Ion Channel Macromolecular Complexes in Cardiomyocytes: Roles in Sudden Cardiac Death

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Abstract: **The movement of ions across specific channels embedded on the membrane of individual cardiomyocytes is crucial for the generation and propagation of the cardiac electric impulse. Emerging evidence over the past 20 years strongly suggests that the normal electric function of the heart is the result of dynamic interactions of membrane ion channels working in an orchestrated fashion as part of complex molecular networks. Such networks work together with exquisite temporal precision to generate each action potential and contraction. Macromolecular complexes play crucial roles in transcription, translation, oligomerization, trafficking, membrane retention, glycosylation, post-translational modification, turnover, function, and degradation of all cardiac ion channels known to date. In addition, the accurate timing of each cardiac beat and contraction demands, a comparable precision on the assembly and organizations of sodium, calcium, and potassium channel complexes within specific subcellular microdomains, where physical proximity allows for prompt and efficient interaction. This review article, part of the Compendium on Sudden Cardiac Death, discusses the major issues related to the role of ion channel macromolecular assemblies in normal cardiac electric function and the mechanisms of arrhythmias leading to sudden cardiac death. It provides an idea of how these issues are being addressed in the laboratory and in the clinic, which important questions remain unanswered, and what future research will be needed to improve knowledge and advance therapy. (*Circ Res*. 2015;116:1971-1988. DOI: 10.1161/CIRCRESAHA.116.305017.)**

Key Words: arrhythmias, cardiac ■ death, sudden, cardiac ■ ion channels ■ multiprotein complexes

Fundamental research conducted over the past 20 years has led to an explosion of knowledge on the genetic and molecular mechanisms that regulate the function of cardiac ion channels. One of the most important outcomes of such new understanding has been the realization that the traditional reductionist view that ionic currents are the expression of

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Nonstandard Abbreviations and Acronyms

AKAP	A kinase–anchoring protein
BrS	Brugada syndrome
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	cyclic adenosine 3′,5′-monophosphate
CASK	Ca^{2+}/calmodulin-dependent serine protein kinase
Cav3	Caveolin-3
FGFs	fibroblast growth factor homologous factors
GPD1L	glycerol-3-phosphate dehydrogenase 1–like protein
hERG	human ether-a-gogo-related
ID	intercalated disc
I_{K1}	inward rectifier potassium current potassium current
I_{Kur}	ultrarapid delayed rectifier K+ current
I_{to}	transient outward current
K_{ATP} channel	ATP-sensitive K+ channel
KChIP	Kv channel–interacting protein
Kir	potassium inward rectifier
K_V	voltage-gated potassium
LQTS	long-QT syndrome
MirPs	minK-related peptides
MOG1	multicopy suppressor of gsp1
PDZ	postsynaptic density protein [PSD95], Drosophila disc large tumor suppressor [Dlg1], and zonula occludens-1 protein [zo-1])
PKA	protein kinase A
PKC	protein kinase C
PKP2	plakophilin-2
SAP97	synapse-associated protein 97
SCD	sudden cardiac death
SIDS	sudden infant death syndrome
SIV motif	serine–isoleucine–valine motif
SUR	sulfonylurea receptor

distinct proteins that are fixed and function independently expressed on an intracellular or surface membrane is no longer tenable. An ion channel protein may encounter and interact with hundreds of other proteins during its lifespan, from biosynthesis until degradation. Such a complex regulation over time and space suggests an important plasticity for these protein complexes which is a major determinant of cardiomyocyte function, including excitability, excitation–contraction coupling, intercellular communication, and the pathogenesis of arrhythmias. This article is part of the *Circulation Research Compendium on Sudden Cardiac Death.* It reviews research on many of the currently known multicomponent assemblies formed by the main cardiac ion channels with their protein partners. It looks also at the possible role that such assemblies may have in the molecular underpinnings of the normal electric function of the cardiomyocyte and the mechanisms of complex cardiac arrhythmias and sudden cardiac death (SCD). We are focusing on *cis*-interacting proteins, that is, within the same cell. Although there is emerging evidence for important roles of proteins, such as the β-subunits, of the voltage-gated sodium channels in transinteractions as cell-adhesion molecules,[1] this aspect is not addressed in this review.

Ion Channel Macromolecular Complexes

Macromolecular complexes consist of a handful to several thousand individual components, including proteins, nucleic acids, carbohydrates, and lipids, and perform a wide array of vital tasks in the cell.[2] As such, they are essential to the proper functioning of all cellular processes, including metabolism, cell signaling, gene expression, trafficking, cell cycle regulation, and the formation of subcellular structures.[3,4] In the cardiac myocyte, macromolecular complexes also play crucial roles in converting energy, generating and propagating electric signals, and mediating contractility, as well as intercellular communication.[5,6] To achieve these functions, complex molecular networks work together with exquisite temporal precision to generate each AP and contraction. The accurate timing of the molecular events demands, in addition, a comparable precision on the location of each molecule within the cell. Indeed, molecular networks assemble and organize within specific subcellular microdomains, where physical proximity allows for prompt and efficient interaction.[7] For example, Petitprez et al[8] described 2 separate pools of ($Na_V1.5$) sodium channels in ventricular cardiomyocytes. One subpopulation localizes at the lateral membrane of the myocytes, whereas the other localizes at the intercalated disc (ID), and a recent study has shown that $Na_V1.5$ and potassium inward rectifier (Kir)2.1 colocalize at both the ID and the lateral membrane, which is important for mutual regulation and the control of cardiac excitability.[9]

Genetic Cardiac Channelopathies

Genetic cardiac channelopathies were identified >20 years ago.[10] As of today, >35 distinct genes encoding ion channel subunits or regulatory proteins are known to be linked to arrhythmogenic syndromes.[11] The estimated prevalence of cardiac channelopathies in the general population remains however difficult to assess.[12] Cardiac channelopathies are likely responsible for about half of sudden arrhythmic cardiac death cases.[13] The most prevalent genetic disorder is the congenital long-QT syndrome (LQTS). LQTS is caused by mutation-induced decrease in repolarizing currents or by increase in depolarizing currents. The second most frequent cardiac channelopathy is Brugada syndrome (BrS).[14] The molecular mechanisms underlying BrS are still matter of controversy.[15] Other important but more recently described forms of inherited arrhythmias caused by channel dysfunction include catecholaminergic polymorphic ventricular tachycardia,[16] congenital short-QT syndrome,[17,18] and mixed phenotypes.[19]

Sodium Channel Macromolecular Complexes

The main voltage-gated sodium channel expressed in cardiac myocytes is $Na_V1.5$;[20] it is encoded by the human gene *SCN5A*. $Na_V1.5$ is a large pore-forming protein, also called α-subunit, with 2016 amino acids and of a molecular weight of ≈220 kDa (Figure 1). The $Na_V1.5$ protein has been shown to assemble with small (≈30–40 kDa), single transmembrane segment proteins called β-subunits.[21] Four of these β-subunits have been described in the human genome.[21] The exact stoichiometry between the α- and β-subunits of the cardiac Na^+ channels is not known. However, the brain α-subunit of the Na^+ channels was copurified with 1 β1 and 1 β2-subunit suggesting a possible 1:2 α to β stoichiometry.[22] Several hundreds of mutations in

SCN5A have been linked to cardiac arrhythmic disorders, such as the congenital and acquired LQTS, BrS, conduction slowing, sick sinus syndrome, atrial fibrillation, and dilated cardiomyopathy.[23] This impressive list of allelic disorders underlines the crucial role of $Na_V1.5$ in physiology and diseases.

$Na_V1.5$ Interacting Proteins

$Na_V1.5$ interacts with and is regulated by a myriad of proteins[6] hence forming macromolecular complexes (Figure 1). These different interacting proteins reside in specific subcellular regions of the cardiac myocytes, such as the lateral membrane domains or the ID, thus defining distinct pools of $Na_V1.5$ channels coexisting in cardiac cells.[24] Importantly, mutations in the genes coding for some of these partner proteins were found in patients with genetic cardiac channelopathies, for example, congenital LQTS and BrS.[17] The proteins interacting with $Na_V1.5$ may have different functions such as anchoring/adaptor proteins involved in trafficking, targeting, and anchoring of the channel protein to specific membrane compartments; as enzymes interacting with and modifying the channel structure via post-translational modifications; and as proteins modulating the biophysical properties of $Na_V1.5$ on binding. For further details see the recent review article.[24]

Among the proteins that have been proposed to be involved in targeting the $Na_V1.5$ channel proteins to specific compartments, α1-syntrophin (Figure 2A) and the membrane-associated guanylate kinase protein, synapse-associated protein 97 (SAP97), play crucial roles (Figure 1). Both proteins have PDZ (postsynaptic density protein [PSD95], Drosophila disc large tumor suppressor [Dlg1], and zonula occludens-1 protein [zo-1]) protein–protein interacting domains allowing the direct interaction with the 3 last C-terminal residues of $Na_V1.5$ (serine–isoleucine–valine or SIV motif). Recent studies using genetically modified mouse models indicated a role of the syntrophin–dystrophin macromolecular complex and the key role of the SIV motif in determining the density of $Na_V1.5$ channels at the lateral membranes of myocytes (Figure 3).[25,26] Although the role of the SIV motif and SAP97 at the ID remains to be clarified, neither truncated channels (ΔSIV) nor the cardiac ablation of SAP97 were sufficient to perturb the expression of $Na_V1.5$ at the ID of mouse cardiac cells (Figure 2A and 2B).[25,26] Two other distinct protein–protein interacting domains are well recognized in the C-terminal sequence of $Na_V1.5$ (Figure 1)[27]: the IQ (isoleucine-glutamine calmoduline binding motif)-motif allowing specific interaction with calmodulin and the PY (proline-tyrosine protein-protein interaction motif)-motif, a domain found in membrane proteins permitting the binding of ubiquitin ligases of the Neural precursor cell Expressed, Developmentally Downregulated 4 (Nedd4)/Nedd4-like family.[28] Although the structural details and roles of the interaction of calmodulin and $Na_V1.5$ have been controversial, a recent study[29] suggested a model where calmodulin may be an essential molecular player in the transitions between the different channel states.[29]

Mutations in Genes Coding for $Na_V1.5$ Channel–Interacting Proteins and Associated Disorders

Among the long list of proteins interacting with $Na_V1.5$ (Figure 1),[30] mutations in the genes coding for 6 of them were reported in patients with altered electric function that may lead to SCD. Also important, >20 naturally occurring mutations have been described in the genes coding for the 4 β-voltage-gated sodium channel subunits.[31] These mutations were found in patients with SCD phenotypes, such as BrS, sudden infant death syndrome (SIDS), sudden unexpected nocturnal death syndrome, and idiopathic ventricular fibrillation. The molecular mechanisms underlying the observed phenotypes were diverse, but the majority of these β-subunit mutations reduced the $Na_V1.5$-mediated I_{Na}.[31]

We review here briefly the evidence demonstrating that mutations of the proteins of the $Na_V1.5$ macromolecular complexes cause severe electric disturbances.

