Sodium channels as macromolecular complexes: Implications for inherited arrhythmia syndromes

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Abstract

Mutations in cardiac ion channels and their auxiliary subunits can lead to life-threatening cardiac arrhythmias. In recent years it has become apparent that ion channels are part of large, multi-protein complexes, comprising not only the ion channels and their auxiliary subunits, but also components of the cytoskeleton, regulatory kinases and phosphatases, trafficking proteins, extracellular matrix proteins, and possibly even other ion channels. Disruption of any member of a particular ion channel complex has the potential to disrupt the function of the associated channels, resulting in paroxysmal disease. Understanding the molecular composition of individual ion channel signaling complexes in heart may yield important insights into the molecular basis of cardiac arrhythmias and may suggest novel therapeutic approaches to treatment of these life-threatening conditions.

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1. Introduction

Cardiac action potentials are generated and propagated through the coordinated activity of multiple ion channels, including voltage-gated sodium channels (Na\textsubscript{v}1s), calcium channels, and potassium channels [1]. Mutations in genes encoding these channels cause familial arrhythmias. Voltage-gated ion channels comprise a central ion-conducting pore associated with one or more auxiliary subunits that regulate channel function. The channel pore can be formed by a single protein, as in sodium and calcium channels, or by four identical or nonidentical \(\alpha\)-subunits, as in potassium channels, the latter being the source of tremendous molecular diversity. It is now clear that ion channels do not exist in isolation in vivo but instead associate in complex with other plasma membrane, extracellular matrix, and intracellular proteins that participate in cell adhesion, cytoskeletal anchoring, and signal transduction. The molecular composition of a particular complex can have significant influence on channel function and localization and consequently on cellular excitability. A given channel pore-forming subunit may behave very differently depending on its associated proteins. Thus, the formation of various channel complexes is predicted to be the source of significant functional diversity. A major challenge to our field is to determine the molecular organization of ion channel signaling complexes in functional microdomains of excitable cells, e.g. t-tubules or intercalated disks of cardiac myocytes. We predict that the molecular composition of channel signaling complexes is tissue-specific and even subcellular domain-specific within a given cell type, resulting in localized differences in channel physiology.

In this review, we will focus on the molecular composition of cardiac Na\textsubscript{v}1 complexes. Na\textsubscript{v}1 \(\beta\)-subunits, in addition to modulating channel gating and voltage-dependence, function as molecular scaffolds to bring other proteins to the channel, resulting in the formation of macromolecular complexes and differential regulation of excitability. In
addition, Na1 1-σ-subunits participate in protein–protein interactions that are β-subunit independent. Because we currently have a more complete understanding of Na1 signaling complexes in neurons compared to cardiac myocytes, we will begin with examples of proteins associated with neuronal Na1s and then discuss those data in terms of cardiac Na1s, cardiac potassium channels, and future lines of investigation in cardiovascular medicine.

2. Na1s are composed of α– and β-subunits

Na1s are responsible for action potential conduction and propagation in excitable cells [2]. Na1s isolated from mammalian neurons and cardiac myocytes are multimeric proteins composed of single, pore-forming α-subunit and one or more β-subunits [2]. In neurons, Na1s are clustered at high density at nodes of Ranvier [3] and are localized at specialized subcellular domains, including axon initial segments, dendrites, and neuronal cell bodies [4], pre- and postsynaptic neuronal membranes [5–7], and the neuromuscular junction [8]. In heart, Na1s are located at intercalated disks, at the cell surface, at the t-tubules, and in the sinoatrial (SA) node [9–12].

The mammalian pore-forming α-subunits encode a gene family of at least nine members (SCN1A–SCN11A) [13]. A number of excellent reviews are available that provide detailed information on α-subunit structure, function, gene expression, and roles in inherited disease [4,14–20]. While some tissue specificity of Na1 gene expression exists, traditional ideas of “brain” vs. “heart” Na1s are no longer valid. Especially interesting to the field of cardiovascular research is the identification of TTX-S “brain” channels (Na1.1, Na1.3, and Na1.6) at t-tubules of cardiac myocytes as well as in the SA node [10–12,21]. While the predominant sodium current in isolated cardiac myocytes is TTX-R, TTX-S currents are detectable following activation with β-scorpion toxin CssIV [11]. In addition to cardiac muscle, the TTX-R “heart” channel, Na1.5, is expressed in the limbic system of the brain [22,23]. These data are summarized in Table 1. It is essential that researchers and physicians consider this data when developing and prescribing new drugs for arrhythmia and epilepsy. It is reasonable to predict that mutations in Na1.5 known to cause arrhythmia may also lead to neurological disorders. Likewise, mutations in Na1.1 known to cause epilepsy may also lead to arrhythmia. There are numerous reports describing patients in which, for example, LQT presents as idiopathic epilepsy, or occipital lobe epilepsy also shows a prolonged QT interval [24]. In cases of Sudden Unexplained Death in Epilepsy, cardiac factors are frequently suspected and death is often attributed to increased autonomic stimulation [24]. An alternative explanation in some cases may be expression of a mutant ion channel gene in both brain and heart such that the patient has epilepsy and arrhythmia. Accurate identification of the underlying genetic components for each patient will be essential to designing effective therapies.

