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Oxidant stress derails the cardiac connexon connection

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Connexin 43 (Cx43) is the major protein component of gap junctions that electrically couple cardiomyocytes at the intercalated disc. Oxidant stress, reduced Cx43 expression, and altered subcellular localization are present in many forms of structural heart disease. These changes in Cx43 lead to alterations in electrical conduction in the ventricle and predispose to lethal cardiac arrhythmias. In their study in this issue of the *JCI*, Smyth et al. tested the hypothesis that oxidant stress perturbs connexon forward trafficking along microtubules to gap junctions (see the related article beginning on page 266). Failing human ventricular myocardium exhibited a reduction in Cx43 and the microtubule-capping protein EB1 at intercalated discs. Oxidant stress in the adult mouse heart reduced N-cadherin, EB1, and Cx43 colocalization. In HeLa cells and neonatal mouse ventricular myocytes, peroxide exposure displaced EB1 from the plus ends of microtubules and altered microtubule dynamics. Mutational disruption of the EB1-tubulin interaction mimicked the effects of oxidant stress, including a reduction in surface Cx43 expression. These data provide important new molecular insights into the regulation of Cx43 at gap junctions and may identify targets for preservation of cellular coupling in the diseased heart.

Rapid propagation of electrical impulses in excitable tissue is essential to processes as diverse as cognition, movement, and the genesis of the heartbeat. Central to rapid conduction in the heart and other organs are gap junctions. Gap junctions are low-resistance conduits between cells, comprised of proteins called connexins. In the heart, connexins are key mediators of electrical conduction and are thus central to excitation and contraction. Connexins hexamerize to form connexons or hemichannels in the membranes of apposing cells that dock head-to-head to form intact gap junction channels (Figure 1).

The major connexin of working ventricular myocardium is connexin 43 (Cx43). Cx43 is richly endowed with protein interaction domains and sites of phosphorylation that contribute to regulation of the functional expression of gap junction channels. The carboxyl terminus contains a PDZ-binding domain, multiple consensus serine and tyrosine phosphorylation sites, and binding sites for tubulins. Post-translational modifications and protein-protein interactions are thought to be important for proper formation and localization of clusters of gap junctions into so-called "plaques," although Cx43 with a truncated carboxyl terminus forms working gap junction channels (1).

Structural complexity of intercalated discs

The cardiomyocyte is a complex and highly structured cell. In normal myocardium, gap junction channels are prominently located at intercalated discs positioned at cell ends, mediating electrical propagation that is preferentially in the direction of the long axis of the cell (Figure 1). A remarkable feature of gap junctions and other ion channels is their extremely short half-life (approximately 1–3 hours) (2, 3). The short half-life of gap junction channels is particularly surprising given the transmembrane topology and the complex structure of the intercalated disc that houses connexin-containing gap junctional plaques and implies highly coordinated and tightly regulated trafficking mecha-

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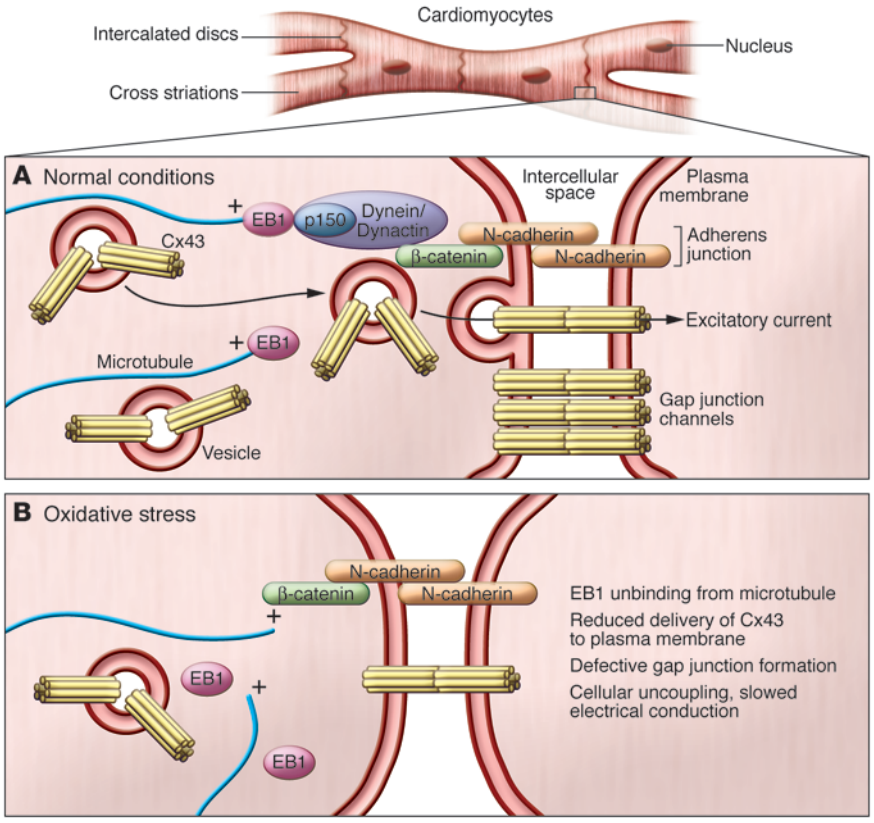

