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Cardiac Na Channels: Structure to Function

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Abstract

Heart rhythms arise from electrical activity generated by precisely timed opening and closing of ion channels in individual cardiac myocytes. Opening of the primary cardiac voltage-gated sodium ($Na_v1.5$) channel initiates cellular depolarization and the propagation of an electrical action potential that promotes coordinated contraction of the heart. The regularity of these contractile waves is critically important since it drives the primary function of the heart: to act as a pump that delivers blood to the brain and vital organs. When electrical activity goes awry during a cardiac arrhythmia, the pump does not function, the brain does not receive oxygenated blood, and death ensues. Perturbations to $Na_V1.5$ may alter the structure, and hence the function, of the ion channel and are associated downstream with a wide variety of cardiac conduction pathologies, such as arrhythmias.

1. Na^+ CHANNELS: I_{Na} THE FAST VOLTAGE-GATED Na^+ CHANNEL

Overview

Voltage-gated sodium (Na_v) channels are transmembrane proteins of approximately 260 kDa that underlie rapid depolarization to initiate action potentials in electrically excitable cells, such as neurons and cardiac myocytes. They are topologically organized into primary and auxiliary subunits, α and β (Fig. 1). The primary α subunit contains pore-forming and voltage-sensing domains (VSDs) that control the gating and ion selection functions of the channel. The auxiliary β subunits modulate gating, regulate expression, and also play a role in the oligomerization of individual channels (Isom, 2001; Namadurai et al., 2015; Yu & Catterall, 2003).

Na_V channels can adopt at least three structurally distinct states: open, closed, and inactivated. In the absence of an electrical stimulus, at the cell membrane resting potential, Na_V channels reside in a functionally deactivated (closed) state. Upon membrane depolarization, they activate (open) but rapidly become inactivated (nonconducting) again (Yellen, 1998). The functional distinction between deactivated and inactivated states is that channels deactivate during cellular hyperpolarization, while an inactivated channel occurs in response to cellular depolarization, rendering the channel unavailable to open and conduct ions. The relative residency in the closed and inactivated states is critical because the upstroke of the action potential depends on the availability of the channels to open (Kleber & Rudy, 2004). Even a subtle depolarization from the cell resting potential may promote

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channel inactivation that may reduce cellular excitability. Recovery from inactivation (RFI) occurs only after membrane repolarization, during which the channel recovers from the inactivated state and in a separate transition, deactivates to a closed available state. The time and voltage dependence of Na_V channel transitions between discrete channel states depends on a complex interplay of structural, chemical, genetic, and electrical factors that determine channel function (Chen-Izu et al., 2015; Herren, Bers, & Grandi, 2013; Marionneau & Abriel, 2015; Rook et al., 2012).

The alpha (α) subunit

There are 10 genes (*SCN1A–SCN10A*) that encode mammalian Na_V α subunit isoforms, each of which exhibits distinct structures related to their distinct physiological function. *SCN5A* is the gene that encodes the α subunit of the primary cardiac isoform ($Na_V1.5$) of the voltage-gated sodium channel. $Na_V1.5$ consists of a continuous chain of four heterologous domains (DI–DIV), each containing six transmembrane spanning segments (S1–S6) organized around a central, ion-selective pore (Fig. 2). A ring of DEKA residues between segments S5 and S6 of each domain forms the ion selectivity filter (SF) (Sun et al., 1997; Yamagishi et al., 2001). Positively charged residues are clustered in the S4 segments that comprise the voltage sensor (Kontis & Goldin, 1997; Stuhmer et al., 1989). In some Nay isoforms, the intracellular linker between domains three and four, DIII/DIV, includes a hydrophobic isoleucine-phenylalanine-methionine (IFM) motif, which acts as a gating mechanism to confer rapid occlusion of the channel pore, resulting in fast inactivation subsequent to channel opening (Stuhmer et al., 1989; West et al., 1992). Other studies also suggest a role for the intracellular DIV carboxy-terminus (C-terminus) in channel inactivation in both the brain and cardiac isoforms ($Na_V1.1$ and $Na_V1.5$, respectively) (Cormier et al., 2002; Mantegazza et al., 2001; Motoike et al., 2004).

Additionally, the resolved structure (using cryoelectron microscopy) of the rabbit voltage-gated skeletal calcium channel, $Ca_V1.1$, provides new insight into the mammalian architecture of voltage-gated ion channels, as sodium and calcium channels are both four-domain channels closely related among the voltage-gated ion channel superfamily (Wu et al., 2015; Yu et al., 2005). This Ca_V structure has the potential to serve as a template to inform more accurate homology models of the human Na_V channels for use in computational studies (Fig. 3).

