

Published in final edited form as:

FEBS Lett. 2014 April 17; 588(8): 1322–1330. doi:10.1016/j.febslet.2014.02.008.

Arrhythmogenic Cardiomyopathy and Brugada Syndrome: Diseases of the connexome

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Abstract

This review summarizes data in support of the notion that the cardiac intercalated disc is the host of a protein interacting network, called “the connexome,” where molecules classically defined as belonging to one particular structure (e.g., desmosomes, gap junctions, sodium channel complex) actually interact with others, and together, control excitability, electrical coupling and intercellular adhesion in the heart. The concept of the connexome is then translated into the understanding of the mechanisms leading to two inherited arrhythmia diseases: Arrhythmogenic Cardiomyopathy, and Brugada syndrome. The cross-over points in these two diseases are addressed to then suggest that, though separate identifiable clinical entities, they represent “bookends” of a spectrum of manifestations that vary depending on the effect that a particular mutation has on the connexome as a whole.

Keywords

Brugada syndrome; Arrhythmogenic right ventricular cardiomyopathy; sodium channel; desmosomes; connexin43; plakophilin-2

The intercalated disc: structure and function

In 1877, Engelman laid the foundation for decades of work when he concluded that “cardiac cells are in direct contact during life but become independent as they die” (quoted by Weidmann, 1952¹). Ultrastructural studies later revealed that this “direct contact” is supported by three structures, present at the site of end-end cell apposition: gap junctions, desmosomes and adherens junctions. In the following paragraph we provide a brief historical review of the discovery of the relation between a structure (gap junctions), its function (to provide a low-resistive pathway between cells) and its primary molecule (connexins). Similar historical perspectives can be found in the literature as it pertains to the structures that provide mechanical coupling between cells (e.g.,²).

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Historical overview: the puzzle of separate but connected

The way in which cardiac cells coordinate their independent activities was a fundamental point of debate in cardiac physiology for the first half of the 20th century. By the early 1950s, two seemingly contradictory results were, on the other hand, strongly supported by the data: cells were individually wrapped in a cell membrane (and therefore electrically separate from their neighbors) and yet, the space constant of the cardiac tissue was much longer than the length of the single cell¹. The advent of electron microscopy opened a large window into the cardiac landscape. After the early description of the intercalated disc as an area of specialization at the site of cell-cell contact³ Dewey, Sjostrand and Andersson proposed (in 1958⁴) that the cardiac intercalated disc was “a connecting surface,” like the one first described by Robertson in the crayfish⁵. Six years later, the structural observation was finally reconciled with functional studies. Vanderkloot and Dane demonstrated that the intercalated disc was a subdomain of low electrical resistance. In their article, they speculated that “One possibility which might account for the low electrical resistance is that the intercalated discs are ‘leaky’ in regions where there are desmosomes or at the regions of membrane fusion”⁶. In 1965, Barr, Dewey and Berger provided the first experimental evidence specifically linking that “region of membrane fusion” (at that time called the nexus) with the propagation of the action potential⁷. The notion that “the nexus” was formed by fused membranes^{7, 8} was challenged by Revel and Karnovsky who, in 1967⁹, demonstrated that the membranes were not fused but separated by a gap with interposed junctions, leading Revel to later coin the term “gap junctions.”

The idea of one molecule-one function versus that of multi-tasking molecules

The discovery in the 1980s of the connexin gene family gave molecular identity to a well-characterized ultrastructure¹⁰⁻¹². A molecule-structure-function association was established and set the foundation for a well-accepted principle: connexins make gap junctions, which allow for the transfer of charge between cardiac cells.

The principle stated above never intended to discard the possibility that connexins have other functions. Yet, with a few exceptions¹³⁻¹⁷, the underlying assumption has been that connexin-related events are gap junction-related (or at most, hemichannel-related) events. In contrast, evidence dating back to the early work of Ross Johnson and his colleagues¹⁸ has consistently pointed to the possibility that connexin, as a molecule, participates in cellular processes that are independent of the existence of a connexin-formed permeable channel. This notion has caught new speed partly through the finding of the perinexus^{19, 20} (reviewed in a separate section below), as well as the evidence that Cx43 can regulate microtubule stability independent of gap junction formation¹⁵. It is in the context of these non-canonical functions of connexin^{14, 21} that we build on the concept of the connexome.

Connexins are far from being the only multi-tasking molecule at the intercellular junction. Multiple examples abound, though perhaps the best studied is the case of beta-catenin, which acts as an intermediary to regulate transcription as well as cell adhesion²². In the case of desmosomes, the data suggest that they also act as signaling platforms². Finally, evidence in cardiac, and non-cardiac cells show that cadherin-rich structures also serve as a point of capture for microtubule anchoring^{23, 24}. Overall, the evidence indicates that individual

molecules, as well as the actual structures of the intercellular junctions exert multiple functions. The idea of silos of one-molecule-one function (or one structure-one function) seems not to apply to the intercalated disc. A communal protein interacting network seems a better description of the way in which the overall function is achieved.

