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Review

Isoform-specific and pan-channel partners regulate trafficking and plasma membrane stability; and alter sodium channel gating properties

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ABSTRACT

Voltage-gated sodium channels are cell membrane glycoproteins responsible for action potential generation and propagation in excitable cells. These large polypeptides which are comprised of 24 transmembrane segments organized into four domains require cellular factors to regulate channel maturation and sorting to different cellular compartments, anchoring the channels at plasma membrane, and modulating gating properties of these channels as effector molecules in the signal transduction pathway. Mutations of sodium channels or their cytosolic partners produce similar pathologies, providing a compelling evidence for the biological significance of channel complexes that form during channel biogenesis and following sorting to different cellular compartments and anchoring at plasma membrane. Genetic, biochemical and bioinformatic approaches have been utilized to identify sodium channel partners. Here we review the important functional role of pan-sodium channel and isoform-specific partners in regulating sodium current density and gating properties.

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Voltage-gated sodium channels are glycoproteins which consist of a large pore-forming α-subunit (referred to as sodium channel hereinafter) and smaller auxiliary β-subunits, which generate inward sodium currents that underlie electrogenesis in neurons and myocytes [10]. Sodium channels are closed at rest and respond to membrane depolarization by conformational changes, cycling through activated (channel opens transiently to allow the flow of sodium ions down their concentration gradient), inactivated (cytoplasmic pore of the channel is closed to stop the flow of sodium ions) and recovery from inactivation (repriming: channel undergoes conformation changes to restore structure at rest) states [35]. The density of sodium channels at the plasma membrane, the voltage-dependence of channel gating, and response to repetitive stimulation of different channels contribute to shaping excitability of neurons and myocytes. The current density and gating properties of sodium channels can be modulated in a cell-type specific manner, partly because of differential expression of channel partners.

Nine different sodium channel isoforms (Nav1.1–Nav1.9) share a common structural motif (Fig. 1) with conserved primary sequences especially of the transmembrane segments, and less

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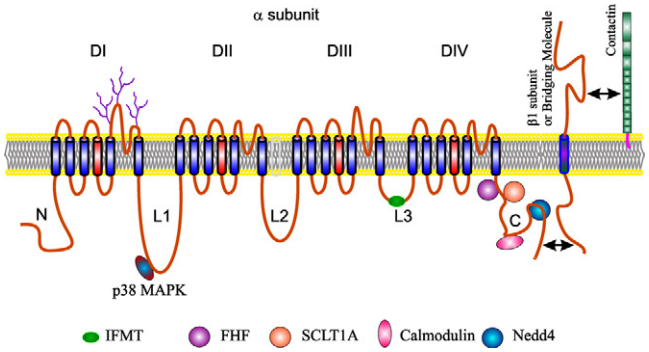

Fig. 1. Schematic of voltage-gated sodium channel showing cytosolic channel partners. A schematic of an extended polypeptide secondary structure of the α-subunit of sodium channels, showing the 24 transmembrane segments, organized into four domains, linked by three cytoplasmic loops, with a cytoplasmic N- and C-termini of the polypeptide. Several sodium channel partners are depicted, indicating their site of interaction with the channel. IFMT, is the tetrapeptide (isoleucine, phenylalanine, methionine and threonine) which acts as the fast-inactivation particle for all sodium channels. Contactin, a GPI-anchored extracellular protein, is depicted in a tripartite complex with the α-subunit which requires either β1 subunit or other transmembrane proteins which serve to simultaneously interact with the cytoplasmic N- and C-termini of the channel and the extracellular domains of contactin. MAPK (mitogen-activated protein kinase), FHF (fiborblast growth factor homologous factor), CaM (calmodulin), SCLT1A (sodium channel clathrin interacting protein 1A), Nedd4 (neural precursor cell expressed developmentally down-regulated, an E3 ubiquitin ligase) are known channel partners.

conserved N- and C-termini and cytoplasmic loops [11]. The α-subunit is necessary and sufficient to produce a functional channel, while β-subunits and other proteins and enzymes are important for (1) channel maturation and sorting to different neuronal compartments, (2) anchoring channels at plasma membrane, (3) modulating biophysical properties of the channels. Mutations of sodium channels that can be rescued by co-expression of β-subunits, chemical chaperones or cell incubation at lower temperatures than 37 °C [66,73,79], as well as mutations of channel partners that mimic pathologies of sodium channelopathies [17,81], provide a compelling reason to identify and functionally characterize components of the channel complexes that form during channel biogenesis and following channels sorting to different cellular compartments and anchoring at plasma membrane.

