HHS Public Access

Author manuscript

Biochim Biophys Acta. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Biochim Biophys Acta. 2018 January ; 1860(1): 40–47. doi:10.1016/j.bbamem.2017.05.018.

Intracellular Trafficking Pathways of Cx43 Gap Junction Channels

Irina Epifantseva[1] and **Robin Shaw**[1,2,*]

[1]Heart Institute and Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, 90048, USA

[2]Department of Medicine, University of California Los Angeles, Los Angeles, CA, 90095, USA

Abstract

Gap Junction (GJ) channels, including the most common Connexin 43 (Cx43), have fundamental roles in excitable tissues by facilitating rapid transmission of action potentials between adjacent cells. For instance, synchronization during each heartbeat is regulated by these ion channels at the cardiomyocyte cell-cell border. Cx43 protein has a short half-life, and rapid synthesis and timely delivery of those proteins to particular subdomains are crucial for the cellular organization of gap junctions and maintenance of intracellular coupling. Impairment in gap junction trafficking contributes to dangerous complications in diseased hearts such as the arrhythmias of sudden cardiac death. Of recent interest are the protein-protein interactions with the Cx43 carboxy-terminus. These interactions have significant impact on the full length Cx43 lifecycle and also contribute to trafficking of Cx43 as well as possibly other functions. We are learning that many of the known non-canonical roles of Cx43 can be attributed to the recently identified six endogenous Cx43 truncated isoforms which are produced by internal translation. In general, alternative translation is a new leading edge for proteome expansion and therapeutic drug development. This review highlights recent mechanisms identified in the trafficking of gap junction channels, involvement of other proteins contributing to the delivery of channels to the cell-cell border, and understanding of possible roles of the newly discovered alternatively translated isoforms in Cx43 biology.

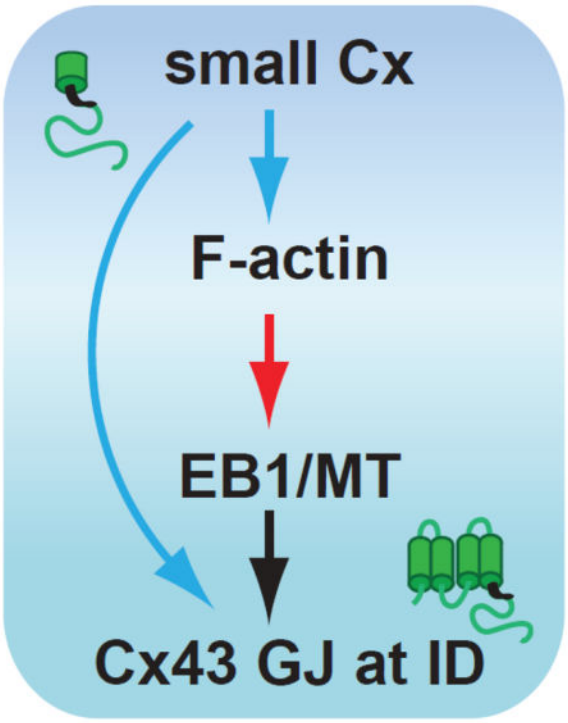
Graphical Abstract

[*]Correspondence to: Robin.Shaw@cshs.org.

[~]Current address: Robin Shaw, MD, PhD, Wasserman Endowed Chair in Cardiology, Cedars-Sinai Medical Center and UCLA, 8700 Beverly Blvd., Suite Davis 1016, Los Angeles, California 90048, Direct: 310.967.3842, Fax: 310.967.3891

Conflict of Interest

The authors have no conflicts of interest to declare.



1. Introduction

Cell-cell communication is critical for all organ systems, and assured by transmembrane channels forming conductive pores in the plasma membranes between adjacent cells [1]. These channels located in so-called gap junctions (GJ) which consist of connexin (Cx) proteins [2, 3]. All human connexins have a common organization: four transmembrane domains separating two extracellular and one intracellular loop. Both the amino (NT) and carboxyl (CT) termini are intracellular [4]. Each transmembrane channel (hemichannel or connexon) is a hexamer of connexins, and connexons from one cell dock to hemichannels from an adjacent cell to form GJ channels [5]. GJs allow the diffusion of small (typically less than 1 kDa) hydrophilic substances molecules and ions without exposure to the extracellular environment [6, 7]. GJs are the only channels which mediate direct cytoplasmic exchange - a process called gap junctional intercellular communication (GJIC) [8–12]. Interestingly, mutations in connexin genes, and therefore, in gap junctional communication, are associated with a large variety of pathologies and inherited connexin-associated disorders affect almost every major organ system [13, 14].

