

MINIREVIEW

Calcium transport across the sarcoplasmic reticulum Structure and function of Ca^{2+} -ATPase and the ryanodine receptor

David L. Stokes¹ and Terence Wagenknecht²

¹Skirball Institute for Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, USA;

²Division of Molecular Medicine, Wadsworth Center and Department of Biomedical Sciences, State University of New York at Albany, USA

Contraction of striated muscle results from a rise in cytoplasmic calcium concentration in a process termed excitation/contraction coupling. Most of this calcium moves back and forth across the sarcoplasmic-reticulum membrane in cycles of contraction and relaxation. The channel responsible for release from the sarcoplasmic reticulum is the ryanodine receptor, whereas Ca^{2+} -ATPase effects reuptake in an ATP-dependent manner. The structures of these two molecules have been studied by cryoelectron microscopy, with helical crystals in the case of Ca^{2+} -ATPase and as isolated tetramers in the case of ryanodine receptor. Structures of Ca^{2+} -ATPase at 8-Å resolution reveal the packing of transmembrane helices and have allowed fitting of a putative ATP-binding domain among the cytoplasmic densities. Comparison of ATPases in different conformations gives hints about the conformational changes that accompany the reaction cycle. Structures of ryanodine receptor at 30-Å resolution reveal a multitude of isolated domains in the cytoplasmic portion, as well as a distinct transmembrane assembly. Binding sites for various protein ligands have been determined and conformational changes induced by ATP, calcium and ryanodine have been characterized. Both molecules appear to use large conformational changes to couple interactions in their cytoplasmic domains with calcium transport through their membrane domains, and future studies at higher resolution will focus on the mechanisms for this coupling.

Keywords: Ca^{2+} -ATPase; calcium transport; muscle; ryanodine receptor; sarcoplasmic reticulum.

Contraction of striated muscle provides one of the best studied examples of calcium signaling. The sarcoplasmic reticulum (SR) is the storage site for the bulk of the calcium and its transport across the SR membrane is managed by two molecules: the ryanodine receptor (RyR) and Ca^{2+} -ATPase. Initially, the signal for calcium release from the SR begins with a depolarization of the cell membrane, which travels into a specialized invagination in this membrane, called the T-tubule. There, voltage-sensitive calcium channels (known alternatively as L-type channels or the dihydropyridine receptor) open and allow small amounts of calcium into the cell. This does not raise intracellular calcium concentrations sufficiently for contraction; rather, the primary role of these L-type calcium channels is to open the RyR, either singly or as small groups. Such discrete openings give rise to localized regions of calcium release, which have been observed and quantified by confocal imaging and dubbed calcium sparks. In cardiac muscle, L-type channels facilitate transport of small amounts of extracellular calcium, which induce nearby RyRs to open by a mechanism termed calcium-induced calcium release. However, in skeletal muscle there is evidence for a physical interaction between the L-type channels and RyRs across the small gap between the plasma membrane and the adjacent SR membrane. Indeed,

these macromolecular units of calcium release, consisting of L-type channels of the T-tubule and RyRs of the SR, are spatially organized and visible by electron microscopy either *in situ* or in isolated vesicle preparations as so-called triad junctions. The sum of a great many calcium sparks, the corresponding chemical unit of calcium release, is the macroscopic rise in calcium concentration that activates muscle contraction.

Unlike release, calcium reuptake into the SR is constitutively active and requires coupling of the energy of ATP hydrolysis to calcium transport. This is accomplished by Ca^{2+} -ATPase, which generates a thousandfold concentration gradient of calcium across the SR in resting muscle. At the molecular level, this pumping is $\approx 10^5$ times slower than release, reflecting the mechanical constraints of energy coupling. In particular, two calcium ions are transported into the SR and two protons are expelled for each ATP hydrolyzed. Other poorly characterized transport molecules in the SR, such as anion exchangers, prevent the build up of electrical potential across the SR membrane. The cell makes up for the relatively slow reaction cycle by providing a vast number of calcium pumps, which literally cover the large surface of SR between triad junctions. It has been estimated that the number of Ca^{2+} -ATPase molecules are roughly equal to the number of calcium ions released for a single muscle twitch and one can therefore imagine each Ca^{2+} -ATPase undergoing, on average, a single cycle of transport to effect muscle relaxation.

