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Connexin43, and the regulation of intercalated disc function

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Gap junctions mediate the passage of ions and small molecules between cells. In the adult working cardiac ventricles, gap junctions are formed predominantly by oligomerization of the 43 kDa protein connexin43 (Cx43). It is generally accepted that gap junction-mediated intercellular communication is modulated by changes in the intracellular environment. The activity of various kinases, the concentration of protons or calcium, or the association of Cx43 with other intracellular components, all converge to determine the filtering capabilities of intercellular channels. The most studied post-translational modification of Cx43 is the phosphorylation of amino acids in its C-terminal domain (see, e.g.^{1,2}). Additional studies have shown that Cx43 is also a substrate for Nε-lysine acetylation.³ Changes in the ionic intracellular milieu can also, directly or indirectly, regulate the conductive state of Cx43. These modulatory mechanisms are likely to be activated in various pathophysiological states.² Given the key role of gap junctions in action potential propagation, Cx43 regulation is a subject of intense research, and it is seen as an important pharmacological target for the treatment and/or prevention of cardiac arrhythmias.³⁻⁶

Gap junction regulation by non-catalytic molecules: mechanical junctions

In addition to the regulation of Cx43 by catalytic molecules, electrical coupling is modulated by non-catalytic components of the cell. Of particular interest is the cross-talk of gap junctions with molecules classically defined as part of “mechanical junctions.” In the heart, mechanical continuity is provided by desmosomes and by adherens junctions. Like the gap junctions, these complexes preferentially reside at the site of end-end contact between myocytes, within the intercalated disc. For many years, gap junctions in the heart were considered independent from the molecules that form the mechanical junctions. An important breakthrough came with a study (which in fact, occupied the first pages of the opening issue of *HeartRhythm*⁷) indicating that connexin43-immunoreactive gap junction plaques were absent in cardiac tissue obtained from the heart of patients expressing mutant desmosomal proteins. Those findings opened the notion of a crosstalk between two intercalated disc components previously considered independent (desmosomes and gap junctions). Additional studies have confirmed this observation. Separately, Glenn Radice and his colleagues demonstrated that loss of N-cadherin, the transmembrane protein of the adherens junctions, leads to severe arrhythmias and to the loss of gap junction plaques.⁸ These studies were followed by others, in cell systems as well as in animal and human

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samples, supporting the notion that disruption of mechanical junctions, be it desmosomes or adherens junctions, disrupts the integrity of the gap junction plaques. Proper electrical coupling was then found to rely on the preservation of mechanical coupling between cells. A different role for adherens junctions proteins on gap junction formation was demonstrated by the work of Robin Shaw and his colleagues.⁹ These authors provided evidence indicating that N-cadherin is necessary for the delivery of de novo connexons to the plasma membrane. These results highlight the importance of ion channel trafficking as an important regulatory step in the life cycle of Cx43.

Gap junction regulation by non-catalytic molecules: Ankyrin-G

Also resident of the intercalated disc are molecular complexes not traditionally considered “junctional,” that is, not involved in providing a physical continuum between neighboring cells. The question arises as to whether “non-junctional” complexes can also interact with gap junctions and modify their function. An intercalated disc-resident complex highly relevant to cardiac electrophysiology is that of the sodium channel. Two key protein components (among others) are $\text{Na}_v1.5$, and the scaffolding protein ankyrin-G (AnkG). Studies in neonatal ventricular myocytes showed that loss of AnkG expression led to a decrease in Cx43 abundance, a loss of Cx43 gap junction plaques, and a reduction of junctional conductance between myocytes.¹⁰ These results led to the hypothesis that AnkG is a scaffolding molecule shared by both, gap junctions and sodium channels. As such, two complexes of highly complementary function share not only a common location but also, a scaffolding molecule that is capable of acting as a point of convergence for other shared regulators. Evidence not discussed here also shows that loss of AnkG expression weakens intercellular adhesion strength, whereas plakophilin-2 is necessary for proper sodium channel function. Overall, the data strongly suggest that the boundaries between the components of one complex or another are in fact much more tenuous than originally thought.

The crowded quarters of a gap junction plaque

For gap junctions to interact with components of other complexes, physical proximity would be required. We have utilized improved methods of tissue preservation (high-pressure freezing and freeze-substitution) to evaluate the type of electron-dense structures that can be found in the immediate vicinity of a gap junction plaque in the intercalated disc. Figure 1 shows some of our results. The low-magnification image in panel A shows the quality of tissue preservation, and the presence of an intercalated disc winding between two cells, from one end to the other. Higher magnifications show the very close physical proximity between structures. In panel B, a mitochondria sits within a few nanometers from both, a gap junction plaque and a desmosome. In panel C, the yellow arrow points to a gap junction plaque can actually make contact with the mitochondrial outer membrane (is this a part of the “mitochondrial Cx43”?). Finally, panel D reveals that, given the turning and twisting of the membrane, a gap junction can be in contact with the inner part of a desmosomal plaque. The close spatial relation between structures is further emphasized in Figure 2. In this case, we present a reconstruction of a tomographic electron microscopy image of an intercalated disc. A movie displaying all the different planes is included in the supplemental data (Suppl. Figure 1). Notice not only the proximity between desmosomes and the gap junction plaque but also, the strong vesicular activity present between the two structures and that seems to involve the mitochondria located at both sides of the intercellular space. These images suggest that Cx43 within a gap junction plaque can be in physical contact with the molecular components of other structures. A close look at these images yields unlikely the possibility that gap junctions are functionally independent from other complexes at the intercalated disc.

