Connexin 43 and CaV1.2 Ion Channel Trafficking in Healthy and Diseased Myocardium

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Overview of Ion Channel Trafficking in the Heart

A remarkable aspect of cardiac ion channel biology is that individual ion channels have half-lives on the order of hours. For example, Connexin 43 (Cx43) gap junction proteins have a half-life of 1 to 3 hours,1,2 whereas potassium channels, calcium channels, and the sodium–calcium exchanger have half-lives that are reported in the 2 to 8 hours range.3,4 The short life span of ion channels suggests that there needs to be efficiency in their life cycle and movements which follow the order of: formation, delivery to the correct subdomain on plasma membrane, behavior once in membrane, and internalization back into the cell. To maintain this efficiency in ion trafficking, thousands of individual proteins contribute to a functional equilibrium. Mutations in a single protein can disrupt this equilibrium and over time manifest as arrhythmogenic substrate or cardiomyopathy.

Cardiomyocytes use common intracellular organelles and machinery to produce and shuttle ion channel proteins to their specific organelles and functional subdomains at the cell membrane. After gene transcription in the nuclei, proteins are translated and subjected to post-translational modification in the endoplasmic reticulum (ER) and then further modified in the Golgi apparatus. For ion channels, sorting and delivery to their subcellular destination begins in the Golgi apparatus. The Golgi complex is usually found adjacent to the lateral side of each nucleus in mammalian ventricular cardiomyocytes. Co-localized with each Golgi is the centrosome at which microtubules form an intricate and dynamic outgoing network,18 which are docked onto molecular motors and delivered to the cell periphery along microtubules.7 These extending microtubules form an intricate and dynamic outgoing network capable of shuttling ion channel–containing vesicles to their destinations. In the context of trafficking, one can consider the Golgi to be the loading dock and microtubules the highways along which packets of channels are delivered to the plasma membrane.

The mechanisms by which microtubules exert their specificity in interacting with the membrane subdomains are now being elucidated. Our report in 200710 and subsequent studies11–13 have led to the Targeted Delivery model of ion channel delivery. The Targeted Delivery model has since been supported by multiple other reports.14–17

Targeted Delivery

Targeted Delivery is the understanding that channels, once formed and exiting the Golgi, can be rapidly directed across the cytoplasm to their respective specific membrane subdomains. The highways for transport are microtubules whose negative ends originate at Golgi-oriented microtubule-organizing centers and whose positive ends are growing outward and can be captured at the plasma membrane by membrane anchor proteins and complexes. Specificity of delivery is a combination of the individual channel, the plus-end-tracking proteins at the positive ends of microtubules which guide microtubule growth and capture, and the membrane-bound anchor complex which captures the microtubule thus completing the highway for channel delivery. Actin cytoskeleton serves as en route rest-stop stations to redirect microtubules,18 providing additional sorting sites. This Targeted Delivery paradigm of ion channel forward trafficking has been generalized to several cardiac ion channels and explored in terms of various cytoskeletal elements and anchor proteins.19

In this review, Targeted Delivery is explored in detail for 2 different types of channels: Cx43 hemichannel trafficking to the intercalated discs and CaV1.2 channel trafficking to T-tubules.

Cx43 Trafficking in the Heart

Connexins are ubiquitous transmembrane proteins which are encoded by ≥20 different genes in human with Cx43 being the most commonly expressed in all organ systems, particularly in the heart.20,21 An extensively studied and well-appreciated function of connexins is their ability to form gap junctions. To form a gap junction, 6 connexin monomers from 1 cell oligomerize to form a transmembrane channel referred to as a connexon or hemichannel. The connexons from 1 cell then dock and couple with apposing connexons on neighboring cells and coalesce into dense gap junction plaques.22–24 Gap junctions are specialized channels that aid in the intercellular exchange
of small metabolites, secondary messengers, and ions carrying electric signals between neighboring cells, therefore allowing cells to cooperate both electrically and metabolically. Data also exist that Cx43 hemichannels can occur as free, nonjunctional channels in the plasma membrane. These hemichannels are normally closed but may open in response to various triggers including cell depolarization, decreased extracellular Ca\(^{2+}\) ion concentration, increased intracellular Ca\(^{2+}\) concentration, and alterations in the phosphorylation or redox status. Generally, the opening of plasma membrane Cx43 hemichannels is considered to be associated with pathological rather than physiological entities, contributing to cell swelling and cell death. In the heart and brain cells, excessive hemichannel opening allows the entry of Na\(^{+}\) and Ca\(^{2+}\) and the escape of K\(^{+}\), adenosine triphosphate (ATP), and other small metabolites, leading to osmotic shifts, energy depletion, Ca\(^{2+}\) overload, and cell death promotion. Therefore, blockage of the Cx43 hemichannels using pharmacological inhibitors can possibly have protective effects against cardiac insult such as in the case of ischemia/reperfusion injury.