Of all 20 murine and 21 human connexins, Connexin 43 (Cx43, from the gene GJA1) is the most commonly expressed GJ protein [15, 16]. Cx43 has a fundamental role in excitable tissues to facilitate low resistance communication and thus rapid action potential transmission between adjacent cardiac cells. Such rapid communication synchronizes the cardiac heartbeat, and propagates electrical signals in the brain [17–19]. Cx43 is particularly enriched in ventricular cardiomyocytes where it is localized to the cardiomyocyte gap junction as part of the intercalated disc (ID) to facilitate action potential propagation [20].

In recent years, it has been reported that Cx43 can be involved in non-canonical events such as cell cycle regulation and cancer progression, wound healing, muscle differentiation, gene regulation and development [21–24]. Cx43 protein, and in particular its C-terminus, has also been implicated in the trafficking of cardiac ion channels such as Nav1.5 and junctional protein N-cadherin [25–28]. These studies identify Cx43 by immunohistochemistry with antibody epitopes at the C-terminus [25], or with a C-terminus truncation of the distal 5 amino acid residues [26, 27], or with co-immunoprecipitation with N-Cadherin [28]. Therefore, it is not a surprise that mutations or any deficiency in Cx43 expression or gap junction formation are associated with diverse pathologies, including heart disease [29–35], connective tissue disease [36], and cancer [21, 24]. Cardiac pathologies are frequently associated with connexin redistribution which is a form of gap junction remodeling [32, 37]. Moreover, impaired Cx43 trafficking contributes to the arrhythmias of sudden cardiac death [17, 30, 32, 38–49]. Decrease in expression and distribution of Cx43 in the cardiomyocytes has been described in the patients with hypertrophic cardiomyopathies [50–52], dilated cardiomyopathies [53, 54], ischemic cardiomyopathies [50, 52] and clinical congestive heart failure [33], and hereditary disorders [55]. Cx43 hemichannels and gap junctions have been implicated in mediating ischemia/reperfusion injury, cardioprotection and neuroprotection [56].

As many of the above studies highlight, several decades of research on Cx43 has revealed that many of these functional roles are attributed to the C-terminal domain (CT) of this protein which can not be explained by the formation and existence of intercellular channels. Recent findings on the existence of endogenous internally translated C-terminal isoforms from Cx43 mRNA [57–60], which can explain general cytoplasmic activity of the proteins, are opening a new understanding of the regulation of gap junction trafficking and function. Roles previously assigned to full length Cx43 may in fact be due to the smaller isoforms instead.

This review is focused on current understandings of the mechanisms of Cx43 trafficking and related regulation of gap junction organization. Suggestions are also provided regarding the directions of future research.

2. The Cx43 trafficking life cycle

2.1 Anterograde trafficking

Like all connexin proteins, Cx43 follows a traditional secretory pathway (Fig. 1A). It is inserted into the membrane of the endoplasmic reticulum (ER) after transcription in the nuclei and translation in ER, where folding and post-translational modifications occur. Checkpoints control exit of hemichannels and if proper channel folding fails, those proteins are transferred to ER-associated degradation by quality control mechanisms [18, 61]. Interestingly, some chemicals such as 4-phenylbutyrate and glycerol can potentially rescue the misfolding of Cx43 and normalize trafficking of retained protein [13]. Following checkpoint regulation, ion channels exit the ER and are packaged into vesicles coated with coat protein complex II (COPII) and are translocated to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) [62]. Further sorting continues within the ERGIC and

posttranslational modifications occur while ion channels progress from cis-Golgi network to trans-Golgi (TGN) to generate functional channels ready for delivery [63].

Oligomerization into hemichannels or connexons usually happens during the transition from rough the ER to the Golgi apparatus [5], with modification and sorting to occur later in the TGN (Fig. 1A) [64]. Even though most connexins are oligomerized in the ER, late oligomerization for Cx43 could have been evolutionary established to control heteromeric hemichannel formation with other connexin isoforms [18].

