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Review

Cytoskeletal roles in cardiac ion channel expression ☆

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ABSTRACT

The cytoskeleton and cardiac ion channel expression are closely linked. From the time that newly synthesized channels exit the endoplasmic reticulum, they are either traveling along the microtubule or actin cytoskeletons or likely anchored in the plasma membrane or in internal vesicular pools by those scaffolds. Molecular motors, small GTPases and even the dynamics of the cytoskeletons themselves influence the trafficking and expression of the channels. In some cases, the functioning of the channels themselves has profound influences on the cytoskeleton. Here we provide an overview of the current state of knowledge on the involvement of the actin and microtubule cytoskeletons in the trafficking, targeting and expression of cardiac ion channels and a few channels expressed elsewhere. We highlight, also, some of the many questions that remain about these processes. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

Contents

1. Introduction

All eukaryotic cells harbor complex scaffolds of filamentous polymers that collectively comprise the cytoskeleton. Classically comprised of the actin, microtubule and intermediate filament cytoskeletons [1], these scaffolds perform a number of functions in addition to providing structural support to the cell. Intermediate filaments vary widely in their composition according to cell type; they function largely as mechanical stress absorbers [2]. In contrast, the actin and microtubule cytoskeletons are comprised of the same fundamental components across cell types, i.e., the α- and γ-actins and α- and β-tubulins, respectively. Like intermediate filaments, they provide structural support, actin bearing tension [3] and microtubules resisting compression [4]. Unlike intermediate filaments, the actin and microtubule cytoskeletons are also intimately involved in and essential to cell motility, intracellular trafficking, organelle localization, cytokinesis, membrane compartmentalization, and other functions [5–7].

In recent years, the degree to which the cytoskeleton influences the functional expression of ion channels has become increasingly

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evident. Channels are transported to and from the cell surface along cytoskeletal highways and they are frequently bound to cytoskeletal elements while at the cell surface. Furthermore, there are many instances in which interactions with the actin or the microtubule cytoskeleton directly or indirectly regulate the channels; likewise for the influence of those membrane-bound proteins on the various cytoskeletons.

In this review, we will highlight both the roles of the actin and microtubule cytoskeletons in the transport, targeting and functioning of cardiac ion channels, as well as, where the cases are particularly illustrative, channels expressed in other tissues. The roles of Rab GTPases in the processes will be discussed, as well. While much of this work has been conducted in heterologous mammalian cell expression systems or cardiomyoblast cell lines, newer approaches involving the transfection of adult cardiomyocytes have begun to confirm and extend findings made in those heterologous systems.

2. Channel interactions with the actin cytoskeleton

2.1. General features of the actin cytoskeleton

The cortical actin cytoskeleton is composed of polymers of "non-muscle" β- and γ-actins, and interacts strongly with interlaced spectrins [8]. The spectrins, in cooperation with molecules such as ankyrins, link the actin cytoskeleton to membrane proteins like adhesion molecules and ion channels [8]. In cardiac myocytes, spectrins are strongly associated with T-tubules and the sarcoplasmic reticulum [9,10].

In addition to serving as an anchor for many membrane proteins and cell adhesion molecules [11,12], the actin cytoskeleton serves as a scaffold from which endocytosis and exocytosis is conducted [13,14]. It is involved in the forward trafficking of connexin 43 to the intercalated disk of cardiomyocytes [15] and, very probably, in the similar trafficking of other membrane proteins. It is responsible also for the cellular motility that is important to tissue formation, wound healing and immune response [16,17], as well as the migration of neoplastic cells [11,17,18]. In cardiomyocytes, there is evidence for its association with costameres, T-tubules and the sarcoplasmic reticulum [8,19]. It is likely that one or both of the β- and γ-actins are also involved with T-tubule maintenance. Disruption of the actin cytoskeleton prevents loss of these structures in cultured rat ventricular myocytes [19]. The β- and γ-actins have been implicated also in the formation and maintenance of the intercalated disk [20]. The general features of both the actin and the microtubule cytoskeleton and their interactions with cardiac ion channels are illustrated in Fig. 1.

2.2. Channel interactions

Several cardiac-expressed ion channels have been demonstrated to interact with the actin cytoskeleton, including potassium channels Kv1.5 [21,22] and Kir2.1 [23], sodium channel $Na_v1.5$ [24], and the L-type Ca^{2+} channel [25]. Mutations that affect linkage to the actin cytoskeleton have been shown to affect ion channel expression *in vivo*. For example, knockout of the actin-linking 4.1R protein is associated with multiple cardiac phenotypes (bradycardia, long-QT syndrome, increased Ca^{2+} transients) in transgenic mice [26]. Perturbations in the expression of ankyrins, which act as chaperones as well as linkers of membrane proteins to the actin cytoskeleton via spectrins, are associated with prolonged QT intervals in both mice and humans [27].