To review all non-canonical functions of junctional molecules would go beyond the scope of this article. On the other hand, to limit the review exclusively to intercellular contact molecules would exclude a very important component of the intercalated disc, namely, the voltage-gated sodium channel (VGSC) complex^{25, 26}. In the next paragraphs we will concentrate on desmosomes, gap junctions and the voltage-gated sodium channel (VGSC) complex, three complexes conventionally associated with three functions: adhesion, electrical coupling and cell excitability. Our goal will be to convey the message that molecules classically defined as belonging to one of the structures are indeed relevant to the function of the others, so that intercellular adhesion is in part controlled by connexins and by components of the VGSC complex, sodium current by desmosomal molecules and by connexins and electrical coupling by the integrity of desmosomes and by the expression of ankyrin-G (AnkG), a molecule conventionally identified with sodium channel function^{27, 28}.

The connexome

Intercellular adhesion strength depends on molecules “of the VGSC complex”

The ability of cells to adhere tightly to each other depends in part on the expression of molecules conventionally ascribed to the VGSC complex. This link was established several years ago by the Isom lab^{29–31}. In 1981, Hartshorne and Catterall purified “the saxotoxin receptor of the sodium channel from rat brain” and identified two polypeptides, which they referred to as “ α ” and “ β .”³² They proposed that these two subunits conformed the functional sodium channel. In 1992, Lori Isom isolated the cDNA, sequenced and functionally expressed the β -1 subunit, concluding that this protein is crucial to the overall function of the sodium channel.³¹ In this manner, this 22,581 dalton protein was labeled as a “ β ” for its “ α ,” a subunit merely accessory to sodium channel function. It was eight years later (in 2000) that the Isom lab demonstrated that “sodium channel beta subunits” also mediate cell adhesion,³³ a fact important not only in the formation of the sodium channel complex^{29, 33} but also in sodium channel-independent functions such as cell migration, cell aggregation and interaction with the cytoskeleton (see³⁰).

The work described above has been extended to show that AnkG, a well-established scaffolding protein for the sodium channel in heart as well as in the nervous system^{27, 34–36}, is necessary for proper cell adhesion. This was shown by experiments using an assay where the contact between the cells and the extracellular matrix is disrupted by the use of dispase. If adhesion between cells is strong, the layer lifts as one sheet (cells remain attached to one another). If cell-cell adhesion is weaker, the sheet fragments. Thus, the more fragments, the weaker the mechanical coupling. Using this method, Sato et al showed that loss of Ank-G expression (causes a decrease in mechanical coupling between cells³⁷).

The precise contribution of sodium channel beta subunits, or of AnkG, to the overall adhesion strength of cardiac cells in situ is not known. But one can speculate that genetic

alterations that displace the beta subunit or AnkG from the intercalated disc could have the potential of altering myocardial structure. This is particularly relevant in the context of data showing that a mutation in the gene coding for the alpha subunit of the sodium channels (SCN5a) associates with dilated cardiomyopathy.³⁸ Separate studies have reported the presence of structural defects in patients with Brugada syndrome^{39, 40}. This important link between adhesion and sodium current function will be extensively discussed in the last sections of this review.

Intercellular adhesion is impaired by loss of “gap junction proteins”

More than twenty years ago Ross Johnson and his colleagues reported that Fab fragments of antibodies to the extracellular domain of Connexin43 (Cx43), the most abundant connexin in the heart, inhibits adherens junction assembly in cells in culture¹⁸. More recently, we implemented the disperse assay described above to compare two cell populations¹⁴: one, HEK293 cells that endogenously express Cx43 and another one, a stable HEK293 line where we used lentivirus to permanently silence Cx43 expression. Three groups were compared: untreated (UNT), treated with a virus that contains a non-silencing construct (ϕ shRNA-Cx43) and a third group where Cx43 expression was prevented (shRNA-Cx43). Loss of Cx43 expression brought about a loss of intercellular adhesion strength, represented by a significant increase in the number of fragments detected 90 minutes after disperse addition¹⁴. These results show that Cx43 expression is relevant to mechanical coupling. Whether this effect is consequent to gap junctions being a physical element that provides intercellular adhesion, or whether the result involves intermolecular interactions between Cx43 and components of the mechanical junctions, remains to be determined. The results do show that intercellular adhesion strength is a function of Cx43 that extends beyond the formation of a low-resistive pathway between cells.

Electrical coupling decreases after loss or mutations in molecules “of the desmosome” or “of the VGSC complex”

Shortly after the link between desmosomal mutations and AC was established, the Saffitz group showed decreased abundance of immunoreactive Cx43 signal at the intercalated disc in heart samples derived from patients with Naxos disease,⁴¹ with Carvajal syndrome⁴² and in general, with AC.⁴³ In 2007, Oxford et al confirmed that the desmosomal protein PKP2 and Cx43 co-exist in the same macromolecular complex⁴⁴. Moreover, shRNA-mediated loss of PKP2 expression in cultured rat ventricular cardiomyocytes led to a reduction in Cx43 abundance at the site of cell-cell contact, as well as decreased dye coupling between cells⁴⁴. The observation of an association between Cx43 and PKP2 was later confirmed by other studies^{45, 46}. Furthermore, recent data using super-resolution fluorescence microscopy have demonstrated that Cx43 and PKP2 are in close physical proximity and in fact, PKP2 clusters are often found within the boundaries of the Cx43 plaque²¹. These results support the notion of direct modulation of Cx43 by PKP2. Yet, it should be noted that complete loss of PKP2 causes only a 50% decrease in gap-junction mediated intercellular coupling⁴⁴, and previous work has demonstrated that this decrease in junctional coupling is not sufficient to significantly affect conduction velocity.^{47, 48}

