The Carboxy Terminal Domain of Connexin43
From Molecular Regulation of the Gap Junction Channel to Supramolecular Organization of the Intercalated Disk

Nicholas J. Severs

Cardiac function depends fundamentally on gap junctions, plaques of transmembrane channels constructed from connexin proteins that bridge the plasma membranes of adjacent myocytes. By electrically coupling the entire myocyte population of the heart, these junctions preside over the cell-to-cell passage of the precisely orchestrated patterns of current flow that synchronize, coordinate, and harness individual cell contractions to generate the heart beat.

In the ventricular myocardium, gap junctions are organized, together with 2 types of adhesion junction, the fasciae adherentes junctions and desmosomes, at the intercalated disks where the cell ends abut.1 The intercalated disk has a characteristic structure, exquisitely specialized for the task of integrating cell-to-cell electromechanical function (Figure). The fasciae adherentes junctions, which transmit mechanical force from cell to cell, link up the myofibrils of adjacent cells in series. They are thus, of necessity, situated in vertical zones of the disk. Because these vertical zones are staggered to varying extents, they are linked by membrane areas that lie parallel to the long axis of the cell. It is in these areas that the gap junctions are typically situated. Desmosomes, into which the desmin intermediate filaments of the cytoskeleton insert, form discrete “press-studs” predominantly (though not exclusively) located in the same membrane zones as the gap junctions. Larger gap junctions are commonly located at the disk periphery.2–4 Each junction type has thus traditionally been regarded as a spatially distinct entity with its own specialized function.

It has long been known, however, that the formation and stability of gap junctions depends on their adhesion junction partners.5,6 Mutations in or suppression of adhesion junction proteins have more recently been found to lead to such profound reductions in gap junction size and quantity that impulse propagation is impaired, emphasizing the critical importance of this relationship.7–10 With the discovery that a number of these proteins—originally regarded as characteristic of and exclusive to adhesion junctions—in fact interact with connexins, the notion of molecular “cross-talk” between the junction types is now very much in vogue.11 The binding sites through which these proteins interact with the gap junction lie in the cytoplasmic carboxy terminal (CT) domain of the connexin molecule. This domain is well known to house a series of functionally important sites, notably those involved in channel gating and those for phosphorylation (a process implicated in diverse roles including trafficking of connexins to the plasma membrane and assembly and degradation of the gap junction plaque). Apart from c-Src and adhesion junction proteins such as the catenins and N-cadherin, a range of other connexin binding partners has been identified, notably, tubulin, caveolins, and zona occludens-1 (ZO-1).12 CT binding sites for tubulin and associated motor transport proteins mediate trafficking of connexins to the correct destination at the cell surface, whereas ZO-1 is intimately involved in regulation of gap junction size.13

To date, what we have learned about the function of the CT domain of connexin43 (Cx43), the principal connexin expressed in the heart, has depended heavily on exogenous expression systems. What has been lacking until now is an opportunity to put the thoughts emerging from this work to the test in the whole heart in vivo. In the article published by Maass et al14 in this issue of Circulation Research, an elegant approach is applied to overcome the obstacles that have hitherto prevented such study. It had previously been found that mice generated to express a CT-truncated form of Cx43 instead of normal Cx43 die shortly after birth, but by decreasing the gene dosage (crossing with mice that are heterozygous for the gene encoding Cx43), animals harboring one allele for the truncated variant (K258stop) and one Cx43 knock-out allele were found to survive to adulthood with no overt abnormalities in the overall morphology or function of the heart.15 By using this model, the authors have now been able to examine directly what happens in the intact functioning heart when Cx43 lacks a major part of the CT tail that houses key functional sites, notably those involved in phosphorylation, ZO-1 binding and linkage to adhesion proteins.

In a nutshell, what Maass and her colleagues found is that lack of this Cx43 CT domain results in an increased size of the gap junction plaques, a reduction in their overall number, and an altered pattern of their spatial organization at the intercalated disk. The CT domain thus appears to play a crucial role in regulation both of gap junction size and its correct positioning within the disk. This immediately prompts the question “how?” Which of the many possible Cx43 binding partners is critically involved in these processes?

A more detailed look at the precise nature of the altered spatial organization provides some clues, and for this we need to refer back to the Figure. We have already seen from this Figure how, in the normal heart, the gap junctions in longitudinal zones of the disk alternate with the adherentes junctions in vertical zones, with desmosomes scattered between the gap junctions. What
happens in the absence of the Cx43 CT is that this alternating pattern of gap junctions and adhesion junctions through the course of the disk largely disappears, the gap junctions becoming concentrated as aggregates at the disk periphery. These gap junctions are not only larger than those normally found at the periphery of the disk, but appear unevenly distributed around it, concentrated as foci at the disk edges.

