Protein assemblies of sodium and inward rectifier potassium channels control cardiac excitability and arrhythmogenesis

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Willis BC, Ponce-Balbuena D, Jalife J. Protein assemblies of sodium and inward rectifier potassium channels control cardiac excitability and arrhythmogenesis. Am J Physiol Heart Circ Physiol 308: H1463–H1473, 2015. First published April 11, 2015; doi:10.1152/ajpheart.00176.2015.—The understanding of how cardiac ion channels function in the normal and the diseased heart has greatly increased over the last four decades thanks to the advent of patch-clamp technology and, more recently, the emergence of genetics, as well as cellular and molecular cardiology. However, our knowledge of how these membrane-embedded proteins physically interact with each other within macromolecular complexes remains incomplete. This review focuses on how the main cardiac inward sodium channel (Nav1.5) and the strong inward rectifier potassium channel (Kir2.1) function within macromolecular complexes to control cardiac excitability. It has become increasingly clear that these two important ion channel proteins physically interact with multiple other protein partners and with each other from early stages of trafficking and targeting through membrane anchoring, recycling, and degradation. Recent findings include compartmentalized regulation of Nav1.5 channel expression and function through a PDZ (postsynaptic density protein, Drosophila disc large tumor suppressor, and zonula occludens-1 protein) domain-binding motif, and interaction of caveolin-3 with Kir2.1 and ankyrin-G as a molecular platform for Nav1.5 signaling. At the cardiomyocyte membrane, Nav1.5 and Kir2.1 interact through at least two distinct PDZ domain-scaffolding proteins (synapse-associated protein-97 and α1-syntrophin), thus modulating reciprocally their cell-surface expression at two different microdomains. Emerging evidence also shows that inheritable mutations in plakophilin-2, ankyrin-G, dystrophin, syntrophin, synapse-associated protein-97, and caveolin-3, among others, modify functional expression and/or localization in the cardiac cell of Nav1.5, Kir2.1 or both to give rise to arrhythmogenic diseases. Unveiling the mechanistic underpinnings of macromolecular interactions should increase our understanding of inherited and acquired arrhythmogenic cardiac diseases and may lead to advances in therapy.

macromolecular complex; Nav1.5; Kir2.1; SAP97; syntrophin

THE CARDIAC ACTION POTENTIAL (AP) depends on the orchestrated voltage- and time-dependent opening and closing of selective ion channels formed by proteins that embed in the lipid bilayer of the cardiomyocyte membrane (10, 60). Most of our current understanding of how these cardiac ion channels function comes from using reductionist experimental approaches, including one in which a single ion channel or protein is genetically or pharmacologically altered to explore its role in cellular excitability, depolarization, repolarization, and rest. While such approaches have been successful in shedding light on the role played by individual ion channels in shaping the AP, alone they provided an overly simplistic view of the function of such channels within their native multiprotein cellular environment. Like other proteins, the functional lifetime of a cardiac ion channel spans several steps that include, but are not limited to, transcription, translation, oligomerization and glycosylation in the Golgi apparatus, vesicular trafficking, membrane retention, posttranslational modification, turnover, ion channel function, and degradation (Fig. 1) (6). At each step the channel might interact with dozens of other different proteins (26). Furthermore, normal cardiomyocyte function requires certain unique physiological characteristics, such as intracellular compartmentalization of protein assemblies and function, electrical excitation, electromechanical coupling, and cell-to-cell communication, all of which depend on the precise function and localization of ion channels interacting with multiple proteins within each individual cell (6).

Na\(^{+}\) and inward rectifier K\(^{+}\) ion channels are of particular interest because the interplay of the ionic currents that flow through them is essential in the control of cellular excitation and AP propagation (80). Evidence is accumulating that from early stages of protein assembly and trafficking, these two channel types physically interact with common partners that...
may include, but are not limited to, anchoring/adapter proteins, enzymes, and regulatory proteins (Table 1). The increased understanding of how these intermolecular interactions occur has begun to shift the traditional paradigm of a channel-to-channel communication that depends solely on the transmembrane voltage. Importantly, mutations in these adapter/anchoring proteins and enzymes are now starting to be linked to inherited arrhythmogenic syndromes in which ion channels remain structurally intact but have altered function, further highlighting the relevant clinical implications that macromolecular ion channel interplay may have in both normal physiology and cardiac diseases.

This brief review article focuses on recent findings on the intermolecular interactions between the main voltage-gated cardiac sodium channel, NaV1.5, and the strong inward rectifier potassium channel, Kir2.1, their protein partners that have been linked to proarrhythmogenic disease, and the potential role that their multiprotein assemblies may have in normal ventricular electrophysiology and arrhythmogenesis.

