

Releasing YAP From an α -Catenin Trap Increases Cardiomyocyte Proliferation

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Adult mammalian cardiomyocytes possess little endogenous proliferative capacity. This innate limitation underlies our inability to reverse heart failure and improve patient outcome after myocardial injury. The mechanisms that restrict adult mammalian cardiomyocyte proliferation have been the intensely scrutinized, and molecular insights are slowly emerging.¹ In this issue of *Circulation Research*, Li et al² add an important new insight by showing that α -catenins, components of cardiomyocyte intercellular junctions, restrain the mitogenic transcriptional coactivator Yes-associated protein (YAP) and thereby inhibit cardiomyocyte proliferation.

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Intercalated disks mechanically couple cardiomyocytes. Located on the ends of adult mammalian cardiomyocytes, intercalated disks are composed of 2 kinds of adherens junctions, fasciae adherens and desmosomes, interspersed with gap junctions (Figure). In epithelia, such as skin, desmosomes and fasciae adherens constitute physically and molecularly distinct complexes. Epithelial fasciae adherens contain classical cadherins, transmembrane adhesion receptors that mediate cell–cell adhesion through homotypic interactions. The cytoplasmic tails of cadherins interact with catenins (α E-catenin [*Cttna1*], β -catenin [*Cttnb1*], and γ -catenin [*Jup*, also known as plakoglobin]), which bind the actin cytoskeleton. Analogously, epithelial desmosomes contain desmosomal cadherins (desmoglein and desmocollin), which interact with γ -catenin (the only protein common to fasciae adherens and desmosomes) and plakophilin. These connect to intermediate filaments through desmoplakin and other plakin family proteins. In heart, the distinction between these 2 types of adherens junctions is blurred: mature mammalian intercalated disks contain hybrid junctional complexes containing proteins characteristic of each type of junction, and hence have been referred to as area composita, with local variations yielding regions with more desmosome, or more fascia adherens-like characteristics.³ Cardiac intercalated disks contain 2 α -catenin isoforms: the widely expressed α E-catenin and the cardiac-restricted α T-catenin (*Cttna3*). Interestingly, α T-catenin may

contribute to the formation of the hybrid area composita because it interacts with the desmosomal protein plakophilin-2.⁴

Cardiac intercellular junctions evolve during heart development, with mature intercalated disks forming only in the postnatal heart.^{5,6} In the fetal heart, desmosomes and fasciae adherens remain more distinct, and both localize widely over the membranes of stellate-shaped cardiomyocytes. As cardiomyocytes mature, they elongate and align their myofibers along the cell's long axis. In the first 2 postnatal weeks, desmosomes and fasciae adherens of murine cardiomyocytes coalesce into area composita that focally cap the cardiomyocyte poles.

The essential function of cardiac adherens junctions to join cardiomyocytes mechanically was vividly exposed by genetic inactivation of several intercalated disk components, including γ -, α E-, and α T-catenins. Loss of γ -catenin caused fetal demise from heart defects and ventricular rupture.⁷ Fetal cardiomyocyte-restricted α E-catenin inactivation was compatible with embryonic survival, but mice had cardiomyocyte apoptosis and ventricular dilatation and thinning.⁸ These mice were also predisposed to ventricular rupture after myocardial infarction (MI). Mice lacking α T-catenin similarly survived to term, developed progressive cardiomyopathy, and were more susceptible to ischemia-induced arrhythmias, associated with mislocalization of gap junctions and loss of plakophilin-2 from area composita.⁹

