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Kir2.1-Na$_V$1.5 channelosome and its role in arrhythmias in inheritable cardiac diseases

Lilian K. Gutiérrez, PhD,[1] Ana I. Moreno-Manuel, MSc,[1] José Jalife, MD, PhD[1,2,3,4]

ABSTRACT

Sudden cardiac death in children and young adults is a relatively rare but tragic event whose pathophysiology is unknown at the molecular level. Evidence indicates that the main cardiac sodium channel (Na$_V$1.5) and the strong inward rectifier potassium channel (Kir2.1) physically interact and form macromolecular complexes (channelosomes) with common partners, including adapter, scaffolding, and regulatory proteins that help them traffic together to their eventual membrane microdomains. Most important, dysfunction of either or both ion channels has direct links to hereditary human diseases. For example, certain mutations in the *KCNJ2* gene encoding the Kir2.1 protein result in Andersen-Tawil syndrome type 1 and alter both inward rectifier potassium and sodium inward currents. Similarly, trafficking-deficient mutations in the gene encoding the Na$_V$1.5 protein (*SCN5A*) result in Brugada syndrome and may also disturb both inward rectifier potassium and sodium inward currents. Moreover, gain-of-function mutations in *KCNJ2* result in short QT syndrome type 3, which is extremely rare but highly arrhythmogenic, and can modify Kir2.1-Na$_V$1.5 interactions in a mutation-specific way, further highlighting the relevance of channelosomes in ion channel diseases. By expressing mutant proteins that interrupt or modify Kir2.1 or Na$_V$1.5 function in animal models and patient-specific pluripotent stem cell–derived cardiomyocytes, investigators are defining for the first time the mechanistic framework of how mutation-induced dysregulation of the Kir2.1-Na$_V$1.5 channelosome affects cardiac excitability, resulting in arrhythmias and sudden death in different cardiac diseases.

KEYWORDS Ion channels; Macromolecular complexes; Channelopathies; Arrhythmogenic mechanisms; Sudden cardiac death

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A. Introduction

The detailed molecular mechanisms that control the electrical function of the cardiomyocyte through the regulation of its sarcolemmal ion channels are still poorly understood, let alone the mechanisms underlying the molecular derangements that lead to life-threatening arrhythmias in inheritable and acquired cardiac diseases. This is an important problem because arrhythmias and sudden cardiac death (SCD) are among the most important causes of morbidity and mortality in the developed world.[1] Among arrhythmogenic diseases, genetic (inheritable) cardiac diseases encompass a large proportion of SCD patients 40 years or younger.[2] These include primary arrhythmogenic disorders, known as ion channel diseases or channelopathies, such as long QT syndrome (LQTS), Andersen-Tawil syndrome (ATS), short QT syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS),[3] as well as inherited cardiomyopathies, such as hypertrophic cardiomyopathy,[2] Duchenne muscular dystrophy (DMD), and arrhythmogenic cardiomyopathy, all of which can lead to arrhythmias and SCD by mechanisms yet unknown.[4–6] Traditionally, such diseases have been treated as "monogenic" on the incorrect assumption that there must be a direct relationship between the protein mutation and the disease phenotype. However, cardiac proteins responsible for excitation and contraction do not function in isolation but are parts of large multiprotein complexes that must be considered for a thorough understanding of the disease.[7–9]

From the [1]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, [2]CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain, [3]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, and [4]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan.

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While the field of protein-protein interactions in cardiac electrophysiology is in its infancy, it is clear that the classical view that ionic currents are a functional expression of distinct ion channel proteins that are statically and independently expressed on an intracellular or surface membrane is no longer tenable. Recent work aimed at dissecting the macromolecular ion channel complexes has revealed that cardiac ion channels are at the center of multiprotein arrays that interact dynamically with other protein components at different life cycle stages of the channel subunit and at distinct intracellular compartments.[10–12] An ion channel protein may encounter hundreds of other proteins during its life span, from biosynthesis until degradation.[13–15] Such a complex regulation over time (turnover) and space (tethering) suggests an important plasticity for these protein complexes, which could be a major determinant of cardiomyocyte electrophysiology, including excitability, excitation-contraction (e-c) coupling, and intercellular communication, as well as in the pathogenesis of cardiac electrical function and arrhythmias. Another recent breakthrough is the heterogeneity of macromolecular channel complexes, which can be composed of α subunits of different ion channel types depending on their membrane localization and their role in activation and propagation of the electrical activity.[16,17] This is complemented by the recent discovery of Kir2.1-Na$_V$1.5 complexes (channelosomes) at the lateral membrane, the t-tubules, and the intercalated disc of the cardiomyocyte.[18,19] The objective of this review article is 2-fold: first, we provide an overview of the structure, function, and regulation of the 2 major cardiac ion channels—Kir2.1 and Na$_V$1.5—that control cardiac excitability and impulse conduction; second, we highlight the integration of those 2 channels into macromolecular complexes, with specific focus on the role of Kir2.1-Na$_V$1.5 channelosome dysfunction in the molecular mechanisms of complex cardiac arrhythmias in 4 selected inheritable cardiac diseases.

Abbreviations

AP1: adaptor protein complex 1

CASK: calcium/calmodulin-dependent serine protein kinase

GPD1-L: glycerol-3-phosphate dehydrogenase 1 like

MAGUK: membrane-associated guanylate kinase

PDZ: postsynaptic density protein 95, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein

PIP_2: phosphatidylinositol-4,5-biphosphate

PKA: protein kinase A

PKC: protein kinase C

PLC: phospholipase C

SEI: serine–glutamic acid–isoleucine motif

B. Strong inward rectifying potassium channel

Cardiac potassium channels play a major role in shaping the action potential (AP). Among the diversity of potassium channels, inward rectifiers are a class of K^+ channels that can conduct much larger inward currents at membrane voltages negative to the K^+ equilibrium (also known as reversal) potential than outward currents at voltages positive to it, even when K^+ concentrations on both sides of the membrane are made equal. Among the superfamily of inward rectifiers (Kir), 3 strong inward rectifier potassium channels—Kir2.1–Kir2.3 subfamily—are responsible for the inwardly rectifying potassium current (I_{K1}) that maintains the resting membrane potential in cardiomyocytes.[20,21] Kir2.x channels were first described in skeletal myocytes by Katz,[22] who called them *anomalous* rectifiers owing to their dual/contrary behavior; later, the current conducted by these channels was known as I_{K1}.[23]

The Kir2.x family is encoded by the subfamily of *KCNJ* genes, which integrates distinct isoforms of Kir2.x potassium channels expressed in different cells and organs.[24,25] The Kir2.x family members hold close homology among subunits and share a similar protein structure. It consists of 2 transmembrane-spanning α-helix domains—M1 and M2—linked by a selectivity ion pore forming a loop between M1 and M2 (also known as the P-loop). The cytoplasmic regions are the amino (NH_2) and carboxyl (COOH) terminals (Figure 1A).[26,27] Both NH_2 and COOH structures will form the cytoplasmic pore, which extends intracellularly, with varying width depending on the channel configuration.

Among the strong inward-rectifying potassium subunits expressed in the human heart (Kir2.1, Kir2.2, and Kir2.3), Kir2.1 is the most abundant in the ventricles followed by Kir2.2 while Kir2.3 is primarily expressed in the atria.[28] In general, Kir2.x subunits assemble in a homotetrameric manner (ie, assembly of 4 identical monomeric subunits); however, it is possible to also find heterotetramers.[24,29,30]

I_{K1} conducted through Kir2.1 channels exhibits unique functions as it helps maintain the different phases of the AP (Figure 1B).[31,32] Such functions may be visualized by exploring its voltage dependence on the current/voltage relationship (Figure 1C). For example, the I_{K1} reversal potential coincides with and controls the resting membrane potential (∼−80 mV). At positive voltages, the current rectifies strongly, developing a negative slope conductance and shutting down completely at potentials positive to −20 mV (see below for more details). Such a nonlinear voltage dependence contributes significantly to shape the temporal evolution of the membrane potential during rest and during the AP (Figure 1B). I_{K1} is the largest conductance during the resting membrane potential (AP phase 4). The current increases briefly upon stimulation, as it controls the approach to threshold during the AP foot. It then shuts down during the AP upstroke (phase 0) and the initial phase of repolarization (phase 1) and increases only upon repolarization to voltages negative to −20 mV, which helps control the *plateau* duration (phase 2) and the final phase of repolarization (phase 3) toward the resting membrane potential.

