Connexin43 regulates sodium current; ankyrin-G modulates gap junctions: the intercalated disc exchanger

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1. Introduction

Intercellular communication is necessary for proper cardiac function. Electrical signals are transmitted via low-resistive pathways (gap junctions) to jolt each myocyte into contraction. As the cells develop force, the muscle needs to move as a unit, with myocytes both contracting and then relaxing in a coordinated fashion. Intercellular adhesion is therefore necessary for transmission of force and for maintenance of the functional syncytium. The mechanical coupling of cardiac myocytes is provided by two primary structures: desmosomes and adherens junctions. The former couples to intermediate filaments (desmin, in the case of the heart), whereas the latter anchors N-cadherins to the actin cytoskeleton. A ‘mixed’ desmosome/adherens junction structure, dubbed the ‘area composita’, is also present in the adult mammalian heart. Whether both desmin and actin filaments anchor at the area composita has not been clearly determined. The intercellular structures that anchor one cell to another and provide a physical cell–cell continuum form electron-dense plaques that are clearly distinguishable by electron microscopy. Their characteristic location at the cells’ end led to the description of the intercalated disc as an electron-dense structure located at the site of end–end cell apposition, harbouring the intercellular junction complexes that provide electrical and mechanical coupling in the heart.

The advent of immunofluorescence techniques brought about the demonstration that other molecules, not classically considered junctional, are also present at the cell end and, in fact, co-localize with ‘junctional’ molecules. Of particular interest are two ion channel proteins fundamental to normal cardiac electrophysiology: the sodium channel α-subunit, NaV1.5, and the potassium channel protein Kv1.5. For a number of years, each of these channels, and their accessory proteins, was studied as an independent entity. Yet, recent data show that cross-talk at the intercalated disc extends to interactions between complexes previously seen as being independent (Figure 1). As such, loss of expression of plakophilin-2, a desmosomal molecule, affects gap junction-mediated coupling as well as sodium channel function; loss of N-cadherin expression affects gap junctions and also the function of Kᵥ1.5 channels; loss of intercellular contact leads to a decrease in sodium current; expression of AnkG, a protein associated with the sodium channel complex, is necessary for proper intercellular adhesion strength and for proper electrical coupling; finally, expression of Cx43, a protein previously associated only with gap junctions, is in fact required for the normal function of sodium and potassium currents. When taken together, the evidence suggests that the intercalated disc is not a site where independent molecules reside, but rather the host of an ‘interactome’—a protein interacting web that involves molecules relevant to excitability, propagation, and mechanical coupling between cells. Due to limitations in space and scope, in the present article, I concentrate on the relationship between sodium channels and gap junctions; in particular, I elaborate on the notion that Cx43 is, in addition to the pore-forming unit of gap junctions, a protein involved in cell excitability, whereas components of the voltage-gated sodium channel complex can actually regulate electrical coupling between cells.

2. Location, location, location . . . and how to get there

Just like people in their neighbourhoods, the behaviour of proteins is greatly affected by the location in which they reside. In fact, data from our laboratory support the notion that the same channel (the

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tetrodotoxin-resistant sodium channel expressed in adult ventricular myocytes; most likely formed by the sodium channel α-subunit Na1.5) functions differently depending on its location within the cell. In our studies, ‘local’ sodium currents were elicited by sealing a patch pipette at a defined location (either the intercalated disc or the mid-section of the cell) and applying voltage clamp protocols in the cell-attached configuration. In this mode, currents were recorded only from the small area of the cell covered by the patch pipette.6,11 Our experiments demonstrated location-specific gating properties: in particular, a significant negative shift in the voltage dependence of steady-state inactivation for channels located in the mid-section of the cell, when compared with those residing in the area of the intercalated disc.6 Analysis of these data led us to propose that at the level of the resting membrane potential, tetrodotoxin-resistant sodium channels in the cell midsection are mostly inactivated, and as such, they are not likely to contribute significant current to the action potential upstroke, thus leaving the burden of excitation to the channels localized at the intercalated disc. These results were consistent with the notion that sodium channels segregate into distinct pools (as demonstrated by the elegant work of Petitprez et al.12); furthermore, our data unveiled a previously undefined correlation between location and function. In addition, we demonstrated that the amplitude of sodium current at the intercalated disc is significantly larger if the cells remain paired, thus suggesting that the integrity of intercellular junctions is actually a major determinant of sodium current amplitude.

The results described above support the idea that the clustering of proteins within the confines of the intercalated disc shapes the electrophysiological profile of the cell. We recently presented direct evidence that Cx43 is in itself relevant to sodium channel function.9 In that paper, we demonstrated that loss of expression of Cx43 leads to a reduction in sodium current amplitude in adult ventricular myocytes and a reduction in the abundance of the Na1.5 signal at the intercalated disc. These results provided the first demonstration that Cx43 (as a molecule, not as a gap junction channel) modulates the excitability of the cardiac myocyte. The role of Cx43 in propagation is therefore dual: to provide a low-resistance pathway and to enhance the amplitude of the depolarizing force.

