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Near-atomic resolution structure of the largest known Ca^{2+} channel: ryanodine receptor

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Ryanodine receptors (RyRs) are the major Ca^{2+} channels that allow the release of Ca^{2+} from the sarcoplasmic reticulum (SR) or the endoplasmic reticulum (ER). There are three known mammalian isoforms of RyRs: RyR 1, 2 and 3, which share 70% sequence identity. RyR1 is predominately expressed in skeletal muscle, while RyR2 is enriched in the heart, where RyR1−2 are directly involved in excitation-contraction coupling. RyR3 was originally identified in the brain. Being permeable to Ca^{2+}, RyRs are involved in various physiological events such as learning and memory, secretion, fertilization, and apoptosis. Mutations in RyRs result in a number of life-threatening genetic conditions such as malignant hyperthermia (MH), central core disease (CCD) and catecholaminergic polymorphic ventricular tachycardia (CPVT) [1].

RyRs are the largest known ion channels, and form a homotetramer (~5,000 residues per subunit, 2.2–2.3 MDa per tetramer). RyRs have about 500,000 $Å^2$ exposed surface area, which provide ample binding sites for ions, small molecules and protein binding partners including Ca^{2+}, Mg^{2+}, calmodulin (CaM), FK506-binding proteins (FKBPs), Protein kinase A (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), calsequestrin (CSQ), and triadin. Because of their large size, membrane protein nature, and dynamic features (i.e., multiple conformational states), full-length RyRs are extremely difficult targets for X-ray crystallography. Only some portions of RyRs have been determined by X-ray crystallography, which cover ~15% of the entire protein [2]. However, their large size made them popular targets for cryo-electron microscopy (cryo-EM). There are about 20 cryo-EM studies of RyRs with a primary focus on RyR1, and the resolutions improved from 34 Å to about 10 Å. The overall structures agree very well with each other: a mushroom with a ~280×280×120 Å cytoplasmic cap (~80% of the entire protein) and a ~120×120×60 Å transmembrane stem (~20%). Both regions form square prisms with a ~40° relative rotation. Due to the low resolution (~10 Å), it is hard to identify secondary structures, and impossible to locate individual amino acids. The exact binding sites for Ca^{2+}, auxiliary proteins and small molecules are still not unambiguously assigned.

In summer 2014, Filip van Petegem [2] predicted that new RyR cryo-EM studies would be well beyond the 10-Å barrier soon. Just a few months later, Dr. Yan and her colleagues [3] determined the structure of RyR1 at near-atomic resolution. This structure clearly illustrates the locations of all the subdomains including nine cytoplasmic domains (the NTD (N-terminal domain), SPRY1-3 (SplA kinase ryanodine receptor domain), P1-2, handle, HD (helical domain), and central domains), one channel region, and one CTD (C-terminal domain) domain. An atomic model has been successfully built. This makes it, for the first time, possible to examine the structural organization as well as the functional and regulatory mechanisms of the largest known high-conductance Ca^{2+} channel.

The RyR pore-forming region shares sequence similarity with that of tetrameric potassium and sodium channels, and the upstream of the pore-forming region contains a few hel-

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ices. The previous consensus was 6–8 helices, while cryo-EM studies showed 5–6 helix candidates [2]. In this near-atomic structure, there are six helices plus a VSC subdomain between helix S2 and S3. In general, the six helices resemble a voltage-gated ion channel superfamily fold. However, three unique features are also present in RyR1: (i) helix S6 extends to the cytoplasm followed by the CTD. At the cytoplasmic border of the membrane, four S6 helices form an activation gate (1 Å opening in the current closed state); (ii) VSC domain between S2 and S3 bridges CTD and the S1–S4 segments; (iii) a negative-charge-enriched hairpin loop between S5 and the pore helix protrudes to the SR lumen, which may attract Ca^{2+} to the channel.

RyRs have enormous exposed area for the binding of ions, small molecules and protein binding partners. The binding sites for FKBP12 and CaM have been studied via cryo-EM [2,4]. Due to the poor resolution in previous cryo-EM reconstructions, the details of the binding sites have not been determined. Here, in this high-resolution RyR1 reconstruction, FKBP12 is bound in a cleft formed by the handle, NTD and SPRY1/3 domains. In addition, three regions showing armadillo repeats have been identified: the helical repeats in subdomain C of the NTD, the handle domain, and the helical repeats in the central domain. Each armadillo repeat is a hairpin structure consisting of a pair of alpha helices. Tandem armadillo repeat units fold together as a superhelix, forming a versatile platform for binding to various protein partners and known for their inherent conformation adaptability.

The cytoplasmic cap of RyRs has ~80% of the entire protein mass and provides the binding sites for various cofactors. How the channel opening is coupled to the conformational changes in the cytoplasmic cap is not well understood. Here, the RyR1 structure provides a possible explanation of the long-range allosteric regulation of channel activities. There exist two superhelical assemblies in the cytoplasmic cap: one is formed by the armadillo repeats from the NTD, handle and central domains, and the other is formed by the helical domain (17 alpha helical hairpins and seven alpha-helices at the carboxyl-terminal end). These superhelical assemblies constitute a network for binding of cofactors and propagation of conformational changes. The extensive interactions between the central domains and the channel domain support the propagation of conformational changes from any location of the cytoplasmic cap to the channel domain.

This near-atomic resolution structure of RyR1 provides a detailed view of the organization of the largest known channel, identified a number of unique and important features of RyR1, and provides an explanation of the propagation of the conformational changes in the cytoplasmic cap to the channel domain. There are still more questions to be answered (e.g., how the binding of various cofactors may affect the allosteric coupling between the cytoplasmic cap and the channel region). High-resolution structures of RyRs in different conformational states are needed.

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