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Mechanisms of Human Arrhythmia Syndromes: Abnormal Cardiac Macromolecular Interactions

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Many cardiac ion channels exist within macromolecular signaling complexes, comprised of pore-forming subunits that associate with auxiliary subunits, regulatory enzymes, and targeting proteins. This complex protein assembly ensures proper modulation of channel activity and ion homeostasis. The association of genetic defects in regulatory and targeting proteins to inherited arrhythmia syndromes has led to a better understanding of the critical role these proteins play in ion channel modulation.

Ion channels are a major class of pore-forming proteins that allow the movement of ions down their electrochemical gradient, which is essential for a wide variety of basic physiological processes, including cardiac excitation-contraction coupling. A characteristic feature of most ion channels is that they do not always conduct ions. Rather, the passage of ions is dynamically modulated by voltage, mechanical force, second messengers, or channel-associated regulatory proteins (34).

Most ion channels are multi-subunit complexes, involving an arrangement of proteins closely packed around a water-filled pore that extends through the plane of the membrane (50). The principal, pore-forming subunits are known as the α-subunits, whereas auxiliary subunits are denoted β, γ, etc. Biochemical purification experiments have revealed such multi-subunit complexes in purified preparations of voltage-gated sodium (Na^+) (32), potassium (K^+) (73), and calcium (Ca^{2+}) channels (31). Another example includes the intracellular Ca^{2+} release channel, known as the ryanodine receptor (RyR2), which is one of the largest ion channel complexes in the heart (combined molecular weight >4 MDa) (104).

Ion channels can also associate with and be regulated by intracellular signaling proteins. For example, most ion channels are substrates for protein kinases and phosphatases, which in many cases are integral components of the channel macromolecular complex (57, 59). Direct targeting of these enzymes to channel complexes allows for rapid and localized regulation of channel activity by phosphorylation and dephosphorylation (103). Specialized anchoring proteins target enzymes to particular ion channels or to restricted subcellular microdomains within cardiac myocytes (18, 58). Recent studies show that specific auxiliary or anchoring proteins make important contributions to ion channel regulation, and in some cases they even act as signaling proteins themselves (13).

During the past few years, it has become clear that abnormal intermolecular interactions within ion channel macromolecular complexes may underlie abnormal channel gating, which in turn leads to cardiac arrhythmias and sudden death. These findings have led to an expanded view of cardiac "channelopathies." This review focuses on several cardiac macromolecular assemblies implicated in the pathogenesis of cardiac arrhythmia syndromes.

Inherited Cardiac Arrhythmia Syndromes

Lethal cardiac arrhythmias in individuals with structurally normal heart are often caused by variants in genes that encode cardiac ion channel α- and β-subunits (102). Common inherited arrhythmia syndromes include the congenital long QT syndrome (LQTS), Brugada syndrome (BrS), short QT syndrome (SQT), and catecholaminergic polymorphic ventricular tachycardia (CPVT) (76). Moreover, inherited mutations in these ion channel subunits may underlie a significant proportion of sudden infant death syndrome (SIDS) cases (93). The physiological consequences of mutations in α- and β-subunits associated with these arrhythmia syndromes have been extensively reviewed in other papers (69, 82).

Recent genetic studies have revealed that inherited mutations in genes encoding additional regulatory and targeting proteins associated with ion channels may also cause cardiac arrhythmias (67, 96). The physiological consequences of inherited mutations in these signaling and targeting proteins may include abnormal channel localization within a cellular microdomain or ion channel-gating defects due to allosteric effects. Recent studies suggest that defective protein-protein interactions within cardiac macromolecular channel assemblies may also contribute to the pathogenesis of arrhythmias in patients with structural heart disease (e.g., cardiomyopathy and heart failure). Therefore, we will also discuss one such example, namely acquired defects in the ryanodine receptor/intracellular Ca^{2+} release channel complex (46, 99).

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Ankyrin Dysfunction in Patients with Cardiac Arrhythmias

Ankyrins are responsible for targeting integral membrane proteins in a host of tissues. Higher vertebrates express three ankyrin gene products termed ankyrin-R (encoded by *ANK1*), ankyrin-B (*ANK2*), and ankyrin-G (*ANK3*). In the heart, all three ankyrin gene products are expressed (12, 25, 41, 49, 51, 66, 70, 95). Although structurally similar, ankyrin polypeptides have nonoverlapping cellular functions (7). In the past 5 years, findings in both humans and mice have illustrated a critical role for ankyrin function in heart. Dysfunction in ankyrin-B has been linked with defective intracellular Ca^{2+} regulation and human arrhythmia (type 4 long QT syndrome or "ankyrin-B syndrome"). Additionally, human gene variants that disrupt ankyrin-G interactions with voltage-gated Na^+ channel have been associated with human Brugada syndrome.