B.1. Strong inward rectifier potassium channel trafficking

The functional lifetime of the Kir2.1 channel spans multiple steps that include transcription in the nucleus, translation by the ribosomes, oligomerization in the sarcoendoplasmic reticulum, and glycosylation by the Golgi apparatus. This is followed by vesicular trafficking, membrane retention, posttranslational modifications, turnover, ion channel function, turnover, and degradation.[33] At each step, the channel

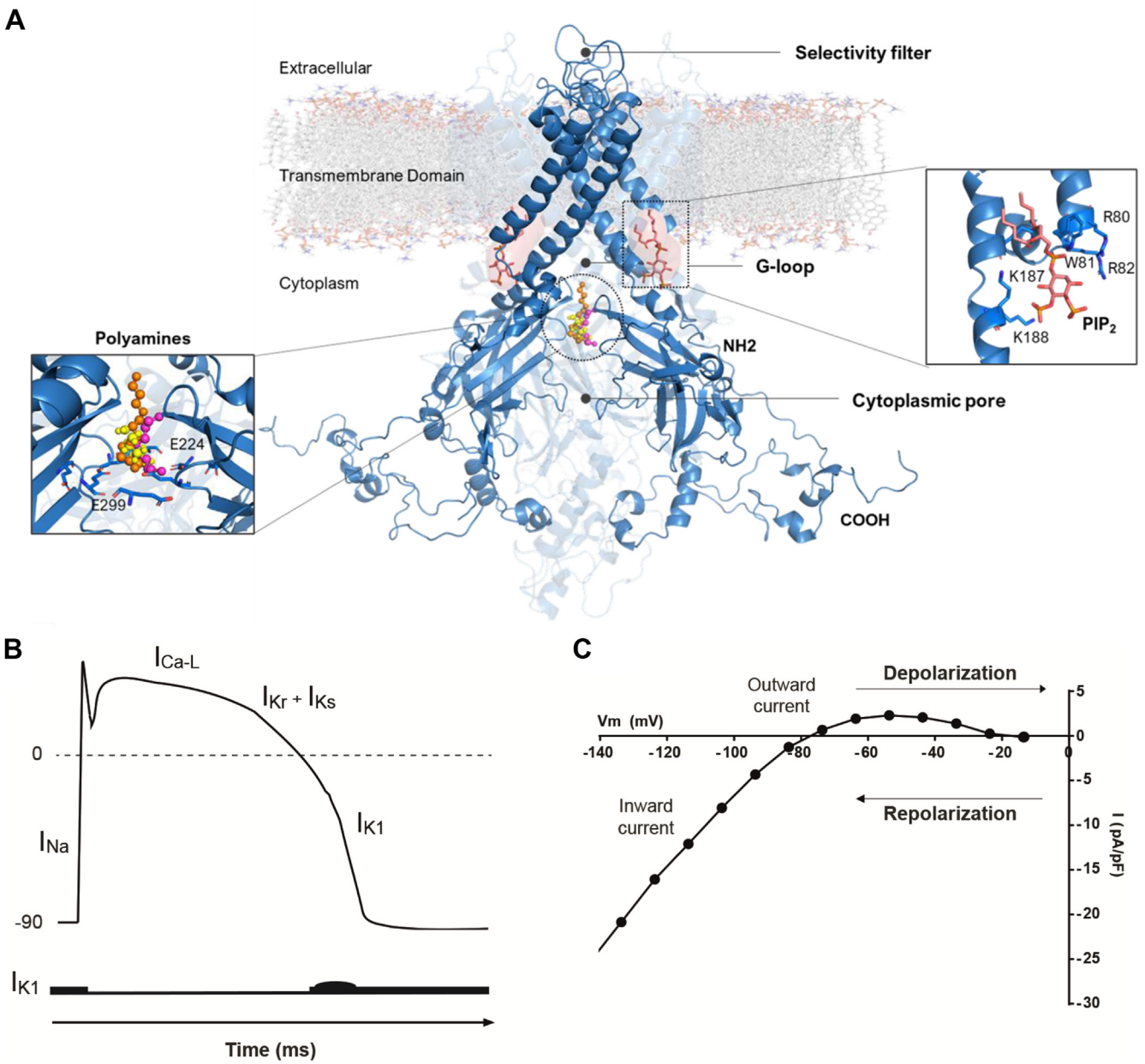

Figure 1

Kir2.1 channel structure and its control of the cardiac action potential. **A:** Homotetrameric Kir2.1 channel structure pointing at some relevant constrictions for K^+ through its central pore. ***Left:*** Kir2.1 residues (ie, E224 and E299) important for polyamines binding (spermine, putrescine, and spermidine) and inward rectification. ***Right:*** Phosphatidylinositol-4,5-biphosphate (PIP_2) binding pocket conformed by an arginine-lysine network embedded in the Kir2.1 channel structure. **B:** Temporal changes in inward rectifier potassium current (I_{K1}) during the cardiac action potential (AP). Upon an external depolarizing stimulus (not shown), I_{K1} first increases from the resting membrane potential to control the approach to threshold leading to the AP upstroke; it then rectifies toward zero during the plateau and then increases again to control the final phase of repolarization. **C:** I_{K1} current/voltage (I/V) relationship shows a reversal potential at −80 mV and strong rectification at voltages between −50 and 0 mV. COOH = carboxyl; $I_{Ca\text{-}L}$ = L-type calcium current; I_{Kr} = fast component of the delayed rectifier current; I_{Ks} = slow component of the delayed rectifier current; I_{Na} = sodium current; NH_2 = amino.

might interact with literally hundreds of other different proteins.[13] Furthermore, normal cardiomyocyte function requires a complex environment that allows 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.[8,11]

An important aspect of Kir2.x channel regulation is precise forward trafficking to specific membrane subdomains.[28,34–36] The Golgi acts as a central biosynthetic sorting station, responsible for directing newly synthesized membrane proteins to their respective subcellular destinations.[37,38] Signal-dependent Golgi export processes control the membrane surface density of Kir2.x channels.[39] Transport of Kir2.1 channels from the Golgi is dictated by residues embedded in the NH_2 and COOH domains of the channel.

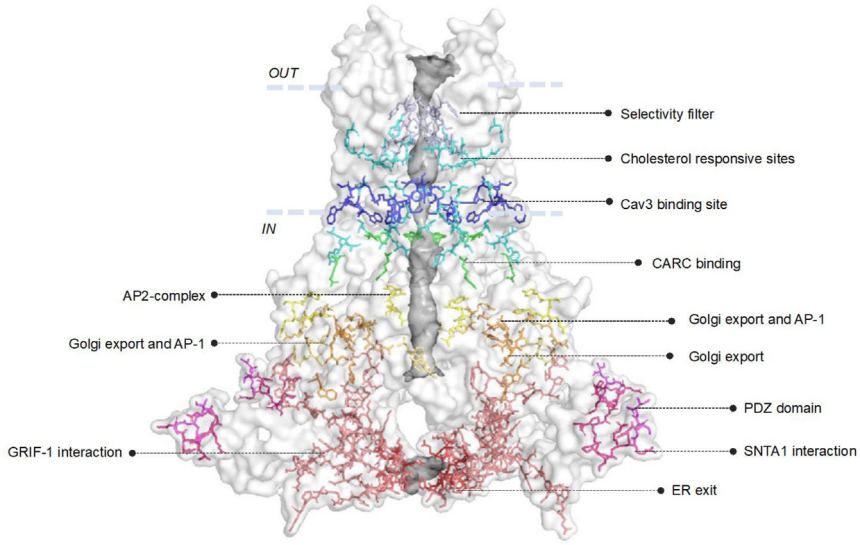

Figure 2
Functional motifs in the Kir2.1 tertiary structure. Kir2.1 molecular structure showing colored signature motifs involved in major Kir2.1 functions and interactions. AP1 = adaptin protein complex 1; AP2 = adaptin protein complex 2; CARC = cholesterol inverted recognition consensus; Cav3 = caveolin-3; ER = endoplasmic reticulum export; GRIF-1 = general regulatory factor-1; PDZ = postsynaptic density protein 95, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein; SNTA1 = α1-syntrophin.

These residues form a recognition site for the interaction with adaptor protein complex 1 (AP1), thereby marking Kir2.1 for incorporation into clathrin-coated vesicles at the *trans*-Golgi network.[39]

Adaptor protein complexes are heterotetrameric protein complexes that mediate intracellular membrane trafficking. Of the 5 different adaptor protein complexes, AP1, AP2, and AP3 are clathrin associated whereas AP4 and AP5 are not.[40] The adaptor protein complexes bind to sorting signals on cytoplasmic tails of cargo proteins. They also recruit clathrin and other accessory proteins and concentrate cargo proteins into vesicular carriers that transport cargo proteins from donor to target membranes.[40–42]

Similarly, amino acid signature motifs along the Kir2.1 primary structure related to principal functions have been identified (Figure 2 and Table 1). Most motifs define the trafficking of Kir2.1 from the sarcoendoplasmic reticulum (SR) to the plasma membrane. The cytoplasmic end of the Kir2.1 amino acid sequence includes the serine–glutamic acid–isoleucine motif, which is the recognition site of the PDZ (postsynaptic density protein 95, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein) domain. This motif is included in the membrane-associated guanylate kinase proteins (such as Synapse-associated protein 97 or SAP97, postsynaptic density protein 95, chapsyn 110, and calcium/calmodulin-dependent serine protein kinase [CASK]). These are mainly scaffolding proteins that simultaneously bind 2 or more other proteins and organize binding partners into a functional unit to regulate trafficking and localization.[43] Although this protein map is customized to Kir2.1, it can be homologated to comparable motif signatures along the Kir2.x family.

Kir2.1 has several phosphorylation sites engaging in post-translational modifications (PTMs) such as protein kinase A (PKA) and protein kinase C (PKC) via tyrosine kinase and casein kinase II, N-myristoylation, and N-glycosylation. Recently, Brown et al[44] performed mass spectrometry–based proteomics to characterize the PTMs of functional Kir2.1, which provided insight into at least 5 novel phosphorylation sites at the NH_2 and COOH terminals. This approach, together with BioID, APEX, or other proximity-labeling screening approaches, may enable complete mapping of Kir2.1 protein function helping to assess the consequences of specific mutations.[45–47]

B.2. Mechanism of inward rectification

The nonlinearity of I_{K1} is given by the strong inward rectification resulting from voltage-dependent blockade of the Kir2.1 channel by intracellular Mg^{2+} and polyamines (eg, putrescine, spermine, and spermidine).[48] These positively charged molecules are forced into the channel from the cytoplasm when the reversal potential of potassium across the membrane is more positive than the resting membrane potential and will electrostatically and physically block the potassium ion flux. In 1994, Lopatin et al[48] were the first to undertake the biochemical and biophysical characterization of polyamines as potential factors behind the inward rectification of Kir2.x channels.

