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Molecular Determinants of Cardiac Transient Outward Potassium Current (I_{to}) Expression and Regulation

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Abstract

Rapidly activating and inactivating cardiac transient outward K^+ currents, I_{to} , are expressed in most mammalian cardiomyocytes, and contribute importantly to the early phase of action potential repolarization and to plateau potentials. The rapidly recovering ($I_{to,f}$) and slowly recovering ($I_{to,s}$) components are differentially expressed in the myocardium, contributing to regional heterogeneities in action potential waveforms. Consistent with the marked differences in biophysical properties, distinct pore-forming (α) subunits underlie the two I_{to} components: Kv4.3/Kv4.2 subunits encode $I_{to,f}$, whereas Kv1.4 encodes $I_{to,s}$ channels. It has also become increasingly clear that cardiac I_{to} channels function as components of macromolecular protein complexes, comprising (four) Kv α subunits and a variety of accessory subunits and regulatory proteins that influence channel expression, biophysical properties and interactions with the actin cytoskeleton, and contribute to the generation of normal cardiac rhythms. Derangements in the expression or the regulation of I_{to} channels in inherited or acquired cardiac diseases would be expected to increase the risk of potentially life-threatening cardiac arrhythmias. Indeed, a recently identified Brugada syndrome mutation in *KCNE3* (MiRP2) has been suggested to result in increased $I_{to,f}$ densities. Continued focus in this area seems certain to provide new and fundamentally important insights into the molecular determinants of functional I_{to} channels and into the molecular mechanisms involved in the dynamic regulation of I_{to} channel functioning in the normal and diseased myocardium.

Keywords

Kv channels; $I_{to,f}$; $I_{to,s}$; repolarization; dispersion; arrhythmia; remodeling

1. Introduction

The rapidly activating and inactivating “transient” outward current has been capturing the interest of cardiac electrophysiologists since it was first described as an early outward current in sheep Purkinje fibers [1,2], in which the activity of this current is evident in the prominent early phase of action potential repolarization. Although initially attributed to Cl^- [1,2], subsequent work revealed that the major component of the transient outward current is blocked

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by 4-aminopyridine (4-AP) and carried by K^+ [3,4]. The Cl^- component, in contrast, is regulated by Ca^{2+} and is insensitive to 4-AP [5]. The focus here is on the K^+ -dependent transient outward current, typically referred to as I_{to} or I_{to1} . Following the early finding in sheep Purkinje fibers, I_{to} has been identified in atrial and ventricular myocytes in most mammals [6–17], including humans [18–21], as well as in sinoatrial [22,23] and atrioventricular nodal [24,25] cells. Biophysical analyses further revealed the presence of two I_{to} components with distinct recovery kinetics: the fast ($I_{to,f}$) and slow ($I_{to,s}$) components [26,27]. In many species, $I_{to,f}$ and $I_{to,s}$ are differentially expressed and contribute to regional heterogeneities in action potential waveforms. In canine and human ventricles, for example, $I_{to,f}$ is more prominent in epicardium and midmyocardium than in endocardium and underlies the “spike and dome” morphology of epicardial action potentials [28,29]. Markedly reduced $I_{to,f}$ densities and pronounced alterations in action potential repolarization are observed in failing human hearts, as well as animal models [21,30], and in chronic atrial fibrillation [31–36]. Interestingly, dysregulation of I_{to} has also been postulated to play a pivotal role in the Brugada syndrome [37].

Considerable effort has been focused on defining the molecular correlates of I_{to} channels and on elucidating the mechanisms that regulate the functional expression of these channels. There is now consensus that pore-forming (α) subunits of the Kv4 subfamily, Kv4.2 (*KCND2*) and Kv4.3 (*KCND3*), encode $I_{to,f}$ and that Kv1.4 (*KCNA4*) encodes $I_{to,s}$ channels [38]. A large number of Kv channel accessory (β) subunits have also been proposed to function in the generation of myocardial I_{to} channels. A critical role for the Kv channel interacting protein, KChIP2, was revealed with the demonstration that the targeted disruption of *Kcnp2* eliminates $I_{to,f}$ in mouse ventricular myocytes [39]. In large mammals, KChIP2 appears to be the primary determinant of the transmural gradient of $I_{to,f}$ [40–42]. Functional roles for Kv β [43] and diaminopeptidyl transferase-like protein 6 (DPP6) [44] subunits in the generation of Kv4-encoded $I_{to,f}$ channels have also been proposed. In addition, quite recently, members of the MinK related peptide (MiRP) subfamily, MiRP1 and MiRP2, have been suggested to function in the regulation of $I_{to,f}$ [45–47] and, interestingly, a mutation in the gene (*KCNE3*) encoding MiRP2 has been identified in Brugada syndrome [47]. Accumulating evidence also suggests that Kv channel pore-forming α and accessory β subunits interact (directly or indirectly) with components of the actin cytoskeleton and with a variety of regulatory and signaling molecules. In addition to summarizing the fundamental molecular physiology of cardiac I_{to} , discussed in previous comprehensive reviews [38,48,49], this review integrates recent findings suggesting novel physiological roles of accessory subunits and modulatory proteins, highlighting the dynamic and complex mechanisms that regulate the functioning of cardiac I_{to} channels.

2. Physiological roles of cardiac I_{to} channels

Myocardial action potentials reflect the coordinated functioning of inward (Na^+ and Ca^{2+}) and outward (K^+ and Cl^-) current carrying channels that open and close in a voltage- and time-dependent manner. Of these, voltage-gated K^+ (Kv) channels are the most diverse, and multiple Kv channels with distinct gating properties determine the heights and durations of myocardial action potentials [38]. Cardiac I_{to} channels activate and inactivate rapidly on membrane depolarization, and in most mammalian cardiomyocytes, including rat [10,50], mouse [51], ferret [12], feline [13], rabbit [14,15], canine [17,28] and human [19–21] ventricular myocytes, as well as in atrial cells from rat [6], mouse [7], rabbit [8], canine [9] and human [18,19], I_{to} is a prominent repolarizing current. In large mammals, including human and canine, I_{to} partially repolarizes the membrane, shaping rapid (phase 1) repolarization [52] and setting the initial plateau (phase 2) potential. The activity of I_{to} channels influences the activation of voltage-gated Ca^{2+} channels and the balance of inward and outward currents during the plateau, thereby mediating the duration and the amplitude of phase 2. In rodents, high I_{to} densities dominate all phases of repolarization and account for the remarkably abbreviated action potentials, which lack a clear plateau phase, in these species [53]. There are, however, exceptions. Macroscopic

I_{to} is absent or very small in guinea pig [54,55] and porcine [56–58] atrial and ventricular myocytes. Although the removal of extracellular Ca^{2+} reportedly revealed the presence of fast activating and inactivating “ I_{to} -like” currents in these cells [59,60], later pharmacological studies suggested that the measured currents were much more likely to be due to monovalent cation flux through L-type Ca^{2+} channels [61,62] or to be mediated by inward rectifier K^{+} channels [63,64].