The interactions between ion channels and the actin cytoskeleton are generally not direct but instead involve intermediary actin-binding molecules. α-Actinin2 has been shown to link Kv1.5, Nav1.5 and $K_{Ca}2.2$ [21,22,24,28] to the actin cytoskeleton. The Kir2.1 inward rectifier potassium channel is linked via filamin-A [23] and the L-type calcium channel binds to the actin cytoskeleton via Ahnak [25,29,30], very likely anchoring the channels in the T-tubules [25]. Ankyrin-G has been shown to be necessary for the normal expression of $Na_v1.5$ and to its localization to the intercalated disk and to T-tubules in cardiomyocytes [31,32]. Indeed, these workers found that direct binding of the channel to Ankyrin-G was required for this normal localization of $Na_v1.5$. Ankyrin-G performs a similar function with the KCNQ potassium channels in neurons [33] and interaction with ankyrin-B is necessary for the maintenance of normal Kir6.1 expression in the heart [34]. Ankyrin-G has been implicated, as well, in intercalated disk structural integrity. Loss of ankyrin-G in cardiac myocytes was found to result in a significant reduction in desmosome and gap junction (Cx43) densities at the intercalated disk along with a decrease in intercellular adhesion strength and electric coupling [35].

Results of experiments in which linkages to the actin cytoskeleton are disrupted indicate that the actin cytoskeleton is very important in regulating channel numbers at the cell surface. The functional expression of several channels is dramatically increased by treatment with actin depolymerizing agents such as cytochalasin D. Inward rectifier potassium currents, underlain by the Kir2.1 and the K_{ATP} channels, are increased by actin cytoskeleton depolymerizing agents [36,37]. The effect of cytochalasin D on the expression of Kv1.5 is similarly potent. Treatment of HEK293 cells expressing Kv1.5 with the drug resulted in a roughly 4-fold increase in functional expression of the channel [21]. Since channel kinetics were unchanged, this was very probably due to the insertion of increased numbers of channels into the plasma membrane. The authors obtained similar results with α-actinin2-antisense RNA. Thus, it is likely that disruption of the linkage of Kv1.5 to the actin cytoskeleton frees intracellular pools for insertion into the plasma membrane. In contrast, surface expression of the Ca^{2+}-sensitive $K_{Ca}2.2$ potassium channel is reduced by co-expression with α-actinin2-antisense RNA [28]. Clearly, α-actinin2 linkage of an ion channel to the actin cytoskeleton can result in anchorage both at the cell surface and away from that surface. In the case of Kv1.5, the linkage may be most important for maintenance of internal pools of channel whereas, for $K_{Ca}2.2$, it appears to be important, in effect, to anchoring that channel at the plasma membrane. Lu et al., [28] found that interference of $K_{Ca}2.2$ binding to actinin led to a rapid internalization of the channel and a strong association with EEA1, indicating that the channel was indeed being retained in early endosomes after internalization. It will be interesting to learn how the binding of the same molecule can affect the surface expression of two potassium channels in opposite ways.

The expression of Kv1.5 is increased also when the channel is co-expressed with SAP97 [38–40], a PDZ-protein that likely also anchors the channel indirectly to the actin cytoskeleton [39]. This seems most likely to be due to an inhibition of the internalization of channels from the plasma membrane. The HCN2 and HCN4 cardiac pacemaker channels also interact with SAP97 [41] and are thus, also likely linked to the actin cytoskeleton. Kir2.1 is another channel that associates with the actin cytoskeleton via PDZ proteins [42]. This channel links via another actin-binding protein, filamin-A, at least in vascular smooth muscle cells [23]. Interestingly, when filamin was over-expressed, channel numbers at the cell surface were increased, indicating that internalization was likely inhibited. There is evidence that another cardiac-expressed channel, Kv4.2, is also affected by the state of the actin cytoskeleton. In this case, however, disruption of the actin cytoskeleton had the opposite effect to that reported for the other channels. The current which this channel underlies, I_{to}, was reduced by cytochalasins and increased by the actin cytoskeleton-stabilizing agent phalloidin [43].

Not all effects of the actin cytoskeleton are due to anchoring of channels at or away from the plasma membrane, however. Results from work with the $Na_v1.5$ sodium channel indicate that the kinetics of those channels are also affected [44]. Treatment of rat and rabbit ventricular myocytes with cytochalasin D resulted in a slowing of inactivation of that channel. In single channel analysis, treatment of the myocytes with the drug resulted in bursts of openings upon

