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Author(s)	Takeshima, Hiroshi; Hoshijima, Masahiko; Song, Long-Sheng
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Review

Ca^{2+} microdomains organized by junctophilins

Hiroshi Takeshima [a,*], Masahiko Hoshijima [b,1], Long-Sheng Song [c,2]

[a] *Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan*
[b] *Department of Medicine and Center for Research in Biological Systems, University of California, San Diego, CA 92093, USA*
[c] *Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA*

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ABSTRACT

Excitable cells typically possess junctional membrane complexes (JMCs) constructed by the plasma membrane and the endo/sarcoplasmic reticulum (ER/SR) for channel crosstalk. These JMCs are termed triads in skeletal muscle, dyads in cardiac muscle, peripheral couplings in smooth and developing striated muscles, and subsurface cisterns in neurons. Junctophilin subtypes contribute to the formation and maintenance of JMCs by serving as a physical bridge between the plasma membrane and ER/SR membrane in different cell types. In muscle cells, junctophilin deficiency prevents JMC formation and functional crosstalk between cell-surface Ca^{2+} channels and ER/SR Ca^{2+} release channels. Human genetic mutations in junctophilin subtypes are linked to congenital hypertrophic cardiomyopathy and neurodegenerative diseases. Furthermore, growing evidence suggests that dysregulation of junctophilins induces pathological alterations in skeletal and cardiac muscle.

1. Junctional membrane complexes in excitable cells

1.1. Coupling between plasma membrane and endo/sarcoplasmic reticulum

Junctional membrane complexes (JMCs) are structural contacts between the plasma membrane (PM) and the endo/sarcoplasmic reticulum (ER/SR), first observed in muscle cells by Porter and Palade in the mid-50s [1]. Subsequent studies have identified several types of JMCs widely in excitable cells [2], including "subsurface cisterns" in neurons [3]. The best-characterized function of JMCs is the coupling of electric excitation of the PM to myofilament contraction (E-C coupling) through SR Ca^{2+} release in muscles [4,5]. Recent studies demonstrate that JMCs are also transiently constructed in nonexcitable cells, and these unstable JMCs play an essential role in the store-operated Ca^{2+} entry mechanism [6].

1.2. Muscle-specific membrane complexes

In striated muscles, JMCs are highly specialized and have been extensively characterized. In adult mammalian ventricular cardiomyocytes, JMCs are located in two distinctive subcellular compartments: "peripheral couplings" occur directly underneath the PM, whereas "dyads" occur between the tubular invaginations of the PM (termed transverse-tubules: T-tubules) and the ER/SR in the cytosol. The terminal ER/SR cisternae in cardiac JMCs are flat (~20 nm in width) and filled with characteristic "beaded granules" comprised of calsequestrin (Fig. 1) [7]. Cardiac T-tubules are ~70–250 nm in diameter in rodents and ~400 nm in large animals and develop along the Z-line of the myofibril while constituting dyads with ER/SR [8–11]. Cardiac T-tubules are thought to play an important role in conducting PM depolarization inward to the cell center and synchronizing dyad activation cell-wide. JMCs in skeletal muscle are generally shaped as "triads", consisting of two ER/SR sacs flanking a flat T-tubule and located at the A-I junction of myofibril assembly (Fig. 1) [12]. T-tubules are mostly generated during postnatal development both in skeletal and cardiac muscles and this is coordinated with the cytoplasmic appearance of JMCs [13,14]. It is, however, not clear whether the tip of T-tubules randomly elongates and forms dyads, or if there is an active mechanism to attract T-tubules to preexisting ER/SR cisterns tethered to the sarcomere *via* obscure molecular mechanisms.

In both cardiac dyads and skeletal triads, the gap between T-tubules and ER/SR cisternae is consistently 12–15 nm in depth and filled with periodical projections termed "feet" that bridge the two membranes. After the initial description of the feet by electron microscopy, it took a couple of decades to realize that the ryanodine receptor (RyR), a ~565 kDa tetramer and a primary Ca^{2+} release channel in the ER/SR membrane, makes up the "foot structure" in