Mutations in ankyrin-B cause type 4 long QT syndrome

Nearly a dozen years ago, Schott and colleagues identified the fourth locus for the congenital long QT syndrome (LQT4) (87). Affected family members displayed an atypical form of the LQTS, with an uncommon T-wave morphology, sinus node bradycardia, and atrial fibrillation (87). Moreover, certain family members displayed stress- or exercise-induced sudden cardiac death (87). Using linkage analysis, LQT4 was associated with an 18 cM region on chromosome 4q25–27 (87). Sequencing of the *ANK2* gene revealed a common missense variant in exon 36 (A4274G), resulting in the substitution of a glutamic acid for a glycine at ankyrin-B residue 1425 (E1425G) (67). The single variant co-segregated with the LQT phenotype in 22 of 24 individuals, and with sinus node dysfunction in 23 of 24 individuals (67). This variant was not found in unaffected family members or in a large panel of control individuals. Thus autosomal-dominant mutations in the gene encoding ankyrin-B were linked to LQT4.

Since the discovery of ankyrin-B variant E1425G, a number of additional *ANK2* human gene variants have been identified (55, 65, 68, 90, 112). Patients harboring *ANK2* variants display sinus node dysfunction, atrial fibrillation, conduction defects, and/or polymorphic ventricular arrhythmias (65, 67, 68). However, unlike E1425G carriers, QT interval prolongation was not a consistent feature in carriers of these *ANK2* nucleotide variants (65, 67, 68). The phenotypic differences in patients likely reflect the severity of the molecular defects associated with the E1425G variant in ventricular myocytes. In fact, recent comparative analyses of all *ANK2* loss-of-function variants revealed that three variants (E1425G, V1516D, and R1788W) exhibited severe cellular phenotypes (65). Consistent with these findings, the same variants were associated with the most severe clinical phenotypes (65). In contrast, *ANK2* variants with minor loss-of-function phenotypes in cardiomyocytes are associated with less severe clinical phenotypes. The low frequency at which these mutations with milder phenotypes have been detected may suggest that many patients remain undetected (65, 90).

Mice that are haploinsufficient in ankyrin-B (ankyrin-$B^{+/-}$) constitute an excellent animal model to study the effects of autosomal-dominant loss-of-function mutations in *ANK2*. Ankyrin-$B^{+/-}$ mice are viable (ankyrin-B-deficient mice die shortly after birth) (89) and share a number of common phenotypes with the original human LQT4 kindred (with the E1425G mutation). Specifically, ankyrin-$B^{+/-}$ mice display sinus bradycardia, conduction defects, and catecholamine-induced polymorphic ventricular arrhythmia associated with syncope and/or death (67). Similar to LQT4 individuals, ankyrin-$B^{+/-}$ mice have structurally normal hearts (67).

Primary cardiac myocytes isolated from ankyrin-$B^{+/-}$ mice were used to demonstrate that the E1425G variant was a loss-of-function variant of ankyrin-B. Neonatal ankyrin-$B^{+/-}$ cardiomyocytes display reduced spontaneous contraction rates, abnormal Ca^{2+} transients, and aberrant localization of the Na/Ca exchanger (NCX) (64, 67, 95). Exogenous expression of ankyrin-B cDNA was sufficient to restore myocyte phenotypes (64, 67, 95). On the other hand,