To date, 3 specific amino acids inside the Kir2.1 channel pore are well known to interact with Mg^{2+} and polyamines.[49] Negatively charged residues D172, E224, and E299 are polyamine-binding positions. The aspartic acid D172 is located inside the transmembrane domain in one of the helices and is known as a *rectification controller*[50]; glutamic acids E224 and E299 are located at the cytoplasmic domain between the G-loop and the cytoplasmic pore (Figure 1A). Mutations in each of these 3 residues have been described to result in short QT syndrome type 3 (SQTS3), where there is

Table 1 Color coded list of Kir2.1 structural motifs and their specific functional role

Motif	Role specificity
R^{44}-R^{46}	Golgi transport and export
$^{NUM}TQTTIGYG^{NUM}$	Selectivity filter
$^{81}WRWMLVIF^{88}$	Binding site for caveolin-3
Y^{68}, L^{69}, A^{70}, C^{76}, V^{77}, I^{79}, L^{85}, V^{93}, S^{95}, F^{159}, S^{165}, I^{166}, V^{167}, I^{175}, M^{183}	Cholesterol-responsive sites
R^{67}, F^{73}, V^{77}	Cholesterol inverted recognition consensus (CARC) motif
R^{82}, F^{88}, L^{90}	Second putative CARC binding motif
Y^{242}	Clathrin-mediated endocytosis
$^{242}YIPL^{245}$	Golgi to membrane trafficking
$^{314}SY^{315}$	Golgi exit and adaptin protein complex 1 (AP1) binding
^{319}EI-W^{322}	Golgi export and AP1 complex binding
$^{341}YSRF^{344}$	Adaptin protein complex 2 complex binding site
$^{348}R…R^{396}$	General regulatory factor-1 interaction
$^{374}FCYENEV^{380}$	Endoplasmic reticulum exit
$^{417}EPRPLRRESEI^{427}$	Interaction with α1-syntrophin (*SNTA1*)
$^{440}SEI^{442}$	Postsynaptic density protein 95, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein domain recognition site

The cytoplasmic region accounts for the largest representation of signature motifs related to trafficking and protein-protein interactions.

a gain of Kir2.1 channel function. Under these conditions, the inward rectification of I_{K1} is reduced. Consequently, more potassium will leave the cell during repolarization and the AP and QT interval will be shortened.[51]

Recently, Lee and Nichols[52] suggested through molecular dynamic simulations that the rectification mechanism of Kir2.2 channels by spermine (with a possible extrapolation to other long polyamines) is the result of weak block arising from movement of the polyamine into the cytoplasmic pore. This is intensified as K^+ ions are displaced from the inner cavity through the selectivity filter, and finally spermine completely occupies the selectivity filter vestibule interrupting the channel conductance. On the contrary, when this K^+ driving force decreases, polyamines exit the channel, to restore K^+ conductance.[52] However, a lack of consensus remains about which is the exact mechanism of inward rectification. Thus, additional experimental data and X-ray crystallographic structures are needed to validate any proposed mechanism.

B.3. Kir2.1–phosphatidylinositol-4,5-biphosphate interactions

Some ion channels such as the voltage-gated sodium channel have an intrinsic gate to enable the channel open state. However, Kir2.1 channel gating is produced by a conformational change in the protein produced by the interaction with the second messenger phosphatidylinositol-4,5-biphosphate (also known as $PI(4,5)P_2$ or PIP_2) at the membrane.[53]

PIP_2 is part of the phosphoinositide family, which includes acidic phospholipids with a characteristic inositol head and 2 acyl chains. The phosphatidylinositol compound can be phosphorylated on the 3, 4, and 5 positions, which leads to a variety of polyphosphoinositides.[54] Specifically, PIP_2 phosphorylation occurs in the 4 and 5 positions and is localized in the inner cytoplasmic leaflet of the plasma membrane (Figure 1A).

Among the plasma membrane components, PIP_2 has minor representation (<1% of the whole cell); however, it has been shown to be involved in multiple functions of ion channels, ion transporters, signal pathways, and protein's recognition sites.[54]

In the 1970s, it was discovered that PIP_2 has a main role in 2 distinct signal pathways.[54] First, PIP_2 serves as a cleavage substrate for the enzyme phospholipase C, which produces 2 classical second messengers: the soluble inositol 1,4,5-triphosphate and the membrane-delimited diacylglycerol. Inositol 1,4,5-triphosphate participates in Ca^{2+} release from intracellular stores, whereas diacylglycerol activates PKC.[55]

PIP_2 regulates the activity of Kir channels as well as channels encoded by the *KCNQ* gene family, transient receptor potential channels, and some ion transporters such as the Na^+/Ca^{2+} exchanger (NCX).[54] Hilgemann and Ball[55] demonstrated that temporary depletion of PIP_2 inhibited NCX and the ATP-sensitive potassium channel. Here, PIP_2 generated by endogenous lipid kinases potently activated the cardiac NCX and ATP-sensitive potassium channels, but when phospholipase C signaling came into play, both channels shut down.[55]

A second approach to demonstrate the Kir channel–PIP_2 interaction was using polyamines and PIP_2 inhibitors (eg, PIP_2 antibodies and the polycation neomycin) to deplete PIP_2 availability in excised inside-out patches of *Xenopus oocytes* expressing the Kir2.1 channel.[56] This finding suggested that long polyamines prevented a steady rundown of I_{K1} in the presence of PIP_2 inhibitors and stabilized the channel activity, possibly by modulating the inward current and increasing Kir2.1-PIP_2 binding affinity. Interestingly, the negatively charged residue D172, which is known to interact with polyamines inside the transmembrane pore, might play a relevant dual role in binding polyamines and serving as an indicator of regulation of PIP_2 interactions.[56]

While some channels have additional regulators to achieve an open state, Kir2.x channels are uniquely activated by PIP_2. This is possible given the specialized binding network between the inner membrane leaflet and the cytoplasmic region of Kir2.x channels. Specific amino acid residues form a binding pocket (Figure 1A), which attracts the negatively charged phosphate groups of the phospholipid and enables it to interact with positively charged amino acids in the Kir2.x protein.

A decade ago, Hansen[57] resolved the X-ray crystallographic structure of chicken Kir2.2 including PIP_2 binding.

This finding revealed a detailed conformation of PIP_2 within the protein channel. Specifically, the glycerol backbone and 1' phosphate of PIP_2 capped the Kir2.2 M1 helix while 5′ inositol phosphate coordinated with the distal end of the M2 helix (Figure 1A).[57]

Besides the intimate arrangement of the principal phosphates with the binding pocket, Kir2.1 has multiple specific PIP_2 binding sites. Through ligand docking simulations of the Kir2.1 homology modeling structure, D'Avanzo et al[58] found 2 relevant clusters of PIP_2 preferential binding. The first cluster corresponded to the general residues, primarily a network of lysins, known to interact closely with PIP_2 (namely, R80, R82, K182, K185, K187, K188, and R189). The second cluster was smaller in size and extended out from the end of the distal transmembrane region (such as K64, R218, K219, K228, and R312). The distribution outside the binding pocket seems to define the role of these residues in regulating channel activity by transducing the ligand binding event into the gating mechanism rather than affecting the direct ligand binding.[59]

The mechanism for this specific process starts with PIP_2 binding to the pocket interface, which locates between the transmembrane domain and the cytoplasmic domain; then, the flexible C-linker contracts to a compact helical structure, as the cytoplasmic domain translates toward and tethers to the transmembrane domain. Finally, the G-loop inserts into the transmembrane domain and the inner helix activation gate opens.[53,60]

The regulation of Kir2.1 channels by PIP_2 is defined by the expression of homo- or heterotetramers, since only 1 functional Kir2.1 subunit is sufficient for the channel to become available and open in some sort of conductance state. The presence of complete functional tetrameric subunits exerts positive cooperativity and promotes the fully open state.[61] This mechanism is observed in Kir2.1 loss-of-function mutations, where heterotetramers have a reduced, but not completely abolished, I_{K1} function; meanwhile, in mutant homotetramers, I_{K1} function is totally suppressed by dysfunctional PIP_2-Kir2.1 interactions.[27]

C. Cardiac sodium channel

Voltage-gated sodium channels mediate fast depolarization during the AP upstroke. They are complex transmembrane structures consisting of a pore-forming α subunit, Na$_V$1.1–Na$_V$1.10, supported by 5 β subunits: Na$_V$β1.1, Na$_V$β1.1B, Na$_V$β2.1, Na$_V$β3.1, and Na$_V$β4.1. The distinct subunits are expressed alone or together in several types of tissue (eg, skeletal muscle, brain, and heart) where their function varies. Interestingly, in cardiac tissue Na$_V$1.5, the α subunit is sufficient to generate sodium current (I_{Na}) but the β subunits may regulate its function. Although Na$_V$1.5 is the principal cardiac sodium channel, other Na$_V$1.x subunits have also been found, including Na$_V$1.4 and Na$_V$1.8.[62,63]

Na$_V$1.5 is encoded by the *SCN5A* gene. It is a large protein formed by 4 homologous domains—D1–D4—with 6 spanning-membrane segments—S1–S6.[64] S5 and S6 of each domain are joined by an extracellular loop, called the P-loop, which forms the pore.[65] S4 has a positively charged amino acid that acts as the transmembrane voltage sensor. Toxin-response assays for the sodium channel have enabled the identification of key amino acids (aspartate-glutamate-lysine-alanine) forming a specific ring known as a "DEKA" motif, which allows ion permeation in the P-loops. Moreover, the lysine in D3 is critical for Na^+ vs K^+ selectivity (Figure 3A).