In the heart, localization of Cx43 gap junctions at the intercalated discs is crucial to provide the intercellular coupling necessary for rapid action potential propagation through the myocardium and synchronized cardiac contraction. Altered Cx43 localization and losses in cell–cell gap junction coupling occur during cardiac disease and contribute to abnormal impulse propagation and arrhythmogenic substrates leading to sudden cardiac death. A large number of studies in recent years have demonstrated a decrease in expression accompanied by lateralization and heterogeneous distribution of Cx43 in the myocardium of patients with hypertrophic cardiomyopathies, dilated cardiomyopathies, ischemic cardiomyopathies, and clinical congestive heart failure. The presence of Cx43 at the lateral cell membrane has been shown in patients with hypertrophic cardiomyopathies and in different animal models to be associated with slowing of conduction velocity in early, compensated stage of hypertrophy that was followed by a reduction of Cx43 and development of arrhythmias. In a canine pacing-tachycardia model of dilated cardiomyopathies, dephosphorylation of Cx43 and its appearance at the lateral cell border were associated with decreased epicardial and endocardial conduction velocities in both ventricles and with prolongation of the ventricular depolarization interval (QRS). In another canine pacing-induced heart failure study, increased dephosphorylation of Cx43 was associated with later stages of heart failure although its presence at the lateral cell membrane occurred in an even later stage when the conduction velocity is significantly reduced.

Localization of Cx43 at the lateral cell membrane regions has also been observed in the border zone of myocardial infarcts and has been correlated with development of re-entrant circuits leading to ventricular tachycardia. It is also not clear whether the presence of lateral localized Cx43 correlated with low resistance cell–cell coupling. Electron microscopy and colabeling studies have suggested that some of the lateral Cx43 may not be in plasma membrane but in nearby autophagosomes, suggesting a role of lateral Cx43 other than cell–cell communication.

In summary, altered Cx43 localization occurs in diseased myocardium, with consequences that include lethal arrhythmias. The mislocalization of Cx43 during disease reflects impaired forward trafficking to the intercalated discs. Exploring the mechanisms of forward trafficking and alterations in disease could pave the way for therapeutic rescue of cell–cell coupling in diseased myocardium.

**Cx43 Forward Trafficking in Normal Heart Physiology**

Cx43 oligomerizes into hemichannels at the TGN which is relatively late for such an event to occur because other connexins oligomerize in the ER. Oligomerization of Cx43 at the TGN may represent a means of controlling heteromeric hemichannel formation with other connexin isoforms. On exiting the TGN, vesicles containing Cx43 hemichannels must navigate the complex cardiomyocyte intracellular environment, a feat they achieve by trafficking along dynamic microtubules.

Trafficking of Cx43 hemichannels to the intercalated discs involves a major plus-end binding protein, EB1, which is known to be necessary for targeted delivery of Cx43 hemichannels to adherens junction complexes (Figure 1). Through interaction with another plus-end protein, p\(_{150}\) (glued), the EB1-tipped microtubule complexes specifically with β-catenin molecules at the adherens junctions of intercalated discs. Vesicular cargo is unloaded and subsequently inserted into the plasma membrane at nearby gap junctions. Other reports propose a less specific paradigm of connexin delivery, whereby connexons are inserted indiscriminately into the lateral membrane of the cell and freely diffuse to gap junction structures. The free diffusion model may serve as an alternative pathway for Cx43 delivery. However, the inefficiency of lateral diffusion to a few specific subdomains, the short half-life of connexins (1–3 hours in the myocardium), and the complex interactions between a single cell with multiple neighboring cardiomyocytes, all suggest that directed targeting can be a more effective form of connexon localization to the intercalated discs. Free diffusion of hemichannels likely does occur, but over a restricted region of membrane. For instance, after delivery to adherens junctions, channels may diffuse to and within a local plaque.

