Cardiac excitation-contraction (E-C) coupling links action potentials to muscle contraction and is in essence a process of calcium ion mobilization. The central mechanism governing this process in ventricular myocytes is Ca\(^{2+}\)-induced Ca\(^{2+}\) release, or CICR. It has been established for more than 20 years that CICR operates in a local control mode, taking place in a restricted junctional space of \(\sim 12\) to 15 nm between the transverse (T)-tubule and sarcoplasmic reticulum (SR) membranes, namely, the junctional membrane complexes or cardiac dyads.\(^{2,3}\) Within this dyadic “fuzzy space,” clusters of ryanodine receptor (RyR) Ca\(^{2+}\) release channels on the SR constitute the calcium release apparatus together with the directly apposed voltage-gated L-type Ca\(^{2+}\) channels (LTCCs) located primarily on the T-tubule membrane.\(^{5}\) On membrane depolarization, a small amount of Ca\(^{2+}\) influx through the opening of LTCCs locally activates adjacent RyRs to release a much larger (\(\sim 10\) times) amount of Ca\(^{2+}\) from the SR.\(^{6,7}\) The normal, functional cross-talk between LTCCs and RyRs depends on a stable local ultrastructure—the cardiac dyad.

The molecular mechanism underlying the formation of cardiac dyads remained a mystery until the pioneering work of Takeshima and colleagues.\(^{8}\) In their study, junctophilins were identified as key molecules that maintain junctional membrane complexes between the plasma membrane and the endoplasmic/sarcoplasmic reticulum (ER/SR) in excitable cells. The junctophilin protein family contains 4 members (JP1-4), and JP2 is the only subtype expressed in cardiac myocytes. Lack of JP2 in mice causes embryonic lethality, and JP2 knockout embryonic myocytes have deficient junctional membrane complexes and abnormal Ca\(^{2+}\) signaling, such as reduced intracellular Ca\(^{2+}\) transients.\(^{8}\) Thus, JP2 provides a structural basis for nanoscopic signaling between LTCCs and RyRs during E-C coupling in ventricular myocytes (Figure).

Impaired cardiac E-C coupling/Ca\(^{2+}\) handling is a hallmark of heart failure.\(^{9-12}\) Gomez and colleagues\(^{13}\) first proposed in 1997 that defective E-C coupling is probably due to a change in the relation between RyRs on the SR and LTCCs on T-tubules, although no direct evidence was provided. In the past 10 years, evidence from isolated ventricular myocytes suggests that T-tubule loss and/or disorganization is a significant and common event in advanced heart failure of different etiologies and results in dysynchronous Ca\(^{2+}\) release and impaired contraction.\(^{14-23}\) More recently, the phenomenon of T-tubule remodeling in response to either pressure overload or myocardial infarction was substantiated using an in situ confocal imaging technique in intact hearts.\(^{24-26}\) The reorganization of T-tubule structure alters the spatial organization between LTCCs and RyRs, leading to an increase in orphaned RyRs, the loss of local control of RyRs by LTCCs, Ca\(^{2+}\) release instability, and E-C coupling deficiency in failing myocytes.\(^{16,18,21}\) In addition to T-tubule remodeling, downregulation of JP2 has been found in a variety of heart failure models as well as in failing human hearts.\(^{12,24,26-28}\) Two recent studies in which JP2 was knocked down in either cultured ventricular myocytes\(^{29}\) or by transgenic expression of a JP2 shRNA in mice\(^{29}\) revealed that JP2 downregulation is a key mechanism underlying T-tubule disruption in failing myocytes. The latter study in mice also suggests that JP2 deficiency disrupts the stability of junctional membrane complexes.\(^{29}\) The next logical question is, what is the mechanism responsible for JP2 dysregulation?