Subunit β

There are only four genes (*SCN1B–SCN4B*) that encode the mammalian Na_V β subunits, all of which are expressed in cardiac tissue (Maier et al., 2004). They are membrane proteins consisting of a single transmembrane segment connecting an extracellular Immunoglobin (Ig) domain to an intracellular C-terminus (Brackenbury & Isom, 2011; Cusdin, Clare, & Jackson, 2008). Studies have shown that subunits β1 and β3 have the highest sequence homology out of the four and that they are noncovalently bound to α subunits, while subunits β2 and β4 bind to α subunits through a disulfide bond (Chen et al., 2012; Namadurai et al., 2015). Recently, the X-ray crystal structures of the human β3 and β4 subunits have been resolved (Gilchrist et al., 2013; Namadurai et al., 2014), and evidence suggests that the presence of membrane-embedded glutamic acid residues in the helical

region of β3 may promote oligomerization (into dimers and trimers) which may play a role in channel localization (Morgan et al., 2000; Namadurai et al., 2015). β4 subunits, on the other hand, lack these residues and have been implicated in "resurgent current" in hippocampal neurons (Lewis & Raman, 2011; Namadurai et al., 2015). Resurgent current arises from Na channel RFI and reopening during repolarization, which results in a transient inward current during the action potential. The proposed mechanism posits that the β4 subunit competes with the inactivation particle upon depolarization, forming a blocked state of the channel that is distinct from the fast-inactivated state. Repolarization favors removal of the β4 particle, resulting in a channel that briefly conducts before inactivating or deactivating, depending on the membrane potential (Lewis & Raman, 2014). Cardiac cells have also been shown to exhibit resurgent currents, termed nonequilibrium gating, although the specific molecular mechanism is not clear, β4 subunits are expressed in cardiac myocytes (Clancy et al., 2003; George, 2009; Moreno & Clancy, 2012).

2. ACTIVATION OF THE CARDIAC Na^+ CHANNEL $Na_V1.5$

Activation of the Na_V family of ion channels depends fundamentally on voltage sensing, whereby changes in the membrane potential promote changes to channel structure. Under resting conditions Na_V channels are closed, but open in response to local membrane depolarization caused by a capacitive current stimulus. If a sufficient number of channels open, then permeation of sodium ions occurs through the channel pore down the electrochemical gradient into the cell, depolarizing it and thus beginning a positive feedback loop in which more sodium channels open in response to the changing membrane potential. This promotes even further depolarization, which amplifies the electrical signal and drives the cellular action potential (Catterall, 2010; Huxley & Kendrew, 1952).

2.1 Structure and mechanism of the Na_V voltage-sensing domains

The S1–S4 helices of each asymmetric domain (DI–DIV) of the α subunit comprise the VSD of the Na_V. The amphiphilic S4 helix contains an alternating motif of a positively charged residue followed by two hydrophobic residues. This well-conserved "310-helical" arrangement serves as the voltage-sensing element of activation in each homologous domain (Schwaiger et al., 2011; Yarov-Yarovoy et al., 2012). The charged residues on S4 are hypothesized to cause an outward motion of the helix, toward the extracellular side of the membrane, which in turn induces conformational changes within the pore (Armstrong, 1981; Capes et al., 2013; Payandeh et al., 2011; Yarov-Yarovoy, Baker, & Catterall, 2006). This is the "helical screw" model, which is supported both by experimental evidence as well as extensive studies involving structural modeling of the Na_V VSD (Catterall, 1986, 2000, 2010; Yarov-Yarovoy et al., 2006, 2012). In this model, the S4 voltage sensor motion is coupled to pore opening and closing via the S4–S5 linker (Long, Campbell, & Mackinnon, 2005). The combination of outward and rotating movement of the S4 segment has been predicted to impose a gating movement of the S4–S5 linker that is roughly orthogonal to the transmembrane orientation of the pore-forming helices in the bacterial NaChBac VSD. Moreover, investigation into charge-neutralized mutants of the mammalian $Na_V1.4$ sodium channel has found that S4, in all four domains, are involved in pore gating, albeit to different extents (Chanda & Bezanilla, 2002; Yarov-Yarovoy et al., 2012). In particular, charges on

DIV S4 seem to have a larger effect on inactivation than activation, whereas the S4 helices in DI–DIII seem to influence activation (Chahine et al., 1994; Chanda & Bezanilla, 2002; Kontis, Rounaghi, & Goldin, 1997; Stuhmer et al., 1989). This may imply that the DIV voltage sensor is tightly coupled to fast inactivation (Chanda & Bezanilla, 2002).

An integral component in the helical screw model of Na_V activation is the existence of negative countercharges that stabilize and shield the positively charged intramembrane S4 voltage-sensing helix as it responds to membrane depolarization. To overcome the energetically unfavorable translocation of charges over a hydrophobic barrier, the S4 helix of the VSD has been predicted, in molecular modeling studies, to favor one or more intermediate steps (Chanda & Bezanilla, 2002). This has been verified for the active states of the bacterial NachBac channel in which disulfide crosslinking and mutant cycle studies have identified molecular interactions between the S4 helix and surrounding S1–S3 helices that facilitate activation (DeCaen et al., 2008, 2009, 2011; Paldi & Gurevitz, 2010; Yarov-Yarovoy et al., 2012). Structurally, the role of countercharges in mammalian sodium channels have been studied using a combination of molecular modeling and molecular dynamics simulations and are predicted to involve three intermediate states of S4 translocation (Gosselin-Badaroudine et al., 2012). Functional investigation into mammalian VSDs using disulfide cross-linking has not been possible because these channels contain native cysteine residues that eliminate the possibility of intuiting disulfide bonding for any specific pair. However, mutagenesis studies of the VSDs of $Na_V1.4$ demonstrate that reversing the charge of the S1–S3 countercharges had domain-specific outcomes including inhibition of activation in DI–DIII but destabilization of fast inactivation in DIV (Groome & Winston, 2013). While these studies thus far do not include the specific structural aspects of the $Na_V1.5$ VSDs, they provide valuable insight into the intricacies of activation of Na_V channels.