The Na1 β-subunit gene family consists of four members: β1 (SCN1B) [25], β2 (SCN2B) [26], β3 (SCN3B) [27], and β4 (SCN4B) [12]. β1A, a splice variant of SCN1B, is expressed in embryonic brain and adult heart in rat [28]. β1, β1A, β2, β3, and β4 are proteins with type I topology, containing an extracellular amino-terminus, a single transmembrane segment, and an intracellular carboxyl-terminus. All five β-subunits contain a single, extracellular, immunoglobulin (Ig) domain that is structurally homologous to the V-set of the Ig superfamily and often found in cell adhesion molecules (CAMs) [27,29,30]. This unique property was first discovered for β2, revealing that its extracellular domain contains an Ig-fold and an extended region with similarity to the CAM contactin [26,29]. The extracellular domains of β1/β1A, and β3 show a similar homology to the CAM myelin P0 [27,31]. Analysis of the β4 extracellular domain reveals a predicted Ig domain with homology to β2 [30].

α-Subunit cDNAs express functional Na1s in heterologous expression systems such as Xenopus oocytes or mammalian fibroblasts. However, for TTX-S α-subunits, the currents characteristic of these channels expressed in isolation are quite different from native currents. Co-expression of the β-subunits with these channels results in cell type-specific shifts in the voltage-dependence of activation and inactivation, changes in channel modal gating behavior resulting in altered rates of activation, inactivation and recovery from inactivation [32], and increased channel expression at the plasma membrane [33]. β1-mediated effects on TTX-R Na1.5 channels, however, remain controversial [10,34–36]. In contrast to TTX-S channels, expression of Na1.5 in oocytes produces channels that inactivate rapidly in the absence of β-subunits [37]. According to some reports, β1 has no observable effect on Na1.5 function [38,39]. Other groups have reported that coexpression of β1 and Na1.5 results in increased current density with no detectable effects on channel kinetics or voltage-dependence [37,40]. Some groups have reported modulation of channel sensitivity to lidocaine block with subtle changes in channel kinetics and gating properties in response to β1 expression [41], while others have reported significant shifts in the voltage-dependence of steady-state

<table>
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<th>Table 1</th>
<th>Mammalian Na1s that are expressed in both brain and heart</th>
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<tr>
<td>Na1 1-σ-subunit (gene)</td>
<td>TTX sensitivity</td>
</tr>
<tr>
<td>Na1.1 (SCN1A)</td>
<td>+</td>
</tr>
<tr>
<td>Na1.3 (SCN3A)</td>
<td>+</td>
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<tr>
<td>Na1.5 (SCN5A)</td>
<td>−</td>
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<tr>
<td>Na1.6 (SCN8A)</td>
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inactivation [10,42,43] or the rate of recovery from inactivation [43,44]. Scn1b null mice provide a unique opportunity to resolve this controversy by investigating the result of β1 deletion on TTX-R sodium currents in heart [61]. Coexpression of Na\(_{v}\)1.5 with β3 in one study resulted in increased current density, depolarizing shifts in the voltage-dependence of inactivation, and increased rate of recovery from inactivation [44] yet, in another study, produced hyperpolarizing shifts in inactivation and slowed recovery from inactivation [43]. Coexpression of β2 [10] or β4 [30] with Na\(_{v}\)1.5 resulted in no obvious effects over α alone, suggesting that these β-subunits may not affect the function of Na\(_{v}\)1.5. Alternatively, β2 and β4 may require the presence of other proteins that are not present in fibroblasts or oocytes to modify sodium currents. It is important to consider that it may be impossible to properly reconstitute in vivo Na\(_{v}\)1 signaling complexes in heterologous expression systems. Because Na\(_{v}\), β-subunits bring critical signaling molecules to the channel pore in vivo, one must take care to avoid over-interpretation of in vitro studies of β-subunit-mediated channel modulation.