**Sodium Channels in Macromolecular Complexes**

The voltage-gated cardiac sodium channel consists of a 260-kDa α-subunit (NaV1.5) coded by the SCN5A gene (12, 31, 61) and two β-subunits that modulate but are not crucial for functional NaV1.5 expression (12, 13). NaV1.5 folds into four homologous domains (I–IV) to form a selective pore that permeates inward sodium current (\(I_{Na}\)), which is the main depolarizing current involved in excitation (phase 0 of the AP) of atrial, Purkinje, and ventricular cells. Voltage-gated sodium channels are normally closed when the cell is at rest. However, upon depolarization above a threshold of \(\sim -65\) mV, NaV1.5 channels open in an all-or-none fashion, generating a large and fast \(I_{Na}\) that further depolarizes the cell. Upon depolarization above \(-40\) mV, the channels quickly inactivate, allowing voltage-gated \(Ca^{2+}\) and \(K^+\) channels to interact in a voltage-dependent manner and contribute to the AP plateau, excitation-contraction coupling, and repolarization (10, 60). Rapid excitation and all-or-none depolarization generated by \(I_{Na}\) activa-
ion are essential for the rapid conduction of the electrical impulse through the atrial and ventricular myocardium. It is now well established that, given the crucial role of Na\textsubscript{v}1.5 in cellular excitability, functional defects in Na\textsubscript{v}1.5 translate into altered AP propagation and conduction, as well as arrhythmia inducibility (2, 16). In addition, in recent years the role of the late I\textsubscript{Na} has become increasingly important in both normal conditions as well as a number of pathologies (3).

Such is the case for mutations in SCN5A, which have been implicated in Na\textsubscript{v}1.5 loss-of-function (decreased I\textsubscript{Na}) (8, 72, 77) and gain-of-function (increased I\textsubscript{Na}) (84) ion channel diseases. Multiple human SCN5A channel mutations have been reported, and the functional phenotype of each mutation depends on the localization and type of mutation. Na\textsubscript{v}1.5 gain-of-function mutations are observed, for example, in the long QT syndrome type 3 (LQTS) (52, 84). On the other hand, loss-of-function mutations in SCN5A are predominant in up to 30% of patients with Brugada syndrome (BrS), a genetically transmissible proarrhythmic disease and one of the major causes of sudden death in the young (11, 66, 71). In some cases, BrS associates with inherited SCN5A mutations that impair Na\textsubscript{v}1.5 trafficking to the cell membrane, resulting in loss of functional expression (7, 72, 77). In other cases, BrS mutations may cause loss of I\textsubscript{Na} through gating defects; e.g., speeding of inactivation and a hyperpolarizing shift in channel availability (58).

To date, more than 15 different non-ion channel proteins have been reported to interact with Na\textsubscript{v}1.5 at any time point from biosynthesis to degradation, half of them associated with arrhythmogenic disease. However, the motifs through which Na\textsubscript{v}1.5 interacts with other proteins have not been fully characterized. While it has been shown that individual Na\textsubscript{v}1.5 \(\alpha\)-subunits interact with each other, it is currently undetermined whether this occurs through their respective NH\textsubscript{2}-terminal or COOH-terminal domains (17). Voltage-gated Na\textsuperscript{+} channel COOH-termini are key in the regulation of channel trafficking through their binding partners (62). Also, there is evidence that each channel interacts with anchoring/adapter proteins, enzymes, and protein modulators mainly through its COOH-terminus, which includes a PDZ (postsynaptic density protein, Drosophila disc large tumor suppressor, and zonula occludens-1 protein) domain (S/T)XV-COOH consensus motif (Fig. 2, A and B). Na\textsubscript{v}1.5 has been shown to interact with syntrophin, synapse-associated protein-97 (SAP97), and PTHPI proteins through its PDZ-binding domain and a VPIAxxSD motif between loops II and III through which it interacts with ankyrin-G (1).

### Some Proteins Partners of Na\textsubscript{v}1.5 Associated with Electrophysiological Disease

**Plakophilin-2.** Plakophilin-2 (PKP2) is an essential protein component of the cardiac desmosome and is mainly located at the intercalated disc (ID). Mutations in PKP2, the gene that codes for PKP2, have been associated with proarrhythmic syndromes such as BrS and arrhythmogenic right ventricular cardiomyopathy (14, 28). The Delmar laboratory has made substantial contributions in this field. They showed that Na\textsubscript{v}1.5 coimmunoprecipitates with PKP2 and that both proteins clearly colocalize at the ID. Furthermore, in vitro silencing of PKP2 decreased I\textsubscript{Na} in cultured cardiac myocytes and led to slow conduction velocity, rate-dependent activation failure, and arrhythmic behavior in neonatal rat ventricular monolayers (64, 65). Also, reduced levels of PKP2 in PKP2\textsuperscript{+/−} mice correlated with a reduction in I\textsubscript{Na} without changing Na\textsubscript{v}1.5 mRNA transcript levels or altering Na\textsubscript{v}1.5 protein localization (15). Finally, in an elegant paper using superresolution microscopy, the same group found different PKP2 mutations in a pool of patients with BrS. They also reported that by decreasing Na\textsubscript{v}1.5 and increasing separation of microtubules from the cell end (14), BrS-associated PKP2 mutations decreased I\textsubscript{Na} at the ID in both HL-1 and human-induced pluripotent stem cell-derived cardiomyocytes. Importantly, the I\textsubscript{Na} deficit was restored by transfection of wild-type PKP2. While the relevance of PKP2 to cardiac electrophysiology is clear, the molecular determinants of PKP2-Na\textsubscript{v}1.5 interaction are still unknown and will undoubtedly be the topic of future research efforts.