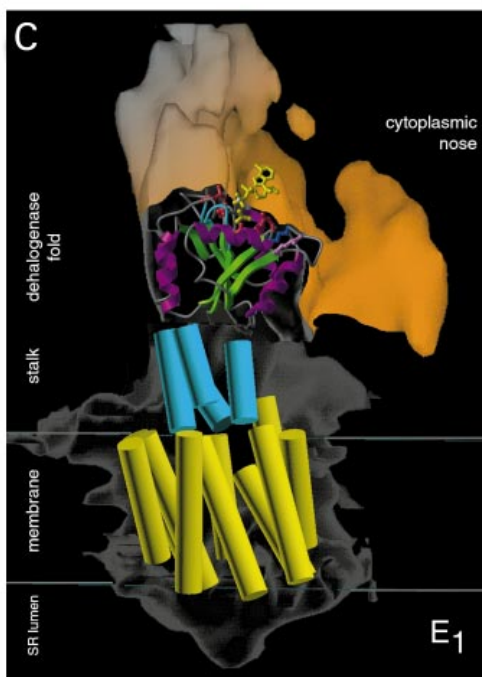
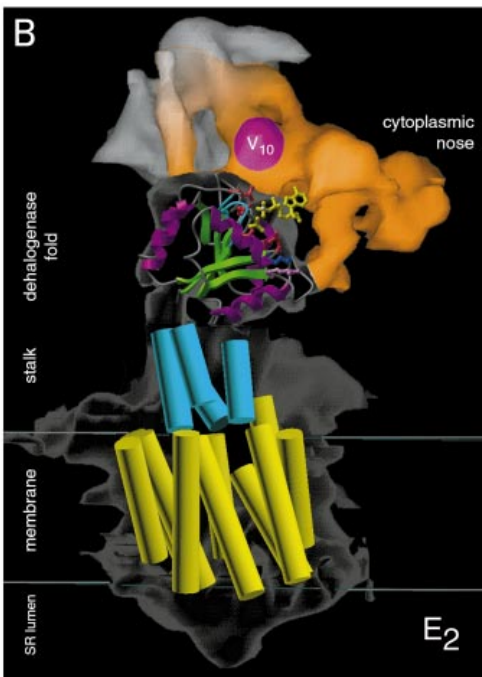
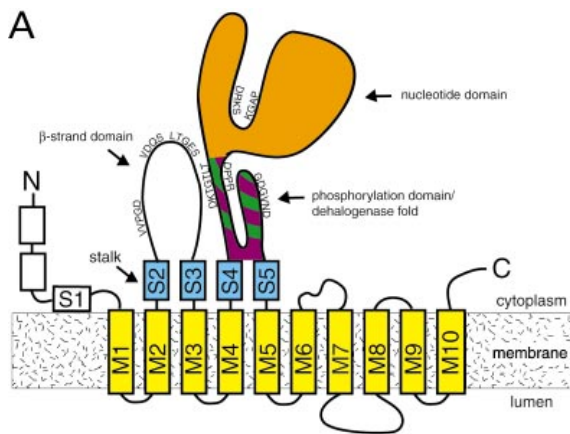
Correspondence to D. Stokes, Skirball Institute for Biomolecular Medicine, New York University School of Medicine, 540 First Ave, New York, NY 10012, USA. Fax: + 1 212 263 1580, Tel.: + 212 263 1580, E-mail: stokes@saturn.med.nyu.edu

Abbreviations: SR, sarcoplasmic reticulum; ER, endoplasmic reticulum; RyR, ryanodine receptor; InsP_3R , inositol 1,4,5-trisphosphate receptor; FKBP, FK 506-binding protein.

Note: a web page is available at <http://saturn.med.nyu.edu/~stokes>
(Received 5 January 2000; accepted 28 March 2000)

FUNCTION OF Ca^{2+} -ATPASE

Ca^{2+} -ATPase is a member of the family of P-type ATPases, most of which couple ATP hydrolysis to cation transport [1]. This family was originally distinguished by its enzymology; that is, by the transient phosphorylation of an aspartate during



the reaction cycle and by the inhibitory characteristics of vanadate. As gene sequences emerged, a high degree of homology was found near the phosphorylated aspartate and at several other key places in the cytoplasmic portion of the molecule [2]. In addition, a consistent pattern of hydrophobicity suggested a conserved transmembrane topology (Fig. 1A) although this pattern was variably interpreted to indicate the presence of 8 or 10 transmembrane helices. Ca^{2+} -ATPases were divided into two subfamilies, those found in plasma membranes (PMCA) and those found in either sarcoplasmic or endoplasmic reticulum membranes (SERCA). Spectroscopic analysis as well as chemical cross-linking have been extensively used to identify regions of the protein involved in the binding and hydrolysis of ATP [3]. More recently, site-directed mutagenesis of the best studied family members (SERCAs, PMCAs, Na^+/K^+ -ATPase, gastric H^+/K^+ -ATPase, yeast H^+ -ATPase, *Escherichia coli* Kdp) has examined the roles of individual residues in various steps of the reaction cycle. This mutagenesis has been most successful for SERCA1 (isoform found in skeletal muscle), in part because it is subjected to relatively little intracellular processing before insertion into the SR or ER membrane. Thus, residues have been classified according to whether they primarily affect calcium transport, phosphorylation, or the coupling of these two functions, which according to spectroscopic studies are separated by $\approx 40 \text{ \AA}$ [4]. In particular, the calcium-binding sites are formed by acidic and hydrophilic residues in the middle of transmembrane helices 4, 5, 6 and, possibly, 8, whereas ATP binding and phosphorylation occur well above the cytoplasmic surface of the membrane.

In addition to the molecular architecture of these binding sites, the nature of the conformational changes thought to couple these two sites is a central issue in understanding the function of these pumps [3,5]. In particular, the binding of calcium to transmembrane sites is known to activate the nucleotide site for phosphorylation. In a subsequent step, the energy of the phosphoenzyme is used to transport the calcium ions, a process that involves dramatically lowering the affinity at the calcium sites and reorienting the accessibility of these sites from the cytoplasmic to the luminal side of the membrane.