Figure 1

Structure of the cardiac intercalated disc. (**A**) A schematic of the intercalated disc under normal conditions shows normal forward trafficking along microtubules of Cx43-containing connexons to the adherens junction for incorporation into gap junction plaques between cardiomyocytes. Connexins mediate electrical conduction between cells and are thus central to cardiomyocyte excitation and contraction. (**B**) In this issue of the *JCI*, Smyth et al. (9) show that in the setting of oxidative stress, the microtubule-capping protein EB1 dissociates from the microtubule plus end, impeding connexon trafficking to the adherens junction and reducing the generation of gap junction channels. This results in cellular uncoupling and slowed electrical conduction in the ventricle and may predispose to lethal cardiac arrhythmias. Adapted with permission from *Cell* (10) and ref. 9.

nisms both to and from the cell membrane. The structure of the intercalated disc itself is heterogeneous, being comprised of two types of cell-cell junctions in addition to gap junctions: adherens junctions and desmosomes (Figure 1). Adherens junctions transmit force from cell to cell, and desmosomes constitute the sites of desmin attachment. The spatial relationships of these specialized junctions within the intercalated disc provide for adjacencies of the structures but are not immutable. Gap junction plaques are semi-crystalline arrays ranging from tens to thousands of gap junction channels. Viewed en face, gap junction plaques have a characteristic appearance, with large plaques at the perimeter of the gap junction and small plaques in central interplicate regions (for a review, see ref. 4). The question of how this structural arrangement comes about in development, as well as how it changes with disease, is an area of active investigation.

Gap junction life cycle

A comprehensive understanding of the regulation of gap junction channel biosynthesis, trafficking, and degradation continues to evolve. The constitutive gap junction life cycle involves multiple steps, including connexin synthesis and oligomerization, hemichannel translocation to the plasma membrane for incorporation into gap junctions, and subsequent removal from the plasma membrane and degradation with continuous intercellular coupling being maintained. The molecular details of connexin trafficking, targeting, and turnover are of great interest because of well-described alterations in functional expression and localization of connexins in the diseased heart (3). Reduced Cx43 expression and altered subcellular localization contribute to changes in electrical conduction in the heart that are proximate causes of potentially lethal ventricular arrhythmias.

Connexins are co-translationally inserted into the ER membrane for transport through the secretory pathway. In order to form hemichannels, connexins must oligomerize into hexamers; in the case of Cx43, oligomerization takes place in the trans-Golgi network (5). The oligomerization of connexins is essential for normal functional expression, particularly subsequent transport to sites in the plasma membrane.

Connexins are transported through the Golgi and packaged into transport vesicles in the trans-Golgi network for delivery to the plasma membrane. The forward trafficking involves Cx43 binding directly to microtubules (6, 7) (Figure 1). However, pharmacological disruption of microtubules reduces the efficiency of Cx43 delivery to the plasma membrane but does not inhibit the process altogether (8). The article published by Smyth, Shaw, and colleagues in this issue of the *JCI* (9) and previous work from the same group (10) further define the molecular machinery involved in Cx43 forward trafficking to the intercalated disc. Gap junctions are known to form in close proximity to N-cadherin–containing adherens junctions, and adherens junctions presage the development of electrical gap junctions in juvenile cardiac tissues (11). In the absence of N-cadherin, gap junctions do not form in the mammalian ventricle. Shaw and colleagues previously examined the role of microtubules and adherens junctions in the trafficking of Cx43 to the plasma membrane in a vital microscopy study of gap junction formation in HeLa cells. They proposed a model of microtubule-based targeted delivery of Cx43 directly to gap junction plaques that involves the interaction of the dimeric microtubule plus end–capping protein, EB1. Using siRNA methods, they showed that EB1 interaction with its binding partner p150(Glued) of the dynein/dynactin complex tethers microtubules at the adherens junction by way of interaction with β-catenin, which interacts with N-cadherin (10) (Figure 1). This model contrasts with another prevailing model of connexin delivery to the plasma membrane, which proposes that connexons are delivered to regions of un-apposed plasma membrane, where they are free to diffuse in the plane of the membrane, coalesce with the edges of existing gap junctions, and subsequently dock with hemichannels in apposing cells

to form new gap junction channels (6). The two models are not mutually exclusive, and it is tempting to speculate that the relative roles of such forward trafficking may change in pathological conditions, contributing to aberrant localization of Cx43 gap junction channels.