Cx43 outside gap junctions

The electron micrographic images demonstrate the proximity between electron-dense structures. As such, they argue that the Cx43 that is present within a gap junction plaque is exposed to molecules in other electron-dense components. It thus seems logical to argue that Cx43 near the plaque (but not within the plaque) would be even more exposed to the surrounding molecular milieu. In that context, it is important to remember that Cx43 at the intercalated disc is not exclusively circumscribed to the gap junction plaque. In an elegant recent study, Robert Gouride and his colleagues demonstrated that the area surrounding a gap junction plaque is populated by non-gap junction forming Cx43 connexons.¹¹ Their studies showed that Cx43 in this area, which they dubbed “the perinexus,” closely associates with the scaffolding protein ZO-1 (zonula occludens-1); more importantly, they demonstrated that the Cx43-ZO-1 association in the perinexus limits the abundance of Cx43 within the gap junction pool. As such, loss of the Cx43-ZO-1 interaction increases the proportion of Cx43 involved in gap junction channel formation, at the expense of a non channel-forming pool.

The studies of Sato et al¹⁰ indicate that two other scaffolding proteins present at the intercalated disc (AnkG and PKP2) also associate with Cx43. It seems reasonable to speculate that this interaction is also “perinexal,” that is, not within (or not *only* within) the gap junction plaque. While disruption of Cx43-ZO-1 association leads to larger gap junction plaques,¹¹ loss of AnkG or PKP2 decreases intercellular communication.^{10,12} Ankyrins are known to be relevant for the proper targeting of proteins to their functional subdomain.¹³ PKP2 is necessary for recruitment of molecules at a junctional site.¹⁴ We speculate that AnkG and PKP2 recruit and retain Cx43 into the perinexus, from where ZO-1 will control its trafficking into the gap junction pool. Failure to recruit or retain (loss of association with PKP2 or AnkG) would therefore cause the reduction and eventual loss of the gap junction plaque. Failure to interact with ZO-1 would have the opposite effect (an increased gap junction plaque size). While the loss of either scaffolding protein would deplete the pool of Cx43 from the perinexus, in one case gap junction plaques would be formed (and in fact, would be larger) and in the other, gap junctions would also be lost. At the end, the perinexus may represent a site with multiple switches, provided in part by the relevant scaffolding proteins, ultimately regulating the size and the function of the gap junction plaque. Yet, while in the perinexus, Cx43 may exert an important effect on the function of other ion channels, as further discussed below.

Cx43 as an ancillary component of other molecular complexes

The finding of a separate pool of Cx43 in the intercalated disc, invites speculation as to its possible function. It is unlikely that the Cx43 molecules in the perinexus are simply on a “standby” mode, waiting idle for the green light to move into the gap junction plaque. It is also unlikely that Cx43 and ZO-1 are the only inhabitants of the perinexal space (see Figures 1 and 2 here, and the discussion in the paragraph above). Rather, we speculate that this pool of Cx43 is in fact exposed to a variety of other molecular complexes that approach -but are not components of- the gap junction plaque. Some of those molecules are likely regulators of Cx43 function (e.g., kinases). Others, we hypothesize, may actually see their function modified by the presence of Cx43. In other words, we speculate that while Cx43 in the perinexus awaits passage into the gap junction plaque, it also acts as an accessory to the function of other molecular complexes. In that context, it is important to mention that several investigators have identified unexpected effects consequent to the loss of Cx43 expression, or to the remodeling of Cx43. For example, Danik et al reported a decrease in the amplitude of potassium currents in Cx43-deficient mice,¹⁵ whereas Lin et al have reported decreased local sodium current amplitude after cell separation (and Cx43

internalization¹⁶), and various laboratories have pointed to functional effects that require connexin expression even if the connexin molecule does not form functional channels (or hemichannels). Overall, while still highly speculative, there is reason to believe that Cx43 is not only a pore-forming subunit of gap junctions but also, a functional component of other molecular complexes. The perinexus may provide a physical space where these Cx43-dependent, gap junction-independent functions take place. A point in case may actually be Ankyrin-G, which would provide an anchoring point for the interaction of Cx43 with other ion channel molecules (e.g., Nav1.5). Future studies may draw a portrait of Cx43 not only as the highly regulatable molecule that has been extensively described but also, as a regulator of the function of other molecular complexes.