2.1a Targeted Delivery—The mechanism of how newly translated oligomerized protein get to their "working" location is still being explored. After exiting the TGN the folded and oligomerized hemichannels are usually transported to the cell surface where, according to classic theory, they freely diffuse within the lipid bilayer to their final functional destination [65–69]. It is possible that post-Golgi hemichannels take a more direct route to a particular membrane-subdomain such as the cell-cell border and IDs. In the paradigm of a more direct delivery system which we call Targeted Delivery, channels exit the Golgi in vesicles to be trafficked along microtubules via motor proteins to a specific subdomain on the membrane hosting anchor proteins (i.e. adherens junctions) which capture the microtubules, allowing for hemichannel offloading from microtubule to membrane (Fig. 1A,B) [70]. Such Targeted Delivery might be commonly utilized by specialized membrane proteins in polarized cells or by cardiomyocytes for trafficking of Cx43 to the ID which are located primarily at the longitudinal ends of cardiomyocytes, perpendicular to the anchoring cytoskeleton, and support functions of cardiomyocytes both mechanically and electrically [71]. IDs consist of Gap Junction plaque, ion channels, adherens junctions and desmosomes [72–75] (Fig. 1A,B). Several cardiomyopathies are driven by disrupted mechanical junctions associated with altered Cx43 localization and dissociation from desmosomes [51, 76–80].

Targeted Delivery is a model preferred by these authors as it is an efficient model compatible with the short life cycle of Cx43 [39, 70, 81–87] and well substantiated by experimental studies. Biochemical and imaging studies reveal that the half-life of many of the connexin family members are within 2–5 hours, with less than 2 hours at the plasma membrane [70]. This highly dynamic turnover suggests that the entire gap junctional content might be renewed several times on a daily basis at plasma membrane of any cell type [70, 88]. It is a particular interesting phenomena for such an important protein in the heart like Cx43 where one would expect much longer life similar to some other ion channels up to 30–40 hours [89]. Therefore, trafficking of channels needs to be efficient, accurate, and robust.

The Targeted Delivery paradigm is based on the observation that newly synthetized Cx43 hemichannels are targeted directly to adhesion junctions [39, 70, 81–87]. Upon exiting the TGN, vesicles with Cx43 hemichannels must navigate the complex intracellular environment as they are transported along the filamentous microtubule and actin networks [90]. The minus ends of microtubules are nucleated in the microtubule-organizing centers (MTOC) which is co-localized with TGN [91] where the main sorting of cargo-proteins occurs [92]. In the TGN cargo proteins are loaded on molecular motors of microtubules and delivered to the cell periphery [90, 93]. Therefore, in the Targeted Delivery model the dynamic process is dependent upon coordinated action of microtubule-based motors

including kinesin [94]. In the context of trafficking, one can consider the Golgi and the TGN to be the “loading dock” and the microtubules dynamic “highways” along which packets of channels are delivered to the plasma membrane. Microtubules-highways pause when they reach and anchor at adherens junction complexes (N-cadherin, β-catenin) in the plasma membrane and deliver cargo to the cell-cell border or IDs.

Microtubule-based vesicle transport has been described for many ion channels and transporters in polarized epithelial cells of kidney and liver and is also capable of delivering vesicles with Cx43 hemichannels to the plasma membrane [66, 69, 70, 95–97]. Capture of microtubules by the appropriate membrane anchor and delivery of cargo to a specific region in the membrane is a critical aspect of Targeted Delivery. For precise Cx43 delivery several members of this paradigm have been identified: a microtubule plus-end binding protein, EB1, is known to be necessary for targeted delivery of connexons to adherens junction [70]. The EB1-tipped microtubule specifically seeks and interacts with β-catenin molecules bound to N-cadherin at the adherens junctions of intercalated discs where vesicles can be unloaded and inserted at the plasma membrane in close proximity to gap junctions (Fig. 1A, B). The presence of $p150_{GLUED}$ is necessary for this process. As details of this paradigm are elucidated, it has been found that the desmosome associated linker protein desmoplakin may also be involved in capturing the EB1-tipped microtubule thereby affecting Cx43 targeted delivery to IDs [98, 99] (Fig. 1B). Interestingly, it was shown that Cx43 and EB1 were diminished at the IDs of patient with ischemic cardiomyopathy and EB1 was displaced from microtubules [39], suggesting that impaired forward trafficking of ion channels contributes to acquired heart failure. Manipulation of EB1 or upstream regulators of EB1 could potentially preserve gap junction during oxidative stress when EB1 is more likely to be displaced from microtubules [39].

