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The cardiac connexome: Non-canonical functions of Connexin43 and their role in cardiac arrhythmias

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Abstract

Connexin43 is the major component of gap junctions, an anatomical structure present in the cardiac intercalated disc that provides a low-resistance pathway for direct cell-to-cell passage of electrical charge. Recent studies have shown that in addition to its well-established function as an integral membrane protein that oligomerizes to form gap junctions, Cx43 plays other roles that are independent of channel (or perhaps even hemi-channel) formation. This article discusses non-canonical functions of Cx43. In particular, we focus on the role of Cx43 as a part of a protein interacting network, a connexome, where molecules classically defined as belonging to the mechanical junctions, the gap junctions and the sodium channel complex, multitask and work together to bring about excitability, electrical and mechanical coupling between cardiac cells. Overall, viewing Cx43 as a multi-functional protein, beyond gap junctions, opens a window to better understand the function of the intercalated disc and the pathological consequences that may result from changes in the abundance or localization of Cx43 in the intercalated disc subdomain.

Keywords

Cx43; gap junctions; sodium channels; desmosomes; intercalated disc

1. Introduction and historical perspective

The heartbeat results from the added output of millions of cells that contract in synchrony. To achieve this function, complex molecular networks work in concert, with exquisite temporal precision. The accurate timing of the molecular events demands a comparable precision on the location of each molecule within the cell. Indeed, molecular networks organize within well-confined microdomains, where physical proximity allows for prompt and efficient interaction. In turn, loss of molecular organization in the nanoscale can be a core component in the pathophysiology of disease.

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The present review focuses on the cardiac intercalated disc, a region of specialization formed at the end-end site of contact between cardiac myocytes. When first observed through light microscopy (in 1866), the intercalated disc was considered “a cementing material” at cardiac cell boundaries. The 1893 article by Przewoski “Du mode de reunion des cellules myocardiques de l’homme adulte” supported the idea that the intercalated disc was necessary for cell-cell adhesion. However, the scientific community at the time was divided on whether cardiac cells were separate from each other, or fused into a single syncytium. The latter hypothesis was in fact favored by most during the early twentieth century. The advent of electron microscopy tilted and eventually settled this debate. The studies of Sjostrand and Andersson [1] and others showed that the intercalated disc consisted of a double membrane, flanked by the termination of myofibrils in dense material. Their observations led Muir [2] to conclude that “the discs represent the junctions between neighboring cardiac muscle cells.” He then wrote: “... there is no valid evidence to contest the statement that the intercalated discs are specialized regions of cellular adhesion.” Sjostrand and colleagues further described an area of specialization in the cardiac intercalated disc composed of “three dark lines with two intervening less dense lines” [3]. This structure, which was similar to the one previously identified in the giant axon of the crayfish, was named the “longitudinal connexion” by these investigators. Years later, Revel coined the term “gap junctions”, thus emphasizing two key features: a gap between the cells and yet a junction between them. Since then, and also as a result of the pioneering electrophysiological experiments of Weidmann [4], the intercalated disc has been recognized as an area of specialization that provides a physical continuum between cardiac cells through mechanical junctions (desmosomes; adherens junctions; area composita [5]) and intercellular channels (gap junctions).

2. Cardiac gap junction ultrastructure

Modern methods of electron microscopy have allowed us to better visualize the intercalated disc, including gap junction plaques and the intercellular space, and to obtain a structural solution in three dimensions [6, 7]. The resulting image departs somewhat from the classical picture of the intercalated disc as composed of separate and independent rigid structures. The tomographic electron micrograph images of Figure 1 show for example the close proximity that can exist between gap junctions and desmosomes (see [7]). Also notice the close association between gap junctions and mitochondria, perhaps facilitating molecular interactions largely unexplored (also see Figure 1 in [6]). Furthermore, the analysis of gap junction plaques in 3D shows that at least in some cases, what could have been considered an internalized gap junction is actually a tubular formation of the intercalated disc that projects into the cell. An example is shown in Figure 2. Data were collected applying Focused Ion Beam Scanning Electron Microscopy to a block of mouse ventricular tissue. A single XY image is shown in panel A, containing a portion of intercalated disc, where a gap junction (GJ) embedded in the plicate region can be seen. Panel B shows another single XY image of the same region but 260 nm deeper into the tissue. Just by looking at the image in panel B, one could think that the circular structure observed near the ID represents an internalized gap junction (label “IGJ?”). Panel C shows a gallery view of 24 XY sections 20 nm apart from each other as we go deeper in the tissue. XY section number 1 corresponds to

panel A, and section number 13 corresponds to panel B. The images clearly show that the “internalized GJ” is in fact part of a finger like protrusion of the intercalated disc that projects along the z axis of the sample. Segmentation of the contours of this “internalized GJ” along all the z planes yielded the 3D reconstruction shown in Figure 3. Note the finger-like projection of the ID and its curved trajectory. The complete structure measures almost 500 nanometers from top to bottom. It seems clear that a single micrograph obtained at plane 13 could have been interpreted as an internalized gap junction, whereas the 3D rendering obtained speaks of a different interpretation. This example is shown as evidence of the power of new visualization methods to study not only structures but also, cell biological processes predicated in part on the bases of 2D imaging data.