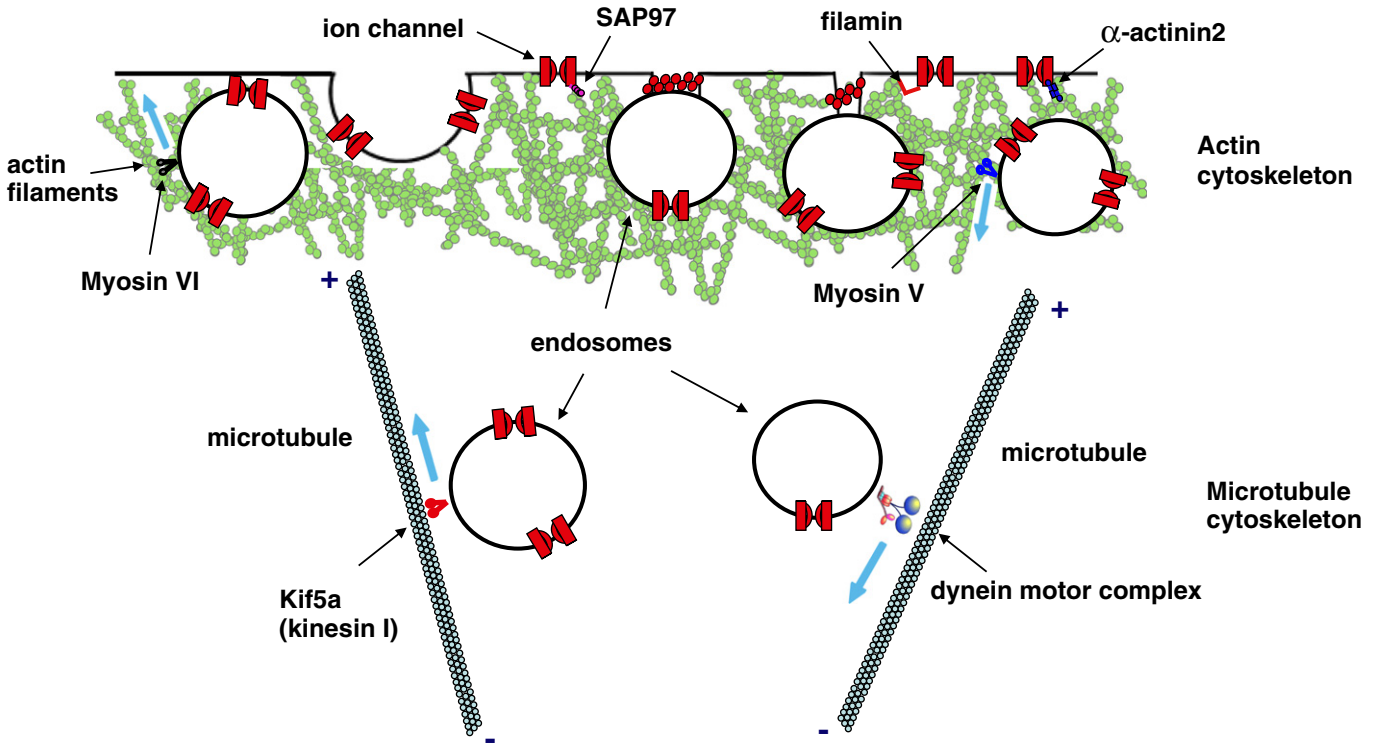

Fig. 1. Key cytoskeletal interactions with cardiac ion channels. Key interactions between cardiac ion channels and the actin and microtubule cytoskeletons are shown. On microtubules, kinesins carry ion channels in vesicles toward the plasma membrane and dynein, away from it. Myosins V and VI are depicted carrying vesicular bound ion channels in the directions indicated. SAP97, filamin and α-actinin2 are shown tethering plasma membrane resident channels to the actin cytoskeleton.

depolarization occurring at time points long after bursts in control myocytes had ceased. The apparent open probability of the channels in the whole cells, however, was paradoxically reduced. Although the authors did not consider the possibility, the result probably indicates a reduction in channel numbers at the cell surface.

Thus, while manipulation of the actin cytoskeleton does in some cases affect channel kinetics [44], it is likely that the main role of the actin cytoskeleton in the modulation of cardiac ion channel expression is in the trafficking and anchoring of those channels. Indeed, even the kinetic effects on the sodium channel are likely related to its anchoring to the actin cytoskeleton. The mechanisms by which the actin cytoskeleton modulates the localization and trafficking of cardiac-expressed ion channels are only beginning to be unraveled.

2.3. Myosins V and VI

As mentioned above, the actin cytoskeleton is essential for immediately submembranous membrane protein trafficking as well as for endocytosis. This trafficking is conducted, at least mainly, by the myosin V and myosin VI motors which track along the actin filaments [5]. While there is little direct evidence as yet on the roles of these motors in the trafficking of cardiac ion channels, their essential roles in the trafficking of other membrane proteins indicates that they are very probably important is this trafficking as well.

Myosin VI, which, unlike other myosins, tracks towards the "pointed" (−) end of the actin filaments [45], has been shown to be important to the endocytosis of plasma membrane-resident CFTR in endothelial [46,47] and in HEK293 cells when heterologously expressed [48]. The motor has been similarly implicated, along with SAP97, in the forward trafficking of AMPA receptors in hippocampal neurons [49], and in clathrin-dependent endocytosis [50–54]. Myosin VI is involved also in the recycling of internalized plasma membrane proteins back to that locale [55] and has been implicated in secretory vesicle fusion with the plasma membrane [56].

The myosin V family, carrying traffic in the opposite direction, is comprised of three closely related motors, myosin Va, b and c. They are typical myosins, in that they track towards the barbed (+) end of actin filaments. Like myosin VI, they carry vesicular traffic through the cortical actin cytoskeleton [57,58]. Myosin V has been shown to work with kinesin to mutually increase the processivity of both motors [59] and myosin V isoforms have been shown to capture vesicles delivered by the microtubule-dependent trafficking system to the cortical actin cytoskeleton in melanosomes [60]. Myosin Vb has been directly implicated in the delivery of CFTR to the plasma membrane in human airway epithelial cells [61]. It is highly likely that these motors will prove very much involved in the trafficking of cardiac ion channels as well.