2.2 Naturally occurring mutations can cause delayed $Na_V1.5$ activation

Isolated cardiac conduction disease (ICCD) is observed on the ECG in a widening of the QRS complex that indicates delays in ventricular excitation (Grant et al., 2002; Tan et al., 2001). Mutations in $Na_V1.5$ have been shown to cause ICCD and typically result from a depolarizing shift of the Na^+ channel activation curve (Grant et al., 2002; Tan et al., 2001). This shift most likely results from a reduction in the rate of channel activation or decreased channel sensitivity to the voltage required for activation. Mutant channels may require a longer time to reach depolarized membrane potentials at which the maximum I_{Na} occurs. The lag in activation of ICCD mutant Na^+ channels results in a reduction of the action potential upstroke velocity, which is a primary determinant of conduction velocity, leading to an increase in the amount of stimulus current required to excite coupled tissue (Rohr, Kucera, & Kleber, 1998; Shaw & Rudy, 1997; Tan et al., 2001). Computational modeling supports the hypothesis that a depolarizing shift in the $Na_V1.5$ activation and corresponding reduced I_{Na} produces right ventricular conduction delay and a transmural activation gradient, which is a mechanism that is consistent with the findings of a study investigating the G514C mutation linked to ICCD (Tan et al., 2001).

3. INACTIVATION

Inactivation of $Na_V1.5$ is critically important because the completeness of the inactivation process influences both action potential duration and action potential frequency (restitution) in the myocardium. There are at least three distinct forms of inactivation that have been identified in $Na_V1.5$ channels: fast, intermediate, and slow (Goldin, 2003; Wang et al., 2000).

4. FAST INACTIVATION

Fast inactivation in $Na_V1.5$ is a time-and voltage-dependent process that occurs via a "hinged lid" mechanism in which the cytoplasmic linking region between DIII and DIV binds into the hydrophobic pore, thereby occluding ion conduction (Kellenberger et al., 1997; McPhee et al., 1998; Smith & Goldin, 1997). An NMR solution structure of this linking region, or inactivation particle, has been resolved as a 53 amino acid peptide, consisting of a single alpha-helix structure flanked by two disordered tail regions (Rohl et al., 1999). Several early mutagenesis studies have implicated the importance of a hydrophobic sequence of isoleucine, phenylalanine, and methionine (IFM) within the DIII–DIV linker as critical for sodium channel inactivation: mutation to glutamine (Q) at one or more of the IFM residues either diminished or abolished inactivation (Eaholtz et al., 1999; Eaholtz, Scheuer, & Catterall, 1994; Eaholtz, Zagotta, & Catterall, 1998; Goldin, 2003; Patton et al., 1992; West et al., 1992) Fig. 4.

Experimental studies have implicated an adjacent threonine (T) residue as being important for the binding of the inactivation particle within the mouth of the pore, since its substation with a methionine (M) residue increased by 24% sustained noninactivating currents (Miyamoto et al., 2001). In $Na_v1.5$, cysteine scanning of the IFM sequence has shown it to be critical for stable binding of the inactivation particle to the pore (Deschenes, Trottier, & Chahine, 1999). Additionally, a recent study has shown that a shortened peptide containing the IFM sequence (KIFMK) can not only block inactivation-deficient open $Na_v1.5$ channels (L409C, A410W) but must be cleared from the pore before it can close, as evidenced by tail currents recorded upon repolarization. The same authors also found that an F1760K mutation will eliminate fast inactivation altogether, suggesting that the inactivation particle itself is important for stabilizing the inactivated state of the channel (Wang & Wang, 2005). Hence, there is ample evidence that fast inactivation occurs via a hydrophobic packing of the DIII–DIV linker within the intracellular mouth of the pore. Additionally, site-directed glutamine mutations in the DIII S4–S5 linker have been shown to lead to a positive shift in the voltage dependence of fast inactivation, suggesting that the abolition of hydrophobic residues in that region functionally corresponds to the loss of interaction with the hydrophobic IFM triad (Smith & Goldin, 1997). There is limited consensus on the mechanism of the "hinged lid" hypothesis of fast inactivation, as initial investigation suggested that the glycine and proline residues in the linker (G1484, G1485, and P1512) form the hinge (Kellenberger et al., 1997), but theoretical models in which the inactivation particle forms a hairpin motif centered around G1505 only implicate P1512 in functioning of the hinge (Sirota, Pascutti, & Anteneodo, 2002).