In Targeted Delivery, specificity of delivery occurs near the membrane, and is a result of a particular membrane-bound anchor protein occurring at distinct membrane subregions. Membrane anchors are critical to capture with specificity a subgroup of microtubules in order to allow channel delivery directly to regions of membrane that happen to contain the particular anchor. For Cx43 delivery to the intercalated disc, EB1-tipped microtubules bind to N-cadherin–associated β-catenin and also p\(_{150}\) (glued). Desmoplakin may also be involved in capturing the EB1-tipped microtubule for Cx43 delivery, although the transmembrane domain still seems to be N-cadherin rather than desmosomal desmoglein. Nonsarcomeric actin has also been shown to be necessary for Cx43 forward delivery. However, it remains to be determined how actin interacts with channels and the microtubule apparatus. At any given point in time, the majority of intracellular Cx43 channels are not moving rapidly on microtubules but rather are stationary and associated with nonsarcomeric actin.
Other than microtubules, there is increasing appreciation for the involvement of nonsarcomeric actin cytoskeleton in Targeted Delivery of Cx43. The fundamental question remains with regard to why actin is involved in Cx43 trafficking. If vesicles containing Cx43 can depart the Golgi and ride a microtubule highway straight to its proper subdomain,
is there a need for actin filaments which seem to slow down vesicle transport? Actin can have at least 2 important roles in forward delivery of Cx43. The first is to help contribute specificity to delivery. Vesicles transported along microtubules on kinesin motors move rapidly, at a rate of ≈1 μm per second.10 Thus, delivery to most locations at a cell membrane can occur within a minute. Association with important accessory proteins and post-translational modification of channels, both of which can affect delivery destination, probably also happen en route between the Golgi and membrane. Hopping off the microtubule highway on an actin rest stop, which is analogous to a highway rest stop with convenience stores for needed supplies, could allow Cx43 and the vesicle containing it to pick up accessory proteins and allow for needed post-translational modification. Such rest stops would occur at Z-disc, subcoronal accessory proteins and allow for needed post-translational modification. Such rest stops would occur at Z-disc, subcortical locations, or other important cytoskeleton intersections in the cytoplasm. These actin rest stops could also allow the Cx43-containing vesicles to use multiple microtubule highways in their delivery path. The Golgi-exiting microtubule could be destined for an actin rest stop, allowing for a different membrane domain–specific microtubule to finish the delivery. The second potential role for actin in microtubule-based forward delivery pertains to the microtubules themselves. In nonmyocyte systems, actin can help stabilize and guide microtubules.51,54 Actin could be the blueprint along which microtubule highways are patterned. In this respect, actin involvement could be an upstream to microtubules in determining location of Cx43 delivery.

Cx43 Forward Trafficking in Heart Pathophysiology

We have found that when isolated cardiomyocytes are subjected to oxidative stress, Cx43 gap junction delivery to intercalated discs is impaired because of disruption of the forward trafficking machinery.12 Specifically, oxidative stress causes the microtubule plus-end protein EB1 to disassociate from the tips of microtubules, impairing microtubule attachment to adherens junction structures and subsequent delivery of Cx43 hemichannels to plasma membrane12 (Figure 1). Manipulation of EB1 as well as the upstream regulators of EB1 localization at microtubules could potentially preserve or enhance gap junction coupling during stress. As many ion channels rely on microtubules for their transport, it is likely that such disruption of microtubule-trafficking machinery inhibits delivery of many essential channels to the sarcolemma.

Such studies provide evidence that forward trafficking of Cx43 is impaired in acquired heart failure. At present, we do not know how oxidative stress causes EB1 displacement and disassembly of the forward trafficking apparatus. We have preliminary investigations on the role of actin in maintaining EB1-based microtubule integrity, and the response of these cytoskeletal fibers to stress conditions. This remains an active area of investigation.

Regulation of Cx43 Forward Trafficking by its Alternatively Translated Isoforms

Ion channel function and trafficking are usually dependent and regulated by auxiliary protein subunits.55 With regard to accessory proteins, Cx43 hemichannels are notable for, despite extensive examination, not being associated with their own unique β-subunits that assist in their trafficking. It turns out that Cx43 mRNA, through alternative translation, encodes its own trafficking subunits56 which are N-terminal truncations of the full protein. These isoforms have potential roles in non-canonical functions of Cx43 as well.

To understand alternative translation, it should be recognized that traditional translation of mRNA begins with the first coding triplet, which is always an AUG (methionine). Most transcribed genes (mRNA strands) have other AUG sites downstream of the first one. The Cx43 protein has 6 methionines, corresponding to the different AUG triplet translation start sites, beyond the first one (Figure 2). Alternative translation occurs when ribosomal translation initiates not at the first triplet but at a downstream triplet. By initiating translation at downstream sites, alternative translation creates truncated proteins that lack the respective nontranslated upstream (N-terminal) portions of the proteins.

