Localization and trafficking of cardiac voltage-gated potassium channels

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Abstract

The proper trafficking and localization of cardiac potassium channels is profoundly important to the regulation of the regionally distinct action potentials across the myocardium. These processes are only beginning to be unravelled and involve modulators of channel synthesis and assembly, post-translational processing, various molecular motors and an increasing number of modifying enzymes and molecular anchors. The roles of anchoring proteins, molecular motors and kinases are explored and recent findings on channel internalization and trafficking are presented.

Introduction

Kv channels (voltage-gated potassium channels) consist of assemblies of four α-subunits plus accessory subunits and are gated by changes in the transmembrane potential. A broad class of channels related in differing degrees to the prototypical Shaker channel of Drosophila, Kv channels are collectively responsible for the regulation of the durations and amplitudes of the cardiac action potentials. Differing substantially in their kinetics of activation and inactivation, specific channels underlie specific currents in the heart. Kv4.x channels, for example, activate and inactivate rapidly and underlie the I\textsubscript{to} (transient outward current), whereas Kv1.5, responsible for the atria-specific I\textsubscript{to} (ultrarapid delayed rectifier K\textsuperscript{+} current), inactivates much more slowly. hERG (human ether-a-go-go-related gene) activates and inactivates rapidly, then passes most of its current as it recovers from inactivation.

In addition to the differences in their kinetics, the various Kv channels expressed in the heart vary also in their distributions and degrees of expression across the myocardium. It is this variation that is responsible for the differences in shape and duration of the atrial and ventricular action potentials etc. The mechanisms behind these variations in expression and localization are now only beginning to be elucidated.

Surface expression may be regulated by a large number of controls. Modulation of gene expression at the transcriptional and translational levels, phosphorylation of mature channel proteins, as well as interactions with accessory subunits and with cellular components that regulate trafficking and targeting to the cell surface can all play important roles. Glucocorticoids have been shown to modulate Kv1.5 at the transcriptional level [1]; changes in the transcription of the Kv1.5 and Kv4.3 genes have also been demonstrated in the molecular remodelling associated with atrial fibrillation [1–3]. It is quite possible, in addition, that differences in channel expression across the myocardium are related to modulation of transcriptional rates of the various channels. This cannot be the whole story, however. The Kv1.5 transcript is comparably abundant in both atrial and ventricular myocytes [4], yet the I\textsubscript{to} is essentially absent from the latter [5]; the Kv1.4 transcript is similarly abundant [6], but, in most species, the protein is apparently not expressed in the heart at all [7]. Post-translational mechanisms must operate in the differential control of channel expression as well.

Assembly and early trafficking events

Once transcribed, the mRNA encoding a channel travels to the cytoplasm for translation and assembly. Kv channels lack a typical membrane-protein signal sequence and are instead targeted to the ER (endoplasmic reticulum) by the second transmembrane domain (referred to as S2) [8]. It is unclear whether the ribosome translocates to the rough ER prior to the initiation of this event. Folding of the nascent T1 domain begins while the constituent amino acid residues are incorporated into the ribosome [9,10], and is complete by the time T1–S1 linker synthesis is achieved [10]. It is possible that the assembly of the nascent channel begins even before the membrane insertion process is initiated, as T1 assembly and tertiary structure formation appear to be coupled [11].

Not all nascent channels fold and assemble properly, so quality control mechanisms exist to prevent trafficking of these channels out of the ER. Most extensively studied for the hERG channel, these mechanisms involve retention
motifs such as RXR (Arg-Xaa-Arg), which, when exposed, prevent trafficking out of the ER, proteosomal degradation of grossly misfolded channels, as well as other less well-defined processes [12]. Conversely, appropriate exposure of forward trafficking signals, such as the VXXSL in the Kv1.4 C-terminus [13], actively promotes transit out of the ER. Chaperones and accessory proteins such as β-subunits, KChIPs (potassium channel-interacting proteins) and KChAP (potassium channel-associated protein) also promote trafficking out of the ER [14–18]. In depth reviews of these processes are available elsewhere [19,20].

Once out of the ER, the newly synthesized protein passes through the Golgi apparatus where glycosylation is completed and preliminary sorting to the plasmalemma or intracellular organelles occurs [21]. There is little evidence that the Golgi is responsible for targeting to specific sarcolemmal domains, however. This targeting is much more likely to occur following channel exit from this organelle [22,23] and is quite specific. Channel localization is quite specific: in atrial myocytes, Kv1.5 is targeted mainly to the intercalated disc, whereas in ventricular myocytes it is present both at the intercalated disc and in proximity to the Z-lines [24,25]. Kv4.2 is similarly found at the intercalated disc [7], and in T-tubules [26], whereas KCNQ1 (KvLQT1) is present in T-tubules and also in the peripheral sarcolemma [27]. A major question is whether the distinct localizations result from channel-specific trafficking mechanisms or instead from specialized retention/exclusion and anchoring at the cell membrane.