The extent of electrical coupling between cells is also modified by loss of expression of AnkG. Indeed, studying neonatal rat ventricular myocytes in culture, Sato et al demonstrated that loss of expression of AnkG led to reduced gap junction conductance³⁷. These results support the notion that AnkG is much more than a scaffold for sodium channels. Its role may be key in defining the exact position of various molecules of the connexome so that they interact with each other in the appropriate subdomain. In fact, AnkG may be a key piece that controls the traffic of molecules between the perinexus and the gap junction plaque (illustrated in Fig. 1).

Sodium current amplitude is modulated by “cell adhesion molecules”

Early studies showed that the VGSC resides preferentially at the cardiac intercalated disc^{25, 26} and that it can be co-precipitated with Cx43 and with N-cadherin⁴⁹. We therefore explored if disruption of desmosomal integrity could alter sodium current (I_{Na}). Our studies focused on the desmosomal protein PKP2, given that a) PKP2 mutations are the most common variant in AC⁵⁰ and b) early-onset ventricular fibrillation and sudden death in the absence of an overt structural cardiomyopathy are often found in PKP2 mutations carriers⁵⁰. In the first demonstration of a relation between PKP2 and I_{Na} , Sato et al⁵¹ showed that in cardiac myocytes lacking PKP2, I_{Na} was significantly decreased. Furthermore, loss of PKP2 expression in cardiac myocytes led to decreased abundance of immunoreactive $Na_v1.5$ (the α -subunit of the VGSC) at sites of cell contact, and optical mapping experiments showed increased reentrant activity and significantly decreased conduction velocity in the absence of PKP2, when compared to controls⁵¹. A subsequent study showed that the cytoskeletal adaptor protein Ankyrin-G (AnkG) may play a key role in allowing for the interaction between three molecular components previously considered independent: the desmosome, the gap junction, and the VGSC complex³⁷. These observations, limited to the cellular/molecular levels, prompted us to investigate the susceptibility to VGSC-dependent arrhythmias in hearts deficient in PKP2.

Patients affected with AC carry loss of function mutations in a heterozygous fashion. We therefore studied a mouse model with PKP2 haplo-insufficiency (PKP2-Hz), which mimics the clinical situation of patients harboring truncating mutations, with an expected ~50% of PKP2 availability⁵². The mouse model did not show signs of structural cardiomyopathy. $Na_v1.5$ protein abundance was not altered and yet, the amplitude of I_{Na} in isolated ventricular cardiomyocytes was significantly decreased. Furthermore, there was a shift in gating and I_{Na} kinetics when compared to wild-type cardiomyocytes⁵². Thus, intrinsic, genetically mediated partial loss of PKP2 was able to affect sodium current amplitude, similarly to what was demonstrated in cells after total loss of PKP2 expression.

To further unmask the consequences of I_{Na} deficit, we challenged the PKP2-Hz mouse model with flecainide. All treated animals showed an increased sensitivity to drug-induced atrial and ventricular conduction prolongation, showing marked increased P wave, PR and QRS interval durations and increased conduction velocities in Langendorff-perfused isolated hearts. Furthermore, flecainide injection in vivo caused ventricular arrhythmias and some cases of sudden death in PKP2-Hz animals but not in the wild-types.⁵² These results

demonstrated that PKP2 haploinsufficiency causes reduced I_{Na} in murine hearts, documenting for the first time the relationship between PKP2 and the VGSC in vivo.

Additional studies on the relation between adhesion molecules and the VGSC complex

Separate studies have confirmed the relation between desmosomal integrity, and the structure and/or function of the VGSC complex. Rizzo et al showed prolonged ventricular activation time, decreased conduction velocity, decreased upstroke velocity and decreased I_{Na} amplitude in mice over-expressing a mutation in desmoglein2.⁵³ Gomes et al⁵⁴ reported that patients with AC harboring desmoplakin mutations showed regional conduction delay and heterogeneous $Na_v1.5$ distribution; moreover, in a collaborative immunohistochemistry study⁵⁵ on heart samples from patients with AC, Noorman and colleagues showed that in most cases, $Na_v1.5$ was reduced at the intercalated disc, even if the distribution of the N-cadherin signal remained normal. Finally, reduced sodium current amplitude has been observed in PKP2-deficient HL1 cells and in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from a patient with PKP2 deficiency^{56, 57}.

Though not a desmosomal molecule, the coxsackievirus and adenovirus receptor CAR is another case of an adhesion molecule that also regulates sodium current. Undoubtedly a receptor for both coxsackievirus and adenovirus, studies have demonstrated that the immunoglobulin extracellular domains of CAR are capable of homophilic binding, and participate in intercellular adhesion in epithelial cells. The role of this molecule on cell adhesion in the heart is less well defined. Interestingly, cardiac-restricted deletion of CAR causes significant slowing of A-V propagation and disruption of gap junctions at the intercalated disc.^{58, 59} Recent studies show that, as in the case of other molecules with immunoglobulin extracellular domains (such as the sodium channel β -subunits), CAR expression modulates sodium current function⁶⁰.