FUNCTION OF RyR

Two families of calcium-release channels have been extensively characterized, the RyRs and the inositol 1,4,5-trisphosphate

Fig. 1. Architecture and conformational change of Ca^{2+} -ATPase. (A) Topology of the amino-acid sequence showing 10 transmembrane helices and four distinct cytoplasmic domains: stalk, β -strand, nucleotide, and phosphorylation. Highly conserved sequences in the cytoplasmic domains are shown by their single-letter code. The phosphorylation domain has been proposed to form a Rossman fold [25] that is closely juxtaposed with the stalk. (B) Interpretation of the 8- \AA structure of Ca^{2+} -ATPase from tubular crystals [21], which is thought to represent the E_2 conformation. Transmembrane and stalk helices are shown as yellow and cyan cylinders, respectively. The dehalogenase fold has been fitted into a domain above the stalk [26], which places ATP (yellow stick model) at a site consistent with the experimentally determined location of CrATP. The putative site of the crystallization agent, decavanadate (V_{10}), is colored purple. (C) Hypothetical structure for the E_1 conformation. This structure is a hybrid of the cytoplasmic domains of H^+ -ATPase, believed to be in the E_1 conformation [23], and the transmembrane/stalk portions of Ca^{2+} -ATPase as shown in (B). The main effects of the conformational change are a separation of the cytoplasmic nose from the main cytoplasmic domains and a 20° tilt of the dehalogenase domain [24].

receptors (InsP₃Rs). Although RyRs are the major calcium-release channels in striated muscle, InsP₃Rs are also present in smaller amounts and both types of channel are present in many other types of mammalian cell (reviewed in [6–8]). RyRs and InsP₃Rs are distantly related, and share some structural properties. In particular, both are tetramers constructed from identical subunits (or homologous isoforms for some InsP₃Rs) of unusually large size, ≈ 290 kDa for InsP₃Rs and ≈ 550 kDa for RyRs; in fact, they are the largest ion-channel proteins known.

It is not clear why RyRs have such unusually high molecular masses (2.3 MDa for the holoreceptor). A clue is perhaps provided by the regulatory activity of numerous endogenous modulatory ligands, both small molecules and macromolecules. Examples of known and putative small-molecule modulators of RyRs include ATP, calcium, magnesium, cyclic ADP ribose, and nitric oxide (reviewed in [9]). Calcium is of particular note because of its likely role in activating calcium release from the SR during excitation/contraction coupling in cardiac muscle, and in propagating release after initiation by sarcolemmal/T-tubule depolarization in cardiac and skeletal muscle. Interestingly, millimolar and higher calcium concentrations deactivate skeletal channel activity, indicating at least two modulatory sites for calcium per receptor subunit. The inhibitory site probably plays a role in terminating calcium release.

Among the macromolecular modulators of skeletal and cardiac RyRs are calmodulin (one to four sites per RyR subunit), a 12-kDa FK506-binding protein (FKBP, one site per subunit), and the above-mentioned L-type voltage-sensitive calcium channel in the T-tubule [10]. Many of the RyR protein ligands are probably resident components of the excitation/contraction coupling apparatus. For example, the role of FKBP may be to mediate interactions between RyRs, enabling them to form small arrays at triad/dyad junctions [11]. Such multi-protein complexes represent the functional unit of excitation/contraction coupling, and the ultimate goal for structural biologists will be to determine the composition and 3D architecture in sufficient detail to elucidate the mechanisms of this coupling.

Although the physiological properties of RyRs have been studied extensively, structure/function correlations for such large integral membrane proteins present experimental challenges. Analyses of the sequences of the three RyR isoforms [RyR1 ('skeletal'), RyR2 ('cardiac'), RyR3 ('brain')] have provided few definitive functional insights [12]. General agreement exists that the transmembrane segments, the number of which is disputed, lie in the C-terminal ≈ 1/5 of the sequence and that the N-terminal 4/5 lies in the cytoplasm (Fig. 2). Many of the ligand-binding sites of the RyR are located in the cytoplasmic region, which probably accounts for its large size. Beyond this, only tentative assignments can be made for the numerous regulatory and protein–protein interaction sites that are ostensibly present. Some assignments of functional activities to specific sequences within RyRs have been proposed,