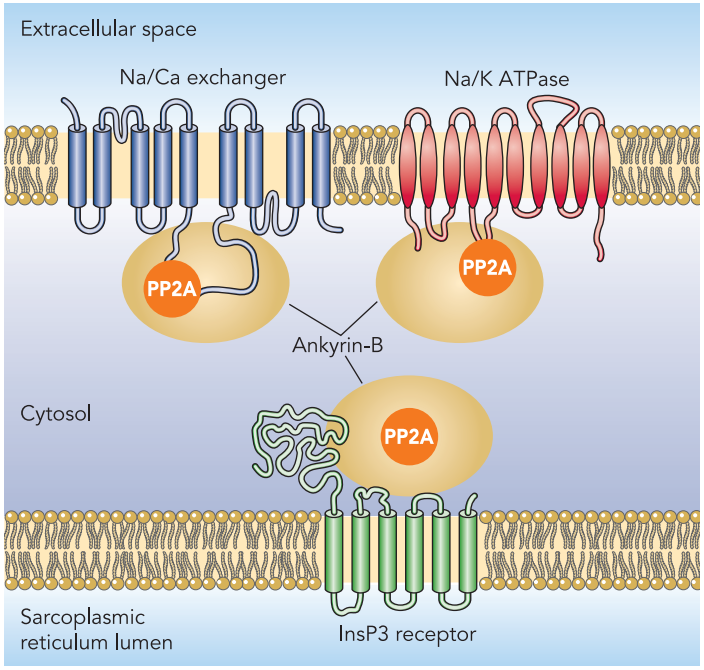

FIGURE 1. **Model of ankyrin-B protein complexes in the cardiac myocyte**
Ankyrin-B is a multivalent adapter protein that directly links structurally unrelated cardiac ion channels and transporters including Na^+-K^+ ATPase, Na^+/Ca^{2+} exchanger, and $InsP_3$ receptor with cardiac cytoskeletal components. Ankyrin-B was also recently implicated in targeting of protein phosphatase 2A (PP2A). Dysfunction in ankyrin-B-dependent pathways results in abnormal ion channel/transporter targeting to specialized cardiac membrane domains and electrical instability.

overexpression of ankyrin-B mutant E1425G in ankyrin-B$^{+/-}$ myocytes was unable to restore abnormal phenotypes, even though the mutant protein was properly synthesized and localized within the cardiomyocyte (67). Based on the role of ankyrins in other tissues, ankyrin-B loss-of-function was hypothesized to affect the targeting of cardiac ion channels.

Evaluation of ankyrin-B$^{+/-}$ mouse cardiac lysates revealed that the Na^+/Ca^{2+} exchanger (NCX), Na^+-K^+-ATPase alpha 1 and 2 (Na^+ pump isoforms), and inositol 1,4,5 trisphosphate receptor ($InsP_3$ receptor) protein levels are significantly reduced (67). Moreover, NCX and Na^+ pump levels are preferentially reduced over transverse tubule (T-tubule) membrane sites in ankyrin-B$^{+/-}$ cardiomyocytes (67). The reduction in channel and transporter levels is most likely a result of posttranslational effects, since Northern analysis revealed no change in channel/transporter mRNA levels (67). Subsequent biochemical analyses revealed that ankyrin-B directly associates with NCX, Na^+ pump, and $InsP_3$ receptor (FIGURE 1) (62). Moreover, overexpression of ankyrin-B in cardiomyocytes with reduced ankyrin expression restored NCX, $InsP_3$ receptor, and Na pump levels to normal (16, 62-64, 67, 68). These data support the current hypothesized role of ankyrin-B as a cellular chaperone for targeting of specific ion channels/transporters at the myocyte T-tubule/sarcoplasmic reticulum (SR) membranes.