All five β-subunits are expressed in juvenile and/or adult heart in rodents and are differentially localized to specialized subcellular domains and cell types: β1 and β2 are localized at both t-tubules/Z-lines and intercalated disks in ventricular myocytes [10,45,46], β1A localizes to the myocyte surface [28], β3 colocalizes with β1 at the t-tubules/Z-lines [21], and β4 colocalizes with β2 at the intercalated disks [21]. In addition, β1, β2, β3, and β4 are colocalized with Na\(_{v}\)1.1 and Na\(_{v}\)1.3 in SA nodal cells where they may contribute to automaticity [12]. β1 and β2 associate with Na\(_{v}\)1.1 and with Na\(_{v}\)1.5 in ventricular myocytes as assessed by coimmunoprecipitation [10,45]. Very little is known about cardiac Na\(_{v}\), α- and β-subunit trafficking in vivo. A study using over-expression of fluorescent-tagged Na\(_{v}\)1.5, β1, and β2 in HEK293 cells suggested that Na\(_{v}\)1.5 and β2 are transported separately to the plasma membrane while Na\(_{v}\)1.5 and β1 form a complex in the endoplasmic reticulum that may facilitate plasma membrane trafficking [47]. Na\(_{v}\)1.5 cell surface density is regulated by Nedd4-2 ubiquitination [48–50]. The availability of mouse models will now allow these ideas to be tested in vivo.

The observation that β4 is expressed in heart may lead to important new advances in the understanding of the regulation of cardiac sodium currents by auxiliary subunits. β4 may serve as the endogenous open-channel blocker that generates resurgent sodium current [51]. While resurgent sodium current in cardiac myocytes has not yet been reported, this information may lead to important new insights into the molecular basis of cardiac excitability and arrhythmia. Finally, a recent study showed β1 mRNA and protein expression in embryonic mouse heart [46]. Thus, Na\(_{v}\), β-subunits may have effects on embryonic cardiac development as well as on postnatal electrical excitability.

3. Na\(_{v}\),1 function in vivo

In heterologous systems, even in the presence of β-subunits, sodium currents expressed by the various SCN genes are difficult to distinguish from one another, suggesting that these channels might have some overlapping functions in vivo. However, studies with genetically engineered null mice clearly show that this is not the case. In most situations, the loss of expression of a single SCN gene, or even reduced gene expression, results in a severe phenotype, demonstrating that each SCN gene product has specialized functions that cannot be compensated by over-expression of one of the remaining SCN genes. Scn1a null mice display motor deficits, ataxia, and seizures with lethality by postnatal day 16 [52]. Scn1a heterozygotes also exhibit a dramatic seizure phenotype that is frequently lethal. This phenotype is remarkably similar to Severe Myoclonic Epilepsy of Infancy caused by truncation mutations in SCN1A that result in its haploinsufficiency [53]. The cardiac phenotype of these mice has not yet been reported. Targeted disruption of Scn5a causes intrauterine lethality with severe deficits in ventricular morphogenesis [54]. Scn5a heterozygotes show normal survival but have several deficits, including impaired atrioventricular conduction, delayed intramyocardial conduction, increased ventricular refractoriness, and ventricular tachycardia with characteristics of reentrant excitation [54]. Null mutations of Scn8a result in ataxia, tremor, and progressive paralysis with lethality by 3 weeks of age [55]. The cardiac phenotype of these mice is not yet known. The hypomorphic allele Scn8a\(^{med1}\) reduces Scn8a mRNA and protein to 10% of normal levels [56]. Scn8a\(^{med1/med1}\) homozygotes exhibit ataxia and tremor and develop a progressive dystonic phenotype that resembles primary torsional dystonia in humans [57,58].

4. Na\(_{v}\),1 signaling complexes

Obviously, the critical difference between in vitro and in vivo studies is channel expression in native cells. In their native environment, Na\(_{v}\),1s are targeted to specialized subcellular domains where they associate with specific signaling and structural proteins, and where they are exposed to second messengers in a regulated fashion. For example, in heart, Na\(_{v}\),1s are differentially localized to t-tubules/Z-lines, intercalated disks, and SA node [9–12,21,45]. To make this situation even more complex, Na\(_{v}\),1.5 colocalizes with β-adrenergic receptors and G-proteins to caveoli in heart [59]. Recreating each individual situation in a heterologous system is nearly impossible, thus limiting our ability to interpret in vitro results in terms of in vivo physiology. If it were possible to exactly recreate these macromolecular complexes in vitro, then we would likely find that a given Na\(_{v}\),1 α-subunit behaves very differently depending on the molecular composition of the signaling
complex. As explained in the following section, we predict that β-subunits play critical roles in the formation of Naᵥ1 signaling complexes in vivo.