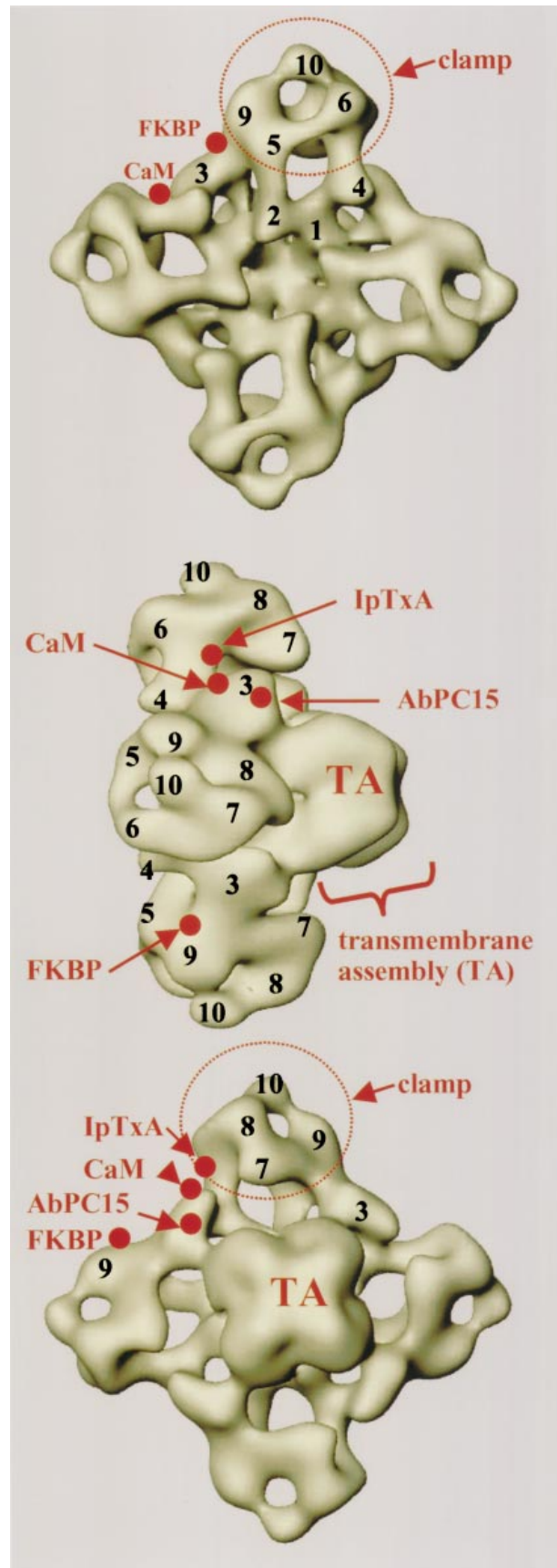


Fig. 2. Solid-body representation of one 3D reconstruction of skeletal RyR [30]. The numbers indicate distinct globular structures that correspond to structural domains (or clusters of domains), all of which are located in cytoplasmic regions of the receptor. The filled red circles indicate the locations of ligands as determined by reconstructions of RyR–ligand complexes [40–42]. Abbreviations: CaM, calmodulin; FKBP, FK506-binding protein; IpTxA, imperatoxin A; AbPC15, monoclonal antibody specific for a fusion peptide corresponding to RyR residues 4425–4621 [43]; TA, transmembrane assembly.

based on properties of cloned segments of the receptor (e.g. see Fig. 3) and on the effects of sequence-specific antibodies on receptor properties (see the reviews cited above for details). However, the pace of research in relating the sequence to function has recently accelerated with the development of expression systems that allow biophysical characterization of genetically altered cloned receptors [13–17].

Ca²⁺-ATPASE STRUCTURE

Both the RyR and Ca²⁺-ATPase have been the subject of structural studies for more than a decade. X-ray-crystallographic studies have not yet been possible, because of difficulties in growing suitable crystals. Nevertheless, cryoelectron microscopy has been used to determine structures at ever increasing resolution, thus defining the molecular architecture and delineating the various domains associated with molecular function. In the case of Ca²⁺-ATPase, 2D crystals can be induced by vanadate within the native SR membrane [18]; these crystalline membranes tend to roll up to form hollow tubes with helical symmetry [19]. The conformation of Ca²⁺-ATPase can be defined by the inhibitors that promote crystal growth [20]; in particular, the requirement for EGTA and the stabilizing effect of thapsigargin indicate that Ca²⁺-ATPase adopts the low-calcium-affinity conformation known as E₂. In contrast, a multilamellar crystal form grows in the presence of saturating calcium concentrations, which presumably stabilizes the high-calcium-affinity conformation known as E₁. As mentioned, conformational changes are central to the coupling of ATP hydrolysis to calcium transport. In particular, the E₂ state has a low affinity for calcium ions from the luminal side of the membrane and the nucleotide site is unreactive to ATP. In contrast, the E₁ state has a high affinity for calcium from the cytoplasm and ATP is readily used to phosphorylate the catalytic aspartate residue. Thus, comparing structures of the pumps in these various conformations will help to define the mechanisms of energy coupling.

At present, the E₂ state is represented by a 3D structure of Ca²⁺-ATPase [21] and the E₁ state is represented by a projection structure of Ca²⁺-ATPase [22] and by a 3D structure of H⁺-ATPase from *Neurospora* [23], all at ≈ 8 Å resolution. Although the H⁺-ATPase is phylogenetically distant, its structure resembles that from Ca²⁺-ATPase in important ways. In particular, both 3D structures define the arrangement of 10 transmembrane helices, and a detailed comparison [24] revealed that the transmembrane helices could be virtually superimposed. In contrast, the cytoplasmic domains were quite diverse and it was suggested that the gross differences in the cytoplasmic domain reflected the conformational change elicited by cation binding to the transmembrane helices, i.e. the E₂ to E₁ transition. This interpretation is consistent with a projection structure of Ca²⁺-ATPase in the E₁ conformation, which more closely resembles the H⁺-ATPase than Ca²⁺-ATPase in the E₂ conformation. Specifically, the cytoplasmic portion of Ca²⁺-ATPase forms a compact head-like structure in the E₂ state, with the crystallization agent, decavanadate, holding several domains together (Fig. 1B). In the E₁ state, the cytoplasmic nose hangs down nearer to the membrane, leaving a gap between the cytoplasmic domains (Fig. 1c). Significantly, the site of CrATP has been localized to this gap, suggesting that these domain movements may be related to ATP binding and hydrolysis. This opening of the ATP site during the E₂ to E₁ transition is consistent with results from a variety of ATP site ligands on Ca²⁺-ATPase, although inconsistent with similar studies on Na⁺/K⁺-ATPase [3].