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**Table 1. Common protein partners of Na\textsubscript{v}1.5 and Kir2.1**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Localization</th>
<th>Protein Function</th>
<th>Na\textsubscript{v}1.5 Interaction</th>
<th>Kir2.1 Interaction</th>
<th>Interaction Sites</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankyrin-G</td>
<td>ID</td>
<td>Anchoring, transport</td>
<td>Yes</td>
<td>?</td>
<td>Domains II and III loop (VPIAXX-ESD) of Na\textsubscript{v}1.5</td>
<td>38, 44, 46, 49–51</td>
</tr>
<tr>
<td>AP-1</td>
<td>Cytosol and TGN</td>
<td>Adaptor, vesicle transport</td>
<td>Yes</td>
<td>?</td>
<td>Tertiary structure between NH\textsubscript{2}-terminal and COOH-terminal Kir2.1 domains</td>
<td>45</td>
</tr>
<tr>
<td>Caveolin-3</td>
<td>LM</td>
<td>Anchoring, caveola formation</td>
<td>Yes</td>
<td>Yes</td>
<td>COOH-terminus PDZ-binding domain</td>
<td>27, 39, 54</td>
</tr>
<tr>
<td>Desmoglein-2</td>
<td>ID</td>
<td>Desmosome structure</td>
<td>Yes</td>
<td>?</td>
<td>COOH-terminus PDZ-binding domain</td>
<td>27, 39, 54</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>LM and TT</td>
<td>Cytoskeletal, anchoring</td>
<td>Yes</td>
<td>Yes</td>
<td>Amino acids 307–326 of COOH-terminus of Kir2.1</td>
<td>57, 63</td>
</tr>
<tr>
<td>Filamin-A</td>
<td>Sarcomere</td>
<td>Cytoskeletal, anchoring</td>
<td>?</td>
<td>Yes</td>
<td>COOH-terminus PDZ-binding domain</td>
<td>27, 39, 54</td>
</tr>
<tr>
<td>FHF</td>
<td>Cytosol</td>
<td>Growth factor</td>
<td>Yes</td>
<td>?</td>
<td>COOH-terminus of Na\textsubscript{v}1.5</td>
<td>32, 42, 83</td>
</tr>
<tr>
<td>GPD1-L</td>
<td>Cytosol and LM</td>
<td>Enzyme, modulator</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>43, 78, 79</td>
</tr>
<tr>
<td>MOG-1</td>
<td>Cytosol and ID</td>
<td>Intracellular traffic</td>
<td>?</td>
<td>?</td>
<td>Domain II and III loop of Na\textsubscript{v}1.5</td>
<td>35, 87</td>
</tr>
<tr>
<td>Plakophilin-2</td>
<td>ID</td>
<td>Desmosome structure</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>14, 15, 28, 64, 65</td>
</tr>
<tr>
<td>SAP97</td>
<td>ID and TT</td>
<td>Scaffolding</td>
<td>Yes</td>
<td>Yes</td>
<td>COOH-terminus PDZ-binding domain</td>
<td>27, 29, 30, 40, 41, 48, 54, 70, 75, 82</td>
</tr>
<tr>
<td>Syntrophin</td>
<td>LM</td>
<td>Cytoskeletal, adaptor</td>
<td>Yes</td>
<td>?</td>
<td>COOH-terminus PDZ-binding domain</td>
<td>34, 39, 48, 54, 74, 86</td>
</tr>
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</table>

**AP-1, adaptor protein complex 1; GPD1-L, glycerol-3-phosphate dehydrogenase-like protein; MOG1, multiplicity suppressor of gsp-1; SAP97, synapse-associated protein 97; ID, intercalated disc; LM, lateral membrane; TT, T tubule; TGN, trans-Golgi network; FHF, fibroblast growth factor homologous factors.**

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**References**

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Desmoglein-2. Desmoglein-2 is another desmosomal protein that coimmunoprecipitates with NaV1.5 and Kir2.1 at the intercalated disc of both channels. Interestingly, decreased $I_{Na}$ and slow conduction velocity (CV) were found in a mouse harboring a N271S mutation in $DSG2$, the gene that codes for desmoglein-2 (59). While further studies are warranted, this further highlights the importance that desmosomal proteins play in retaining/modulating NaV1.5 at the ID and consequently the potential mechanistic roles in cardiac electrophysiological diseases characterized by impaired CV such as BrS.

Caveolin-3. The caveolin family of proteins assist in the formation of particular lipid domain or rafts called caveolae. Caveolae are specialized membrane microdomains enriched in cholesterol and sphingolipids that are present in multiple cell types including cardiomyocytes (5). Functionally, caveolin-3 (Cav3) is both an anchoring protein for molecules within caveolae and a regulatory element for protein signaling. Mutations in Cav3, the gene coding Cav3, which is also the main cardiac isoform, have been associated with hypertrophic cardiomyopathy, LQT9, and sudden infant death syndrome (SIDS) (18, 19, 81). Cav3 coimmunoprecipitates with NaV1.5 in both heterologous expression systems and adult mammalian cells, and both proteins colocalize at the lateral membrane (68, 81, 88). Furthermore, coexpressing wild-type NaV1.5 in HEK293 cells with either V14L, T78M, or L79R mutant Cav3 found in SIDS patients increased the persistent inward $I_{Na}$, which could also provide a potential mechanism for increased AP duration leading to LQT9 (19). However, this has not been proven in vivo, and further studies are warranted.

