at the AIS (Figure 2) (Vacher et al., 2008, Clark et al., 2009). Kv1 channels at the AIS play crucial roles in determining action potential initiation and propagation (Kole & Stuart, 2012). These channels generally contain Kv1.1 and Kv1.2 α subunits and auxiliary Kv β subunits (Van Wart et al., 2007, Lorincz & Nusser, 2008, Ogawa et al., 2010, Duflocq et al., 2011, Vacher et al., 2011), although Kv1.4 is found at the AIS in younger animals (Ogawa et al., 2008). Delayed rectifier-type Kv2.1 and Kv2.2 channels (Lim et al., 2000, Johnston et al., 2008, Sarmiere et al., 2008) and M-type Kv7.2 and Kv7.3 channels (Cooper et al., 2001, Cooper, 2011, Klinger et al., 2011) are present at high density at the AIS. Other Kv channel subunits have not been described at the AIS (Vacher et al., 2008).

I.2- Kv channel trafficking and clustering determinants and mechanisms

Specific amino acid sequence motifs acting as trafficking determinants (TDs) have been identified within Kv1, Kv2 and Kv7 α and Kv β auxiliary subunits, and direct the establishment and maintenance of expression and localization of these channels at the AIS. Many of these TDs are located within the intrinsically disordered C-terminal domains, which are thought to act as “intermolecular fishing rods” for interacting proteins important in Kv channel trafficking (Magidovich et al., 2006). These C-terminal domains are highly conserved in mammalian channel orthologs (*e.g.*, human and rat Kv1.2 are 100% identical in the cytoplasmic C-terminus and 99% identical overall), yet are the most divergent regions within the α subunits in mammalian paralogs (*e.g.*, human Kv2.1 and Kv2.2 are only 39% identical in the C-terminus, yet are otherwise 92% identical, and 62% identical overall).

A number of TDs regulate biogenic trafficking of Kv channels through the endomembrane system, from their sites of translation in the ER, to their sites of action in the plasma membrane. These include an anterograde TD sequence VXXSL present within the Kv1.4 cytoplasmic C-terminus, but not in other Kv1 family members (Li et al., 2000), and a potent ER retention TD in the turret domain external to the Kv1.1 channel pore that is dominant to the VXXSL TD (Manganas et al., 2001b), and which acts dictate the subunit composition-dependent trafficking of heteromeric Kv1 channels (Manganas & Trimmer, 2000). These TDs can act interdependently to regulate trafficking and plasma membrane expression of Kv1 channels (Zhu et al., 2003).

The same amino acids that form the potent turret TD confer sensitivity to dendrotoxin or DTX, which suggests that a DTX-like molecule within the ER could mediate ER retention by binding to this TD (Manganas et al., 2001b). Expressing soluble DTX within the ER lumen enhances cell surface expression of homomeric and heteromeric channels containing wild-type DTX-sensitive Kv1.1, but not those containing Kv1.1 mutants with reduced DTX binding (Vacher et al., 2007). The matrix metalloprotease MMP23 has a domain similar to a toxin with a Kv1 channel binding selectivity distinct from DTX. Overexpression of MMP23 leads to intracellular retention of Kv1 channels in order of the strength of their MMP23-binding (Rangaraju et al., 2010), suggesting that other MMP23-like mammalian proteins that contain a DTX-like domain could mediate intracellular trafficking by binding to the turret TD. Other Kv1 channel TDs include an acidic motif in a membrane-proximal region of the cytoplasmic C-terminus of Kv1 α subunits (Manganas et al., 2001a), and a specific motif within the extracellular loop between transmembrane segments S1 and S2 (McKeown et al., 2008), although the impact of altering these TDs is likely due to folding defects and ER quality control (Braakman & Bulleid, 2011).

The Kv1.2 α subunit contains weak versions of these TDs, and as such has trafficking characteristics sensitive to modulation by coassembly with Kv1 α subunits containing stronger TDs (Manganas & Trimmer, 2000) and with auxiliary Kv β subunits (Shi et al., 1996, Campomanes et al., 2002, Gu et al., 2003). Phosphorylation of Kv1.2 also regulates its trafficking, for example upon acute suppression of Kv1.2 current upon muscarinic stimulation (Huang et al., 1993), mediated by endocytosis triggered by phosphorylation of specific C-terminal tyrosine residues (Nesti et al., 2004), and leading to disruption of Kv1.2 interaction with the cytoskeletal protein cortactin (Williams et al., 2007). Note that phosphorylation at a different C-terminal tyrosine residue regulates Kv1.2 clustering (Gu & Gu, 2011, Smith et al., 2012). C-terminal serine phosphorylation sites regulate biogenic trafficking of Kv1.2-containing channels (Yang et al., 2007), and PKA-mediated enhancement of Kv1.2 currents (Johnson et al., 2009).