Cx43 is a product of the GJA1 gene, and we have recently reported that the coding region of GJA1 mRNA occurs as a polycistronic molecule with different N-terminal truncated isoforms of Cx43 protein arising from internal translation of the same mRNA molecule.56 We have found that the GJA1 mRNA produces the expected full-length 43-kDa protein as well as proteins that are ≈32, 29, 26, 20, 11, and 7 kDa in size (Figure 2) with the 20-kDa isoform (GJA1-20k) being the predominate isoform in human heart tissue and several other cell lines.56 This is the first evidence that alternative translation is possible for human ion channels and in human heart. These results have since been supported by a separate report showing that the GJA1-20k isoform is expressed in many cell lines.
that express high levels of full-length Cx43.\(^5\) In addition, it has also been reported that this 20-kDa isoform is induced by hypoxic stimuli in the mouse brain and is the result of internal translation from an internal ribosome entry site (IRES) element.\(^5\)

We have found that at least one of the alternatively translated isoforms, GJA1-20k, is important for increasing trafficking of Cx43 to the plasma membrane.\(^5\) Loss of all 4 of the following Cx43 isoforms: GJA1-32k, GJA1-29k, GJA1-26k, and GJA1-20k, severely abrogated the formation of Cx43 gap junctions at the membrane.\(^5\) Interestingly, reintroduction of the GJA1-20k isoform was sufficient to rescue the transport of Cx43 to the cell surface. The majority of ectopically expressed GJA1-20K remained localized primarily at cytoplasmic reticular structures which were confirmed to be the ER/Golgi network. The interaction between full-length GJA1-43k and GJA1-20k in the ER was confirmed using coimmunoprecipitation assays where Brefeldin A (an inhibitor of protein transport from the ER to the Golgi) resulted in increased interactions between these 2 peptides. These data suggest a role of GJA1-20k isoform early in the Cx43 vesicular transport pathway and that this isoform may function as a cytoplasmic chaperone auxiliary protein for trafficking of de novo GJA1-43k molecules through the ER/Golgi complex to the membrane.\(^5\) We also found that the mammalian target of rapamycin (mTOR) signaling pathway increases expression of the GJA1-20k isoform and Cx43 trafficking.\(^5\) It remains to be determined how GJA1-20k contributes specificity to trafficking delivery. We expect that GJA1-20k is involved in cytoskeleton organization.

**Cx43 Internalization in Healthy Cardiomyocytes**

Endocytosis of Cx43 can occur either through internalization of uncoupled hemichannels or entire through gap junctions, which requires engulfment of gap junctions from the opposing neighboring cell plasma membrane as well. The internalized double-membrane intracellular structures are known as non-functional annular gap junctions. Both the lysosome and the proteasome have been implicated in degradation of Cx43,\(^5\) and interestingly, autophagy is now known to be involved in degradation of annular gap junctions in failing hearts.\(^5\) Studies have shown that recycling of gap junctions occur during cell cycle progression in cell lines,\(^5\) but whether gap junctions are recycled in cardiomyocytes remains a controversial issue. It is exciting to consider the possibility that there exist a delicate balance and competition between the various post-translational modifications of Cx43, including phosphorylation\(^5\) and ubiquitination\(^5\) that may act as checkpoints within the same connexin molecule, or connexon hemichannel. This would then allow specific series of events to permit internalization and degradation of the hemichannel, or annular gap junction.

In the case of Cx43, phosphorylation is most well studied, and the importance of phosphorylation has been highlighted by recent findings that casein kinase–dependent phosphorylation alters gap junction remodeling and decreases arrhythmic susceptibility.\(^5\) Many residues on the C terminus of Cx43, specifically 22 serines, 5 tyrosines, and 4 threonines, are potentially subjected to phosphorylation. To make matters even more complex, Cx43 exists as a hexamer on the plasma membrane, and it is currently not known how phosphorylation differs between individual connexins of the same connexon. It is likely that a cascade of phosphorylation events occurs preceding ubiquitination of Cx43, which then leads to channel internalization and degradation.\(^6\) For example, it has been shown that Cx43 phosphorylation by epidermal growth factor and 12-O-tetradecanoylphorbol-13-acetate promotes interaction between Cx43 and the E3 ubiquitin ligase Nedd4 (neuronal precursor cell-expressed developmentally downregulated 4), leading to the subsequent ubiquitination of Cx43.\(^6\)