In addition to their essential roles in maintaining the mechanical integrity of the heart, adherens junctions are ideally positioned as signaling centers that transduce mechanical forces. The signaling functions of adherens junctions are best highlighted by their role in the pathogenesis of arrhythmogenic cardiomyopathy, in which desmosome gene mutations cause fibrofatty infiltration of the ventricular wall and a high risk of ventricular arrhythmia. Arrhythmogenic cardiomyopathy mutations destabilize desmosomes and diminish γ -catenin localization to intercalated disks. The liberated γ -catenin has been proposed to compete with its closely related cousin, β -catenin, and thereby dampen Wnt/ β -catenin signaling.¹⁰ This altered canonical Wnt signaling may contribute to the adipogenic changes characteristic of arrhythmogenic cardiomyopathy. More recently, desmosome mutations in arrhythmogenic cardiomyopathy were linked to modulation of Hippo/YAP signaling, an important pathway that regulates organ size and cellular proliferation and survival¹¹ and that also modulates canonical Wnt signaling.¹² Desmosome mutations increased activation of NF2, an upstream regulator of the Hippo kinase cascade. As a result, Hippo kinase phosphorylation of the YAP transcriptional coactivator increased, resulting in YAP sequestration near the membrane and reduced transcriptional activity.¹¹

The study of Li et al² defines a new inter-relationship between intercalated disks and YAP activity. They studied mice with fetal, cardiomyocyte-specific inactivation of both α E- and

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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(*Circ Res*. 2015;116:9-11.

DOI: 10.1161/CIRCRESAHA.114.305496.)

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Circulation Research is available at <http://circres.ahajournals.org>

DOI: 10.1161/CIRCRESAHA.114.305496

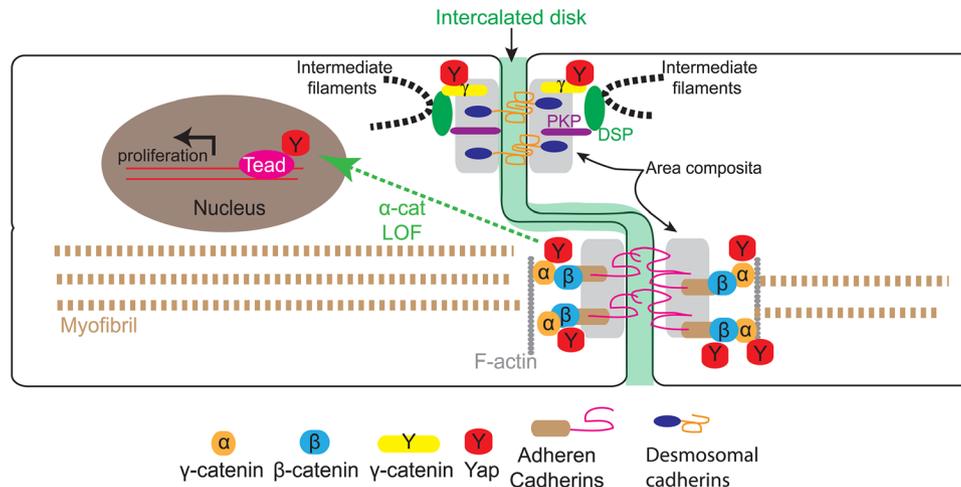


Figure. Yes-associated protein (Yap) sequestration at the area composita of adult cardiomyocytes. In the intercalated disk, thin filaments from the terminal sarcomeres attach to the ID through the area composita via α -, β -, and γ -catenins. α -, β -, and γ -catenins each interact with Yap in the area composita, preventing Yap from translocating into the nucleus thus making the area composita a Yap reservoir. Extracellular signal stimulation or α -catenin inactivation can release Yap, allowing it to enter the nucleus, where it stimulates mitogenic gene expression. DSP indicates desmoplakin; LOF, loss of function; and PKP, plakophilin 2.