4.1. β-subunits are key players in Naᵥ1 signaling complexes

β-subunits do not form the ion-conducting pore, but are multifunctional proteins that play critical roles in modulation of channel function, regulation of channel expression levels at the plasma membrane, and cell adhesion [32]. This feature makes Naᵥ1s unique among the superfamily of voltage- and ligand-gated ion channels. In vitro evidence suggests that β-subunits serve as critical communication links between adjacent cells, the extracellular environment, and intracellular signaling mechanisms, possibly including other ion channels, in Naᵥ1 complexes. The availability of β1 and β2 null mice now allows this novel hypothesis to be tested in vivo [60,61].

β1 and β2 have functional roles in addition to modulation of channel function in vitro [62]. β1 and/or β2 interact with the extracellular matrix molecules tenascin-C and tenasin-R [63,64], proteins that are expressed in both brain and heart [65,66]. β1 and β2 participate in homophilic cell adhesion, resulting in cellular aggregation and recruitment of the cytoskeletal anchoring protein ankyrin to the plasma membrane at sites of cell–cell contact [67]. AnkyrinR and ankyrinG are expressed in both brain and heart and are critical to the regulation of electrical excitability [68–72]. β1 and β2 associate with ankyrinG [45,67,73,74] and ankyrinB [45], β1-ankyrin interactions are regulated by phosphorylation of β1Y181, located in the carboxyl-terminus [73], resulting in its differential localization in cardiac myocytes [45]. β1Y181E, a mutant that does not interact with ankyrin, also does not modulate Naᵥ1 function despite efficient association with α, suggesting that regions of the β1 intracellular domain are critical for channel modulation as well as cytoskeletal association [74]. β1 associates with the cardiac intercalated disk proteins N-cadherin and connexin-43 [45]. β1 also interacts with other CAMs found at the node of Ranvier, paranode, and axon initial segment, including contactin, Naᵥ β2, neurofascin-186, neurofascin-155, and NrCAM [74–80]. The interaction of β1 with contactin or neurofascin-186 results in significant increases in channel cell surface expression. Cardiac β-subunit-interacting Ig-CAMs have not yet been identified. NCAM is expressed at intercalated disks in heart and is upregulated in myocardial ischemia and in Chagas’ disease myocarditis [81,82]. Thus, it is a candidate for β1 interaction in heart. Finally, β1 associates with the intracellular phosphatase domain of receptor phosphotyrosine phosphatase β [83], potentially providing a mechanism for regulated tyrosine phosphorylation.

β1, in particular, appears to be essential for the interaction of Naᵥ1s with multiple CAMs and cytoskeletal proteins in vitro and in vivo [61,74–76]. β1-mediated cell adhesive and cytoskeletal interactions occur in the presence or absence of the pore-forming α-subunit. In the presence of α, some β1-mediated cell adhesive and cytoskeletal association events ultimately result in changes in channel activity in vitro [64,74,84]. We propose that β1-subunits act as molecular scaffolds to bring cytoskeletal, cell adhesion, and signaling molecules to the ion conducting pore, thus affecting channel function, channel subcellular localization, and channel cell surface expression in a cell-specific or subcellular domain-specific manner.

4.2. What does this mean in terms of cardiovascular physiology?

Previous work from our laboratory and others has made significant contributions to the understanding of Naᵥ1 signaling complexes in neurons. Do similar interactions occur between Naᵥ1 β1-subunits, extracellular matrix, cell adhesion, and cytoskeletal proteins in heart? While we have less information on the identities of cardiac specific Naᵥ1 interacting proteins, the answer is yes. Naᵥ1s form macromolecular complexes in heart as they do in brain. More interestingly, while some of the proteins involved in Naᵥ1 complexes may be similar in brain and heart, e.g. ankyrin and tenascin, we predict that many proteins will be specific to particular myocyte subcellular domains, e.g. connexin-43.

Tyrosine phosphorylation of the intracellular domain of some Ig-CAMs abolishes their ability to interact with ankyrin, establishing specialized microdomains in polarized cells [85–88]. For example, non-phosphorylated neurofascin interacts with ankyrinG at nodes of Ranvier while tyrosine-phosphorylated CAMs in the same gene family are found at other specialized sites of cell–cell contact such as paranodes, neuromuscular junctions, adherens junctions, and regions of neuronal migration and axon extension [85,89]. Similarly, tyrosine-phosphorylated β1 (pYβ1) and non-phosphorylated β1 are differentially localized to specific subcellular domains (intercalated disks vs. t-tubules/Z-lines, respectively) in cardiac myocytes where they interact with different cytoskeletal and signaling proteins [45].