A case for anchoring

Supporting the hypothesis that channel distributions result at least in part from specific anchoring rather than specialized transport, among the first proteins found to affect Kv channel surface expression were MAGUKs (membrane-associated guanylate kinases). Consisting of an inactive guanylate kinase domain, an SH3 domain (Src homology 3 domain) and one or more PDZ domains, MAGUK proteins were first identified as components of neuronal synapses where they act as scaffolds upon which proteins are anchored and localized [28]. PSD-95 (postsynaptic density 95) was the first MAGUK shown to bind to Kv channels, specifically Kv1.1, Kv1.2, Kv1.3 and Kv1.4 [29], via their extreme C-terminal canonical PDZ-binding domains (S/T-X-V). The surface expression of at least Kv1.4 [29] and, as demonstrated later, Kv1.5 [30], is increased by this interaction. One possibility is that PSD-95 serves to anchor newly arrived Kv channels at the plasma membrane, preventing their recycling and thus increasing their surface expression. PSD-95, however, is not expressed in heart, but its close relative SAP97 (synapse-associated protein 97) is.

Like PSD-95, SAP97 binds to most Kv1 channels. Unlike PSD-95, however, this binding causes retention of most Kv1 channels in the ER, at least in heterologous cells [31]. Kv1.5 is the sole exception. Its surface expression is instead dramatically increased when co-expressed with SAP97 [32–34]. This difference in Kv1.5 behaviour from that of other Kv1 channels is likely to be due to fundamental differences in the way Kv1.5 interacts with SAP97 in comparison with the SAP97 interactions with other members of the Kv1 family. Kv1.1–Kv1.4 channels bind SAP97 via their canonical C-terminal PDZ-binding domains and this binding is essential to SAP97’s effect [31]. Kv1.5 binding to SAP97, on the other hand, is irrelevant to its up-regulation by SAP97; deletion of the Kv1.5 C-terminal PDZ-binding domain is without effect [33,35]. Whereas Kv1.5 can be found to bind SAP97 when overexpressed in transiently transfected heterologous expression systems [32,34,36], no evidence of any such binding can be found in extracts of rat and canine cardiac myocytes [25,33]. Thus Kv1.5 up-regulation by SAP97 must occur via an indirect mechanism.

A role for phosphorylation

Perhaps SAP97 affects Kv1.5 expression via an intermediary molecule. One potential candidate is α-actinin-2. Known to bind both SAP97 and Kv1.5 [37], one function of this molecule is to link membrane-resident proteins to the cortical actin cytoskeleton. Perhaps, phosphorylation is important in the process: an intact threonine residue at position 15 in Kv1.5 is required for SAP97 co-expression to have its effect [35]. Although there is no evidence as yet that this threonine residue is indeed subject to phosphorylation, it is part of a consensus protein kinase C phosphorylation site.

There is a great deal of evidence that phosphorylation does affect the expression of a number of Kv1 channels. Tyrosine phosphorylation has been implicated in the down-regulation of Kv1.5 [38] and Kv1.3 [39] as well as other channels, but it is its role in the regulation of Kv1.2 expression that has been best elucidated. As demonstrated by Nesti et al. [40], phosphorylation of a specific Kv1.2 N-terminal tyrosine residue causes rapid internalization of the channel. Blocking of endocytosis by overexpression of a dominant-negative dynamin mutant wholly eliminated the down-regulation of Kv1.2 currents. Dynamin catalyses the scission of endocytic vesicles from the plasma membrane [41] and is required for clathrin-dependent and most clathrin-independent endocytoses [42].

Interestingly, like Kv1.5, Kv1.2 binds an actin-binding protein, in this case, cortactin [43], and Hattan et al. [43] speculate that cortactin may both anchor the channel at the plasma membrane and co-ordinate its interactions with the proteins involved in tyrosine-kinase-dependent endocytosis. It is tempting to speculate that actinin plays a similar role for Kv1.5 and filamin, an actin-binding protein that binds Kv4.2 [44], in the regulation of these channels. Whatever the case, as for Kv1.2, endocytosis is important in the modulation of Kv1.5 surface expression. Kv1.5 currents are dramatically increased when endocytosis is blocked by a dynamin-inhibitory peptide [45]. Internalization of the channel requires an intact N-terminal proline-rich SH3-binding domain, consistent with the possibility that tyrosine
phosphorylation may be a regulator of Kv1.5 endocytosis, as well.