Polarized trafficking of Kv1 channels to axons is dependent upon the T1 domain within the cytoplasmic N-terminus (Rivera et al., 2005) that serves as a binding site for Kif5b subunits

of the microtubule motor kinesin 1 (Rivera et al., 2007). Another kinesin subunit, KIF3A, a component of kinesin 2, is involved in polarized trafficking of Kv1.2 channels to axons (Gu et al., 2006, Gu & Gu, 2010). Kinesin-based trafficking of Kv1 channels is strongly influenced by cytoplasmic Kv β auxiliary subunits, which also bind to the T1 domain. All three Kv β subunit isoforms (Kv β 1-3) can promote cell surface expression of associated Kv1 channel complexes *via* effects on biogenic ER export (Shi et al., 1996, Campomanes et al., 2002), while co-expression of Kv β 2 with intact Kv1.2 (Campomanes et al., 2002) or reporter constructs containing the Kv1.2 T1 domain (Gu et al., 2003) increases their polarized expression in axons versus dendrites. Kv β 2 directly interacts with the microtubule binding protein EB1 (Gu et al., 2006, Vacher et al., 2011), and knockdown of EB1 suppresses Kv1.2 trafficking to axons (Gu & Gu, 2010). Phosphorylation of Kv β 2 regulates its interaction with EB1, and alters trafficking of Kv1 channels to axons and specifically to the AIS (Vacher et al., 2011), suggesting a mechanism for the dynamic regulation of ion channel localization at the AIS (Kuba et al., 2006, Grubb & Burrone, 2010a, Kuba et al., 2010, Grubb et al., 2011). Kv1 α subunits contain a PDZ-binding motif (Figure 1) on their C-termini (Kim et al., 1995), and specific PDZ domain-containing proteins of the MAGUK family, specifically PSD-93, are found associated and colocalized with Kv1 channels at the AIS (Ogawa et al., 2008). The precise targeting mechanisms that generate the observed colocalization of these proteins to the AIS are not known.

Unlike Kv1 channels, biogenic intracellular trafficking of Kv2.1 channels to the plasma membrane is not strongly influenced by TDs (Shi et al., 1994, Lim et al., 2000). However, increased insertion of Kv2.1 into the plasma membrane occurs acutely in response to stimuli that induce neuronal apoptosis (Yu et al., 1999, Pal et al., 2003, Pal et al., 2006, Redman et al., 2007) *via* increased Kv2.1 phosphorylation at specific C-terminal sites (Redman et al., 2007), and in response to monocular deprivation, leading to changes in neuronal excitability and intrinsic plasticity in visual cortical neurons (Nataraj et al., 2010). Changes in Kv2.1 phosphorylation at other C-terminal sites regulate whether Kv2.1 is clustered or is dispersed across the somatodendritic membrane (Misonou et al., 2004, Misonou et al., 2005, Misonou et al., 2006), although Kv2.1 channels at the AIS of cultured neurons are somewhat refractory to dephosphorylation-dependent dispersal (Misonou et al., 2004). The clustering of Kv2.1 in neurons is mediated by a specific C-terminal domain (the PRC domain, Figure 1) that also restricts the localization of clustered Kv2.1 to proximal dendrites, and to the AIS (Lim et al., 2000). Phosphorylation of Kv2.1 by CDK5 is required for maintenance of AIS clustering (Cerdeira & Trimmer, 2011). Recent studies suggest that Kv2.2 exists in somatodendritic clusters in certain brain neurons (Hermansteyne et al., 2010), in some cases associated with Kv2.1 (Kihira et al., 2010). Kv2.2 has been proposed to act at the AIS of auditory neurons (Johnston et al., 2008). The precise mechanism of Kv2 channel clustering at the AIS has not been determined, although compelling data have been provided supporting a role for a perimeter fence-based mechanism underlying the somatodendritic clustering of Kv2.1 (O'Connell et al., 2006, Tamkun et al., 2007).

Neuronal M-type Kv7 channels at the AIS exist as heteromers of Kv7.2 and Kv7.3 α subunits (Figure 2) (Cooper, 2011). Coassembly of Kv7.2 and Kv7.3 is crucial to their efficient trafficking, as the respective homomeric channels exhibit poor cell surface expression due to ER retention (Schwake et al., 2003, Rasmussen et al., 2007). Heteromeric Kv7.2/Kv7.3 assembly is also required for efficient localization at the AIS (Rasmussen et al., 2007). Trafficking of homomeric Kv7.3 is especially deficient, a characteristic that has been related to a Kv7.3-specific Ala at position 315 (as opposed to a Thr at this position in all other Kv channels) that acts as a potent pore TD, such that its mutation to Thr allows for expression of homomeric Kv7.3 channels (Gomez-Posada et al., 2010)(Figure 1). The location of this residue deep within the channel pore makes it unlikely that retention is mediated by interaction with a luminal ER protein, and more likely due to misfolding of

homomeric Kv7.3 channels and retention *via* ER quality control (Braakman & Bulleid, 2011).

Numerous protein-protein interactions are mediated by the C-terminus of Kv7 α subunits (Haitin & Attali, 2008) (Figure 1). This includes interaction of Kv7.2 and Kv7.3 with ankyrin-G (Ank-G), *via* a C-terminal binding motif, which localizes Kv7 channels to the AIS (Cooper, 2011), and of Kv7.2 with calmodulin (CaM), which plays a particularly critical role in trafficking (Etxeberria et al., 2008, Alaimo et al., 2009). The C-terminus of Kv7 α subunits is also extensively modified by phosphorylation, with proteomic analyses identifying at least fourteen *in vivo* sites in Kv7.2, and four sites in Kv7.3 (Cerdeira et al., 2011), providing a potential mechanism for dynamic regulation of Kv7 channel trafficking. Trafficking of Kv7 channels is dynamically regulated in epithelial cells, with intracellular retention in unpolarized cells, and plasma membrane expression upon establishment of epithelial polarity (Andersen et al., 2011).