Forward trafficking and oxidant stress

The present work (9) extends previous findings and has implications for possible mechanisms of gap junction remodeling in heart disease. Oxidant stress is present in many forms of heart disease and, given the rapid kinetics of Cx43 turnover, altered trafficking is likely to affect gap junction channel formation. Smyth et al. hypothesized that oxidant stress perturbs connexon forward tracking (9). In human ventricular myocardium from end-stage ischemic myopathic hearts, the authors demonstrated a reduction in Cx43 and EB1 at intercalated discs. Oxidant stress induced by ischemia-reperfusion and peroxide perfusion of adult mouse heart similarly reduced N-cadherin and Cx43 colocalization as well as co-immunoprecipation of Cx43 and EB1 by N-cadherin. In HeLa cells and neonatal mouse ventricular myocytes, peroxide exposure displaced EB1 from the plus ends of microtubules, altered microtubule dynamics, and inhibited microtubule approach toward the cell membrane. A mutation in EB1 that disrupted the EB1-tubulin interaction produced a phenocopy of oxidant stress, including a reduction in surface Cx43 expression in the heterologous expression system. In zebrafish, colocalization of a labeled connexin with N-cadherin was reduced with oxidant stress, as was cellular coupling and conduction velocity in the myocardium. Although the mechanism of forward trafficking of labeled Cx48.5 in zebrafish is not defined, these experiments provide a nice proof of concept for relevance in the intact heart.

The study by Smyth et al. (9) makes a compelling case for a defect in forward Cx43 trafficking in the presence of oxidant stress and that EB1 unbinding from microtubules is involved in the faulty trafficking (Figure 1). As with all provocative work, a number of questions are raised by these data. It is a certainty that the changes in Cx43 and gap junction disposition in the setting of ischemia reperfusion or other interventions that induce oxidant stress are multifactorial. Changes in the rate and site of delivery are important, but stabilization of gap junction channels in the plaque, as well as altered rates of internalization and degradation, are likely to be equally important. The role of other interacting proteins such as zona occludens 1 (ZO-1) in this process continues to be debated (12–15). A prominent feature in diseased myocardium is lateralization of immunoreactive Cx43. The mechanism by which this change in protein distribution occurs is an area of active investigation that may or may not be related to aberrant delivery of Cx43 to the plasma membrane. The mechanism of the oxidant-induced disruption in the EB1-tubulin interaction, central to defective delivery of Cx43 to the intercalated disk, is not revealed by these data and is likely to be important in developing therapeutic targets that restore normal trafficking of Cx43.

Connexin trafficking in heart disease

Oxidant stress in the diseased heart varies over time and thus only intermittently interferes with forward trafficking of Cx43. The rate and extent of reversibility of the effects of oxidant stress on forward trafficking are likely to affect the degree of gap junction remodeling. Furthermore, the changes in forward trafficking need to be considered in the context of the alterations in network properties of the myocardium that accompany structural heart disease and oxidant stress. It is likely that targeting the oxidation-reduction imbalance, which admittedly has proven to be challenging, may be the more effective course, given the cascade of effects resulting from oxidant stress. Remodeling of the heart in ischemia, hypertrophy, and heart failure involves regionally disparate alterations of active membrane properties (i.e., ion channels, transporters, and pumps) as well as changes in cellular coupling. Disease-associated cellular coupling defects are in part due to changes in functional expression of connexins but, importantly, additional effects of remodeling of sodium current, changes in the extracellular matrix, and alteration of cell size and shape may undermine cell-cell communication. The latter changes are often associated with disruptions in cellular structure including the cytoskeleton, having significant implications for trafficking of connexins and other proteins to the membrane. Finally, the role of alterations in the expression of other connexin isoforms in compensating or complicating defective Cx43 delivery to the plasma membrane should be considered.

It is difficult to overstate the importance of maintenance of normal gap junction function in the prevention of arrhythmias and maintenance of health. The work by Smyth et al. (9) provides important new molecular insights into the homeostatic regulation of Cx43 at gap junctions, providing yet another piece in the complex puzzle of regulation of cell-cell communication in the heart.

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