Action potential waveforms are variable in different regions of the heart [38,65], reflecting, at least in part, differences in the densities and properties of I_{to} . Markedly higher densities of I_{to} , together with the expression of the ultrarapid delayed rectifier, for example, accelerate the early phase of repolarization, leading to lower plateau potentials and shorter action potentials in atrial cells [14,19,66,67]. In addition, in human (as well as canine and feline) ventricles, I_{to} densities are much higher in epicardium and midmyocardium, than in endocardium [29, 68,69]. The high density of I_{to} is reflected in the prominent phase 1 notch and the “spike and dome” morphology of epicardial and midmyocardial action potentials, that are largely absent in endocardium [13,28,69]. I_{to} densities are also reportedly higher in right than in left (midmyocardial and epicardial) myocytes, consistent with the more pronounced spike and dome morphology of right, compared with left, ventricular action potentials [70,71].

Regional heterogeneities in cardiac action potential waveforms also reflect the presence of two functionally distinct components of I_{to} . Although both components activate and inactivate quite rapidly, the rates of recovery from inactivation are distinct (Figure 1). The fast component, $I_{to,fast}$ ($I_{to,f}$), for example, recovers very rapidly with time constants in the range of 60 to 100 ms [48,49]. In contrast, the slow component, $I_{to,slow}$ ($I_{to,s}$), recovers slowly with time constants on the order of seconds [26,27]. $I_{to,f}$ and $I_{to,s}$ can also be distinguished based on differential sensitivities to several spider toxins, including the *Heteropoda* toxins [26,27,72,73] and phrixotoxins [74], that selectively block $I_{to,f}$, and have no measurable effects on other Kv channels, including $I_{to,s}$ channels.

In human and ferret left ventricles, I_{to} in epicardial myocytes recovers rapidly, whereas a slowly recovering component of I_{to} dominates in endocardial myocytes, suggesting that $I_{to,f}$ densities are higher in epicardial cells, whereas $I_{to,s}$ predominates in endocardial myocytes [26,29,68]. In rat ventricles, although $I_{to,f}$ and $I_{to,s}$ are coexpressed, $I_{to,f}$ densities are higher in epicardial, than in endocardial and septum, myocytes, and $I_{to,s}$ densities are similar [75,76]. In mouse ventricles, $I_{to,f}$ densities are significantly higher in right, than in left, ventricular myocytes and are low in the interventricular septum, whereas $I_{to,s}$ is only detected in the septum [27,77,78] (Figure 1). In rabbit atrial and ventricular myocytes, I_{to} recovers slowly (time constants $\approx$ 600 ms to 8 s) [79,80], suggesting that $I_{to,s}$ is the primary transient Kv current [81]. In human, canine and mouse atrial myocytes, in contrast, only $I_{to,f}$ appears to be expressed [7,80,82].

3. Molecular correlates of cardiac I_{to} channels

Functional Kv channels reflect the assembly of four (4) pore-forming Kv (α) subunits [83], each of which has six transmembrane segments (S1 to S6), including the S4 voltage sensor [84], a single pore (H) region between the S5 and S6, and cytoplasmic N- and C-termini (Figure 2). The H (pore) loop contains the G(Y/F)G signature motif and functions as the K^{+} selectivity filter [85]. In the Kv1 to Kv4 subfamilies, N-terminal tetramerization (T1) domains immediately preceding S1 (Figure 2) play critical roles in Kv α subunit assembly [86,87]. In heterologous expression systems, Kv α subunits can assemble as homomultimers or as heteromultimers, containing different α subunits from the same subfamily [88–90].

Considerable evidence suggests that Kv4 α subunits underlie $I_{to,f}$ channels. Expression of Kv4.3 predominates in human and canine ventricles [91–94], whereas both Kv4.2 and Kv4.3 are expressed in rodent and ferret ventricles [26,75,95,96]. In rodents, Kv4.2 expression is

correlated with regional heterogeneities in $I_{to,f}$ [75,95]. Heterologous expression of Kv4 α subunits gives rise to rapidly activating, inactivating and recovering (time constants ≈ 50 to 380 ms) Kv currents [75,91,97–99], and the currents are sensitive to 4-AP [97,98], as well as to the heteropodatoxins [72] and phirotoxins [74]. Genetic manipulations *in vitro* and *in vivo* have provided direct evidence that Kv4 α subunits underlie $I_{to,f}$ channels. Expression of mutant Kv4 α subunits that function as dominant-negatives, for example, attenuated $I_{to,f}$ in rat ventricular myocytes *in vitro* [100], and eliminated $I_{to,f}$ in mouse ventricular (Figure 1) [101] and atrial [7] myocytes *in vivo*. Selective gene silencing using antisense oligonucleotides (AsODNs) targeted against Kv4.2 and Kv4.3 reduced $I_{to,f}$ in cultured rodent ventricular myocytes [102, 103]. In rat atrial myocytes, AsODNs targeted against Kv4.2, but not Kv4.3, attenuated $I_{to,f}$ [7], whereas in human atrial myocytes, $I_{to,f}$ was significantly attenuated by Kv4.3, but not by Kv4.2, AsODNs [82]. In addition, targeted gene deletion of *Kcnd2* (Kv4.2) in mice eliminates ventricular $I_{to,f}$, further revealing the critical role of Kv4.2 in the generation of $I_{to,f}$ channels in rodents [104].

Consistent with differences in biophysical properties, considerable evidence suggests that myocardial $I_{to,s}$ channels are molecularly distinct from I_{to} channels and are encoded by the Kv1 α subunit, Kv1.4. Heterologous expression of Kv1.4 yields slowly recovering (time constants of seconds), 4-AP sensitive transient Kv currents [75,105–107] that closely resemble $I_{to,s}$ [26,27,80,82]. In addition, expression of Kv1.4 has been demonstrated at the transcript level in rat [75,105,108], ferret [26], canine [91] and human [109] hearts, and at the protein level in mouse, rat and ferret ventricles [26,75,110] and rabbit atrium [82]. In ferret left ventricles, the Kv1.4 protein is predominantly expressed in endocardial myocytes, but is essentially absent in epicardium, consistent with the observed gradient in $I_{to,s}$ [26]. A close relationship between Kv1.4 protein expression and $I_{to,s}$ density has also been described in rat [75] and mouse [111] ventricles. Definitive evidence for a role of Kv1.4 was provided with the demonstration that $I_{to,s}$ is eliminated in intraventricular septum myocytes from (Kv1.4 $^{-/-}$) animals in which the *Kcna4* locus was disrupted by homologous recombination [111]. In addition, the upregulated $I_{to,s}$ in left ventricular apex cells expressing the mutant Kv4.2 subunit (Kv4.2DN), that functions as a dominant negative, is eliminated in the Kv1.4 $^{-/-}$ background (Figure 1) [111].