Ankyrin-G. Ankyrin proteins facilitate the anchoring and transport of other proteins including ion channels to the cell cytoskeleton. Mutations in the two main cardiac isoforms, ANK2, the gene coding for ankyrin-B, and ANK3, which codes for ankyrin-G, have been associated with cardiac proarhythmogenic syndromes that include LQT4, atrial fibrillation, and BrS (20, 49–51). Currently, only ankyrin-G has been shown to interact with NaV1.5, and this occurs directly via the linker loop VPIAXX-ESD between domains II and III of NaV1.5, an ankyrin-binding motif that was originally described in neurons (38). In the cardiac myocyte, ankyrin-G has been found to primarily localize at the ID and the T tubules (33). Additionally, the NaV1.5 E1053K mutation was found in a patient with BrS and was shown to impair the binding of NaV1.5 to ankyrin-G, which in turn prevented the accumulation of...
Nav1.5 at the cell surface, without altering protein folding or Golgi-mediated trafficking (44, 50). Interestingly, this effect was only observed in cardiomyocytes, as mutant Nav1.5 E1053K channels were correctly targeted to the membrane in HEK293 cells, further highlighting the importance of using cardiac myocytes for future studies on macromolecular complex interplay. In a recent report, ankyrin-G deficiency in mice resulted in loss of Na\(^+\) channels, membrane reorganization of PKP2, and lethal arrhythmias in response to β-adrenergic stimulation. Thus ankyrin-G seems critical for in vivo membrane recruitment and regulation of Nav1.5 at the ID (46).

**Dystrophin-associated protein complex.** The dystrophin-associated protein complex (DAPC) includes dystrophin, which is a cytoskeletal protein that plays a major structural role in muscle cells. It links the cytoskeleton to the extracellular matrix by binding its NH\(_2\)-terminus to actin and its COOH-terminus to the glycoprotein complex at the sarcolemma (24). As illustrated in Fig. 2A, Nav1.5 interacts with dystrophin via syntrophin adapter proteins through its PDZ-binding motif [serine-isoleucine-valine (SIV)] (27, 54). Lack of dystrophin was shown to modulate Nav1.5 in the mdx mouse model of Duchenne muscular dystrophy. In addition to reduced dystrophin, mdx mice have reduced Nav1.5 protein expression, as well as reduced I\(_{\text{Na}}\) and CV, whereas mRNA levels of SCN5A transcript are unchanged (27). Importantly, syntrophin does not localize to the ID. Therefore, syntrophin-dependent Nav1.5 modulation most likely occurs at the lateral membrane (54). To date, two mutations in SNTA1, the gene coding α\(_{I}\)-syntrophin, have been associated with LQTS (74, 86). While macromolecular disruption has been proposed as a mechanism of these mutations, further studies are warranted.

**Multiplicity suppressor of gsp-1.** Multiplicity suppressor of gsp-1 (MOG1) is a protein involved in regulating intranuclear protein trafficking. Nav1.5 has been shown to coinmunoprecipitate with MOG1, and both proteins colocalize at the ID. Additionally, the two proteins interact via the intracellular loop between domains II and III of Nav1.5 (87). Furthermore, two mutations in RANGRF, the gene that codes for MOG1, have been recently associated with BrS (35). One of these mutations, E83D, failed to regulate Nav1.5 trafficking to the cell membrane and silencing MOG1 decreased I\(_{\text{Na}}\) by roughly 50% (35).

**Glycerol-3-phosphate dehydrogenase-like protein.** Glycerol-3-phosphate dehydrogenase-like protein (GPD1-L) shares more than 80% sequence homology with GPD. While it displays glycerol dehydrogenase activity, it is slower than GPD. Pull-down assays have shown that GPD1-L interacts with Nav1.5 (78). More importantly, mutations in GPD1-L have been associated with BrS and three cases of SIDS (43, 79). The A280V mutation in GPD1-L that was linked with BrS as well as all three SIDS-associated GPD1-L mutations (E83K, I124V, R273C) produced significant reductions in I\(_{\text{Na}}\) in both heterologous expression systems and neonatal mouse myocytes when coexpressed with wild-type Nav1.5 (43, 79). The molecular mechanisms of Nav1.5-GPD1-L have not been elucidated; thus further work is warranted.

**Fibroblast growth factor homologous factors.** Fibroblast growth factor homologous factors (FHFIs) are nonsecreted intracellular modulators of ion channels that bind to COOH-terminal domain of Na\(^+\) channels (42). Knockdown of FHF13 (FGF13), was shown to decrease I\(_{\text{Na}}\) density and channel availability in cardiac myocytes (83). It also reduced wave propagation velocity in myocyte monolayers. In addition, FGF13 knockdown-reduced Ca\(_{\text{v}}\)1.2 current density and substantially altered subcellular targeting of Ca\(_{\text{v}}\)1.2 channels (83). More recently, FGF12 was reported to be the major FHF expressed in the human ventricle and that a single missense mutation in FGF12-B (Q7R-FGF12) reduced binding to the Nav1.5 COOH-terminus, yielding a BrS phenotype. In adult rat cardiac myocytes, Q7R-FGF12, but not wild-type FGF12, reduced I\(_{\text{Na}}\) channel availability and AP amplitude without affecting Ca\(^{2+}\) channel function (32).