α1-Syntrophin

$Na_V1.5$ is part of the dystrophin multiprotein complex; Gavillet et al[32] demonstrated that $Na_V1.5$ interacts with dystrophin via adaptor syntrophin proteins (see also Figure 2A).[32] Similar to the binding with SAP97, this interaction is dependent on the last 3 residues (SIV) of the $Na_V1.5$ protein. Two missense mutations in *SNTA1*, encoding α1-syntrophin, have been described in patients with congenital LQTS.[33] The *SNTA1* mutation, p.A390V, was reported to disrupt a

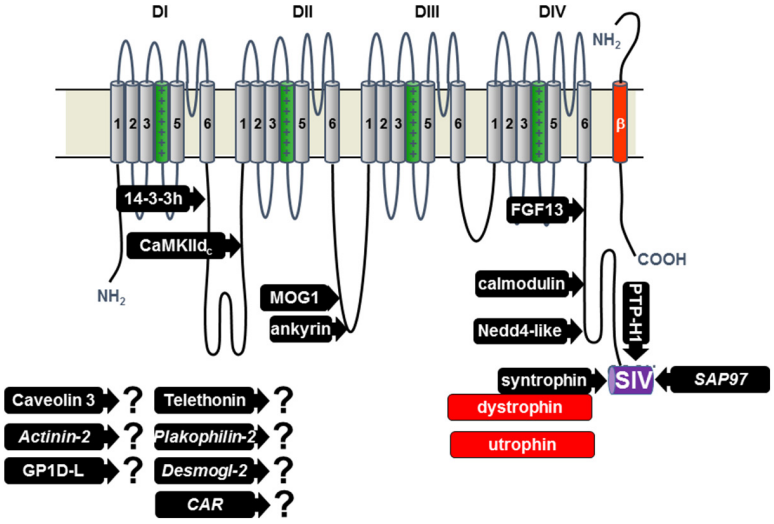

Figure 1. Topography of $Na_V1.5$ channel and its interacting proteins. The proteins for which a binding site has been mapped are represented: 14-3-3 protein η-isoform, calmodulin-dependent protein kinase II (CaMKII) δ-c, multicopy suppressor of gsp1 (MOG1), ankyrin-g, fibroblast growth factor like 13 (FGF13), calmodulin, Neural precursor cell Expressed, Developmentally Downregulated 4-2 (Nedd4-2)-like ubiquitin ligases, syntrophin proteins adapting either dystrophin or utrophin, protein tyrosine phosphatase-H1, synapse-associated protein 97. The proteins with question marks were found to interact with $Na_V1.5$ but the sites of interaction are not yet known (CAR is coxsackie and adenovirus receptor and Desmogl-2 is desmoglein-2). Only 1 of the 4 β-subunits is represented (red). SIV indicates serine–isoleucine–valine.

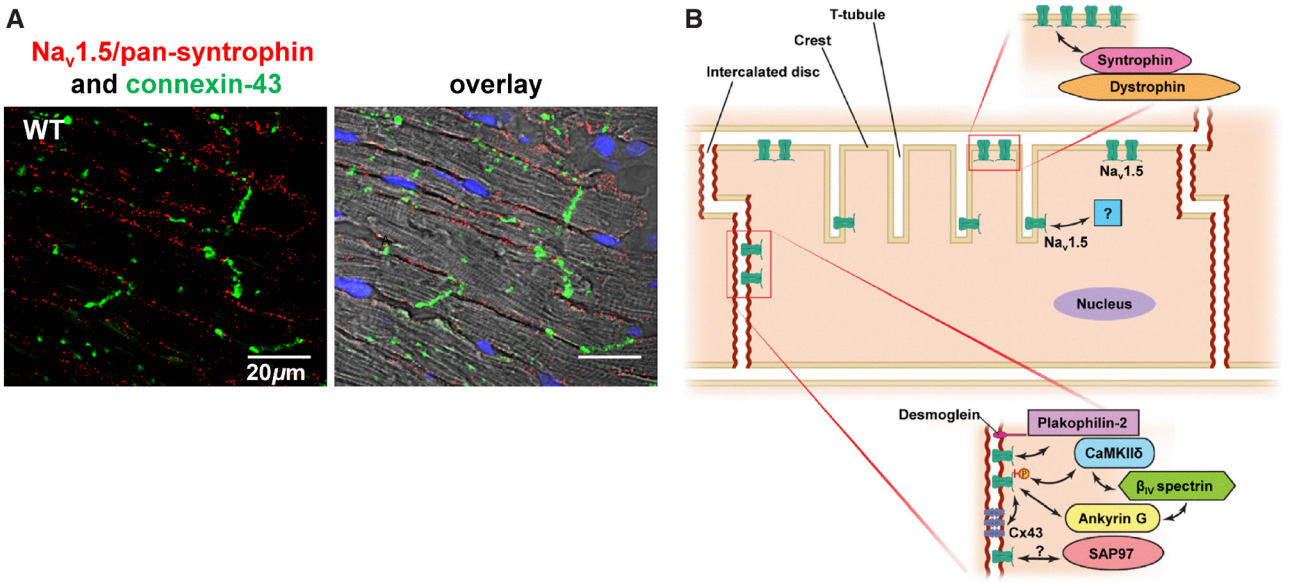

Figure 2. A, Proximity ligation assay staining using antibodies for Na$_v$1.5 and pan-syntrophin demonstrating the specific location of the interaction between these 2 proteins at the lateral membranes of mouse cardiac cells (red dots). In green, immunofluorescence staining demonstrating the presence of connexin43 at the intercalated disc (ID). B, Depending on the partner proteins, they interact with, Na$_v$1.5 is found either at the ID region, or at the lateral membrane (composed of crest regions and t-tubules) of cardiomyocytes. Along the crests, functional sodium channels do not distribute homogenously, but segregate in densely populated clusters, coexisting with areas devoid of functional channels. Reprinted from Shy et al[25] with permission of the publisher. Copyright ©2014, Wolters Kluwer Health.

macromolecular complex comprising neuronal nitric oxide synthase, plasma membrane Ca-ATPase type 4b, and Na$_v$1.5 with syntrophin.[33] The mutant syntrophin protein increased the late Na^+ current, a finding that is consistent with the LQTS phenotype. Increased nitrosylation of Na$_v$1.5, when the mutant syntrophin was coexpressed in HEK293 cells was observed. Further, the mutation *SNTA1* p.A257G was found in 3 unrelated LQTS probands.[34] Although no increase in the late I_{Na} was observed with this variant, significant increase in peak I_{Na} and slowed fast inactivation resulted from the co-expression of this mutant syntrophin. The gene *SNTA1* was also found to be mutated in 8 cases of patients with SIDS and these variants caused an increase of the Na$_v$1.5-mediated late I_{Na} which was inhibited by neuronal nitric oxide synthase inhibitors.[34]

Caveolin-3

Caveolin proteins are important components of caveolae, which are cholesterol-rich plasma membrane invaginations where signaling molecules and ion channels are enriched. Caveolin-3 (Cav3) is encoded by the gene *CAV3*; it is the predominant caveolin isoform expressed in cardiac cells. *CAV3* was found to be mutated in patients with congenital LQTS and SIDS.[35,36] Cav3 was coimmunoprecipitated with Na$_v$1.5 in rat cardiac tissue and HEK293 cells.[35,37] Immunofluorescence stainings showed that the 2 proteins are colocalized at the

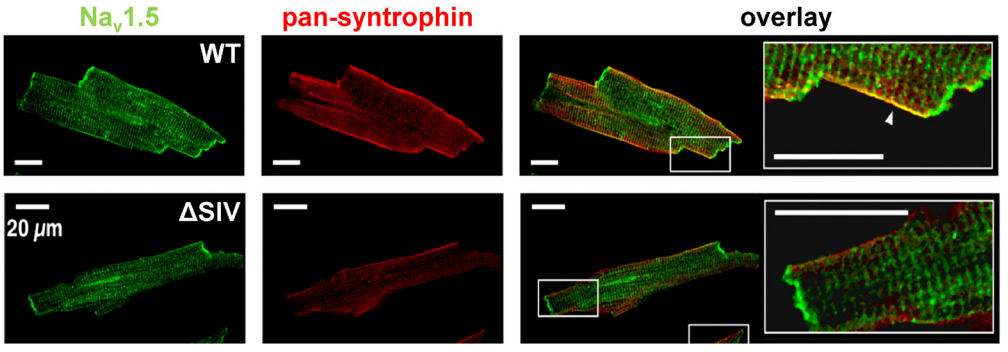

Figure 3. Upper, Isolated mouse ventricular myocyte with double immunofluorescence staining (imaged with confocal microscopy). Na$_v$1.5 (green) is expressed at the intercalated discs, lateral membrane. The punctate staining most likely represents the expression at the t-tubules. Syntrophin is only expressed at the lateral membrane where it colocalizes with Na$_v$1.5 (see arrow in merge showing the yellow region of colocalization). **Lower**, Stainings of myocytes from genetically modified mice (truncation of the last 3 residues of Na$_v$1.5 interacting with syntrophins and synapse-associated protein 97 [SAP97], ΔSIV) illustrating the reduction of Na$_v$1.5 expression exclusively at the lateral membrane. Reprinted from Shy et al[25] with permission of the publisher. Copyright ©2014, Wolters Kluwer Health. SIV indicates serine–isoleucine–valine.