A more detailed architecture for the cytoplasmic domains has been suggested by a homology between P-type pumps and L-2 haloacid dehalogenases, which indicate an α/β fold for the highly conserved sequences at the core of the ATP site of P-type ATPases [25]. According to this homology, this dehalogenase domain would be located directly above the ‘stalk’ that connects cytoplasmic domains to the transmembrane helices (Fig. 1). Interestingly, the cytoplasmic densities directly above this stalk are a conserved structural feature in Ca²⁺-ATPase, H⁺-ATPase, and Na⁺/K⁺-ATPase; when the dehalogenase fold is fitted into the density map for Ca²⁺-ATPase [26], the ligand-binding site is positioned at the gap where CrATP was observed to bind [27]. The homology also suggests a large (240 residues) insert in one of the loops of the dehalogenase fold, which most likely occupies much of the remaining cytoplasmic densities (orange domains in Fig. 1), including the cytoplasmic nose. This insert is connected to the α/β fold by a flexible hinge, which could provide a pivot point for the observed movement of the cytoplasmic domains during the transition between E₂ and E₁ states. Indeed, a group of residues in the inserted domain of P-type ATPases are implicated in ATP binding, by either mutagenesis or chemical modification, supporting the idea that ATP is bound at the interface between the two domains [26]. The structural mechanism for coupling such domain movements to the calcium sites within the membrane is currently unclear, but more details should come from an X-ray crystal structure of Ca²⁺-ATPase in the E₁ conformation, which was published shortly after this review was written [28].

RyR STRUCTURE

No laboratory has reported ordered arrays of RyR suitable for structural analyses; thus, our knowledge of its structure has come mainly from electron microscopy of isolated detergent-solubilized receptors. Thus far, two laboratories have determined 3D reconstructions of RyRs at resolutions of ≈ 30 Å from frozen-hydrated receptors [29–32]. As shown in Fig. 2, the receptor comprises two major components: a larger multi-domain assembly resembling a square prism, and a smaller ‘baseplate’ projecting from one face of this prism. The reconstruction is concordant with interpretations of the amino-acid sequence (Fig. 3) if we equate the larger component with the carboxy ≈ 4/5 of the sequence, which has been assigned as cytoplasmic, and the smaller baseplate to the transmembrane N-terminal portion of the sequence. This assignment of cytoplasmic and transmembrane assemblies is almost certainly correct because it is supported by electron-microscopical images of skeletal muscle and SR (see References cited in [6]). Furthermore, a number of ligands that are known to bind to cytoplasmic sites have been localized on the 3D structure to the cytoplasmic assembly (discussed below and in Fig. 2). The cytoplasmic assembly is sometimes referred to as the ‘foot’ region, a term that was applied to the structure based on its appearance in electron micrographs of sectioned muscle, and before its molecular identity was known.

The cytoplasmic assembly is composed of numerous distinct regions (Fig. 2), which is somewhat surprising given the limited resolution of the reconstruction. These regions presumably correspond to independently folded domains or clusters of closely packed domains, and 10 such regions (labeled in Fig. 2) have been reproducibly resolved in reconstructions of not only skeletal RyR, but the other two isoforms as well [33,34]. Apparently, the globular regions are rather loosely packed such that solvent occupies ≈ 50% of the volume enclosed by the cytoplasmic assembly.

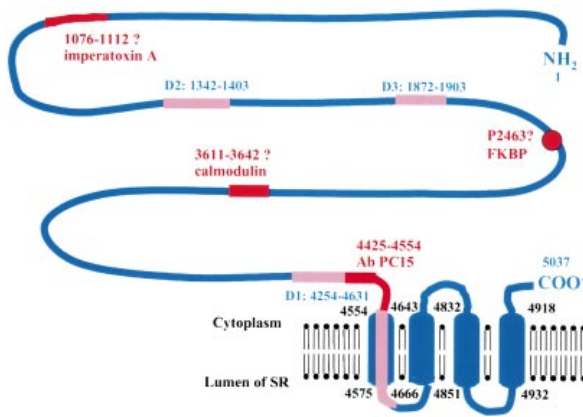


Fig. 3. Schematic representation of skeletal RyR sequence. The pink rectangles D1–D3 denote regions (with residue numbers indicated) that are highly variable among the three known RyR isoforms. The regions in red indicate sequences that are putatively involved in binding the indicated modulators and which have been mapped on to the 3D architecture of the receptor (indicated by the red circles in Fig. 2). Four transmembrane regions are indicated near the C-terminus, but the actual number might be higher. Imperatoxin A is believed to mimic an interaction of the dihydropyridine receptor with RyR [44]. The FKBP-interaction site involving Pro2463 was determined by Cameron *et al.* [45], the calmodulin site by Moore *et al.* [46], and the residues recognized by the monoclonal antibody PC15 by Treves *et al.* [43].