**Membrane-associated guanylate kinases protein complexes.** Membrane-associated guanylate kinase (MAGUK) proteins are characterized by their modular domain structure, which contains up to three PDZ domains, an Src homology-3 domain, and a catalytically inactive guanylate kinase-like domain. Such domains allow MAGUK proteins to function as intracellular scaffolding molecules, playing important roles in the assembly of macromolecular signaling complexes and determining the subcellular distribution of different ion channels (25, 36).

Recently, an increasing number of studies have centered on macromolecular complexes that include SAP97, a MAGUK protein that is expressed abundantly in the heart (30, 41, 48, 75). SAP97 is important for the function and localization of a number of ion channels (54). Of interest, a gain-of-function mutation in SAP97 (M861T) was observed in a human patient with BrS (Ackerman M, personal communication, 2015).

In the past few years, studies addressing the role of SAP97 with regard to the function and localization of Nav1.5 channels have led to fascinating results. The interaction of Nav1.5 with SAP97 is very important for the localization of Nav1.5 at the ID, as was first shown in the dystrophin-deficient mdx mouse and confirmed in wild-type adult rat heart (48, 54). Both proteins have been shown to colocalize at both the ID and at the T tubules (48) and to interact through PDZ binding (Fig. 2B) (27). However, in a recent report, Nav1.5 expression at the ID and I\(_{\text{Na}}\) density were shown to be unaffected in knock-in mice lacking the Nav1.5 SIV domain (ΔSIV) (70). Also, the Abriel group (29) has shown that cardiac-specific ablation of SAP97 in a transgenic mouse had no effect on I\(_{\text{Na}}\), although the authors did observe an increase in SCN5A mRNA expression levels and drastic reduction in potassium currents. These surprising results bring attention to the fact that our current understanding of the role of SAP97 in the regulation of Nav1.5 channel expression and function remains preliminary, and further studies are warranted.

**Potassium Channels in Macromolecular Complexes**

Evidence accumulated over the last 20 years strongly indicates that K\(^+\) channel function depends on complicated, multicomponent protein complexes that help in the assembly and delivery of the right channel subunits to the right place in the cell membrane at the right time. Growing interest on the molecular composition of these complexes and how they influence the function and localization of the different K\(^+\) channels is leading to novel insights into how different K\(^+\) channel isoforms and accessory proteins within the complexes participate in the control of cardiac excitability and the mechanisms of arrhythmias.

The breadth of the K\(^+\) channel field is enormous, and it would take a compendium to do it justice. Therefore, here we
will limit the discussion to the inward rectifier potassium (Kir2.x) channels responsible for $I_{K1}$, which is the strongly rectifying current that controls the resting membrane potential, the depolarization toward threshold and the final phase of AP repolarization (53, 69, 80). Kir2.x ion channel proteins are distinguished by their ability to strongly rectify, that is, to pass $K^+$ current in the inward direction much more readily than outward, a characteristic that is critically important in the modulation of cardiac excitability. The Kir2.x family of proteins includes five isoforms; Kir2.1 (IRK1/KCNJ2), Kir2.2 (IRK2/KCNJ12), Kir2.3 (IRK3/KCNJ4), Kir2.4 (IRK4/KCNJ14), and Kir2.6 (IRK/KCNJ18) (37). $I_{K1}$ density is substantially larger in the ventricles than the atria, yielding functionally important AP differences; these dissimilarities are due in part to different expression density and subcellular localization of the channels subunits that form $I_{K1}$ (21). Kir2.1 is the major isoform underlying $I_{K1}$ in the human ventricular muscle, with Kir2.2 expressing to a lesser extent. In contrast, in atrial cells, $I_{K1}$ is mainly conducted through Kir2.3 channels (47, 85).

Clinically, KCNJ2 gene mutations leading to loss of function of Kir2.1 have been linked with Andersen-Tawil syndrome (ATS) (23, 55). On ECG, patients with ATS show QT prolongation and predisposition to ventricular tachyarrhythmias such as torsades de pointes. On the other hand, Kir2.1 gain-of-function mutations give rise to the type-3 variant of the short QT syndrome, which results in significant QT shortening and increased risk of sudden cardiac death (56).

Expression, trafficking, localization, and function of Kir2.x channels are all regulated by interactions with other proteins. However, in contrast with other ion channels, the information available on protein-protein interactions involving Kir2.x channels is limited. As illustrated in Fig. 2, Kir2.1, Kir2.2, and Kir2.3 contain a COOH-terminal motif [the last 3 amino acids sequence (SXI), where X is any amino acid] that enables interaction with PDZ domain containing proteins (41). Lleonoudakis et al. showed that cardiac Kir2.x channels interact with SAP97, CASK, Veli-4, and Mint1 proteins, all members of the MAGUK family, through a PDZ-binding motif. They also demonstrated that components of the DAPC, including $\alpha_1$- , $\beta_1$-, and $\beta_2$-syntrophin, dystrophin and dystrobrevin, interact with Kir2.x channels through the PDZ-binding motif (21, 39–41).