3. Channel interactions with the microtubule cytoskeleton

3.1. General features of the microtubule cytoskeleton

Microtubules are long tubular structures, approximately 250 Å in diameter, composed of repeated heterodimers of α- and β-tubulin, assembled in a polar, head-to-tail manner. The plus end of the microtubule faces outward towards the plasma membrane and the minus end, towards the nucleus, i.e., the centriole [62]. The two tubulins are closely related globular proteins, each with a molecular weight of approximately 55 kD. The great majority of microtubules form as single tubes and, collectively, constitute the microtubule cytoskeleton. The mitotic spindle is also composed of these structures, and microtubules are the main component of cilia, as well. The microtubule cytoskeleton is essential to vesicular trafficking from the ER, through the Golgi, on to the plasma membrane and back into the interior of

the cell. In adult cardiomyocytes, microtubules are essential for the normal structure of the cells and for the proper localization of organelles [5,6]. The tubules are generally aligned along the cell's longitudinal axis [63]. Microtubule depolymerizing agents increase beating rates in neonatal hearts [64–66]; increased microtubule density underlies the reduced contractility common in cardiac remodeling [67].

Microtubules are highly dynamic and grow by the addition of α/β dimers to the plus end of the tube and retract by the dissociation of dimers from the same site. Nucleation occurs at the centriole with the aid of a third tubulin isoform, γ-tubulin in a γ-tubulin ring complex [68,69]. In polymerizing, both members of the tubulin dimer are bound to individual GTP molecules. The α-tubulin-bound GTP remains stable after polymerization, but the β-tubulin-bound molecule may quickly hydrolyze. If GTP hydrolysis to GDP occurs at the tip of the microtubule, that dimer will dissociate. And, if, as is likely, the adjacent tubulin molecules are also GDP-bound, the microtubule will undergo depolymerization. Depolymerization can be extreme or limited; addition of a GTP-bound tubulin will stop the process and re-initiate elongation [70,71].

Tubulins are subject also to a number of post-translational modifications [72], most of which occur after polymerization [72,73]. Among the best studied are detyrosination/tyrosination of the α-tubulin C-terminus, acetylation of an internal lysine in that subunit, and polyglutamylation and glycylation of both α- and β-tubulins. These modifications can influence microtubule stability as well as the interactions of microtubules with themselves and with the dynein and kinesin motors that track along them. That the modifications influence motor interactions, especially, seems potentially relevant to the trafficking of ion channels. For instance, it is possible that differential post-translational modifications may mark subsets of microtubules that lead to specific cellular locales. Consistent with this possibility, there is evidence that components of the dynein–dynactin complex preferentially interact with the "+" ends of tyrosinated microtubules [74,75]. Similarly, various kinesin isoforms exhibit differing affinities for detyrosinated, acylated and polyglutamylated microtubules [76–78]. What this means is unclear, though. To date, there is no evidence, for example, for the orientation of differentially modified microtubules towards specific cardiomyocyte structures (e.g., T-tubules or intercalated disks), a phenomenon one might expect if these microtubule modifications are marking routes to specific cellular locales. Indeed, since these post-translational modifications occur post-assembly, accumulate in an age-dependent manner and may directly stabilize microtubules [79], more extensively modified microtubules may simply signal that they are stable microtubules. Motors may preferentially bind them simply to reduce the probability of microtubule depolymerization interfering with the delivery of their cargoes. (For more on dynein and kinesin see Sections 3.4 and 3.5, below).

3.2. A dramatic example: TRPV channels and the microtubule cytoskeleton

Among the most dramatic interactions described to date between the microtubule cytoskeleton and ion channels involve TRPV channels. Although not expressed at significant levels in the heart, they are expressed in arterial smooth muscle [80] and other cell types, in addition to their prominent roles in nociceptive neurons [81]. TRPV channels are non-selective cation channels, with high Ca^{2+} permeability, involved in the detection and/or transduction of chemical or physical stimuli [82].

At least two members of the TRPV family, TRPV1 [83] and TRPV4 [84], interact directly with the microtubule cytoskeleton. TRPV1 is a thermosensitive channel that opens also in response to endogenous N-arachidonoyl-dopamine [85] and to capsaicin [86]. TRPV4 is also sensitive to heat [87] and is mechano- [88] and osmosensitive [89]. Both TRPV1 and TRPV4 bind to the microtubule cytoskeleton [83,84], via motifs in the intracellular C-terminus of the channels. Indeed, TRPV1 channels are reportedly anchored at their sites of action by the microtubule cytoskeleton [82]. In the case of TRPV1, microtubule binding has been shown to be with the E-hook domains of both α- and β-tubulins, with the affinity for β-tubulin being higher [90]. Binding is to the plus end of intact microtubules but not to tubulin dimers [91]. TRPV4 interacts also with the actin cytoskeleton [84], whereas TRPV1 evidently does not [83,90].

The interactions of the microtubule cytoskeleton with TRPV1 have been more thoroughly studied than those with TRPV4. Upon activation of TRPV1, peripheral microtubules are rapidly depolymerized [92,93]. This rapid microtubule depolymerization leads to growth cone retraction in neurons [82] and has been shown to increase the motility of other cell types [93,94]. The effect is apparently not dependent on a substantial calcium influx. Neither chelation of external Ca^{2+} nor depletion of internal Ca^{2+} stores prevents depolymerization upon TRPV1 activation [92,95]. While the mechanism by which this occurs is unknown, it has been postulated that phosphorylation of microtubule-associated proteins (MAPs) may be involved or that calmodulin may be a part of the process, initiating a microtubule-depolymerizing enzymatic cascade in response to even small increases in intracellular Ca^{2+} [82].