* Corresponding author. Tel.: +81 75 753 4572.
  E-mail addresses: takeshim@pharm.kyoto-u.ac.jp (H. Takeshima),
mhoshijima@ucsd.edu (M. Hoshijima), long-sheng-song@uiowa.edu (L.-S. Song).
  [1] Tel.: +1 858 822 3422.
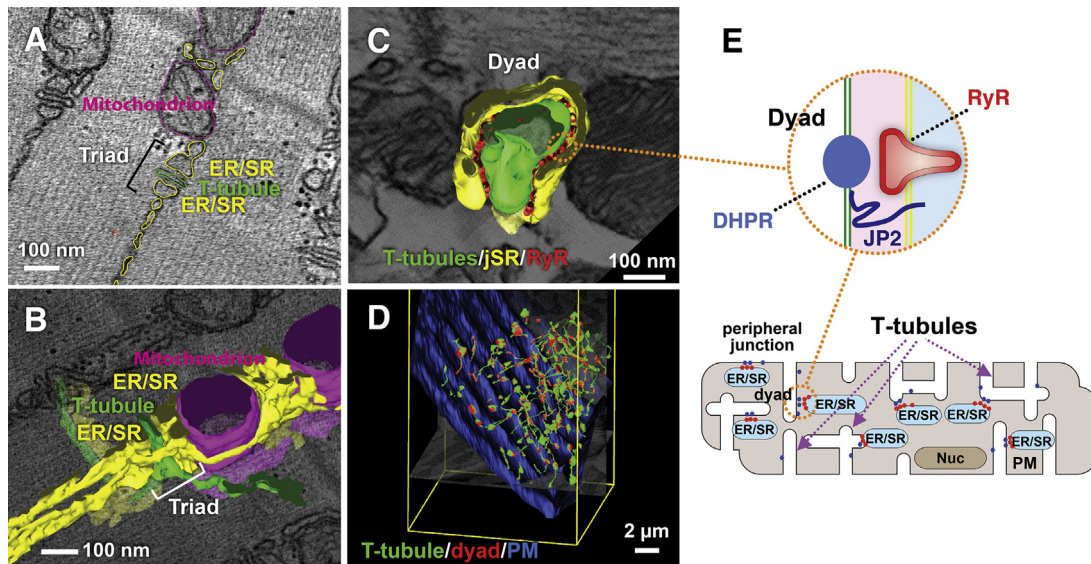 [2] Tel.: +1 319 384 2890.

Fig. 1. Junctional membrane complexes (JMCs) in striated muscles. Two types of three-dimensional (3D) electron microscopy (EM) technologies (EM tomography and serial block-face scanning EM) are used to demonstrate JMC nano 3D architectures in tibia anterior muscle (A, B) and in ventricular muscle (C, D) obtained from adult mice. Triads consisting of the terminal cisternae of the SR and T-tubules are located at the junction of A-band and I-band in fast skeletal muscles (A). The triad structure is depicted as a 3D geometric model reconstructed in EM tomography in B. In contrast, cardiac dyads formed by T-tubules and SR, are located near Z-lines (C, EM tomography) [8] and are widely distributed in the cytoplasm, associating with the T-tubule network (D, serial block-face scanning EM) [24]. E schematically shows the structure of JMCs and distribution of Ca^{2+} channel molecules in JMCs in cardiac muscle. DHPR, dihydropyridine receptor; RyR, ryanodine receptor; JP2, junctophilin 2; ER/SR, endo/sarcoplasmic reticulum; jSR, junctional SR; PM, plasma membrane; Nuc, nucleus.

muscular JMCs (reviewed in [15]). We now understand that dyads and triads anatomically represent "couplons", which are theoretically proposed cellular nano-units to explain the two conflicting natures of muscle regulation: the robustness and the dynamism of the E–C coupling gain control [16,17]. In cardiac muscle, dihydropyridine receptors (DHPRs), voltage-dependent L-type Ca^{2+} channels, and RyRs are functionally linked in a diffusion-limited narrow JMC space *via* a mechanism termed Ca^{2+}-induced Ca^{2+} release (CICR) [5,18]. In skeletal muscle, on the other hand, the direct interaction between DHPRs and RyRs converts the depolarization signal into SR Ca^{2+} release through a voltage-induced Ca^{2+} release (VICR) mechanism [4]

1.3. Molecules maintaining JMC structures in muscles

There was early speculation that RyRs constitute JMCs through their physical interaction with PM proteins, such as DHPRs, or T-tubule membrane. However, this working hypothesis was refuted by studies in skeletal muscles obtained from mutant mice lacking either skeletal DHPR ("dysgenic") or RyR1 ("dyspedic") [19]. The size and the frequency of JMCs are reduced in these mutant mice, but junctions remain structured. Structural changes in JMCs have also been reported in other mutant mouse strains in which RyR-interacting proteins, such as calsequestrin and triadin, are genetically ablated. In *Casq1*-null mice, skeletal muscle triads are significantly deformed and unique multilayered JMCs are observed in fast-twitch muscles [20]. In contrast, the frequency and extent of JMCs are largely preserved in *Casq2*-null cardiomyocytes, although SR cisternae are significantly enlarged in these cells [21]. In *Trdn*-null mice, the number and the size of dyadic junctions are reduced in cardiomyocytes [22], while triad alteration is subtler in skeletal muscles [23]. Specifically, triad orientation is modified and calsequestrin is occasionally mis-localized in triadin-deficient skeletal muscle cells [23].