In summary, Cx43 regulation has been extensively documented, and its importance for the function of gap junctions is well accepted.^{1,2} Yet, the present evidence lead us to suggest that the function of Cx43 goes beyond that of making gap junctions. Improvements in the preservation of samples, and the resolution of optical images gives us a glimpse into a world where Cx43 is not confined to the inside of a gap junction plaque and in fact, it may share nano-domains with other ion channel complexes, thereby affecting their function. The gap junction-independent role of Cx43 as a regulator of cell electrophysiology emerges as a novel and exciting area for future investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Marquez-Rosado L, Solan JL, Dunn CA, Norris RP, Lampe PD. Connexin43 phosphorylation in brain, cardiac, endothelial and epithelial tissues. *Biochim Biophys Acta*. 2011
2. Remo BF, Qu J, Volpicelli FM, et al. Phosphatase-resistant gap junctions inhibit pathological remodeling and prevent arrhythmias. *Circ Res*. 2011; 108(12):1459–1466. PMID: 3126103. [PubMed: 21527737]
3. Colussi C, Rosati J, Straino S, et al. Nepsilon-lysine acetylation determines dissociation from GAP junctions and lateralization of connexin 43 in normal and dystrophic heart. *Proc Natl Acad Sci U S A*. 2011; 108(7):2795–2800. PMID: 3041095. [PubMed: 21282606]
4. Qu J, Volpicelli FM, Garcia LI, et al. Gap junction remodeling and spironolactone-dependent reverse remodeling in the hypertrophied heart. *Circ Res*. 2009; 104(3):365–371. PMID: 2652889. [PubMed: 19096029]
5. Verma V, Larsen BD, Coombs W, et al. Novel pharmacophores of connexin43 based on the "RXP" series of Cx43-binding peptides. *Circ Res*. 2009; 105(2):176–184. PMID: 2737470. [PubMed: 19556520]
6. O'Quinn MP, Palatinus JA, Harris BS, Hewett KW, Gourdie RG. A peptide mimetic of the connexin43 carboxyl terminus reduces gap junction remodeling and induced arrhythmia following ventricular injury. *Circ Res*. 2011; 108(6):704–715. PMID: 3076085. [PubMed: 21273554]
7. Kaplan SR, Gard JJ, Protonotarios N, et al. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). *Heart Rhythm*. 2004; 1(1):3–11. [PubMed: 15851108]
8. Li J, Patel VV, Kostetskii I, et al. Cardiac-specific loss of N-cadherin leads to alteration in connexins with conduction slowing and arrhythmogenesis. *Circ Res*. 2005; 97(5):474–481. [PubMed: 16100040]

9. Shaw RM, Fay AJ, Puthenveedu MA, von Zastrow M, Jan YN, Jan LY. Microtubule plus-end-tracking proteins target gap junctions directly from the cell interior to adherens junctions. *Cell*. 2007; 128(3):547–560. PMID: 1955433. [PubMed: 17289573]
10. Sato PY, Coombs W, Lin X, et al. Interactions between ankyrin-G, Plakophilin-2, and Connexin43 at the cardiac intercalated disc. *Circ Res*. 2011; 109(2):193–201. PMID: 3139453. [PubMed: 21617128]
11. Rhett JM, Jourdan J, Gourdie RG. Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol Biol Cell*. 2011; 22(9):1516–1528. PMID: 3084674. [PubMed: 21411628]
12. Oxford EM, Musa H, Maass K, Coombs W, Taffet SM, Delmar M. Connexin43 remodeling caused by inhibition of plakophilin-2 expression in cardiac cells. *Circ Res*. 2007; 101(7):703–711. [PubMed: 17673670]
13. Lowe JS, Palygin O, Bhasin N, et al. Voltage-gated Nav channel targeting in the heart requires an ankyrin-G dependent cellular pathway. *J Cell Biol*. 2008; 180(1):173–186. PMID: 2213608. [PubMed: 18180363]
14. Bass-Zubek AE, Hobbs RP, Amargo EV, et al. Plakophilin 2: a critical scaffold for PKC alpha that regulates intercellular junction assembly. *J Cell Biol*. 2008; 181(4):605–613. PMID: 2386101. [PubMed: 18474624]
15. Danik SB, Rosner G, Lader J, Gutstein DE, Fishman GI, Morley GE. Electrical remodeling contributes to complex tachyarrhythmias in connexin43-deficient mouse hearts. *FASEB J*. 2008; 22(4):1204–1212. PMID: 2726820. [PubMed: 17984180]
16. Lin X, Liu N, Lu J, et al. Subcellular heterogeneity of sodium current properties in adult cardiac ventricular myocytes. *Heart Rhythm*. 2011 In Press.

Figure 1.

Transmission electron microscopy image of adult murine heart. Tissue prepared by high-pressure freezing and freeze-substitution method. D, desmosome. GJ, gap junction. M, mitochondria. Panel A: low magnification highlighting the degree of tissue preservation, and the entire intercalated disc as it meandered between two cells. B and C show the proximity (and, in C, the contact; yellow arrow) between mitochondria, gap junctions and desmosomes. D shows an example of a very close approximation of a gap junction, to a desmosome.

Figure 2. Compiled three-dimensional tomographic electron microscopy reconstruction of an intercalated disc. Notice the proximity between mitochondria, desmosomes and gap junctions, and the extensive vesicular activity that can be observed between the three structures.