4.1 Structural and functional aspects of fast inactivation gating

A recent study has revealed that fast inactivation in $Na_V1.4$ strictly depends on the motion of the DIV voltage sensor. The authors hypothesize that the opening of the sodium channel actually occurs in two steps: one in which the DI, DII, and DIII voltage sensors open the pore and allow ion permeation and another in which activation of the DIV voltage sensor renders the channel in an intermediate state (S2) and induces a conformational change in the pore itself, which serves as a necessary precursor to the binding of the inactivation particle (Goldschen-Ohm et al., 2013). Using an inactivation-deficient mutant (L435W/L437C/A438W, DI-S6), they argue that the frequent observation of subconductance states in electrophysiological recordings of the inactivation-deficient $Na_v1.4$ channel may indicate a discrete open state that occurs following DIV voltage sensor movement albeit before the fast inactivation particle enters the pore. These secondary conductance states are normally hidden by rapid channel inactivation. The implications for this gating paradigm are significant in that fast inactivation may be a mechanism in which a two-step structural process is translated, functionally, into a single step. In addition, this finding may help to explain conflicting data supporting high-affinity block of both open and inactivated states by local anesthetics (Bennett et al., 1995a; Goldschen-Ohm et al., 2013; Wang et al., 2004). While this study was conducted using WT and mutant forms of $Na_V1.4$, the proposed existence of the preinactivated S2 state should be applicable to $Na_V1.5$ given the high level of homology between both isoforms.

4.2 A role for the carboxy-terminus and allosteric proteins in inactivation

The intracellular C-terminus of the α subunit of $Na_V1.5$ plays an important role in modulating fast inactivation (An et al., 1998; Cormier et al., 2002; Mantegazza et al., 2001; Tateyama et al., 2004). In this channel an uncoupling of the C-terminus from the DIII–DIV inactivation loop has been shown to increase inward current during repolarization leading to delays in cellular repolarization. These studies have suggested that the C-terminus is involved in the structural stabilization of the fast-inactivated state (Motoike et al., 2004). An S1885stop deletion experiment further revealed that the highly charged portion of the C-terminal S6 is likely involved in electrostatic interactions with the inactivation particle (Cormier et al., 2002). One possibility is that the C-terminal stabilization of the DIII–DIV inactivation loop constitutes a mode of intermediate inactivation (Cormier et al., 2002; Kass, 2006; Mantegazza et al., 2001), although there is lack of consensus regarding the precise structural determinants of such an intermediate inactivated state.

Fast inactivation in $Na_V1.5$ can also be modulated by accessory proteins such as fibroblast growth factor homologous factors (FHFs), which are a family of intracellular signaling proteins that interact with the C-terminus. It has been suggested that a tethered FHF-derived N-terminal particle competes for the induction of the intrinsic fast inactivation particle of the Na_V at depolarized transitions near the open state (Dover et al., 2010). One study has found that FGF12, an FHF that is the most abundant in human heart, has diminished interaction with a C-terminal H1849R mutant of $Na_V1.5$, resulting most notably in a depolarizing (~ −9 mV) shift in fast (steady state) inactivation, which is a gain-of-function phenotype associated with arrhythmia and long QT syndrome (Musa et al., 2015). Another study has identified the acidic-rich C-terminal region (1773–1832) of $Na_V1.5$ as critical for FGF12 binding, and that

the presence of FGF12 modulates the voltage dependence of inactivation, with no effect on activation of the channel (Liu et al., 2003). Congenital long QT syndrome (LQT3) is a disorder characterized by a delay in cardiac cellular repolarization, a condition that can lead to cardiac arrhythmias and sudden death. Other mutations in this C-terminal region, such as E1784K, 1795insD, and Y1795C interfere with fast inactivation and evoke small, sustained currents associated with LQT3 as well (An et al., 1998; Rivolta et al., 2001; Veldkamp et al., 2000).

4.3 Channelopathies arising from altered fast inactivation

The first naturally occurring mutation that was identified in $Na_V1.5$ is ΔKPQ: a 3 amino acid deletion (lysine, proline, glutamine at positions 1505–1507) in the DIII–DIV linker that was shown to affect fast inactivation and was causally linked to LQT3 (Bennett et al., 1995b). This motif is known to be critical for fast inactivation of the Na^+ channel, and the DKPQ mutation results in a small (<1% of peak current amplitude) persistent noninactivating current (“bursting”) during normal cellular repolarization. This mutation was also the first to be modeled in a study of an in silico mutation that linked genotype to phenotype (Clancy & Rudy, 1999). Model simulations showed that delayed ventricular repolarization promotes a substrate for triggered activity via early afterdepolarizations (EADs), which result from reactivation of L-type Ca^{2+} channels. Since that first study, numerous modeling and simulation studies have been used to predict effects of arrhythmia-linked mutations in genes underlying ion channels and accessory and regulatory proteins (Noble & Rudy, 2001).