In general, Targeted Delivery is an efficient general model of delivery of membrane proteins and channels to the cell-cell border. Regarding Cx43 gap junctions, and taking into account the short half-life of Cx43 protein and the constant need to maintain cellular coupling in the heart, the Targeted Delivery is a fast and efficient way to control GJ coupling at the intercalated discs. Post membrane delivery, free lateral diffusion of connexons within the plasma membrane could also take place, but either for delivery as nonjunctional protein or occurring within a restricted region, for example, within and around the local plaque. There is an urgent need to develop techniques which allow for tracking of a single particle such as connexons in live cells. This technology could greatly assist investigators in understanding the behavior of the highly dynamic Cx43 protein once is already in the plasma membrane.

2.1b Role of actin in Targeted Delivery—Non-sarcomeric actin (filamentous or F-actin) is an important participant in Targeted Delivery of Cx43 in additional to microtubules, regulating vesicular transport at early (ER to Golgi) and later (TGN to sarcolemma) stages [90, 100]. There are three forms of actin in cardiomyocytes: α-actin comprising the thin filaments of the sarcomere [101], and β- and γ-actin forming F-actin. F-actin is not associated with generating contractile force [102]. However, it participates in maintenance of membrane subdomains such as intercalated discs, T-tubules, caveolae and in regulating intracellular vesicular transport through motor protein-based trafficking and vesicular fusion with the cell membrane [71, 103–105]. Several studies identified the dependence of GJ

formation and maintenance on actin [106–108]. We have found that Cx43 colocalizes with non-sarcomeric actin structures (β-actin) along the vesicular transport pathway at the perinuclear region, as well as intercalated disc region (Fig. 1A). Cx43 cargo transport is slowed down when associated with actin [109]. This finding is consistent with previous studies of Cx32 pausing on actin structures en route to the cell border of hepatocytes [110]. It is not yet clear why actin slows hemichannel transport, or whether there is a benefit to actin's involvement in trafficking.

We presume that actin can have at least two important roles in forward delivery of Cx43. The first possible role of actin is to provide a pool of already formed hemichannels ready to be delivered to the ID. While paused at the actin and in reserve, the hemichannels could associate with important accessory proteins and undergo post-translational modification. Thus microtubule highways could use actin as a "way station", in analogy with a highway rest stop. 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. Therefore, the second potential role for actin in microtubule based forward delivery is to confer specificity and directionality to delivery. Vesicles transported along microtubules on kinesin motors move rapidly, at a rate of about 1 μm per second [70]. Thus, delivery to most locations at a cell membrane can occur within a minute. Actin can also help stabilize and guide microtubules, redirect moving microtubules to tailor cargo delivery and protein insertion into sarcolemma surface destination. Interestingly, in the context of axonal neuronal growth, there are are examples of actin directing microtubules [111]. Also actin can form the scaffold, like a blueprint for navigation of microtubules in plants and neurites [112, 113] and human cells [114, 115]. In this case actin navigates and plays the role of a road signs for the microtubule highways in myocyte and non-myocyte system. Therefore, in our current hypothesis, non-sarcomeric actin acts upstream of microtubule-based delivery in trafficking.

2.2 Retrograde trafficking (Internalization)

Endocytosis of Cx43 as either hemichannels or whole gap junction channels (with the opposing plasma membrane) takes place right after ubiquitination of Cx43 prior to lysosomal, proteasomal degradation [116, 117] or autophagy (which is involved in degradation of this protein during heart failure) (Fig. 1) [76, 118]. These events are thought to be triggered by posttranslational modifications of Cx43 at the C-terminus in consecutive steps, such as binding of 14-3-3 motif [119], phosphorylation [40, 118], ubiquitination [120, 121], and SUMOylation [122]. Internalization is a very important regulatory step in the Cx43 lifecycle as it is the second determinant of gap junction coupling after forward delivery [123]. Typically, posttranslational modifications occur as a sequential cascade [118, 119, 124, 125] of events resulting in the formation of connexosomes or annular gap junction channels (Fig. 1A) [64, 126]. Connexosomes are destined either for degradation or recycling. Recycling of gap junctions from an "older pool" of Cx43 rather than *de novo* Cx43 synthesis can occur after the completion of mitosis in cell lines [127, 128]. Whether gap junctions are recycled in cardiomyocytes remains controversial.

In the case of Cx43, phosphorylation at multiple sites are the most well studied post-translational modifications. The importance of phosphorylation has been highlighted by recent findings that casein kinase-dependent phosphorylation alters gap junction remodeling and decreases arrhythmic susceptibility [40]. 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, it is currently unclear how phosphorylation differs between the individual six connexins of the same connexon.