TTX-S and TTX-R Naᵥ1s are differentially localized to t-tubules/Z-lines and intercalated disks, respectively, in cardiac myocytes [10,11] (Fig. 1). The TTX-S channels Naᵥ1.1, Naᵥ1.3, and Naᵥ1.6 localize to t-tubules/Z-lines, while TTX-R Naᵥ1.5 is found at intercalated disks. Points made by Brette and Orchard [90], however, are well taken: immunolocalization of Naᵥ α- and β-subunits at Z-lines does not automatically imply t-tubule function. In addition, if Naᵥ1s are located at the t-tubules, they may be restricted to specialized subdomains that are separate from the calcium channel-containing dyad. It is clear that additional work is required to investigate these issues. pYβ1 colocalizes with connexin-43, N-cadherin, and Naᵥ1.5 at intercalated disks but is not detected at the t-tubules/Z-lines. An antibody that does not differentiate
Thus, Kvs involved in Ito and Nav1s are likely localized at both t-tubules and at intercalated disks and it is possible that TTX-S as well as TTX-R Nav1s may interact with Kvs involved in Ito.

Physiology experiments designed to determine the relative distributions of INa and Ito at t-tubules vs. intercalated disks using diffusion or detubulation studies estimated that these currents are approximately uniformly distributed between the two subcellular domains (reviewed by [90]). Thus, Kvs involved in Ito and Nav1s are likely localized at both t-tubules and at intercalated disks and it is possible that TTX-S as well as TTX-R NaVs may interact with Kvs involved in Ito.

between the phosphorylated and non-phosphorylated forms of β1 stains both intercalated disks and t-tubules/Z-lines. Anti-pYβ1 immunoprecipitates N-cadherin from heart membranes and from cells transfected with β1 and N-cadherin in the absence of other NaVs subunits. pYβ1 does not associate with ankyrinβ in heart membranes while ankyrinβ associates with an immune complex precipitated using the non-discriminating β1 antibody. N-cadherin and connexin-43 associate with β1 and with Nav1.5 in heart membranes. These results suggest that Nav1 complexes at intercalated disks of ventricular myocytes are composed of Nav1.5 and pYβ1 in close association with both N-cadherin and connexin-43 [45]. Ankyrinβ has recently been shown to localize at intercalated disks as well [69]; however, because pYβ1 does not interact with ankyrin, it is unlikely that these two proteins associate directly. Non-phosphorylated β1, ankyrinβ, and TTX-S NaVs associate at t-tubules/Z-lines. β1 phosphorylation ("P") appears to regulate its localization to differential subcellular domains in myocytes and thus its availability to associate with differential signaling molecules. NaVs may associate with Kvs involved in Ito through molecules. Nav1s may associate with Kvs involved in Ito through interaction with N-cadherin and connexin-43, while non-phosphorylated β1, ankyrinβ, and TTX-S NaVs are associated at t-tubules/Z-lines. β1 phosphorylation appears to regulate its localization to differential subcellular domains in myocytes and thus its availability to associate with differential signaling molecules.

In adult heart, cardiomyocytes are linked together at intercalated disks, where adherens junctions, gap junctions, and desmosomes make up the cardiac intercellular junctions that mediate mechanical and electrical coupling throughout the heart [91]. A close association of adherens junctions with mechanical junctions at the intercalated disks is necessary for conduction, and expression of N-cadherin at adherens junctions is a prerequisite for subsequent gap junction formation [92,93]. Our results demonstrate that pYβ1 interacts with NaV1.5 and N-cadherin, an adherens junctional protein, at intercalated disks and that the NaV1.5 signaling complex also associates with connexin-43, a gap junctional protein. The intracellular domain of cadherin interacts with the catenins, proteins that ultimately communicate with the actin cytoskeleton and play roles in the regulation of cell adhesion and intracellular signaling [94]. Disruption of the F-actin based and microtubular cytoskeleton in myocytes modulates the coupling between availability and activation of cardiac NaVs, suggesting that cytoskeletal integrity may be a requirement for normal propagation of cardiac action potentials and thus regulation of excitability [95,96]. In addition to N-cadherin, pYβ1 may interact with cytoskeletal anchoring proteins located at intercalated disks such as the PDZ domain containing molecule syntrophin-γ2, which also interacts with NaV1.5 [97], or ZO-1, a PDZ-domain containing molecule that associates with connexin-43 [98,99].