lateral membrane of the cardiomyocytes.[35] The coexpression of $Na_V1.5$ and the LQTS and SIDS mutants of Cav3 in HEK293 cells were also shown to increase the late Na^+ inward current.[35,36] It has been proposed that both *CAV3* and *SNTA1* mutations share a common mechanism in releasing inhibition of neuronal nitric oxide synthase, leading to an increase in $Na_V1.5$ S-nitrosylation and, as a result, augmented late I_{Na}.[30]

Glycerol-3-Phosphate Dehydrogenase 1–Like Protein

Glycerol-3-phosphate dehydrogenase 1–like protein (GPD1L) is an enzyme homolog to GPD1, a key enzyme of the respiratory chain. In 2002, a locus on chromosome 3 in a family with BrS was detected, and later a missense mutation in the gene coding for GPD1L was observed.[38,39] Coexpression experiments showed that mutant GPD1L reduced the $Na_V1.5$-mediated I_{Na}. Three other *GPD1L* mutations have been described in babies that died of SIDS.[40] Expression of these SIDS variants in neonatal mouse cardiomyocytes also decreased the I_{Na}, demonstrating that patients with SIDS may have decreased I_{Na} similarly to BrS. The mechanisms by which the mutations of GPD1L reduce the I_{Na} have been investigated in expression systems.[41] It is proposed that Ser-1503 of $Na_V1.5$ is phosphorylated by protein kinase C (PKC) and that this reduces the I_{Na}. It has been shown that the activity of PKC depends on GPD1L function, and that the mutant GPD1L variants lead to a further decrease in the I_{Na} after a diacylglycerol-dependent stimulation of PKC.[41] Another possible mechanism is that the GPD1L mutant increases nicotinamide adenine dinucleotide phosphate and via PKC effects on mitochondria, and this decreases reactive oxygen species, which then reduce the I_{Na} by yet unknown mechanisms.[42]

MOG1

Multicopy suppressor of *gsp1* (MOG1) is a 29-kd protein encoded by the *RANGRF* gene. MOG1 interacts with the intracellular loop of $Na_V1.5$ between domains II and III.[43] The 2 proteins also colocalize at the IDs in mouse ventricular cells. MOG1 coexpression in HEK293 cells increased the $Na_V1.5$-mediated current without altering its biophysical properties, suggesting that MOG1 is a cofactor for optimal channel expression at the cell membrane. A human study described 1 BrS variant of MOG1 that reduced the expression of $Na_V1.5$ at the cell membrane of rat atrial cardiomyocytes and decreased the I_{Na}.[44] The details about how MOG1 regulates the expression of $Na_V1.5$ are still to be investigated.

Plakophillin-2

Plakophilin-2 (PKP2) is found at the IDs of cardiomyocytes. Delmar's group demonstrated that $Na_V1.5$ interacts not only with PKP2 at the IDs, but also in a complex with ankyrin-G and connexin43.[45,46] Whether the interactions between these different proteins of the IDs are direct or indirect and the site of interaction with $Na_V1.5$ remain to be determined. In a recent study with 200 BrS patients, 5 missense mutations in the gene *PKP2* were demonstrated.[47] The I_{Na} and the density of $Na_V1.5$ channels at the ID were reduced when PKP2 mutant proteins were coexpressed.[48] This experimental evidence strongly supports a role for PKP2 in targeting and regulating the density of $Na_V1.5$ at the IDs and also its implication in BrS.

Fibroblast Growth Factor Homologous Factors

Fibroblast growth factor homologous factors (FGFs) are cytosolic proteins that can modulate both cardiac Na^+ and Ca^{2+} channels.[49] The proximal part of the C-terminal domain of $Na_V1.5$ has been shown to bind to murine FGF13 and human FGF12 (Figure 1).[50,51] Knockdown of FGF13 in murine ventricular myocytes decreased I_{Na} and channel availability.[50] Interestingly, a genetic variant of the gene coding for human FGF12 (p.Q7R) was identified in 1 BrS patient.[51] When expressed in rat myocytes, this variant reduced I_{Na} and channel availability, hence leading to a loss of function which is consistent with the BrS phenotype.

Synapse-Associated Protein 97

SAP97 regulates the targeting, localization, and function of cardiac K^+ and sodium channels via their PDZ domain–binding motifs located in the C termini. The interaction between SAP97 and $Na_V1.5$ has been demonstrated independently by the Abriel and the Jalife laboratories.[8,9,25] Although there is strong experimental evidence for a direct interaction between these 2 proteins via the $Na_V1.5$-SIV motif and a SAP97 PDZ domain, the exact role of SAP97 on the regulation of $Na_V1.5$ function remains to be clarified.[26] A mutation found in 1 patient with BrS modified the valine of the SIV motif of $Na_V1.5$ into a methionine. This mutation was shown to specifically reduce the interaction with SAP97, but not α1-syntrophin. In parallel, the mutation decreased the number of $Na_V1.5$ channels and I_{Na}.[25] It seems likely that genetic variants in *DLG1*, the gene coding for SAP97, will be found in patients with inherited channelopathies associated to SCD.

Calcium Channel Macromolecular Complexes

The voltage-gated L-type calcium channel, $Ca_V1.2$, is the main pathway for the entry of calcium into cardiac cells.[52] The pore-forming $Ca_V\alpha_1$ subunit carries the main biophysical and pharmacological properties of the channel that plays a key role in excitation–contraction coupling and AP duration. The $Ca_V\alpha_1$ subunit is modulated by interactions with different accessory subunits (Figure 4). It is associated with 4 different β-isoforms ($Ca_V\beta1$–4) and 4 different α_2-δ-isoforms ($Ca_V\alpha_2$-δ1–4). Both $Ca_V\beta$ and $Ca_V\alpha_2$-δ have been shown to play dual roles in regulating both the biophysical properties and trafficking of Ca_V channels. In addition to these regulatory subunits, the γ subunits (8 isoforms) have been described as a third class of accessory subunits.[53] In cardiomyocytes, the fully functional $Ca_V1.2$ channel, which is composed of at least $Ca_V\alpha_1$, $Ca_V\beta$, and $Ca_V\alpha_1$-δ subunits (Figure 4) can be considered as the main core macromolecular calcium channel complex.

$Ca_V1.2$ Interacting Proteins

All 4 $Ca_V\beta$s increase the Ca^{2+} current when they are coexpressed in heterologous expression systems along with a $Ca_V\alpha_1$ subunit. $Ca_V\beta$s also alter the voltage dependence and kinetics of activation and inactivation. Furthermore, $Ca_V\beta$ subunits either regulate or are needed for the modulation of $Ca_V\alpha_1$ by protein kinases, G proteins, ubiquitin ligases of the Neural precursor cell Expressed, Developmentally Downregulated 4 family, and small RGK (Rem, Rem2, Rad, and Gem/Kir) proteins (Figure 4).[54,55] $Ca_V\beta$ proteins have also been shown to

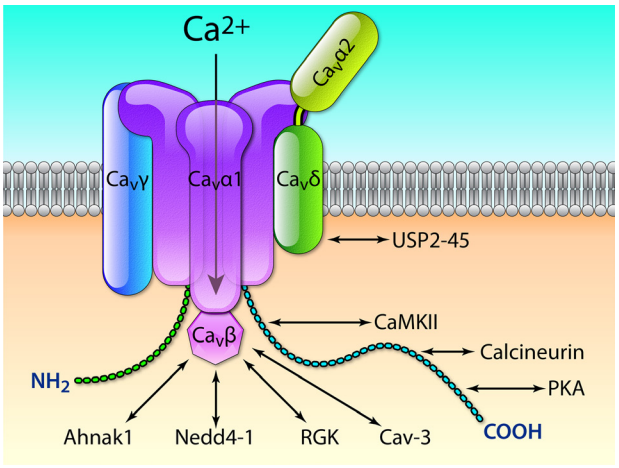

Figure 4. $Ca_v1.2$ channel subunits ($Ca_v\alpha_1$, $Ca_v\beta$, $Ca_v\alpha_2$-δ, and $Ca_v\gamma$) and their major interacting proteins. Ahnak1, Nedd4-1(Neural precursor cell Expressed, Developmentally Downregulated 4-1), RGK (Rem, Rem2, Rad, and Gem/Kir), caveolin-3 (Cav-3), protein kinase A (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and ubiquitin carboxyl-terminal hydrolase 2 isoform 45 (USP2-45). Illustration credit: Ben Smith.

interact with Ahnak1,[56] a protein involved in the protein kinase A (PKA)-mediated control of the $Ca_V1.2$ channel. Altogether, these data demonstrate that $Ca_V\beta$ subunits play a pivotal role in the localization and regulation of cardiac calcium channels.

