Editorial

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Controlling the Gap
Myocytes, Matrix, and Mechanics

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Effective myocardial contraction depends in part on the efficient propagation of electrical signals through the tissue. The principal structure allowing for such rapid myocardial depolarization is the gap junction plaque, a collection of intercellular pores located primarily at intercalated discs. There, at myocyte poles, gap junctions function in close proximity to the complexes responsible for cell–cell tethering—desmosomes and fascia adherens junctions. Thus, the intercalated disc is the site of both intercellular coupling and adhesion. Gap junctions are formed by clusters of paired hexamers, one from each cell, which bridge to form a low-resistance intercellular channel facilitating the transmission of ions and small molecules (<1000 kD). Each hexamer (or connexon) is comprised of six individual connexins, a diverse protein family found in vascular, neural, and cardiac tissue. In humans, ventricular gap junctions are formed primarily from connexin43 (Cx43), a 43-kD protein encoded by chromosome 6.1

Cx43 is a dynamic molecule, with rapid turnover (t\textsubscript{1/2} roughly 1 hour)2 and responsiveness to a variety of mechanical and chemical stimuli. Myocyte connectivity, as mediated by gap junctions, depends not only on net expression of Cx43, but on effective translocation of Cx43 to the cell surface, the phosphorylation state of both Cx43 and associated proteins,3 and intracellular pH.4 Ventricular Cx43 net protein expression is downregulated in various cardiac disease conditions such as end-stage ischemic,5 idiopathic,5 hypertrophic,1,6 and tachycardia-induced induced cardiomyopathies,7 myocardial ischemia and hibernation,8 and allograft rejection.9 Less is known, however, about posttranslational modification of Cx43 as a regulatory mechanism of gap junction function. Cx43 is normally phosphorylated at serine residues on the C-terminal region, and this appears regulated by PKC, PKA, and the MAP kinases. Gap junction conductance declines with Cx43 hypophosphorylation, which is mediated by PP2a in nonischemic and ischemic cardiomyopathies.2 Dephosphorylation of Cx43 has recently been shown to be mediated by PP2a in nonischemic cardiomyopathies.11 However, precisely how phosphorylation state alters gap junction function remains unclear.3

The mechanisms by which Cx43 protein expression levels are downregulated in disease (including decompensated hypertrophy),1 yet perhaps upregulated in adaptive hypertrophy, represent new intriguing areas of research. Recent studies have implicated the MAP kinases as constitutive activation of JNK in vivo results in reduced Cx43 protein expression by nearly 10-fold compared with normals.12 Overexpression of calcineurin in mice results in reduced Cx43 expression, phosphorylation, and protein redistribution.13 Conversely, Cx43 expression can be upregulated in settings of adaptive hypertrophy, leading to improved myocyte connectivity and increased tissue conduction velocity.1 cAMP can mediate this process in cultured myocytes, although it involves enhanced protein but not gene expression.14,15 More recently, uniaxial stretch has been demonstrated to strikingly upregulate Cx43 expression in stretched, cultured myocytes,16 mediated in part through autocrine secretion of VEGF.15,17

One of the missing links in this cascade is the outside-in signaling linking alterations in extracellular matrix (ECM) mechanics and/or chemistry to gap junction expression and activity. In this issue of Circulation Research, Shanker et al approach this problem18 and shed new insight into such pathways. Their principal findings are that matrix components rich in arginine-glycine-aspartate (RGD) motifs as well as mechanical stretch both stimulate myocyte expression of Cx43, and that both stimuli act through outside-in β1-integrin signaling (Figure). This highlights an intriguing common nodal entry point for diverse inputs controlling Cx43 expression. Plating cultured myocytes on various matrix substrates such as type I collagen or fibronectin (the latter rich in RGD motifs), they found increased Cx43 with the RGD-rich matrix. This was not further enhanced by stretch or exogenous VEGF, suggesting a shared pathway for both RGD- and matrix. This was not further enhanced by stretch or exogenous VEGF, suggesting a shared pathway for both RGD- and stretch-induced signal transduction. Importantly, altered Cx43 levels enhanced myocyte coupling, as assessed by dye-transfer assays.