Sodium current depends on “the gap junction protein” Connexin43

In the classical description of the electrophysiology of the adult ventricle, sodium channels provide the current that is necessary for the generation of an action potential, whereas Cx43 forms gap junctions to allow transfer of charge between one cell and the next. This description then separates the type of channel, with its function: sodium channels are responsible for cell excitability; gap junctions allow cell-cell passage of charge. In a recent study, however, we reported that loss of Cx43 expression brings about a loss of sodium current amplitude⁶¹. In other words, the molecule necessary for making the gap junctions is actually necessary to maintain the complex in charge of generating the action potential. This means that loss of Cx43 expression is in fact a double-edge sword: not only will the path between cells be disrupted but also, the amount of charge that is generating by the excited cell will decrease. These results were consistent with others obtained in fetal atrial myocytes⁶² and led to the conclusion that loss and/or redistribution of Cx43 expression, as it happens in a number of pathological cardiac conditions (e.g.,^{63–65}), can have complex deleterious effects on propagation.

Recent studies confirmed and expanded the importance of Cx43 in the control of sodium current amplitude. Lubkemeier et al⁶⁶ developed a conditional knock-in murine model

where, upon tamoxifen injection, the wild-type Cx43 was replaced, specifically in the heart, by a truncated form lacking the last five amino acids of the protein (Cx43D378stop). Interestingly, the Cx43D378stop protein forms gap junctions of normal junctional conductance, unitary conductance and permselectivity. Localization of Cx43 was not affected by the mutation, nor did it affect the localization of the ZO-1 protein (known to associate to Cx43 via its C-end). Yet, the animals died within 21 days after tamoxifen injection, and Holter recordings showed a transition from normal sinus rhythm to complex ventricular arrhythmias culminating in ventricular fibrillation and sudden death. Electrophysiological analysis revealed that, among the observed changes, there was a significant reduction in the amplitude of I_{Na} . This result clearly demonstrated that a key function of Cx43 is to maintain normal cell electrophysiology and that, even if gap junctions are present, a loss of Cx43-dependent, gap junction-independent functions can lead to severe arrhythmias and death. Studies are in progress to determine the molecular mechanism that links Cx43 and the function of the VGSC complex in the heart.

Visualizing interacting complexes at the intercalated disc

Electron microscopy

The results described above provide convincing evidence of a functional interaction between the various molecules that reside at the intercalated disc. Furthermore, it is likely that these interactions are, in fact, far more complex. Using tomographic electron microscopy (TEM) we have observed structural features that likely carry unknown functions. For example, it is common to see actual physical contact between gap junctions and the outer membrane of mitochondria⁶⁷ (Fig. 2). It is also common to observe extensive vesicular activity localized specifically at the space separating desmosomes and gap junctions⁶⁷ (Fig. 3) and in fact, the presence of small membranous particles in the intercellular space. The intercalated disc is, structurally and functionally, not a simple aggregate of independent, separate complexes but the host of a protein interacting network.

Super-resolution fluorescence microscopy

A limitation of electron microscopy is that complexes that do not form electron-dense structures cannot be visualized. On the other hand, the resolution of light microscopy is limited by the diffraction of light^{68, 69} reaching to approximately 250 nm in the best of conditions and therefore not sufficient to assess the actual distances between molecules. To overcome this limitation, we have implemented super-resolution fluorescence microscopy methods^{21, 69}. We built a set-up on a conventional inverted microscope using commercially available optics and applied laser illumination, reducing, and oxygen scavenging conditions to manipulate the blinking behavior of individual fluorescent reporters. Movies of blinking fluorophores were reconstructed to generate subdiffraction images at ~20 nm resolution. We mapped a molecule classically defined as a component 'of the desmosome' (PKP2) and another one defined as a 'gap junction molecule' (Cx43), and defined their spatial organization. As shown in Figure 4,²¹ in about half of Cx43 clusters we observed an overlay of Cx43 and PKP2 at the Cx43 plaque edge. SiRNA-mediated loss of Ankyrin-G expression yielded larger Cx43 clusters, of less regular shape, and larger Cx43-PKP2 subdomains. 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 our studies led us to conclude that super-resolution fluorescence microscopy, complemented with Monte-Carlo simulations and PLAs, allows the study of the nanoscale organization of an interactome in cardiomyocytes. Our results further showed that PKP2 and Cx43 share a common hub that permits direct physical interaction. These results allowed us to fully support the notion that not only are molecules functionally interacting into what we dubbed a connexome, but they also occupy a common space at the intercellular junction²¹. The actual location of this space within the context of the overall intercalated disc architecture may be related to the studies characterizing the perinexus, as described below.