11.3- Defects in Kv trafficking in epilepsy

Among the Kv1 and Kv2 α and Kv β subunits known to be associated with the AIS, only the KCNA1 gene (<http://omim.org/entry/176260>) encoding the Kv1.1 α subunit has mutant alleles associated with neurological disorders, specifically episodic ataxia type 1 or EA-1, often associated with seizures, which arises due to loss-of-function of Kv1.1-containing channels. Numerous missense mutations have been identified that reduce or eliminate trafficking of Kv1.1-containing channels, as well as those that affect channel gating (Kullmann, 2010). A truncated mutant generated from a specific EA-1 nonsense mutation “RX” (Rea et al., 2002) lacks the membrane proximal acidic TD in the Kv1.1 C-terminus (Figure 1), causing misfolding and intracellular retention of the mutated Kv1.1 α subunits (Manganas et al., 2001a). Importantly, co-assembly of these mutated RX subunits with otherwise normal Kv1 α subunits acts as a dominant negative for their folding and trafficking (Manganas et al., 2001a, Rea et al., 2002), and expression of RX in neurons leads to reduced Kv current levels, and increased glutamate release, consistent with loss of expression of RX-containing channels, including those at the AIS (Heeroma et al., 2009), and providing a possible basis for the severe EA-1 symptoms associated with this mutation (Eunson et al., 2000).

The KCNA2 gene encoding Kv1.2 is not known to harbor mutations resulting in neurological or other human diseases (<http://omim.org/entry/176262>), however, Kv1.2 knockout (KO) mice have enhanced susceptibility to flurothyl-induced seizures at postnatal day 14 (P14), at P15 begin to have spontaneous running-bouncing seizures with tonic extension, and by P19 are dead from seizures (Brew et al., 2007). A mouse ENU-induced mutant that exhibits chronic motor uncoordination, named *Pingu*, carries a KCNA2 missense mutation yielding an I402T mutation in the S6 transmembrane segment, resulting in decreased Kv1.1 and Kv1.2 expression in cerebellum, and heterologous cells expressing this mutant have decreased expression, suggesting a trafficking defect (Xie et al., 2010). The KCNB2 gene encoding Kv β 2 does not harbor disease-causing mutations (<http://omim.org/entry/601142>), but certain patients with 1p36 deletion syndrome lack the chromosomal region containing the Kv β 2 gene (Shapira et al., 1997), and there exists a strong association between seizure severity and deletion of KCNB2 (Heilstedt et al., 2001, Kurosawa et al., 2005). Disease-associated mutations in Kv2.1 and Kv2.2 (<http://omim.org/entry/600397> and <http://omim.org/entry/607738>, respectively) have not been described.

Eleven distinct allelic variants of Kv7.2 are associated with Benign Familial Neonatal Epilepsy (BFNE) (<http://omim.org/entry/602235>), as are mutations in Kv7.3 (<http://omim.org/entry/602232>). Kv7 channels are also important as targets for retigabine, a novel anti-epileptic drug that acts as a positive allosteric modulator in promoting the enhanced

opening of neuronal Kv7 channels (Gunthorpe et al., 2012). One set of BFNE-associated mutations cluster in the Kv7.3 primary sequence just up- (D305G, W309R, G310V) and down- (R330C) stream of the critical pore Ala-315 TD (Figure 1). It is not known whether these mutants exhibit reduced current due to reduced single channel conductance or reduced expression (Maljevic et al., 2010), although the G310V mutant has reduced levels of axonal surface expression in cultured neurons (Chung et al., 2006). A mutation in the pore region of Kv7.2 results in misfolding and reduced trafficking, however the channels that appear at the cell surface have normal single channel conductance (Maljevic et al., 2011). It is intriguing that a large number of BFNE mutations cluster within the Kv7.2 C-terminus, including missense, nonsense and frame shift mutations. Not surprisingly, many of these yield reduced expression *via* effects on trafficking of homomeric and heteromeric Kv7.2-containing channels (Maljevic et al., 2010). Some of these (Figure 1) appear to act through decreasing binding of CaM (Richards et al., 2004) such as Kv7.2 mutations R353G (Etzeberria et al., 2008) and L339R (Alaimo et al., 2009), which impact the exit of Kv7.2-containing channels from the ER, as well as L619R (Richards et al., 2004), whose trafficking properties have not been determined. While the role of CaM in Kv7 channel function is complex (Haitin & Attali, 2008), and includes effects on gating, and binding to other interacting proteins such as AKAPs (Bal et al., 2010), CaM binding, and its disruption by mutations found in epilepsy, impact trafficking. BFNE mutations have also been found to alter the polarized localization and plasma membrane expression of Kv7.2/Kv7.3 channels in axons (Chung et al., 2006).