4. Roles of accessory subunits in the generation of I_{to} channels

Although heterologous expression of Kv4.3/4.3 or Kv1.4 α subunits generates Kv currents with biophysical properties similar to those of myocardial $I_{to,f}$ and $I_{to,s}$, there are clear differences. Expressed Kv4 channels, for example, inactivate slower and recover from inactivation much more slowly than native $I_{to,f}$ channels. These observations have long been interpreted as suggesting that additional subunits are required for the generation of cardiac I_{to} channels (Figure 3). Indeed, a number of molecularly diverse accessory subunits have been demonstrated to modulate the biophysical properties and the cell surface expression of heterologously expressed Kv4 channels [112,113]. Of these accessory subunits, only the cytoplasmic KCHIP2 protein has been shown directly to be an essential component of myocardial $I_{to,f}$ channels [39]. The KCHIPs, first identified in brain using the Kv4 N-terminus as bait in a yeast two-hybrid screen, belong to the neuronal calcium sensor (NCS) family of Ca^{2+} binding proteins with four EF-hand (Ca^{2+} binding) motifs [114,115]. Co-expression with KCHIP subunits dramatically increases the cell surface expression of Kv4 channels, slows current inactivation and markedly accelerates recovery [114,116]. In addition, KCHIP2 co-immunoprecipitates with Kv 4.2 (and Kv4.3) α subunits from adult mouse ventricles [103], and the targeted deletion of the KCHIP2 locus (*Kcnip2*) eliminates (mouse) ventricular $I_{to,f}$ [39]. Interestingly, KCHIP2 protein expression is dramatically reduced in Kv4.2 $^{-/-}$ ventricles [104], suggesting that expression of the Kv4 and KCHIP2 proteins is reciprocally regulated. In human and canine, there is a marked transmural gradient in KCHIP2 expression across the ventricular wall [40–

42] that parallels the gradient in $I_{to,f}$ density. A modest epi/endocardial gradient in KChIP2 transcript expression has also been reported in mouse ventricles [117].

The molecular mechanisms underlying the enhancement of cell surface expression and the modulation of the gating properties of Kv4 channels by KChIP2, however, have not been elucidated. The proximal N-terminal residues preceding the Kv4.3 T1 domain (Figure 2) are essential for KChIPs binding [114,118]. It has been suggested that KChIP binding to the N-termini of Kv4 α subunits masks a retention signal, and releases channels from the endoplasmic reticulum (ER), allowing forward trafficking to the membrane [116,118]. To date, however, no functional ER retention signals in Kv4 N-termini have been identified. It has also been reported that KChIP1 stabilizes Kv4 protein expression [118] and drives Kv4 subunit tetramerization [119]. Other studies, however, suggest critical roles for the Kv4 C-terminus [120] and the T1 domain [121] in mediating the interaction(s) with KChIPs. Recent structural analyses of KChIP1 with Kv4.3 N-termini revealed that each KChIP1 laterally binds to two neighboring Kv4.3 N-terminal domains, the proximal N-terminus in one Kv4.3 subunit and the T1 domain of the adjacent Kv4.3 subunit [122,123]. Another EF-hand motif-containing Ca^{2+} binding protein, neuronal calcium sensor-1 (NCS-1), also co-immunoprecipitates with Kv4 α subunits from adult mouse ventricles [124]. Like the KChIPs, co-expression with NCS-1 increases Kv4 current densities and affects the rates of Kv4 current inactivation [124,125].

The diaminopeptidyl transferase-like protein 6 (DPP6 or *DPPX*) (Figure 3), first identified as a protein co-immunoprecipitating with Kv4.2 from rat brain [126], has also been suggested to be an integral component of brain [126] and heart [44] Kv4 channels. Reportedly lacking enzymatic activity [127], DPP6 co-expression increases the cell surface expression of Kv4 α subunits [126], shifts the voltage dependences of activation and inactivation of heterologously-expressed Kv4 currents to more negative potentials, and accelerates the rates of current activation, inactivation and recovery [44,126,128]. Interestingly, heterologous co-expression of DPP6 with Kv4.3 and KChIP2, produces Kv currents that closely resemble native cardiac $I_{to,f}$ [44]. Gene silencing of DPP6 in neurons, however, produces only very subtle effects on the voltage- and time-dependent properties of Kv4-encoded currents [129]. Another member of the DPP-like subfamily of proteins, DPP10, has also been demonstrated to associate with Kv4.2 and KChIP3 in rat brain [130] and to have regulatory effects similar to DPP6 on heterologously expressed Kv4 (with and without KChIP) channels [130–133], and DPP10 has also been shown to be expressed in normal and failing human ventricles [134].

The MinK and MinK-related peptide (MiRP) subfamily of transmembrane accessory subunits, modulate the properties of multiple types of Kv channels, including Kv4 channels [135]. Co-expression with MiRP1, for example, affects the kinetics and the voltage-dependent properties of Kv4 and Kv4-KChIP2 channels [45,136–138], and induces an “overshoot” in peak current amplitude during current recovery [45,137,138], a phenomenon evident in human epicardial $I_{to,f}$ [29]. MiRP1 is expressed at the protein level in human, canine, rat and mouse ventricles [46,139,140] and co-immunoprecipitates with Kv4.2 from adult mouse hearts [46]. Although MiRP1 does not measurably affect the densities of expressed Kv4.2-KChIP2 currents [137], targeted deletion of the gene (*Kcne2*) encoding MiRP1 resulted in reduced (~25%) ventricular $I_{to,f}$ densities with no measurable changes in total or surface Kv4.2 expression [46]. Another member of this family, MiRP2 (Figure 3), co-immunoprecipitates with Kv4.3 from human atria [47]. Interestingly, a missense mutation (R99H) *KCNE3* (which encode MiRP2) was recently identified in an individual with Brugada syndrome and in phenotype-positive (but not phenotype-negative) family members [47]. Heterologous expression experiments revealed that MiRP2 decreases Kv4.3- (with and without KChIP) encoded current densities [47,139,141]. In addition, co-expression of the Brugada syndrome MiRP2 mutant reversed the inhibitory effects of wild type MiRP2, leading to markedly increased Kv4.3 currents [47]. Taken together, these intriguing observations suggest that MiRP2 is required for the physiological functioning

of human $I_{to,f}$ channels and that gain of function mutations in MiRP2 predispose to Brugada syndrome through augmentation of $I_{to,f}$ [47].