There is increasing evidence that Cx43 ubiquitination plays an important role in regulating gap junction internalization and degradation, and several E3 ubiquitin ligases have been shown to regulate Cx43 internalization from the plasma membrane including TRIM21,\(^6\) Smurf2,\(^7\) and Nedd4.\(^6,68\) Because these studies were performed in cell culture systems, it still remains largely unclear how Cx43 ubiquitination is regulated in the cardiomyocytes. Nedd4 was the first described E3 ubiquitin ligase to be implicated in regulating Cx43 ubiquitination, internalization, and autophagic degradation through a mechanism involving recruitment of the endocytic adaptor Eps15 (epidermal growth factor receptor substrate 15) and the autophagic receptor p62 in cell lines.\(^6,67\) In neonatal rat cardiomyocytes, Nedd4 has also been reported to interact with Cx43 possibly regulating its ubiquitination and internalization in response to norepinephrine.\(^7\) Further evidence implicating the role of Nedd4 in regulating Cx43 in myocytes was recently reported showing that only under basal conditions, silencing of Nedd4 in the HL-1 mouse atrial cell line led to increased Cx43 protein with a decrease in its ubiquitination levels.\(^64\) Wwp1, which is a close family member to Nedd4, has been recently shown to interact with ubiquitinate and degrade Cx43 in cell lines.\(^65\) In addition, cardiomyocyte-specific overexpression of Wwp1 in an inducible transgenic mouse model led to a significant reduction in Cx43 protein levels in the heart, thus highlighting the importance of Wwp1 in regulating Cx43 turnover in the myocardium.\(^65\)

**Cx43 Internalization in Diseased Cardiomyocytes**

Our experience with Cx43 protein is that post-translational modification preferentially affects ion channel internalization. Pathological gap junction remodeling is strongly associated with altered phosphorylation of Cx43.\(^10,73,74\) Rather than individual independent phosphorylation events of singular residues at the C terminus, it is likely that internalization results from a sophisticated cascade of post-translational modifications. The Cx43 C terminus contains a phosphorylation-dependent 14-3-3 binding motif at Serine 373 (within 10 amino acids of the end of the protein). 14-3-3 proteins are known to regulate protein transport and have been implicated in facilitating de novo Cx43 transport from ER to Golgi apparatus.\(^75,76\) Phosphorylation of Ser373 and subsequent 14-3-3 binding provide a gateway to a signaling cascade of downstream phosphorylation of Ser368, leading to gap junction...
ubiquitination, internalization, and degradation during acute cardiac ischemia.2

The C terminus of Cx43 is the main protein–protein interaction domain responsible for Cx43 binding to its partners within the cell.77 In close proximity to the Cx43 14-3-3 binding motif is a PDZ protein interaction domain at the distal end of the C terminus. It is through this PDZ domain that Cx43 interacts with zonula occludens protein 1 (ZO-1),78 and this interaction has been demonstrated to regulate Cx43 gap junction plaque size and assembly.79,80 Disruption of Cx43/ZO-1complexing has been reported to increase gap junction plaque size in cultured cells.81,82 Phosphorylation of Cx43 Serine373 can disrupt interaction with ZO-1,83 and indeed, it would be sterically unlikely for both 14-3-3 and ZO-1 to bind the same Cx43 protomer simultaneously. However, increased Cx43/ZO-1 interaction has also been associated with gap junction remodeling, highlighting the complex nature of these dynamic post-translational and protein complexing events.84,85

Acute cardiac ischemic injury in isolated rat hearts has been shown to cause increased ubiquitination of Cx43 at the intercalated discs accompanied by increased interaction between Cx43 and Nedd4.86 However, silencing of Nedd4 in HL-1 mouse atrial cells subjected to ischemic conditions did not have any significant effect on Cx43 ubiquitination nor degradation, and only under basal conditions did the knockdown of Nedd4 prevent ubiquitination and degradation of Cx43.87 This suggests that other E3 ubiquitin ligases besides Nedd4 may regulate Cx43 ubiquitination and degradation in cardiac injury. Indeed, it has been recently reported that cardiomyocyte-specific overexpression of Wwp1 in an inducible transgenic mouse model caused a significant reduction in Cx43 protein levels in the heart, leading to the development of lethal left ventricular arrhythmias.65

CaV1.2 Channel Trafficking in the Heart

The calcium-handling proteins that are important in cardiac excitation–contraction coupling, in particular the voltage-gated L-type calcium channels (LTCCs), are mostly enriched in T-tubules. Enrichment of the LTCCs (with pore-forming subunit CaV1.2) at the T-tubules helps bring these channels in close proximity (=15 nm) to intracellular sarcoplasmic reticulum–based calcium sensing and releasing channel ryanodine receptors (Figure 3). This is important for efficient calcium-induced calcium release process during each heartbeat. Upon membrane depolarization, initial calcium influx occurs through CaV1.2 channels, and the close association between CaV1.2 and ryanodine receptors permits efficient calcium-induced calcium release and subsequent sarcomeric contraction.65 In addition, a recent report showed that the membrane scaffolding protein, bridging integrator 1 (BIN1), which organizes T-tubule microfolds,87 is important to bridge the dyadic cleft spanning between CaV1.2 channels at the T-tubules and phosphorylated ryanodine receptors at the sarcoplasmic reticulum membrane, thus maintaining the LTCC–ryanodine receptor coupling at the dyads and regulating calcium transient development.88

