



A bridge from the endoplasmic reticulum to the plasma membrane comes into view

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Every breath of life and every beat of the heart relies on the faithful translation of fast voltage signals into a dynamic influx of Ca^{2+} ions that initiates the contraction of skeletal and cardiac muscle. This process, known as excitation–contraction coupling, requires the precise geometric arrangement and functional coordination of two large ion channel macromolecular complexes that reside in two opposing membrane compartments which are separated by a narrow cleft—the L-type voltage-gated Ca^{2+} channels (Ca_v1) in the traverse-tubular (t-tubule) membrane and the ryanodine receptor (RyR) in the sarcoplasmic reticulum (SR). In the heart, this compartment is known as a dyad based on its bipartite organization (Fig. 1). The propagation of the cardiac action potential into the t-tubule results in the activation of dyadic L-type channels ($\text{Ca}_v1.2$) that convey a local influx of Ca^{2+} into the cleft. The freely diffusing Ca^{2+} , in turn, binds RyR2 across the dyadic cleft to elicit a much larger release of Ca^{2+} from intracellular stores (1). In addition to the two ion channel behemoths, a wide range of Ca^{2+} -effector proteins such as calmodulin-dependent kinases and calcineurin also reside in this space and couple changes in Ca^{2+} signals to various cellular functions, rendering the dyadic junction a privileged Ca^{2+} -signaling domain (1). In the skeletal muscle, the analogous subcellular compartment is known as a triad owing to its tripartite arrangement (Fig. 1). In the triad, voltage-dependent activation of the $\text{Ca}_v1.1$ L-type channel is conformationally coupled to RyR1, thus obviating the need for Ca^{2+} ions in evoking Ca^{2+} release from the intracellular store (2). Beyond striated muscle, close apposition of the endoplasmic reticulum and the plasma membrane (ER–PM junctions) is found in many cell types including neurons, where they play a vital role in Ca^{2+} homeostasis and signaling and lipid exchange (3, 4). The function and formation of ER–PM junctions can be static or dynamic, involving a rich repertoire of proteins. For example, depletion of intracellular Ca^{2+} stores causes the coaccumulation of stromal interaction molecule and Orai channels in ER–PM junctions (5). Ensuing localized Ca^{2+} entry through Orai replenishes the intracellular stores (5). By comparison, dyadic and triadic junctions are preassembled. In this broader context, the junctophilin family of proteins (Jph1 to Jph4) have been shown to play an essential role in both the formation of ER–PM junctions in muscle and neurons as well as to serve as a scaffold that recruits relevant ion channels to this specialized Ca^{2+} -signaling compartment (6). In PNAS, the paper by Yang et al. (7) presents high-resolution atomic structures of a key Jph domain alone and associated with an isolated cytosolic segment of the $\text{Ca}_v1.1$ channel. This study opens new frontiers in understanding the structure and mechanisms underlying the formation of ER–PM junctions and the recruitment of ion channels to this subcellular domain.