Three homologous Kv β accessory subunits, Kv β 1, Kv β 2 and Kv β 3, as well as alternatively spliced variants of each, have been identified [142,143]. The Kv β subunits are low molecular weight, cytosolic proteins that co-localize with Kv α subunits in brain [142–151]. In heterologous cells, co-expression of Kv β subunits affects the properties and densities [148, 149] of Kv α subunit-encoded channels. Although initially isolated using a Kv1 channel toxin [144] and suggested to be specific for Kv1 channels [145,148,149], both Kv β 1 and Kv β 2 co-localize with Kv4 α subunits in (rat) brain [146,147] and co-expression of Kv β subunits alters the properties of heterologously expressed Kv4.2 currents [152]. In addition, Kv β 2 reportedly associates with Kv4.3 in (rat) brain [153] and heterologous co-expression with Kv β 1, Kv β 2 or Kv β 3 increases Kv4.3-encoded Kv current densities [136,153,154].

In spite of the postulated link between Kv β subunits and the generation of Kv1-encoded channels [144–151], studies on Kv β 1.1 deficient, Kv β 2 deficient and combined Kv β 1.1/Kv β 2 deficient mice did not reveal an essential role for these subunits in regulating the expression of Kv1.1 or Kv1.2 subunits/channels in the cerebellum or in peripheral nerve [155,156]. It is possible that Kv1 α subunit expression is altered in other parts of the central nervous system and/or in other tissues (including the heart) and/or that Kv β 3 expression is upregulated in these animals to compensate for the loss of Kv β 1.1 and/or Kv β 2; these possibilities were not explored [155,156]. These mouse lines do, however, display neurological phenotypes, including seizures [155,156], suggesting that loss of Kv β 1.1 and/or Kv β 2 affects neuronal excitability. Consistent with this suggestion, it has been reported that K^+ currents and action potential waveforms are altered in hippocampal CA1 pyramidal neurons in (an independently generated line of) Kv β 1.1-deficient mice [157,158]. The observed reductions in rapidly inactivating K^+ currents were interpreted as reflecting the loss of Kv β 1.1-mediated inactivation of Kv1-encoded A-type (I_A) K^+ currents [157,158]. Given the documented role for Kv4 α subunits in the generation of I_A , however, the observed effects may suggest a role for Kv β subunits in the generation of Kv4-encoded I_A channels. Consistent with this hypothesis, it has been reported that deletion of Kv β 1 markedly attenuated Kv4-encoded mouse ventricular $I_{to,f}$, whereas Kv1-encoded currents were unaffected [43]. Interestingly, structural data suggests that Kv β subunits can interact with Kv4 subunits in the presence of KChIP (Figure 3), resulting in the formation of multimeric Kv4-channel complexes.

Biochemical experiments on extracts from neonatal rat ventricles suggest that the voltage-gated Na^+ (Nav) channel accessory subunit, Nav β 1, associates with Kv4.2/Kv4.3 α subunits [159]. It has also been reported that co-expression of Nav β 1 increased Kv4.3-encoded current densities in heterologous cells [137], and gene silencing of Nav β 1 dramatically reduced $I_{to,f}$ densities and decreased KChIP2 transcript expression in neonatal rat ventricular myocytes [159]. These data suggest the intriguing possibility that cardiac $I_{to,f}$ and Nav channels are functionally coupled through accessory (Nav β) subunits. The K^+ channel-associated protein, KChAP, also increases the cell surface expression, without influencing the time- and/or voltage-dependent properties, of Kv4-encoded channels [160].

5. Interactions with the actin cytoskeleton

Similar to other ion channels, accumulating evidence suggests that Kv channel α and accessory (β) subunit assemblies associate with the actin cytoskeleton. These interactions likely are important in determining channel stability, trafficking, localization and biophysical properties, but also for providing microdomains for regulatory molecules. Consistent with this suggestion, the properties of rat ventricular $I_{to,f}$ channels are regulated by the actin cytoskeleton, particularly under pathological conditions. Pharmacological disruption of actin microfilaments

by cytochalasin D, for example, reportedly decreased $I_{to,f}$ densities in hypertrophied myocytes, whereas stabilization of actin microfilaments by phalloidin increased the currents [161]. Neither treatment had effects on $I_{to,f}$ densities in non-hypertrophied cells [161]. Heterologously expressed Kv4.2 currents, however, are augmented on exposure to cytochalasin D [162]. These seemingly disparate observations might suggest important roles for Kv4 channel accessory/regulatory proteins in mediating interactions between Kv4 channels and the actin cytoskeleton and that these accessory/regulatory proteins are differentially expressed in different cell types [162]. It has also been shown that the insulin-induced recovery of $I_{to,f}$ densities (to normal) in diabetic rat ventricular myocytes requires an intact cytoskeleton, actin microfilaments and microtubules, presumably to traffic newly synthesized channels to the membrane [163].

Several actin-binding proteins and other scaffolding proteins have been suggested to mediate the interactions between Kv channels and the actin cytoskeleton. Filamin, for example, interacts directly with the Kv4.2 C-terminus through a C-terminal filamin binding domain (that is also present in Kv4.3 (Figure 2)) and increases the cell surface expression of Kv4.2 channels [164]. The actin binding protein, α -actinin-2, in contrast, binds to the Kv1.4 N-terminus [165]. A number of scaffolding proteins, including the PDZ-domain-containing membrane-associated guanylate kinases (MAGUKs), have been implicated in the clustering and localization of ion channels (and neurotransmitter receptors) in brain [166–168], and more recently, in the heart [169]. Of these, the 95 kDa post-synaptic density protein (PSD-95) and the synapse-associated protein 97 (SAP97) have been shown to interact directly with Kv α subunits. PSD-95, for example, binds directly to the PDZ-binding domains in the C-termini of Kv1 α subunits and co-immunoprecipitates with Kv1.4 from brain [167]. Interactions with PSD-95 increase the clustering and cell surface expression of Kv1 channels in heterologous cells [167,170,171]. Co-expression with PSD-95 localizes Kv1.4 channels to specific membrane microdomains known as “lipid rafts” [172–174] and interaction with PSD-95 appears to stabilize Kv1.4-encoded channels in the membrane [175], presumably by suppressing internalization [176]. PSD-95 also reportedly binds to C-terminal PDZ-binding motifs in Kv4 α subunits [177], although the functional impact of this binding on Kv4 channel surface expression and clustering is not clear [167,177]. Although not detected in rat heart [178,179], PSD-95 is reportedly expressed in human atria [180].