In addition to SR/ER-associated proteins, several PM proteins have reported roles in JMCs. A part of peripheral JMCs are associated with the ~70–90 nm vesicular invaginations termed caveolae in

cardiac muscle [24]. Consistently, mouse deficiency in caveolin-3, which is an essential protein for caveolae formation in striated muscles, deforms T-tubules and mis-localizes JMC channel molecules in skeletal muscle [25] and degenerate cells in cardiac muscle [26]. Bridging integrator 1 (BIN1, also termed amphiphysin 2) is a conserved member of the BAR-domain family that has multiple splicing isoforms with diverse biological functions [27] and loss of Bin1 has been documented in failing hearts [28]. Heterologous expression of BIN1 forms narrow tubular extensions of the PM into the cytoplasm in Chinese hamster ovary cells [29]. A recent study genetically suppressed BIN1 expression in mouse cardiomyocytes and found structurally altered T-tubules [30]. The study also implicated that a particular BIN1 isoform, BIN1+13+17, contributes membrane foldings in T-tubules [30]. Finally, mouse genetic ablation of MG29, which is also termed synaptophysin-like 2 and is a synaptophysin-like protein expressed in skeletal muscle selectively [31], causes enlarged T-tubules, vacuolar ER/SR, and triad disarray in muscles [32].

Although these SR/ER and PM proteins have their clear roles in the regulation of JMCs, established evidence suggests that the association of the T-tubule and SR/ER membranes is primarily established by junctophilins, as reviewed in detail in the following sections.

2. Junctophilin subtypes

2.1. Structure of junctophilin (JP) and its JMC-forming function

Junctophilin type 1 (JP1), the inaugural member of the family proteins, was first identified in the junctional SR fraction of rabbit skeletal muscle [33]. After cloning rabbit JP1, database searches and homology cloning identified and clarified tissue-specific JP family members, namely JP1-4 [34]. The JP subtype genes are currently designated as *JPH1-4* in the human and *Jph1-4* in the mouse genomes, respectively. The primary structure of JP subtypes is largely hydrophilic and possesses a large cytoplasmic domain (Fig. 2A). JP subtypes share conserved sequences of 14 amino acid residues, termed MORN motifs, which repeat eight times in the N-terminal region composed of ~330 amino acid residues [33]. The
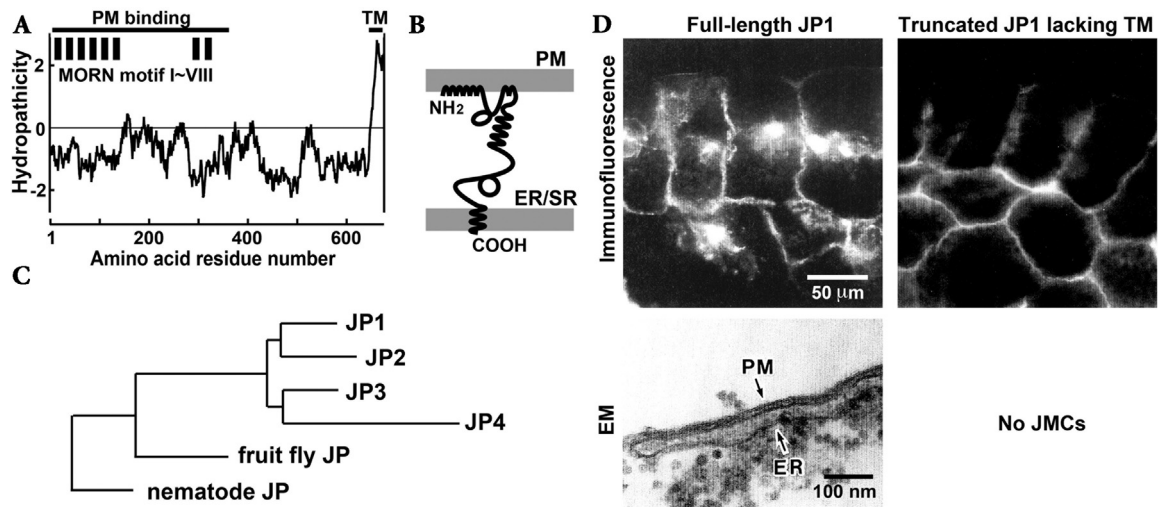
Fig. 2. Structure and function of junctophilin. (A) Hydropathicity profile of rabbit JP1 [48]. Repeated MORN motifs and an SR membrane-spanning segment are indicated. (B) Proposed JP structure in junctional membrane complex. (C) Phylogeny tree of junctophilin proteins. Invertebrates contain a single junctophilin gene, while there are four genes encoding tissue-specific junctophilin subtypes (JP1–JP4) in vertebrates. The simplified tree is adapted from a previous publication [86]. (D) Functional expression of rabbit JP1 in amphibian embryonic cells [48]. Exogenous JP1 proteins are localized to the cell periphery and form ER-PM complexes (upper left panel). Mutant JP1 lacking the C-terminal transmembrane segment remains tethered to the PM (upper right panel), but no junctional membrane complex is formed (lower panel).