Overall, the ultrastructural information shows that cardiac gap junction plaques have a complex three-dimensional structure, likely allowing for protein interactions with various molecular partners. The spatial organization of these intermolecular interactions remains an important area of active investigation (see also [8, 9]).

3. Gap junctions and connexins: not the same thing

Gap junctions were originally defined as an anatomical structure. It was then discovered that they are primarily composed of intercellular channels that provide a low-resistance pathway for direct cell-to-cell passage of electrical charge between cardiac myocytes. Subsequent work demonstrated that each gap junction channel is composed of two hexameric structures called connexons that dock across the extracellular space and form a permeable pore. Each connexon results from oligomerization of an integral membrane protein, connexin. The most abundant connexin isotype in the heart, brain and other tissues is the 43-kD protein, connexin 43 [10]. The importance of Cx43 expression in the propagation of the cardiac action potential is well established. If Cx43 is not expressed, normal propagation is disrupted and lethal arrhythmias can ensue [11, 12]. These and other studies have indicated that Connexin43 plays a fundamental role in cardiac electrophysiology (see also [13–18]).

3.1 Cx43 also localizes outside the gap junction plaque and interacts with “non-gap junction” molecules

It was a long-held assumption that the role of Cx43 on propagation was directly associated with its ability to form gap junction channels. Yet, it is not until recently that this assumption has been challenged. Indeed, there is no data supporting the notion that connexins are single-function molecules. In fact, structural evidence indicates that Cx43 is not only located in the gap junction plaque but also in the plicate region of the intercalated disc, likely as a component of the area composita. The Gourdie laboratory was first to demonstrate the existence of a pool of connexons that localize to the periphery of a gap junction plaque (a structural domain called “the perinexus”), where they do not form gap junctions but maintain functionally relevant intermolecular interactions [8]. Subsequently, we utilized immunoelectron microscopy to demonstrate that Cx43 is not only present along the interplicate region of the cardiac intercalated disc, forming gap junction plaques but also in the plicate region (the region perpendicular to the direction of the actin fibers), coinciding with structures associated with mechanical coupling (see Figure 4, reproduced from [19]). In a separate study [20] we utilized single molecule imaging and analysis tools to demonstrate

the co-localization of Cx43 with a specific desmosomal molecule, PKP2. A set-up using commercially available optics was built on a conventional inverted microscope. Laser illumination, reducing, and oxygen scavenging conditions were used to manipulate the blinking behavior of individual fluorescent reporters. Movies of blinking fluorophores were acquired and processed to generate subdiffraction images at 20 nm resolution (based on direct stochastic optical reconstruction microscopy, or dSTORM). In about half of Cx43 clusters, we observed overlay of Cx43 and PKP2 at the Cx43 plaque edge (Figure 5). SiRNA-mediated loss of Ankyrin-G expression yielded larger Cx43 clusters, of less regular shape, and larger Cx43-PKP2 subdomains, further showing that the Cx43-PKP2 complex is affected by a scaffolding protein classically considered a member of a third-party: the voltage-gated sodium channel complex [21]. The Cx43-PKP2 subdomain was validated by a proximity ligation assay (PLA) and by Monte-Carlo simulations indicating an attraction between PKP2 and Cx43. Overall, the data show that PKP2 and Cx43 share a common hub that permits direct physical interaction. This structural paper demonstrated for the first time the co-localization (in the nanometric range) between Cx43 and a molecule conventionally described as belonging to a mechanical junction, and led us to propose that Cx43 is part of a protein interacting network, separate and independent from the gap junction plaque, that we dubbed “the connexome” (see also [22]).

3.2 Non-electron dense molecular complexes at the intercalated disc and their interaction with Cx43

The presence of Cx43 outside the gap junction plaque opens the possibility that as a molecule, Cx43 takes part in functions classically ascribed to other molecular complexes. It is therefore important at this juncture to mention that at least some channel protein complexes involved in both depolarization and repolarization localize preferentially to the intercalated disc. This physical proximity allows for a key functional consequence: molecules traditionally defined as junctional, such as connexin43 (Cx43) actually regulate the function of ion channels responsible for the action potential. In turn, molecules accessory to ion channels are also relevant for gap junction function [6, 23, 24]. Below we will describe evidence supporting the notion that the intercalated disc is not just the site of residence of independent “junctional” and “non-junctional” complexes that are oblivious to the presence and function of the others. It is, rather, the home of a protein interacting network (the connexome, as introduced above) where molecules multi-task to achieve, jointly, intimately-related functions: the entry and exit of charge into the cell, the transfer of charge between cells, and the anchoring of cells to each other, which provides a mechanically stable environment critical to ion channel function.

3.3 Connexin43 regulates potassium currents

The first report correlating Cx43 expression to non-junctional currents was by Danik et al. [25]. These authors noted that the action potential duration recorded from the ventricle of Cx43 conditional knockout animals were significantly shorter than control. This shortening associated with higher levels of sustained repolarizing current and higher levels of inward rectifier current in myocytes from the right ventricle. These data were first to show that Cx43 is not only a gap junction-forming molecule in the heart but also, a component of a molecular network that regulates excitability and repolarization. A relation between Cx43

primary structure and potassium current function was also demonstrated by Lubkemeier et al. [26].