The junctophilin family of proteins were identified in 2000 as an important component of junctional membrane complexes by Takeshima et al. (8). Four different subtypes of Jph have been identified, with Jph1 primarily expressed in skeletal muscle, Jph2 in cardiac and skeletal muscle, and Jph3 and Jph4 in neurons (see reviews in refs. 6 and 9). In terms of molecular architecture, the Jphs are composed of eight membrane occupation and recognition nexus (MORN) domains, a long α -helical region, thought to be a spacer between the ER and PM, a long divergent region, and a transmembrane domain. The first six MORN domains are linked to the remaining two via a joining region that is largely disordered. The MORN domains are thought to associate with the PM through interactions with phosphoinositol-containing phospholipids, palmitoylation, or association with adapter proteins, while the transmembrane domain spans the ER (6, 9). The α -helical domain has been proposed to function as a molecular spacer that determines the size of the cleft. Beyond its structural role, Jph1 to Jph4 have emerged as versatile modulators of various ion channels, recruiting and fine-tuning the function of RyR1 to RyR3 (10–12), $\text{Ca}_v1/2$ (11, 13), and KCNQ channels (10). In skeletal muscle, Jph1 recruits $\text{Ca}_v1.1$ to triads through a physical interaction with the channel carboxy terminus (13) and serves as one of the minimal requisite components necessary for reconstituting voltage-dependent excitation–contraction coupling (the others being the $\text{Ca}_v1.1$ β_{1a} subunit and the adaptor protein stac3) (14). In the heart, the interaction of Jph2 with RyR2 stabilizes channel function and prevents diastolic Ca^{2+} leak (12). In the brain, Jph3/4 recruits Ca_v1 and Ca_v2 channels to ER–PM junctions and can selectively modify the inactivation kinetics of Ca_v2 channels (11). Not surprisingly, constitutive knockout of Jph1 and Jph2 is lethal, while knockout of Jph3 and Jph4 results in deficits in motor coordination, learning, and memory (6). In the heart, acute loss of Jph2 has been shown to result in heart failure and increased mortality, presumably due to deficits in t-tubule maturation and altered Ca^{2+} homeostasis (12). By contrast, Jph2 overexpression promotes t-tubule formation and is protective against heart failure in mice (15). Human mutations in

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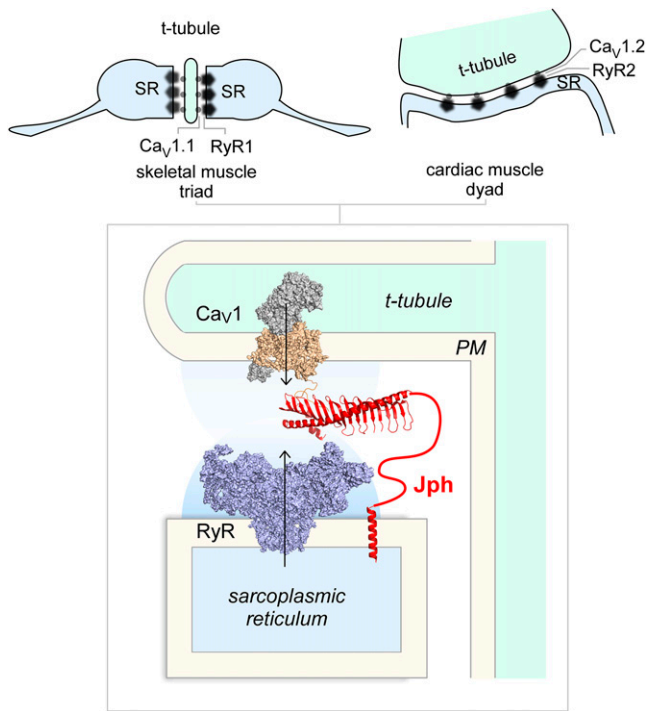


Fig. 1. Cartoon illustrates the excitation–contraction machinery in skeletal and cardiac muscle. The Ca_v1 channels (PDB: 5GJV) are localized to the t-tubular membrane, while the RyR (PDB: 6W1N) is in the SR membrane. Jph plays a key role in formation of the junction and is proposed to recruit both Ca_v1 and RyR to this exquisite Ca^{2+} -signaling compartment.

Jph have been linked to various diseases. Specifically, Jph2 mutations have been linked to hypertrophic cardiomyopathies (HCM), dilated cardiomyopathies, and atrial fibrillation. Jph1 mutations have been identified in Charcot-Marie-Tooth disease, while trinucleotide expansion mutations in Jph3 have been linked to Huntington disease-like 2 syndrome (6). The pathophysiological mechanisms of these human mutations are likely complex, involving changes in ER–PM structure and regulation of multiple ion channels—a seemingly intractable problem.