The $Ca_V\alpha_1$-δ auxiliary subunits are the product of a single gene that is post-translationally cleaved into α2 and δ peptides and remain associated via disulfide bonds.[57] Only $Ca_V\alpha_1$-δ1 and $Ca_V\alpha_1$-δ2 proteins have been found to be expressed in mouse heart.[58] Coexpression of the $Ca_V\alpha_1$-δ subunit, along with $Ca_V\alpha_1$ and $Ca_v\beta$ subunits, accelerated activation and deactivation kinetics and significantly increased I_{Ca}.[58] Animals lacking the $Ca_V\alpha_1$-δ1 subunit demonstrated reduced basal myocardial contractility and relaxation and decreased L-type Ca^{2+} peak current amplitude.[59] $Ca_V\alpha_1$-δ1 has been recently shown to be essential in the regulation of the $Ca_V1.2$ channel cell surface density mediated by the deubiquitylase USP2-45.[60]

The $Ca_V\gamma$ proteins consist of 4 transmembrane domains with intracellular N- and C-terminal ends. In the human heart, only $Ca_V\gamma4$, 6, 7, and 8 have been found to be expressed.[53] The $Ca_V\gamma$ subunits differentially modulate calcium channel function when coexpressed with the $Ca_V\beta1b$ and $Ca_V\alpha_1$-δ1 subunits, altering both activation and inactivation properties.[53] The effects of $Ca_v\gamma$ on $Ca_v1.2$ function are dependent on the subtype of $Ca_v\beta$ subunit.[53]

In cardiac myocytes, $Ca_v1.2$ channels are mainly localized in the t-tubular system (Figure 5).[61] L-type calcium channels are 3- to 9-fold enriched in the t-tubule membrane than on the extratubular surface sarcolemma. Within the t-tubule, studies have estimated that ≈75% of the L-type calcium channels are localized in the dyad domains (Figure 5), thus constituting the main $Ca_v1.2$ macromolecular complex in cardiac cells. The dyad represents a small area where $Ca_v1.2$ channels, situated on the cytoplasmic side of the plasma membrane, are in opposition to the ryanodine receptor type 2 that is situated on the membrane of the sarcoplasmic reticulum. An essential component of the dyadic cleft is junctophilin 2 (Figure 5). Junctophilin 2 is a cleft protein that anchors the t-tubular membrane to the sarcoplasmic reticulum membrane; doing so it plays a key role in maintenance and function of that space.[62] In junctophilin 2 knockdown mice, reduction in the number of dyads was observed,[63] suggesting that junctophilin 2 is responsible for maintaining the dyadic structure. A calcium-binding

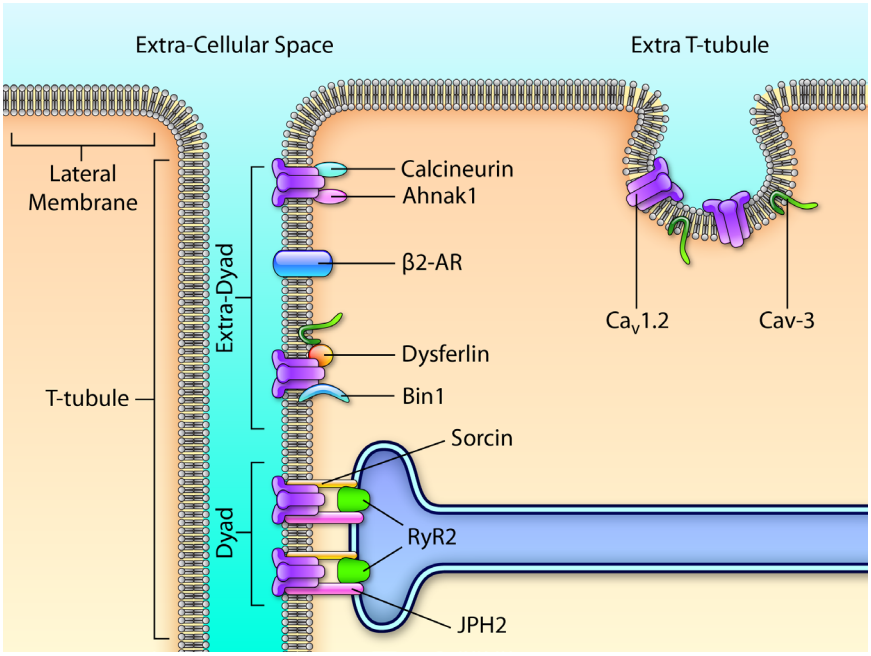

Figure 5. Scheme showing the protein composition of the 3 $Ca_V1.2$-macromolecular complexes (dyad, extradyad, and extra t-tubule). (1) The extra t-tubule; $Ca_V1.2$ channels, and caveolin-3 (Cav-3); (2) the extradyad: $Ca_V1.2$ channels, bridging integrator 1/amphiphysin 2 (Bin1), dysferlin, β2-adrenergic receptor (β2-AR), ahnak1, and calcineurin; and (3) the dyad: $Ca_V1.2$ channels, type 2 ryanodine receptor (RyR2), sorcin, and junctophilin 2 (JPH2). Illustration credit: Ben Smith.

protein, sorcin, expressed in cardiac cells, has also been shown to interact with both $Ca_v1.2$ and ryanodine receptor type 2.[64] Finally, PKA and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which are known to mediate the regulation of $Ca_v1.2$ channel activity via their interactions with the C-terminal of the $Ca_V\alpha_1C$ subunit, have also been shown to be part of the dyad.[65]

Recently, other partner proteins have been described to be important in regulating $Ca_V1.2$ expression at the t-tubule. Amphiphysin 2, also called bridging integrator 1, belongs to the BAR domain proteins superfamily and is involved in membrane invagination.[66] Hong et al[66] have shown that bridging integrator 1 is expressed at the t-tubules, initiates t-tubule genesis, and delivers CaV1.2 to t-tubules.[66] Ahnak1 is indirectly associated with the L-type Ca^{2+} channel via its β_2-subunit and has been shown to be located at the sarcolemma and t-tubules of cardiomyocytes.[56] Similar to bridging integrator 1, the exact localization within the t-tubule system is not known. Nevertheless, its implication in the regulation of I_{Ca} via the β-adrenergic pathway suggests the presence of Ahnak1 and $Ca_V1.2$ channels in extradyad complexes. Dysferlin, a member of the ferlin family, has recently been shown to be expressed mainly at the ID of cardiomyocytes and is also present at the t-tubules.[67] These observations suggest that other $Ca_V1.2$ macromolecular complexes, which may be Cav3-dependent, also exist in the extradyadic compartment (Figure 5). Recently, a subpopulation of $Ca_V1.2$ channels that is located in the caveolae has been found to be part of a macromolecular signaling complex, including β2-adrenergic receptor, adenylate cyclase, protein phosphatase 2A, and PKA.[68] Calcineurin, another interacting/regulating protein of $Ca_V1.2$ channels, has also been shown to be present in caveolae and the t-tubule system.[69] Via its association with the adapter protein A kinase–anchoring protein (AKAP5), calcineurin interacts with Cav-3.[70] Altogether, these findings suggest the presence of t-tubular distinct $Ca_V1.2$ macromolecular complexes that are also present in extradyadic compartments (Figure 5).

In parallel, other groups have demonstrated that a subpopulation of $Ca_V1.2$ channels is localized to caveolae in the extra T-tubular lateral membrane of ventricular cardiomyocytes (Figure 5), thus suggesting that at least a third $Ca_V1.2$ macromolecular complex exists.[68]

Mutations in Genes Coding for Calcium Channel–Interacting Proteins and Associated Disorders

Mutations in genes coding for calcium channel accessory subunits have been linked to BrS and short-QT syndrome type 6.[71,72] A loss-of-function mutation (p.S481L) of the *CACNB2* gene, encoding the $Ca_V\beta2$ subunit, was found in a BrS patient.[71] Despite the large I_{Ca} mutation-induced decrease in heterologous expression systems, no reduction of $Ca_V1.2$ channel number has been observed at the plasma membrane. This suggests another mechanism of Cav regulation than the traffic defect that is generally observed in BrS. Templin et al[72] reported a mutation (p.S755T) in *CACNA2D1*, the gene encoding the $Ca_V\alpha_2$-δ1 subunit, in a SCD patient with short-QT syndrome. An important decrease of the I_{Ca} was observed with the expression of the mutant variant without any modification of the protein expression, thus suggesting that the single channel biophysical properties of the L-type channel were altered.

The p.I5236T mutation of Ahnak1, identified in patients with hypertrophic cardiomyopathy,[73] increased the I_{Ca} as well as shifted slightly leftward its voltage dependence,[73] similar to what has been observed after PKA activation. It is proposed that Ahnak1 may be an important target of PKA-mediated phosphorylation in the enhancement of L-type I_{Ca} by the β-adrenergic receptor type 2. Furthermore, 3 other Ahnak1 variants were identified in hypertrophic and dilated cardiomyopathy patients.[74] Contrary to what has been proposed in the former study, it was recently found that Ahnak1 is not essential for β-adrenergic upregulation of I_{Ca} in mice. Instead, Ahnak1 interacts with the $Ca_V\beta$ subunit to modulate the β-adrenergic response of I_{Ca}.[74]

Potassium Channel Macromolecular Complexes and Associated Disorders

Cardiac potassium channel proteins are coded by >40 different genes.[75] In addition, several auxiliary subunits and associated proteins are involved in the trafficking, distribution, and anchoring of potassium channels at specific microdomains at the plasma membrane, and contribute to their organization in macromolecular complexes.[76] Such partners help in the control of potassium channel expression and biophysical properties, thus regulating the plasticity of cardiac electric activity both under normal conditions and in disease states. In this section, we review the interactions of the major potassium channels as part of macromolecular assemblies and the role of such assemblies in cardiac excitation and repolarization.