3.4 Connexin43 regulates sodium current

The biochemical interaction between Cx43 and other ion channel complexes came from the work of Malhotra et al, showing co-precipitation of Nav1.5 with Cx43 [27]. The physical proximity of these molecules was later observed by Rhett et al. [28] Functional evidence of cross-talk between Cx43 and the sodium channel complex was provided by Jansen et al. [29]. As shown in Figure 6, these authors showed that siRNA-mediated loss of Cx43 expression in adult ventricular myocytes leads to a decrease in the amplitude of the sodium current (I_{Na} [29]). The functional effect coincided with decreased co-localization of Cx43 and Nav1.5 at the intercalated disc. A similar decrease in I_{Na} was later reported by Desplantez et al in fetal atrial myocytes of Cx43-deficient mice [30]. Overall, the data demonstrate that Cx43 expression is necessary for proper sodium current function, and for the accumulation of Nav1.5 at the cardiac intercalated disc [31].

3.5 Loss of the Cx43 PDZ binding domain: sudden cardiac death with normal gap junctions

The observations by Danik et al. [25] and by Jansen et al. [29] related to the complete loss of Cx43 expression. More recently, Lubkemeier et al reported that a cardiac specific, tamoxifen-activated knockin murine model of a Cx43 mutation caused sudden death in the absence of a structural disease. The mutation in question was a loss of the last five amino acids of the Cx43 sequence (i.e., truncation Cx43D378stop). The original thought was to create a murine model where Cx43 could not bind to a PDZ domain-containing protein (particularly, ZO-1). The results were surprising in two fronts: First, the association of Cx43 and ZO-1 was unaffected by the mutation; second, mice died in ventricular fibrillation within three weeks of the tamoxifen injection, and the gap junctions were normal. Patch clamp analyses of isolated adult Cx43D378stop cardiomyocytes revealed a significant decrease in sodium and potassium current densities. Furthermore, we also observed a significant loss of Nav1.5 protein from intercalated discs in Cx43D378stop hearts. The phenotypic lethality of the Cx43D378stop mutation was very similar to the one previously reported for adult Cx43 deficient (Cx43KO) mice. Yet, in contrast to Cx43KO mice, the Cx43 gap junction channel was still functional in the Cx43D378stop mutant. We concluded that the lethality of Cx43D378stop mice is independent of the loss of gap junctional intercellular communication, but most likely results from impaired cardiac sodium and potassium currents. The Cx43D378stop mice revealed for the first time that Cx43 dependent arrhythmias can develop by mechanisms other than impairment of gap junction channel function.

4. The connexome as a site of microtubule capture in the intercalated disc

A follow up study further explored the mechanisms underlying the loss of sodium current in the Cx43D378stop mice. The elegant work of Robin Shaw and his colleagues had clearly documented the concept of targeted delivery, showing that microtubule-mediated trafficking of Cx43 was targeted to N-cadherin rich subdomains [32–34]. The presence of Cx43 at N-

cadherin-rich domains (likely the area composita; [5] see also Figure 4 in this review, reproduced from [19]) led us to propose that once at the area composita, Cx43 itself, via its PDZ binding domain, may facilitate the anchoring of microtubules that are delivering Na_v1.5 to the cell end [35]. To assess this hypothesis required specific localization of relevant molecular players, and the latter was prevented by the resolution limit of fluorescence microscopy. By the application of the nanoscale imaging methods introduced above, it was possible to establish: (i) the morphology of clusters formed by the microtubule plus-end tracking protein 'end-binding 1' (EB1), (ii) their position, and that of sodium channel alpha-subunit Na_v1.5, relative to N-cadherin-rich sites, and (iii) the role of Cx43 C-terminus on the above-mentioned parameters and on the location-specific function of I_{Na}. Super-resolution fluorescence localization microscopy in murine adult cardiomyocytes revealed EB1 and Na_v1.5 as distinct clusters preferentially localized to N-cadherin-rich sites (see Figure 7). The extent of co-localization decreased in Cx43D378stop cells. Functional studies using macropatch [36] and scanning patch clamp [37] showed reduced I_{Na} exclusively at cell end, without changes in unitary conductance. Experiments in Cx43-modified HL1 cells confirmed the relation between Cx43, I_{Na}, and microtubules. From this study we concluded that Na_v1.5 and EB1 localization at the cell end is Cx43-dependent. Cx43 is part of a molecular complex that determines capture of the microtubule plus-end at the ID, facilitating cargo delivery. These observations firmly linked excitability and electrical coupling through a common molecular mechanism.

5. The connexome and the intracellular signaling platforms

A function-beyond-adhesion well known to mechanical junctions is that of supporting intracellular signaling platforms. While this has been well described for the cadherin-catenin axis [38], it is not until recently that the role of plakophilin-2 in intracellular signaling has been brought to light. In particular, previous studies show that PKP2 is necessary for the proper function of RhoA [39] and of PKC α [40] signaling. The latter may play a key role in the activation of the Hippo pathway and of Wnt signaling, both potentially involved in the pathogenesis of arrhythmogenic cardiomyopathy [41, 42]. Separate work has shown that the ankyrin/spectrin core at the intercalated disc scaffolds a signaling platform to regulate cardiac excitability [43]. Interestingly, a cross-talk between the AnkG and the PKP2-supported platforms was unveiled by those studies. Given the relation of Cx43 both with the sodium channel complex and with the mechanical junctions discussed above, we speculate that Cx43 also forms part of the scaffolding for these intracellular signaling platforms. The role of Cx43 in scaffolding intracellular signaling molecules remains an area still poorly explored and yet likely to be of high functional relevance.