II- Voltage-gated sodium channels

II.1- Structure and expression of principal and auxiliary subunits of native Nav channels in mammalian brain

Nav channels consist of a highly posttranslationally modified α subunit, approximately 260 kDa in size, associated with auxiliary β subunits (~30 kDa) through either covalent ($\beta 2$, $\beta 4$) or non-covalent ($\beta 1$, $\beta 3$) linkages (Catterall, 2000). Nav α subunits have four internally repeated homologous domains (I-IV), each resembling a Kv channel α subunit (Figure 3). The voltage-sensing and pore-forming α subunit is sufficient for functional expression, but the kinetics and voltage-dependence of channel gating are impacted by β subunits. Auxiliary subunits are also involved in channel trafficking, localization, and interaction of Nav channels with cell adhesion molecules, extracellular matrix, and intracellular cytoskeleton (Qu et al., 2001, McEwen et al., 2004). Nav1.1 (Duflocq et al., 2008, Lorincz & Nusser, 2008), Nav1.2 (Boiko et al., 2003, Lorincz & Nusser, 2008) and Nav1.6 (Caldwell et al., 2000, Krzemien et al., 2000, Boiko et al., 2003) are present at the AIS (Figure 4). Specific functions have been attributed to the different AIS Nav channels (Van Wart & Matthews, 2006, Hu et al., 2009). Nearly 700 mutations of Nav1.1 (<http://omim.org/entry/182389>) have been identified in patients with inherited and sporadic epilepsy, making this the most commonly mutated gene in human epilepsy (Catterall et al., 2010, Meisler et al., 2010). A small number of mutations have been found in Nav1.2 (<http://omim.org/entry/182390>) and Nav1.3 (<http://omim.org/entry/182391>) (Meisler et al., 2010), and studies in mice suggest that Nav1.6 (<http://omim.org/entry/600702>) may contribute to seizure disorders (Meisler et al., 2010). Of the four β subunit genes (SCN1B-4B), to date only mutations in SCN1B (<http://omim.org/entry/600235>) are associated with epilepsy (Patino & Isom, 2010).

II.2- Nav channel trafficking determinants and mechanisms

In mammalian neurons, dense clusters of Nav channels at the AIS underlie action potential generation (Clark et al., 2009, Kress & Mennerick, 2009). Similar to Kv7 channels, Nav clustering at the AIS occurs *via* binding to AnkG (Leterrier et al., 2011), mediated by phosphorylation of the ankyrin-binding motif of Nav by the protein kinase CK2 (Garrido et

al., 2003, Brechet et al., 2008), suggesting a mechanism for dynamic changes in AIS localization (Grubb & Burrone, 2010a, Grubb et al., 2011). β subunits, especially $\beta 2$, increase sodium current density in some heterologous systems by enhancing α subunit cell surface expression (Isom et al., 1995). Compared to wild type, *Scn2b* null hippocampal cultures have an 50% reduction in cell surface ^3H -saxitoxin binding (Chen et al., 2002), and the CA3 region of *Scn1b* null hippocampus expresses decreased levels of Nav1.1 and increased levels of Nav1.3 compared to wild type (Chen et al., 2004). However, the ability of $\beta 1$ and $\beta 2$ to increase Nav current density appears to be cell-type specific (Patino & Isom, 2010).

II.3- Defects in Nav trafficking and epilepsy

Improper localization or expression of Nav channels has been implicated in a number of pathological conditions linked to altered neuronal action potential generation and conduction (Mantegazza et al., 2010). Strikingly, defects in Nav channel function can lead to either neurological hyperexcitability (Meisler et al., 2010) or conduction failure/axonal degeneration (Smith, 2007) depending on the pathological context. Analyses of Nav mutations performed in non-neuronal cells or in mouse genetic models reveal a mixture of loss-of-function and gain-of-function effects due to altered channel gating (Catterall et al., 2010). Other mutations lead to loss-of-function resulting from folding and/or trafficking defects that reduce channel expression, which is exacerbated in the absence of auxiliary β subunits (Rusconi et al., 2007, Misra et al., 2008, Rusconi et al., 2009). However in contrast to Cav channels (Pietrobon, 2010), mutated Nav1 channels do not seem to impair expression or function of wild-type Nav1 channels. A recent study showed that coexpression of two Dravet syndrome Nav1.1 truncation mutants (R222* and R1234*) did not impact expression of wild-type Nav1.1, in either heterologous cells or neurons (Bechi et al., 2012). Moreover, mutations in $\beta 1$ subunits also impair cell surface expression of Nav channels (Patino & Isom, 2010).