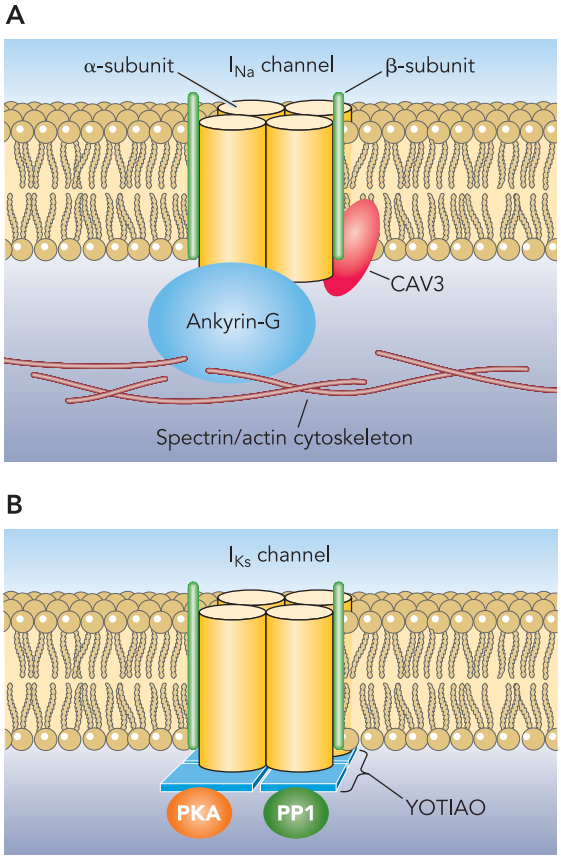

FIGURE 2. **Model of proposed interactions in cardiac Na^+ and K^+ channel complexes**
A: $Na_v1.5$ channel complex consist of *SCN5A*-encoded pore-forming α-subunits (yellow), associated with one or more β-subunits (green). Ankyrin-G (blue) has recently been implicated in targeting $Na_v1.5$ to the cardiomyocyte intercalated disc membrane domains. Caveolin-3 (CAV3) also associates with the cardiac Nav1.5 protein complex and is speculated to compartmentalize $Na_v1.5$ with signaling proteins in membrane microdomains termed "caveolae." *B*: the I_{Ks} channel complex consists of *KCNQ1*-encoded α-subunits (yellow) and *KCNE1*-encoded β-subunits (green). The targeting protein yotiao anchors protein kinase A (PKA) and protein phosphatase (PP1) to the channel complex, which is a requirement for K^+ current regulation by β-adrenergic signaling.

At the level of the single myocyte, loss of ankyrin-B-dependent targeting of NCX and Na^+ pump isoforms mimics the activity of cardiac glycosides (8, 81). Specifically, isolated ankyrin-B$^{+/-}$ cardiomyocytes display increased SR Ca^{2+} load and elevated SR Ca^{2+} transients (67). Consistent with findings in LQT4 patients and the phenotype of ankyrin-B$^{+/-}$ mice, ankyrin-B$^{+/-}$ cardiomyocytes display normal action potentials at rest (67). However, exercise or stress induces QT interval prolongation in ankyrin-B$^{+/-}$ mice, consistent with the induction of cellular after-depolarizations by isoproterenol in ankyrin-B$^{+/-}$ myocytes (67). Therefore, reduced expression or abnormal function of ankyrin-B acts as a substrate for human arrhythmia susceptibility due to abnormal myocyte electrical stability.

Defects in ankyrin-G-based pathways cause Brugada syndrome

For nearly a decade, ankyrin-G has been implicated in the trafficking of voltage-gated Na^+ channel isoforms in the central nervous system (6). Ankyrin-G is co-expressed with neuronal voltage-gated Na^+ channel isoforms ($Na_v1.2$, $Na_v1.6$) at specialized membrane domains, including axon initial segments, nodes of Ranvier, and at the neuromuscular junction (21, 41, 42, 106). Moreover, ankyrin-G directly associates with neuronal voltage-gated Na^+ channels (FIGURE 2A) (17, 41). In 2003, two independent groups identified the ankyrin-G-binding sequence (ABS) in a cytoplasmic loop that connects $Na_v1.2$ domain II and III (28, 48). This nine-residue motif is conserved across species and is found in most Na^+ channel gene products, including $Na_v1.1$, $Na_v1.2$, $Na_v1.4$, $Na_v1.5$, and $Na_v1.6$. Based on the role for ankyrin-G in Na^+ channel targeting in the nervous system and conserved ankyrin-binding sequence between $Na_v1.2$ and the primary cardiac Na^+ channel isoform ($Na_v1.5$), Mohler and colleagues (66) hypothesized that this ankyrin-G pathway was conserved in excitable cells. Immunoblots and immunostaining demonstrated that ankyrin-G was expressed in vertebrate heart and was concentrated at cardiomyocyte membrane domains enriched with $Na_v1.5$ (66). $Na_v1.5$ and ankyrin-G directly associated using purified proteins and in co-immunoprecipitation assays, and deletion of the ABS renders $Na_v1.5$ unable to bind ankyrin-G (66).

In 2004, Priori and colleagues screened a large cohort of patients with Brugada syndrome (a

syndrome characterized by precordial ST segment elevation, right bundle branch block, and fatal cardiac arrhythmias) for mutations in the ABS domain of the *SCN5A* gene, which encodes the $Na_v1.5$ channel (66). A single *SCN5A* missense variant was identified, which resulted in the substitution of a highly conserved glutamic acid with a lysine in the $Na_v1.5$ ABS (E1053K). Subsequent biochemical analysis revealed that $Na_v1.5$ E1053K was unable to associate with ankyrin-G (66). Moreover, $Na_v1.5$ E1053K was not properly targeted to the membrane surface of adult cardiomyocytes (66). These data strongly implicate an ankyrin-G-based pathway for proper $Na_v1.5$ trafficking in cardiomyocytes. Furthermore, they further support a critical role for the ankyrin family of polypeptides in the regulation of normal cardiac rhythm.