T-tubules, which are continuously extended from surface sarcolemma, are lipid bilayers embedded with transmembrane- or lipid-associated proteins.89 Cardiac T-tubules occur at regular intervals along the lateral sides of the cell, closely coincident with the sarcomeric Z-discs. The physiological function of cardiac T-tubules depends on the proteins that are localized at and within the vicinity of the T-tubules, including transmembrane ion channels and ion-handling proteins. Specific membrane scaffolding proteins and cytoskeletal structural proteins are required to localize to T-tubules for the organization and regulation of T-tubule network and structure. By differentially compartmentalizing proteins involved in ion handling and signaling, T-tubules serve as a signaling hub-like organelle to regulate myocyte function. The expression of transmembrane ion channels, ion transporters, and pumps have been well characterized in cardiac T-tubules.90

It has also been reported that a subset of CaV1.2 channels is localized within caveolae to aid in calcium signaling.91 Caveolae are distinct membrane microdomains "little caves" that exist in both T-tubules and the lateral sarcolemma of ventricular cardiomyocytes. A caveola is a flask-shaped structure enriched with cholesterol and sphingolipids formed by the cholesterol-binding scaffolding protein caveolin-3. Biochemical fractionation and electron microscopy studies have identified a subpopulation of many ion channels at caveolae, and loss of caveolae is associated with arrhythmogenesis.92 The precise role of caveolae on ion channel regulation and its significance still await further investigation. In addition, the mechanisms affecting CaV1.2 enrichment at caveolae are unknown, but close interactions between caveolae and the cytoskeleton present an appealing possibility of targeted ion channel delivery to these sarcolemmal microdomains.92

CaV1.2 Forward Trafficking in Normal Heart Physiology

Enrichment of CaV1.2 channels in the T-tubules is essential for the efficient contractile function of the myocardium. We found that trafficking of CaV1.2 vesicles from the TGN to T-tubules also occurs in a microtubule-dependent manner (Figure 3). Moreover, consistent with the Targeted Delivery model just as with Cx43 connexons, dynamic microtubules preferentially interact with a specific membrane anchor protein, BIN1, to insure targeted delivery of CaV1.2 to the T-tubules (Figure 3). BIN1 contains a membrane curvature Bin, Amphiphsin, and Rvs (BAR)domain (which confers the ability to form membrane curvature), a coiled-coil domain, and a SRC homology 3 (SH3) protein–protein interaction domain. Perhaps most compelling evidence for BIN1 using the cytoskeleton is the finding that deletion of the coiled-coil and SH3 domains does not affect membrane invagination but abrogates CaV1.2 colocalization with these structures. Therefore, it is through interaction specifically with the BIN1 membrane scaffolding protein, and not T-tubule structures, that targeting of CaV1.2 delivery is achieved.11 The specificity of Targeted Delivery is also contributed by the microtubule plus end tracking proteins (+TIP) at the plus ends of growing microtubules. For example, EB1 works in concert with p34Glued (glued) to target Cx43 channels to adherens junctions at intercalated discs,90 whereas the other +TIP protein CIIP170 has been reported to interact with BIN1,93 possibly facilitating BIN1-directed delivery of LTCCs to T-tubules.
A subpopulation of CaV1.2 channels, on the contrary, can be delivered to caveolae through interaction between sub-units of LTCC channel complex and the caveolae structural protein caveolin. In addition, the fibroblast growth factor homologous factors have been shown to be potent regulators of CaV1.2 localization to the sarcolemmal membrane by interacting with C-terminal domains of ion channels.

Figure 3. CaV1.2 trafficking in healthy and failing hearts. CaV1.2, a voltage-gated L-type calcium-channel (LTCC) protein, is synthesized by ribosomes, translocated to the rough endoplasmic reticulum, transported through the Golgi apparatus, and then to the trans-Golgi network. CaV1.2 proteins are then sorted into vesicular carriers, docked onto microtubules, and subsequently delivered to their subcellular destinations (T-tubules). The association of microtubules with bridging integrator 1 (BIN1), a membrane scaffolding protein, warrants the delivery of CaV1.2 to the T-tubules. Top: In the healthy heart, BIN1 is responsible for creating T-tubule folds thus affecting extracellular ion diffusion and controlling the driving force of CaV1.2 channel activity. BIN1-folded subdomains within T-tubules also limit LTCC lateral diffusion once the channels are inserted into T-tubule membrane, to maintain functional LTCC–ryanodine receptor (RyR) dyads and healthy excitation–contraction coupling. Bottom: In the failing heart, BIN1 expression is reduced, and the dense membrane folds in T-tubules are lost due to low expression of BIN1. This leads to defective CaV1.2 delivery to the T-tubules, decreased LTCC–RyR dyads, and altered excitation–contraction coupling. LTCC indicates L-type calcium channel.