3.3. Microtubules and cardiac ion channels

While the effects of TRPV channel activation on the microtubule cytoskeleton are dramatic and powerful, the interactions of other ion channels (and probably of TRPV channels in their journeys to and from the cell surface) are also very important. It is along microtubules that, upon exit from the endoplasmic reticulum, newly formed ion channels take their first steps towards the plasma membrane. This trafficking is dependent, of course, on the dynein and kinesin motors which track along the microtubules. While it is not clear whether the transport of newly synthesized ion channels through the endoplasmic reticulum (ER) to the ER exit site(s) requires cytoskeletal elements or not [96], exiting membrane proteins require both intact microtubules and dynein function to reach the Golgi apparatus [97], and thereafter, to travel through it and on to the plasma membrane. The microtubule cytoskeleton is important, also, once the channels have reached the plasma membrane, in the endocytosis and post-internalization trafficking of these proteins.

The depolymerization of microtubules by treatment with colchicine or nocodazole has potent effects on the surface expression of several cardiac-expressed ion channels. In adult rat atrial myocytes, nocodazole roughly doubled the I_{kur} currents underlain by the voltage-gated potassium channel Kv1.5 [98]. Surface expression of the channel, assayed immunocytochemically, was also increased. The voltage-gated Kv4.2-associated I_{to} and the hERG-associated I_{kr} currents were similarly both increased by nocodazole in ventricular myocytes, [99], as was the functional expression of the ClC-2 chloride channel [100]. Interestingly, nocodazole treatment resulted in reduced functional expression of the Kir2.1 inward rectifier channel [99].

Stabilization of the microtubule cytoskeleton with taxol has been shown to reduce $Na_v1.5$ sodium channel density at the sarcolemma in neonatal rat cardiomyocytes [101]. Sodium current density was halved in neonatal myocytes by this treatment. Surface expression of KCNQ1, on the other hand, is not affected by either microtubule depolymerization or stabilization but depolymerization abolishes regulation of the channel by Protein Kinase A [102].

Although changes in sodium channel kinetics have been reported in response to microtubule depolymerization [103], for other channels tested, to date, channel kinetics appear to be unaffected [98,99]. Thus, in most cases the evidence favors instead interference with normal channel trafficking. A great deal has been learned in recent years about the mechanisms and precise players involved in this trafficking. This traffic is carried by the dynein and kinesin motors. Several studies have appeared on the roles of these motors in the trafficking of cardiac-expressed ion channels:

3.4. Dynein

Dyneins are retrograde motors, traveling along microtubules from the "+" to the "−" ends of the tubules. Three major isoforms exist, two of which are involved in trafficking; the third is important in ciliary/flagellar movement [104]. Dynein I carries the bulk of retrograde vesicular traffic in the cytoplasm. The movement-generating portion of the motor consists of two identical 520 kD heavy chains which utilize ATP for energy plus several intermediate and light chains [105]. Some of these intermediate and light chains are involved in dynein function in general and others are specific to different intracellular trafficking processes, such as endosomal transport or ER to Golgi transport [106]. Another large complex, dynactin, acts as an essential bridge between the motor and cargo [105].

The dynactin complex is highly sensitive to the stoichiometry of its subunits in the surrounding cytoplasm. Over-expression of individual dynactin subunits such as p50 results in a dissociation of the dynactin complex [107]. This property has been extensively exploited as a method to experimentally unlink dynein from its cargoes, effectively eliminating dynein function [107–109]. The involvement of dynein in the trafficking of ion channels has been studied by this method.

The first ion channel shown to interact with dynein *in vivo* was a chloride channel, ClC-2 [100]. In the heart, this channel underlies the hyperpolarization-activated inwardly rectifying chloride current [110]. When dynein function was disrupted by over-expression of p50 or by pharmacological block with erythro-9-[3-2-(hydroxynonyl)]adenine (EHNA), ClC-2 currents were dramatically increased [100]. These increased currents were associated with reduced numbers of channels in early endosomes, indicating that internalization of the surface-expressed channel was probably reduced. In the same study, a physical interaction between dynein and the ClC-2 channel was demonstrated by co-immunoprecipitation and by affinity chromotography combined with MALDI-TOF mass spectrometry.

Choi et al. [98] employed similar methods to determine that dynein is also involved in Kv1.5 trafficking. p50 over-expression in HEK293 cells expressing the channel roughly doubled Kv1.5 current densities. Immunocytochemistry and experiments testing the susceptibility of the channel in intact cells to external protease digestion confirmed that channel numbers at the cell surface were dramatically increased. Kv1.5 co-immunoprecipitated with dynein intermediate chain and with p50. Like ClC-2, a subpopulation of the channel localized to early endosomes and block of endocytosis greatly reduced the number of Kv1.5-positive early endosomes.