N-terminal MORN motif region is followed by a putative α-helical region (~100 residues), a divergent region of 215–292 residues with no homology between the subtypes, and a C-terminal transmembrane segment that spans the SR/ER membrane (Fig. 2B). JP family proteins are found across animal species; vertebrates including human and mouse share four JP subtype genes and there is a single JP gene in invertebrates (Fig. 2C).

In a study using amphibian embryos, overexpressed JP1 was detected at the cell periphery by immunohistochemistry and efficiently formed JMCs reminiscent of peripheral couplings and subsurface cisternae (Fig. 2D). In contrast, a deletion mutant of JP1, which lacks the C-terminal transmembrane segment, localized along the cell membrane but did not generate JMCs. These data suggest that ectopically expressed JP1 binds to the cell membrane, spans the SR/ER membrane, and thereby forms JMCs. The N-terminal region of both JP1 and JP2, which includes the eight MORN motifs, tightly interacts with phosphatidylinositol phosphate species as evidenced by biochemical assays [33,35,36]. The MORN motif region is also necessary for the binding of plant phosphatidylinositol monophosphate kinase to phosphatidic acid and phosphatidylinositol phosphate species [37]. Therefore, the common function of repeated MORN motifs seems to be the recognition of phospholipids, and the attachment of JPs with the cell membrane may be due to a direct interaction of the MORN motifs with membrane lipids. Interestingly, in contrast with the embryonic cells, exogenous JP expression has failed to form JMCs in several commonly used mammalian cell lines (Takeshima et al., unpublished observations). This cell-type specificity may be due to the abundance of PM-associated cytoskeletal proteins in mature eukaryotics. The PM-associated cytoskeletal proteins are largely absent in cleavage cells in fertilized eggs and it remains unclear how JP preferentially generate JMCs at the PM in excitable cells. Recent studies suggest the direct interaction of JP subtypes with caveolins, DHPRs, and RyRs [38–41], contributes to the formation of JMCs and thereby to the regulation of Ca^{2+} handling in muscle cells. The biological role of these protein–protein interactions in the synthesis and maintenance of JMCs remains to be fully elucidated.

2.2. JP-mediated Ca^{2+} microdomains

Previous studies using knockout mice demonstrated that JP subtypes are necessary to establish RyR-mediated channel crosstalk in JMCs in excitable cells (Fig. 3). In adult mice, JP2 is expressed throughout muscle cell types, including skeletal, cardiac and smooth muscle cells, and is localized in dyads in the cardiac muscle and in triads in the skeletal muscle. *Jph2*-null mice die during the early embryonic development due to cardiac failure [33]. Cardiomyocytes from *Jph2*-null embryos have defective peripheral coupling, abnormal SR Ca^{2+} releases, and impaired CICR during E–C coupling [33]. Therefore, JP2 is essential for both peripheral coupling formation and functional crosstalk between DHPRs and RyRs in the embryonic heart. Consistent with the findings in the *Jph2*-null embryos, JP2 was shown to contribute to dyad formation and its stabilization in neonatal and adult cardiomyocytes (see Section 3.1). Indeed, one mechanism for the E–C coupling dysfunction in *Jph2*-null hearts is impaired maturation of the T-tubule membrane network [42,43].

Both JP1 and JP2 are abundantly expressed in adult skeletal muscle. While JP2 is first expressed *in utero*, JP1 expression is highly inducible during the postnatal development. *Jph1*-null mice display sucking failure and die shortly after birth [44]. The skeletal muscle of the *Jph1*-null neonates is characterized by poor triad formation and lowered VICR efficiency. Therefore, during skeletal muscle development, JP2 mediates peripheral coupling and dyad formation in embryonic and perinatal stages, and then JP1 likely plays an essential ole in the postnatal triad formation and the establishment of efficient VICR [45]. It is noteworthy that JP1 is not able to mediate the conversion of dyads to triads, as shown in a JP1 overexpression study in cardiomyocytes [46].