CaV1.2 Forward Trafficking in Heart Pathophysiology

In failing heart, forward trafficking of CaV1.2 channels to T-tubules is also impaired (Figure 3). Biochemical assessment of CaV1.2 channel content in failing heart indicates no difference in total channel content compared with healthy muscle, yet channel localization to T-tubules is impaired.
difference between impaired forward delivery of Cx43 channels and CaV1.2 channels in failing hearts exists with their respective anchor proteins. Even in diseased heart muscle, the adherens junction structures for Cx43 delivery to intercalated discs remain intact, whereas transcription of BIN1 protein, needed to anchor microtubules for CaV1.2 delivery to T-tubules, is reduced by half. In animal models, successful treatment of heart failure and recovery of function correlates with recovery of muscle BIN1 levels.

Accessory Proteins Involved in CaV1.2 Targeted Delivery

As mentioned before, ion channel function and trafficking are usually dependent and regulated by auxiliary protein subunits including their own unique β-subunits that assist in their trafficking. In the case of LTCC, accessory β-subunits exist with the expression of 4 different isofoms (β1–β4) varying across species in the myocardium. In the mouse hearts, only β2 subunit (with 5 splice variants β2a–2e) has been detected, whereas all of the 4 isoforms have been detected in canine myocardium. By masking the ER retention signal at the intracellular I-II loop of CaV1.2 protein, β-subunits are critical in facilitating the ER exiting of CaV1.2 channel. Because of the essential role of the LTCC β subunits in regulating trafficking and surface expression of the calcium ion channels, different β subunit mutations have been implicated in human disease. More specifically, 2 point mutations in the β2b subunit, which is the most abundant LTCC β subunit isoform in the heart, have been implicated in disease. A S481L mutation, which occurs in the C terminus of β2b, contributes to a sudden death syndrome characterized by a short QT interval and an elevated ST segment. A T111 mutation occurs in the β2b N terminus and causes accelerated inactivation of cardiac L-type channels and is linked to Brugada syndrome.

The role of β-subunit in targeted delivery of LTCCs remains unclear. We speculate that the β-subunit may be the one directly binding to membrane anchor proteins to facilitate delivery of LTCCs to membrane subdomains. We also speculate that the specificity of LTCCs delivery can be determined by binding of BIN1 or caveolin–3–like membrane anchor proteins with different β-subunit isoforms and splice variants.

T-tubules and CaV1.2 Regulation in Normal Heart Physiology

A recent development in cardiac membrane biology is the finding that T-tubule invaginations are not simply straight and planar, but instead contain complex folds which are tight and narrow enough to limit the free flow of extracellular ions. We found that BIN1 is responsible for these minifolds within the T-tubules, thus affecting extracellular ion diffusion and controlling the driving force of CaV1.2 channel activity. BIN1-folded subdomains within T-tubules may also limit LTCC lateral diffusion once the channels are inserted into T-tubule membrane, to maintain functional LTCC-ryanodine receptor dyads. Therefore, BIN1-like membrane scaffold proteins may help localize particular pools of ion channel proteins to membrane subdomains for compartmentalized regulation of ion channel activity and function.

T-tubules and CaV1.2 Regulation in Heart Pathophysiology

In failing hearts, L-type calcium channels also have diminished forward trafficking resulting in intracellular accumulation of the channels. There already exists significant evidence that gross T-tubule network remodeling occurs in failing heart. It is an area of active research with regard to the mechanisms of T-tubule remodeling in failing hearts. Junctophilin-2 trafficked by microtubules has been implicated in impaired T-tubule maintenance during heart failure. However, the role of junctophilin-2 in T-tubule remodeling during heart failure has been questioned because of a lack of decrease with heart failure because T-tubule structures are diminished or return with recovery of T-tubule structures in treated heart failure. In these same studies, BIN1 decreased with decrease in T-tubule density in heart failure and then, BIN1 recovered along with T-tubule density during functional recovery of the myocardium.