What is the explanation for this disrupted spatial organization? Immediate suspicion falls on disturbance of adhesion junction-gap junction "cross-talk", in other words the inability of adhesion junction protein partners to interact with Cx43 in the absence of the CT domain. Consistent with this is the finding that the binding of β-catenin and p120-catenin to Cx43 is abolished by the truncation. As the authors point out, it is unclear whether this leads to a defect in cotrafficking or in coassembly of Cx43 with adhesion junction proteins. The problem is that we do not really know how gap junctions become correctly positioned in the normal myocyte. One theoretical possibility is that adhesion junction proteins link the periphery of the gap junction to a neighboring adhesion junction, thereby tethering the gap junction in position. We can see in panel C of the Figure, for example, that the gap junction in this example touches a fascia adherens (arrows). However, not all gap junctions make such direct contact with adhesion junctions; the longitudinal zone of disk membrane shown by freeze-fracture electron microscopy in D extends over 3 sarcomeres, with 3 gap junctions (areas outlined with dashes). To the right (between arrowheads), the membrane turns up at right angles toward the viewer into a fascia adherens-containing plicate zone. Each ventricular myocyte is linked to ~10 other myocytes by intercalated disks. Although all disks are constructed according to a common plan, they vary considerably in size and morphology. The largest disk occupies the entire maximal width of the myocyte body whereas the smallest is formed from single abutting myofibrils (extending as side branches from the cell bodies). The disk may appear as a series of steps that change course at the position of consecutive sarcomeres of adjacent myofibrils; the longitudinal zones may extend for 1, 2, 3, or more sarcomeres in length. The steps may form a continuous series or they may go back and forth so that each end of the disk is in line. Within any given disk, there may be many steps or few, (B, from Severs et al16 with permission from Elsevier; C from Severs17).
far as one can judge, the same applies to gap junction–desmosome contact, most desmosomes being scattered in the vicinity of but at some distance from gap junctions. Perhaps contact sites between gap junctions and adhesion junctions play a role in positioning of the gap junction during a certain stage in its life cycle only. Or perhaps the contact sites seen by electron microscopy are entirely fortuitous, and the interacting protein forms an entirely separate pool from that located in the adhesion junctions. We simply do not know.

High-resolution immunogold labeling at the electron-microscopical level may usefully contribute to these issues and help clarify the nature of apparently overlapping localizations reported from fluorescence microscopy.

Regarding the increased size and decreased number of gap junctions, these changes neatly parallel the effects observed in in vitro cell systems when binding of ZO-1 to the Cx43 CT is blocked; as such, they are in keeping with the idea that ZO-1 regulates accretion of new channels to the gap junction periphery. In contrast to the adhesion proteins, however, Maass et al found that binding of ZO-1 to the truncated Cx43 was not completely abolished, raising the possibility of additional sites in the Cx43 molecule, other than the well documented PDZ domain, to which ZO-1 may bind. If such alternative sites do, in fact, permit ZO-1 to function correctly in regulation of plaque size, then, as the authors suggest, gap junction enlargement might arise through inhibition of degradation via inability of ubiquitin protein ligases to interact with the truncated Cx43.

Turning from what goes wrong in the absence of the Cx43 CT to what goes right, the fact that gap junction plaques are formed at all, have functional channels, and come to be situated in approximately the right place lead the authors to conclude that neither phosphorylation of Cx43 nor the PDZ/ZO-1 binding domain are essential for trafficking and targeting Cx43 to the appropriate destination and its assembly into functional gap junctions. Successful trafficking may be explained in part by the retention of the microtubule binding site in the truncated Cx43 but, in this context, it would also be of interest to know whether the binding of ZO-1 to truncated Cx43 that was observed is restricted to fully formed gap junctions or occurs in the nonjunctural pool of Cx43 (eg, that en route to the plasma membrane). Another thought is whether the presence of other connexins may partially “rescue” what might otherwise be more extreme defects arising from lack of the Cx43 CT domain. Ventricular myocytes, in addition to Cx43, express traces of Cx45 which is coassembled with the Cx43 into the same gap-junctional plaques. Could the presence of the Cx45 CT partially offset the consequences of lack of the Cx43 CT? And what about myocyte types with other connexin coexpression patterns? For example, atrial myocytes typically coexpress Cx40 with Cx43 (as well as lower amounts of Cx45), and because of their narrow diameter, have less clearly demarcated intercalated disks than do ventricular myocytes, ie, the gap junctions and desmosomes are often spread along the cell sides. It would be of interest to know whether, in this rather different setting, the absence of the Cx43 CT impacts significantly on gap junction supramolecular organization.

The work by Maass et al marks a turning point in which in vitro–derived theory on connexin CT function and binding partners is put to the test in the living organism. This may open the way to the generation of new mouse models lacking a series of specific protein partner binding sites. At the same time, we still need to clarify the CT binding partners of connexins other than Cx43 using existing approaches. And even with Cx43, we are still only at the early stages of unraveling which protein partner interacts with which, when, where, and why.

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References


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