Genetic, biochemical and bioinformatic approaches have been utilized to identify sodium channel partners. We have used yeast two-hybrid (Y2H), affinity purification methods, and bioinformatics to identify channel partners that bind to the C-terminus of several sodium channels, and have also identified isoform-specific channel partners (Fig. 1). Here we review the important functional role of pan-sodium channel and isoform-specific partners in regulating sodium current density and gating properties.

Fibroblast Growth Factor Homologous Factors (FHF1-4)

Four prototype members of the fibroblast growth factor homologous factor family (FHF1–4) comprise an intracellular fibroblast growth factor (iFGF11-14) subfamily but are distinguished from other FGF proteins by their inability to interact with FGF receptors [38]. The genes encoding the four FHF subfamily members have a similar structure of five exons, and each undergo alternative splicing of exon 1 which encode N-terminus of different lengths in the different FHF proteins, for example, exon 1 of FHF2B encodes only 9 residues while exon 1 of FHF2A encodes 62 residues [28]. We identified FHF proteins as sodium channel partners in a Y2H screen using the C-terminus of Nav1.6 and Nav1.9 channels as bait, and confirmed their interaction with sodium channels by co-localization, co-immunoprecipitation in vivo and in vitro, and patch-clamp recordings [45,48,69,84]. FHF proteins are expressed in central and peripheral nervous systems at all developmental stages [30], but show a differential tissue-specific expression pattern, for example, while FHF2 is expressed in peripheral sensory neurons and hippocampal neurons, it is not detectable within motor neurons in the spinal cord [84].

The crystal structure FHF2 has recently been shown to consist of a beta-trefoil core, analogous to that of FGFs and exhibits an FGF-characteristic heparin-binding surface, and unstructured N- and C-termini [29]. Despite sequence divergence among the four FHF isoforms, structural and functional studies have identified a conserved sodium channel binding surface on the FHF core domain which is essential for modulation of sodium channel properties and co-localization with sodium channels at axon initial segments of hippocampal neurons [29,44]. FHF-A and -B isoforms with different N-termini have been shown to differentially modulate sodium channels suggesting a binary effect, with the binding of the core FHF protein to the C-terminus of the channel regulating current density and voltage-dependence properties of fast-inactivation, and a further effect on slowing repriming or enhanced slow-inactivation induced by the extended N-terminus of the "A" isoforms [45,51,69,84].

Importantly, however, FHF proteins modulate sodium channels in an isoform-dependent manner (Table 1 and [45,49,48,51,69,84]). The co-expression of FHF1B with Nav1.5 channels hyperpolarizes voltage-dependence of fast-inactivation without altering current density [49]. In contrast, FHF4B depolarizes fast-inactivation and reduces current density of Nav1.5 by 45%, whereas FHF4A hyperpolarizes fast-inactivation and reduces current density by 90% [51]. The co-expression of FHF2A with Nav1.6 in a DRG-derived cell line (ND7/23) increases current density and depolarizes fast-inactivation but slows repriming and enhances frequency-dependent inhibition of the channel [69], whereas FHF4A has no effect on Nav1.6 current density, but depolarizes fast-inactivation, slows repriming and enhances frequency-dependent inhibition of the channel [45]. FHF2B increases Nav1.6 current density and depolarizes fast-inactivation but does not impair repriming or enhances frequency-dependent inhibition of the channel [69,84], whereas FHF4B reduces current density of Nav1.6 by 80% and depolarizes fast-inactivation [45].

The FHF2B-induced increase in Nav1.6 current density and depolarized shift of fast-inactivation, which increases the fraction of channels that are available to open, would be expected to increase neuronal excitability [45,84]. In contrast, the interaction between FHF2A with Nav1.6 is more complicated, because the increase in current density is accompanied by slower repriming and enhanced frequency-dependent inhibition of the channel, especially at high stimulation frequency [69]. Similarly, FHF4A-induced effects on Nav1.6 would be expected to reduce neuronal excitability because of enhanced frequency-dependent inhibition [45]. Paradoxically, however, neurons from a FHF1–FHF4 double knockout mouse, or the expression of a mutant FHF4 protein that does not bind sodium channels manifest a reduction in excitability [31,44]. In the aggregate, these findings show that the Nav1.6–FHF interaction could have profound effects on neuronal excitability, depending upon co-localization of the specific FHF isoforms with the channel, and may also depend on the cell background where this interaction is being investigated.

Sodium Channel Clathrin Interacting Protein 1A (SCLT1A)

Endocytosis plays a central role in the polarized distribution of membrane proteins in neuronal somatodendritic and axonal compartments [83]. While selective sorting and delivery may be key mechanisms in the selective distribution of membrane proteins