Caveolin-3 Variants are Associated with Human Arrhythmia

Another example of an inherited cardiac arrhythmia syndrome caused by mutations in an ion channel-anchoring protein is long QT syndrome type 9 (LQT9), which is associated with genetic variants in the gene encoding caveolin-3 (96). Caveolins are the primary coat proteins required for the assembly of ~50- to 100-nm flask- or spherical-shaped sphingolipid-/cholesterol-rich plasma membrane invaginations termed caveolae ("little caves") (91). In vertebrates, caveolae activity is associated with a number of cellular processes, including vesicular transport (transcytosis, endocytosis), cholesterol homeostasis, compartmentalized cell signaling, and tumorigenesis (reviewed in Ref. 78). Three unique genes (*CAV1*, *CAV2*, *CAV3*) encode the ~21-kDa polypeptides named caveolin 1, 2, and 3. Although caveolin-1 and -2 are expressed in most tissues, caveolin-3 is primarily expressed in muscle tissue, including heart, skeletal and smooth muscle, and diaphragm (92).

In cardiac tissue, caveolae have been associated with a number of critical ion channels, transporters, and receptors, as well as key signaling molecules. Specifically, the primary voltage-gated Na^+ channel ($Na_v1.5$) (FIGURE 2A), the voltage-dependent K^+ channel $K_v1.5$, HCN4 pacemaker channels, the Na^+/Ca^{2+} exchanger, $InsP_3$ receptor, plasma membrane Ca^{2+} ATPase, TRP channels, and the L-type Ca^{2+} channel ($Ca_v1.2$) have been localized with caveolae (or caveolin) in heart (3, 9, 10, 23, 24, 54, 56, 94, 111). Additional signaling proteins localized to caveolae include β_2-adrenergic receptor, PP2A, PKA (RII), $G_{\alpha s}$, eNOS, protein kinase C, and adenylyl cyclase (2, 5, 15, 71, 83, 84, 108). Thus caveolin serves both to compartmentalize and to regulate ion channel function and associated intracellular signaling pathways.

Findings in both humans and mice demonstrate the importance of caveolin-3 for normal vertebrate muscle organization and function. Mice lacking caveolin-3 display skeletal muscle myopathy (26, 33), mild to moderate cardiomyopathy (107), and abnormal transverse-tubule organization (26, 97). These findings in mice are paralleled by human muscle disease associated with CAV3 variants. Specifically, CAV3 dominant-negative variants are associated with human limb-girdle muscular dystrophy (LGMD-1C) (27, 61). Additional human CAV3 variants are associated with rippling muscle disease, distal myopathy, and hyperCKemia (107). Elevated caveolin-3 expression is also associated with human Duchenne muscular dystrophy (80).

Recent findings have linked dysfunction in caveolin-3 with human ventricular arrhythmias and sudden cardiac death. In 2006, Vatta and colleagues identified four CAV3 nucleotide variants in a large cohort of LQTS patients (96). Probands with CAV3 variants displayed a variety of cardiac phenotypes, including nonexertional syncope, sinus bradycardia, and prolonged QT_c intervals (96). In agreement with previous findings by Yarbrough et al. in rat (111), Vatta and colleagues demonstrated that caveolin-3 and $Na_v1.5$ were co-localized in human heart and were associated in co-immunoprecipitation assays (96). Co-expression of recombinant $Na_v1.5$ channels with human caveolin-3 mutants in HEK293 cells resulted in a two- to threefold increase in late (sustained) Na^+ current (96) that likely contributes to QT prolongation and cardiac arrhythmias in carriers of CAV3 mutations. Since CAV3 mutations did not result in a loss of association between $Na_v1.5$ and caveolin-3 in co-immunoprecipitation experiments, it has been proposed that abnormal subcellular targeting may not be the mechanism for the increase in late Na^+ current. It remains to be determined whether genetic mutations in caveolin-3 allosterically affect $Na_v1.5$ channels or whether CAV3 variants cause cardiac dysfunction by altering the localization of other critical ion channels and transporters to the sarcolemma. Nonetheless, these exciting findings (along with recent ankyrin studies) clearly demonstrate that targeting proteins that associate with ion channel macromolecular complexes are essential for normal cardiac function. In further support of these findings, two recent reports demonstrate an association between human CAV3 variants and sudden infant death syndrome (1, 14).