6. The intercellular space

The size of the space separating two cardiac cells at the intercalated disc changes depending on the proximity to the various structures, as well as the vesicular activity between the two cells (see Figure 1). It is a common view that the intercellular space is not relevant for electrophysiology. This view, however, is changing. Mathematical modeling studies (e.g., [44, 45]) and experimental evidence [46] support the idea that the intercellular space may be critical to propagation via an electric field mechanism [47]. This model will be discussed

later in this review. Of note, increased size of the intercellular space has been reported in animal models of arrhythmogenic cardiomyopathy [48–51], though the opposite (an actual reduction) was found by Leo-Macias et al in a comprehensive, three-dimensional analysis of the intercellular space by tomographic electron microscopy of thin sections of ventricular tissue [7]. The findings further showed that at the sites occupied by area composita, the intercellular membranes of wild-type murine adult ventricular tissue separate by a gap of approximately 63 nm. This measure may be relevant to modeling studies exploring the possibility of electric field-mediated propagation in the heart [9, 44, 47, 52].

7. The connexome, visual proteomics and the possibility of “mini-nodes of Ranvier” at the intercalated disc

The observations described throughout this article support the notion that Cx43 is part of a protein interacting network where molecules work together to collectively achieve electrical coupling, cell excitability and intercellular adhesion strength. Because of its functional relevance, and its association with Cx43 [27], the precise location of the alpha subunit of the sodium channel molecule (Nav1.5) is a subject of particular interest. Using a “visual proteomics” approach [53] that combined correlative light-electron microscopy, angle-view scanning patch clamp, three-dimensional single molecule localization microscopy, focused ion beam scanning electron microscopy, Monte-Carlo and random-walk simulations, we have gathered preliminary evidence suggesting that Nav1.5 organizes into clusters that reside adjacent to N-cadherin rich domains, likely at the area composita. These nodes containing both adhesion and excitability molecules are reminiscent of nodes of Ranvier, where Nav channels are packed into clusters in close association with adhesion molecules [54]. As in the case of neurons [55, 56], AnkG may be critical for the organization of the structure and the retention of channels within the tightly packed node.

8. The mini-node of Ranvier, and the possibility of ephaptic transmission

Several mathematical models of cardiac action potential propagation assume that gap junctions are the only path for transfer of charge between cells. Accordingly, they predict that decreases in junctional conductance bring about decreases in conduction velocity. This notion contrasts sharply with actual data showing that only extreme reductions in Cx43 abundance (and electrical coupling) lead to significant changes in conduction velocity [11, 57, 58]. These results have given new impetus to the notion that, under poor gap junction-mediated coupling, propagation can be maintained via a separate “electric field mechanism” [44, 45, 47, 52] also referred to as “ephaptic transmission” [9, 47, 52]. This alternative postulates that the large I_{Na} in the proximal side of an intercellular cleft generates a negative extracellular potential within the cleft, which depolarizes the distal membrane and activates its sodium channels. Thus, propagation can continue downstream in the absence of gap junctions provided there is a large I_{Na} at the intercalated disc, and a narrow intercellular cleft separating the two apposing cells.

The success or failure of propagation under the model described above depends heavily on the dimensions of the intercellular cleft at the site where the sodium channel clusters are located, and on the dimensions of the sodium channel cluster itself (and hence the current

density that can generate). It is not until recently that a three dimensional reconstruction of the intercellular space at the intercalated disc was generated [7]. The results show that the intercellular cleft in the vicinity of the adhesion complexes is approximately 63 nm. Unpublished observations from our laboratory indicate that this is also the position of the mini-node of Ranvier, described above. A quick analysis of published literature suggest that 63 nm may be too large a cleft to allow for ephaptic transmission. On the other hand, Veeraraghavan et al [9] have used a different experimental approach to explore sodium channel localization; these authors reached the conclusion that sodium channels organize in the periphery of gap junction plaques, separated by gaps of 30 nm or less and therefore, within distances permissive of electric field mediated transmission of charge. These seemingly discrepant results invite to further studies. Most importantly, they highlight the fact that solving the molecular organization of the intercalated disc is crucial to advance our understanding of cell-cell communication and action potential propagation in the heart.

9. Conclusions

Through the previous sections, we have summarized evidence showing that molecules classically defined as belonging to the gap junctions, interact with other molecular complexes and in particular with those forming sodium channels as well as mechanical junctions. We have also described how through these interactions, the function of one complex is altered by changes in the expression of molecules of a different group. The evidence suggests that intercalated disc molecules are not necessarily constrained to a single function (e.g., Cx43 is not only limited to making gap junctions). Rather, we propose that molecules of the intercalated disc multi-task within a protein interacting network (the connexome), working in concert toward one common function: the propagation of excitatory current from one cell to the next. From this perspective, Cx43 is a molecule relevant to cell excitability (by modulating I_{Na}) [29, 30], sodium channels can support cell-cell electrical coupling and intercellular adhesion strength [44, 45], and “adhesion molecules” are actually required for the function of electrical complexes [51, 59, 60]. The results have important consequences in translational medicine. They revive the question of whether the anatomy of the intercalated disc is compatible with the notion of ephaptic transmission. Furthermore, they bring about the idea that Brugada syndrome and arrhythmogenic cardiomyopathy (a ‘sodium channelopathy’ and a “desmosomal disease,” respectively) are not completely separate diseases but rather bookends of a common spectrum [19, 61, 62]. Separately (though not discussed in this review) we also propose that cardiomyopathies consequent to mutations in the gene coding for $Na_v1.5$ may result, at least in part, from a deficiency in intercellular adhesion strength. Non-canonical functions of intercalated disc molecules, and the concept of the connexome, can help in our understanding of the molecular bases of disease. Overall, the implication of these interactions to the understanding of arrhythmia mechanisms and arrhythmia treatment emerges as an exciting area of future investigation.