Some Additional Partners of Kir2.x

MAGUK protein complexes. A model has been proposed in which Kir2.x channels associate with distinct MAGUK proteins (SAP97, CASK, Veli, Mint1), forming different complexes (40). However, the association of Kir2.x channels with CASK, Veli-4, and Mint1 has not been explored in detail, and their role in cardiac cells has not been clarified. On the other hand, it has been shown that SAP97 regulates $I_{K1}$ by modulating surface expression of Kir2.x channels. Binding to SAP97 is important to anchor Kir2.x channels at the plasma membrane, mainly through their interaction with their PDZ-binding domains (Fig. 2B) (48, 75, 82). In addition, data suggest that SAP97 binding to the Kir2.3 COOH-terminal domain results in conformational modifications in the channel structure with consequent modification of the unitary conductance (82). It has also been shown that SAP97 assembles a signaling complex involved in $\beta_1$-adrenergic receptor ($\beta_1$-AR) regulation of $I_{K1}$. The SAP97-binding site on the COOH-terminus of Kir2.x channels overlaps a putative protein kinase A phosphorylation site (RRXS) at the extreme COOH terminus of Kir2.x channels. If this site is phosphorylated, SAP97 will fail to bind to the Kir2.x channel protein (75). Furthermore, SAP97 with its Src homology-3 and guanylate kinase-like domains (Fig. 2) can interact with A-kinase anchoring protein and help assemble kinases and phosphatases. Taken together, the data suggest that the interaction between the SAP97 and Kir2.x is a dynamic process that can be regulated by the phosphorylation state of the Kir2.x channel. Finally, further data suggest that SAP97 actively participates in the localization of Kir2.1 and Kir2.2 channels at the T tubules and of Kir2.1 and possibly also Kir2.3 at the ID (48, 75, 82). Therefore, the above studies indicate that SAP97 contributes to the function and localization of the Kir2.x channels and that it is central for the assembly of macro-signaling complexes and their distribution within precise cellular subdomains of the cardiac myocyte.

Caveolin-3. Recently, it was reported that Kir2.1 may associate with Cav3 in human cardiac cells. The interaction seems to play a pathophysiological role in Cav3 mutations related with LQT9, where there is decreased $I_{K1}$ density, mainly due to reduced cell-surface expression of Kir2.1 channels (76). Even though an important relationship between Cav3 and Kir2.1 has been demonstrated, further studies are needed to elucidate the molecular mechanisms involved in the interaction and to identify other molecular components of the Cav3-Kir2.1 macromolecular complex.

Dystrophin-associated protein complex. While Kir2.x channels do interact with $\alpha_1$, $\beta_1$, and $\beta_2$-syntrophin, dystrophin, and dystrobrevin through their respective PDZ-binding motifs (Fig. 2A) (39), the physiological relevance of these interactions in cardiac cells remains poorly understood. For example, cardiomyocytes from the dystrophin-deficient mdx mouse have a small, albeit statistically significant, decrease in Kir2.1 protein without modification in KCNJ2 mRNA (27). On the other hand, a transgenic mouse model that overexpresses Kir2.1 channels also shows significantly increased membrane levels of SAP97, Nav1.5, and syntrophin proteins, without changes in the mRNA levels for their respective genes (DLG1, SCNA5, and SNTA1) (34, 48). Conversely, in the ventricles of heterozygous KCNJ2 knockout (Kir2.1$^{-/-}$) mice, the relative membrane protein levels of SAP97, Nav1.5, and syntrophin are significantly reduced, whereas the genes coding these proteins are unchanged (34, 48).

Altogether, the foregoing findings suggest that in cardiac cells, the DAPC may be important in the regulation of Kir2.x channel expression/function. It is also possible that DAPC proteins contribute to determining the subcellular localization of Kir2.x channels in cardiomyocytes, similar to what has been demonstrated for Nav1.5 channels. Thus more studies are warranted to further elucidate this potential interaction.

Filamin-A. Filamins are actin–cross-link proteins. They directly interact with diverse other proteins and are involved in multiple cellular processes including cell-cell and cell-matrix adhesion, mechanoprotection, actin remodeling, and intracellular signaling pathways (89, 90). Filamin-A has been shown to increase the number of functional resident Kir2.1 channels within the membrane in arterial smooth muscle cells by binding to a region located between amino acids 307 and 326 on the...
Kir2.1 COOH-terminus. Thus filamin-A appears to act as a cytoskeletal-anchoring protein for the Kir2.1 channel within cells, stabilizing its surface expression and potentially recruiting it to signaling complexes within the membrane (63). Altogether, there is growing evidence supporting the significant role of filamin as an interacting partner of membrane channels in cardiac cells (57). Further experiments will be required to pinpoint the role of filamin on the function of Kir2.x channels in cardiomyocytes. Additionally, while filamin has been shown to localize at the Z lines in cardiac cells, more work will be needed to decipher whether pools of Kir2.x channels are localized in this region of the myocytes as a result of their interaction with filamin.