In the case of generalized epilepsy with febrile seizures (GEFS+), two mutations in the Nav1.1 C-terminal cytoplasmic domain cause improper folding/trafficking. The M1841T mutant (Rusconi et al., 2007) exhibits impaired trafficking, which can be partially rescued by coexpression of $\beta 1$ subunits, yielding channels with biophysical properties identical to those of wild-type Nav1.1. Incubation of cells at permissive temperatures (temperatures $<30^\circ\text{C}$) and interactions with CaM, G-protein $\beta\gamma$ subunits and pore blocker drugs (phenytoin) all partially rescue M1841T cell surface expression (Rusconi et al., 2007). The R1916G mutation (Rusconi et al., 2009) occurs within the IQ motif critical to CaM binding (Bahler & Rhoads, 2002) and yields loss of function due to folding and trafficking defects (Bernier et al., 2004). Cell surface trafficking of R1916G can be rescued by $\beta 1$ subunit coexpression, revealing channels with altered gating properties, but not by other β subunits, CaM or G $\beta\gamma$ (Rusconi et al., 2009). Although numerous mutations of Nav1.2 channels (M252V, V261M, A263V, R233Q, R1319Q, L1330F, and L1563V) associated with benign familial neonatal epilepsy (BFNIS) affect Nav1.2 gating, leading to a gain-of-function and consequently neuronal hyperexcitability, only a few of these mutations are also associated with changes in trafficking (Scalmani et al., 2006, Xu et al., 2007b, Liao et al., 2010a, Liao et al., 2010b). To date, only one study shows that R1319Q, L1330F, and L1563V mutations yield reduced plasma membrane trafficking in addition to loss- (R1319Q, L1330F) or gain- (L1563V) of-function gating phenotypes (Misra et al., 2008). These findings have led to several hypotheses regarding how the developmental expression of Nav1.2 impacts the age dependence of BFNIS.

All epileptic syndromes associated with $\beta 1$ subunit mutations are included in GEFS+ as well as in Dravet Syndrome. The majority of *SCN1B* epilepsy mutations are loss-of-function, with many yielding reduced Nav channel plasma membrane trafficking. Interestingly, the

majority of reported disease-causing mutations in $\beta 1$ (C121W, R85C, R85H, R125C) occur within the extracellular immunoglobulin-like domain (Meadows et al., 2002, Xu et al., 2007a, Patino et al., 2009). This domain is important in mediating interaction of $\beta 1$ with cell adhesion molecules and extracellular matrix. $\beta 1$ (C121W) knock-in mice exhibit a complete loss of $\beta 1$ targeting to the AIS, although the subcellular localization of Nav channel α subunits appears surprisingly unaffected (Wimmer et al., 2010a), consistent with previous studies showing that AnkG-binding motifs in Nav α subunits are both necessary and sufficient for their AIS targeting. This suggests that the increase in excitability in $\beta 1$ (C121W) neurons is mediated by a “gain-of-function” in channel gating, due to loss of $\beta 1$ subunits in the AIS channels. It is intriguing that these results from the $\beta 1$ (C121W) knock-in mice (Wimmer et al., 2010a) conflict with those from heterologous cells, where $\beta 1$ -C121W mutants exhibit normal cell surface trafficking (Meadows et al., 2002). The R125C mutation results in $\beta 1$ subunits that are synthesized normally but not trafficked to the cell surface, although this can be rescued by lower temperatures, or by expression in *Xenopus* oocytes maintained at 18°C (Patino et al., 2009). Other GEFS+ $\beta 1$ mutations (R85C and R85H) yield reduced plasma membrane expression, and coexpressed Nav channels exhibit altered gating relative to wild-type $\beta 1$ (Xu et al., 2007a). A recently identified GEFS+ mutation G257R occurs in a soluble secreted splice variant of $\beta 1B$ that promotes neurite outgrowth *in vitro*, and based on its prominent expression during embryonic development, has been suggested to play a role in axonal pathfinding, although subtle effects on Nav1.3 gating are observed (Brackenbury & Isom, 2011). The G257R mutant has defective trafficking resulting in its intracellular retention, generating a functional null cellular phenotype that may impact neuronal function *via* effects on Nav channel gating, or through altered neuronal pathfinding (Patino et al., 2011).

Increases in Nav α subunit expression and channel activity have been observed after *status epilepticus* in the rat model of temporal lobe epilepsy (TLE), due to increased expression of specific Nav mRNAs (Aronica et al., 2001, Ketelaars et al., 2001). For example, entorhinal cortex layer II neurons have increased Nav channel expression and activity (Hargus et al., 2011), leading to their hyperexcitability, and a resultant increase in excitatory input into hippocampus *via* the perforant path (Kumar & Buckmaster, 2006). Interestingly, these neurons exhibit increased staining of Nav1.6 at the AIS and Nav1.2 (Hargus et al., 2011). Expression of Nav1.6 and Ank-G, but not Nav1.1, is upregulated in the CA1 region of the hippocampus following *status epilepticus*, while Nav1.1 expression remains similar to the age-matched controls, however, the staining presented was not at normal sites of expression of Nav1.6 and Ank-G at the AIS, but was intracellular (Chen et al., 2009), such that the relationship of the increased expression to neuronal excitability remains unclear. Note that Nav α subunits are extensively phosphorylated (Berendt et al., 2010), providing a potential mechanism for dynamic changes in expression, localization, and function.

Conclusions

Trafficking of Kv and Nav channels to the AIS is a crucial determinant of neuronal excitability. Many of the Kv and Nav channels associated with epilepsy mutations are localized at the AIS, making this a hotspot for epileptogenesis (Wimmer et al., 2010b), and many of these mutations yield defects in trafficking. Moreover, dynamic regulation of the expression and localization of Kv and Nav channels at the AIS results in altered excitability (Kuba et al., 2006, Grubb & Burrone, 2010a, Kuba et al., 2010), suggesting that activity-dependent modulation of trafficking to these sites could impact neuronal and network function.