Voltage-gated sodium channels are characterized by 3 conformational states: open, inactivated, and closed.[66] In the heart, Na$_V$1.5 channels open upon depolarization, allowing the rapid flow of I_{Na} during the AP upstroke (Figures 3B and 3C). However, the channels rapidly inactivate as the membrane depolarizes and become unavailable to open and conduct. When the cell repolarizes to the resting membrane potential, Na$_V$1.5 "deactivates" and remains in the closed state unless the cell is electrically stimulated. This differential process is relevant because subtle depolarization from the resting membrane potential may promote channel inactivation and reduce cellular excitability.[67] This form of reduced cellular excitability was postulated by Singer et al[68] as an explanation for the so-called "phase IV" (bradycardia-dependent) paroxysmal atrioventricular block, suggesting that phase 4 depolarization in Purkinje fibers may be a significant factor in the reduced cellular excitability seen clinically in patients with advanced atrioventricular conduction disease. However, more recent studies have demonstrated that phase 4 depolarization in Purkinje fibers may in fact increase excitability and facilitate conduction by bringing the cell closer to the threshold for I_{Na} activation.[69,70]

Na$_V$1.5 channel inactivation and time of recovery from inactivation determine the absolute refractory period, during which it is impossible to initiate a new AP. In contrast, the relatively refractory period is dictated by Na$_V$1.5 inactivation, recovery from inactivation, and deactivation toward the closing state. During this time, if the cell is sufficiently repolarized after a previous AP and there are enough Na$_V$1.5 channels available, the cell will be able to initiate a new AP if it receives a sufficiently large amount of depolarizing current from its surroundings.[71]

Under abnormal conditions, a late (or persistent) I_{Na} may occur when sodium channels do not inactivate completely, and a small remnant current remains active (~0.5% of the total I_{Na}). This current may be abnormally present in large mammalian hearts and has been shown to prolong the AP duration (APD) by disrupting the balance between inward and outward currents during the AP plateau (Ca^{2+} and K^+ fluxes, respectively).[72,73] This phenomenon could be threatening since an anomalous prolongation of phase 2 could lead to cardiac arrhythmias in the form of early afterdepolarizations. An abnormal late I_{Na} (its presence or its abnormally increased amplitude) is responsible for long QT–related arrhythmias in LQTS type 3 and in the Brugada/LQTS type 3 combined phenotype.[74–76] The window current occurs within a specific voltage range (~−70 and −40 mV) where the sodium activation and inactivation curves overlap, which might prolong the APD and generate a new propagated

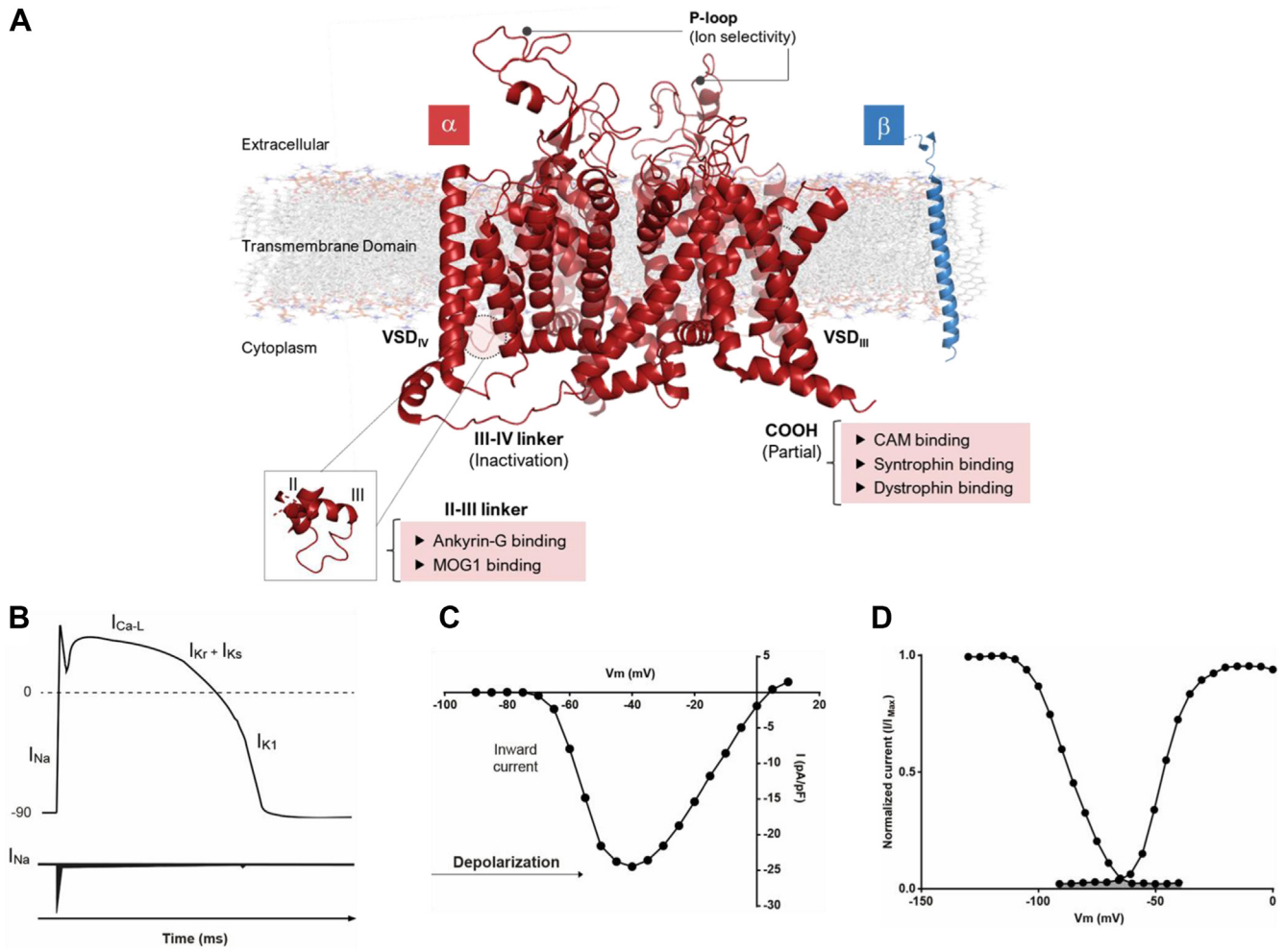

Figure 3

$Na_V1.5$ structure and function. **A:** Molecular structure of $Na_V1.5$, indicating its functional domains through the spanning-membrane segments embedded in the plasma membrane. The regulatory β subunit is shown in blue. **B:** Representation of the human ventricular action potential (AP), showing depolarizing and repolarizing currents; the middle trace shows the temporal changes in sodium current (I_{Na}) throughout the AP. **C:** I_{Na} current/voltage relationship shows a depolarizing inward current whose peak at −40 mV coincides closely with the maximum AP upstroke velocity. **D:** Voltage-dependent I_{Na} activation and inactivation curves. The window current is highlighted in gray. CAM = calmodulin; COOH = carboxyl; MOG1 = multicopy suppressor Of ts Gsp1; $I_{Ca\text{-}L}$ = L-type calcium current; I_{Kr} = fast component of the delayed rectifier potassium current; I_{Ks} = slow component of the delayed rectifier potassium current; VSD_{III} = voltage-sensing domain III; VSD_{IV} = voltage-sensing domain IV.

response at the end of phase 3 of the AP (Figures 3B and 3D).[72–74]

C.1. $Na_V1.5$ regulation and molecular complexes

The cardiac $Na_V1.5$ channel interacts with a diversity of regulatory proteins, in some cases forming micro- and macromolecular complexes inside the cardiomyocyte. The multiple interactions depend on the specific localization of $Na_V1.5$, since the channel has been suggested to reside in subcellular compartments such as the lateral membrane, the intercalated discs, and the t-tubular network. For example, Gavillet et al[75] showed that $Na_V1.5$ is regulated by syntrophin, which is part of the dystrophin multiprotein complex (DPC), but dystrophin and syntrophin are present only in the lateral membrane, suggesting the existence of $Na_V1.5$ in this compartment. In contrast, Makara et al[76] reported that ankyrin-G is a specific key player to target $Na_V1.5$ to the intercalated discs. At the same time, this $Na_V1.5$ pool seems to be dependent on the expression of other intercalated disc proteins such as connexin-43, plakophilin-2, and desmoglein-2.[77,78]

Experimental assays have enabled the screening of $Na_V1.5$ interacting proteins and their role in I_{Na} regulation. Such proteins can be categorized as anchoring/adaptor proteins involved in targeting and trafficking to the plasma membrane, enzymes that interact with the channel via PTM, and finally binding proteins modulating the biophysical properties of the channel.[25]

Certain domains in the $Na_V1.5$ intracellular COOH terminus are relevant for the interplay with auxiliary proteins. Such is the case of the ^{1908}IQ^{1909} motif, which binds to calmodulin and the PDZ domain (just like Kir2.1), among others. The PDZ domain–binding motif in the sodium channel (^{2014}SIV^{2016} for the COOH terminal and ^{20}SLA^{22} for the NH_2

terminal) interacts with PDZ proteins such as α1-syntrophin and SAP97 at different locations within the cardiomyocyte, thus defining distinct pools of the Na$_V$1.5 protein complex.[66,79,80]

One of the most characteristic molecular complexes involving Na$_V$1.5 is formed by its interaction with the DPC component α1-syntrophin coded by the gene *SNTA1*. Cardiomyocytes deficient in dystrophin/utrophin have a significantly reduced I_{Na}. Similarly, dominant-negative mutations in *SNTA1* exhibit a loss of function of I_{Na}.[81] Meanwhile, a novel macromolecular complex involving neuronal nitric oxide synthase Ca^{2+}-ATPase with syntrophin and Na$_V$1.5 has been described, where an increased expression of defective syntrophin increases the late I_{Na},[82] with possible proarrhythmic consequences.

