Minireview

Three-dimensional architecture of the skeletal muscle ryanodine receptor

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Abstract Recent advances in determining the three-dimensional architecture of the skeletal muscle ryanodine receptor/calcium release channel (RyR) by cryo-electron microscopy and three-dimensional reconstruction are discussed. The tetrameric receptor is characterized by a large 4-fold symmetric cytoplasmic assembly that consists of many domains separated by solvent-containing crevices and holes. Experimental evidence suggests that at least one regulatory ligand, calmodulin, binds to sites on the cytoplasmic assembly that are at least 10 nanometers from the transmembrane channel.

Key words: Ryanodine receptor; Calcium release; Cryo-electron microscopy; Three-dimensional reconstruction; Excitation–contraction; Muscle; Image processing

1. Introduction

Ryanodine receptors (RyRs) are intracellular calcium release channels found in a variety of vertebrate tissues (for reviews see [1–4]). In mammals, three isoforms of the gene have been identified, one of which (RyR1), is expressed primarily in skeletal muscle [5,6]. The skeletal RyRs are located principally at triad junctions [7–9], specialized regions where the sarcoplasmic reticulum and transverse tubules are closely apposed [10]. The transverse tubules are invaginations of the sarcolemma and contain dihydropyridine receptors which function as voltage sensors in excitation-contraction coupling [11,12]. RyRs release Ca^{2+} from the lumen of the sarcoplasmic reticulum to the myoplasm in response to nerve impulses that are detected by the dihydropyridine receptors. The mechanism by which the voltage sensors and RyRs communicate with one another is not understood.

RyRs are unusually large ion channels, both in terms of their unitary conductances and physical dimensions. They comprise four large polypeptide subunits, each of molecular mass 565,000 Da in skeletal muscle. Intriguingly, the purified skeletal RyR also contains four tightly associated copies of a much smaller protein, FK-506 binding protein, an immunophilin, whose role in receptor function remains to be clarified [13–16] The focus of this review is the three-dimensional architecture of the RyR from skeletal muscle.

Being both structurally complex and integral membrane proteins, RyRs are poor candidates for high resolution structural characterization by X-ray crystallography or NMR. A powerful approach for attacking such structures is the combination cryo-electron microscopy of frozen-hydrated macromolecular solutions and computer analysis to generate three-dimensional reconstructions [17,18]. Although molecular levels of resolution are a reasonable goal only when highly-ordered arrays are available (e.g. two-dimensional crystals), accurate determinations of the structure can be determined at lower resolution (typically 20–40 Å) even from micrographs containing images of isolated complexes that have little or no symmetry [19–25]. A goal of this review is to demonstrate the utility of three-dimensional reconstructions of RyRs determined by these methods for understanding the mechanisms of intracellular calcium release and excitation–contraction coupling in muscle.

Two groups, including our own, have described three-dimensional reconstructions of the rabbit skeletal muscle RyR that were determined from electron micrographs of frozen-hydrated, detergent-solubilized receptors [26,27]. The two reconstructions appear to be in good agreement, confirming both the validity of the reconstructions and the two differing methods of data collection and analysis that were used. The few minor differences between the two reconstruction could be due to slightly different resolutions or, since different buffer conditions were used, to real structural differences. Fig. 1A shows a surface representation of our reconstruction with the approximate position of the bilayer indicated schematically. The receptor in this orientation resembles a mushroom, with the stem and cap corresponding to the transmembrane and cytoplasmic assemblies respectively. Fig. 2A,B shows orientations of the cytoplasmic and transmembrane assemblies from which the 4-fold symmetry of the receptor is readily apparent.

2. The transmembrane assembly

Our model shows a channel-like feature emerging at the distal end of the transmembrane assembly (Figs. 1b and 2b) that probably corresponds to the mouth of the ion-conducting channel (the reconstruction of Serycheva et al. [27] differs from ours in this region in that a channel does not appear to be resolved). Near its cytoplasmic end the channel appears to be blocked by a mass of density we refer to as the 'plug' (Figs. 1b and 2a,d). We have estimated the mass of the transmembrane assembly (isolated from the RyR as shown in Fig. 2d) to be 360 kDa (90 kDa per subunit) by computing its volume relative to the whole receptor and scaling to a total mass of 2.3×10^6 for the RyR. Thus, the estimated mass should not be substantially affected by errors in magnification or the threshold density chosen to represent the surface of the receptor.