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 [18, 30, 129]. 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 [130, 131]. Phosphorylation of Ser373 and subsequent 14-3-3 binding provide a gateway to downstream phosphorylation of Ser368, leading to gap junction ubiquitination, internalization and degradation during acute cardiac ischemia [119].

Acute cardiac ischemic injury in isolated rat hearts has also been shown to cause increased ubiquitination of Cx43 at the intercalated discs accompanied by increased interaction between Cx43 and Nedd4 [121]. 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. Only under basal conditions did the knockdown of Nedd4 prevent ubiquitination and degradation of Cx43 [132]. This suggests that other E3 ubiquitin ligases besides Nedd4 may regulate Cx43 ubiquitination and degradation in cardiac injury. Indeed, it has been recently reported in an inducible transgenic mouse model that cardiomyocyte specific overexpression of the ubiquitin ligase Wwp1 caused a significant reduction in Cx43 protein levels in the heart leading to the development of lethal left ventricular arrhythmias [120].

3. Alternative translation initiation sites within Cx43

Alternative translation has not been extensively studied in eukaryotic systems, but seems to be a source of considerable biologic diversity. Less than 20,000 genes encode more than 80,000 protein-coding transcripts and a rough estimation of the number of proteins synthesized from these transcripts is in the range of 250,000 to 1 million in mammalian species. This suggests a substantial regulation at the transcriptional, post-transcriptional, and translational level [133]. In support of this, a recent study confirmed that there is no strong correlation between transcript and protein levels in mammals [134]. Indeed, the use of pre-existing mRNAs through regulation of translation is beneficial in many circumstances, because mRNA biogenesis is time consuming, whereas synthesis of protein is a fast process for many genes (including ion channels). Therefore, translational control plays an important role in a key biological processes. Translation defines not only the amount of protein produced, but ribosomal translation can be initiated from downstream AUG start sites by activation of alternative, internal AUG start codons. As a result distinct proteins can be generated from a single mRNA molecule (Fig. 2) [135].

Translation is initiated on most mRNAs by a canonical cap-dependent mechanism which involves binding of 10–13 different eukaryotic initiation factors (eIFs) at the mRNA 5′-UTR. Once recruited to the ribosomes, this initiation complex starts to scan the transcript from the 5′-UTR to locate the AUG start codon with a specific nucleotide context (Kozak sequence) thereby initiating translation [136]. However, several other non-canonical mechanisms were recently described [137]. An internal ribosome entry site (IRES) in viral and some cellular mRNAs was identified in close proximity to AUG start sites. Non-canonical internal intiation is generally observed under stressful physiological conditions (such as starvation, hypoxia, inflammation, or apoptosis) when cap-dependent translation is diminished [137, 138]. Other non-IRES alternative mechanisms of translation have been proposed. They include either direct internal binding of initiation translation factor eIF4E to the coding site of mRNA, or a mechanism called leaky ribosomal scanning [137, 139]. In second case translation can be initiated at the first AUG start site, but it will fail if the Kozak consensus sequence context is not optimal enough to start translation [136, 140, 141]. Translation will then be initiated at the subsequent internal AUG start site. To date, cap-independent translation and leaky ribosomal scanning are thought to represent the two basic mechanisms explaining the existence of alternative translation.

3.1 Alternative translation of Cx43

GJA1 mRNA is the first example of polycistronic molecule and an example of alternative translation observed in human ion channels and in the human heart. After the first AUG (Methionine), there are 6 additional downstream AUG codons within Cx43 mRNA, which means that ribosomal translation can be initiated at multiple internal sites to produce truncated proteins that lack the corresponding non-translated upstream (N-terminal) portions of the protein. A total of 6 additional N-terminally truncated protein isoforms (in addition to a full length Cx43) can be generated from Cx43 mRNA, resulting in a total of 7 different Cx43 proteins [58]. The truncated isoforms are detected as distinct bands on the western blot lysate from the left ventricle of non-failing human heart tissue (Fig. 2). In addition to 43kDa full length isoform, the largest 3 isoforms (32kDa, 29kDa and 26kDa in size) contain 2 transmembrane domains, part of intracellular loop, and the C-terminus. The smallest 3 isoforms are 20kDa, 11kDa, and 7kDa in size and include part of the C-terminal tail with the 20kDa isoform also containing part of the last transmembrane domain. The nomenclature for the smaller protein isoforms has been adopted by using the Cx43 gene name (GJA1), reflecting the origin of the mRNA, in combination with the size of the truncation protein produced. For instance, ribosomal translation beginning at the coding methionine residue 213 of GJA1 produces a 20kDa protein named GJA1-20k (Fig. 2) [58].