Yotiao-Based Signaling Complex is Required for I_{ks} Modulation

Inherited channelopathies associated with cardiac arrhythmias may also result from defective interactions between pore-forming and regulatory (targeting) subunits in the macromolecular channel complex. These regulatory subunits include channel subunits referred to by β, γ, δ, but also protein kinases, phosphatases, and anchoring proteins. A well studied example includes the channel complex conducting the I_{KS} current, which is a slowly activating and

deactivating potassium current essential for normal cardiac repolarization (36). In the heart, expression of both *KCNQ1* (α-subunit) and *KCNE1* (*minK*, β-subunit) are required to form a functional I_{KS} channel complex (4, 85). Humans heterozygous for gene variants in *KCNQ1* or *KCNE1* may develop type 1 or type 5 long QT syndrome (LQT1, LQT5), respectively. In these patients, arrhythmias and sudden cardiac death occur typically in the face of stimulation of the sympathetic nervous system (88).

I_{KS} activity is highly regulated by the activity of protein kinase A (PKA). PKA stimulation via β-adrenergic agonists increases I_{KS} current amplitude to hasten the repolarization of the cardiac action potential (53, 98). In 2002, Marx and colleagues identified yotiao as an essential targeting protein for the I_{KS} channel complex in heart (57). Previous findings in brain demonstrated that yotiao is an A-kinase anchoring protein (AKAP) (20, 105). In addition to scaffolding PKA near critical membrane effector proteins (e.g., KCNQ1), yotiao is a binding partner for the protein phosphatase PP1 (104). Consistent with these findings, Marx and colleagues demonstrated that the cardiac I_{KS}/yotiao protein complex associated with both the regulatory subunit of PKA and PP1 (FIGURE 2B) (57). It was further demonstrated that normal regulation of I_{KS} required yotiao-associated activity (57). Moreover, Kurokawa et al. (43, 44) revealed that, in addition to forming a channel scaffolding platform for I_{KS} subunits, yotiao/I_{KS} interactions directly regulate the I_{KS} channel phosphorylation state and channel gating.

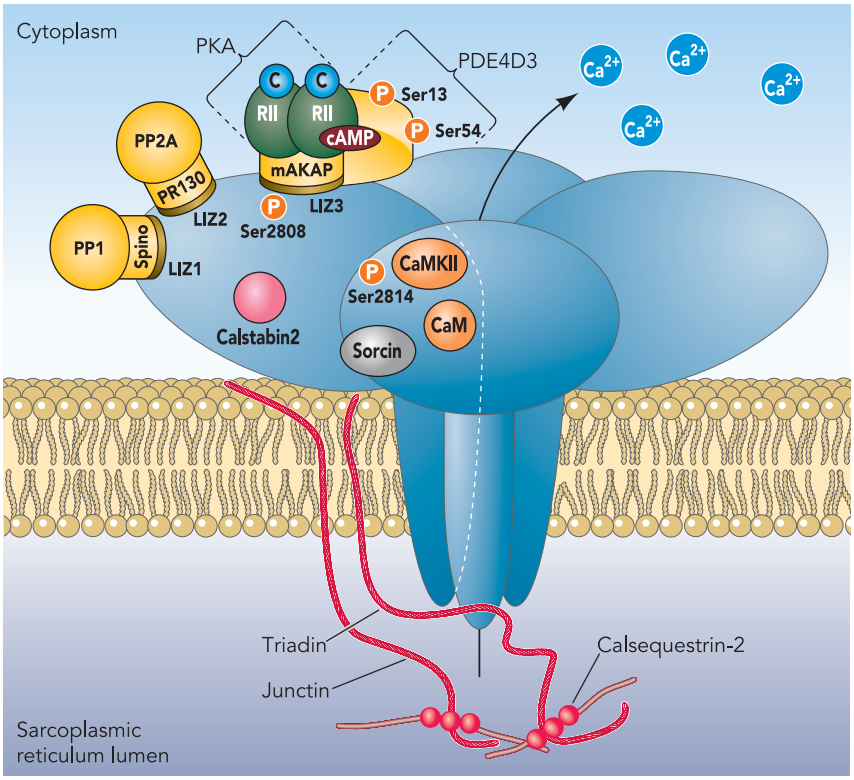

FIGURE 3. **The ryanodine receptor macromolecular signaling complex**
Four RyR2 monomers (*left*) contribute to the tetrameric Ca^{2+} release channel macromolecular complex. Regulatory proteins and enzymes associate with the large cytoplasmic RyR2 domains protruding into the cytosolic space. Calmodulin (CaM), FKBP12.6 (calstabin2), and sorcin are thought to bind directly to the RyR2 monomers, whereas binding of other subunits is mediated by specific targeting proteins. Leucine-isoleucine zipper (LIZ) motifs on RyR2 bind to corresponding LIZ peptides in anchoring proteins spinophilin (targeting PP1), PR130 (targeting PP2A), and mAKAP (targeting PKA and PDE4D3). The mechanism for CaMKII binding to RyR2 is currently unknown. Triadin and junctin interact with RyR2 on the luminal side of the channel complex.