Taken together, these results reinforce the well-established notion that targeting the right protein subset to the right subcellular domain is key to normal cell function. The mechanisms responsible for the precise subcellular distribution of intercalated disc proteins continue to be actively investigated. In 2007, Shaw et al.13 published their studies showing that microtubule plus-end tracking proteins target gap junctions from the cell interior to adherens junctions. These investigators proposed a model whereby microtubules are tethered to N-cadherin-containing mechanical junctions via a complex that involves the microtubule plus-end tip tracking protein EB1, p150(Glued) of the dynein/dynactin complex, and β-catenin. In a follow-up paper, Smyth et al.14 reported that the oxidative stress that associates with an ischaemic event leads to loss of Cx43-containing plaques as well as a displacement of EB1. A point mutation in the EB1-binding region of tubulin also caused EB1 displacement and diminished connexon delivery. These and other observations led to the conclusion that the microtubular network is directly involved in connexin transport and that EB1 displacement may be a key component in the loss of Cx43 from the intercalated disc that occurs in various pathological conditions. Interestingly, the motor protein involved in the delivery of Cx43 to the intercalated disc in the adult cardiac myocyte has not been identified. In vitro studies have shown that linker connexin vesicles are moved along microtubules by kinesin motors.15 Whether a similar mechanism applies to the cardiac ventricular myocyte and its most common gap junction protein Cx43 remains to be defined. In addition, the work of Shaw et al. focused on the role of N-cadherin as the anchoring point of entry for Cx43 into the junction. Yet, gap junctions are often found in the immediate vicinity of desmosomes.16 It therefore seems reasonable to speculate that desmosomal cadherins (desmocollin, desmol-glein) could be another point of entry for Cx43 into the intercalated disc.

While the studies of Shaw et al. have begun to unveil the mechanisms of Cx43 delivery, less is known about the mechanisms that deliver sodium channels to the intercalated disc. Yet, a recent study, conducted in cultured rat hippocampal neurons, points to the possibility that Cx43 and Na1.5 may share a common path. Indeed, Leterrier et al. have shown that both EB3 and EB1 interact with the sodium channel scaffolding protein, AnkG.17 The investigators further showed that loss of EB3 led to a reduction in AnkG concentration and in the concentration of Nav channels. A similar, though less extensive, effect was observed after EB1 knockdown. The investigators concluded that EB3 and EB1 coordinate a molecular interplay between AnkG and the microtubular network in the axon initial segment and that this interaction plays a key role in the maintenance of neuronal polarity. Their data also showed that the EB expression is relevant to the abundance of sodium channel proteins. While these results were obtained in neurons, and the formation of protein clusters may be cell-specific, speculation as to whether a similar process may apply to the cardiac myocyte seems reasonable. Indeed, work from the Mohler laboratory previously demonstrated that cardiac Na1.5 targeting in the heart does require an AnkG-dependent pathway.6 It is therefore possible that this pathway is established, at least in part, via the interaction of AnkG with EB proteins and a consequent stabilization of the microtubular network.17 Moreover, it is worth noting that loss of AnkG expression also leads to a decrease in Cx43 abundance, a reduction in
Cx43 plaques from the site of intercellular contact, and a decrease in junctional conductance between myocytes, in the light of the studies of Leterrier et al. I speculate that the effects of AnkG silencing on Cx43 and on electrical coupling are consequent, at least in part, to the disruption of microtubule-mediated delivery of Cx43. Altogether, I postulate that the microtubular network provides a common pathway for the delivery of intercalated disc proteins, including Na\textsubscript{1,5} and Cx43 in the heart, with the AnkG–EB1 interaction being necessary for proper function. Of note, additional studies have implicated the microtubules in the trafficking of the cardiac sodium channel α-subunit Na\textsubscript{1,5}.

### 3. The reciprocal regulation of Cx43 and the voltage-gated sodium channel complex

Reciprocity is not uncommon in biology. While the studies of Shaw et al. indicate that the microtubular network is fundamental to Cx43 trafficking and for gap junction assembly, recent work of the Lo laboratory shows that Cx43 is, in turn, a regulator of microtubular dynamics, at least in mouse embryonic fibroblasts. Indeed, their experiments demonstrated that Cx43 deficiency causes cell polarity defects such as failure of the Golgi apparatus and the microtubule-organizing center to reorient in the direction of a wound closure. Similar results were observed when cells expressed a Cx43 mutant lacking its tubulin-binding domain. On the other hand, cell motility was unaffected by expression of a Cx43 mutant that retained the tubulin-binding domain but was incapable of forming gap junctions. Overall, these and other experiments led to the conclusion that Cx43 regulates the tubulin cytoskeleton. Whether the Cx43-dependent regulation of the microtubular network occurs in cardiac myocytes remains to be defined; yet, it is tempting to speculate that Cx43 is both transported by the microtubules and also necessary for their stability. In that case, proteins that traffic through the microtubular network may fail to reach the membrane if Cx43 is not properly expressed or localized. Combining these observations with those of others, including Casini et al., I propose that Cx43-mediated microtubular stabilization is required for the formation of functional sodium channel complexes at the intercalated disc. The latter may explain why loss of expression of Cx43 leads to a reduction in sodium current amplitude in adult ventricular myocytes and a reduction in the abundance of the Na\textsubscript{1,5} signal at the intercalated disc.

### 4. Conclusion

I have described recent evidence supporting the notion that the intercalated disc represents a site of extensive cross-talk between molecular complexes previously considered to be independent. I have also proposed that part of this cross-talk involves shared mechanisms of microtubule-based trafficking in which Cx43 down-regulation may disturb trafficking of sodium channel components and, conversely, loss of AnkG expression may prevent the arrival of Cx43 to its final destination. This mutually dependent mechanism of trafficking may constitute, at least in part, the molecular bases for the reciprocal regulation of two fundamental components of the local circuit current: the sodium channels and the gap junctions. Cx43 emerges as a molecule that is involved not only in forming gap junctions for the cell—cell transfer of electrical charge but also as an organization centre for the establishment of proper sodium channel function. The gap junction-independent roles of Cx43 emerge as an exciting new area of future investigation.

#### Conflict of interest

none declared.

#### References

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