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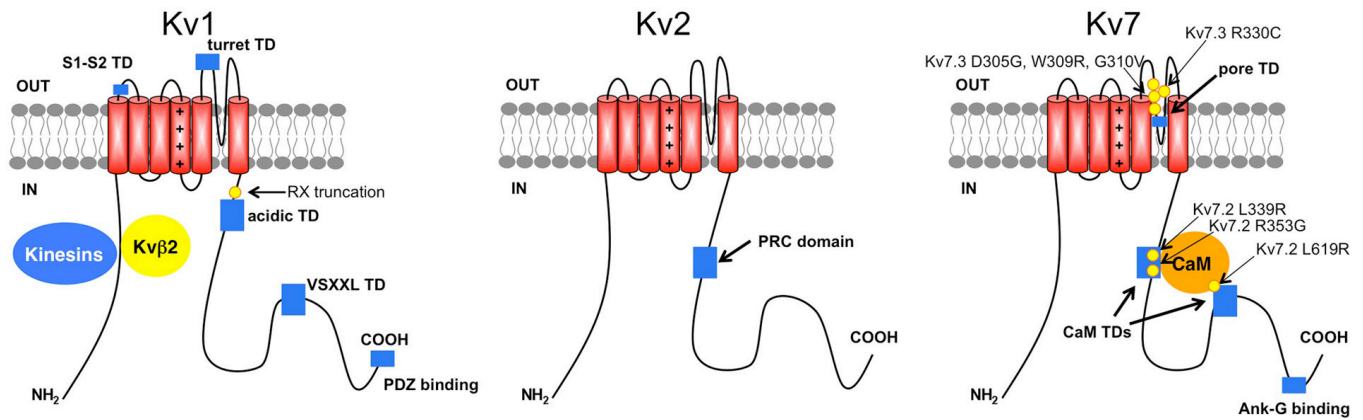


Figure 1.

Cartoon showing transmembrane topology of a prototypical Kv1 (left) and Kv7 (right) channel α subunit, with the voltage-sensing module comprising transmembrane segments S1-S4 (positive charges shown in S4) to the left, and the pore module comprising S5-S6 to the right. Colored rectangles show approximate locations of identified trafficking determinants or TDs. Colored ovals show approximate binding sites of interacting proteins implicated in trafficking to and/or scaffolding at the AIS. Yellow circles denote specific sites of epilepsy-associated mutations known to impact trafficking.