The dimensions of the transmembrane assembly are more consistent with predictions of 10 or more transmembrane segments, as opposed to those suggesting only four [32]. However, the transmembrane region of solubilized receptors contains an unknown amount of bound detergent micelles which, combined with the effects of limited resolution, could cause the mass of the transmembrane region to be overestimated. Thus, the precise number of transmembrane segments remains an unsettled issue.

Even at these relatively low resolutions, the structural changes that accompany switching of RyR between functional states can be detected. Serysheva *et al.* [29,35] determined reconstructions of skeletal RyR in the presence of EGTA, which should favor the closed state of the receptor, and in the presence of either ryanodine or calcium/ATP which are expected to induce open states. Unexpectedly, not only were changes detected in the transmembrane regions between ‘open’ and ‘closed’ reconstructions, but also in the most distal regions of the cytoplasmic assembly, well over 100 Å from the membrane. In the reconstruction of the ryanodine-treated RyR, the entire transmembrane assembly appeared to be rotated by 4° about the receptor’s fourfold symmetry axis relative to its orientation in the closed receptor. With calcium/ATP as activators, the rotation was not apparent, but a more subtle change in the relative orientations of the subunits in the transmembrane regions was seen. In the cytoplasmic assembly, the most striking change occurred in the so-called ‘clamps’, the assemblages of domains (numbered 5–10 in Fig. 2) that form the corners. In particular, the relative dispositions of domains 6, 9 and 10 were altered. Independent support for global conformational changes had been reported by Ikemoto and colleagues [36] who employed a conformationally sensitive fluorescent probe that was attached to the cytoplasmic region of functional RyRs. Recently, our group has investigated the open and closed states of another RyR isoform (RyR3) by 3D cryoelectron microscopy, and similar changes to those described for the skeletal isoform have been found [34].

The global nature of the conformational differences between open and closed states of the RyR probably has functional significance. The locations of three modulatory ligands (calmodulin, FKBP, and imperatoxin A) on the cytoplasmic assembly of RyR have been mapped (Fig. 2). Imperatoxin A is thought to mimic one of the sites of interaction between the dihydropyridine receptor and RyR which, together with these other ligands, influences channel conductance. All three ligands bind at sites that are 100 Å or more from the presumed location of the transmembrane ion-conducting pore running down the center of the transmembrane region.

FUTURE PROSPECTS

Given such large distances between cytoplasmic sites for ligands and the ion-transport pathways, both Ca²⁺-ATPase and RyR are likely to rely on long-range allosteric interactions to couple the relevant domains. The current structures define the relative disposition of these domains and hint at the domain motions associated with protein function. For both molecules, higher resolution will allow refinement of the mechanisms for structurally coupling the domains. In the case of Ca²⁺-ATPase, recent atomic structure of the E₁ conformation [28] should allow molecular modeling of the 8-Å structure for the E₂ conformation. The tubular crystals used for this 8-Å structure will also likely yield higher resolution, as recently demonstrated by the 4.6-Å structure for the nicotinic acetylcholine receptor [37]. In the case of RyR, significant improvement in resolution should be forthcoming using the ‘single particle’ approach. Current image processing technology allows images of tens of thousands of individual receptors to be combined for the determination of 3D reconstructions [38]. These methods, when applied to rapidly frozen non-crystalline specimens imaged with the newest generation of field emission gun transmission electron microscopes, can produce resolutions of 10 Å or higher [39], thereby revealing elements of secondary structure. Thus, by continuing our efforts, we hope to eventually reveal the structural mechanisms of excitation/contraction coupling, which will ultimately be generalized to include mechanisms of calcium signaling by related molecules.

ACKNOWLEDGEMENTS

The authors are grateful for financial support from the NIH (AR40997 and GM56960 to D. L. S., AR40615 to T. W.) and from the Muscular Dystrophy Association (to T. W.).

REFERENCES

- Moller, J.V., Juul, B. & le Maire, M. (1996) Structural organization, ion transport, and energy transduction of ATPases. *Biochim. Biophys. Acta* **1286**, 1–51.
- Axelsen, K.B. & Palmgren, M.G. (1998) Evolution of substrate specificities in the P-type ATPase superfamily. *J. Mol. Evol.* **46**, 84–101.
- McIntosh, D. (1998) The ATP binding sites of P-type ion transport ATPases. *Adv. Mol. Cell Biol.* **23A**, 33–99.
- Andersen, J.P. (1995) Dissection of the functional domains of the sarcoplasmic reticulum Ca²⁺-ATPase by site-directed mutagenesis. *Biosci. Rep.* **15**, 243–261.
- MacLennan, D.H., Rice, W.J. & Green, N.M. (1997) The mechanism of Ca²⁺ transport by sarco (endo) plasmic reticulum Ca²⁺ ATPases. *J. Biol. Chem.* **272**, 28815–28818.
- Franzini-Armstrong, C. & Protasi, F. (1997) Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions. *Physiol. Rev.* **77**, 699–729.