In 2001, Piippo et al. (72) identified a missense variant in *KCNQ1* resulting in the substitution of a glycine for an aspartic acid in the COOH-terminal region of KCNQ1 (G589D) associated with LQT1. Large-population analyses revealed that this single variant was responsible for ~30% of Finnish cases of LQTS (likely due to founder effects) (22, 72). Further analyses of carriers of the *KCNQ1* G589D variant revealed a high prevalence of arrhythmia associated with exercise (72). Electrophysiological analysis demonstrated that the G589D mutation displayed a significantly increased threshold of activation compared with wild-type channels, and the expression efficiency of functional channels was <20% compared with wild-type channels (72).

In a set of elegant experiments, Kass and colleagues demonstrated that the *KCNQ1* G589D variant abolished the interaction between the AKAP yotiao and the I_{KS} channel complex (57). Furthermore, the authors demonstrated that, due to an inability of *KCNQ1* G589D to interact with the yotiao/PKA/PP1 complex, the I_{KS} complex was insensitive to β-adrenergic regulation (57). Subsequent modeling of this mutation by Saucerman et al. supported these findings (86). Specifically, in silico modeling indicated that the *KCNQ1* variant was unlikely to cause QT instability (prolongation) at rest (86). However, this variant, in combination with increased sympathetic activity, could result in QT_c prolongation and extrasystoles (86). Together, these data suggest that the *KCNQ1* accessory protein yotiao is required for normal sympathetic regulation of human cardiac electrical activity. Furthermore, these data suggest that human gene variants that affect the yotiao/I_{KS} channel complex are a likely cause of potentially fatal human arrhythmias.

The Ryanodine Receptor Macromolecular Complex

Genetic mutations are responsible for a relatively small fraction of all ventricular arrhythmias in the general population. Ventricular arrhythmias occur more commonly in patients with structural heart diseases, such as congenital heart failure (74). Recent studies have demonstrated acquired defects in the ryanodine receptor (RyR2) macromolecular complex (FIGURE 3) that could each by themselves, or by acting together, increase the likelihood of abnormal channel gating and cardiac arrhythmias (103).

Ryanodine receptors are critically involved in the regulation of cardiac excitation-contraction coupling, during which opening of plasmalemmal L-type Ca^{2+}

channels triggers a much greater release of Ca^{2+} from the sarcoplasmic reticulum (SR). Each channel complex consist of large tetramers of RyR2 monomers, each comprised of a large regulatory domain protruding into the cytosol and a much smaller transmembrane domain containing the channel pore (19). It is now well accepted that RyR2 channels exist as large macromolecular complexes, comprised of numerous regulatory subunits including calmodulin (CaM), the FK506-binding protein FKBP12.6 (also known as calstabin2), protein kinase A (PKA), Ca^{2+}/calmodulin-dependent kinase II (CaMKII), protein phosphatases 1 and 2A (PP1, PP2A), phosphodiesterase (PDE4D3), junctin, triadin, and calsequestrin (103) (FIGURE 3). Gating behavior of RyR2 channels can be regulated by many of these accessory proteins (103, 110).

In 2001, inherited mutations in RyR2 were linked to an autosomal-dominant form of inherited cardiac arrhythmias, known as catecholaminergic polymorphic ventricular tachycardia (CPVT) (45, 77). Single-channel recordings of CPVT-mutant RyR2 channels in planar lipid bilayers (37–39, 47, 99) revealed gain-of-function defects following PKA phosphorylation (47, 99) or caffeine stimulation (30, 40). Moreover, it has been demonstrated that CPVT-associated variants sensitize RyR2 to activation by cytosolic Ca^{2+} (39, 99) and delay Ca^{2+}-dependent channel inactivation (60). Alternative mechanisms for diastolic SR Ca^{2+} leak through CPVT mutant RyR2 have been proposed and reviewed elsewhere (30, 37). Further support for a gain-of-function defect in RyR2 in CPVT was obtained in cardiomyocytes isolated from knock-in mice heterozygous for the CPVT-associated mutations R4496C or R176Q ($R4496C^{+/-}$ and $R176Q^{+/-}$ mice, respectively) (40, 52), in which catecholamine-induced spontaneous Ca^{2+} release events were observed.