3.2 GJA1-20k as a chaperone protein in Targeted delivery

So far, studies mentioned above have also shown that the 20 kDa isoform (GJA1-20k) expression is predominant in human heart tissue, in the heart of zebrafish and in many cancer cell lines (Fig. 2) [58, 59, 142]. Experiments with the mutated 20kDa isoform showed that formation of Cx43 gap junctions was severely reduced, with restoration upon re-introduction of the 20kDa isoform [58]. Co-immunoprecipitation and immunostaining experiments with Brefeldin A (a common inhibitor of transport from ER to Golgi) confirmed interactions between the full length GJA1-43k and GJA1-20k [143]. Therefore,

this suggests that GJA1-20k isoform contributes to vesicular trafficking of the 43kDa full length isoform from ER/TGN to the intercalated disc and may function as a protein-chaperone. Furthermore, it was found that the inhibition of the mammalian target of rapamycin (mTOR/PI3K/AKT) pathway increases internal translation and protein levels of the GJA1-20k isoform and the Cx43 gap junction plaque size at the cell-cell borders in cardiomyocytes (Fig. 3) [58]. It is known that the mTOR pathway promotes cap-dependent translation [144]. Therefore, it is possible that Cx43 can be rescued or enhanced with mTOR inhibitors which are common therapeutic immunosuppressants used in organ transplantation. Interestingly, the mTOR pathway is involved in cardiovascular physiology and pathology, and inhibition of this pathway has cardioprotective effect [145].

It remains unclear at what location in the forward pathway that GJA1-20k facilitates Cx43 trafficking. We believe that GJA1-20k might be involved in a trafficking of Cx43 channel-containing vesicles through actin stabilization to guide microtubules for targeted delivery and/or by mediating actin-to-microtubule transfer to assist in the delivery of Cx43 channels as well as multiple ion channels and membrane proteins (Fig. 3). As mentioned earlier, Cx43 or at least portions of the C-terminus of Cx43 have been implicated in the trafficking of other cardiac ion channels (Nav1.5) and junctional proteins (N-cadherin) to the intercalated disc [25–28]. The short half-life of Cx43 may reflect a continuous need for a prepared and sufficient pool of GJA1-20k isoforms. Previous work has shown that F-actin integrity is necessary for *de novo* Cx43 delivery to cell-cell borders in neonatal ventricular cardiomyocytes. We identified that Cx43 expression at the ID was significantly reduced either in hearts subjected to acute ischemia or F-actin disruption by LatrunculinA. The two effects were not additive, suggesting that a disarray of F-actin was common to both interventions [146]. Therefore, non-contractile F-actin “rest stops” may provide a reservoir of Cx43 channels, which the 20k isoforms could help mobilize during stress, providing a bolus of Cx43 channels in response to stressful conditions (Fig. 3). While we know that GJA1-20k (CT-tail of Cx43) contains a microtubule-binding domain [147, 148], and that CT-tail directly interacts with α- and β-tubulin and co-localize with GJs at intercalated discs [149–152], it is not clear how Cx43 or the smaller isoforms interact with F-actin. We also don’t know whether F-actin and the microtubule machinery interact in directing Cx43 delivery. In future studies, it will be interesting to learn whether or how GJA1-20k regulates actin-to-microtubule movement of Cx43 hemichannel vesicles.

4. Concluding remarks

It is highly likely that the internally translated GJA1-20k isoform is a protein-chaperone or “beta-subunit” that serves and autoregulates the full length Cx43 trafficking to the cell-cell border. Production of isoforms and trafficking are tightly regulated and also directly involved in cytoskeleton based trafficking. Since EB1-tipped microtubules and actin are both impaired in stressed myocardium [39, 109], GJA1-20k could stabilize the cytoskeleton delivery apparatus during the injury or cell stress. Further understanding of the role of GJA1-20k in cytoskeleton-based vesicular transport may lead to introducing new therapeutic drugs such as mTOR inhibitors or others for preserving the abundance of Cx43 and regulating cell coupling at the intercalated disc.

