Defining the Complexity of the Junctional Membrane Complex

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Each heart beat in every cardiac myocyte begins with the surface membrane depolarization during an action potential that opens L-type Ca^{2+} channels and allows the influx of a small amount of extracellular Ca^{2+} that triggers a larger release of Ca^{2+} from the intracellular store, the sarcoplasmic reticulum (SR), through Ryanodine Receptors (RyR2).[1–2] This process, known as Ca^{2+}-induced Ca^{2+}-release (CICR), is the primary mechanism underlying excitation-contraction coupling (ECC) in the heart. Cardiac myocytes are relatively large cells, and efficient cardiac function requires not only the rapid sequential activation of contraction between cells but also the simultaneous activation of the contractile apparatus within each cell. To accomplish this, adult cardiac myocytes localize L-type Ca^{2+} channels in deep membrane invaginations known as T-Tubules, located along the Z-lines adjacent to the SR Ca^{2+} release channels in highly ordered structures known as dyads. The potential importance of these organized junctional membrane complexes has been defined during the last decade using in situ imaging techniques that show their loss in pathological conditions including myocardial infarction and heart failure.[3]

Additional proteins essential for the structure and function of the junctional membrane complex have been identified. Junctophilin-2 (JPH2) connects the T-tubular and SR membranes and is downregulated in a number of models of heart failure.[4–6] Of greater note, JPH2 disruption leads to heart failure in rodent models, JPH2 overexpression protects against the transition from hypertrophy to failure in a rodent model with thoracic aortic constriction., and mutations in JPH2 cause hypertrophic cardiomyopathy. More recently, the protein bridging integrator-1′ (Bin1), also known as M-amphiphysin-2, has been shown to be essential for the development and stability of T-tubules.[7] The identity and roles of other proteins in the junctional membrane complex are less clear.

Striated Muscle Preferentially Expressed Protein Kinase (SPEG) is a serine/threonine kinase in the myosin light chain kinase family.[8] SPEGβ and SPEGα, a shorter isoform lacking 854 amino acids at the N-terminal end, are both expressed in the heart. SPEG interacts with

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myotubularin (MTM1), and SPEG mutations are rare causes of dilated cardiomyopathy.[9] Targeted deletion of SPEG in the mouse leads to dilated cardiomyopathy and early postnatal mortality.[10] In addition, cardiac progenitor cells of Speg$^{-/-}$ mice are abnormal, and the neonatal defects in Speg$^{-/-}$ mice can be rescued through the intrauterine injection of wild type cardiac progenitor cells into the developing heart.[11] Thus, although SPEG clearly plays a role in cardiac development and function, a role in Ca^{2+} handling has not previously been identified.

In this issue of *Circulation Research*, Quick et al. use MS/MS mass spectrometry to search for novel proteins that co-immunoprecipitate with both RyR2 from the hearts of wild type mice and Jph2 from the hearts of transgenic mice overexpressing Jph2.[12] They successfully identified Spegα and Spegβ as novel components of the junctional membrane complex with isoform-specific binding to RyR2 and Jph2. They go on to show that SPEG is downregulated in human heart failure, and to engineer a tamoxifen-inducible cardiac-specific Speg knockout mouse that demonstrates the rapid onset of T-tubular disarray followed by heart failure, a lower SR Ca^{2+} content with increased spark frequency with no change in Serca2a-mediated SR Ca^{2+} uptake, and no change in Jph2 expression but a marked decrease in Jph2 phosphorylation. Taken together, these studies provide strong evidence that SPEG plays a critical role in both the structure and function of cardiac T-tubules and the RyR2-mediated Ca^{2+} release that they direct.

This well-conceived and elegant study is important for several reasons. First, it identifies a novel and important role for SPEG in the junctional membrane complex, and suggests a previously unknown functional role for JPH2 phosphorylation. Second, the work provides further evidence that T-tubular disarray can causally contribute to the pathogenesis of heart failure. Finally, it provides insight into Ca^{2+}-dependent mechanisms leading to heart failure that are independent of changes in SERCA2a expression and function, and that may not be amenable to therapies aimed at raising SERCA2a such as AAV-SERCA2a gene delivery to the heart.[13]

As would be expected, the identification of SPEG in the junctional membrane complex raises as many questions as it answers. What (if any) are the specific roles of the SPEGα and SPEGβ isoforms? Which residue(s) of JPH2 is (are) phosphorylated, and is the decrease in JPH2 phosphorylation causally related to the development of T-tubular disarray and heart failure (as opposed to an indirect effect through phosphorylation of another target)? Does phosphorylation of JPH2 alter its proteolytic cleavage? Does SPEG interact with BIN1, and does myotubularin play a role in the junctional membrane complex?[7,9] Do polymorphisms in SPEG predispose to heart failure and/or arrhythmias? Do mutations in SPEG cause inherited forms of sudden death?

While SPEG was the only new protein identified by the MS/MS screen using Jph2 and RyR2, it is likely that other unidentified proteins are playing a role in the junctional membrane complex. These proteins may bind to either JPH2 or RYR2. Alternately, future studies could now use SPEG as bait to identify other members of the protein complex. During the last several years, our understanding of the junctional membrane complex has increased. The manuscript of Quick et al. has contributed to our improved understanding.

That said, we do not yet know just how complex the junctional membrane will turn out to be.

Acknowledgments

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