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Review

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.

Contents

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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.

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 Fig. 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 Fig. 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 Fig. 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

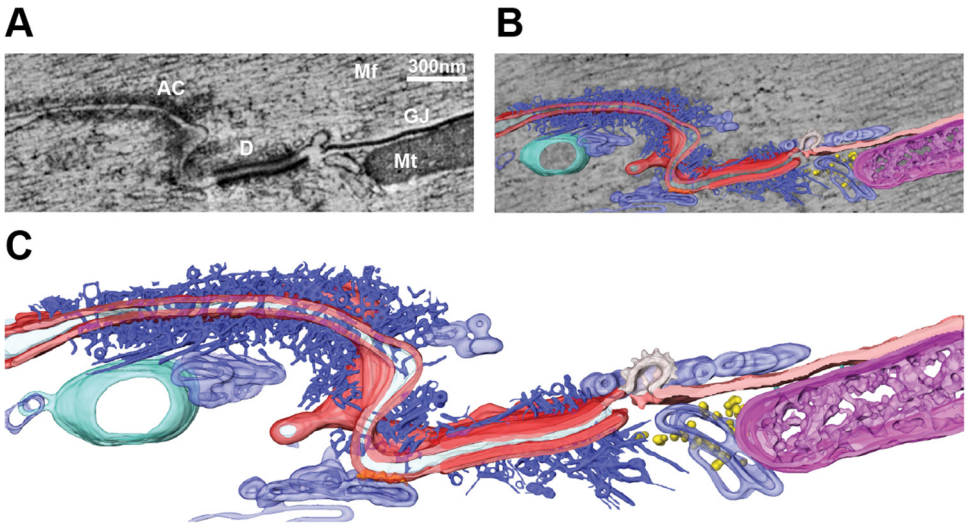

Fig. 1. Tomographic electron microscopy section and 3D rendered representation of a portion of intercalated disc of mouse ventricular tissue. (A) An XY virtual section of a tomogram is shown. Different typical intercalated disc structures (desmosome (labeled as D), gap junction (GJ) and area composita (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.

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