The PDZ-binding motifs in the C-termini of Kv1 α subunits also interact directly with another MAGUK protein, SAP97 [170] and, in contrast with PSD-95, this interaction results in retention of Kv1 channels in the endoplasmic reticulum [170,171,181]. It has also been reported that SAP97 regulates the functional expression of Kv4 channels in neurons [168] and in the heart [169]. In rat ventricles and human atria, for example, Kv4.2/Kv4.3 α subunits interact with SAP97 via C-terminal PDZ-binding motifs, and the Kv4-SAP97 proteins are co-localized at the plasma membrane in rat atrial myocytes [169]. Heterologous co-expression with SAP97 enhanced Kv4.3 channel clustering at the membrane surface, increased current densities and slowed inactivation in heterologous cells, and similar effects were observed on rat atrial $I_{to,f}$ by the overexpression of SAP97 [169]. Conversely, SAP97 RNA interference significantly decreased $I_{to,f}$ densities in rat atrial myocytes, consistent with modulatory effects of SAP97 on endogenous $I_{to,f}$ channels [169]. In addition, recent work suggests that SAP97 functions as a molecular adaptor of the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), mediating phosphorylation-dependent regulation of cardiac (and neuronal) Kv4 channels [168,169]. Consistent with this hypothesis, it was recently demonstrated that SAP97 and CaMKII co-immunoprecipitate and that the expression level of SAP97 affects the interaction between CaMKII and Kv4.3 in rat heart [169]. Additional studies in heterologous cells revealed that reduced expression of SAP97 led to loss of CaMKII-induced modulation of Kv4.3-encoded currents and eliminated the effects of CaMKII inhibitors [169].

Syntaxin 1A, a membrane associated protein involved in neurotransmitter exocytosis, has also been shown to interact with Kv4.2 α subunits. In addition, syntaxin 1A co-immunoprecipitates with Kv4.2 from rat heart and co-expression of syntaxin 1A with Kv4.2 decreases cell surface channel expression and modulates gating kinetics [182]. It appears that syntaxin 1A shares the KChIP2 binding site (Figure 2) in the Kv4.2 N-terminus and affects KChIP2-mediated modulation of Kv4.2 channels [182]. Accumulating evidence also suggests that ion channels are functionally modulated by cell-cell and cell-extracellular matrix interactions. In neonatal rat ventricular myocytes in culture, for example, cell-cell contact results in down-regulation of Kv4.2 expression [183]. Although a relatively unexplored area in cardiac physiology, it seems certain that there will be increased emphasis on defining the physiological role(s) of extracellular matrix components, as well as components of the intracellular actin cytoskeleton, in the regulation of myocardial I_{to} (and other Kv) channels and on delineating the underlying molecular mechanisms controlling these pathways under physiological and pathophysiological conditions.

6. Other regulatory mechanisms

Also first described in Purkinje fibers [184], it is now well recognized that ventricular and atrial $I_{to,f}$ densities are modulated physiologically by adrenergic receptor activation [185]. It has also been suggested that adrenergic stimulation is an important determinant of $I_{to,f}$ densities in the diseased myocardium [185]. The effects of myocardial α -adrenergic and β -adrenergic receptor stimulation are mediated through the activation of intracellular second messenger cascades, culminating in the activation of protein kinases and resulting in the regulated phosphorylation and dephosphorylation of downstream targets, including ion channels. Indeed, several protein kinases and phosphatases have been linked to the regulation of myocardial Kv channels, particularly I_{to} channels [185], and multiple potential phosphorylation sites for cAMP-dependent protein kinase (PKA), protein kinase C (PKC), extracellular signal regulated kinase (ERK) and CaMKII have been identified in Kv4 (Figure 2) and Kv1.4 α subunits. Of these, CaMKII has emerged as a pivotal regulator of both cardiac $I_{to,f}$ (Kv4.2/4.3) and $I_{to,s}$ (Kv1.4) channels. A serine-threonine kinase, CaMKII, is activated by binding of Ca^{2+} -bound calmodulin (Ca^{2+}/CaM), resulting in CaMKII autophosphorylation [186]. In heterologous cells, CaMKII activation slows the inactivation and accelerates the recovery from inactivation of Kv1.4 channels via phosphorylation of an N-terminal consensus CaMKII phosphorylation site, suggesting a physiological role for CaMKII in the regulation of $I_{to,s}$ [187].

Considerable evidence also suggests a role for CaMKII in the regulation of $I_{to,f}$ channels. CaMKII, for example, co-immunoprecipitates with Kv4.2/Kv4.3 subunits from rat heart [169,188]. In addition, inhibition of CaMKII accelerates $I_{to,f}$ inactivation in human atrial and rat ventricular myocytes [188,189]. Modulatory effects of CaMKII on heterologously expressed Kv4 channels have also been described and, interestingly, the effects on Kv4.2- and Kv4.3-encoded channels are distinct [188,190]. In HEK-293 and CHO cells, inhibition of CaMKII accelerates the inactivation of Kv4.3 currents [169,188,190]. The opposite effect is produced by autophosphorylated (activated) CaMKII [190] and is mediated by phosphorylation of a CaMKII consensus motif in the C-terminus (Figure 2) of Kv4.3 [190]. The association of CaMKII with Kv4.3 occurs in the absence of increased intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), and the Kv4.3 protein is phosphorylated at low $[Ca^{2+}]_i$ [188]. In contrast, co-transfection of activated CaMKII with Kv4.2 and KChIP3 in COS-7 cells increased total and cell surface Kv4.2 expression via phosphorylation of two CaMKII consensus motifs in the C-terminus of Kv4.2, resulting in increased current amplitudes without measurable changes in gating [191]. In HEK-293 cells, increasing $[Ca^{2+}]_i$ reportedly resulted in Kv4.2 phosphorylation and a slowing of inactivation [188]. The latter effect is prevented by inhibition of CaMKII, suggesting that increased $[Ca^{2+}]_i$ results in the modulation of Kv4.2 channels by endogenous CaMKII [188].