The authors chose the integrin family of signaling molecules as likely mediators of the stretch/ECM effects on Cx43 expression. In nonstretched myocytes plated on type I collagen, addition of a nonspecific integrin activator (MnCl2) induced similar Cx43 upregulation as with stretch and exposure to RGD motifs. This was abrogated by blockade of β1 but not β3-integrins. Interestingly, myocyte stretch itself augmented β1-integrin protein expression, perhaps acting in a feed-forward manner to stimulate Cx43 expression. Furthermore, the intercellular adhesion molecule N-cadherin was also augmented by both stretch and exposure to RGD-rich ECM components, supporting the functionality of this regulation.
Two-dimensional myocyte layers, of course, are sufficiently different from the complex in vivo setting that extrapolation to intact hearts must remain speculative. Myocyte behavior (including MAP kinase activation) in response to stretch itself depends on the phase of the cardiac cycle when stretch is applied,\textsuperscript{19} and may further be influenced by wall stress.\textsuperscript{1,20} It is difficult to impose specific stresses on isolated myocytes, thus little remains known as to how stress itself might impact Cx43 expression and/or gap junction physiology. As noted by the authors, matrix-driven changes in Cx43 expression could be adaptive, maladaptive, or both, depending on the specific setting. In settings of cardiac injury, deposition of fibronectin by fibroblasts and degradation of collagen by tissue metalloproteinases could trigger augmented Cx43 expression through increased myocyte exposure to RGD motifs. Changes in cardiac stretch linked to remodeling might enhance Cx43 and help improve conduction velocity through the enlarged tissue.

Another question is whether the present studies might shed light into mechanisms of arrhythmia. Normal ventricular tissue is heterogeneous, and studies have shown that transmural disparities in Cx43,\textsuperscript{7} calcium-handling proteins including SERCA2a and phospholamban,\textsuperscript{21} and potassium channels\textsuperscript{22} are important for normal excitation-contraction coupling. Disruption and/or augmentation of this carefully orchestrated heterogeneity may occur with ischemia, infarction, or altered loading conditions, thereby increasing arrhythmia susceptibility. This phenomenon appears particularly noteworthy in failing hearts with dyssynchronous contraction, where endocardial Cx43 hypoexpression has been shown in the high stress (late contracting) wall.\textsuperscript{23} Patients with such hearts display an increased risk of arrhythmia and sudden death.\textsuperscript{24} Whether this too reflects outside-in regulation by matrix/mechanical interactions remains to be determined.

It may prove that the observations made by Shanker et al\textsuperscript{18} do indeed represent a homeostatic mechanism designed to offset the effects of injury and intercellular fibrosis (which likely reduce myocyte coupling and conduction velocities) through augmented Cx43 expression. To be effective, though, such a mechanism would ideally preserve regional patterns of myocyte connectivity and conduction. Heterogeneous stretch (eg, ventricular dyssynchrony) or fibrosis (eg, focal infarction) could conceivably exacerbate arrhythmia susceptibility through Cx43 dysregulation, rather than preserving normal conduction patterns.

More important than speculation about the consequences of Cx43 regulation by ECM components and stretch, however, is the fact that these two inputs share a common mode of signal transduction. Further, the current study provides evidence that \(\beta1\)-integrin pathways may impact a variety of adhesion and coupling proteins. This economy of signaling obviously provides both an enticing opportunity and a daunting challenge. Modulation of \(\beta1\) signaling (through modification of ECM components pharmacologically, for example) appears to be a potentially powerful tool to impact a variety of cellular processes. At the same time, achieving a specific desired effect in the setting of shared signaling pathways will undoubtedly prove difficult. Nonetheless, the new findings of Shanker et al\textsuperscript{18} provide an intriguing look at the importance of ECM signaling, and at common nodes that transduce information from outside the myocyte to within.

References


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