In this context, the study by Yang et al. (7) represents a major leap forward by furnishing a molecular understanding of Jph and a structural blueprint to dissect the complex effects of human Jph mutations. In this work, the authors have solved the structures of the tandem MORN motifs and α -helix rich domain (ARD) of Jph1 and Jph2 in isolation as well as that of Jph2 bound to a cytosolic fragment of the $Ca_v1.1$ channel (7). These structures provide an in-depth view at the bridge linking the plasma membrane and SR and reveal that the β -sheet-rich MORN motifs of Jph1 and Jph2 adopt an exquisite “rib cage”-like repetitive structure, the convex side of which cradles the long contiguous α -helix of the ARD, while the concave side forms a narrow basic groove. In the structure of the Jph2– $Ca_v1.1$ complex, the $Ca_v1.1$ peptide was found to interact with this groove, suggesting that it may serve as a site of interaction for other plasma membrane-localized binding partners. Interestingly, although the structures of the MORN and ARD regions of the two Jph isoforms were nearly identical in isolation, or when bound to the $Ca_v1.1$ peptide, the region that joins the sixth and seventh MORN repeats was found to adopt different conformations in the three structures. In

all four Jph isoforms, the joining region between the sixth and seventh MORN motifs is considerably longer than that found between the other MORN motifs and has low sequence complexity, which has led to the prediction that it is intrinsically disordered. While this joining region was truncated in the Jph constructs used by Yang et al. for crystallization, the conformational differences observed in the three structures may provide a glimpse into the ensemble of states sampled by this region. Complementing their structural studies, the authors also undertake functional analysis to demonstrate that disruption of the interaction between Jph1 and the $Ca_v1.1$ carboxy terminus through structure-informed mutagenesis diminished $Ca_v1.1$ clustering and weakened excitation–contraction coupling, consistent with previous studies (13). As noted, the structures also shed light on how human mutations in Jph1 to Jph3 may ultimately contribute to human disease by disrupting Jph folding and interactions with target proteins. Notably, a number of variants have been found at the interface between the MORN motifs and ARD. As these mutations either remove stabilizing interactions or introduce steric hindrance between these regions, it is expected that they would weaken or prevent this interaction, greatly perturbing the tertiary structure of Jph2. More concretely, biochemical studies revealed that an HCM-linked mutation in Jph2 may weaken its binding to Ca_v1 channels, potentially informing on pathophysiological mechanisms.

The structures by Yang et al. (7) also raise many new questions. First, the Jph MORN domains have been classically thought to anchor Jph to the PM by binding phosphatidylserine (PS)- and phosphatidylinositol (PI)-containing phospholipids. Surprisingly, the authors found no density corresponding to PS or PI in crystals grown in the presence of these phospholipids. This suggests that the Jph MORN domains may only weakly bind lipids. While it is possible that the high ionic strength utilized for crystallization weakened the Jph–phospholipid interaction, it is also possible that the MORN domain may require additional partners for robust association with the membrane. Second, the authors clearly demonstrate that the association of Jph with the Ca_v1 channel carboxy terminus is important for determining channel localization. Yet this domain is also a hub for many regulatory proteins that tune channel gating. If so, are there as-yet-unknown effects of Jph2 on channel activity? Superresolution scanning patch-clamp experiments in rat ventricular myocytes hint at spatially altered activity profile of $Ca_v1.2$ upon overexpression of Jph2 (16). Third, the present structures provide an unparalleled view of the Jph interfaces involved in binding Ca_v1 channels. However, this is only one part of the full picture. Jph is also capable of recruiting other ion channels, including the RyR (6). Are there conserved motifs that support binding to the Jph MORN domain? It is also possible that Jph may use multiple interfaces to glue together various channel complexes. Indeed, both the joining region and the carboxyl-terminal divergent regions of Jph have been reported to bind RyR (11, 12).

Excitation–contraction coupling is one of the most fundamental phenomena in physiology, and disruption of this process is at the epicenter of many life-threatening human

diseases. Since the early electron micrographs that revealed the ultrastructure of the dyad and the triad (17), identifying the molecular structures and events that

translate membrane depolarization to Ca^{2+} release has been a high priority. Pieces of this elaborate puzzle are now falling into place, one domain at a time.

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