It has also been reported that increased CaMKII activity down-regulates *KCND3* (Kv4.3) transcript expression, resulting in decreased $I_{to,f}$ densities in isolated canine ventricular myocytes [192]. Interestingly, rapid pacing increases CaMKII activity in Langendorff-perfused rabbit hearts [193] and in isolated canine ventricular cells [192]. Accumulating evidence suggests that calcineurin and its downstream target, the nuclear factor of activated T-cells (NFAT), transcription factor, are important in the regulation of $I_{to,f}$ channels. In isolated adult rat ventricular myocytes, for example, increased Ca^{2+} influx results in decreased $I_{to,f}$ densities as a result of down-regulation of Kv4.2 transcript expression mediated by calcineurin, and independent of CaMKII [194]. In mouse left ventricles, transmural differences in $[Ca^{2+}]_i$ appear to underlie the differential activation of calcineurin and NFAT across the ventricular wall [195]. Higher activity of calcineurin/NFAT in the endocardium down-regulates Kv4.2 expression, resulting in reduced $I_{to,f}$ densities [196]. In a mouse model of acute myocardial infarction, $I_{to,f}$ density is decreased as a result of activation of the calcineurin/NFAT pathway and the transcriptional down-regulation of Kv4.2 [197]. In isolated neonatal rat ventricular myocytes, however, it has been reported that expression of a constitutively active form of calcineurin increases $I_{to,f}$ densities through the up-regulation of Kv4.2 transcript expression [198].

It has been reported PKA activation by cAMP inhibits I_{to} in rat ventricular myocytes [199], although another study revealed no effects of cAMP/PKA on I_{to} [200]. Nevertheless, there are two consensus PKA phosphorylation sites in the N- and C-terminal cytoplasmic domains of Kv4.2 (Figure 2), and both are phosphorylated in heterologous cells by PKA [201]. Interestingly, PKA-mediated phosphorylation of serine 552 in Kv4.2 modulated the properties of Kv4.2-encoded currents only in the presence of KChIP3 [202]. It has also been reported that (serine 552) phosphorylation of the Kv4.2 protein is increased with KChIP co-expression [118]. In embryonic rat neurons, PKA activation (and serine 552 phosphorylation) leads to Kv4.2 internalization [203]. Although there are also PKA phosphorylation sites in KChIP3, phosphorylation of these sites is not necessary for PKA modulation of Kv4.2-KChIP3 channels [202]. Similarly, Kv1.4 is a substrate for PKA, and an N-terminal PKA phosphorylation site in Kv1.4 has been identified [204]. In diabetes, angiotensin II (AT-II) activation suppresses PKA activity, resulting in inhibition of $I_{to,f}$ in (rat) ventricular myocytes [205].

Inhibitory effects of PKC have been observed on heterologously expressed Kv4.2/4.3-[206] and Kv1.4-encoded [207] currents and activation of PKC reduces I_{to} amplitudes in rat and canine ventricular myocytes [206,208–211]. Two alternatively spliced variants of Kv4.3 have been identified in rat [212,213] and human [93,214], and the full-length variant contains consensus PKC phosphorylation sites (Figure 2) [93,214] that have been suggested to underlie PKC-dependent down-regulation of $I_{to,f}$ mediated by α -adrenergic receptor activation [215]. It has also been suggested that PKC-mediated phosphorylation underlies endothelin-induced inhibition of Kv1.4- and Kv4.3-encoded currents [216]. Two PKC phosphorylation sites have also been identified in the C-terminus of Kv4.2 [217], and inhibition of PKC phosphorylation increases Kv4.2 surface expression and the amplitudes of Kv4.2-encoded currents [217].

In rat ventricles, activation of PKC negatively regulates Kv4.2 protein [210] and KChIP2 transcript [218] expression. In neonatal rat ventricles, PKC-dependent transcriptional down-regulation of KChIP2 appears to be mediated by the activation of the mitogen-activated protein kinase/ERK kinase (MEK) pathway [218]. Inhibition of MEK increased KChIP2 transcript expression [218]. In brain, basal ERK phosphorylation of the Kv4.2 C-terminus is observed [219,220], and inhibition of MEK increases neuronal Kv4.2-encoded current (I_A) amplitudes and shifts the voltage-dependences of current activation and inactivation [221,222]. There are three ERK phosphorylation sites in the Kv4.2 C-terminus [219]. Of these, phosphorylation at threonine 607 mimics the effects of ERK activation on Kv4.2-KChIP3 encoded currents [220]. Interestingly, co-expression with KChIP3 appears necessary for the modulatory effects

of ERK-mediated phosphorylation of Kv4.2 [220]. It has also been suggested that the MEK/ERK pathway acts downstream of PKA and PKC [222,223].

Other protein kinases also reportedly modulate $I_{to,f}$ channels. Cardiac-specific overexpression of p90 ribosomal S6 kinase (p90RSK), for example, attenuates $I_{to,f}$ densities in mouse ventricular myocytes [224], and activation of p90RSK appears to mediate hydrogen peroxide-induced modulation of heterologously expressed Kv4.3- (with and without KChIP2) encoded currents [224]. In addition, inhibition of protein tyrosine kinase (PTK) activity suppresses $I_{to,f}$ in human atrial [225] and rat ventricular [226] myocytes. It has also been reported, however, that inhibition of PTK attenuates $I_{to,f}$ only in ventricular myocytes from diabetic animals [205]. In heterologous cells, inhibition of PTK reduced, and inhibition of tyrosine phosphatase augmented, Kv4.3-encoded currents [227]. In addition, Kv4.3 co-immunoprecipitates with a non-receptor PTK, c-Src, and is phosphorylated on tyrosine [227]. It was also reported that the phosphorylation of KChIP3 by G-protein-coupled kinase (GRK)2 negatively regulates the membrane trafficking of Kv4.2-KChIP3 channels in heterologous cells [228].

Other post-translational modifications of Kv4- and Kv1.4-channels, although suggested, have been much less well studied. In heterologous cells, for example, glycosylation of Kv1.4 increases channel stability, trafficking and cell surface expression [229]. Palmitoylation, in contrast, appears to regulate Kv channel membrane expression through effects on accessory subunits. Palmitoylation of PSD-95, for example, has been shown to be required for the trafficking and clustering of Kv1.4 channels [230,231]. In addition, palmitoylation of KChIP2 appears to play a role in regulating the membrane expression of Kv4 channels [232].