Na$_V$1.5 is also a target for phosphorylation by multiple proteins such as PKA and PKC, Ca^{2+}/calmodulin kinase II (CaMKII), and glycerol-3-phosphate dehydrogenase 1-like gene (*GPD1-L*), all capable of regulating I_{Na}. PKA is a downstream effector of the β-adrenergic receptor–signaling pathway. However, the effects of PKA on I_{Na} remain to be fully defined.[83] In contrast, PKC activation reduces I_{Na} by decreasing the open probability of the channel.[84] It also promotes a hyperpolarizing shift in steady-state inactivation.[85] Direct evidence for CaMKII interaction with Na$_V$1.5 was obtained from transgenic mice expressing CaMKIIδC and rabbit ventricular myocytes with an acute overexpression of CaMKIIδC. In both models, this effect increased Na$_V$1.5 phosphorylation, which slowed fast inactivation, created a hyperpolarizing shift in steady-state inactivation, and increased late I_{Na} and the number of Na$_V$1.5 channels undergoing intermediate inactivation along with slowed recovery from inactivation.[86] *GPD1-L* has been shown to affect trafficking of the cardiac Na^+ channel to the cell surface. A *GPD1-L* mutation decreases Na$_V$1.5 membrane expression, reduces I_{Na}, and causes BrS.[87] CASK, a membrane-associated guanylate kinase protein, interacts with the C terminus of Na$_V$1.5 at the lateral membrane and regulates its surface expression. CASK silencing causes an increase in I_{Na} both in vitro and in vivo by enlarging the pool of Na$_V$1.5 channels at the lateral membrane.[88]

C.2. Kir2.1-Na$_V$1.5 channelosome

Kir2.1 and Na$_V$1.5 are 2 of the most important ion channels controlling cardiac excitability and impulse propagation. They form macromolecular complexes with other proteins, and their respective currents are reciprocally modulated. Recently, molecular studies in animal models and heterologous expression systems have consistently demonstrated this protein-protein interaction, with increasing evidence supporting the importance of the Kir2.1-Na$_V$1.5 channelosome in cardiac electrical function.[18,19,31,89,90]

Reciprocal I_{K1}-I_{Na} modulation was first demonstrated by Milstein et al[19] in single adult rat ventricular myocytes (ARVMs), in which adenoviral transfer of Na$_V$1.5 increased both I_{Na} and I_{K1}. Similarly, Kir2.1 transfection in human embryonic kidney 293 cells expressing Na$_V$1.5 increased both I_{K1} and I_{Na}, synergistically.[31] Subsequent studies in transgenic mice overexpressing Kir2.1 showed exaggerated increase in peak I_{Na} density.[19] In contrast, in heterozygous Kir2.1 knockout mice, Na$_V$1.5 protein was significantly reduced.[19] The same effect was observed in studies with Na$_V$1.5 defective mice where there was a reduction in both I_{Na} and I_{K1}.[90] In addition, by modifying excitability and conduction velocity, I_{K1}-I_{Na} interactions are important for stabilizing and controlling the frequency of the reentrant arrhythmic activity (rotors) that is responsible for lethal cardiac arrhythmias, such as ventricular tachycardia (VT) and ventricular fibrillation (VF).[91]

Kir2.1 and Na$_V$1.5 both have in common, in their respective COOH terminal, a PDZ binding domain to enable the interaction with scaffolding proteins such as SAP97 and α1-syntrophin. Milstein et al,[19] working with ARVM, described that the SAP97 knockdown resulted in a 50% reduction in I_{K1} and a 60% reduction in I_{Na}. Moreover, the same study showed that Na$_V$1.5 and Kir2.1 channels coimmunoprecipitated in both forward and reverse coimmunoprecipitation reactions.[19]

The same association seen for SAP97 has been shown for α1-syntrophin. Matamoros et al[89] studied the impact of the Kir2.1-Na$_V$1.5 macromolecular complex by knocking down α1-syntrophin via lentiviral transfection in ARVMs. This led to significantly reduced I_{K1} and I_{Na} densities (44% and 60% reduction, respectively).[89] These studies provide evidence that at least 2 scaffolding proteins—SAP97 and α1-syntrophin—are actively participating in the Kir2.1-Na$_V$1.5 macromolecular complex.

The precise mechanism underlying reciprocal Kir2.1-Na$_V$1.5 interactions has not been fully elucidated, but recent data showed that 1 common feature is that both proteins traffic together to the cell membrane.[31,90] Studies of the trafficking-deficient Kir2.1$^{\Delta 314\text{-}315}$ mutant ($\Delta S^{314}Y^{315}$) showed both reduced Kir2.1 and Na$_V$1.5 channel expression at the membrane and reduced I_{K1} and I_{Na} densities, which suggested that impairment of Kir2.1 trafficking also impairs Na$_V$1.5.[18,31] Results in isolated ARVMs demonstrated that Na$_V$1.5 and Kir2.1 channels preassemble early in the forward trafficking pathway, enabling them to traffic together more efficiently to the membrane as part of a Kir2.1-Na$_V$1.5 complex rather than alone.[31] A coimmunoprecipitation assay with Na$_V$1.5^{WT} + Kir2.1^{WT} (WT stands for wild type) incubated with endoglycosidase H (a target that shows an association with the SR-Golgi apparatus) suggested that the Kir2.1-Na$_V$1.5 complex assembly occurred (at least partially) when the channels trafficked simultaneously through the SR-Golgi (Figure 4).[31,92]

Transport of Kir2.1 channels to the membrane follows the canonical anterograde pathway and is dependent on signal-mediated processes occurring at the Golgi. The Kir2.1 interaction with the AP1 ϒ-adaptin subunit marks the incorporation of Kir2.1 channels into clathrin-coated vesicles at the trans-Golgi network to allow forward trafficking to the plasma membrane.[39] Na$_V$1.5 also has at least 4 AP1 binding motifs in its cytoplasmic terminus. Ponce-Balbuena et al[31]

demonstrated colocalization of AP1 ϒ-adaptin, $Na_V1.5$, and Kir2.1 at the t-tubular region and intercalated discs. The possible interaction of the Kir2.1-$Na_V1.5$ complex with AP1 ϒ-adaptin was assessed with a human induced pluripotent stem cell–derived cardiomyocyte (hiPSC-CM) model by knocking down the expression of AP1 ϒ-adaptin. The results showed that silencing the AP1 ϒ-adaptin subunit reduced I_{Na} and I_{K1} while also modifying rate-dependent APD adaptation, establishing the AP1 complex as a contributor to the formation and transport to the membrane of the Kir2.1-$Na_V1.5$ channelosome.[31]

An alternative Kir2.1 and $Na_V1.5$ trafficking pathway to the plasma membrane has been proposed. In this case, channels would interact with Golgi reassembly stacking proteins at the SR and traffic together to the specific microdomains at the plasma membrane, without passing through the Golgi.[90] This idea arises from the study of trafficking defective Kir2.1 mutant channels (Kir2.1$^{\Delta 314\text{-}315}$), which specifically mediate one of the trafficking recognition sites. As one would expect, in this type of alterations, proteins are retained along the SR and Golgi system, and their respective membrane currents are analogously altered. However, some Kir2.1 and $Na_V1.5$ channels are able to properly traffic to and incorporate in the plasma membrane. Once again, the reciprocal modulation of I_{K1} and I_{Na} was seen throughout this unconventional pathway supporting the interaction of the Kir2.1-$Na_V1.5$ macromolecular complex.

Some functionally regulated ion channels present coordinated expression, and recent evidence indicates that they form molecular complexes even at the messenger RNA (mRNA) level. So, apparently, they would already be establishing regulatory connections at the transcriptional level. This is of interest because cosynthesis of ion channel mRNA and other proteins may enable detailed electrical regulation and cardiac sarcomere maintenance.[93] Using molecular biology techniques and imaging, Jameson et al[94] demonstrated that mRNA of channels that control the AP, such as *KCNH2*, *SCN5A*, *CACNA1C*, and *KCNQ1*, pertains to the same translational complex and is associated mainly in pairs. Thus, it would be interesting to study the possible functional interaction between *SCN5A* and *KCNJ2* already at the mRNA level and whether the effects of gain- or loss-of-function mutations on either channel and its counterpart already occur transcriptionally, for example, by showing that when 1 transcript is silenced the other transcripts are codepleted.