The perinexus and the connexome

Recent studies have shown that the association of Cx43 with its scaffolding protein ZO1 occurs in the periphery of the Cx43 plaque (the “perinexus”^{19, 20}) and that loss of the interaction with ZO-1 leads to larger gap junction plaques, with a loss of the perinexal domain. In a recent study, the Gourdie group further proposed that the perinexus could be a site for the interaction of Cx43 with Nav1.5¹⁹. From that perspective, the perinexus could be the site of the connexome. This is an interesting possibility, which in fact suggests that changes in the stability of the Cx43-ZO-1 association could actually affect the properties of I_{Na} . Defining the confines of the perinexus in adult cardiac tissue, and the molecular constraints that regulate its composition and its properties represent an important challenge for the future. It is worth noting that an additional structure in the intercalated disc that could host multiple molecules is the area composita⁷⁰. Various investigators have demonstrated that these structures represent a combination of molecules classically defined as belonging to the desmosome or to the adherens junctions^{71, 72}. We speculate that Cx43 and Nav1.5 may also be found in the area composita, and interact within that domain. These two locations (the perinexus or the area composita) are not mutually exclusive but rather, complementary sites that could ensure the proper integration of the connexome with both, molecules involved in mechanical function, and in electrical coupling between cells.

Brugada syndrome and AC: Two diseases with a common substrate

Through the writing above, we have sought to establish that the intercalated disc hosts a common protein interacting network (the connexome) that includes molecules of the desmosome and of the VGSC complex. In the clinical realm, desmosomal mutations lead to ARVC⁷³ and sodium channel dysfunction, to Brugada syndrome^{74, 75}. We surmised that if the molecular substrates (desmosomes; VGSC) are part of a common network, then the diseases (AC; BrS) should also share some common features. In the following section, we first give a brief description of the two diseases and then, discuss their similarities.

Arrhythmogenic Cardiomyopathy

It is generally accepted that loss of integrity of the desmosome leads to “arrhythmogenic cardiomyopathy” or “AC.” (Also called “arrhythmogenic right ventricular cardiomyopathy,” or “arrhythmogenic right ventricular dysplasia,” hence the abbreviations “ARVD” “ARVC” or ARVC/D” found in the literature to refer to this disease^{76, 77}). Approximately 50–70% of the cases of familial AC associate with a mutation in a desmosomal gene^{78, 79}. This

condition presents with a progressive fibro-fatty infiltration, often more prominent in the right ventricular myocardium, and with a high propensity to life-threatening ventricular arrhythmias and progression toward heart failure⁷⁶. It is considered one of the most relevant causes of juvenile sudden cardiac death, especially in competitive athletes, and its prevalence ranges from 1:2000 to 1:5000⁸⁰. It is important to emphasize, however, that a number of cases of unexpected cardiac sudden death occur during the early or “concealed” phase of the disease, in the absence of overt structural manifestations.⁸¹

Brugada syndrome

Brugada syndrome (BrS) is an inherited channelopathy characterized by ST segment elevation of coved morphology in right precordial leads, increased risk of ventricular tachycardia and ventricular fibrillation and absence of cardiac structural disease⁷⁵. Mutations in the gene coding for Nav1.5, the most abundant sodium channel protein in the heart, account for ~20–25% of genotype-positive subjects⁸², and about 4% of patients carry mutations in the *CACNA1c* gene. Several other genes have been associated with sporadic cases of BrS, but each one accounts for <2% of patients; as such, current guidelines do not support routine screening on them in the general BrS population⁸². Overall only 25–30% of patients with clinical diagnosis of BrS have a known genotype, implying that additional, still undiscovered genes may be linked to this disease.

The intersection of BrS and AC

When BrS was initially described, some investigators proposed that this condition shared features with arrhythmogenic cardiomyopathy (AC), thus opening the possibility that they represent two poles of a common spectrum ultimately leading to increased risk of sudden death⁸³. In fact, some BrS patients show minor RV structural abnormalities;⁴⁰ furthermore, mutations in *SCN5A* have been associated with cases of dilated cardiomyopathy,^{38, 84} while desmosomal mutation carriers can experience ventricular fibrillation and sudden death without overt structural disease.^{41, 54, 81, 85, 86}

PKP2 mutations and the BrS phenotype

This phenotypical and molecular crossover led us to investigate whether desmosomal mutations exist in cases of Brugada syndrome⁵⁷. We screened by direct sequencing the *PKP2* gene in a cohort of 200 patients with clinical diagnosis of BrS and no mutations on the most prevalent genes. We discovered five single amino acid substitutions in five unrelated patients⁵⁷. In order to assess if this missense variant in *PKP2* could affect the cardiac I_{Na} , we used an HL-1 cell line, stably silenced for the endogenous *PKP2*. In the absence of *PKP2*, these cells showed a decrease in the native I_{Na} . Cells transiently transfected with each one of the *PKP2* mutants associated with the BrS phenotype showed significantly decreased I_{Na} , when compared with cells transfected with wild type *PKP2*⁵⁷. Similar results were obtained when we used a line of human iPSC-derived cardiomyocytes from a patient lacking *PKP2* at the cell membrane^{56, 87}. In these cells, I_{Na} increased upon transfection with wild type *PKP2*. Transfection with one of the *PKP2* mutants associated with BrS was not able to restore normal I_{Na} ⁵⁷.