Seehoohm et al. [46] have studied the mechanism by which another kinase, the SGK1 (serum- and glucocorticoid-induced protein kinase 1), regulates the KCNQ1/KCNE1 channel, composed of KCNQ1 α-subunits and KCNE1 β-subunits. Underlying the $I_{\text{ks}}$ (slowly activating K$^+$ current), KCNQ1/KCNE1 mutants are associated with forms of long QT syndrome, and functional expression of the channel can be up-regulated by SGK1 activation [47]. Using Xenopus oocytes and COS-7 expression systems, Seehoohm et al. [46] found that activated SGK1 phosphorylates a phosphatidylinositol kinase, PIKfyve, leading to the formation of PtdIns(3,5)P$_2$. This, in turn, stimulates Rab11a-dependent trafficking of KCNQ1/KCNE1 from the recycling endosome to the cell surface. In this first study to implicate Rab GTPases in Kv channel trafficking, Seehoohm et al. [46] further found that endocytosis of KCNQ1/KCNE1 required another Rab GTPase, Rab5. The Rab GTPases are major regulators of intracellular vesicular transport, and individual Rab proteins are associated with specific endosomal compartments. Rab5 is responsible for organizing and maintaining the early endosome; Rab11 is essential to the function of the recycling endosome, as well as for the trafficking of vesicles newly released from the trans-Golgi network [48].

**Molecular motors**

However a channel is targeted, and inserted into and removed from the plasmalemma, it must somehow be carried to and from the sites of those activities. Recent studies have demonstrated that the microtubule cytoskeleton is essential to these processes. Kif17, a kinesin isoform, has been shown to be essential to the forward trafficking of Kv4.2 in neurons [49]. Kinesins track along the microtubule cytoskeleton, generally acting as anterograde transporters, carrying cargo from the interior of the cell to the extremities. Expression of a dominant-negative Kif17 construct in the neurons blocked surface expression of the channel. Kif17 is not expressed in heart [50]. Thus it will be very interesting to determine whether another kinesin isoform is involved in Kv4.2 trafficking in cardiomyocytes.

Our laboratory has shown recently that dynein, the motor responsible for retrograde trafficking along the microtubule cytoskeleton, is also involved in the trafficking of Kv channels, specifically Kv1.5 [45]. Dynemin and Kv1.5 co-immunoprecipitate, and blocking of dynein motor function by overexpression of p50/dynamitin causes a greater than 2-fold increase in Kv1.5 currents when the channel is heterologously expressed. These increases in Kv1.5 functional expression matched those observed when endocytosis was blocked using the dynamin-inhibitory peptide. One interpretation is that interference with retrograde trafficking prevents the trafficking of newly formed endosomes. Unable to internalize further, these endosomes either reintegrate into the sarcolemma, interfere with the further endocytosis of the channel, or both, thereby increasing total Kv1.5 surface expression.

Interestingly, p50/dynamitin overexpression takes time to be effective. As shown in Figure 1, p50/dynamitin overexpression has no effect on Kv1.5 currents 24 h post-transfection but, again, doubles current density after 48 h. Perhaps, at early times post-transfection, p50/dynamitin expression is too low to interfere significantly with dynein motor function. Alternatively, perhaps, it takes time for Kv1.5 to accumulate at the cell surface once retrograde trafficking is blocked.

We have begun work to test directly whether the dynein motor is required for normal trafficking of Kv1.5 and other channels in cardiac myocytes. We have previously shown in rat cardiac myocytes that disruption of the microtubule cytoskeleton by nocodazole dramatically increases $I_{\text{kur}}$, the current underlaid by Kv1.5 [45], and experiments are under way to test the effects of specific dynein inhibition on this cardiomyocyte current.

**Drawing a map**

Despite all of the work that has been done on ion channel trafficking, no general road map has been derived. Therefore, in addition to studies of the effects of interference with channel trafficking, we are currently mapping the pathways followed by Kv1.5 as it travels to and from the plasma membrane. As previously shown via immunocytochemistry/confocal microscopy, Kv1.5 localizes to early endosomes as well as to the cell surface when expressed in HEK-293 cells (human embryonic kidney cells) [45]. Using a Kv1.5 construct tagged externally with GFP (green fluorescent protein) or an HA (haemagglutinin) tag, that finding has been extended to

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**Figure 1** Effect of p50 expression at 24 and 48 h on stably expressed Kv1.5
(A) Twenty-four hour current tracings; (B) current densities at 24 h; (C) 48 h current tracings; (D) current densities at 48 h. Cells were held at $-90$ mV and pulsed from $-80$ to $80$ mV in $10$ mV steps for $1000$ ms.
Kv1.5 and EEA1 co-localize in early endosomes

HEK-293 cells (left) and COS-7 cells (right) were transiently transfected with Kv1.5-HA and, 24 h later, washed and incubated with rabbit anti-HA on ice for 1 h, washed three times with unlabelled medium, and incubated at 37 °C for 10 min to allow the internalization of anti-HA-labelled Kv1.5. Cells were fixed with 4% (w/v) paraformaldehyde, permeabilized and blocked with 2% (w/v) BSA and then incubated with mouse anti-EEA1. Cells were then washed and stained with Alexa Fluor® 549-conjugated anti-mouse and Alexa Fluor® 488-conjugated anti-rabbit secondary antibodies, washed and mounted with DABCO (1,4-diazadiyclo[2.2.2]octane)/glycerol. Visualization was on a Zeiss Apotome microscope. Kv1.5 appears as green, EEA1 appears as red, and co-localization appears as yellow. Scale bar, 5 μm.

References

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