Hormonal regulation of I_{to} channels has also been described. In pregnancy-induced cardiac hypertrophy (in mice), for example, increased estrogen was associated with reduced $I_{to,f}$ densities [233]. A subsequent study reported that MiRP1 expression is up-regulated in response to elevated estrogen [234], further suggesting a role for MiRP1 in the regulation of $I_{to,f}$. In diabetes, however, estrogen appears to prevent the reduction of $I_{to,f}$ and Kv4.2 expression in female rat ventricular myocytes through inhibitory effects on rennin-angiotensin system by suppression of oxidative stress, PKA inhibition and receptor PTK activation [200,235,236]. In contrast, testosterone significantly increases Kv4.3 expression, leading to marked shortening of ventricular repolarization in canine heart [237]. Importantly, sex hormone-dependent regulation of $I_{to,f}$ channels has been suggested to underlie gender differences in arrhythmia susceptibility, such as the observed male predominance in the Brugada syndrome [238,239]. Thyroid hormone treatment also reportedly differentially regulates I_{to} channels and Kv4.2/Kv4.3 expression in rodent ventricular myocytes by a transcriptional mechanism [240–243], whereas Kv1.4 expression is negatively regulated [240–242,244]. Indeed, it has been suggested that thyroid hormone plays a role in postnatal up-regulation of $I_{to,f}$ channels and the developmental change (Kv1.4 to Kv4.2/Kv4.3) in relative Kv α subunit expression levels [240,241,245].

Autocrine/paracrine factors also regulate I_{to} channels. AT-II, for example, decreased $I_{to,f}$ densities in ventricular myocytes isolated from neonatal rats [246] and in adult canine epicardial myocytes [247] via the activation of the angiotensin II type-1 receptor (AT1R) [247]. In addition, the inhibitory effect of AT-II on $I_{to,f}$ is implicated in the initiation of short-term cardiac memory [248]. Incubation with AT II induced transient reductions of Kv4.3 transcript levels via AT1R activation [246], an effect mediated by superoxide and the downstream signaling, apoptosis signal-regulating kinase 1 (ASK1)-p38 kinase pathway [249,250]. In diabetic rat, AT-II levels are elevated [200] and ventricular $I_{to,f}$ densities are reduced, reflecting AT-II-dependent inhibition of PKC and PKA, the activation of receptor PTK and reduced Kv4.2 expression [200,210]. It has also been shown that AT-II and AT1R control the surface expression of $I_{to,f}$ channels [251]. In canine ventricles, AT1R forms a

complex with Kv4.3, and in heterologous cells, application of AT-II leads to the internalization of Kv4.3-AT1R complexes, resulting in marked reductions in $I_{to,f}$ densities [251]. It has also been suggested that AT-II-mediated (inhibitory) regulation of $I_{to,f}$ contributes to the transmural gradient of $I_{to,f}$ in canine ventricles [247]. Consistent with this hypothesis, AT-II transcript expression is substantially higher in the endocardium, than in the epicardium, in human ventricles [252]. It has also been demonstrated that AT-II and AT1R activation modulate $I_{to,f}$ gating in canine and mouse ventricular myocytes [247,253] and Kv4.3-KChIP2-encoded currents in heterologous cells [251].

Considerable evidence now suggests that alterations in cellular metabolism also have marked effects on the expression/functioning of cardiac I_{to} channels, although the underlying molecular mechanisms are poorly understood. In the streptozotocin-induced model of type 1 diabetes, for example, ventricular $I_{to,f}$ amplitudes are reduced [254–257] and the expression Kv4.2/Kv4.3 transcripts and proteins are decreased, whereas Kv1.4 expression is increased [257–259]. Electrophysiological studies, however, did not reveal measurable changes in I_{to} recovery kinetics [254–256]. Reduced ventricular I_{to} density was also documented in (male) *db/db* mice, a genetic model of type 2 diabetes [235,260], although no changes in $I_{to,f}$ densities/properties were reported in another (rat) model of type 2 diabetes produced by high fructose feeding [261]. In the diabetic heart, >90% of the ATP is generated from oxidation of fatty acids [262, 263], and, incubation with insulin [163,261,264], as well as agents that enhance glucose oxidation [264], normalize $I_{to,f}$ densities in ventricular myocytes isolated from diabetic rats. In addition, *in vivo* insulin administration partially prevents diabetes-induced down-regulation of $I_{to,f}$ and Kv4.3 expression in canine ventricles [257]. Interestingly, ventricular $I_{to,f}$ densities are also decreased in a mouse model of diabetic cardiomyopathy in which fatty acid uptake and utilization are increased as a result of overexpression of peroxisome proliferator-activated receptor α (PPAR α) [265], although $I_{to,f}$ is not affected in another mouse model of lipotoxic diabetic cardiomyopathy produced by overexpression of the fatty acid transport protein 1 [266]. In addition, increased intracellular fatty acid metabolites reportedly suppress $I_{to,f}$ densities in rat ventricular myocytes [267]. It has also been suggested that insulin signaling via the MEK/ERK pathway plays a role in the regulation of $I_{to,f}$ expression in ventricular myocytes from diabetic rats [261].

Metabolic changes associated with diabetes, including hyperglycemia, impaired glucose metabolism and increased AT-II, are all associated with an oxidized cellular environment [236,268], and oxidative stress has been implicated in the remodeling $I_{to,f}$. It has, for example, been shown that thioredoxin and glutathione reductase activities are decreased in the diabetic (rat) heart and that inhibition of thioredoxin and glutathione reductase actually prevents the up-regulation of $I_{to,f}$ in diabetic rat ventricular myocytes exposed to insulin [269,270]. Interestingly, atrial $I_{to,f}$ is not suppressed in experimentally-induced diabetes, presumably reflecting an inhibitory action of atrial natriuretic peptide on the renin-angiotensin system (and oxidative stress) [271]. In experiments on left ventricular myocytes from non-diabetic rats, experimentally-induced oxidative stress decreased $I_{to,f}$ densities, and down-regulated Kv4.2 expression [272], an effect mediated by receptor tyrosine kinase signaling [273] and the activation of phosphatidylinositol 3 (PI3)-kinase, MEK and p38 MAP kinases [272].