Decreased FKBP12.6 subunit binding to RyR2 channels causes arrhythmias

Multiple mechanisms have been proposed to explain the decreased stability of the closed conformational state of mutant RyR2 channels. Ikemoto et al. (109) proposed the "domain unzipping" model to explain abnormal RyR2 regulation in CPVT. In this model, the NH2-terminal and central domains of RyR2 form an interacting domain pair, and zipping or unzipping of this domain pair is involved in the opening and closing of the RyR2 channel complex, respectively. Wehrens et al. (99) proposed another model in which the binding of the channel-stabilizing subunit FKBP12.6 (calstabin2) is reduced for CPVT-linked mutations in RyR2 (99). This concept is supported by the finding that the binding affinity of FKBP12.6 is reduced for CPVT-mutant RyR2 channels (47, 99). Moreover, several studies have shown that FKBP12.6 dissociation from RyR2 destabilizes the closed state of the channel [reviewed by Chelu et al. (11)].

Additional evidence for a link between altered FKBP12.6-RyR2 interactions in the pathogenesis of ventricular arrhythmias was obtained in $FKBP12.6^{-/-}$ mice, in which β-adrenergic stimulation induced ventricular tachycardia (99, 101). The electrophysiological phenotype observed in $FKBP12.6^{-/-}$ mice (99) was very similar to those observed in mice with CPVT mutations in the *RyR2* gene (40, 52). Moreover, increasing FKBP12.6 binding to RyR2 using transgenic overexpression of FKBP12.6 suppresses the vulnerability to ventricular arrhythmias (35, 75). An experimental drug (JTV519) that enhanced FKBP12.6 binding to RyR2 also inhibits arrhythmias in $FKBP12.6^{+/-}$ mice (101). Nevertheless, some investigators have disputed the role of FKBP12.6 dissociation in the pathogenesis of CPVT, and additional biochemical experiments are needed to resolve this controversy in the field (29).

Dysfunction of proteins in the RyR2 macromolecular complex and arrhythmias

An important finding by Marks and colleagues was that RyR2 channels are hypersensitive to Ca^{2+}-induced activation in failing hearts (59). Subsequent studies have started unraveling defects in the RyR2 subunit assembly that could explain the observed gating defects. Increased PKA phosphorylation of the RyR2 subunits has been proposed as an important consequence of abnormal channel complex regulation (46, 59, 100). Recent studies have shown that RyR2 phosphorylation is locally regulated by protein kinases and phosphatases bound to the macromolecular channel complex (58). Accordingly, decreased binding of protein phosphatases PP1 and PP2A are thought to affect RyR2 gating in failing hearts (79). Moreover, we recently demonstrated that a phosphodiesterase (PDE4D3) associates with the cardiac RyR2 receptor via the same anchoring protein (mAKAP) that also targets PKA to the channel complex (46). PDE4D3 reduces cAMP level in the RyR2 microdomain, which indirectly suppresses the activity of PKA and phosphorylation levels of the target (RyR2). Therefore, the reduced binding of PDE4D3 to RyR2 in failing human hearts is expected to increase PKA phosphorylation of RyR2, which enhances Ca^{2+} leakage from the SR and promotes arrhythmogenesis (46). This progress in the understanding of the structure and regulation of cardiac ion channels has markedly expanded the repertoire of possible targets for the therapy of cardiac arrhythmias.

Concluding Remarks

The identification of genetic mutations in regulatory and targeting proteins associated with cardiac ion channels has provided new insights into the cellular and molecular basis of arrhythmias. Inherited variants in targeting proteins may cause improper localization of ion channels within their microdomain in cardiac myocytes, or they might directly affect ion channel

gating. Inherited or acquired defects in channel regulatory subunits may also perturb cardiac excitation-contraction coupling and contribute to an increased vulnerability to cardiac arrhythmias. Since most pharmacological studies so far have principally targeted cell-surface ion channels or receptors, studies of key subunits and intracellular signaling molecules binding to ion channels have provided multiple new targets for the treatment of cardiac arrhythmias. ■

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