It is clear that Cx43 affects cell proliferation, its trafficking and trafficking of other ion channels through numerous cell signaling and protein-protein interaction pathways, which are demonstrated in many connexin-associated diseases. Cx43 is a dynamic protein and interaction with so many different proteins is necessary to regulate every step of Cx43 life cycle: formation, forward trafficking to the correct subdomain on the plasma membrane, assembly on the plasma membrane, retrograde trafficking, and degradation. Cx43 does not function alone but rather in concert with other proteins and smaller isoforms from the same gene and mRNA. Greater understanding how Cx43 isoforms affect the trafficking and localization of full length Cx43 gap junction channels can provide new targets for regulating gap junction coupling. Furthermore, it is like that the smaller isoforms affects the trafficking of other ion channels, thus broadening the scope of products of Cx43 alternative translation to general regulation of cardiac electrophysiology.

Acknowledgments

We acknowledge our funding sources from the American Heart Association (13EIA4480016) and the National Institute of Health/National Heart, Lung, and Blood Institute (RO1 HL094414). We are very grateful to Dr. Shaohua Xiao and Dr. Shan-Shan Zhang for their significant contributions to figure's preparation. The authors have no conflicts of interest to declare.

Abbreviations

GJs	Gap Junctions
GJIC	Gap Junctional Intercellular Communication
CT	Carboxy-tail (C-terminus domain, CT-tail, Cx43-CT)
Cx43	Connexin 43
CDS	Coding Domain Sequence
F-actin	Filamentous actin
kDa	Kilodalton
ID	Intercalated Disc
IRES	Internal Ribosome Entry Site
ORF	Open Reading Frame
ER	Endoplasmic Reticulum
TNG	Trans-Golgi Network
ERGIC	Endoplasmic Reticulum-Golgi Intermediate Compartment
eIFs	Eukaryotic Initiation Factors

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Highlights

- Gap junction channels are essential aspects of cardiomyocyte coupling which provide synchronous cardiac contraction.
- The trafficking of gap junction channels to the precise subdomain in the cell known as targeted delivery and its regulation is important for maintenance of cardiac synchrony.
- Six endogenous truncated Cx43 isoforms are produced from the same full-length GJA1 mRNA molecule by means of alternative translation.
- 20kDa alternatively translated isoform of Cx43 is a protein-chaperone and can autoregulate trafficking of the full length protein.

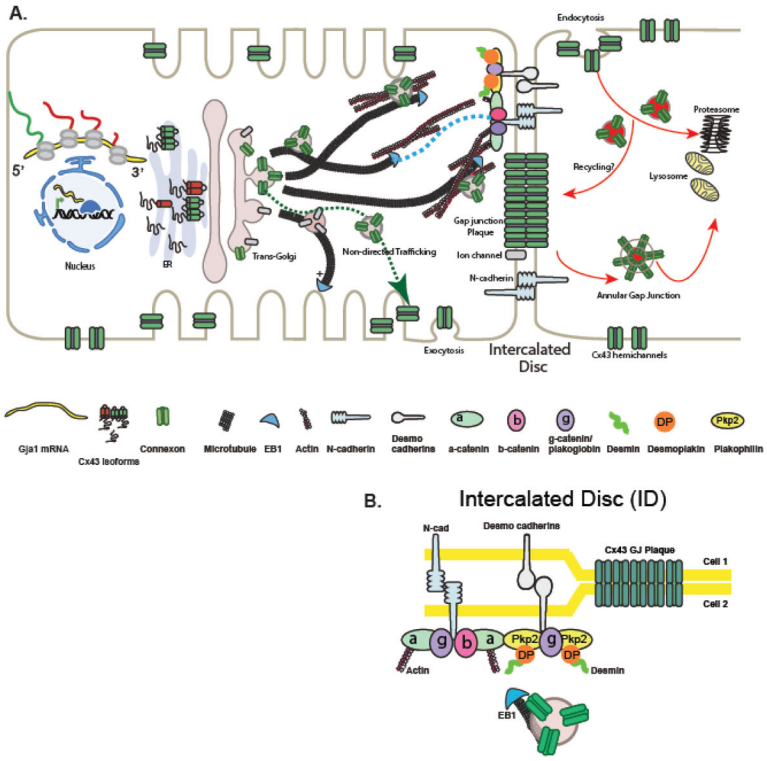

Figure 1. The Cx43 trafficking life cycle and Targeted Delivery to the cell-cell border

(A). The Cx43 lifecycle, comprising anterograde trafficking, lateral diffusion and retrograde trafficking, and highlighting cytoskeletal-based directed targeting of Cx43 to form gap junctions (GJs) at the intercalated disc (ID). Cx43 is targeted to adhesion complexes, containing N-cadherin and catenin-containing regions of the intercalated disc, along EB1-tipped microtubules to form GJ plaques at the ID. Actin is required for GJ localization, but the underlying mechanism is currently unknown.