Dynein has been implicated in the trafficking of several additional ion channels that are expressed in the heart. Loewen et al. [99] showed that the surface expression of hERG, Kv2.1, Kv3.1, Kv4.2 and Kir2.1 are all increased by over-expression of p50 in heterologous cells. The results with Kir2.1 were particularly interesting because they represent the only case to date where the effects of p50 over-expression on a cardiac ion channel do not reflect those obtained with nocodazole. That microtubule-depolymerizing agent instead reduced Kir2.1 expression. The authors speculated that this could be due to a greater dependence of Kir2.1 on the microtubule cytoskeleton for delivery of the channel to the plasma membrane. This would be likely if maintenance of Kir2.1 surface expression is more dependent than other channels on new channel synthesis or if intracellular pools of the channel are outside the actin cytoskeleton (and those of other channels are under the control of the actin cytoskeleton). It will be interesting to learn whether either of these hypotheses is correct.

3.5. Kinesins

Forward trafficking along microtubules is largely dependent on kinesins. The kinesins are a large family of motor proteins, encoded by 45 genes in mammals, that carry anterograde traffic along microtubules [111]. Much smaller than dynein, the various kinesins have varying cargo-specificities. Like dynein, their movement along microtubules is ATP-dependent [112]. It is highly likely that much of the forward trafficking of ion channels beyond the Golgi and on to the cell surface is kinesin-dependent. It is also likely that different ion channels are trafficked by different kinesins.

Among ion channels that are expressed in the heart, Kv4.2 was the first to be shown to be trafficked by a specific kinesin, Kif17, although that demonstration was made in rat cortical neurons [113]. Transfection with a dominant negative isoform of Kif17 blocked Kv4.2 trafficking out of the cell body; transfection with dominant negative isoforms of several other kinesins had no such effect. Similarly, the channel colocalized with Kif17 but not with the other kinesins tested, and the two proteins co-immunoprecipitated as well. While it is unknown whether Kif17 is involved in Kv4.2 trafficking in cardiomycytes, this kinesin has been shown to be involved in Kv1.5 trafficking in the HL-1 atrial cardiomyoblast cell line [114]. Expression of a Kif17 dominant negative in these cells significantly reduced Kv1.5 surface expression but expression of a dominant negative kinesin I isoform did not have a significant effect.

Interestingly, in a ventricularly-derived cardiomyoblast line, H9c2 cells, a kinesin I isoform, Kif5b, has been shown to be required for Kv1.5 trafficking to the cell surface [115], similar to the dependence of Kv1 channels on Kif5b for axonal transport in neurons [116]. In the ventricular myocytes, expression of a Kif5b dominant negative for 24 h prior to induction of Kv1.5 expression almost completely blocked Kv1.5 surface expression as assayed both immunocytochemically and electrophysiologically [115]. Over-expression of wild-type Kif5b dramatically increased expression of the channel. It seems that the kinesin isoforms involved in the trafficking of even a single channel type may vary even between such similar cell types as atrial and ventricular myoblasts. It will be of great interest to learn which kinesin isoforms perform this role in adult cardiomyocytes.

It will also be important to determine how the various cardiac ion channels are identified and carried by kinesins in those cells. Results from work in neurons may prove instructive in designing experiments probing these questions. In rat cortical neurons, the interaction of Kv1.3 with Kif5B requires an intact N-terminal T1 domain in the channel [116]. In contrast, the interaction of Kv4.2 with Kif17 in the same cell type involves that channel's C-terminus [113]. Trafficking of Kv1.2 channels has been shown, in hippocampal neuronal axons, to conducted by yet another kinesin, Kif3 [117]. The targeting process in that case involves the, likely sequential, binding of a beta subunit of the channel, Kvβ2, to both Kif3 and to the EB1 microtubule "+" end tracking protein. The authors hypothesize that Kif3 may carry the channel/β-subunit complex to the microtubule + end where either EB1 might somehow assist with the transfer of the cargo-moving complex to a neighboring microtubule or, if the axonal membrane is in close vicinity, facilitate delivery of the vesicle harboring the channel. Clearly, kinesin-dependent transport in neurons is complex. Undoubtedly, the binding and delivery of ion channels in the heart will prove similarly complicated. Channel-dynein/dynactin interactions will likely prove complicated as well.

4. Roles of small GTPases in cytoskeleton-dependent ion channel trafficking

The cardiac ion channels that are trafficked along actin and microtubules are not carried as free molecules. Instead, like other membrane proteins, they are carried in small vesicles, i.e., endosomes. The endosomes involved in retrograde and anterograde trafficking are regulated by a group of over 60 small GTPases known as the Rab GTPases [118]. Dynein and kinesins [119] and myosins V and VI [57] have all been shown to interact with members of this group.

Rab GTPases cycle between active vesicle-associated GTP-bound and inactive free cytoplasmic GDP-bound states [120] and regulate the budding, tethering, delivery and fusion of membrane vesicles [118,121].

Specific Rab GTPases are involved in specific steps in the trafficking pathways. The sequential binding and unbinding of the various Rab GTPases couples each stage of vesicle transport to the next, conferring directionality on the traffic. Several Rab GTPases have been demonstrated to be involved in the transport of cardiac-expressed ion channels. Among those implicated in cardiac ion channel trafficking, most are known to interact with cytoskeleton-associated motors. Rab4, Rab5, Rab9 and Rab11 interact with isoforms of one or both of dynein and kinesin, and Rab 7 has been shown to bind dynactin [119,122].