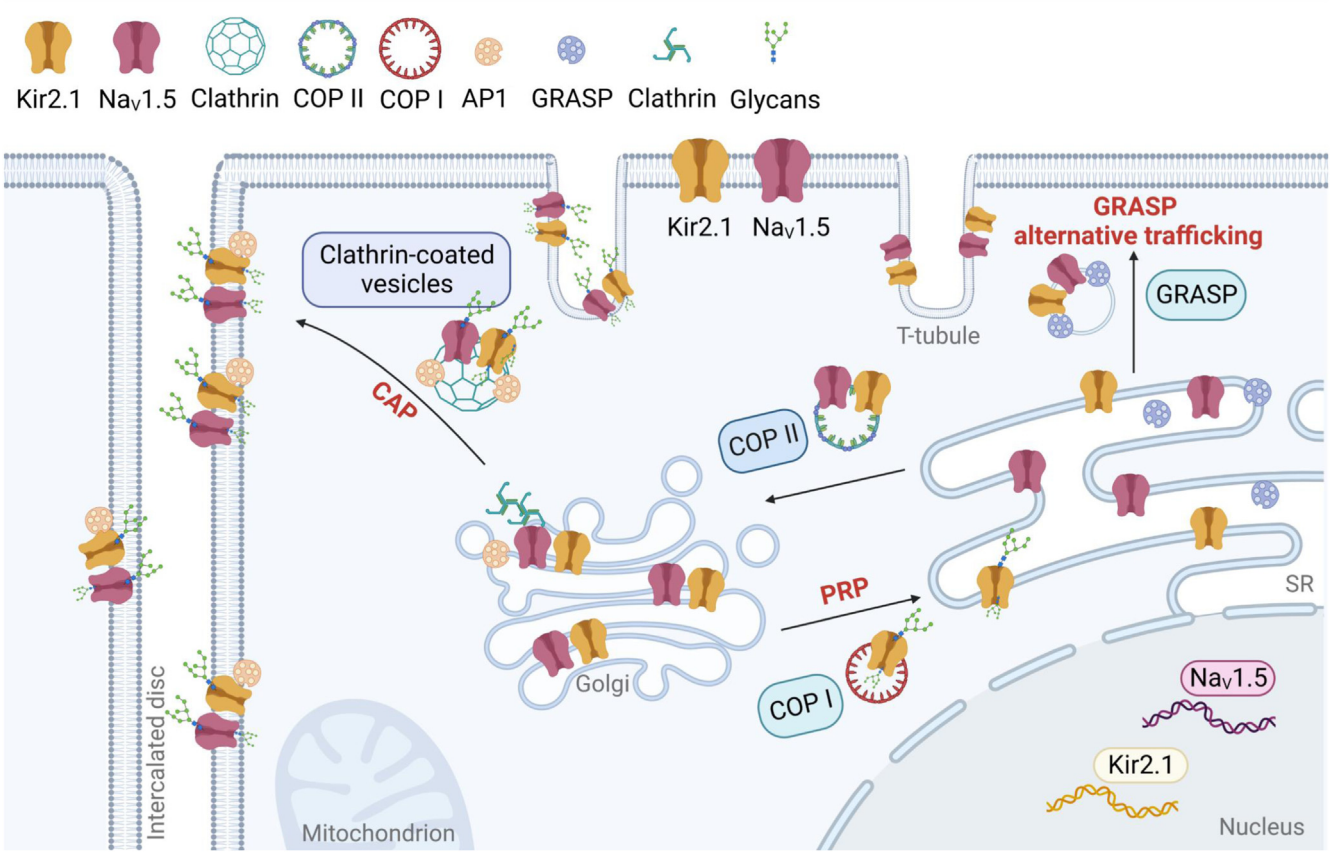

Figure 4
Kir2.1-$Na_V1.5$ channelosome trafficking pathways. The Kir2.1 and $Na_V1.5$ sequences are independently translocated to the sarcoplasmic reticulum (SR), where they are translated to membrane proteins. Following the canonical anterograde pathway (CAP), Kir2.1 and $Na_V1.5$ reach the Golgi network in Coat Protein Complex (COP) II–coated vesicles to be posttranslationally modified. Adaptin protein complex 1 (AP1) recognizes trafficking sequences of both channels and allows their packaging into clathrin-coated vesicles. From the Golgi, posttranslationally modified Kir2.1 channels may return to the SR following the potential retrograde pathway (PRP) to be functional there. A fraction of Kir2.1-$Na_V1.5$ channelosomes could get to the membrane following a Golgi reassembly stacking protein (GRASP)–mediated alternative traffic.

C.3. Kir2.1-Na$_V$1.5 channelosome dysfunction in inheritable cardiac diseases

SCD is a major health problem worldwide. It is often defined as unexpected death, usually caused by a cardiac arrhythmia, within less than 1 hour from the onset of symptoms, and without any prior condition that would seem lethal.[1,95] While by far SCD occurs most commonly in older patients with structural heart disease, inheritable cardiac diseases encompass an important proportion of SCD cases in individuals 40 years or younger. Although inheritable arrhythmogenic diseases are considered rare and with a low prevalence, the importance and concern arise from the complex diagnosis and the great potential to trigger life-threatening arrhythmias and SCD. As of 2012, more than 35 distinct genes encoding crucial cardiac ion channel pore-forming subunits or regulatory proteins had been suggested to be linked to ion channel diseases,[96] each one defining a different inherited cardiac electrical disorder. However, many of such associations have been questioned recently by *ClinGen Gene*-Disease Clinical Validity *curation* groups.[97–99]

It is often hard to associate a dysfunction of a given ion channel/protein with the specific arrhythmia phenotypes of channelopathies.[100–107] For example, in a recently published mouse model of ATS type 1 (ATS1) caused by a trafficking-deficient mutation (Δ314-315) in *KCNJ2*, cardiomyocytes proved to have not only reduced I_{K1} but also reduced I_{Na} and probably alterations in other membrane proteins because of the disruption of the Kir2.1-Na$_V$1.5 channelosome.[18] Similarly, trafficking-deficient Na$_V$1.5 mutations associated with BrS disturb trafficking and organization of associated proteins in the Kir2.1-Na$_V$1.5 complex.[89,90] In contrast, SQTS3 results from gain-of-function mutations in *KCNJ2*. Recently, it has been shown that certain SQTS3 mutations modify Kir2.1-Na$_V$1.5 interactions in a cardiac chamber–specific manner, resulting in different electrophysiological phenotypes in atrial, Purkinje, and ventricular cardiomyocytes. Finally, it has been shown that both Na$_V$1.5 and Kir2.1 associate with the dystrophin-associated protein complex via syntrophin.[108] Therefore, it seems reasonable to expect that the loss of dystrophin that occurs in patients with DMD should disrupt the Kir2.1-Na$_V$1.5 channelosome mediated by syntrophin and result in altered Na$_V$1.5 and Kir2.1 localization and function, potentially leading to arrhythmogenesis and SCD. In this section, we briefly review the Kir2.1-Na$_V$1.5 channelosome involvement in 4 inheritable cardiac diseases (Figure 5 and Table 2).

D. Short QT syndrome

Short QT syndrome (SQTS) is a rare and lethal arrhythmogenic disease characterized by an abnormally short QT interval on the electrocardiogram (ECG) in patients with structurally normal hearts.[109–111] The clinical presentation of the syndrome is diverse because of its variable expressivity even

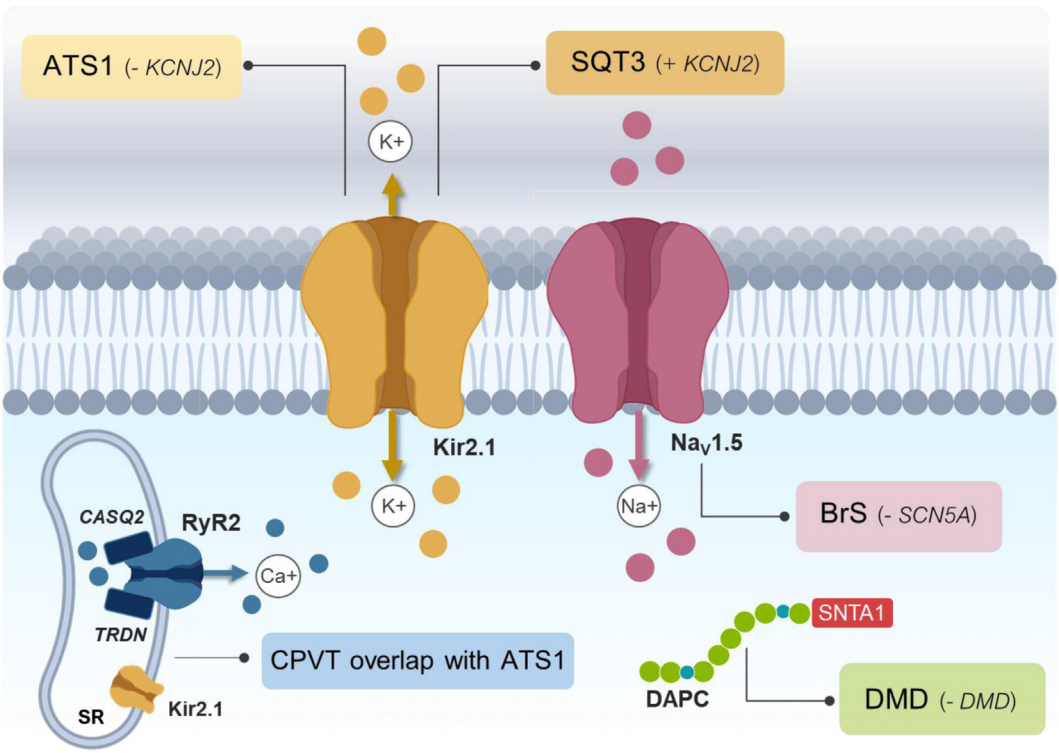

Figure 5
Principal inheritable cardiac diseases related to Kir2.1-Na$_V$1.5 channelosome dysfunction. Cartoon illustrating roles played by Kir2.1 and/or Na$_V$1.5 in the electrophysiological phenotypes in specific inheritable cardiac diseases. ATS1 = Andersen-Tawil syndrome type 1; BrS = Brugada syndrome; CASQ2 = calsequestrin 2; CPVT = catecholaminergic polymorphic ventricular tachycardia; DAPC = dystrophin-associated protein complex; DMD = Duchenne muscular dystrophy; RyR2 = ryanodine receptor type 2; SQTS3 = short QT syndrome type 3; SR = sarcoplasmic reticulum; TRDN = triadin.

Table 2 Kir2.1-$Na_V1.5$ channelosome dysfunction in inheritable cardiac diseases

Disease variant	Gene	Protein/functional effect	Kir2.1-$Na_V1.5$ relation
SQT3	*KCNJ2*	Kir2.1/gain of function	Differential chamber-specific effects on the Kir2.1-$Na_V1.5$ channelosome
ATS1	*KCNJ2*	Kir2.1/loss of function	Reciprocal reduction in I_{K1} and I_{Na} densities
BrS	*SCN5A*	$Na_V1.5$/loss of function	$Na_V1.5$ loss of function results in reduction in I_{K1}
DMD	*DMD*	DAPC/loss of function	Reduction in I_{K1} and I_{Na} densities

To date, 4 different inheritable cardiac diseases have been shown to be associated with Kir2.1-$Na_V1.5$ channelosome dysfunction.
ATS1 = Andersen-Tawil syndrome type 1; BrS = Brugada syndrome; DAPC = dystrophin-associated protein complex; DMD = Duchenne muscular dystrophy; I_{K1} = strong inward rectifier current; I_{Na} = sodium current; SQTS3 = short QT syndrome type 3.

among members of the same family. Nevertheless, in some patients, usually the first symptoms are cardiac arrest, palpitations, or syncope. Atrial fibrillation has been reported in patients with SQTS.[51] However, VT/VF and SCD are frequently the first manifestations, fatal events that may have a devastating impact on society.[112]

SQTS is considered a genetically heterogeneous disease with an autosomal dominant inheritance. Follow-up of patients with SQTS showed that only 20%–30% may be explained by identified mutations.[112] Contrary to LQTS, gain-of-function mutations in potassium channel genes (*KCNH2*, *KCNQ1*, and *KCNJ2*) enhance repolarization, resulting in the abnormal shortening of the cardiac AP defining SQTS (Figure 5 and Table 2).[97] Three genes encoding subunits of the L-type calcium channel (*CACN1Ac*, *CACNB2b*, and *CACNA2D1*) have also been linked to few cases of SQTS. However, these genes have been disputed for SQTS by a recently published ClinGen curation framework.[97] The gene encoding the cardiac chloride–bicarbonate exchanger AE3 (*SLC4A3*) has been related to SQTS cases in recent years.[113] Unfortunately, mechanisms and treatment strategies for SQTS are scarce since the number of patients described worldwide is too low to allow consensus.