Intracellular interactions between β1 and ankyrin are critical for channel cell surface expression as well as for β1-mediated modulation of channel function in vitro [74]. This has been postulated to be especially critical for NaV1.5 [100]. The significance of these interactions is illustrated by a study characterizing a mutation in SCN1A identified in a family with dominantly inherited Generalized Epilepsy with Febrile Seizures Plus (GEFS+2, OMIM 604233) [101]. The mutation, D1866Y, located in the carboxyl-terminal domain of NaV1.1, decreases modulation of α by β1. Direct interaction between the carboxyl-terminal domains of wild-type α and β1 was demonstrated by yeast two-hybrid and coimmunoprecipitation analyses. The D1866Y mutation weakened this interaction, strengthening the hypothesis that the β1 intracellular domain is critical for channel modulation, and demonstrating a novel molecular mechanism leading to seizure susceptibility. NaV1.1 is expressed in heart as well as in brain [10]. Thus, mutations in this channel that are known to lead to epilepsy may also be responsible for generating cardiac arrhythmias.
5. Na\textsubscript{v} \(\beta\)-subunits and arrhythmias

Because \(\beta\)-subunits are critical for maintaining channel association with cell adhesion, signal transduction, and cytoskeletal molecules in vitro, we predict that mutations in \(\beta\)-subunit genes may lead to human disease. Although several mutations in Na\textsubscript{v}\textsubscript{1.1} \(\alpha\)-subunits have been associated with inherited diseases, including epilepsy (SCN1A, SCN2A), skeletal muscle myopathies (SCN4A), LQT and Brugada Syndromes (SCN5A), and erythromelalgia (SCN9A) \cite{110,104,102}, to date only one human disease has been associated with a gene encoding a Na\textsubscript{v} \(\beta\)-subunit. A mutation (C121W) in the extracellular Ig domain of \(\beta\)1 (SCN1B) causes generalized epilepsy with febrile seizures (GEFS+1) (OMIM 604233) \cite{104}. Similarly, a five amino acid deletion in the Ig domain of \(\beta\)1 was found in a family with febrile seizures and early onset absence epilepsy \cite{105}. Compared to wild-type \(\beta\)1, \(\beta\)1C121W increases the fraction of available Na\textsubscript{v}1s at resting membrane potentials and reduces sodium current rundown during high frequency channel activity \cite{84}. The \(\beta\)1C121W mutation also disrupts \(\beta\)1–\(\beta\)1 cell adhesion. These potential effects on channel gating and cell adhesion in vivo may bias cells toward hyperexcitability. \(\beta\)1 is expressed in myocytes as well as in neurons \cite{25}. Although GEFS+1 individuals manifest a clear neuronal phenotype, defects in cardiac function have not been investigated. Examination of the ECG of GEFS+1 individuals may yet reveal abnormalities in cardiac function resulting from the presence of this mutant \(\beta\)-subunit.

The predominant Na\textsubscript{v}1 in cardiac tissue is Na\textsubscript{v}1.5 and many mutations in SCN5A result in LQT-3 (OMIM 603830) or Brugada (OMIM 601144) syndromes \cite{19}. Brugada syndrome has been proposed to be caused by a reduction in the number of functional Na\textsubscript{v}1s leading to decreased sodium conductance in the myocardium. Interestingly, \(\beta\)1 may play a role in this disease. This is illustrated by a study investigating functional expression in HEK cells of a double mutant underlying idiopathic ventricular fibrillation characterized by ST-segment elevation (Na\textsubscript{v}1.5-D1866Y/GEFS+1) \cite{106}. Expression of the mutant \(\alpha\)-subunit alone resulted in robust inward currents, however, coexpression of the mutant \(\alpha\) with wild-type \(\beta\)1 resulted in a four-fold reduction in current density and a corresponding decrease in \(\alpha\)-subunit protein expression. Thus, loss of function caused by the mutation in \(\alpha\) was dependent on the presence of \(\beta\)1. The authors proposed that the addition of \(\beta\)1 results in formation of a mutant \(\alpha\)–\(\beta\) complex with reduced stability.

