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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.

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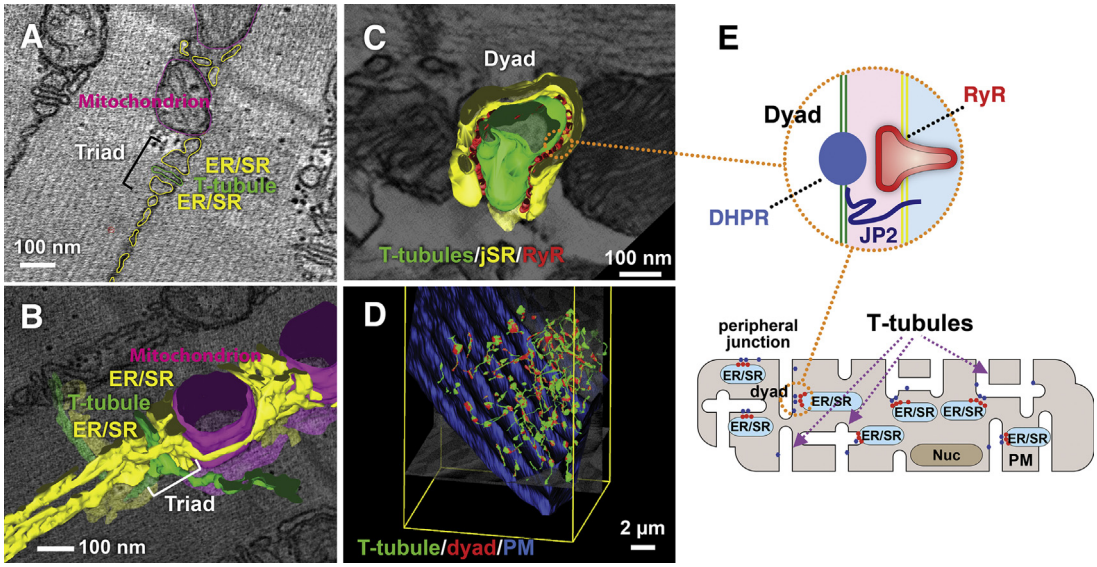

Fig. 1. Junctional membrane complexes (JMCs) in striated muscles. Two types of three-dimensional (3D) electron microscopy (EM) technologies (EM tomography and serial block-face scanning EM) are used to demonstrate JMC nano 3D architectures in tibia anterior muscle (A, B) and in ventricular muscle (C, 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

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