**Adaptor protein-1 complex.** Up to this point, we have dealt with proteins that associate with Kir2.x channels at the plasma membrane. However, critical though less well understood are the interactions of Kir2.x with partner proteins encountered during early trafficking. Recently, it was demonstrated that the adaptor protein-1 adaptin complex interacts with Kir2.1 through an unusual Golgi exit signal dictated by a tertiary structure, localized within the confluence of the cytoplasmic NH2 and COOH terminal domains. The signal creates an interaction site that allows properly folded Kir2.1 channels to insert into clathrin-coated vesicles at the trans-Golgi for export to the cell surface (45). This study uncovered a critical regulatory step for controlling cell-surface expression of the Kir2.x channels from the Golgi and highlighted the importance of understanding whether alterations in the Golgi export process can also account for cardiac disorders that may arise from Kir2.1 mistrafficking.

In summary, in addition to normal density and biophysical properties, Kir2.x channels require proper localization at specialized macromolecular membrane subdomains. Clearly, the molecular and structural characteristics of such subdomains are central in the regulation of local Kir2.x channels and a vastly unexplored field in cardiac electrophysiology.

**Na\textsubscript{v}1.5-Kir2.1 Channels in Channelosomes**

As illustrated in Fig. 2, in the heart, Na\textsubscript{v}1.5 and Kir2.1 independently interact with at least two distinct PDZ domain-scaffolding proteins, SAP97 and \(\alpha_1\)-syntrophin (27, 41, 82). Recent work has demonstrated that SAP97 mediates Na\textsubscript{v}1.5-Kir2.1 interactions (48). It was shown that Kir2.1 overexpression increased Na\textsubscript{v}1.5 and excitability. Conversely, Kir2.1 downregulation decreased \(I_{Na}\). Moreover, virally mediated transfer of SCN5A in adult rat ventricular myocytes increased Kir2.1 protein at the membrane and also increased \(I_{K1}\) density. Those results suggested that these two channel proteins interact dynamically and reciprocally at the molecular level (48). It also appears that common molecular mechanisms might be involved in the regulation of Kir2.1 and Na\textsubscript{v}1.5 functional expression, which is mediated at least in part, through SAP97 PDZ binding within a macromolecular complex (27, 39, 48). Figure 1 illustrates potential interacting pathways that might potentially start from early protein biosynthesis or traffic. Common protein partners associated with Na\textsubscript{v}1.5 and Kir2.1 are listed in Table 1.

A 70 to 80% reduction in \(I_{Na}\) and \(I_{K1}\) density was recently demonstrated in a zebrafish model of arrhythmogenic cardiomyopathy (ACM) with cardiac myocyte-specific expression of the human 2057del2 mutation in the gene encoding plakoglobin (4). Reduction of \(I_{Na}\) and \(I_{K1}\) density produced marked changes in the AP morphology: resting membrane potential was significantly depolarized, AP duration was significantly prolonged, and maximum rate of phase 0 rise was markedly reduced. The myocytes expressing 2057del2 plakoglobin showed a marked decrease in the amount of SAP97 signal at the cell surface. Together, these observations suggested that the aberrant SAP97 distribution in mutant plakoglobin expressing myocytes resulted in altered trafficking of Na\textsubscript{v}1.5 and Kir2.1 channels. ACM is generally associated with mutations in genes that code proteins in the desmosome, a specialized intercellular-junction complex. However, the above study highlights the importance of additional proteins, such as Kir2.1, Na\textsubscript{v}1.5 and SAP97, as potential mechanistic players in arrhythmias linked to ACM (4).

The in vivo role of SAP97 in the heart was assessed by Gillet et al. (29), using a genetically modified mouse model of cardiomyocyte-specific deletion of SAP97. The mice showed expected functional reductions in potassium currents \(I_{K1}\), \(I_{Na}\), and \(I_{Kur}\). However, a surprising finding was that \(I_{Na}\) was not altered, even though Na\textsubscript{v}1.5 protein expression was slightly increased, which could reflect some type of compensatory effect. The role of SAP97 in the function and localization of Na\textsubscript{v}1.5 channels thus remains a topic of further experiments and discussion.

As already discussed, associations of Na\textsubscript{v}1.5 via its COOH-terminal SIV motif allow at least two coexisting pools of Na\textsubscript{v}1.5 channels in cardiomyocytes, one targeted to the lateral membrane through association with syntrophin and the other to the ID through SAP97 (54). Recently, a patient with BrS5 was reported to have a missense substitution that altered the last three amino acids of the COOH terminal (V2016M), highlighting the clinical relevance of the SIV motif in Na\textsubscript{v}1.5 channels (70). However, in the same study, an unexpected finding was that cardiac-specific deletion of the PDZ SIV motif in mice reduced Na\textsubscript{v}1.5 expression at the lateral cardiomyocyte membrane but not the ID (70). Two possible alternatives have been put forth to explain these surprising results: either SIV-dependent regulation of Na\textsubscript{v}1.5 expression is not essential to the ID region or Na\textsubscript{v}1.5 localization within this region is so crucial that compensatory mechanisms exist to protect Na\textsubscript{v}1.5 expression and function (70). Another possible scenario is that Na\textsubscript{v}1.5 channels present other interacting motifs that are still undetermined.