4.4 Modulation of fast inactivation by β subunits

Fast inactivation can also be modulated by associated β subunits. In particular, the β3 subunit, which is normally expressed in ventricular and Purkinje cardiac cells, shifts steady state (fast) inactivation leftward, indicating reduced channel availability. When a ΔECD mutation is made in β3, abolition of this gating shift in $Na_V1.5$ and a persistent current is observed, implicating its interference with the DIII–DIV inactivation particle, as has previously been suggested for the β1 subunit (McCormick et al., 1998; Yu et al., 2005).

5. SLOW INACTIVATION

Slow inactivation of $Na_V1.5$ is a process spanning seconds or tens of seconds that can occur during prolonged or repetitive depolarization (Kass, 2004). Slow inactivation is independent of the DIII–DIV linker associated with fast inactivation and is more likely a result of the structural deformation of the pore, such as with C-type inactivation in potassium channels, although the exact mechanism is not yet known (Liu, Jurman, & Yellen, 1996; Vassilev, Scheuer, & Catterall, 1989). Early experiments involving the transfer of all four $Na_V1.5$ P-loop regions into the $Na_V1.4$ transmembrane backbone conferred the cardiac type of slow inactivation (Russ et al., 1996; Vilin et al., 1999). These regions include the DEKA SF, as well as the highly conserved P-loop region that forms the extracellular funnel of the pore domain. For example, a single residue difference between $Na_V1.4$ (V754) and $Na_V1.5$ (I891) in the DII P-loop region caused a higher probability of slow inactivation in the cardiac isoform (Vilin & Ruben, 2001), and a tryptophan mutation (W53C) in the DI P-loop region has been shown to diminish single channel conductance (Tomaselli et al., 1995).

Furthermore, a mutation in the DEKA SF itself has been reported to increase the probability of Na_V channels entering a slow inactivated state (Hilber et al., 2001). The many regions of the Na_V channel that have been implicated in slow inactivation may contribute to an as yet poorly understood coordinated gating process. A study has correlated the onset of slow inactivation in $Na_V1.4$ with immobilization of the VSDs of DI and DII (Silva & Goldstein, 2013), suggesting that slow inactivation may involve additional protein regions and not be solely be due to deformation of the pore domain.

6. RECOVERY FROM INACTIVATION

Repeated regular firing of cardiac action potentials depends crucially on the process of RFI of $Na_V1.5$ channels. RFI is an I process that indicates the fraction of Na_V channels that are available for activation. The recovery process occurs on a millisecond timescale, with exponential kinetics that are accelerated with increased hyperpolarization (Bezanilla & Armstrong, 1977; Huxley & Kendrew, 1952). Both antiarrhythmic drugs and naturally occurring mutations have been shown to target and modulate RFI. In a physiological setting, RFI participates in a tightly orchestrated relationship with deactivation, ensuring minimization of leak current during channel repriming (Kuo & Bean, 1994).

6.1 DIV voltage sensor movement is rate limiting

The kinetics of recovery from fast inactivation has been studied in both wild-type $Na_V1.5$ sodium channels and in mutants in which the positive S4 arginine gating charges of each domain of the VSD have been neutralized by mutation to glutamine. Results demonstrate that compared to the wild type, while all $Na_V1.5$ S4 mutants slowly recover, the charge-neutralized DIV mutant exhibited the largest effect, with a pronounced recovery lag of almost 0.5 ms; suggesting that the motion of the DIV VSD may be rate limiting for RFI (Capes et al., 2013). Such findings are consistent with the fact that site-3 toxins, small peptide toxins derived from venoms that bind with high specificity to the extracellular surface of Na_V channels inhibit the outward motion of the DIV S4 voltage sensor, consequently inducing a faster RFI (Hanck & Sheets, 2007). During RFI, the voltage sensors of DI–DIII are likely required to deactivate first, closing the channel and preventing conduction, after which the slower inward motion of DIV S4 acts to drive the inactivation particle from its vestibule (Armstrong, 2006; Kuo & Bean, 1994). Furthermore, an accelerated RFI and slower onset of fast inactivation has been found to result from mutations in DIII S1–S2 linker (R1232W) and the DIV S3–S4 linker (T1620M) resulting in idiopathic ventricular fibrillation (Vilin, Fujimoto, & Ruben, 2001), suggesting again an important role for DIV VSD in RFI.

6.2 Modulation of recovery from inactivation by β subunits

RFI of $Na_V1.5$ channels is modulated by at least two of β subunits. One study has shown that the transmembrane helix, plus either the intracellular or extracellular region of β1 is necessary to accelerate its RFI (Fahmi et al., 2001). β1 is known to accelerate RFI, not only in $Na_V1.5$, but also in the skeletal muscle and neuronal isoforms of the channel (Zimmer & Benndorf, 2002). β3 also accelerates RFI of $Na_V1.5$ in oocytes, which may be

physiologically significant because β3 is expressed in cardiac ventricular cells and Purkinje fibers (Fahmi et al., 2001).