7. Ogawa, Y., Kurebayashi, N. & Murayama, T. (1999) Ryanodine receptor isoforms in excitation-contraction coupling. *Adv. Biophys.* **36**, 27–64.
8. Shoshan-Barmatz, V. & Ashley, R.H. (1998) The structure, function, and cellular regulation of ryanodine-sensitive Ca^{2+} release channels. *Int. Rev. Cytol.* **183**, 185–271.
9. Zucchi, R. & Ronca-Testoni, S. (1997) The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmacol. Rev.* **49**, 1–51.
10. Mackrill, J.J. (1999) Protein–protein interactions in intracellular Ca^{2+} -release channel function. *Biochem. J.* **337**, 345–361.
11. Marx, S.O., Ondrias, K. & Marks, A.R. (1998) Coupled gating between individual skeletal muscle Ca^{2+} release channels (ryanodine receptors). *Science* **281**, 818–821.
12. Sorrentino, V. (1995) Molecular biology of ryanodine receptors. In *Ryanodine Receptors* (Sorrentino, V., ed.), pp. 85–100. CRC Press, Boca Raton, FL.
13. Bhat, M.B., Zhao, J.Y., Hayek, S., Freeman, E.C., Takeshima, H. & Ma, J.J. (1997) Deletion of amino acids 1641–2437 from the foot region of skeletal muscle ryanodine receptor alters the conduction properties of the Ca^{2+} release channel. *Biophys. J.* **73**, 1320–1328.
14. Chen, S.R.W., Ebisawa, K., Li, X. & Zhang, L. (1998) Molecular identification of the ryanodine receptor Ca^{2+} sensor. *J. Biol. Chem.* **273**, 14675–14678.
15. Du, G.G. & MacLennan, D.H. (1999) Ca^{2+} inactivation sites are located in the COOH-terminal quarter of recombinant rabbit skeletal muscle Ca^{2+} release channels (ryanodine receptors). *J. Biol. Chem.* **274**, 26120–26126.
16. Nakai, J., Sekiguchi, N., Rando, T.A., Allen, P.D. & Beam, K.G. (1998) Two regions of the ryanodine receptor involved in coupling with L-type Ca^{2+} channels. *J. Biol. Chem.* **273**, 13403–13406.
17. Zhao, M., Li, P., Li, X., Zhang, L., Winkfein, R.J. & Chen, S.R.W. (1999) Molecular identification of the ryanodine receptor pore-forming segment. *J. Biol. Chem.* **274**, 25971–25974.
18. Dux, L. & Martonosi, A. (1983) Two-dimensional arrays of proteins in sarcoplasmic reticulum and purified Ca^{2+} -ATPase vesicles treated with vanadate. *J. Biol. Chem.* **258**, 2599–2603.
19. Toyoshima, C., Sasabe, H. & Stokes, D.L. (1993) Three-dimensional cryo-electron microscopy of the calcium ion pump in the sarcoplasmic reticulum membrane. *Nature (London)* **362**, 469–471.
20. Stokes, D.L. & Lacapere, J.-J. (1994) Conformation of Ca^{2+} -ATPase in two crystal forms: effects of Ca^{2+} , thapsigargin, AMP-PCP, and Cr-ATP on crystallization. *J. Biol. Chem.* **269**, 11606–11613.
21. Zhang, P., Toyoshima, C., Yonekura, K., Green, N.M. & Stokes, D.L. (1998) Structure of the calcium pump from sarcoplasmic reticulum at 8 Å resolution. *Nature (London)* **392**, 835–839.
22. Ogawa, H., Stokes, D.L., Sasabe, H. & Toyoshima, C. (1998) Structure of the Ca^{2+} pump of sarcoplasmic reticulum: a view along the lipid bilayer at 9-Å resolution. *Biophys. J.* **75**, 41–52.
23. Auer, M., Scarborough, G.A. & Kühlbrandt, W. (1998) Three-dimensional map of the plasma membrane H^{+} -ATPase in the open conformation. *Nature (London)* **392**, 840–843.
24. Stokes, D.L., Auer, M., Zhang, P. & Kühlbrandt, W. (1999) Comparison of H^{+} -ATPase and Ca^{2+} -ATPase suggests that a large conformational change initiates P-type ion pump reaction cycles. *Curr. Biol.* **9**, 672–679.
25. Aravind, L., Galperin, M.Y. & Koonin, E.V. (1998) The catalytic domain of the P-type ATPase has the haloacid dehalogenase fold. *Trends Biol. Sci.* **23**, 127–129.
26. Stokes, D.L. & Green, N.M. (2000) Modeling a dehalogenase fold into the 8-Å density map for Ca^{2+} -ATPase defines a new domain structure. *Biophys. J.* **78**, 1765–1776.
27. Yonekura, K., Stokes, D.L., Sasabe, H. & Toyoshima, C. (1997) The ATP-binding site of Ca^{2+} -ATPase revealed by electron image analysis. *Biophys. J.* **72**, 997–1005.
28. Toyoshima, C., Nomura, H. & Ogawa, H. (2000) Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* **405**, 647–655.