Kir2.x channels also interact with syntrophin through their respective PDZ-binding motifs (39). However, whether Na\textsubscript{v}1.5 and Kir2.1 form a “channelosome” with syntrophin and whether they are an integral part of the dystrophin proteins complex currently remain undetermined. Even though in the knock-in \(\Delta SIV\) Na\textsubscript{v}1.5 mouse, Kir2.1 expression and current density were unaltered (70), it is important to emphasize that a decrease in the level of Kir2.1 channels was found in the dystrophin-deficient \(mdx\) mouse in addition to impaired expression and function of Na\textsubscript{v}1.5 channels (27). Also, in the mouse model that overexpresses Kir2.1, increase of Kir2.1 expression led to significant increase in SAP97, Na\textsubscript{v}1.5, and syntrophin (34, 48). Moreover, the opposite results were observed in Kir2.1\(^{--}\) mutant mice in which significant decreases in relative membrane protein levels of SAP97, Na\textsubscript{v}1.5, and syntrophin were reported in addition to the expected decreased levels.
of Kir2.1 (34, 48). These results suggest that the dystrophin protein complex might participate in the reciprocal regulation of Na\textsubscript{v}1.5 and Kir2.1 channels. Additional experiments are warranted to further pinpoint the mechanisms of such participation.

On the other hand, while there is increasing evidence that suggests Na\textsubscript{v}1.5 and Kir2.1 interact from early targeting and anterograde trafficking steps, this is still not completely understood. Elucidating the role of Na\textsubscript{v}1.5-Kir2.1 macromolecular complexes in channel function will require knowledge of whether the two channel proteins use common retrograde trafficking pathways, since the balance between anterograde and retrograde trafficking determines steady-state, cell-surface expression of channel proteins. Recent data suggest that by interacting with dynamin-2, which is thought to play a role in expression of channel proteins. Recent data suggest that by interacting with dynamin-2, which is thought to play a role in retrograde vesicular formation, Na\textsubscript{v}1.5 and Kir2.1 might share a common mechanism for retrograde trafficking (Fig. 1) (67). However, several other clathrin-dependent or clathrin-independent mechanisms of endocytosis (6) might also be involved. Evidently, further studies are warranted on this exciting area of research. Taken together, the above studies underscore the complex organization that underlies Kir2.1 and Na\textsubscript{v}1.5 channel function and the different scaffolding proteins with which both channels interact.

Clinical Correlations

The results discussed in the previous sections provide direct experimental evidence that Kir2.1 and Na\textsubscript{v}1.5 channels share common partner proteins that are important in trafficking and membrane targeting of both channels. The pathophysiological implications of the (im)balance between I\textsubscript{K1} and I\textsubscript{Na} are of particular relevance in the context of inherited or acquired arrhythmogenic syndromes in which Kir2.1 and Na\textsubscript{v}1.5 sarcotubular protein functional density is modified. Such is the case of ATS and BrS, since both inherited syndromes have been associated with traffic-deficient mutations in Kir2.1 and Na\textsubscript{v}1.5, respectively.

ATS mutations are widespread along the Kir2.1 protein, and the identified mutated residues may abolish function, affect assembly, and/or disrupt channel trafficking (9, 22, 23, 55, 73). Given the fact that Kir2.1 and Na\textsubscript{v}1.5 channels share common partner proteins that control trafficking, it is reasonable to surmise that trafficking-deficient mutations in Kir2.1 that give rise to ATS will reduce Nav1.5 expression and that this should contribute to exacerbate the arrhythmogenic phenotype by decreasing I\textsubscript{Na} and cellular excitability and by slowing cardiac conduction. Conversely, given how BrS is associated with reduced I\textsubscript{Na}, it is reasonable to speculate that decreased Na\textsubscript{v}1.5 cell-surface expression is linked to reduced Kir2.1 and thus I\textsubscript{K1}. This would depolarize the cell membrane, decrease I\textsubscript{Na} through partial Nav1.5 voltage deactivation, and further impair conduction velocity and increase arrhythmia incidence.

Overall, the molecular interplay between Nav1.5 and Kir2.1 channels with other proteins emerges as a complex and dynamic process in which the channels share common partners and subdomains in the cardiac cell. Additionally, it is likely that the macromolecular interplay among multiple such partners changes dynamically through time and space within each individual cell. Future work should focus on further understanding the role of macromolecular complexes in arrhythmogenic conditions due to alterations that involve not only the defective/altered channels but also proteins that interact with them as part of macromolecular assemblies. In the same context, one may speculate that mutations in undiscovered scaffolding and/or adaptor proteins which interact with cardiac ion channels through macromolecular interplay could also be involved in the pathophysiology of idiopathic arrhythmias. Therefore, unveiling the mechanistic underpinnings of such macromolecular interactions should increase our understanding of inherited and acquired arrhythmogenic diseases and may increase our ability to prevent sudden and premature cardiac death.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

B.C.W., D.P.-B., and J.J. prepared figures; B.C.W., D.P.-B., and J.J. drafted manuscript; B.C.W., D.P.-B., and J.J. edited and revised manuscript; B.C.W., D.P.-B., and J.J. approved final version of manuscript; J.J. conception and design of research.

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