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References

1. Sjostrand FS, Andersson E. Electron microscopy of the intercalated discs of cardiac muscle tissue. *Experientia*. 1954; 10:369–370. [PubMed: 13210335]
2. Muir AR. An electron microscope study of the embryology of the intercalated disc in the heart of the rabbit. *J Biophys Biochem Cytol*. 1957; 3:193–202. [PubMed: 13438902]
3. Sjostrand FS, Andersson-Cedergren E, Dewey MM. The ultrastructure of the intercalated discs of frog, mouse and guinea pig cardiac muscle. *J Ultrastruct Res*. 1958; 1:271–287. [PubMed: 13550367]
4. Weidmann S. The electrical constants of Purkinje fibres. *The Journal of physiology*. 1952; 118:348–360. [PubMed: 13000763]
5. Franke WW, Borrmann CM, Grund C, Pieperhoff S. The area composita of adhering junctions connecting heart muscle cells of vertebrates. I. Molecular definition in intercalated disks of cardiomyocytes by immunoelectron microscopy of desmosomal proteins. *Eur J Cell Biol*. 2006; 85:69–82. [PubMed: 16406610]
6. Delmar M, Liang FX. Connexin43 and the regulation of intercalated disc function. *Heart rhythm : the official journal of the Heart Rhythm Society*. 2012; 9:835–838. [PubMed: 22056332]
7. Leo-Macias A, Liang FX, Delmar M. Ultrastructure of the intercellular space in adult murine ventricle revealed by quantitative tomographic electron microscopy. *Cardiovasc Res*. 2015; 107:442–452. [PubMed: 26113266]
8. Rhett JM, Jourdan J, Gourdie RG. Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol Biol Cell*. 2011; 22:1516–1528. [PubMed: 21411628]
9. Veeraraghavan R, Lin J, Hoeker GS, Keener JP, Gourdie RG, Poelzing S. Sodium channels in the Cx43 gap junction perinexus may constitute a cardiac ephapse: an experimental and modeling study. *Pflugers Archiv : European journal of physiology*. 2015; 467:2093–2105. [PubMed: 25578859]
10. Beyer EC, Paul DL, Goodenough DA. Connexin43: a protein from rat heart homologous to a gap junction protein from liver. *J Cell Biol*. 1987; 105:2621–2629. [PubMed: 2826492]
11. Danik SB, Liu F, Zhang J, Suk HJ, Morley GE, Fishman GI, et al. Modulation of cardiac gap junction expression and arrhythmic susceptibility. *Circulation research*. 2004; 95:1035–1041. [PubMed: 15499029]
12. Yao JA, Gutstein DE, Liu F, Fishman GI, Wit AL. Cell coupling between ventricular myocyte pairs from connexin43-deficient murine hearts. *Circulation research*. 2003; 93:736–743. [PubMed: 14500334]
13. Kaplan SR, Gard JJ, Carvajal-Huerta L, Ruiz-Cabezas JC, Thiene G, Saffitz JE. Structural and molecular pathology of the heart in Carvajal syndrome. *Cardiovasc Pathol*. 2004; 13:26–32. [PubMed: 14761782]
14. Kaplan SR, Gard JJ, Protonotarios N, Tsatsopoulou A, Spiliopoulou C, Anastakis A, et al. Remodeling of myocyte gap junctions in arrhythmogenic right ventricular cardiomyopathy due to a deletion in plakoglobin (Naxos disease). *Heart rhythm : the official journal of the Heart Rhythm Society*. 2004; 1:3–11. [PubMed: 15851108]
15. Akar FG, Nass RD, Hahn S, Cingolani E, Shah M, Hesketh GG, et al. Dynamic changes in conduction velocity and gap junction properties during development of pacing-induced heart failure. *Am J Physiol Heart Circ Physiol*. 2007; 293:H1223–H1230. [PubMed: 17434978]
16. Qu J, Volpicelli FM, Garcia LI, Sandeep N, Zhang J, Marquez-Rosado L, et al. Gap junction remodeling and spironolactone-dependent reverse remodeling in the hypertrophied heart. *Circulation research*. 2009; 104:365–371. [PubMed: 19096029]
17. Remo BF, Qu J, Volpicelli FM, Giovannone S, Shin D, Lader J, et al. Phosphatase-resistant gap junctions inhibit pathological remodeling and prevent arrhythmias. *Circulation research*. 2011; 108:1459–1466. [PubMed: 21527737]
18. Oxford EM, Everitt M, Coombs W, Fox PR, Kraus M, Gelzer AR, et al. Molecular composition of the intercalated disc in a spontaneous canine animal model of arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Heart rhythm : the official journal of the Heart Rhythm Society*. 2007; 4:1196–1205. [PubMed: 17765621]