Rab GTPases are involved in all stages of vesicular trafficking in cells. As indicated above, the roles of several in the trafficking of cardiac ion channels have been investigated. While only Kv1.5 and Kv4.2 trafficking have been studied in cardiac-derived cells or cardiomyocytes themselves, the trafficking of other cardiac channels have been shown to depend on Rab GTPases when they were expressed in heterologous or other cell systems. Among the Rab GTPases so investigated are Rab1, Rab4, Rab5, Rab7, Rab9 and Rab11 plus a more distantly related small GTPase, Sar1. Rab1 and Sar1 are involved in ER to Golgi trafficking [123–125]. Rab11 is involved in traffic from the Golgi to the plasma membrane and, especially, in the recycling of internalized plasma membrane proteins back to that locale [126,127]. Rab5 is important in clathrin-mediated endocytosis and early endosome formation and maturation [128]. Rab4 binds to early endosomes, directing portions of them and their contents back to the plasma membrane [129]. Rab7 is associated with the late endosome directing its trafficking to the lysosome [130,131] and to the proteasome [132], whereas Rab9 which also associates with late endosomes, directs those with which it is bound to the trans-Golgi network instead [133]. Fig. 2 schematically summarizes the roles of Rab GTPases known to be involved in cardiac ion channel trafficking.

Most studies to date have investigated the post-internalization trafficking of surface-expressed channels. Two studies have examined the forward trafficking pathways that carry newly synthesized cardiac ion channels to the plasmalemma. Included in the first study were tests for the involvement of several Rab GTPases and Sar1 in the trafficking of Kir3.1/Kir3.4 heterotetramers in HEK293 cells [134]. This heterotetramer underlies the cardiac I_{KAch} current. The authors found that functional Rab1 and Sar1 were required for surface expression of the channel but that none of Rab2, Rab6, Rab8 or Rab 11 were required for that expression. The second study to investigate forward trafficking of cardiac ion channels was conducted in transfected adult ventricular myocytes [135]. In that study, Rab1 and Sar1 were both found, similarly, to be necessary for the normal trafficking of Kv4.2, indicating that the channel traffics in a conventional manner from the ER to the Golgi in