6.3 Recovery from inactivation channelopathy and disease phenotypes

Examples of LQT-3 mutations that do not result in a classical gain of function as measured during square wave voltage clamp experiments, include D1790G, E1295K, and I1768V. Interestingly, these mutations are not localized to a single region of the channel, rather they are found in varied regions including the C-terminal domain and in the DIII S3–S4 linker region. Studies have shown that these mutations rather result from an acceleration of RFI. Experiments and mathematical simulations have revealed that under nonequilibrium conditions during repolarization, channel reopening results from faster RFI at membrane potentials that facilitate the activation transition (Clancy et al., 2003). Mathematical modeling and simulation approaches predicted that the additional inward current through the defective channels was sufficient to cause prolonged repolarization, consistent with the long QT syndrome phenotype (Clancy et al., 2003).

Additionally, reduction in $Na_V1.5$ have been attributed to changes in RFI that can lead to Brugada syndrome (diagnosed by ST segment elevation on the ECG) by several distinct alterations to ion channel gating, including reduced rates of RFI as well as faster inactivation subsequent to channel opening and protein trafficking defects (Clancy & Rudy, 2002). For example, the 1795insD mutant channel showed a rightward shift in the recovery curve, indicating a delayed RFI in $Na_V1.5$ of 100 ms or more. The resulting loss of current was shown to be sufficient to explain the Brugada phenotype.

7. REGULATION OF CARDIAC $Na_V1.5$

There has been work focused on the regulation of Nay1.5 by post-translational processes (Marionneau & Abriel, 2015). There is also a growing literature on how posttranslational modifications of $Na_V1.5$ are facilitated through the presence of macromolecular complexes that allow selective and specific protein modification to confer functional effects (Abriel, Rougier, &Jalife, 2015). Multiple phosphorylation mechanisms through a variety of kinase pathways as well as glycosylation, arginine methylation, S-nitrosylation, ubiquitylation, and ion homeostatic mechanisms are all implicated as functional regulators of $Na_V1.5$ (Marionneau & Abriel, 2015).

7.1 Phosphorylation

One of the key functional modulators of $Na_V1.5$ is phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) that results in changes to macroscopic $Na_V1.5$ gating including a reduction in channel availability, increased intermediate inactivation, and slowed RFI (Wagner et al., 2006). Multiple phosphorylation sites in the intracellular loop linking $Na_V1.5$ domains I and II have been identified (S516, S571, and T594) and implicated in the CaMKII-induced changes to I_{Na} gating (Ashpole et al., 2012; Hund et al., 2010), although, how each of the three sites confers functional effects is still not clear.

While all of the effects of CaMKII on macroscopic $Na_V1.5$ current result in peak current reduction, CaMKII activation also results in substantial current increases in the late or

persistent component of current that can cause $Na_V1.5$ gain of function associated proarrhythmia (Anderson, Brown, & Bers, 2011; Bers & Grandi, 2009; Erickson & Anderson, 2008; Grandi et al., 2007; Horvath et al., 2013; Hund et al., 2008; Ma et al., 2012; Maier, 2011; Wagner et al., 2011). During AP-clamp experiments in canine ventricular myocytes, inhibition of CaMKII has been shown to reduce $Na_V1.5$ late current (Horvath et al., 2013; Maltsev et al., 2008). In addition to phosphorylation by CaMKII, increased in intracellular Ca^{2+} modifies and promotes $Na_V1.5$ late current (Mori et al., 2000; Wingo et al., 2004) through a variety of mechanisms including activation of CaMKII (Wagner et al., 2006), increased mitochondrial oxidative phosphorylation (Belardinelli, Shryock, & Fraser, 2006), and consequent increased reactive oxygen species (ROS) (Kohlhaas et al., 2010; Liu et al., 2010). Interestingly, an increase $Na_V1.5$ late current can also activate CaMKII, thereby resulting in a positive feedback cycle between $Na_V1.5$ late current and CaMKII (Yao et al., 2011). The effects conferred by CaMKII on both peak and late $Na_V1.5$ current has been suggested to occur through a complex with βIV-spectrin that positions CaMKII to phosphorylate the channel (Hund et al., 2010).

In addition to CaMKII, in rodent heart cells cAMP-dependent protein kinase (PKA) has been shown to phosphorylate serine residues at positions 525 and 528 and confer functional effects such as reducing channel availability, promoting $Na_V1.5$ trafficking, and increasing $Na_V1.5$ macroscopic current (Hallaq et al., 2006; Marionneau & Abriel, 2015; Matsuda, Lee, & Shibata, 1992; Murphy et al., 1996; Tateyama et al., 2003; Zhou et al., 2002). These effects are similar to those observed in expressed $Na_V1.5$ channels following PKC-dependent phosphorylation of S1503, which additionally promotes the $Na_V1.5$ late current (Qu et al., 1994; West et al., 1991).