19. Agullo-Pascual E, Lin X, Leo-Macias A, Zhang M, Liang FX, Li Z, et al. Super-resolution imaging reveals that loss of the C-terminus of connexin43 limits microtubule plus-end capture and Nav1.5 localization at the intercalated disc. *Cardiovascular research*. 2014; 104:371–381. [PubMed: 25139742]
20. Agullo-Pascual E, Reid DA, Keegan S, Sidhu M, Fenyo D, Rothenberg E, et al. Super-resolution fluorescence microscopy of the cardiac connexome reveals plakophilin-2 inside the connexin43 plaque. *Cardiovascular research*. 2013; 100:231–240. [PubMed: 23929525]
21. Lowe JS, Palygin O, Bhasin N, Hund TJ, Boyden PA, Shibata E, et al. Voltage-gated Nav channel targeting in the heart requires an ankyrin-G dependent cellular pathway. *The Journal of cell biology*. 2008; 180:173–186. [PubMed: 18180363]
22. Agullo-Pascual E, Cerrone M, Delmar M. Arrhythmogenic cardiomyopathy and Brugada syndrome: diseases of the connexome. *FEBS letters*. 2014; 588:1322–1330. [PubMed: 24548564]
23. Delmar M. Connexin43 regulates sodium current; ankyrin-G modulates gap junctions: the intercalated disc exchanger. *Cardiovascular research*. 2012; 93:220–222. [PubMed: 22180603]
24. Agullo-Pascual E, Delmar M. The Noncanonical Functions of Cx43 in the Heart. *J Membr Biol*. 2012; 245:477–482. [PubMed: 22825715]
25. 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; 22:1204–1212. [PubMed: 17984180]
26. Lubkemeier I, Requardt RP, Lin X, Sasse P, Andrie R, Schrickel JW, et al. Deletion of the last five C-terminal amino acid residues of connexin43 leads to lethal ventricular arrhythmias in mice without affecting coupling via gap junction channels. *Basic Res Cardiol*. 2013; 108:348. [PubMed: 23558439]
27. Malhotra JD, Thyagarajan V, Chen C, Isom LL. Tyrosine-phosphorylated and nonphosphorylated sodium channel beta1 subunits are differentially localized in cardiac myocytes. *J Biol Chem*. 2004; 279:40748–40754. [PubMed: 15272007]
28. Rhett JM, Ongstad EL, Jourdan J, Gourdie RG. Cx43 Associates with Na(v)1.5 in the Cardiomyocyte Perinexus. *J Membr Biol*. 2012; 245:411–422. [PubMed: 22811280]
29. Jansen JA, Noorman M, Musa H, Stein M, de Jong S, van der Nagel R, et al. Reduced heterogeneous expression of Cx43 results in decreased Nav1.5 expression and reduced sodium current that accounts for arrhythmia vulnerability in conditional Cx43 knockout mice. *Heart rhythm : the official journal of the Heart Rhythm Society*. 2012; 9:600–607. [PubMed: 22100711]
30. Desplantez T, McCain ML, Beauchamp P, Rigoli G, Rothen-Rutishauser B, Parker KK, et al. Connexin43 ablation in foetal atrial myocytes decreases electrical coupling, partner connexins, and sodium current. *Cardiovascular research*. 2012; 94:58–65. [PubMed: 22287588]
31. Sato PY, Coombs W, Lin X, Nekrasova O, Green KJ, Isom LL, et al. Interactions between ankyrin-G, Plakophilin-2, and Connexin43 at the cardiac intercalated disc. *Circulation research*. 2011; 109:193–201. [PubMed: 21617128]
32. 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; 128:547–560. [PubMed: 17289573]
33. Smyth JW, Hong TT, Gao D, Vogan JM, Jensen BC, Fong TS, et al. Limited forward trafficking of connexin 43 reduces cell-cell coupling in stressed human and mouse myocardium. *J Clin Invest*. 2010; 120:266–279. [PubMed: 20038810]
34. Hong TT, Smyth JW, Gao D, Chu KY, Vogan JM, Fong TS, et al. BIN1 localizes the L-type calcium channel to cardiac T-tubules. *PLoS biology*. 2010; 8:e1000312. [PubMed: 20169111]
35. Casini S, Tan HL, Demirayak I, Remme CA, Amin AS, Scicluna BP, et al. Tubulin polymerization modifies cardiac sodium channel expression and gating. *Cardiovascular research*. 2010; 85:691–700. [PubMed: 19861310]
36. Lin X, Liu N, Lu J, Zhang J, Anumonwo JM, Isom LL, et al. Subcellular heterogeneity of sodium current properties in adult cardiac ventricular myocytes. *Heart rhythm : the official journal of the Heart Rhythm Society*. 2011; 8:1923–1930. [PubMed: 21767519]