The Strong Inward-Rectifying Potassium Channels

Among the 3 strong inward-rectifying potassium channels (Kir2.1, 2.2, and 2.3) that express in the heart, Kir2.1 is the most abundant in the ventricles. Kir channels are responsible for inward rectifier potassium current (I_{K1}) and are involved in the depolarization, repolarization, and resting phases of the cardiac AP.[77] Kir subunits assemble to form tetrameric channels in many cell types, including cardiac myocytes.[78,79] I_{K1} contributes significant repolarizing current between −30 and −80 mV, and thus is responsible for the terminal phase of the AP.[80] In addition, it serves as the primary conductance controlling the resting membrane potential in ventricular myocytes.[81] These channels show strong rectification between −50 and 0 mV, which means that they remain closed during the AP plateau; they only open when the membrane potential repolarizes to levels between −30 and −80 mV, which in the normal AP occurs during the late phases of the AP. Rectification is achieved by a voltage-dependent blockade by intracellular magnesium and polyamines, such as putrescine, spermine, and spermidine,[82] which interact with at least 3 amino acid residues located inside the pore of the channel.[79]

Loss-of-function mutations of Kir2.1 have been identified in patients affected by Andersen–Tawil syndrome, which is also referred to as LQTS type 7 and is characterized by delayed repolarization.[83] Because in addition to the heart Kir2.1 is also expressed in other organs, such as skeletal muscle, Andersen–Tawil syndrome is associated with hypokalemic

periodic paralysis and skeletal developmental abnormalities.[83] In the heart, reduction of I_{K1} leads to QT prolongation and predisposes to arrhythmias; yet QT prolongation is less prominent in patients presenting Andersen–Tawil syndrome than in other types of LQTS.[84] Moreover, although Andersen–Tawil syndrome patients do develop ventricular tachyarrhythmias, including torsades de pointes, SCD is rare in these patients.[85]

Only 3 cases of Kir2.1 gain-of-function mutation have been reported. In 2005, Xia et al[86] reported on a Kir2.1 gain-of-function mutation (V93I) in a large Chinese family with atrial fibrillation. Subsequently, 2 different gain-of-function mutations (D172N and E299V) in the *KCNJ2* gene were reported in patients with short-QT syndrome type 3.[17,87] Increased I_{K1} shortens repolarization and the QT interval and exerts a proarrhythmic effect both in the atria and the ventricles.[88]

Kir2 Channels Have Multiple Functional Partners

In 2001, Leonoudakis et al[89] identified a direct association of Kir2.1, Kir2.2, and Kir2.3 with SAP97. They further demonstrated that a complex composed of members of the membrane-associated guanylate kinase protein family (SAP97, Ca^{2+}/calmodulin-dependent serine protein kinase [CASK], Veli, and Mint1) associates with Kir2 channels via the C-terminal PDZ-binding motif.[90] Also using in vitro protein interaction assays they showed that SAP97, Veli-1, or Veli-3 binds directly to the Kir2.2 C terminus and recruits CASK and proposed a model whereby Kir2.2 associates with distinct SAP97–CASK–Veli–Mint1 complexes. Subsequently, using immunoaffinity purification and affinity chromatography from skeletal and cardiac muscle and brain, they discovered that α1-, β1-, and β2-syntrophin, dystrophin, and dystrobrevin, all members of the dystrophin-associated protein complex, also interact with Kir2.x channels.[76] In this regard, cardiomyocytes from the dystrophin-deficient mdx mouse show a small but significant decrease in Kir2.1 protein.[32] It is also possible that dystrophin-related proteins contribute to determining the subcellular localization of Kir2.x channels in cardiomyocytes, similar to what has been demonstrated for $Na_V1.5$ channels.[32] As demonstrated by affinity pull-down experiments, Kir2.1 to 3 and Kir4.1, all bind to scaffolding proteins but with different affinities for the dystrophin-associated protein complex, as well as SAP97, CASK, and Veli.[90]

In 2001, Dart and Leyland[91] showed that Kir2.1 associates with AKAP5, which is a multivalent-anchoring protein that binds PKA, PKC, and calcineurin. AKAP5 is targeted to the intracellular N- and C-terminal domains of Kir2.1 to anchor kinases close to key channel phosphorylation sites and is required for appropriate modulation of channel function.[91] More recently, it was suggested that both Kir2.1 and AKAP are part of a macromolecular signaling complex that includes the β1-adrenergic receptor and SAP97.[92] Kir2.1 may also associate with Cav3 in human cardiac cells. Cav3 mutations have been shown to reduce cell surface expression of Kir2.1 with consequent reduction of I_{K1} density. Such an effect may add to the previously described late I_{Na} increase[35] and contribute to delayed repolarization and arrhythmia generation in Cav3-mediated LQT9.[93]

Filamin-A increases the number of functional Kir2.1 channels on membrane in arterial smooth muscle cells.[94] It seems to act as a cytoskeletal anchoring protein for the Kir2.1 channel, stabilizing its surface expression. However, although filamin has been shown to localize at the Z lines in cardiomyocytes,[95] it is unknown whether pools of Kir2.x channels colocalize with filamin-A. Finally, it has been demonstrated that Kir2.1 interacts with the AP1 adaptin complex through an unusual Golgi exit signal dictated by a tertiary structure, localized within the confluence of the Kir2.1 cytoplasmic NH3 and COOH terminal domains.[96] The signal allows properly folded Kir2.1 channels to insert into clathrin-coated vesicles at the *trans*-Golgi for export to the cell surface, which is a critical regulatory step for controlling trafficking and cell surface expression of the Kir2.x channels.[96]

Kir2.1 and the $Na_V1.5$/Kir2.1 Channelosome

There is a strong relationship between the inward I_{Na} and the I_{K1}, the 2 most important ionic currents controlling ventricular excitability: by controlling the resting membrane potential, I_{K1} modifies Na^+ channel availability and, therefore, cell excitability.[80] In addition, I_{K1}–I_{Na} interactions are important in stabilizing and controlling the frequency of the electric rotors that are responsible for the most dangerous cardiac arrhythmias, including ventricular tachycardia and ventricular fibrillation.[88] Recent data demonstrated that the I_{Na}–I_{K1} interplay involves a reciprocal modulation of expression of their respective channel proteins (Kir2.1 and $Na_V1.5$) forming a channelosome within a macromolecular complex (Figure 6).[9] Furthermore, evidence suggests that conditions that result in $Na_V1.5$ protein reduction, such as it occurs in the dystrophin-deficient *mdx*^{5cv} mice, are accompanied by a concomitant reduction in Kir2.1 protein levels.[32] Importantly, the finding that coexpression of $Na_V1.5$ may reduce internalization of Kir2.1 was a central mechanistic observation, with important implications in the control of cardiac excitability and SCD.[9]

Recently, Gillet et al[26] investigated in vivo the interactions of SAP97 with Kir2.1 and $Na_V1.5$ by generating a genetically modified mouse model in which SAP97 expression was constitutively suppressed in cardiomyocytes. As expected, I_{K1} was reduced in the SAP97 knockout mice (Figure 7).[9,97] Unexpectedly, I_{Na} and $Na_V1.5$ localization at the ID were unaffected by the loss of SAP97 expression. Ostensibly, the data presented by the papers of Gillet et al[26] and of Shy et al[25] about $Na_V1.5$ seem to contradict previous work.[8,9,97] Yet there are substantial differences between the 2 mouse models and the previous studies that need to be considered. Most important in both mouse models,[25,26] genetic modification is present early in development, whereas in the other studies,[9] SAP97 expression was silenced in adult myocytes that were kept in culture. Therefore, it is conceivable that the consequences on I_{Na} could be different in an inducible SAP97 knockout mouse model. However, $Na_V1.5$ is known to interact with other regulatory proteins at the IDs, such as connexin43,[98–100] PKP2,[45] and ankyrin-G.[101] Furthermore, recent studies suggest that there are microdomains of $Na_V1.5$ at the IDs. In particular, a population of $Na_V1.5$ is located at the periphery of gap junctions in a so-called perinexus

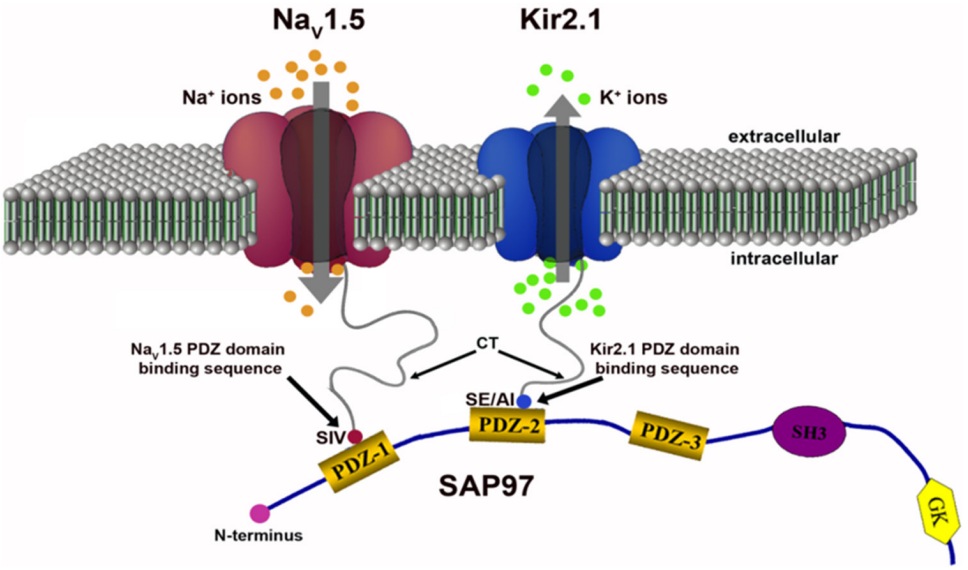

Figure 6. Na$_V$1.5 and potassium inward rectifier (Kir)2.1 form a macromolecular complex (a channelosome). The subcellular localization and channel activity of both Na$_V$1.5 and Kir2.1 are regulated by protein–protein interactions by their respective carboxyl terminal (CT) PDZ (post synaptic density protein [PSD95], Drosophila disc large tumor suppressor [Dlg1], and zonula occludens-1 protein [zo-1]) binding motifs with such PDZ domain–containing proteins as synapse-associated protein 97 (SAP97) and syntrophin. The CTs of 1 Na$_V$1.5 and Kir2.1 molecule each bind to the same SAP97 molecule but at different PDZ domains. These interactions result in changes in the expression of Na$_V$1.5 and Kir2.1 and thereby, influence their function in the cell membrane. Guanylate kinase (GK)-like domain of SAP97; SE/AI, last 3 residues of the Kir2.1 CT, which can be serine and glutamic acid or alanine and isoleucine; SH3, src kinase homology domain of SAP97; SIV, serine, isoleucine, Q:5 valine (last 3 amino acids of the Na$_V$1.5 CT). Reprinted from Milstein et al[9] with permission of the publisher. Copyright ©2012, PNAS.