These data represent the first evidence that missense mutations in PKP2 can cause a decrease in cardiac I_{Na} and facilitate arrhythmias, even in the absence of a structural cardiomyopathy. We propose that PKP2 mutations provide at least part of the molecular substrate of BrS. The inclusion of PKP2 as part of routine BrS genetic testing remains premature; yet, the possibility that some patients showing signs of disease may harbor PKP2 variants should be considered when the genotype is negative for other genes associated with BrS.

ARVC, BrS and the diseases of the connexome

In summary, the experimental data support the notion that rather than controlled by “individually wrapped” separate molecular complexes, excitability, electrical coupling and intercellular adhesion are controlled by a common protein interacting network called the connexome. As the edges of the molecular complexes are blurred, so are the clinical syndromes that associate with them: Brugada syndrome is not purely arrhythmogenic (but includes a structural component), arrhythmias in AC are not only consequent to alterations in macrostructure (molecular changes in the intercalated disc subdomain, and in sodium currents, can be found), and in fact, PKP2 mutations can also be the substrate for BrS. While clinically distinguishable as individual entities, we propose that they share a common origin as diseases of the connexome.

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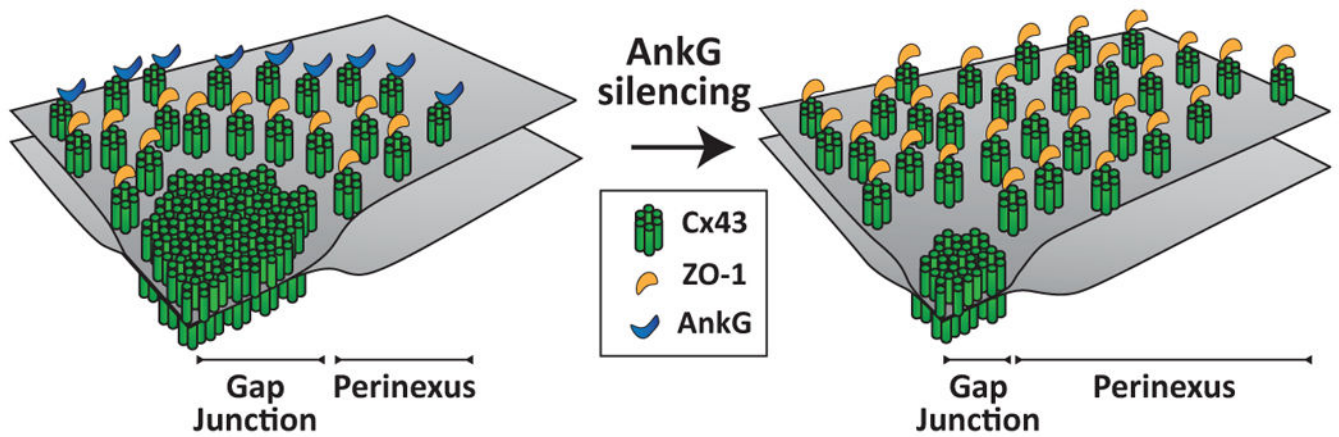


Figure 1.

One possible model of connexome organization. The gap junction is surrounded by the perinexus where Cx43 hemichannels interact with ZO-1, which regulates the transition of connexons to the gap junction. AnkG may be localized at the border of the perinexus restricting the plaque size. When AnkG is silenced (right panel), the perinexal area expands at the expense of the actual pore-forming gap junction. This yields to larger Cx43 plaques but a reduced channel-forming domain and gap junction conductance. From²¹ with permission.