A particularly interesting LQT mutation is Na\textsubscript{v}1.5-D1790G that disrupts the ability of Na\textsubscript{v}1.5 to be modulated by \(\beta\)1 \cite{107}. D1790G is located in the carboxyl-terminus of Na\textsubscript{v}1.5, similar to Na\textsubscript{v}1.1-D1866Y that also disrupts \(\alpha\)–\(\beta\)1 association \cite{101}. Another LQT mutation in the carboxyl-terminus of Na\textsubscript{v}1.5 (Na\textsubscript{v}1.5-E1784K) results in a defect in fast inactivation characterized by a small, persistent current during long membrane depolarizations \cite{108}. Coexpression of this mutant channel with \(\beta\)1 results in an exaggeration of the shifts in the voltage dependence of steady-state inactivation. All three of these mutations support the hypothesis that the carboxyl-terminus of \(\beta\)1 is critical for channel modulation in vivo. Because the intracellular domain of \(\beta\)1 is critical for Na\textsubscript{v}1 ankyrin interactions as well as channel modulation in vitro \cite{74,100}, we predict that diseases resulting from these mutations are a consequence of disrupted channel-cytoskeletal interactions in addition to altered channel electrophysiological behavior. It is not known whether Na\textsubscript{v}1.1-D1866Y and/or Na\textsubscript{v}1.5-D1790G completely abolish \(\alpha\)–\(\beta\)1 association, as extracellular \(\alpha\)–\(\beta\)1 sites of interaction have been identified \cite{109,110}. However, if so, then channel association with other molecules that require \(\beta\)1 may be abolished as well. \(\beta\)1 null mice \cite{61} exhibit a significantly lengthened QT interval compared to their wild-type littermates \cite{111}. Together, these observations suggest that mutations resulting in loss of \(\beta\)1 expression may result in disruption of normal cardiac Na\textsubscript{v}1 function. It remains to be seen whether mutations in \(\beta\)1, or indeed any Na\textsubscript{v} \(\beta\)-subunit, can result in arrhythmia or other cardiac disorders in humans.

6. Cardiac multi-ion channel complexes?

It is clear that cardiac ion channels participate in complexes with cytoskeletal, signal transduction, and extracellular matrix proteins. Is it possible that multiple ion channels associate in the same complex? Na\textsubscript{v}1s and voltage-gated potassium channels (K\textsubscript{vs}) may physically associate in heart. K\textsubscript{4.3} contributes to transient outward current (I\textsubscript{to}) in the human ventricle \cite{1,112}. Interestingly, K\textsubscript{4.3} can be modulated by coexpression of Na\textsubscript{v} \(\beta\)1, resulting in increased current density, shifts in the voltage-dependence of inactivation, and altered channel kinetics \cite{113}. Inhibition of \(\beta\)1 transcription in ventricular myocytes results in the reduction of mRNA and/or protein levels of Na\textsubscript{v}1.5, K\textsubscript{4.2}, K\textsubscript{4.3}, and KChIP2, a calcium-binding accessory subunit that modulates K\textsubscript{4.2}-encoded currents \cite{114}. The observed reductions in Na\textsubscript{v}1 and K\textsubscript{4}, protein levels were consistent with functional reductions in both I\textsubscript{Na} and I\textsubscript{to}. These data agree with previous studies in which inhibition of KChIP2 transcription resulted in reductions of I\textsubscript{Na} and I\textsubscript{to}, reductions in Na\textsubscript{v}1.5 and \(\beta\)1 mRNA levels, and reductions in K\textsubscript{4.2} and K\textsubscript{4.3} protein \cite{115}. Coimmunoprecipitation studies revealed association of K\textsubscript{4.2} or K\textsubscript{4.3} with \(\beta\)1 in ventricular myocardium \cite{114}. Together, these intriguing observations suggest that cardiac Na\textsubscript{v}1.5 may physically associate with I\textsubscript{to} channels via \(\beta\)1 and KChIP2 to form a macromolecular complex.

7. Other Na\textsubscript{v}1 molecular interactions

A number of key Na\textsubscript{v}1 molecular interactions are dependent on the presence of \(\beta\)-subunits. However, this is...
not the complete story. There are multiple proteins that interact directly with Na\(_v\)1 \(\alpha\)-subunits in heart, independent of \(\beta\).

### 7.1. Ankyrins

Na\(_v\)1 \(\alpha\)-subunits interact indirectly with ankyrin via \(\beta\)-subunits [73]. Na\(_v\)1s also interact directly with ankyrin via a conserved 9-amino acid motif located within LII–III [116]. The Na\(_v\)1.5-E1053K mutation associated with Brugada syndrome is localized to the LII–III ankyrin-binding motif [117]. This mutation disrupts the association of Na\(_v\)1.5 with ankyrinG, resulting in decreased channel cell surface expression. These data suggest that the interaction of Na\(_v\)1.5 with ankyrinG is critical to the proper localization of Na\(_v\)1.5 in cardiomyocytes, as it is for Na\(_v\)1 localization to the initial segments of CNS axons [71]. Na\(_v\)1.5-E1053K channels also exhibit shifts in voltage-dependence and altered gating kinetics, suggesting that interaction of Na\(_v\)1.5 with ankyrinG modulates channel function in addition to localization.