region that has been proposed to be involved in ephaptic conduction.[102] It might be possible that constitutive deletion of SAP97 led to compensatory modifications in the expression and organization of 1 more partner proteins that contributed to maintain Na$_V$1.5 expression.

The K_{ATP} Channel Macromolecular Complex

ATP-sensitive K^+ (K_{ATP}) channels function as metabolic sensors in many cell types.[103] They are octameric assemblies of a sulfonylurea receptor (SUR) and an ion-conducting subunit (Kir6.x).[104] This enables them to directly couple their energy metabolism to cellular excitability and function as a crucial regulatory mechanism in the cell response to metabolic demand.[103] Genetic manipulation of cardiac K_{ATP} subunits has revealed a role of these channels in arrhythmia generation.[105] Human K_{ATP} mutations underlie different K_{ATP} channelopathies and can substantially increase the risk of heart disease.[106]

The pore-forming subunit of the K_{ATP} channel is 1 of 2 members of the inwardly rectifying family of K^+ channels, Kir6.1 and Kir6.2 coded by *KCNJ11* and *KCNJ8*, respectively. The 2 SUR subunits (SUR1 and SUR2) are respectively coded by *ABCC8* and *ABCC9*.[106] Although several SUR splice variants have been described, the most commonly studied are SUR1, SUR2A, and SUR2B.[107,108] Like other Kir channels, Kir6.x subunits have a cytoplasmic N and C terminus with 2 transmembrane domains and a pore-forming H5 loop.[79,106] SUR has multiple transmembrane domains with 2 large intracytoplasmic loops, the first and second nucleotide-binding domains (NBD1 and NBD2), which contain consensus sequences for the hydrolysis of nucleotides (Walker A and B motifs).[109]

Coexpression of the 2 types of subunit is necessary to achieve functional expression of K_{ATP} channels and the assembly of a specific Kir6.x with a specific SUR generates currents with a particular single-channel conductance, nucleotide regulation, and pharmacology.[103] However, accumulating evidence suggests that the K_{ATP} channel protein complex is part of a multisubunit macromolecular complex that may also include additional metabolically active protein subunits, including adenylate kinase, creatine kinase, and lactate dehydrogenase.[110] In addition, it has been demonstrated that 14-3-3 proteins promote the cell-surface expression of heterologously expressed and native K_{ATP} channels by functionally antagonizing the arginine-based endoplasmic reticulum localization signal that many ion channels and proteins require to reach the cell surface, and that is present in SUR1.[111] Recently it was shown that K_{ATP} channels are stalled in the Golgi complex of ventricular, but not atrial, cardiomyocytes.[106] It was also demonstrated that PKA-dependent phosphorylation of the C terminus of Kir6.2 by sustained β-adrenergic stimulation leads SUR1-containing channels to reach the plasma membrane of ventricular cells by silencing the arginine-based retrieval signal. Therefore, it was suggested that sympathetic nervous stimulation might enable adaptation to metabolic challenges by releasing K_{ATP} channels from storage in the Golgi.[106]

In a recent proteomics study, glycolytic enzymes previously described for the K_{ATP} channel complex were shown to coimmunoprecipitate with K_{ATP} channel subunit from heart, endothelium, and pancreas,[108] suggesting that glycolytic ATP

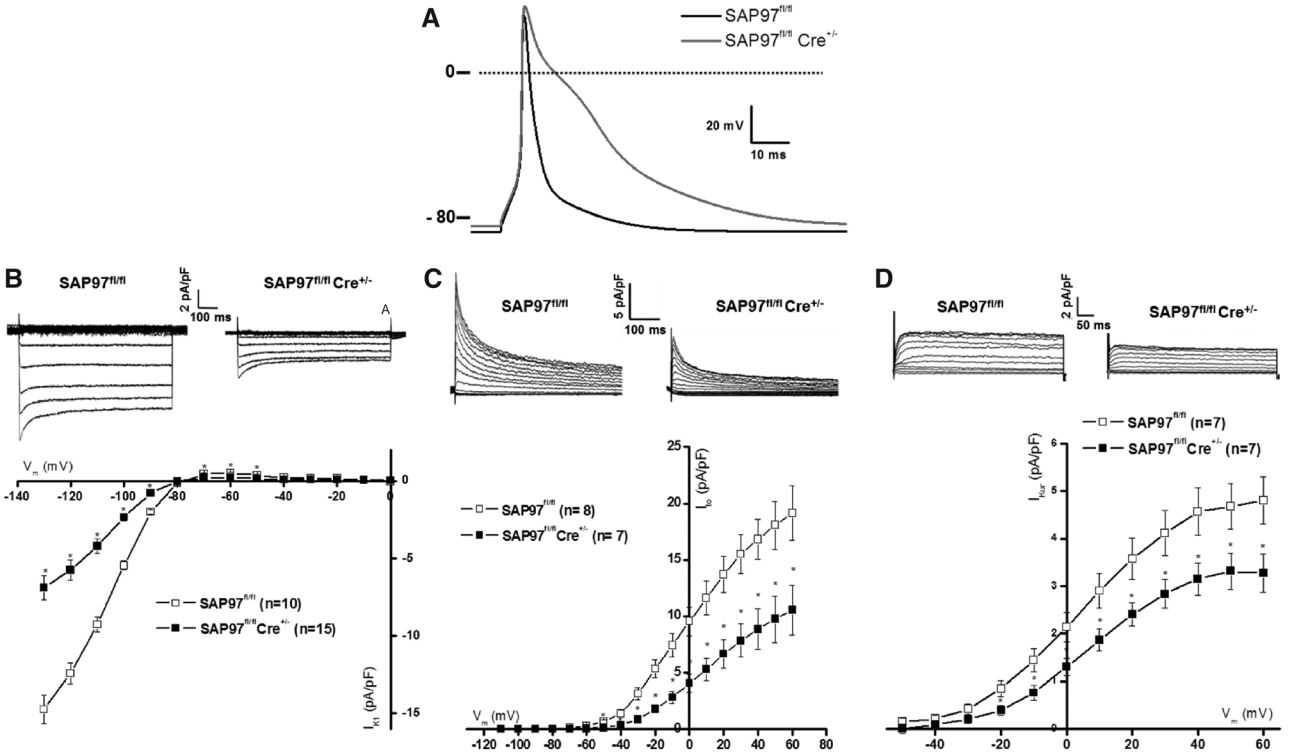

Figure 7. Electrophysiological alterations in synapse-associated protein 97 (SAP97) knockout (KO) ventricular cardiac cells. A, Marked prolongation of the mouse cardiac AP in SAP97-deficient cardiac cells (KO in red and wildtype in black). Decrease of whole-cell potassium current (I_{K1}; **B**), transient outward current (I_{to}; **C**), and ultrarapid delayed rectifier K^+ current (I_{Kur}; **D**) currents in SAP97-deficient cardiac cells. These decreased repolarization currents are the main causes of the AP prolongation. Phase 0 of the AP, that is, the d*V*/d*t*, was not altered consistent with observation that I_{Na} was not modified in the absence of SAP97. Reprinted from Gillet et al[26] with permission of the publisher. Copyright ©2015, Elsevier.

production contributes to fine tuning of K_{ATP} channel opening in these tissues.