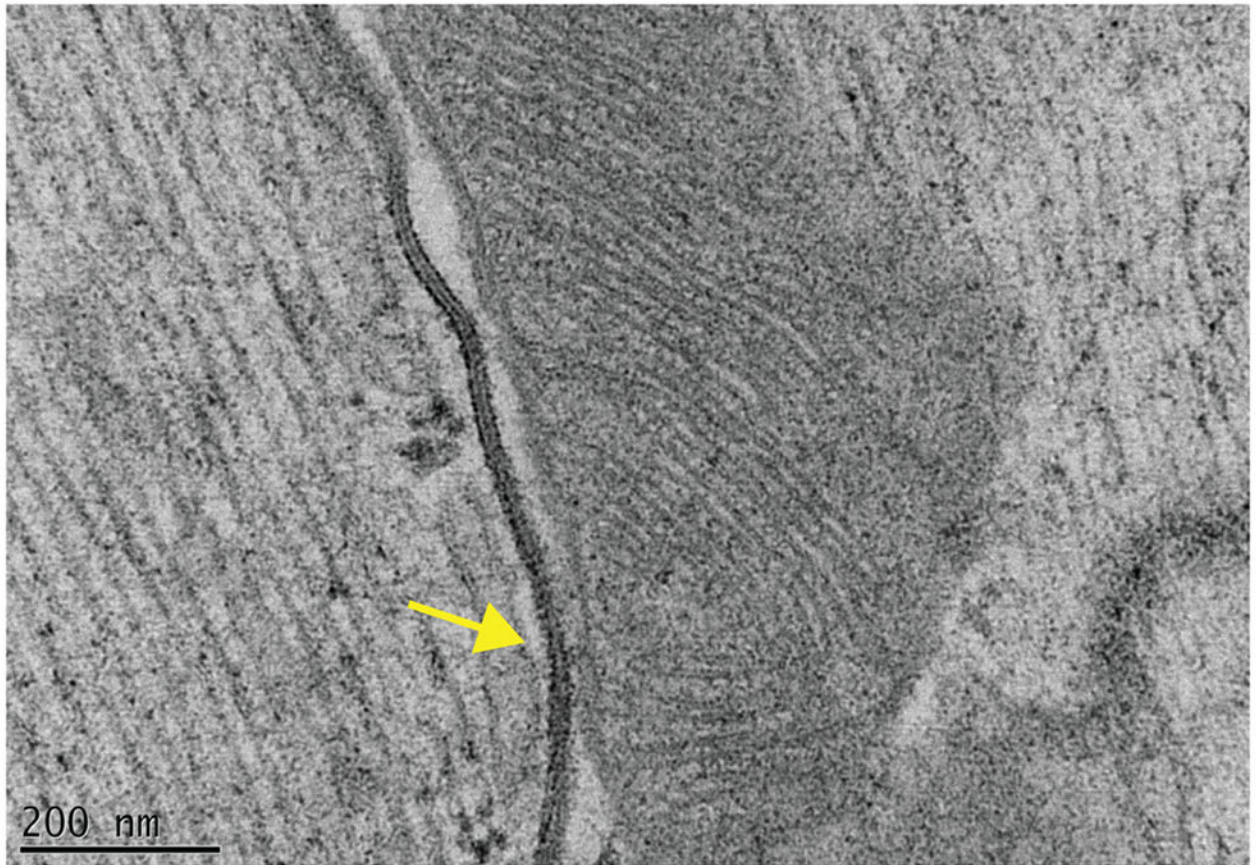


Figure 2. Proximity between gap junctions and mitochondria. Transmission electron micrograph image of adult murine heart tissue. The high preservation of structures was achieved by high-pressure freezing and freeze-substitution methods. Notice the close approximation and contact (yellow arrow) between the gap junction and a mitochondria. From⁶⁷ with permission.

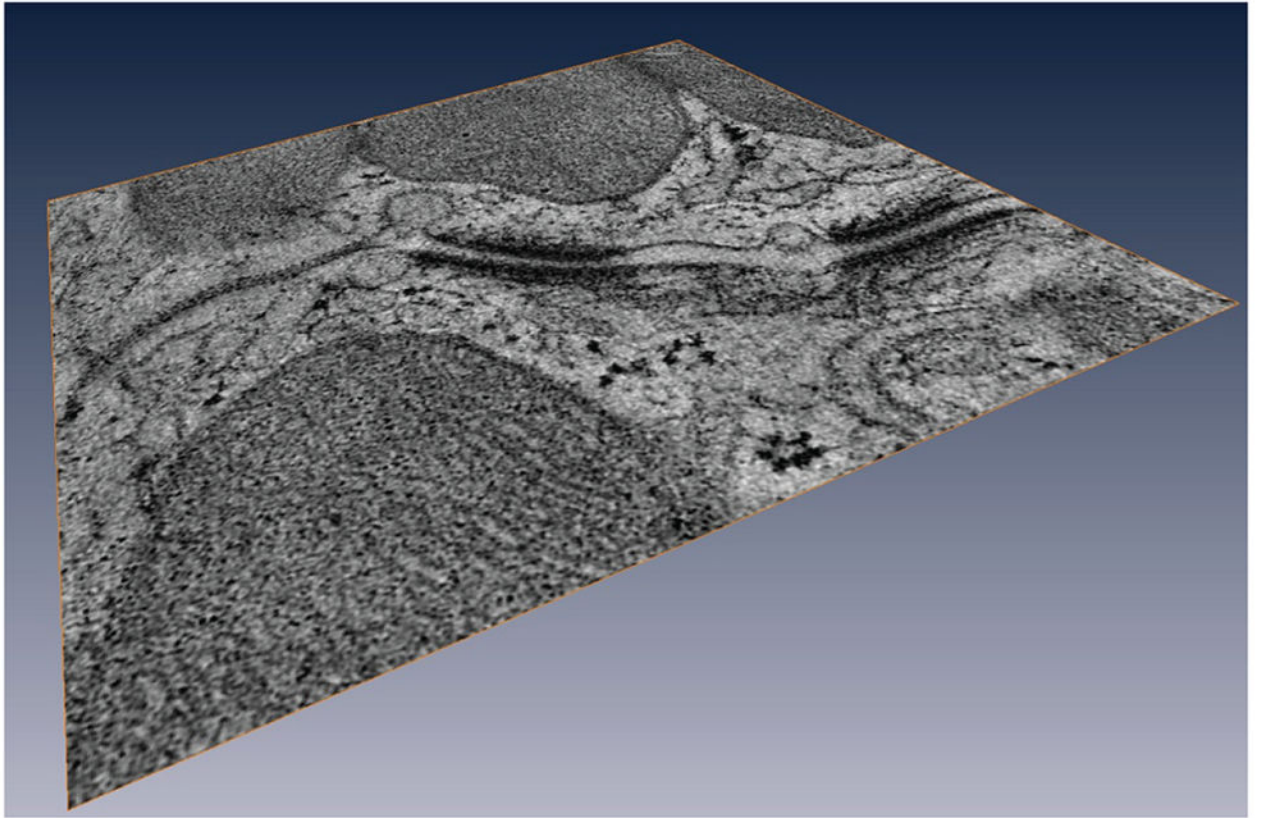


Figure 3. Vesicular activity at the intercalated disc. Tomographic electron microscopy image of an intercalated disc region. Notice the vesicular activity in the space between the desmosomes and the gap junction, as well as in the intercellular space. From⁶⁷ with permission.