JP3 and JP4 are widely expressed in neurons in the brain [34]. Knockout mice lacking either JP3 or JP4 show no obvious abnormalities, suggesting functional redundancy between JP3 and JP4. However, double-knockout mice lacking both *Jph3* and *Jph4* (*Jph3/4*-DKO mice) have severe growth retardation and die within 3–4 weeks after birth under normal housing conditions [47]. *Jph3/4*-DKO mice are rescued when the diet is switched from normal pellets to hydrated paste. Therefore, the lethality of weaning *Jph3/4*-DKO mice is probably caused by a feeding defect associated with the functional impairment of the neuronal circuit controlling mastication or salivary secretion. *Jph3/4*-DKO mice also have impaired performance in learning and memory tasks and abnormal excitability and synaptic plasticity in hippocampal neurons. In hippocampal CA1 neurons, which receive excitatory inputs from CA3 neurons, afterhyperpolarization (AHP) is generated by Ca^{2+}-dependent
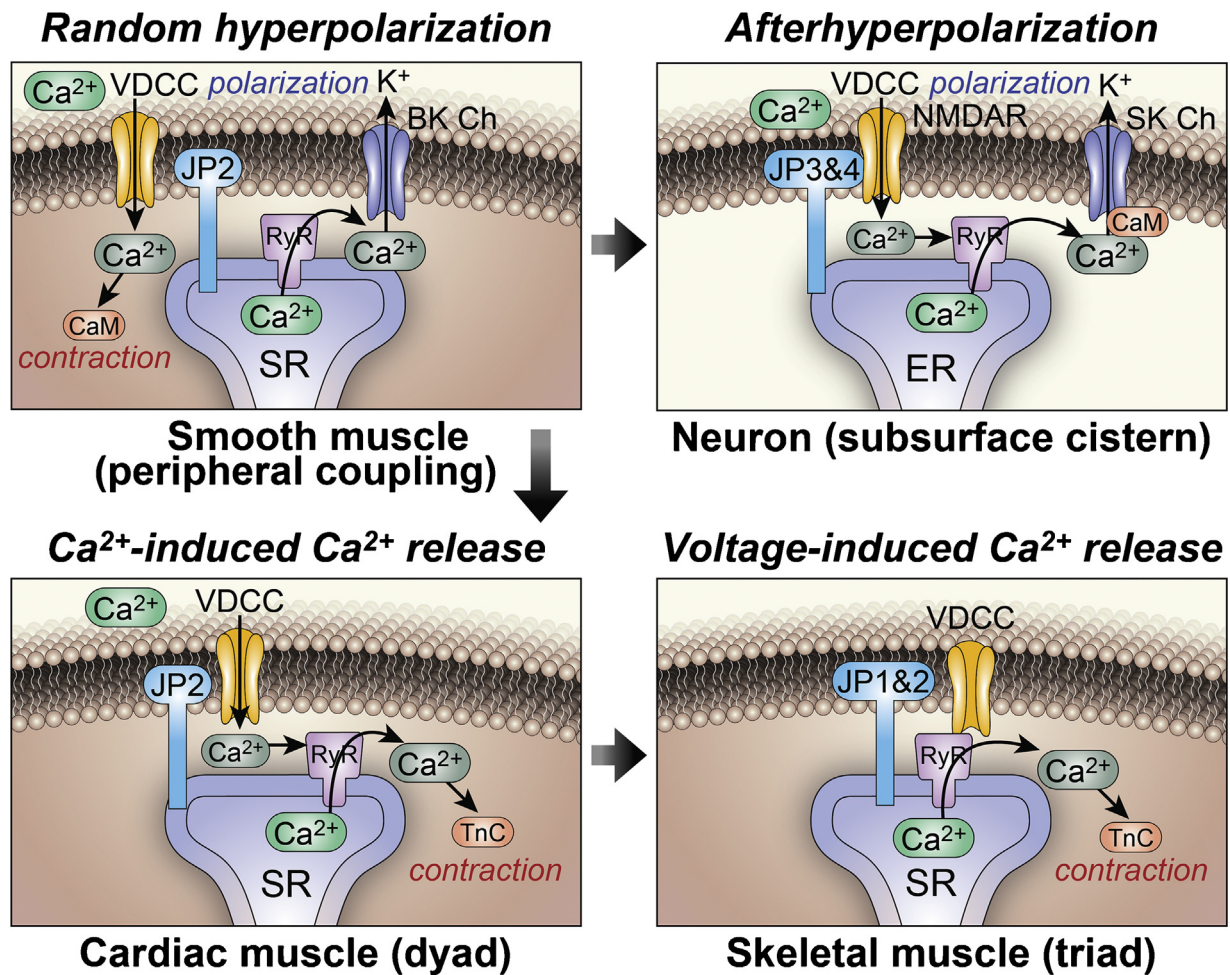
Fig. 3. Channel signalosomes in JMCs organized by JP subtypes. The hypothetical mechanisms are based on the data from knockout mice lacking ryanodine receptor or junctophilin subtypes. Channel communication in junctophilin-mediated membrane complexes may have gained functional diversity during the development of excitable cells. VDCC, voltage-dependent Ca^{2+} channel; RyR, ryanodine receptor channel; BK Ch, big-conductance Ca^{2+}-dependent K^+ channel; SK Ch, small-conductance Ca^{2+}-dependent K^+ channel; NMDAR, N-methyl-D-aspartate receptor channel; CaM, calmodulin; TnC, troponin C.