7. Summary and concluding remarks

Considerable progress has been made in efforts focused on understanding the properties and the roles of cardiac I_{to} channels, as well on defining the molecular mechanisms that regulate the functioning of these channels. In atrial and ventricular myocardium, I_{to} channels play a prominent role in the early phase of repolarization. The two functionally distinct cardiac I_{to} channels, $I_{to,f}$ and $I_{to,s}$, are encoded by Kv4.3/Kv4.2 and Kv1.4 a subunits, respectively. A number of Kv channel accessory (β) subunits have been suggested to function in the generation

of cardiac I_{to} channels. Of these, a crucial role has been definitively demonstrated only in the case of KChIP2 [39]. Recently, however, a potentially important role for MiRP2 has been suggested with the identification of a Brugada syndrome mutation in *KCNE3* (MiRP2) and the demonstration that co-expression of the mutant MiRP2 protein augments $Kv4.3$ current densities [47]. Studies in rodents also suggest functional roles for the accessory $Kv\beta1$ [43] and MiRP1 [46] subunits in the regulation of ventricular $I_{to,f}$ channel densities. In addition, several recent studies suggest that cardiac I_{to} channels interact with components of the actin cytoskeleton and that these interactions contribute to the regulation of I_{to} . The SAP97 scaffolding protein, for example, appears to regulate the surface expression of $Kv4$ channels and to mediate interactions between $Kv4.3$ and CaMKII [169]. A number of other protein kinases (as well as phosphatases) have been postulated to function in the physiological and/or pathophysiological regulation of I_{to} channel expression and/or properties [199,205,215,224–226].

In spite of the progress made in defining the molecular determinants of cardiac I_{to} , the functional roles of the many putative accessory subunits and other regulatory proteins in the regulation of native I_{to} channels remain poorly understood. In addition, although recent structural information on $Kv4$ N-terminus-KChIP1 complexes has provided new insights into the sites of interaction of these two proteins [122,123], the molecular mechanisms underlying KChIP2-mediated regulation of $Kv4$ channel cell surface expression and functioning remain to be defined. Similarly, the mechanisms involved in the regulation of I_{to} channels by actin cytoskeletal/membrane-associated proteins or the extracellular matrix (and cell-cell contact) are poorly understood. In addition, a number of regulatory proteins and intracellular second messenger signaling cascades have been implicated in the regulation of I_{to} channels under physiological, as well as pathological, conditions, contributing to the “remodeling” of I_{to} channels. These signaling mechanisms, however, are quite complex reflecting the interplay between a variety of regulatory proteins, any one of which may be altered in the diseased myocardium. Clearly, an important emphasis of future research will be on deciphering these mechanisms and detailing the molecular determinants of I_{to} channel functioning in the (normal and diseased) myocardium.

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Figure 1. Functionally distinct components of I_{to} , $I_{to,f}$ and $I_{to,s}$, are differentially expressed in ventricular myocytes

Representative whole-cell voltage-clamp recordings from adult mouse left ventricular apex (A) and interventricular septum (B) myocytes isolated from a wild type (WT) animal and a left ventricular apex cell (C) from a transgenic animal expressing a mutant Kv4.2 subunit, Kv4.2DN, that functions as a Kv4 subfamily specific dominant negative (and eliminates $I_{to,f}$). The recording conditions were optimized to allow the isolation of voltage-gated K^+ (Kv) currents, and the voltage-clamp paradigm is illustrated to the right of the current records. The marked differences in the rates of recovery from inactivation of $I_{to,f}$ and $I_{to,s}$ and the predominance of $I_{to,f}$ in WT apex cells (A) are clear in the current records shown; both the fast and the slow I_{to} components are expressed in WT septum cells (B). In contrast, $I_{to,f}$ is not detected in Kv4.2DN left ventricular apex myocytes (C), and, in addition, $I_{to,s}$ up-regulated in

these cells. **(D)** The mean $\pm$ SEM normalized recovery data for $I_{to,f}$ and $I_{to,s}$ are plotted as function of the recovery time: $I_{to,f}$ recovers very rapidly, whereas $I_{to,s}$ recovery is slow. The amplitudes of the peak Kv currents and of $I_{to,f}$ and $I_{to,s}$ in individual cells were determined and normalized to the whole cell membrane capacitance (measured in the same cell) to obtain current densities and mean $\pm$ SEM peak **(E)**, $I_{to,f}$ **(F)** and $I_{to,s}$ **(G)** densities are plotted. In Kv4.2DN myocytes, $I_{to,f}$ is eliminated and only $I_{to,s}$ is expressed, demonstrating the critical role for Kv4 α subunits in the generation of $I_{to,f}$ channels. When the Kv4.2DN protein is expressed in the hearts of (Kv1.4 $^{-/-}$) mice in which the *Kcna4* (Kv1.4) locus has been disrupted, $I_{to,s}$ (in addition to $I_{to,f}$) is also eliminated, demonstrating the critical role for Kv1.4 in the generation of ventricular $I_{to,s}$ channels. (Adapted with permission from [27,78,111])

Figure 2. Amino acid sequence and membrane topology of human Kv4.3

The linear amino acid sequence of human Kv4.3 is illustrated; the C-terminal insert in the long version of the protein (Kv4.3L) is highlighted. The tetramerization (T1) domain, the KChIP2 binding domain and Filamin C binding domain are also indicated. As discussed in the text, it has been reported that Kv β subunit-mediated modulation of Kv4-encoded currents requires both the Kv4 N-terminus and C-terminus, although no specific information about putative Kv β subunit binding domains is available. Several known and potential phosphorylation sites in the Kv4.3 sequence and the specific serine-threonine protein kinases, including protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), calcium-calmodulin dependent protein kinase II (CaMKII) and extracellular signal regulated kinase (ERK), mediating phosphorylation of these residues are also indicated; the color code is provided in

the key the upper right corner of the Figure. Serine and threonine residues that are known or potential targets of multiple protein kinases are indicated accordingly, in multicolor.

Figure 3. Schematic illustration of a Kv4.3 channel with multiple accessory subunits

Cross section of a Kv4.3 channel in the membrane with the N-terminal tails and the S5-H loop-S6 (pore) assemblies (in blue) of (two) Kv4.3 α subunits drawn based on the published crystal structures of Kcsa [85,274], Kv1.2 [275,276] and the Kv4.3N terminus-KChIP1 complex [121]. Similarly, the KChIP2 structure and the KChIP2-Kv4.3 interaction illustrated are based on the published crystal structure of Kv4.3N terminus-KChIP1 complexes [122,123] and the DPP6 and minK structures are based on published DPP6 [277] and minK [278] structures, respectively. The Kv β 1 structure, derived from published crystallographic Kv β 2 data [279, 280], is illustrated docked on the Kv4.3 N-terminus at a site distinct from (and non-overlapping with) the KChIP2 binding site. This configuration suggests that both the KChIP2 and the Kv β 1 subunits could be complexed to cardiac Kv4.3 α subunits in a 1:1:1: (4:4:4) stoichiometry in the generation of functional myocardial $I_{to,f}$ channels.