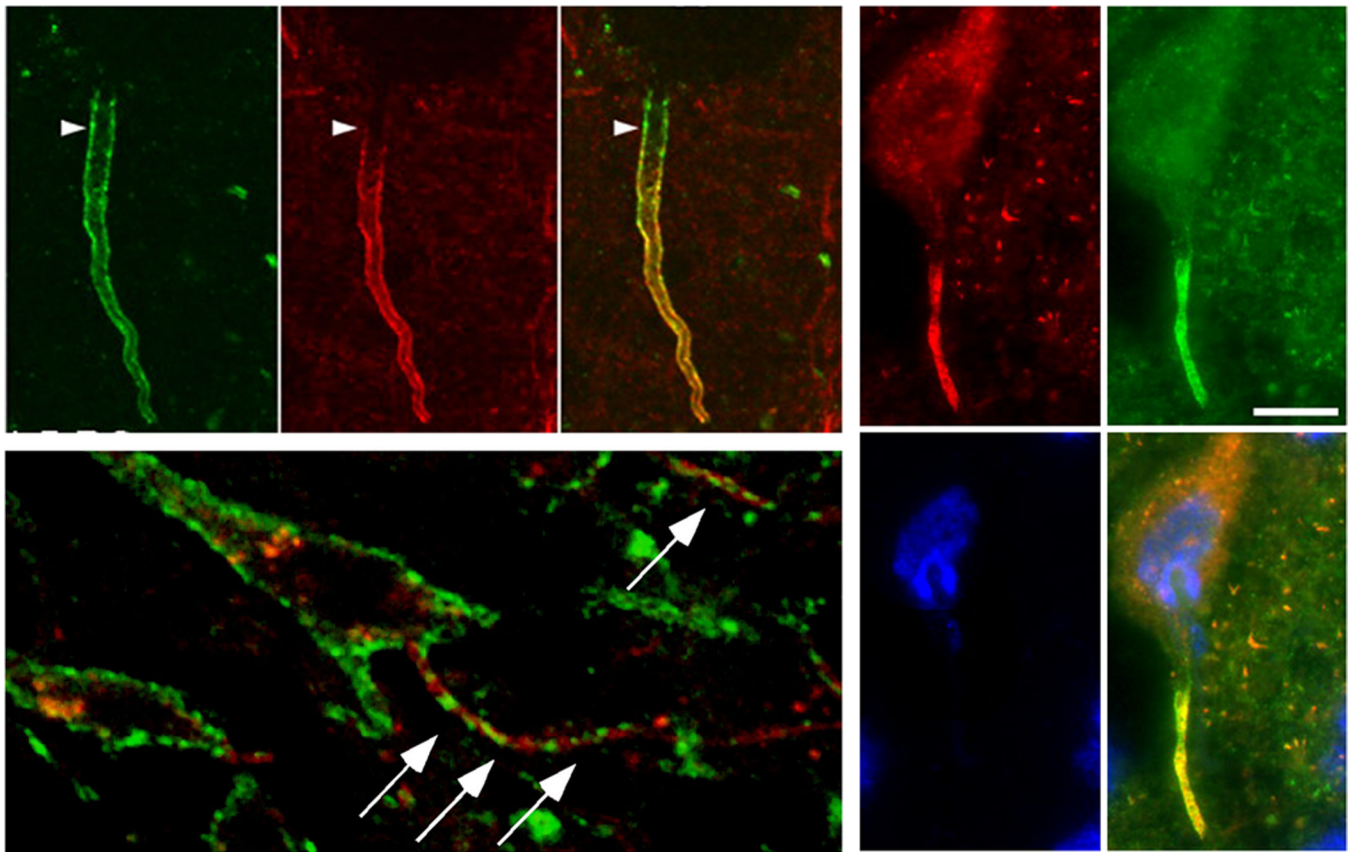


Figure 2.

Localization of Kv1 α subunits at the AIS in the mammalian nervous system. Top left panel: Nav1.6 (green) and Kv1.2 (red) α subunits in a layer 5 pyramidal neuron in rat neocortex (Lorincz & Nusser, 2008). Bottom left panel: Kv2.1 (green) α subunits and ANK-G (red) in neuron within the CA1 region of rat hippocampus (Sarmiere et al., 2008). Right panel. KCNQ2 (red) and KCNQ3 (green) α subunits in the axon initial segment of a mouse spinal cord ventral horn motoneuron; blue is the nucleic acid stain DAPI (Pan et al., 2006).

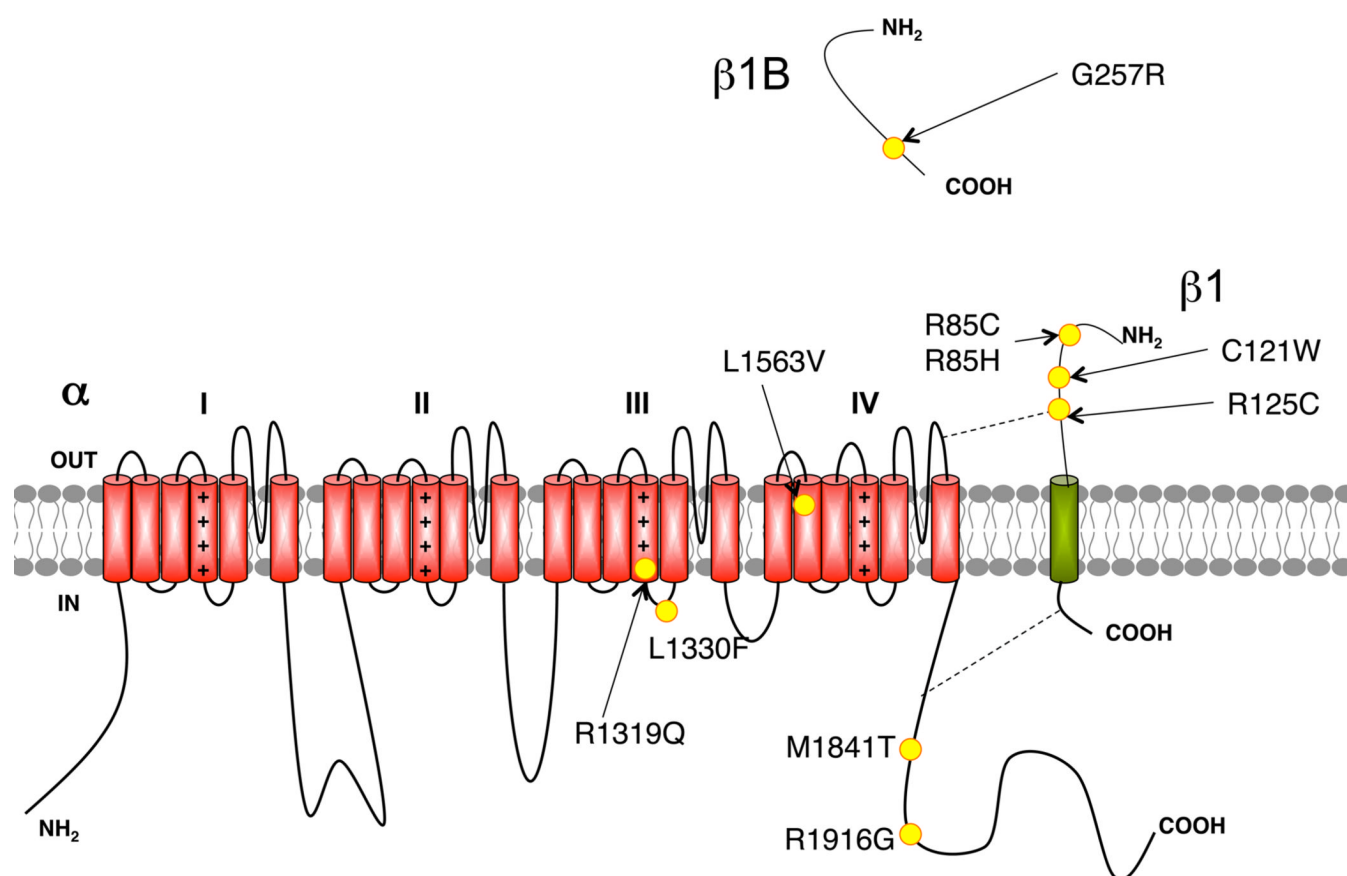


Figure 3.