We are generating mouse models of SQTS3 and have already characterized the arrhythmogenic consequences of the gain-of-function Kir2.1^{E299V} mutation in murine cardiomyocytes.[114] Compared with Kir2.1^{WT}, mice with SQTS3 reproduced the patients' electrical phenotype by having significantly abbreviated QT intervals and being highly inducible for atrial but not ventricular arrhythmias. In patch-clamp experiments, Kir2.1^{E299V} cardiomyocytes had increased I_{K1} density with loss of inward-going rectification, which led to extreme APD shortening. However, unlike mutant ventricular cardiomyocytes, atrial Kir2.1^{E299V} cardiomyocytes presented a reduced I_{K1} slope conductance.

In contrast, I_{Na} was unchanged in atrial Kir2.1^{E299V} cardiomyocytes but in ventricular myocytes the mutation increased excitability by shifting I_{Na} activation and inactivation in the hyperpolarizing direction.[114] Moreover, cardiac conduction system cells from Kir2.1^{E299V} mice manifested substantially higher I_{Na} density than WT cells, explaining the shortening in the PR interval. Therefore, the observed voltage shifts in I_{Na} activation and inactivation in ventricular cardiomyocytes, together with the higher I_{Na} density in the conduction system, increased excitability and protected the ventricles against arrhythmias, which explained in part why supraventricular arrhythmias were predominant in Kir2.1^{E299V} mice. Hence, unexpectedly, the same mutation differentially affected each of the cardiac chambers and cell types, which explains the atrial arrhythmia predisposition and absence of ventricular arrhythmias in the mouse and the patient with SQTS3 with the Kir2.1^{E299V} mutation.[114] Most likely, specific Kir2.1 interactors in each of the cardiac regions modify the electrical properties differently, which should explain the dissimilar outcomes with the same mutation. In this regard, some of the $Na_V1.5$ β subunits are also chamber specific and predictably modify sodium activation and inactivation in cardiomyocytes.[115–119] $Na_V1.5$ β2 and β4 are expressed mainly in ventricles.[120] The coexpression of Kir2.1^{E299V} and $Na_V1.5$ β4 in human embryonic kidney 293 cells that stably expressed $Na_V1.5$ partially reproduced the hyperpolarizing shift of the sodium activation curve seen in mutant ventricles.[114] However, further studies are needed to explore yet unidentified but possible Kir2.1 interactions with $Na_V1.5$ partner proteins that may explain these defects.

E. Andersen-Tawil syndrome

ATS1 is an autosomal dominant channelopathy predominantly characterized by loss-of-function mutations in the *KCNJ2* gene leading to reduced I_{K1} (Figure 5 and

Table 2).[121,122] The clinical features of ATS1 are characteristic of a real syndrome because they affect cardiomyocytes, skeletal muscle cells, and neurons, indicating the ubiquitous role of I_{K1} in all excitable cells. The full triad of clinical manifestations, including dysmorphias, intermittent paralysis, and cardiac arrhythmias, is present in 58%–78% of mutation-positive patients,[100] while 32%–81% manifest involvement of 2 of the 3 organ systems.[100,123] Skeletal abnormalities during development are a relevant characteristic of the ATS phenotype. Some typical manifestations are hypoplastic mandible, hypotelorism, low-set ears, or digit clinodactyly. These ATS-related bone alterations are mainly explained by osteoclasts expressing Kir2.1 in their plasma membrane,[124] which stabilizes the resting membrane potential and keeps the osteoclasts functioning. Importantly, when Kir2.1 is altered, the reduced potassium inward current in the osteoclasts causes an imbalance between osteoblastogenesis[125] and resorption.[126]

The high burden of ventricular arrhythmias in this syndrome raises concern that they may degenerate into lethal arrhythmias and SCD. Recent data from Mazzanti et al[127] reported a 9.3% incidence of SCD in ATS, whereas Delannoy et al[128] previously estimated a 2.3%. About 40% of patients with ATS1 show a phenotypic overlap with catecholaminergic polymorphic ventricular tachycardia, an inherited disease related to alterations in calcium channels and mutations associated with ryanodine receptor type 2.[129]

To date, 95 ATS1 mutations localized throughout the Kir2.1 structure have been reported.[27] A recent classification of ATS1 mutations according to their specific consequences describes that some mutations alter channel assembly, while others are trafficking deficient with Kir2.1 protein accumulation at the SR or Golgi. An example of trafficking-deficient mutation is Δ314-315,[39] which retains both Kir2.1 and Na$_V$1.5 at the Golgi, reducing both I_{K1} and I_{Na} and increasing VF inducibility in a mouse model.[18] However, the majority of mutant Kir2.1 channels reach the plasma membrane but fail to function correctly.[27] The principal mechanism underlying this situation is proposed to relate to binding defects with PIP_2. Mutations that localize in non-PIP_2 binding sites can disrupt the transduction of PIP_2 binding to channel opening, while mutations at PIP_2 binding sites can alter PIP_2 directly. A fourth category includes mutations with defects in 2 or more such mechanisms.[27]

Arrhythmia susceptibility in ATS1 is closely related to reduced Kir2.1 channel function leading to membrane depolarization and generation of spontaneous ectopic ventricular activity. Early studies selectively blocking I_{K1} of isolated Purkinje fibers resulted in AP prolongation and an increased frequency of abnormal spontaneous APs.[130] Other studies in isolated guinea pig ventricular cardiomyocytes with Kir2.1 dominant-negative viral transfection also exhibited QT prolongation and spontaneous discharges on the ECG.[131] Computer simulations testing the consequences of a reduced Kir2.1 scenario in human ventricular substrate showed that a greater reduction in I_{K1} also resulted in delayed afterdepolarizations–mediated triggering of spontaneous APs.[132] Interestingly, in those simulations, the fast component of the delayed rectifier current contribution was dramatically increased upon I_{K1} reduction.

The phenotypic defects of some ATS1 causative mutations have also been linked to an additional functional role of Kir2.1. Working with an ATS1 mouse model expressing trafficking-deficient mutation Kir2.1$^{\Delta 314\text{-}315}$, Macías et al[18] demonstrated that, in addition to the Kir2.1 channels forming channelosomes with Na$_V$1.5, a unique population of Kir2.1 channels localize intracellularly at the SR. Such SR Kir2.1 channels were shown to contribute countercurrent for sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase–mediated Ca^{2+} reuptake, helping to maintain Ca^{2+} homeostasis and e-c coupling (Figure 5 and Table 2). In WT myocytes, such a countercurrent, also observed in skeletal myocytes,[133] helps to maintain an SR Ca^{2+} equilibrium potential and prevent excessive Ca^{2+} release.[134,135] In sharp contrast, Kir2.1$^{\Delta 314\text{-}315}$ cardiomyocytes showed a prolonged recovery of SR calcium transients and multiple abnormal spontaneous calcium release events, suggesting that a slower Ca^{2+} reuptake would result in higher cytoplasmic Ca^{2+} lifetime and higher activity of NCX.[18] Altogether, the mutant SR Kir2.1 should promote a higher probability of spontaneous Ca^{2+}-induced Ca^{2+} release events and spontaneous APs, similarly to the CPVT scenario.[136] The results provided a robust mechanistic explanation for the overlap between arrhythmias produced by ATS1 and those associated with CPVT, including bidirectional tachycardia, polymorphic VT, and risk of SCD.[18]

F. Brugada syndrome

Loss-of-function mutations in *SCN5A* lead to diminished or defective Na$_V$1.5 proteins and cause BrS, an ion channel disease that also increases the risk of arrhythmias and SCD.[137] BrS is diagnosed by a characteristic ECG pattern of ST elevation in leads V_1–V_3 with a right bundle branch block configuration.[138] There may be evidence of slow conduction, particularly at the right ventricular outflow tract.[104] The differential expression of ion channels and regulatory subunits between the cardiac chambers, and even between regions in the same chamber (such as epicardium and endocardium), confers distinguishable depolarization and repolarization patterns that underlie the spatial effect of the BrS causative mutations.[105,139]

Other genes have been linked to cases of BrS, such as *SCN1Bb*, a gene encoding the Na$_V$ β1 subunit. Hu et al[63] showed that Na$_V$ β1 loss of function altered I_{Na} and transient outward current densities, demonstrating the functional and structural association between Na$_V$1.5 and K$_V$4.3. However, a recent evidence-based review article indicated that of 21 genes tested routinely in the clinic, only *SCN5A* has sufficient genetic evidence to support causality for BrS.[98] Many Na$_V$1.5 mutants fail to properly traffic to the sarcolemma or generate channels with gating defects, both resulting in reduced I_{Na} density. Some such mutations also produce a dominant-negative effect; that is, they decrease the expression/function of native subunits (Figure 5 and Table 2).