In this issue of Circulation Research, Wang and colleagues (Xu et al\(^{30}\)) define miR-24 as a novel direct regulator of JP2.
homeostasis in the heart. Bioinformatic analysis backed up by experimental data revealed 2 binding sites for miR-24 in the 3’ UTR of JP2 mRNA, either of which was sufficient for maximal repression of JP2 expression. Extending these in vitro studies to models of heart failure demonstrated that miR-24 was upregulated in compensated hypertrophy and in decompensated heart failure, concomitant with loss of JP2 expression and decreased size and volume density of the cardiac dyads. The authors next tested whether overexpression of miR-24 in cultured adult cardiomyocytes could recapitulate the phenotype observed in heart failure model. They found that a 150% increase in miR-24 levels resulted in the anticipated decrease in JP2 expression, which led to a decrease in Ca\(^{2+}\) transient amplitude and E-C coupling gain but no alterations in expression of other E-C coupling proteins. Thus, this study provides novel mechanistic insights into the regulation of JP2 expression in heart cells, adding an important piece to the puzzle of the events that culminate in E-C coupling defects.

The work by Wang and colleagues in this issue of Circulation Research was built on another recent study from the same group (Wu et al\(^{29}\)). The objective of the previous study was to understand the ultrastructural mechanism underlying the defective LTCCs-RyRs signaling and compromised contractility in heart failure. Using electron microscopy, the authors found that in response to pressure overload, the size and the volume density of the dyads were significantly reduced. The authors went on to show that knockdown of JP2 replicated the dyadic remodeling observed in the heart failure model, thus suggesting that downregulation of JP2 mechanistically contributes to ultrastructural alterations and loss of E-C coupling in failing hearts. The present study extends these findings to identify miR-24 as a mediator of JP2 downregulation in heart failure.

Over the past few years, miRNAs have gained increasing recognition as important regulators of normal cellular function and disease pathogenesis in many tissues, including the heart.\(^{32}\) An miRNA typically has multiple targets; interestingly, Wang and colleagues (Xu et al\(^{30}\)) found that miR-24 overexpression had no impact on other E-C coupling components, and genome-wide scanning did not identify putative miR-24 binding sites in the 3’ UTR of mRNAs encoding other E-C coupling proteins.

The miR-24 binding sites are evolutionarily conserved in the 3’ UTR of JP2 mRNAs of mouse, rat, and human origin.\(^{30}\) One important question is whether miR-24 is a physiological regulator of JP2, or if it is only overexpressed in pathological conditions. Cardiac-specific inducible overexpression or knockdown of miR-24 in genetically modified mice should further shed light on this subject. A second question is whether there are other miRNAs that target JP2 for translational silencing. Furthermore, are there posttranslational mechanisms responsible for downregulation of JP2 protein, such as calpain-mediated proteolysis or modifications such as SUMOylation and ubiquitination that target JP2 for degradation? Are there other proteins involved in maintaining the dyadic ultrastructure and T-tubule organization? Answering these questions will further enhance our understanding of cardiac E-C coupling regulation and dysregulation.

Another related question is, how is miR-24 increased in heart failure? Hypertrophic signaling through the calcineurin-NFAT pathway is a well-established mechanism of heart failure.\(^{33}\) Based on a recent report (Lin et al\(^{34}\)), Wang and colleagues hypothesized that this pathway induces transcription of the miR-23a/27a/24 cluster as an upstream event in JP2 silencing in heart failure. New studies with genetic modification of the calcineurin-NFAT signaling cascade are necessary to determine whether this pathway indeed mediates the increase of miR-24 and subsequent JP2 loss.

An important future experiment will be to examine whether introduction of an antago-miR against miR-24 protects against development or progression of heart failure. These data would not only confirm the role of miR-24 in JP2 downregulation in heart failure but would also indicate whether antago-miR should be pursued as a novel therapeutic strategy to augment JP2 expression in the treatment of heart failure.

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**Disclosures**

None.

**References**


MicroRNA: A Toolkit Fine-Tuning the Dyadic "Fuzzy Space"?
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