Based on hydropathy profiles of the RyR sequence, two laboratories have proposed topological models of the trans-

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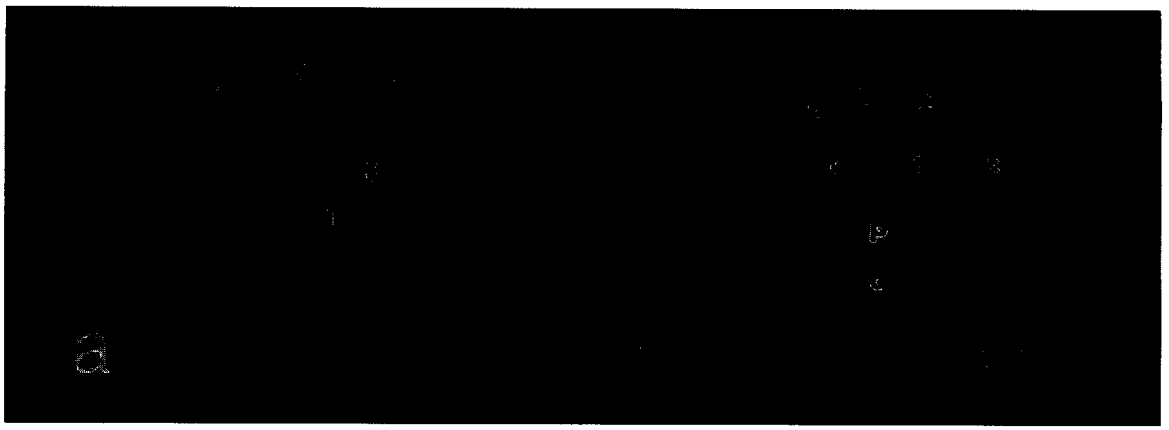

Fig. 1. Solid-body representations of the reconstructed RyR viewed from a direction parallel to the plane of the bilayer. (a) Complete RyR. (b) RyR sliced in half to reveal internal structure. A putative location for the bilayer is indicated by the horizontal lines (4 nm spacing between lines). Abbreviations: TA, transmembrane assembly; CA, cytoplasmic assembly; p, plug; c, solvent filled channel; the globular structures comprising the cytoplasmic assembly (presumably corresponding to structural domains) have been assigned numerals (see [26] for details).

membrane segments of the channel [5,6]. Both models contain α-helical transmembrane segments derived from carboxyl terminal segments of the sequence, but one model has 10 such segments distributed throughout the terminal 1/5 of the sequence, whereas the other postulates only 4 transmembrane segments confined to the terminal 1/10 of the sequence. Both models show few amino acid residues (<175) on the lumenal side of the membrane, and accordingly, we show in Fig. 1 a plausible location for the boundaries of the bilayer on the transmembrane assembly.

Can the three-dimensional reconstruction be used to distinguish between the 4- and 10-transmembrane segment models? The volume occupied by the transmembrane and lumenal parts of the model corresponds to a molecular mass of about 56 kDa per subunit; for this purpose we do not consider protein mass present on the cytoplasmic side of the membrane because, in addition to the cytoplasmic loops between transmembrane segments, it could contain contributions of unknown magnitude from parts of the sequence 'upstream' from the transmembrane segments. The value of 56 kDa is in much better agreement with the 10-transmembrane segment model, which predicts a mass of 43 kDa per subunit for transmembrane and luminal segments. The 4-helix model predicts a value of only 23 kDa. Serycheva et al. [27] also favor the 10-transmembrane segment model based on the apparent mass of the transmembrane assembly in their reconstruction.

The distinctly tapered appearance of the transmembrane assembly (Fig. 1) suggests to us that both of the transmembrane topology models might be overly simplified. A bundle of 10 close-packed transmembrane helices would have a cross-sectional area of about 1,000 Å^2. The transmembrane assembly in our reconstruction has cross-sectional areas of 900, 1,700, and 2,400 Ä^2 at the bottom, middle and top of the bilayer as it is positioned in Fig. 1. One possible interpretation of the increasing cross-sectional area across the bilayer is that the transmembrane helices splay apart from the 4-fold symmetry axis on the cytoplasmic side, and that the volume thereby created between the helices is filled in by additional peptide, not necessarily α-helical, from the cytoplasmic loops that connect the transmembrane helices or from other parts of the sequence.