Other kinases have also been implicated in posttranslational modification of $Na_V1.5$ current. Included are the phosphatidylinositol kinase (PI3K), whose inhibition promotes $Na_V1.5$ late current (Lu et al., 2012), while Fyn and Adenosine Monophosphate-activated Protein Kinases (AMPK) promote $Na_V1.5$ current by increasing channel availability and promoting RFI (Ahern et al., 2005; Light, Wallace, & Dyck, 2003).

7.2 Glycosylation

In additional to kinase modulation of $Na_V1.5$ via phosphorylation, glycosylation of $Na_V1.5$ is an additional presumed mode of posttranslational modification. Multiple putative asparagines have been identified in $Na_V1.5$ as potential N-linked glycosylation sites (Ahmad et al., 2007). However, thus far only asparagines in the ancillary $Na_V\beta1$ subunit have been demonstrated to functionally modify the cardiac Na^+ current, by causing leftward shifts of the inactivation and activation curves, promoting fast inactivation, and slowing RFI (Ednie et al., 2013; Stocker & Bennett, 2006; Ufret-Vincenty et al., 2001).

7.3 Methylation and ubiquitylation

A set of arginine residues in $Na_V1.5$ located in the DI–DII linker has been shown to undergo arginine methylation (Beltran-Alvarez et al., 2014). The effect of methylation of these residues is to increase the $Na_V1.5$ surface expression and hence current density (Beltran-Alvarez et al., 2013). Exactly the opposite effects on channel function have been shown to

result from ubiquitylation, which results in $Na_V1.5$ internalization and reduction in current (van Bemmelen et al., 2004; Laedermann, Decosterd, & Abriel, 2014). The ubiquitylation sites are presently unknown.

7.4 Reactive oxygen species regulation

There are multiple modes of redox regulation of cardiac $Na_V1.5$ through various signaling mechanism, both direct and indirect. A prominent indirect mechanism is by ROS activation of CaMKII, which confers the gain-of-function effects on $Na_V1.5$ late current described earlier (Marionneau & Abriel, 2015). Nitric oxide (generally derived from endothelial nitric oxide synthase (eNOS)), also potentiates $Na_V1.5$ late current, which can occur via direct S-nitrosylation of the channel (Ahern et al., 2000; Cheng et al., 2013). Changes in the NAD(H) balance has been shown to promote mitochondrial ROS production, which leads to a reduction in $Na_V1.5$ peak current, which may provide a link between altered metabolism, excitability, conduction block, and arrhythmic risk (Liu et al., 2013, 2010). Indeed, naturally occurring disease-linked mutations that have been shown to prevent the protein kinase A potentiation of $Na_V1.5$ during oxidative stress, leading to loss of Na channel function and manifestations of Brugada syndrome (Aiba et al., 2014).

8. SUMMARY

Understanding how $Na_V1.5$ structure underlies channel function is continually improving due the development of improved experimental and computational approaches that allow more concrete links between structure and function. Connecting ion channel function to emergent behavior in the heart to cause disease is a critical area of research. The ubiquity of disruption of $Na_V1.5$ channels in cardiac disorders of excitability emphasizes the importance of fundamental understanding of the associated mechanisms and disease processes to ultimately reveal new targets for human therapy.

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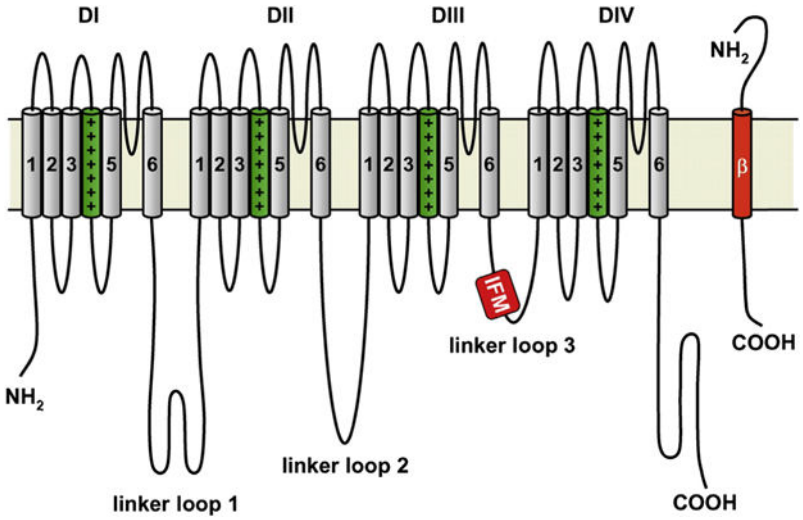

Figure 1. Membrane topology of $Na_V1.5$

Illustration of the four domains (DI–DIV) of the α subunit along with an associated β subunit. The transmembrane S1–S4 helices that comprise the voltage sensing domains are depicted with the charged S4 helix highlighted in green (dark gray in print versions). The S5 and S6 helices form the central pore domain. The DIII–DIV inactivation loop is highlighted with the critical hydrophobic isoleucine, phenylalanine, and methionine (IFM) sequence. The N-terminal and C-terminal domains of each subunit also labeled. Copyright Hugues Abriel, Oxford University Press, 2007. http://dx.doi.org/10.1016/j.cardiores.2007.07.019