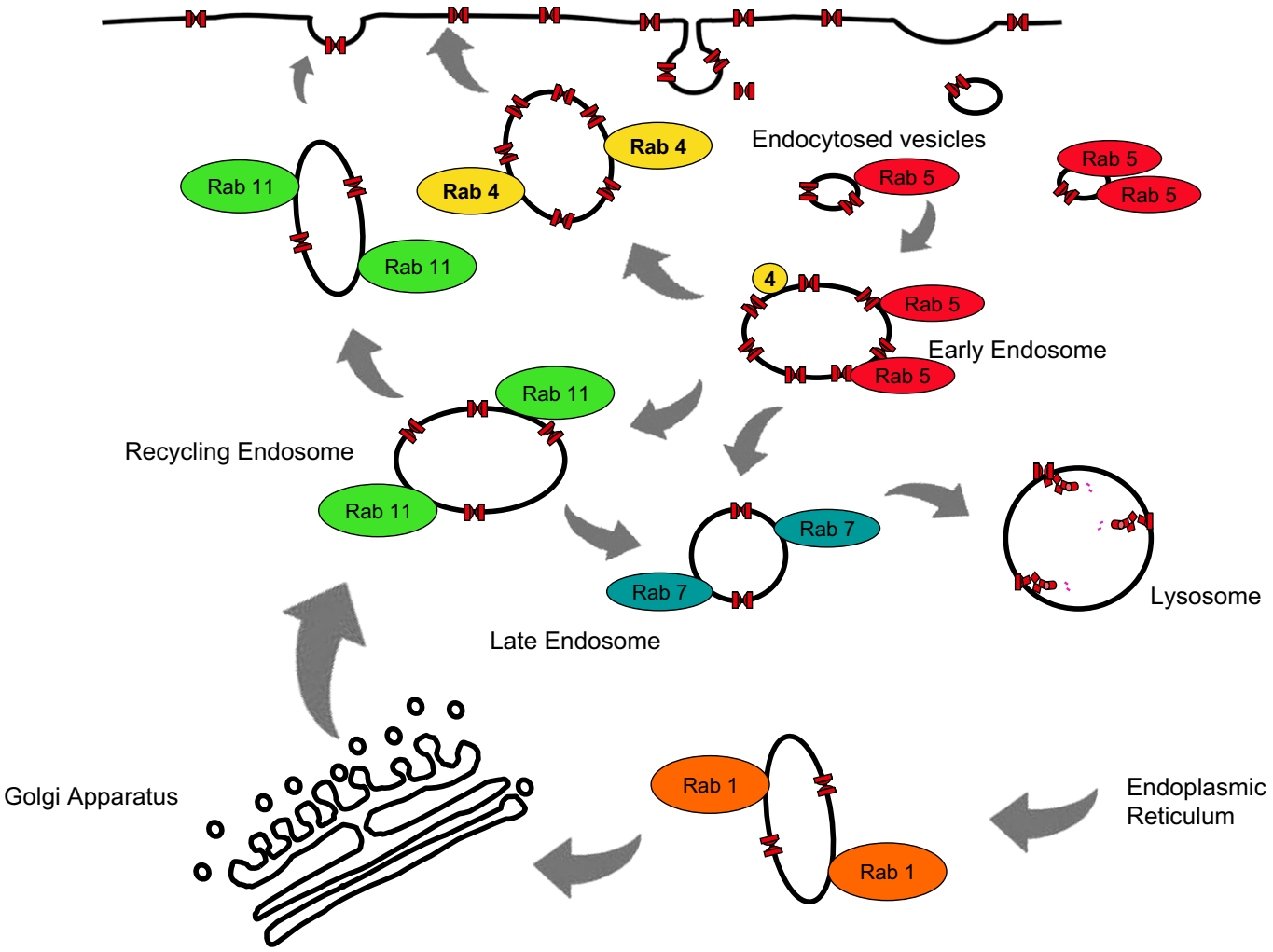

Fig. 2. Involvement of Rab GTPases in cytoskeleton-dependent ion channel trafficking. Rab GTPases known to be involved in cardiac ion channel trafficking are shown. Rab1 and Sar1 (not shown) are required for delivery of newly synthesized channels from the endoplasmic reticulum to the Golgi apparatus. Rab11 is involved in movement of channels from the Golgi to the plasma membrane and in the recycling of channels internalized from the plasma membrane back to that locale. Rab5 is involved in the endocytosis of plasma membrane-resident channels and Rab4 redirects internalized channels back to the plasma membrane. Rab7 is important in the shunting of internalized channels for degradation. Not shown is Rab9, involved in the maintenance of intracellular pools of at least one channel. Rab4, Rab5 and Rab11 have all been shown to interact with both dynein and kinesin isoforms. Rab9 binds a kinesin, and Rab 7 interacts with dynein via dynactin.

the heart. This is quite different from Kv4.2 trafficking in some other cell types, where an unconventional pathway, independent of Sar1, is operative [136,137]. The involvement of these GTPases in the trafficking of other cardiac ion channels has yet to be investigated.

In studies of post-internalization trafficking, Rab5 has been shown to be involved for all cardiac-expressed ion channels tested to date. These include Kv1.5 [138], Kv4.2 [135], CFTR [139], and KCNQ1/KCNE1 [140], although Kv1.5 endocytosis may be independent of Rab5 in HL-1 cells [141]. Recycling of a subset of internalized channels back to the plasma membrane, via both Rab4- and Rab11-dependent pathways, has been demonstrated, also, for all of these channel types [115,135,139–141]. In the cases of Kv1.5 and Kv4.2, the Rab 4-dependent recycling pathway appears to be dominant [135,138,141], whereas for CFTR, the Rab11-dependent recycling pathway predominates [139].

Internalized Kv1.5 localizes also in part to Rab7-positive vesicles in H9c2 cells [138], implicating the lysosomal degradation pathway in the maintenance of normal expression of that channel. While expression of a Rab7 dominant negative had little effect on Kv1.5 currents in these cells, over-expression of wild type Rab7 led to significant decrease in those currents. Similarly, over-expression of wild type Rab7 has also been shown to decrease the amount of CFTR protein in BHK-21 cells [139]. A portion of the internalized CFTR channel is known to be shunted instead to intracellular pools. Over-expression of Rab9, which regulates transport of internalized vesicles to the trans-Golgi network [133], caused a substantial in intracellular CFTR protein [139]. Whether other channels similarly traffic in part via Rab9 remains to be determined.

There is clearly a great deal yet to be learned about the involvement of the many Rab GTPases in cardiac ion channel trafficking. How are the fates of channels chosen by the various Rabs and their effectors? To what degree do ion channels share trafficking pathways and in what ways to the pathways differ? All of this Rab-dependent trafficking is likely carried on microtubule and actin rails.

5. Conclusions and prospects

The actin and microtubule cytoskeleton are essential to the proper expression of ion channels in the heart. These cytoskeletal scaffolds carry the channels to and from the plasmalemma and are probably involved in the localization of specific channels to specific domains on the cardiomyocyte surface. Motor proteins and associated Rab GTPases play major roles in this trafficking, yet we have very little understanding of just how their ion channel cargoes are selected and targeted. A great many questions remain.

Both Kv1.5 and Nav1.5 localize primarily to the intercalated disk in cardiomyocytes. Is this localization due to anchoring to cytoskeletal elements there, to specific delivery to that locale and/or by selective uptake of the channels from other locales on the cell? Are cardiac ion channels, as occurs for G-protein coupled receptors [142,143], organized by the actin cytoskeleton into functional units with other membrane proteins? To what extent are the actin and microtubule cytoskeletons involved in the dramatic changes in ion channel expression [144] that occurs in cardiac remodeling after infarction or congestive heart failure? Changes in microtubule dynamics are well documented in this latter case [145,146]. Can manipulations of the microtubule and actin cytoskeletons, or the proteins that connect them to ion channels, be employed in novel treatments of heart disease? The answers to these and other questions will greatly improve our understanding of cardiac functioning and may well lead, in the long run, to improved treatment and prevention of cardiac disease.

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