Cartoon showing transmembrane topology of an Nav channel, with the domains 1–4 arranged from left to right. The colored oval shows approximate binding sites of ANK-G that mediates localization at the AIS. Yellow circles denote specific sites of epilepsy-associated mutations known to impact trafficking.

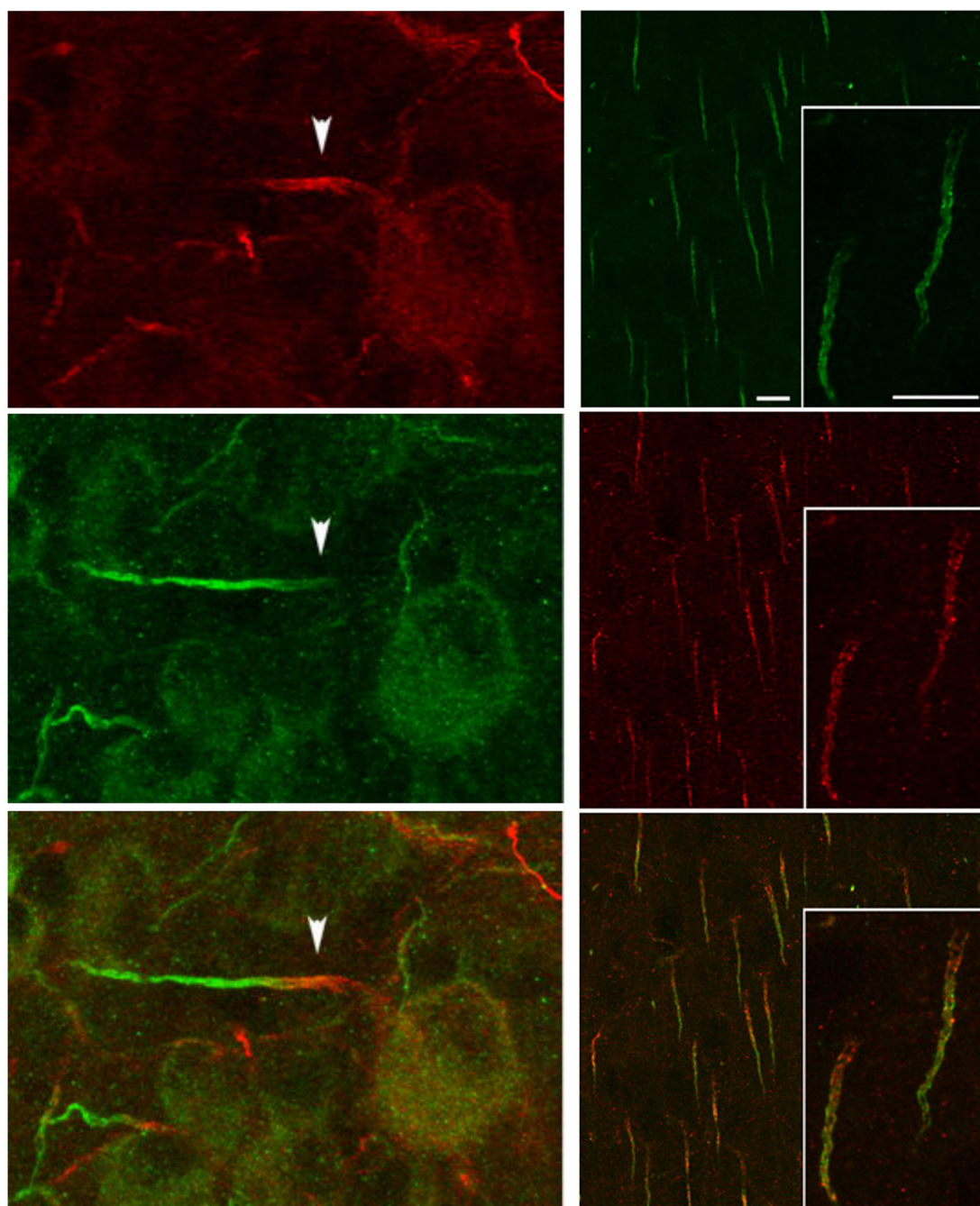


Figure 4. Localization of Nav α subunits at the AIS in the mammalian nervous system. Left. Nav1.1 (red) and Nav1.6 (green) in the AIS of rat retinal ganglion cells. (Van Wart et al., 2007). Right. Nav1.6 (green) and ANK-G (red) in layer 2/3 pyramidal neurons in rat neocortex. (Lorincz & Nusser, 2008).