(B). The Cx43 GJ plaque formation at the ID.

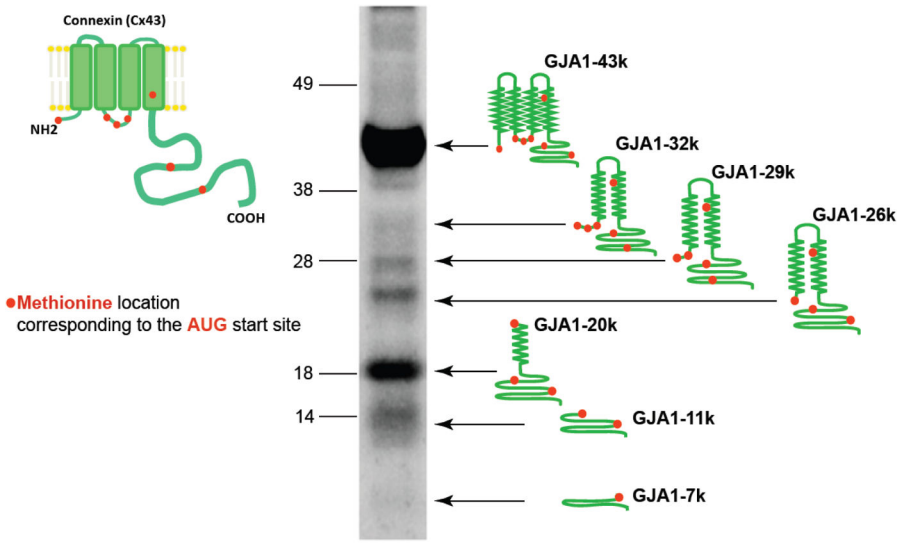

Figure 2. One exon of *GJA1* mRNA produces seven proteins

Western blot of nonfailing heart probed to monoclonal antibodies against the Cx43 C-terminus. (Western blot is taken from Smyth and Shaw, Cell Reports, 2013).

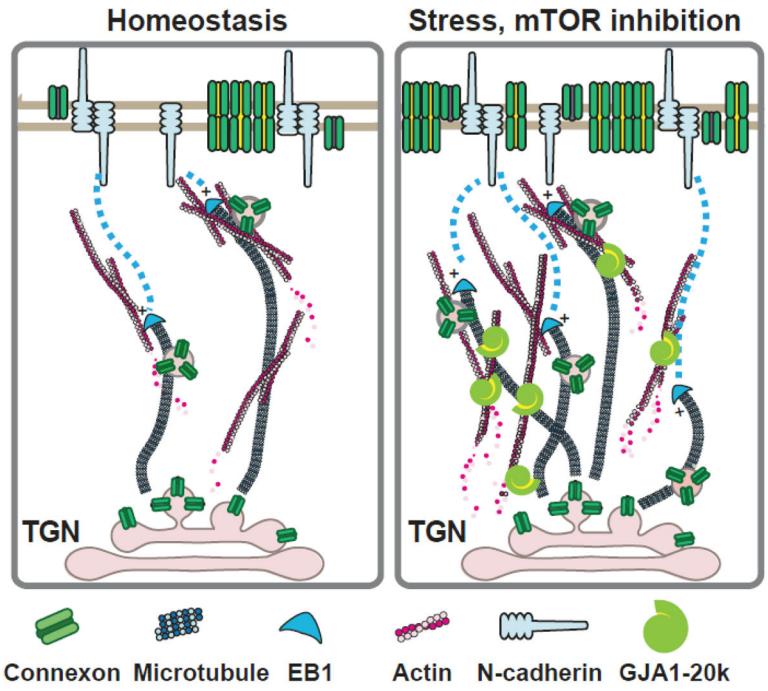

Figure 3. Prospective mechanism of action for GJA1-20k truncated isoform

The internally translated CJA1-20k isoform is associated with the ER and dynamic vesicles, forming highly organized contacts with F-actin and microtubule networks. GJA1-20k interacts with F-actin, provides stabilization of filamentous actin and microtubules. F-actin disruption by oxidative stress or Latrunculin A impairs growth trajectories of the EB1/microtubule-based trafficking machinery, and decreases GJ localization to the cellular border, which can be rescued by exogenous GJA1-20k.