37. Bhargava A, Lin X, Novak P, Mehta K, Korchev Y, Delmar M, et al. Super-resolution scanning patch clamp reveals clustering of functional ion channels in adult ventricular myocyte. *Circulation research*. 2013; 112:1112–1120. [PubMed: 23438901]
38. Benham-Pyle BW, Pruitt BL, Nelson WJ. Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and beta-catenin activation to drive cell cycle entry. *Science*. 2015; 348:1024–1027. [PubMed: 26023140]
39. Godsel LM, Dubash AD, Bass-Zubek AE, Amargo EV, Klessner JL, Hobbs RP, et al. Plakophilin 2 couples actomyosin remodeling to desmosomal plaque assembly via RhoA. *Mol Biol Cell*. 2010; 21:2844–2859. [PubMed: 20554761]
40. Bass-Zubek AE, Hobbs RP, Amargo EV, Garcia NJ, Hsieh SN, Chen X, et al. Plakophilin 2: a critical scaffold for PKC alpha that regulates intercellular junction assembly. *The Journal of cell biology*. 2008; 181:605–613. [PubMed: 18474624]
41. Garcia-Gras E, Lombardi R, Giocondo MJ, Willerson JT, Schneider MD, Khoury DS, et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. *J Clin Invest*. 2006; 116:2012–2021. [PubMed: 16823493]
42. Chen SN, Gurha P, Lombardi R, Ruggiero A, Willerson JT, Marian AJ. The hippo pathway is activated and is a causal mechanism for adipogenesis in arrhythmogenic cardiomyopathy. *Circ Res*. 2014; 114:454–468. [PubMed: 24276085]
43. Makara MA, Curran J, Little SC, Musa H, Polina I, Smith SA, et al. Ankyrin-g coordinates intercalated disc signaling platform to regulate cardiac excitability in vivo. *Circ Res*. 2014; 115:929–938. [PubMed: 25239140]
44. Mori Y, Fishman GI, Peskin CS. Ephaptic conduction in a cardiac strand model with 3D electrodiffusion. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:6463–6468. [PubMed: 18434544]
45. Tsumoto K, Ashihara T, Haraguchi R, Nakazawa K, Kurachi Y. Roles of subcellular Na⁺ channel distributions in the mechanism of cardiac conduction. *Biophys J*. 2011; 100:554–563. [PubMed: 21281569]
46. Veeraraghavan R, Salama ME, Poelzing S. Interstitial volume modulates the conduction velocity-gap junction relationship. *Am J Physiol Heart Circ Physiol*. 2012; 302:H278–H286. [PubMed: 22021331]
47. Sperelakis N. An electric field mechanism for transmission of excitation between myocardial cells. *Circulation research*. 2002; 91:985–987. [PubMed: 12456483]
48. Kant S, Krull P, Eisner S, Leube RE, Krusche CA. Histological and ultrastructural abnormalities in murine desmoglein 2-mutant hearts. *Cell and tissue research*. 2012; 348:249–259. [PubMed: 22293975]
49. Kostetskii I, Li J, Xiong Y, Zhou R, Ferrari VA, Patel VV, et al. Induced deletion of the N-cadherin gene in the heart leads to dissolution of the intercalated disc structure. *Circulation research*. 2005; 96:346–354. [PubMed: 15662031]
50. Rizzo S, Lodder EM, Verkerk AO, Wolswinkel R, Beekman L, Pilichou K, et al. Intercalated Disc Abnormalities, Reduced Na⁺ current density and Conduction Slowing in Desmoglein-2 Mutant Mice Prior to Cardiomyopathic Changes. *Cardiovascular research*. 2012; 95:409–418. [PubMed: 22764152]
51. Cerrone M, Noorman M, Lin X, Chkourko H, Liang FX, van der Nagel R, et al. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. *Cardiovascular research*. 2012; 95:460–468. [PubMed: 22764151]
52. Kucera JP, Rohr S, Rudy Y. Localization of sodium channels in intercalated disks modulates cardiac conduction. *Circulation research*. 2002; 91:1176–1182. [PubMed: 12480819]
53. Nickell S, Kofler C, Leis AP, Baumeister W. A visual approach to proteomics. *Nature reviews Molecular cell biology*. 2006; 7:225–230. [PubMed: 16482091]
54. Zhang Y, Bekku Y, Dzhashishvili Y, Armenti S, Meng X, Sasaki Y, et al. Assembly and maintenance of nodes of ranvier rely on distinct sources of proteins and targeting mechanisms. *Neuron*. 2012; 73:92–107. [PubMed: 22243749]

55. Brachet A, Leterrier C, Irondelle M, Fache MP, Racine V, Sibarita JB, et al. Ankyrin G restricts ion channel diffusion at the axonal initial segment before the establishment of the diffusion barrier. *J Cell Biol.* 2010; 191:383–395. [PubMed: 20956383]
56. Leterrier C, Dargent B. No Pasaran! Role of the axon initial segment in the regulation of protein transport and the maintenance of axonal identity. *Seminars in cell & developmental biology.* 2014; 27:44–51. [PubMed: 24239676]
57. Morley GE, Vaidya D, Jalife J. Characterization of conduction in the ventricles of normal and heterozygous Cx43 knockout mice using optical mapping. *J Cardiovasc Electrophysiol.* 2000; 11:375–377. [PubMed: 10749364]
58. Kirchhoff S, Kim JS, Hagendorff A, Thonissen E, Kruger O, Lamers WH, et al. Abnormal cardiac conduction and morphogenesis in connexin40 and connexin43 double-deficient mice. *Circulation research.* 2000; 87:399–405. [PubMed: 10969038]
59. Beyder A, Strege PR, Reyes S, Bernard CE, Terzic A, Makielski J, et al. Ranolazine decreases mechanosensitivity of the voltage-gated sodium ion channel Na(v)1.5: a novel mechanism of drug action. *Circulation.* 2012; 125:2698–2706. [PubMed: 22565935]
60. Beyder A, Rae JL, Bernard C, Strege PR, Sachs F, Farrugia G. Mechanosensitivity of Nav1.5, a voltage-sensitive sodium channel. *J Physiol.* 2010; 588:4969–4985. [PubMed: 21041530]
61. Cerrone M, Delmar M. Desmosomes and the sodium channel complex: Implications for arrhythmogenic cardiomyopathy and Brugada syndrome. *Trends in cardiovascular medicine.* 2014
62. Cerrone M, Delmar M. Desmosomes and the sodium channel complex: implications for arrhythmogenic cardiomyopathy and Brugada syndrome. *Trends in cardiovascular medicine.* 2014; 24:184–190. [PubMed: 24656989]