channel crosstalk between N-methyl-D-aspartate receptors, RyRs, and small-conductance Ca^{2+}-dependent K^+ channels (SK channels) (Fig. 3). This three-channel crosstalk is functionally disintegrated in *Jph3/4*-DKO CA1 neurons, thereby preventing the induction of long-term potentiation triggered by a high-frequency stimulation [47]. These observations, together with pathological phenotypes found in the cerebellar Purkinje neurons and striatum neurons of *Jph3/4*-DKO mice [48,49], indicate that both JP3 and JP4 coordinately form subsurface cisternae to establish Ca^{2+}-dependent crosstalk between PM and intracellular channels as a machinery for AHP in neurons. Similar machinery is found in hyperpolarization signaling of smooth muscle [50]. In smooth muscle cells, spontaneous opening of RyRs activates big-conductance Ca^{2+}-dependent K^+ (BK) channels to induce PM hyperpolarization. Interestingly, this reverse-mode signaling seems to require JP2-mediated peripheral coupling, while Ca^{2+} influx *via* DHPRs does not directly activate RyRs in smooth muscle cells [50]. Cellular and molecular mechanisms supporting the crosstalk between RyRs and BK channels in smooth muscle and their relationship with peripheral coupling warrant further investigations.

Depletion of the intracellular Ca^{2+} store in the ER/SR activates so-called store-operated Ca^{2+} entry (SOCE), a physiological mechanism involved in maintaining Ca^{2+} influx through the PM and refilling the ER/SR Ca^{2+} store in various cell types. SOCE is coordinated through a direct interaction between two distinct membrane proteins, the ER Ca^{2+} sensor STIM and the Ca^{2+} channel Orai expressed in PM [51,52]. In response to store depletion, STIM undergoes a dynamic redistribution into discrete ER domains juxtaposed closely with the PM. Orai then becomes tethered by clustered STIM proteins within the ER–PM junctions and mediates Ca^{2+} entry. Although SOCE is largely preserved under JP2 deficiency in embryonic cardiomyocytes [53], recent studies indicate that JPs facilitate SOCE by stabilizing the ER/SR–PM junctions in excitable cells. In skeletal muscle, JP1 and JP2 seem to coordinately enhance SOCE activity [54,55]. Furthermore, JP1, which is expressed weakly in neurons, seems to similarly enhance SOCE in this cell type [56].

3. Junctophilin-related diseases

3.1. JP2 and cardiac diseases

Of the JP subtypes, JP1-3 have been found to play important roles in human pathology. In addition to its role in normal development of cardiac E–C coupling machinery, JP2 is a critical safeguard of cardiac ultrastructure and function in response to cardiac stress. JP2 dysregulation has been associated with variety of heart diseases. In human failing heart samples such as those from patients with hypertrophic, dilated or ischemic cardiomyopathy, JP2 is severely downregulated as compared to trauma or "healthy" donor hearts [57,58] (and Song et al., unpublished

observations). Moreover, the expression of JP2 is markedly reduced in response to cardiac stress in multiple animal models of cardiac disease, *e.g.*, pressure overload or myocardial infarction-induced rodent hypertrophy/heart failure models and genetically modified murine models of hypertrophic or dilated cardiomyopathy [10,38,59–64]. Cardiac stress can also cause mis-trafficking of JP2 in failing myocytes, including loss of localization in the T-tubule/Z-line region and redistribution to the peripheral plasma membrane [65].

Emerging evidence suggests that both loss of JP2 expression and mis-localization contribute to structural and functional alterations of cardiomyocytes and the development of heart failure. First, induced reduction in JP2 expression in cultured cardiomyocytes using viral transfection of JP2 shRNA results in a significant decrease in the coupling or contact area between T-tubules and the junctional SR [66], which is consistent with the primary role of JP2 in the formation of JMCs. Second, JP2 knockdown results in a concomitant disorganization of the T-tubule network in cardiomyocytes [60,67]. The interpretation of these data is that JP2 is also essential in maintaining the stability of the organized T-tubule network by tethering the T-tubule membrane with the junctional SR [68]. Third, in line with its effects on E–C coupling architecture, *in vitro* JP2 silencing ablates myocyte CICR function, with decreased and dyssynchronous Ca^{2+} transients [58,66]. Fourth, acute JP2 knockdown in adult transgenic mice expressing JP2 shRNA leads to rapid development of heart failure and a high incidence of sudden cardiac death, which is associated with a disrupted T-tubule system, decreased frequency of JMCs, increased variance of the coupling distance within JMCs and compromised E–C coupling function [69]. Furthermore, recent studies provide compelling evidence that altered JP2 localization also contributes to T-tubule remodeling and Ca^{2+} handling defects in both *in vitro* cultured cardiomyocytes and pathological heart failure models [64,65,70]. These findings are in accordance with the fundamental function of JP2 in the T-tubule/junctional SR regions. On the other hand, cardiac-specific overexpression of JP2 doubles the contact length and frequency of T-tubules/junctional SR-coupled dyads and protects against T-tubule remodeling and heart failure development in response to pressure overload stress [71].