3. The cytoplasmic assembly

Probably the most striking property of the cytoplasmic assembly is the large amount of solvent-occupied volume contained within the various channels, holes and grooves that are present. The protein appears as discrete, interconnected lumps which we interpret as a loosely packed assemblage of domains. The functional significance of this organization is not clear; we have suggested that it might be an efficient design for a scaffolding to withstand the stresses associated with muscle contraction while still permitting ions to diffuse towards and away from the mouth of the transmembrane assembly.

The RyR is thought to be regulated and modulated by numerous ligands [1–4]: e.g. transverse tubule components, Ca^{2+}, Mg^{2+}, calmodulin, FK-506 binding protein, adenine nucleotides, phosphorylation. Some of the binding sites for these ligands are located on the cytoplasmic assembly. For example, Wagenknecht et al. [28] found that calmodulin, which regulates the RyR by direct interaction in vitro [29,30], binds to a site on each subunit (Fig. 2) that is farther than 10 nm from the transmembrane channel. If this site is physiologically relevant, then it seems that very long-range allosteric coupling occurs between sites on the cytoplasmic assembly and the ion-conducting channel. The peripheral regions of the cytoplasmic assembly may also interact with the dihydropyridine receptors. The face of the RyR that would interact with the transverse-tubule in the myofiber contains 4 large (diameter ≈4.5 nm), symmetrically situated cavities which extend all the way through the assembly (asterisks in Fig. 2a). These peripheral cavities are surrounded by three domains (labeled '4', '5', '6') that form the most distally extending parts of the receptor relative to the sarcoplasmic membrane (Fig. 1a), and, hence, one or more of these domains are likely involved in forming contacts with t-tubule components. An intriguing correlation can be made of the peripheral cavities in the reconstructed RyR with the electron microscopy results obtained using the freeze-fracture technique by Block et al. [9] on the organization of triad junctions. They observed 4-fold symmetric structures termed 'tetrads' projecting from the cytoplasmic surface of the transverse tubule that appeared to be precisely positioned in arrays so as to interact with the RyRs