Physical interaction between cardiac K_{ATP} channels and the Na^+/K^+ ATPase has also been suggested, which might provide mechanistic insight into their functional interaction with regards to possibly sharing or competing for the same local pool of submembrane ATP/ADP.[112] Finally, Kir6.2 and SUR2A are expressed at a higher density at the IDs in mouse and rat hearts, where they colocalized with PKP2 and plakoglobin. The disruption of the desmosomal complex in PKP2-deficient mice results in downregulation of K_{ATP} channels, suggesting a possible role of these channels in cell-to-cell communication.[113]

Diversity of Voltage-Gated Potassium Channels

Voltage-gated potassium (K_V) channels are members of the Shal subfamily of voltage-gated K^+ channel pore-forming α-subunits.[114] K_V channels are formed by assemblies of 4 α-subunits plus accessory subunits.[115] They function to control resting membrane potentials, shape AP characteristics and influence the responses to neurotransmitters and neurohormones. There are extensive differences in their kinetics of activation and inactivation among the various K_V channels, and specific channels underlie specific currents in the heart. For example, K_V4.x channels, including K_V1.4, K_V4.2, and K_V4.3, coded by *KCNA4*, *KCND2*, and *KCND3*, respectively, activate and inactivate rapidly and underlie the transient outward current (I_{to}).[114] K_V1.5, which is coded by *KCNA5* and forms the atria-specific ultrarapid delayed rectifier K^+ current (I_{Kur}), inactivates much more slowly.[116] K_V7.1 is coded by *KCNH2,*[117] the human ether-a-go-go-related gene (*hERG*). In contrast to K_V4.1 to 3, hERG activates and inactivates rapidly, then conducts most of its current during its recovery from inactivation. K_V2.1, coded by *KCNB1*, is a slow delayed rectifier K^+ channel that underlies $I_{k,slow2}$ in rat cardiomyocytes.[118,119] Targeted elimination of K_V2 channels in mouse ventricular myocytes leads to prolongation of the action potential duration and the QT interval.[120] K_V2.1 may have distinct physiological roles in atrial and ventricular myocytes.[119] Finally, I_{Ks}, the α-subunit (*KCNQ1*) of the slowly activating delayed rectifier K^+ current activates and deactivates slowly.[121]

Functional Interactions of K_V Channels in their Microenvironment

In the heart, K_V4.2, K_V4.3, and K_V1.4 may assemble to generate transient outward currents. K_V4.3/K_V4.2 subunits form the rapidly recovering $I_{to,f}$ channels, whereas K_V1.4 forms the slowly recovering I_{to} channel, both of which underlie the early phase of AP repolarization and contribute to the AP plateau.[122] Both channel types are differentially expressed in the ventricles, contributing to regional heterogeneities in AP shape and duration.[123]

Substantial evidence indicates that these channels function as integral components of macromolecular protein complexes,[114] and that expressed K_V channels can be regulated

by post-translational modifications, including phosphorylation.[114] Also coexpression with accessory or regulatory proteins in heterologous expression systems modifies cell surface expression, subcellular distribution, channel stability, and biophysical properties of K_V4 channels.[124] Hence, the specificity of channel-mediated signal transduction is most likely the result of association of these integral membrane proteins with discrete sets of partner proteins or from their assembly into stable macromolecular complexes.[125] However, the information available about the functioning of accessory subunits and other regulatory proteins in the generation and regulation of native cardiac K_V channels is limited.[126]

β-Subunits and Voltage-Dependent K^+ Channels

The β-subunits of K_V channels ($K_V\beta$) are cytoplasmic proteins that have a mass of ≈40 kDa. Nine $K_V\beta$-subunits are encoded by 4 genes. They have been shown to associate with K_V α-subunits. The $K_V\beta1$, $K_V\beta2$, and $K_V\beta3$ proteins, which are coded by different genes, are the only $K_V\beta$ proteins expressed in the mammalian heart.[114] Additional variability is produced by alternative splicing on the N-terminal region. $K_V\beta$ subunits are localized in the cytosol with a conserved carboxyl terminal and a variable amino terminal; they form a tetrameric structure and are associated in a 1:1 ratio with the α-subunit. $K_V\beta1$ and $K_V\beta3$ associate with α-subunits early during their biosynthesis in the endoplasmic reticulum and exert a chaperone-like effect enabling their stable expression at the plasma membrane.[127] Notably, this chaperone-like property of $K_V\beta$-subunit does not apply to all K_V channels.[115]

The most important effect of $K_V\beta1$ on the voltage-dependent outward current is to accelerate its rate of inactivation, an effect that is mediated through a ball-and-chain like process whereby the $K_V\beta1$ N-terminal domain blocks the inner cavity of the K_V α-subunit pore.[128] In addition, by binding to the C terminus of the K_V channel, $K_V\beta$ can accelerate the C-type inactivation.[129] In heterologous expression systems, coexpression of $K_V\beta1.3$ with $K_V1.5$ is necessary for the cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent PKA-mediated increase in K^+ current.[130] Moreover, consistent with the presence of multiple phosphorylation sites on the α- and β-subunits, PKC reduces the K^+ current of $K_V1.5$ channels only when coexpressed with $K_V\beta1.2$,[131] which may provide an explanation for the effects of the β-adrenergic or PKC stimulation on I_{Kur} in human atrial myocytes.[132] The duration and the frequency of membrane depolarization can significantly modify the rate of inactivation of I_{Kur} in human atrial myocytes. This effect is modulated by the activation of CaMKII and may also involve the interaction between $K_V\beta$ and the $K_V\alpha1.5$ subunits.[133] The contribution of I_{Kur} to the abbreviation of the AP duration during atrial fibrillation[134] and the fact that the $K_V1.5$ channel is more abundantly expressed in atrial than ventricular myocardium are additional examples of the important role played by $K_V\beta$ subunits in cardiac pathophysiology.[116] Finally, $K_V\beta$ subunits have been shown to confer sensitivity to redox modulation and hypoxia to $K_V4.2$ channels.[135]

Other Ancillary Subunits of K_V channels

The best-known partner of K_V4 channels is the cytoplasmic K_V channel–interacting protein, KChIP2, which has been shown directly to be an essential component of $I_{to,f}$ channels in myocardium. The KChIPs were first identified in brain using the K_V4 N terminus as bait in a yeast 2-hybrid screen.[136] They were shown to have 4 EF (EF hand calcium binding motif)-hand-like domains and bind calcium ions. The expression of KChIP and K_V4 together reconstitutes several features of native A-type currents by modulating the density, inactivation kinetics, and rate of recovery from inactivation of K_V4 channels in heterologous cells.[136] All 3 KChIPs were shown to colocalize and coimmunoprecipitate with brain K_V4 α-subunits, and therefore to be integral components of native K_V4 channel complexes.[136] KChIP2 assembles with the N terminus of the pore-forming K_V4 α-subunit and acts as a chaperone to regulate both surface expression and electrophysiological properties of the channel.[137] In heart, KChIP2 coimmunoprecipitates with α-subunits of $K_V4.2$ and $K_V4.3$ from adult mouse ventricles, and the targeted deletion of the mouse KChIP2 locus (*Kcnip2*) abolishes ventricular $I_{to,f}$.[138] In addition, KChIP2 protein expression is highly reduced in the ventricles of homozygous $K_V4.2$ knockout mice, suggesting that K_V4 and KChIP2 proteins reciprocally regulate each other's expression.[138,139] In mouse ventricles, the KChIP2 mRNA level is somewhat larger in the epicardium than the endocardium.[140] In contrast, large transmural gradients in KChIP2 expression together with large $I_{to,f}$ density gradients have been demonstrated across the human and the dog ventricular walls.[139]

Dipeptidyl peptidase–like protein 6 is a protein that regulates the activation and inactivation properties of cardiac K_V4 channels.[114,141] Dipeptidyl peptidase–like protein 6 increases heterologously expressed K_V4 α-subunits at the cell surface,[142] shifts the voltage dependences of activation and inactivation currents to more negative potentials, and accelerates the rates of current activation, inactivation, and recovery.[141–143] Notably, when dipeptidyl peptidase–like protein 6 is coexpressed with $K_V4.3$ and KChIP2, it yields K_V currents that closely resemble native cardiac $I_{to,f}$.[141]

Transient outward K^+ currents can be modulated by protein kinases.[144] The nonreceptor protein tyrosine kinase c-Src is a member of a family of 9 closely related membrane-bound kinases defined by a common structure with a catalytic kinase domain and amino-terminal regulatory regions termed Src homology 2 (SH2) and 3 (SH3) domains.[145] These modular domains mediate intramolecular and intermolecular interactions that are important in signal transduction. The $K_V4.3$ sequence contains SH2 and SH3 domain-binding motifs, making $K_V4.3$ a strong candidate for direct interaction with and phosphorylation by c-Src. Gomes et al[146] have shown through glutathione *S*-transferase pull-down assays and coimmunoprecipitation, that $K_V4.3$ protein associates with c-Src and that the SH2 and SH3 domains of the kinase mediate this interaction, which may result in enhanced efficiency of $K_V4.3$ phosphorylation by c-Src leading to rapid modulation of $K_V4.3$ channel activity.

SAP97 and $K_V1.5$ subunits can interact, directly or indirectly, both in the heart and in heterologous systems.[137,147] Adenoviral overexpression of SAP97 in neonatal rat atrial myocytes leads to clustering of endogenous $K_V1.5$ subunits at myocyte–myocyte contacts and an increase in both I_{Kur} and the number of 4-aminopyridine–sensitive potassium channels in

cell-attached membrane patches.[148] However, pull-down and coimmunoprecipitation assays in cardiac myocytes showed that the K_V4 channel C terminus, SAP97, and CaMKII interact together, and that the interaction is suppressed by SAP97 silencing and enhanced by SAP97 overexpression.[137] In HEK293 cells, SAP97 silencing reproduced the effects of CaMKII inhibition on current kinetics and suppressed K_V4/CaMKII interactions. Altogether, the above data suggest that SAP97 is a major partner for surface expression and CaMKII-dependent regulation of cardiac K_V4 channels.