Mutations in the *JPH2* coding region have been implicated in JP2 dysregulation and associated with familial hypertrophic cardiomyopathies. Seven distinct mutations (S101R, Y141H, S165F, E169K, A405S, R436C and G505S) have been identified in patients with cardiac hypertrophy [41,72,73]. Patients with the *JPH2* mutation E169K also present with paroxysmal atrial fibrillation or supraventricular tachycardia [41]. These mutations most likely perturb intracellular Ca^{2+} handling of myocytes. In HL-1 cells, adenovirus-mediated expression of JP2 mutants (S101R, Y141H or S165F) causes a marked reduction in the frequency and amplitude of spontaneous Ca^{2+} release and cardiomyocyte hyperplasia [73]. In skeletal myotubes, expression of JP2 mutants (Y141H, S165F) similarly leads to a dramatic decrease in peak amplitude of Ca^{2+} transients and a significant increase in resting cytosolic Ca^{2+} concentration as well as an increase in myotube diameter [74,75]. What remains unclear, however, is how mutations of a structural protein, especially the mutations located in MORN or joining regions, result in disrupted Ca^{2+} signaling in affected cells. Studies from Wehrens group suggest that JP2 binds to and stabilizes RyR2 under normal conditions [69], and the E169K mutation causes a reduced binding of JP2 to RyR2 and thus decreased JP2-mediated stabilization of RyR2 and increased RyR2-mediated SR Ca^{2+} leak [41]. The JP2 S101R mutation results in a conformational change in the secondary structure, which alters its affinity to certain lipids, with an increased affinity for phosphatidylserine [36]. While it has been postulated that this increased affinity alters other JP2–protein interactions, how this type of mutation-associated JP2 modification contributes
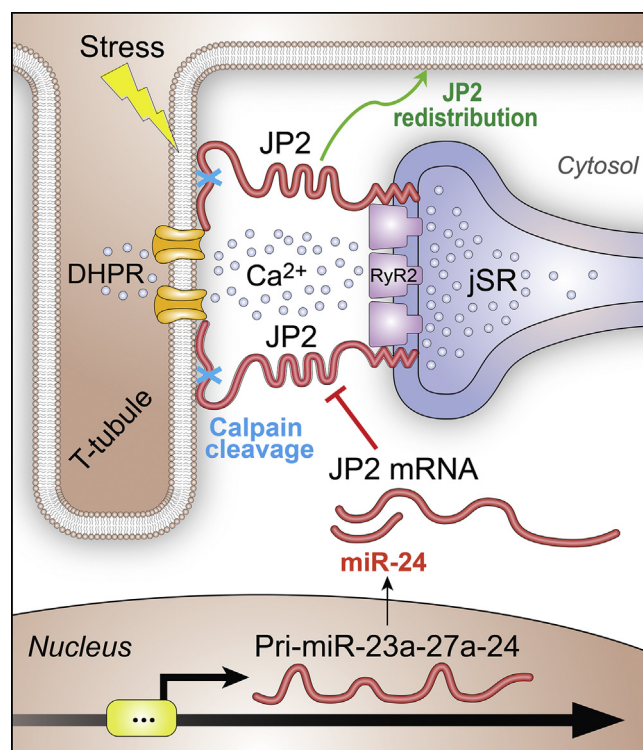

Fig. 4. Mechanisms underlying JP2 dysregulation in heart disease. Depicted are miRNA-mediated gene silencing by miR-24, calpain-mediated proteolysis, and redistribution to the cell periphery in response to microtubule polymerization.

to altered E–C coupling function in cardiomyocytes is completely unknown.

3.2. Mechanisms of JP dysregulation in cardiac and skeletal muscle diseases

Control of JP2 expression is complex, and several mechanisms have been reported in the literature to explain the loss of JP2 in failing hearts (Fig. 4). The first report demonstrated that JP2 is targeted by the microRNA mir-24, which may be responsible for downregulation of JP2 during pressure overload-induced hypertrophy [61]. miR-24 is one of the miRNAs that is upregulated in failing hearts [61,76], and its upregulation coincides with downregulation of JP2 [57,61]. Confirming the relationship between miR-24 and JP2, *in vivo* silencing of miR-24 with an antagomir prevents progression of pressure overload-induced heart failure through maintenance of JP2 expression and T-tubule/junctional SR integrity [77].