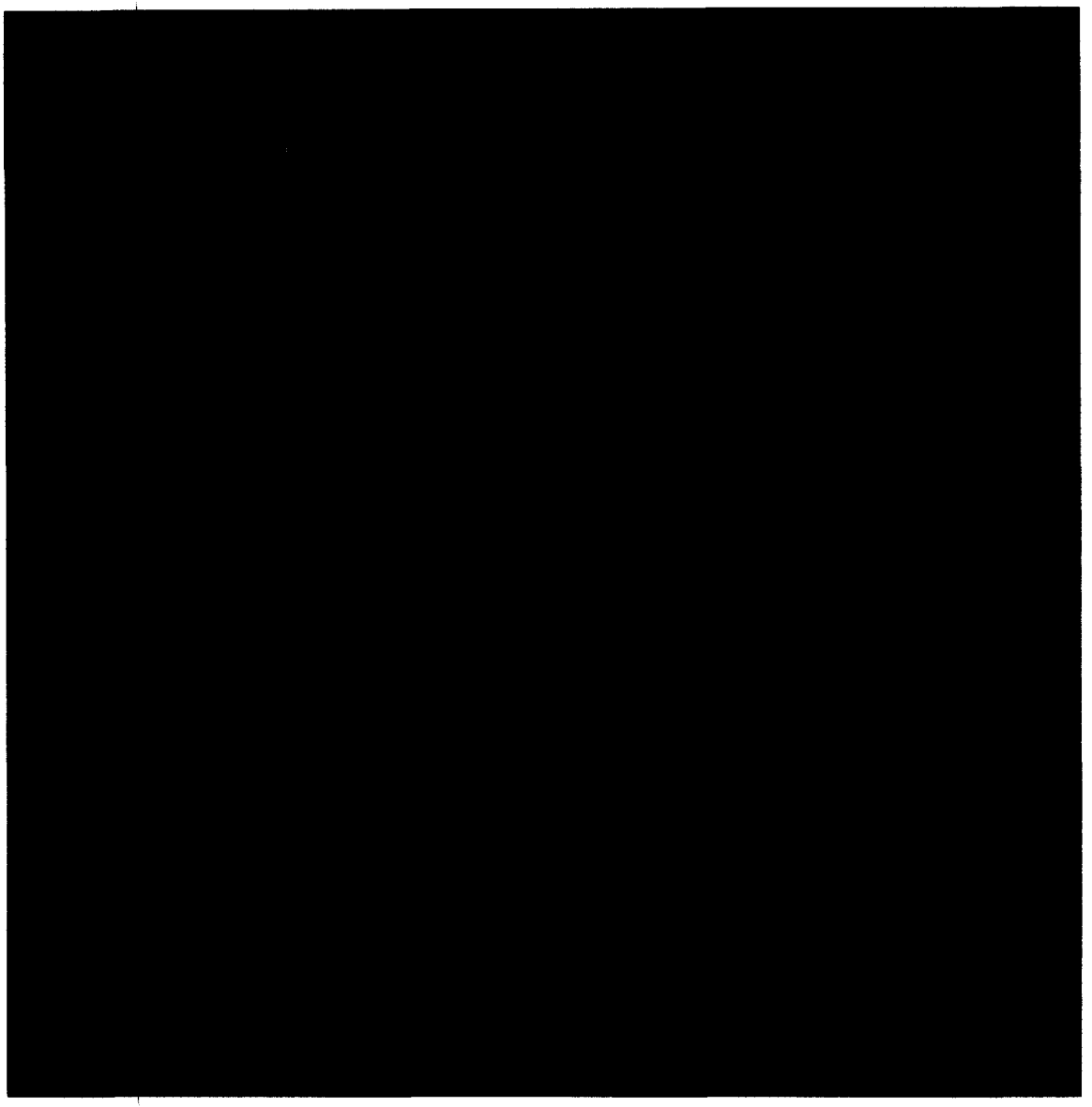

Fig. 2. (a,b) Solid body representations of the RyR viewed from directions that would face the sarcolemma/transverse tubule and sarcoplasmic reticulum membrane systems, respectively. (c,d) The cytoplasmic and transmembrane assemblies as separate structures. In (c) the view is tilted by 30 degrees about a vertical axis from that in (b). (d) shows principally the surface of the transmembrane assembly that interacts with the cytoplasmic assembly. Abbreviations: m, calmodulin binding site; *, peripheral cavities; others as in Fig. 1.

in the adjacent junctional sarcoplasmic reticulum. The center-to-center spacing between adjacent subunits in the tetrads is 13–14 nm, and the subunits are aligned so as to superimpose over the corners of the apposing RyRs. If we orient our three-dimensional reconstruction of the RyR into the triad as prescribed by the model of Block et al., the cytoplasmic portions of each tetrad subunit would align with, and likely project into, the peripheral cavities of the apposing RyR. More recently, strong evidence that the tetrads correspond to dihydropyridine receptors, the voltage sensors of excitation–contraction coupling, has appeared [31]. If dihydropyridine receptors interact with the RyRs in the vicinity of their peripheral cavities as we suggest, then it follows that long-range interactions are involved in the molecular events by which channel gating of the RyR is regulated by voltage changes across the sarcolemma/transverse tubule system.

4. Communication between transmembrane and cytoplasmic assemblies of the RyR

Modulation of calcium release by allosteric effectors acting at remote sites on the cytoplasmic assembly implies that the connections between the transmembrane and cytoplasmic assemblies must be involved in mediating communication between the two. In our reconstructions, four symmetrically related masses of density (labeled '1' in Figs. 1 and 2a,c) join the two assemblies. Each of these 'domains' appears to contact the transmembrane assembly in at least three locations – two inter-

actions with adjacent '2' domains and one interaction with the '3' domain. The '1' domains appear to terminate at or near the 'plug' which appears to be obstructing the transmembrane channel (visible at the distal end of the transmembrane assembly in Figs. 1b and 2a,d).

It is tempting to speculate that ligand-induced conformational changes in the cytoplasmic assembly induce a movement of the '1' domains, which in turn affect the positioning of the plug so as to modulate the conductance of the channel. Such hypothetical schemes should be testable by further structural studies.

For the near future, we envision modest improvements in the resolution of three-dimensional reconstructions of the RyR, but probably not sufficient to resolve secondary structural elements such as the putative transmembrane helices suggested by the hydropathy analyses. A preliminary study has indicated that this level of detail may be sufficient to reveal the nature of conformational changes accompanying changes in the conductance states of the receptor [32]. Perhaps the most fruitful line of investigation will involve the three-dimensional mapping of site-specific macromolecular ligands, because the accuracy with which such ligands can be localized exceeds substantially the resolution of the reconstruction. At the present resolution, ~3 nm, it is already feasible to determine the locations of modulators such as calmodulin with precisions of ~1 nanometer [33].

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