Recent studies have reported that dysfunction of channelosomes formed by Na$_V$1.5 and Kir2.1 may be an

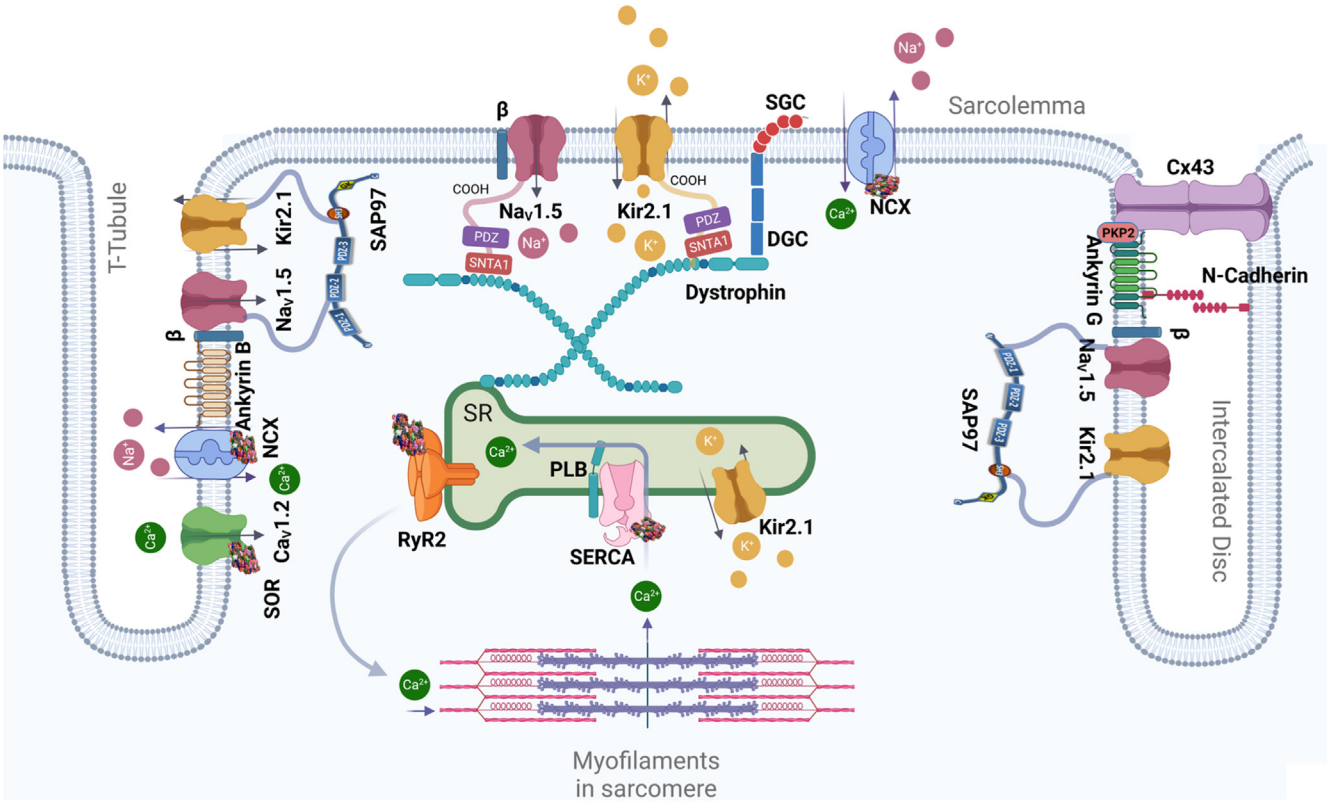

Figure 6
Macromolecular ion channel complexes control excitation and e-c coupling. Graphical illustration of different cardiac ion channels and their interacting proteins working in functional macrocomplexes placed at specific cellular regions. COOH = carboxyl; Cx43 = connexin-43; DGC = dystrophin glycoprotein complex; NCX = Na^+/Ca^{2+} exchanger; PDZ = postsynaptic density protein 95, *Drosophila* disc large tumor suppressor, and zonula occludens-1 protein; PKP2 = plakophilin-2 gene; PLB = phospholamban; RyR2 = ryanodine receptor type 2; SAP97 = synapse associated protein 97; SERCA = sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; SGC = sarcoglycan complex; *SNTA1* = α1-syntrophin gene; SOR = sorcin; SR = sarcoplasmic reticulum.

arrhythmogenic mechanism in BrS.[90] As discussed above, the functional interaction between I_{Na} and I_{K1} is reinforced by positive and reciprocal modulation between the $Na_V1.5$ and Kir2.1/2.2 channels in the control of ventricular excitability. Results obtained using mouse models of *SCN5A* haploinsufficiency and overexpression of native and mutated $Na_V1.5$ channels in heterologous expression systems, and hiPSC-CMs demonstrated that SR trafficking–defective mutant $Na_V1.5$ channels significantly reduced both I_{Na} and I_{K1} densities.[89] Furthermore, Golgi trafficking defective $Na_V1.5$ mutant channels produced a dominant-negative effect on Kir2.1/2.2 and therefore further reduced I_{K1}. However, defective I_{Na} currents produced by $Na_V1.5$ mutant channels that disrupt trafficking from the SR can be partially rescued by Kir2.1/2.2 channels via the unconventional secretory pathway involving Golgi reassembly stacking proteins. Therefore, cardiac excitability would be greatly affected in patients harboring $Na_V1.5$ trafficking-deficient mutations, since these mutants can concomitantly trap Kir2.1/2.2 channels, unexpectedly decreasing I_{K1} in addition to I_{Na}.[90]

G. Duchenne muscular dystrophy

DMD is caused by null mutations in the Dp427 isoform of the *dystrophin* gene.[140] It is an X-linked recessive disease that primarily affects adolescent males, causing progressive skeletal muscle deterioration, with negative effects in the central nervous system.[141] DMD is also characterized by cardiac muscle impairment,[142] which usually starts with ECG abnormalities,[143] but the disease progresses and dilated cardiomyopathy, arrhythmias, and congestive heart failure are the most important life-limiting signs. Most patients with DMD will develop cardiomyopathy by 20 years of age. DMD is caused by mutations in dystrophin, a protein that provides structural support for the myocyte by its interaction with a glycoprotein complex spanning the muscle sarcolemma, effectively linking the actin cytoskeleton to the extracellular matrix (Figure 5 and Table 2). The complex also helps to control the function of ion channels at the cardiomyocyte membrane, particularly the lateral membrane and is therefore important in maintaining normal cardiac electrical function. Consequently, its defects are not only restricted to the skeletal muscle but also affect the cardiac contractility and electrophysiology, and many patients with DMD are at high risk of arrhythmia and SCD, which contributes considerably to the morbidity and mortality of the affected patients.[144] However, the mechanisms are poorly understood and management of DMD diagnosis and prevention of arrhythmia are challenging in patients with DMD.[145]

Several ion channels are part of the DPC, including Kir2.1 and $Na_V1.5$,[75] as has been demonstrated in the transgenic DMD mouse model (*mdx*), where dystrophin mutation causes

a relevant cardiac reduction in both I_{K1} and I_{Na}.[75,146] Recently, Jimenez-Vazquez et al[147] used matured ventricular-like hiPSC-CMs derived from 2 genetically distinct hemizygous DMD males, a heterozygous DMD female and 2 unrelated healthy subjects (controls) to investigate the mechanisms underlying the arrhythmias associated with loss-of-function dystrophin mutations.[147] Their results showed that hiPSC-CMs from patients with DMD cardiomyopathy had a dysfunction of the Kir2.1-Na$_V$1.5 channelosome with a reduction in both I_{Na} and I_{K1}, reduced excitability, slow conduction, and reduced contractility. As such, DMD hiPSC-CMs recapitulated the complex patterns of reentrant arrhythmias seen in patients.[147] Most important, both conduction defects and arrhythmias were restored by α1-syntrophin overexpression, which proved that the underlying mechanisms resided in dysfunction of the Na$_V$1.5–α1-syntrophin–Kir2.1 channelosome, which reduced the cardiac excitability and conduction, predisposing patients to life-threatening arrhythmias and SCD.[147]

Future perspectives

Inherited cardiac channelopathies present with particular pleiotropic phenotypes and cardiac electrical alterations that often overlap with other arrhythmogenic diseases. It is completely accepted and demonstrated that most proteins work in macromolecular complexes.[11] In these macromolecular complexes, proteins interact to form functional units that control essential processes for cardiac physiology, such as excitation and e-c coupling (Figure 6). Increasingly, scientists are beginning to decipher the global scenario of interactors of the main cardiac ion channels and their relation with inherited channelopathies. These studies reinforce the idea of proteins working in macromolecular complexes where they might interact synergistically in some cases, antagonistically in others, contributing to arrhythmic phenotypes and potential overlaps among different arrhythmogenic diseases. It is therefore urgent to understand these complex syndromes as entities that affect ion channels and their interactomes at different stages of their life cycle. Given the complexity of and lack of effective treatments of such potentially lethal diseases, it is highly relevant that research should focus on the identification of specific mechanisms underlying arrhythmias associated with dysfunction of cardiac ion channel complexes; such is the case of Kir2.1-Na$_V$1.5 channelosomes.

Clearly, many more studies are needed to establish new paradigms of cardiac electrophysiology integrating the large diversity of molecular interactions involved in the formation and regulation of cardiac ion channels as well as their function. Progress likely will come from the development of new tools to study protein processing, assembly, and interactions in native myocytes from animal models as well as in hiPSC-CMs. Such knowledge, together with collaboration among fundamental and clinical scientists, and the development of novel strategies for improved risk stratifications are vital to move the field of inheritable arrhythmogenic diseases forward. Such strategies should consider the molecular underpinnings that link each mutation to its specific arrhythmic consequences toward the generation of more effective, mechanistically based therapies to the benefit of patients worldwide.

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Address reprint requests and correspondence: Dr José Jalife, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Calle de Melchor Fernández Almagro, 3, Fuencarral-El Pardo, 28029 Madrid, Spain. E-mail address: jjalife@cnic.es

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