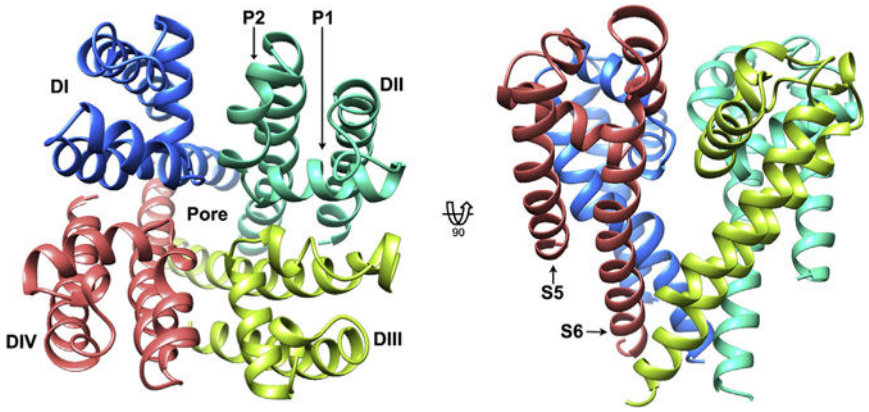

Figure 2. Homology model of the $Na_V1.5$ channel pore domain

Top view of the extracellular face (left), and transmembrane view (right), of the pore domain of $Na_V1.5$, based on the bacterial template Na_VRh (PDB ID code 4DXW). Structure is shown in ribbon representation with each domain colored from DI (blue (dark gray in print versions)) to DIV (red (light gray in print versions)). Pore helices P1 and P2 are labeled in DII, and transmembrane helices S5 and S6 are labeled in DIV for representative purposes. *Kevin R. DeMarco.*

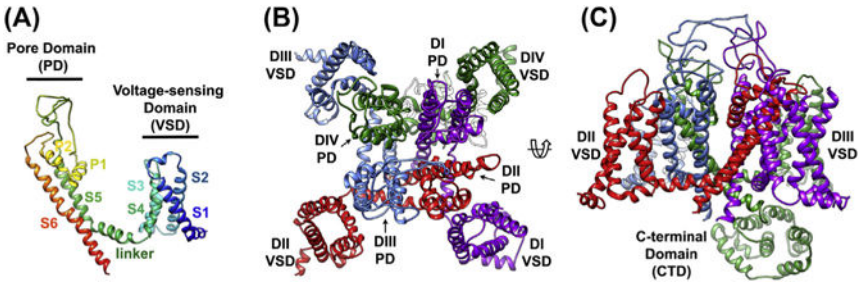

Figure 3. Cryoelectron microscopy reveals structural elements of $Ca_V1.1$

(A) Transmembrane view of a single subunit of $Ca_V1.1$ (PDB accession 3JBR) to illustrate the architecture of the pore domain (P1, P2, S5, and S6), voltage-sensing domain (S1–S4), and linker regions common to voltage-gated sodium and calcium channels. The N-terminus is colored blue, and the C-terminus is colored red in ribbon representation. (B) Top view of $Ca_V1.1$ colored to highlight the heterotetrameric arrangement of the voltage-sensing and pore domains, with the ion-conducting pore in the center. (C) Transmembrane view of $Ca_V1.1$ highlighting the C-terminal domain important for ion channel regulation, as well as a transmembrane fenestration important for local anesthetic ingress. *Kevin R. DeMarco.*

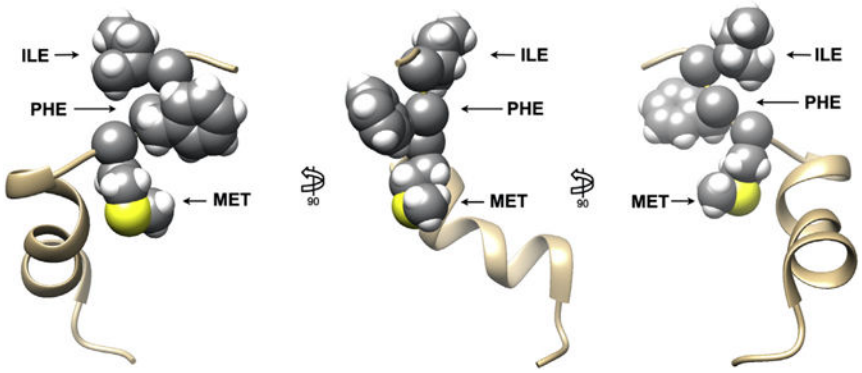

Figure 4. Solution NMR structure of the DIII–DIV inactivation loop

Three views of the putative sodium channel inactivation loop segment (PDB ID code 1BYY). The hydrophobic sequence of residues (IFM), demonstrated to be critical for $Na_V1.5$ inactivation, are highlighted in space-filling representation. The remainder of the segment is shown in ribbon representation. *Kevin R. DeMarco.*