Of the seven gene loci associated with LQT, six encode ion channel subunits, while LQT-4 (OMIM 106410) results from mutations in ankyrinB [1,68]. In cardiac myocytes, ankyrinB is localized to the t-tubules. Mice heterozygous for the ankyrinB null allele exhibit a similar LQT phenotype to humans, with reduced t-tubular localization of the inositol-1,4,5-triphosphate receptor, Na\(^+\)/Ca\(^{2+}\) exchanger, and Na\(^+\)/K\(^+\) ATPase, all of which bind ankyrin B [68]. Calcium signaling in myocytes is severely disrupted, resulting in extrasystoles. Surprisingly, localization and cell surface expression levels of Na\(_v\)1.5 and Na\(_v\)1.6 are unaffected [68,117]. No significant differences in the cardiac action potential upstroke or duration were detected in ventricular myocytes isolated from ankyrinB heterozygotes compared to their wild-type littermates. In contrast, examination of sodium currents in primary cultures of ankyrinB-null neonatal cardiomyocytes revealed prolonged action potentials and subtle differences in channel kinetics [70], suggesting that, while ankyrinB haploinsufficiency has no observable effects on Na\(_v\)1s, the complete loss of ankyrinB expression may be required before Na\(_v\)1 expression and localization are disrupted. Ventricular myocytes express both ankyrinG and ankyrinB. AnkyrinG is localized at both the t-tubules and intercalated discs, with a similar staining pattern to that of Na\(_v\)1s [117]. In mice heterozygous for the ankyrinG-null mutation, the continued expression of ankyrinG may be sufficient for maintenance of normal Na\(_v\)1 localization and expression. Generation of mice specifically lacking ankyrinG in heart would allow further examination of the role of ankyrin isoforms in the localization of cardiac Na\(_v\)1s.

### 7.2. Syntrophins

Syntrophins are cytoplasmic peripheral membrane proteins of the dystrophin associated protein complex (DAPC) [118] that connect to the actin cytoskeleton. Na\(_v\)1.5 copurifies with syntrophins and dystrophin in mouse, suggesting that this channel may interact with the cytoskeleton independent of, or in addition to, ankyrin binding [119]. \(\alpha\)-1, \(\beta\)-1, and \(\beta\)-2-syntrophin contain PDZ-domains that bind to the carboxyl-terminus of Na\(_v\)1.5. A similar PDZ binding motif is located at the carboxyl-termini of a number of inward rectifier potassium channels, including K\(_v\)2.1, K\(_v\)2.2, K\(_v\)2.3, and K\(_v\)4.1 [120]. K\(_v\)2 and K\(_v\)4.1 interact via their PDZ-binding motifs with components of DAPC (including \(\alpha\)-1, \(\beta\)-1, and \(\beta\)-2-syntrophin, dystrophin, and dystrobrevin) in heart. Interaction of sodium or potassium channels with dystrophin via syntrophin may contribute to channel localization, as has been proposed for the interaction of Na\(_v\)1s with ankyrin [85]. Channel function may also be modulated by syntrophin binding: the carboxyl-terminus of K\(_v\)1.1 contains the SXV sequence and the interaction of this region with the cytoskeleton regulates the degree of modulation by K\(_v\) \(\beta\) subunit [85].

### 8. Summary

Cardiac action potentials are formed from the coordinated activation of multiple ion channels whose function and localization are tightly regulated. Altered or loss of function of any of these channels can disrupt action potential conduction, resulting in desynchronization and arrhythmia. Ion channel pore-forming \(\alpha\)-subunits associate with \(\beta\)-subunits that regulate their expression and function. We now know that ion channels also interact with multiple signaling proteins, including cytoskeletal molecules, regulatory kinases and phosphatases, components of cellular trafficking, and extracellular matrix molecules, as part of large, multi-protein complexes. \(\beta\)-subunits appear to play key roles in this intermolecular communication. We propose that proper formation of channel protein–protein interactions is critical to channel function and localization and thus to cellular excitability in vivo.

In the past, enormous progress has been made toward understanding the molecular basis of ion channel structure and function. These experiments necessarily made use of heterologous expression systems, in which channels could be studied in isolation, and thus precluded the identification of cell-specific regulatory and structural interacting proteins that are critical to the modulation of cellular excitability in vivo. It is now essential that we understand how ion channels function as members of cell-specific, multi-protein complexes and how disruption of a particular complex can lead to disease. To accomplish this goal, it is necessary to study channels in their native cellular environments. Tissue-specific gene targeting experiments, especially those utilizing inducible promoters, are currently the gold standard for the study of in vivo functioning of ion channel complexes. Understanding the molecular composition of ion channel complexes in specific subcellular domains will be critical to
the future development of innovative and effective therapies to treat cardiac arrhythmias.

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