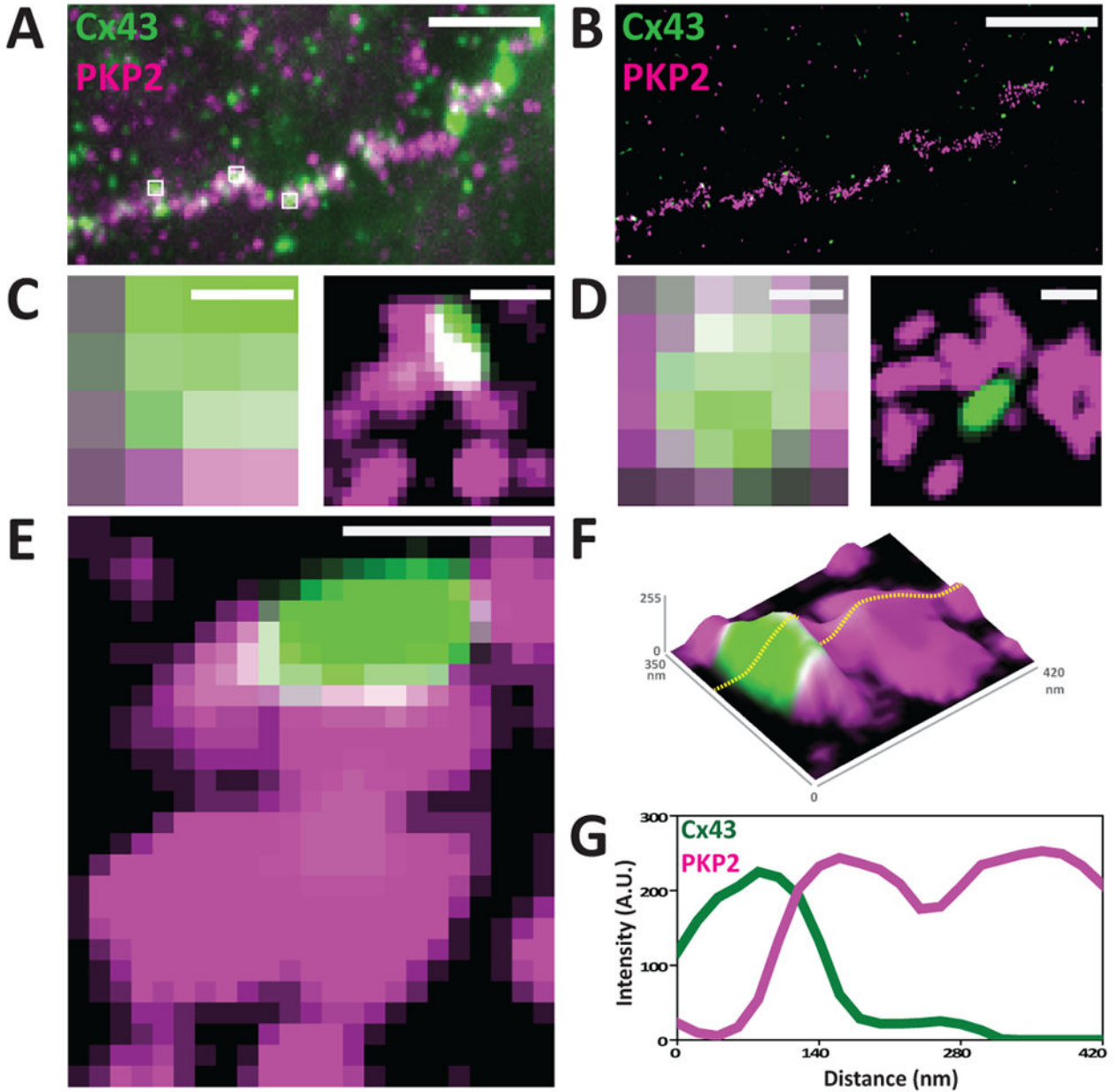


Figure 4. Super-resolution fluorescence microscopy: NRVMs stained for Cx43 (green) and PKP2 (magenta). Panels A and B show the same region visualized by TIRF (A) or by SRFM (B). Enlargement of the small white squares show improved resolution after reconstruction (C-E). Panel E shows a Cx43 cluster surrounded by PKP2. Same image is shown as a topological image in F (z-axis: signal intensity). Intensity plot of the dotted line across the image (G) shows the intersection of both signals. Scale bars: 5 μ m (A and B) and 200nm (C-E). Reproduced from²¹ with permission.