During extended in vitro culture, isolated mature ventricular myocytes lose T-tubules in 3 days. Interestingly, actin stabilization by cytochalasin D can preserve T-tubules in cultured myocytes. To that end, the cardiac isoform of BIN1, which we described recently, was found to be able to promote Neural Wiskott–Aldrich syndrome protein (N-WASP)–dependent actin polymerization. Exogenous BIN1 introduced by adenovirus not only rescued T-tubule membrane intensity but also surface CaV1.2 channels in isolated cardiomyocytes cultured in vitro. Taken together, we have found that T-tubule inner folds are rescued only by the BIN1 cardiac-specific isoform, which promotes N-WASP–dependent actin polymerization to stabilize T-tubule membrane at cardiac Z-discs to help recruit CaV1.2 channels.

In mice with cardiac Bin1 deletion, T-tubule folding is decreased, which does not change overall cardiomyocyte morphology, but frees diffusion of local extracellular calcium and potassium ions, prolonging action potential duration, and increasing susceptibility to ventricular arrhythmias. In addition, these cardiac-specific BIN1-deficient mice exhibit T-tubule remodeling similar to what is observed in failing hearts. Thus, BIN1 cardiac-specific isoform recruits actin to fold T-tubule membrane, creating a fuzzy space that protects against activation of N-WASP. When cardiac BIN1 is decreased, as occurs in acquired cardiomyopathy, T-tubule morphology is altered, and arrhythmias can result.

CaV1.2 Internalization

General internalization of LTCCs is poorly understood with particular lack of studies in cardiomyocytes. In oocytes, the LTCC β-subunit can enhance dynamin-dependent internalization, and in neurons CaV1.2 channels may undergo depolarization and calcium-dependent internalization. We found in cardiomyocytes that a dynamin GTPase inhibitor dysore can increase surface LTCC expression, indicating dynamin-dependent endocytosis of cardiac CaV1.2 channels. Furthermore, a small GTPase Rab11 is implicated in endosomal
transport of LTCCs, thereby limiting surface expression of LTCCs.114

Channelopathies as a Result of Altered Trafficking in Heart Pathophysiology

Numerous channelopathies in heart disease are caused by mutations negatively affecting trafficking. For instance, Anderson et al115 have found that of 28 clinically relevant mutations in Kv11.1, most reduce potassium voltage-gated channel subfamily H member 2 (hERG) current not by altering Kv11.1 expression or kinetics but by diminishing Kv11.1 trafficking to the membrane. In accordance with this finding, different trafficking-deficient mutations in several regions of the hERG channel protein have been identified to cause LQT2 syndrome. Such mutations include T65P in the N-terminus region, N470D and A561V in the transmembrane region, G601S, Y611H, V612L, T613M, and L615V in the pore region, and R752W, F805C, V822M, R823W, and N861I in the C terminus.116 In addition, the missense mutation A558P in hERG has been shown to exert a dominant negative effect causing trafficking deficiency of the channel and fever-induced QT interval prolongation in patients.117

Defective cardiac ion channel trafficking in inherited arrhythmia has also been reported for KCNQ1 (LQT1 syndrome), KCNE1 (LQT5 syndrome), and SCN5A (Brugada syndrome). Such SCN5A trafficking-deficient mutations in Brugada syndrome include T351I, R367H, R1232W, R1432G, and G1743R.116 Also mutations in Nav1.5 that limit binding of Nav1.5 to a membrane anchor protein ankyrin-G have been shown to cause aberrant Nav1.5 trafficking to the intercalated discs and result in human Brugada syndrome.118 An additional ankyrin isoform found in ventricular cardiomyocytes, ankyrin-B, has been described to be associated with targeting and maintenance of the Na+/Ca2+ exchanger (NCX), Na+/K+ ATPase (NKA), at T-tubules where they proximate with the IP3 receptor (InsP3) of the sarcoplasmic reticulum and regulate Ca2+ export. Mutations in ankyrin-B ablating its interaction with NCX/NKA/InsP3 result in arrhythmogenic cardiac disorders in humans, including type-4 long-QT syndrome.119

Conclusions

The individual cardiomyocyte is a highly complex and dynamic system with internal organization designed to maintain efficient cell–cell communication and excitation–contraction coupling. To maintain intracellular homeostasis as well as overall synchrony across the myocardium, cardiomyocytes regulate ion channel intracellular movement and localization through highly sophisticated and highly efficient protein-trafficking machineries. In diseased hearts, cardiomyocyte structures and organization are negatively affected by environmental conditions of stress, affecting channel trafficking and function. Because the physiological movements of cardiac channels are elucidated, and then disease-related changes of these movements are understood, interventions can be designed to promote positive intracellular remodeling. Therefore, new therapies for failing heart should focus on the specific organelles and pathways that regulate cardiomyocyte channel trafficking.

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Disclosures

None.

References


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