Downregulation of JP2 in failing hearts has also been attributed to cleavage by calpain [64], a protease that is activated by elevated intracellular Ca^{2+} in many different forms of cardiac stress and stress-response models [78,79]. In a transgenic mouse with inducible activation of Gαq, loss of T-tubule integrity is associated with a pronounced decrease in full-length JP2 and the appearance of a faster-migrating cleavage fragment, whereas cessation of Gαq activation restores full-length JP2 expression and T-tubule integrity [64]. The ability of a calpain inhibitor to prevent Gαq-mediated JP2 downregulation *in vivo* identifies calpain as the protease that is responsible for generation of the JP2 cleavage fragment. Further analysis with mass spectrometry revealed that cleavage occurs at amino-terminal site 201–202 (leucine/leucine). These data are in line with a previously reported Ca^{2+}-dependent mechanism for JP1 and JP2 proteolysis in skeletal muscle [80]. In this study by Lamb and associates, increasing [Ca^{2+}] promotes proteolysis of JP1, which corresponds with increased μ-calpain (calpain I) autolytic activity.

JP1 proteolysis is accelerated in the *mdx* model of muscular dystrophy. Extending studies to JP2, *in vitro* exposure of ventricular cardiomyocytes to increasing [Ca^{2+}] or *in vivo* ischemia-reperfusion injury results in a decrease in full-length JP2 levels, though no cleavage fragment was detected [80].

Another mechanism underlying dysregulation of JP2 is loss of normal subcellular localization due to microtubule polymerization [65]. Alterations in microtubules, namely microtubule densification (an increase in microtubule density due to enhanced microtubule polymerization), are commonly observed in human failing hearts and animal models of heart failure [81,82]. The recent study by Zhang et al. [65] reveals that the subcellular distribution of JP2 is mechanistically linked to microtubule dynamics. In response to cardiac stress, the densification of the microtubule network results in redistribution of JP2 within the membrane system, thereby inducing T-tubule remodeling and E–C coupling dysfunction and contributing to the development of heart failure. Furthermore, this study indicates that the microtubule motor protein kinesin 1, which is critical for protein anterograde trafficking, is responsible for microtubule densification-mediated JP2 mis-trafficking [65].

3.3. JPs and neurological diseases

Huntington's disease-like 2 (HDL2), like the authentic Huntington's disease, is a progressive neurodegenerative disorder clinically characterized by abnormal movements, dementia and psychiatric syndromes. Studies have linked triplet-repeat expansion mutations in the *JPH3* locus to HDL2, which results in the expansion of polyglutamine encoded by the *JPH3* antisense strand and disruption of the *JPH3* sense strand [83,84]. Both JP3 and JP4 likely exert compensatory roles in neural sites of the brain, although their distributions and expression densities are slightly different [34]. As described in Section 2.2, *Jph3*-null mice show no obvious phenotype in young adult stages. However *Jph3*-null mice exhibit behavioral and pathological defects in old age. These mouse observations support the idea that JP3 deficiency and certain aging effects, such as accumulative tissue oxidation caused by the production of reactive oxygen species, synergistically leads to the late-onset neurodegeneration observed in HDL2 patients [85]. The neural phenotype of *Jph3/4*-DKO mice is discussed in Section 2.2.

Charcot-Marie-Tooth disease (CMT) is an umbrella term for a range of certain inherited genetic disorders that affect the peripheral nervous system. A recent study found that JP1 is likely involved in the pathogenesis of CMT [56]. This study found that the *JPH1* gene is genetically associated with *ganglioside-induced differentiation-associated protein 1 (GDAP1)*, mutations of which are found in a subset of CMT patients. JP1 serves as a potential modifier of the clinical variability of *GDAP1* mutation-associated CMT [56].

4. Perspective

Recent studies have defined basic functions of JMCs organized by JP subtypes in excitable cells, and also unveiled associations and causal roles for JPs in cardiac and neurological diseases. However, there remain many questions to be addressed in future studies: how is the structure of T-tubules maintained by JPs in muscles? How are JPs concentrated in the junctional cisternae of ER/SR? Are there any JP-binding partners other than phospholipids to assist in JMC formation? Which protein factors determine dyad and triad formation in striated muscle? What are pathophysiological mechanisms of hypertrophic cardiomyopathy caused by *JPH2* mutations? Are there any genetic and environmental factors that cooperate with *JPH3* mutations to induce HDL2? These and other questions inevitably provide a future challenge in understanding the physiological and pathological roles of JPs in health and disease.

Conflict of interest

Acknowledgements

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