29. Orlova, E.V., Serysheva, I.I., van Heel, M., Hamilton, S.L. & Chiu, W. (1996) Two structural configurations of the skeletal muscle calcium release channel. *Nat. Struct. Biol.* **3**, 547–552.
30. Radermacher, M., Rao, V., Grassucci, R., Frank, J., Timmerman, A.P., Fleischer, S. & Wagenknecht, T. (1994) Cryo-electron microscopy and three-dimensional reconstruction of the calcium release channel ryanodine receptor from skeletal muscle. *J. Cell Biol.* **127**, 411–423.
31. Serysheva, I.I., Orlova, E.V., Sherman, M.B., van Heel, M., Chiu, W. & Hamilton, S.L. (1995) 3D structure of the skeletal muscle Ca^{2+} release channel in its open and closed states by electron cryomicroscopy and angular reconstruction. *Biophys. J.* **68**, A128.
32. Wagenknecht, T. & Radermacher, M. (1997) Ryanodine receptors: structure and macromolecular interactions. *Curr. Opin. Struct. Biol.* **7**, 258–265.
33. Sharma, M.R., Penczek, P., Grassucci, R., Xin, H.-B., Fleischer, S. & Wagenknecht, T. (1998) Cryoelectron microscopy and image analysis of the cardiac ryanodine receptor. *J. Biol. Chem.* **273**, 18429–18434.
34. Sharma, M.R., Jeyakumar, L.H., Fleischer, S. & Wagenknecht, T. (2000) Three-dimensional reconstruction of the ryanodine receptor isoform three in two conformational states visualized by cryoelectron microscopy. *J. Biol. Chem.* **275**, 9485–9491.
35. Serysheva, I.I., Schatz, M., van Heel, M., Chiu, W. & Hamilton, S.L. (1999) Structure of the skeletal muscle calcium release channel activated with Ca^{2+} and AMP-PCP. *Biophys. J.* **77**, 1936–1944.
36. Kang, J.J., Tarcsfalvi, A., Carlos, A.D., Fujimoto, E., Shahroksh, Z., Thevenin, B.J.M., Shohet, S.B. & Ikemoto, N. (1992) Conformational changes in the foot protein of the sarcoplasmic reticulum assessed by site-directed fluorescent labeling. *Biochemistry* **31**, 3288–3293.
37. Miyazawa, A., Fujiyoshi, Y., Stowell, M. & Unwin, N. (1999) Nicotinic acetylcholine receptor at 4.6 Å resolution: transverse tunnels in the channel wall. *J. Mol. Biol.* **288**, 765–786.
38. Frank, J. (1996) *Three-dimensional electron microscopy of macromolecular assemblies*. Academic Press, New York.
39. Malhotra, A., Penczek, P., Agrawal, R.K., Gabashvilli, I.S., Grassucci, R.A., Junemann, R., Burkhardt, N., Nierhaus, K.H. & Frank, J. (1998) *Escherichia coli* 70 S ribosome at 15 Å resolution by cryo-electron microscopy: localization of fMet-tRNA^{fMet} and fitting of L1 protein. *J. Mol. Biol.* **280**, 103–116.
40. Benacquista, B.L., Sharma, M.R., Samsó, M., Zorzato, F., Treves, S. & Wagenknecht, T. (1999) Amino acid residues 4425–4621 localized on the three-dimensional structure of the skeletal muscle ryanodine receptor. *Biophys. J.* **78**, 1349–1358.
41. Samsó, M., Trujillo, R., Gurrola, G.B., Valdivia, H.H. & Wagenknecht, T. (1999) Three-dimensional location of the imperatoxin A binding site on the ryanodine receptor. *J. Cell. Biol.* **146**, 493–499.
42. Wagenknecht, T., Radermacher, M., Grassucci, R., Berkowitz, J., Xin, H.-B. & Fleischer, S. (1997) Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J. Biol. Chem.* **272**, 32463–32471.
43. Treves, S., Chiozzi, P. & Zorzato, F. (1993) Identification of the domain recognized by anti-(ryanodine receptor) antibodies which affect Ca^{2+} -induced Ca^{2+} release. *Biochem. J.* **291**, 757–763.
44. Leong, P. & MacLennan, D.H. (1999) Complex interactions between skeletal muscle ryanodine receptor and dihydropyridine receptor proteins. *Biochem. Cell Biol.* **76**, 681–694.
45. Cameron, A.M., Nucifora, F.C., Fung, E.T., Livingston, D.J., Aldape, R.A., Ross, C.A. & Snyder, S.H. (1997) FKBP12 binds the inositol 1,4,5-trisphosphate receptor at leucine-proline (1400–1401) and anchors calcineurin to this FK506-like domain. *J. Biol. Chem.* **272**, 27582–27588.
46. Moore, C.P., Rodney, G., Zhang, J.-Z., Santacruz-Tolozza, L., Strasburg, G.M. & Hamilton, S.L. (1999) Apocalmodulin and Ca^{2+} calmodulin bind to the same region on the skeletal muscle Ca^{2+} release channel. *Biochemistry* **38**, 8532–8537.