As reviewed comprehensively elsewhere,[114] *KCNE* genes encode a family of single transmembrane domain proteins called minK-related peptides (MiRPs) that function as accessory β-subunits of K_V channels. When coexpressed in heterologous systems, MiRPs confer changes in K_V channel conductance, gating kinetics, and pharmacology.[114] Coexpression of K_V4 and K_V4-KChIP2 channels with MiRP1 affects the kinetics and the voltage-dependent properties and recapitulates the overshoot in peak current amplitude during current recovery,[149] that is evident in human epicardial $I_{to,f}$.[114,150] Inherited mutations in *KCNE* genes are associated with diseases of cardiac and skeletal muscle and the inner ear.[151,152] For example, aspartate to asparagine substitution to yield p.D76N-MinK is linked to cardiac arrhythmia and deafness. Mutation of arginine to histidine (p.R83H) in MiRP2 is associated with periodic paralysis.[151] Finally, targeted deletion of *Kcne2,* which encodes MiRP1, reduced (≈25%) ventricular $I_{to,f}$ densities by 25% with negligible changes in total or surface $K_V4.2$ expression.[153]

The*KCNE1* gene encodes a 129 amino acid protein in mouse and human that modifies the currents generated by hERG or K_VLQT1. The delayed rectifier K^+ currents resulting from expression of K_VLQT1 alone are small and activate rapidly, but I_{Ks} is reconstituted when minK is coexpressed with K_VLQT1.[154] Evidence suggests that *KCNE1* may have preferential expression in the conduction system.[155] Mutations in *KCNE1* have been reported to cause LQTS.[156]

MiRP2 is a member of the MinK-related peptide family that is coded by *KCNE3*. It coimmunoprecipitates with $K_V4.3$ from human atria.[157] Interestingly, a missense mutation (p.R99H) in K*CNE3* was identified in a family with BrS.[157] Cotransfection of MiRP2 (with and without KChIP) decreases $K_V4.3$ current densities in heterologous expression systems.[157,158] In addition, coexpression of the p.R99H MiRP2 mutant reversed the inhibitory effects of wild-type MiRP2 on $K_V4.3$ currents.[157] Altogether, the above data suggest that MiRP2 is required for normal functioning of human $I_{to,f}$ channels and that gain-of-function mutations in MiRP2 predispose to BrS through augmentation of $I_{to,f}$.[114,157]

KCNQ1 (*K_VLQT1*)

The *KCNQ1* gene, encodes the $K_V7.1$ channel protein, which can form heteromultimers with 2 other potassium channel proteins, KCNE1 and KCNE3.[159] In the human heart, the *KCNQ1* encodes the pore-forming α-subunit, and *KCNE1* (also known as minK) encodes the regulatory β-subunit of the KCNQ1–KCNE1 complex responsible for I_{Ks}, the slowly activating delayed rectifier K^+ repolarizing current.[160] Mutations in *KCNQ1* are associated with hereditary LQTS1 (also known as Romano–Ward syndrome), Jervell and Lange-Nielsen syndrome, and familial atrial fibrillation.[161] In 2002, Marx et al[121] showed that modulation of I_{KS} β-adrenergic receptor stimulation requires targeting of cAMP-dependent PKA and protein phosphatase 1 to hKCNQ1 through the AKAP-9, also known as yotiao. These authors elegantly demonstrated that yotiao binds to the human KCNQ1 by a leucine zipper motif, which is disrupted by an LQTS mutation (hKCNQ1-G589D). Identification of the hKCNQ1 macromolecular complex reveals a mechanism for sympathetic nervous system modulation of cardiac action potential duration (APD) through I_{KS}.[121] These data provided compelling evidence that the cardiac I_{Ks} potassium channel is a macromolecular complex consisting of α-(KCNQ1) and β-subunits (KCNE1) and yotiao (AKAP-9), which recruits PKA and protein phosphatase 1 to the channel.[121]

$K_V11.1$–HERG

The human erg protein (hERG or $K_V11.1$) is the pore-forming subunit of the rapid component of the cardiac delayed rectifier potassium current (I_K) responsible for AP repolarization.[162] It is encoded by the *hERG* gene,[163] which comprises 3 members, *erg1*, *erg2*, and *erg3*, displaying varying expression patterns in different tissues;[164] *herg1* is the best characterized.[163] Structurally, hERG has 6 transmembrane domains (S1–S6), S4 being the voltage sensor, with cytosolic N and C termini. The N terminus, which contains a PAS (Per-Arnt-Sim) domain, strongly affects the biophysical properties of the channel. Functional hERG channels are tetramers with a pore region responsible for K^+ current flow through the plasma membrane.[162] As reviewed elsewhere, alternative transcription of hERG1 results in 2 identical proteins, hERG1a and hERG1b, that diverge only in their N termini.[165] hERG1b can form channels alone or coassemble with hERG1a. A third variant of hERG1, also identical to hERG1a but with a modified C terminus is termed hERGuso.[166] Expression of hERGuso reduces the number of channels at the sarcolemma and the current density. In contrast, coassembly with hERG1b alters channel kinetics increasing channel availability current magnitude.[167] Mutations in hERG lead to long-QT syndrome type 2, a major cause of arrhythmias,[168] as well as to short-QT syndrome type 2, which results in atrial and ventricular arrhythmias.[169]

Even though heterologously expressed hERG channels are largely indistinguishable from native cardiac I_{Kr}, a role for KCNE1 in this current was suggested by the diminished I_{Kr} in an atrial tumor line subjected to minK antisense suppression.[170] Subsequently, McDonald et al[171] showed that hERG and minK formed a stable complex and that the heteromultimerization regulated I_{Kr} activity. This provided additional support for the idea that minK, through the formation of heteromeric channel complexes, is central to the control of the heart rate and rhythm. hERG has been shown to also coimmunoprecipitate with PKA,[172] and similar to other cardiac K_V channel subunits hERG interact with SNAREs (soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptors), which are proteins that are critical for synaptic vesicular secretion and possibly membrane protein trafficking.[173] Recently, Ma et al[168] identified 23 potential interacting proteins that may regulate

cardiac I_{Kr} through cytoskeletal interactions, G-protein modulation, phosphorylation, and downstream second messenger and transcription cascades. Fifteen such proteins were identified as hERG amino terminal (hERG-NT)-interacting proteins, including the caveolin-1, the zinc finger protein FHL2, and protein tyrosine phosphatase nonreceptor type 12 (PTPN12). The other 8 proteins were identified as hERG carboxylic terminal (hERG-CT)-interacting proteins, including the NF-κB–interacting protein myotrophin.[168] Several unexpected binding partners were identified which greatly enhanced the dynamic modulation of I_{Kr} as part of a macromolecular complex.[168]

KCNQ1–HERG Interactions

After the pioneering studies of Sanguinetti et al,[154] it became clear that I_K, the delayed rectifying K current responsible for cardiac repolarization, is mediated by 2 distinct currents, I_{Kr} and I_{Ks}, which work together to produce cardiac repolarization and control the APD. Recent results suggest that in addition to their voltage-dependent interactions, these 2 channels also interact at the molecular level.[174] For example, studies in both transgenic LQT rabbit cardiomyocytes and stable, heterologous cell lines reported that hERG and KCNQ1 underwent reciprocal, functional downregulation in that coexpression of wild-type or dominant-negative pore mutants of KCNQ1 significantly reduced hERG currents, and vice versa.[175] More recently, the same laboratory conducted acceptor photobleach Förster resonance energy transfer experiments and demonstrated that the intermolecular KCNQ1–hERG interactions are direct and mediated by their respective COOH termini.[174] In agreement with the above results, another group showed that KCNQ1 preferentially coimmunoprecipitated with mature hERG channels that were localized on the plasma membrane of HEK293 cells.[176] However, the latter group demonstrated that although hERG channels undergo rapid endocytic degradation on exposure to hypokalemia, KCNQ1 channels are relatively insensitive to extracellular K^+ reduction.[176] Thus, when hERG and KCNQ1 were expressed separately, exposure to 0 mmol/L K^+ for 6 hours completely eliminated the mature hERG channel expression but had no effect on KCNQ1. However, contrary to the transgenic rabbit data,[174] the latter investigators showed that when hERG and KCNQ1 were coexpressed, KCNQ1 significantly delayed the hypokalemia-induced hERG loss.[176] Also, hERG degradation led to a significant reduction in KCNQ1 in hypokalemia.[176] Therefore, although biophysical and pharmacological analyses conducted by both groups indicate that hERG and KCNQ1 closely interact with each other, their respective results seem to go in opposite directions: the former group concluded that coexpression of KCNQ1 significantly reduced hERG currents and vice versa,[174] whereas the latter group concluded that coexpression of KCNQ1 protected hERG against hypokalemia, and hERG reduction reduced KCNQ1.[176] Clearly additional studies will be necessary to resolve this controversy.

Perspectives and Conclusions

We have briefly reviewed research conducted over the past 20 years showing that cardiac ion channels may function as part of large macromolecular complexes. Such complexes play crucial roles the transcription, translation, oligomerization, trafficking, membrane retention, glycosylation, post-translational modification, turnover, function, and degradation of all cardiac ion channels known to date. In fact, macromolecular complexes are vital to a wide collection of cellular tasks. Some of these require physical contact among partner proteins, others do not. Understanding the structure and signaling dynamics of multiprotein assemblies is vital to understanding their function and is likely to shed light on how the heart functions in health and disease. However, we are still lacking a detailed knowledge of such processes, and of the role played by the myriad of ion channel molecular assemblies in the compartmentalization of ion channel function and the mechanisms underlying ion channel dysregulation, life-threatening cardiac arrhythmias, and SCD. This is a significant problem because both arrhythmias and SCD are among the most important causes of cardiovascular morbidity and mortality in the developed world. Clearly, many more studies are needed to establish new paradigms of cardiac electrophysiology integrating the large diversity of molecular interactions involved in the formation, targeting, and regulation of cardiac ion channels and their function, as well as the tissue-specific expression of the components of ion channel complexes not only in the working cardiac muscle of the atria and ventricles, but also the specialized pacemaking and conduction systems. Progress likely will come from the use of systems biology approaches, from the nanoscale all the way to the cellular and organ levels. Progress should also derive from the development and application of modern technologies enabling adequate spatiotemporal resolution to visualize and quantify the processes involved in the assembly and dynamic interactions of ion channel macromolecular complexes in living native myocytes from animal models, as well as in human stem-cell derived cardiomyocytes.

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Disclosures

None.

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