Figure 1.

Tomographic electron microscopy section and 3D rendered representation of a portion of intercalated disc of mouse ventricular tissue. A) An XY virtual section of a tomogram is shown. Different typical intercalated disc structures (desmosome (labeled as D), gap junction (GJ) and area composita (AC)) as well as other structures typically present in myocytes (myofibrils (Mf) and mitochondria (Mt)) are visible. B) Overlay of the tomographic slice and 3D rendered models resulting from segmentation of the different structures of interest: cellular membranes forming the ID (red), a gap junction (light pink), a budding vesicle (white) with a rough surface (possibly clatherin coating) between desmosome and gap junction, a complex network of filaments adjacent to desmosome and area composita (dark blue), tubular and cisternae structures forming an intricate connected network in close proximity to ID (light blue), often decorated with electron dense particles, of dimensions compatible with ribosomes (yellow), a multivesicular body (green) and a mitochondria (magenta) in close contact with the gap junction. C) 3D rendered model of all structures of interest segmented in the tomogram, where all spatial interrelations are observed. Adapted from [7] with permission.

Figure 2.

Tracking of a gap junction plaque through different z-planes expanding a length of 500 nm. A) Single XY section containing a portion of intercalated disc, where a gap junction (GJ) is apparent. Other structures typically present in myocytes (myofibrils (Mf), Z-discs (ZD), lipid droplets (LD) and mitochondrias (Mt)) are also visible. B) Another single XY image of the same region separated 260 nm along the Z-axis of the tissue volume. A circular structure (labeled “IGJ?”) that could be considered an internalized (annular) gap junction is observed near the intercalated disc. C) Gallery view of 24 XY sections 20 nm apart from each other

along the Z-axis. XY section number 1 corresponds to panel A) and section number 13 corresponds to panel B). The images clearly show that the “internalized GJ” is in fact part of a finger-like protrusion of the intercalated disc. Red and green arrows have been added to highlight the visualization of the structure through the different planes. Scale bar is 1 μ m.

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Figure 3. 3D reconstruction of the annular gap junction in Figure 2. The contours of the structure were segmented along all the z planes. The structure was revealed to be a finger-like projection of the ID with a curved trajectory, measuring almost 500 nanometers in the z plane.

Figure 4.

Immuno-electron microscopy of adult ventricular ID decorated with gold particles targeting Cx43. Notice abundant clustering of gold particles coated with a Cx43 antibody along the intercalated regions where gap junction plaques are known to be located; notice also gold particles marking the position of Cx43 in structures of morphology compatible with that of mechanical junctions (see arrows; From [19] with permission).

Figure 5. TIRF vs. SRFM. NRVMS stained for Cx43 and PKP2. A and B) show same region of intercellular contact visualized by TIRF (A) and by super-resolution microscopy (B). Small white squares in (A) are enlarged in (C–E). C and D) show improved resolution after reconstruction (right). E) Cx43 cluster surrounded by PKP2, also shown in (F) as a topological image (z-axis: signal intensity). Dotted line across image is plotted in (G) to show intersection of both signals. Scale bars: 5 μ m (A and B) and 200 nm (C–E). From [20] with permission.

Figure 6.

Standard single-electrode patch clamp data measured in two populations of isolated adult rat ventricular myocytes. One population was treated with an oligonucleotide targeting Cx43 expression and the other with a non-targeting construct. Peak sodium current density was decreased in cells lacking Cx43 (black dots and lines; labeled “Cx43sil”) when compared to cells treated with the non-targeting construct (red lines and symbols; labeled “Cx43scr”). From [29] with permission.

Figure 7. Localization of N-cadherin, EB1 and Nav1.5 in adult murine ventricular myocytes. A,B) TIRF image formed by the projection of 2000 frames of the movie collected during data acquisition. A',B') Corresponding Super Resolution Fluorescence Microscopy images. A'',B''). Enlarged views of the white-boxed areas in A' and B', showing the super resolved protein clusters. Overlapping areas are depicted in white. C) Enlargement showing proximity between Nav1.5 (green) and N-cadherin (magenta) clusters in Cre⁻ (left) and

Cx43D378stop (right) cells. Scale bars: 5 μ m (A, A', B, B'), 1 μ m (A'', B'') and 200 nm (C).
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