α T-catenin driven by Myh6-Cre.² These double knockout (DKO) mice survived to postnatal life. At 6 weeks, the hearts were normal in size, but cardiomyocyte number was higher and cardiomyocyte size was lower than controls. Increased cardiomyocyte number was linked to greater cell cycle activity. Previously, α E-catenin was shown to mediate contact inhibition of keratinocyte proliferation via sequestration of YAP at adherens junctions.¹³ Therefore, Li et al² investigated YAP activity in the α E/ α T DKO hearts and found increased nuclear YAP. Enhanced YAP activity was essential for the proliferative effect of α -catenin loss of function because increased cell cycle activity observed in cultured neonatal cardiomyocytes with siRNA-mediated α E/ α T knockdown was blunted by simultaneous YAP knockdown. Interestingly, the mechanism of YAP activation seems to differ from what was previously reported for α E-catenin regulation of YAP in keratinocytes, where α E-catenin inhibited YAP dephosphorylation by the phosphatase PP2Ac.¹³ In contrast, in DKO hearts, both phospho- and total YAP levels were increased, leading to more activated YAP but only a slight reduction of the phospho:total YAP ratio.² Additional studies are needed to define the mechanistic link between α E/ α T-catenin ablation in DKO hearts and YAP activation.

Li et al² also examined the effect of α E/ α T-catenin ablation in adult cardiomyocytes driven by Myh6-MerCreMer. Although fetal inactivation of either α E- or α T-catenin caused adult onset cardiomyopathy,^{8,9} interestingly, adult stage inactivation of both α E/ α T-catenin (IN-DKO) was compatible with survival for >1 year and did not cause histological abnormalities. Cardiomyocyte proliferation was not elevated in unstressed hearts. However, MI of IN-DKO mice showed that the inactivation of α E/ α T-catenins had a surprising protective effect: IN-DKO mice had better cardiac function and reduced cardiac remodeling at 9 and 12 weeks post MI. These changes were associated with increased cardiomyocyte cell cycle activity in the border and ischemic zones, and with an increased fraction of cardiomyocytes with YAP nuclear localization.

These data suggested that YAP activity in unstressed adult cardiomyocytes is restrained by multiple mechanisms in addition to binding to α -catenins. Myocardial injury releases these additional restraints and unmasks a rate-limiting role for α -catenin trapping of YAP at area composita to limit cardiomyocyte proliferation. Thus, the combination of myocardial injury and α -catenin ablation unfetters YAP, leading to increased cardiomyocyte cell cycle activity and improved recovery from myocardial injury.

These results raise the interesting possibility that modulation of intercalated disk signaling through YAP may be a means to enhance cardiac regeneration. However, many critical questions must be addressed before pursuit of such a strategy. First, this study provided scant data on the overall phenotype of either DKO or IN-DKO models, such as ventricular function, intercalated disk structure, or electric conduction. Individual fetal knockout of either α E- or α T-catenin caused cardiomyopathy.^{8,9} The authors did not report the functional phenotype of the DKO model, but it also likely caused ventricular dysfunction. Given these phenotypes, it is surprising that adult α -catenin inactivation in IN-DKO mice seemed to be well tolerated. This might relate to dynamic developmental remodeling of intercalated disk structure,^{5,6} and suggests that α -catenins are required for normal intercalated disk assembly but are dispensable for their maintenance in the adult heart. Extensive additional data are necessary to provide reassurance that modulation of intercalated disk structure in adult hearts does not cause deleterious effects. In light of potential complications of manipulating intercalated disk structure, perhaps it may be more attractive to target the mechanisms downstream of α -catenins that lead to improved myocardial outcome in IN-DKO hearts after MI. It will be necessary to establish that YAP release from α -catenin inhibition accounts for improved outcome after MI in IN-DKO and then to understand the molecular link between α -catenin and YAP activation better. Given the complex interwoven relationships between Hippo/YAP, Wnt/ β -catenin signaling, and

intercalated disk integrity, it will be important to show that interventions downstream of α -catenin do not provoke deleterious, cardiomyopathic changes.

In summary, the work of Li et al⁹ revealed a new mechanism for intercalated disk signaling, in which α -catenin modulates the subcellular localization and transcriptional activity of YAP. This α -catenin trap restrains YAP-driven cardiomyocyte proliferation, and conceivably springing YAP from this trap will be a useful strategy to increase cardiac repair and regeneration.

Sources of Funding

W.T. Pu was supported by grants from the American Heart Association and National Institutes of Health (R01 HL116461 and U01HL098166).

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KEY WORDS: Editorials ■ desmosomes ■ regeneration