Ryanodine receptors (RyRs) mediate the rapid release of calcium (Ca\(^{2+}\)) from intracellular stores into the cytosol, which is essential for numerous cellular functions including excitation–contraction coupling in muscle. Lack of sufficient structural detail has impeded understanding of RyR gating and regulation. Here we report the closed-state structure of the 2.3-megadalton complex of the rabbit skeletal muscle type 1 RyR (RyR1), solved by single-particle electron cryomicroscopy at an overall resolution of 4.8 Å. We fitted a polyalanine-level model to all 3,757 ordered residues in each protomer, defining the transmembrane pore in unprecedented detail and placing all cytosolic domains as tertiary folds. The cytosolic assembly is built on an extended α–solenoid scaffold connecting key regulatory domains to the pore. The RyR1 pore architecture places it in the six-transmembrane ion channel superfamily. A unique domain inserted between the second and third transmembrane helices interacts intimately with paired EF–hands originating from the α–solenoid scaffold, suggesting a mechanism for channel gating by Ca\(^{2+}\).

**Structure of a mammalian ryanodine receptor**

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Ryanodine receptors (RyRs) are intracellular Ca\(^{2+}\) release channels on the sarcoplasmic and endoplasmic reticula required for fundamental cellular functions in most tissues, including skeletal and cardiac muscle excitation–contraction coupling, synaptic transmission and pancreatic beta cell function1. The type 1 ryanodine receptor (RyR1) mediates excitation–contraction coupling in skeletal muscle. It is a homotrimer of four ~565-kilodalton (kDa) channel-forming protomers, as well as regulatory subunits, enzymes and their respective targeting/anchoring proteins, forming a macromolecular complex that exceeds 3,000,000 daltons. In most tissues, RyR channels are activated by the inward flow of Ca\(^{2+}\) via plasma-membrane Ca\(^{2+}\) channels, resulting in a massive and rapid release of Ca\(^{2+}\) from intracellular stores (a process known as Ca\(^{2+}\)-induced Ca\(^{2+}\) release). By contrast, in skeletal muscle, approximately 50% of RyR1 channels are mechanically activated by direct interaction with voltage-gated Ca\(^{2+}\) channels (VGCCs) on the plasma membrane. RyR1 is also activated by Ca\(^{2+}\) in vitro, and hence those RyR1s not associated with VGCCs are presumably activated by Ca\(^{2+}\), possibly together with coupled gating between adjacent RyRs, amplifying Ca\(^{2+}\) release.

RyR channels are subject to stress-induced Ca\(^{2+}\) leak, which is critically involved in heart failure2, cardiac arrhythmias2, post-traumatic stress disorder4, age-dependent loss of muscle function5, and muscular dystrophy6. RyRs have recently become therapeutic targets for some of these disorders11,12.

Previous single-particle electron cryomicroscopy (cryo-EM) studies revealed that RyR1 adopts a four-fold symmetric mushroom-like superstructure, with about 80% of the mass in the cytosol (the ‘cap’ of the mushroom) and a ‘stalk’ embedded in the sarcoplasmic and endoplasmic reticula membrane. The highest-resolution reconstruction obtained for intact RyR1 before this study was 9.6 Å (ref. 13), which, although giving a good picture of the overall dimensions of the molecule and approximate protomer boundaries, was insufficient to identify secondary structural elements, or locate key domains.

**Architecture of RyR1 at 4.8 Å resolution**

The calcium-free (low nanomolar free Ca\(^{2+}\)) RyR1–calstabin2 (also known as FKBP12.6) complex was purified from rabbit (*Oryctolagus cuniculus*) skeletal muscle by calstabin-affinity chromatography (see Methods and Extended Data Fig. 1 for details). Recent advances in cryo-EM detector technology and data processing4 have allowed us to obtain three-dimensional reconstructions at overall resolutions as high as 4.8 Å (Extended Data Fig. 2).

Three-dimensional classification using RELION 1.2 (ref. 15) identified distinct classes of particles (Extended Data Fig. 3) differing in the cytosolic assembly conformation but not in the transmembrane pore. The best-resolved class yielded a reconstruction with C\(_4\) symmetry and a resolution of 4.8 Å according to the Fourier shell correlation (FSC) = 0.143 gold standard criterion15 (Extended Data Fig. 4a).

The quality of the density map was excellent in the transmembrane region, and lower in the cytoplasmic region. A reconstruction obtained from dephosphorylated (see Methods for details) RyR1 at 5.0 Å resolution had considerably improved density in regions that were poorly defined in the original sample (Fig. 1a and Extended Data Figs 4b–e and 5). This map has been used for most of the interpretation and model building (model–map correlation in Extended Data Fig. 6).

The architecture of RyR1 consists of four protomers surrounding a central transmembrane pore coincident with the four-fold symmetry axis of the tetramer. Each protomer is built around an extended scaffold of α–solenoid repeats16. This scaffold is extraordinary in scale, comprising 37 repeats in three contiguous segments, totaling 2,217 residues, or 56% of the ordered residues in the polypeptide. The α–solenoid scaffold is capped at the amino terminus by two β-trefoil domains, N-terminal domain (NTD)-A and NTD-B, which form a central cytosolic vestibule, and at the carboxy terminus by the transmembrane pore that adopts a fold placing it in the six-transmembrane (6TM) superfamily of ion channels, which includes the voltage-gated sodium and potassium channels and the transient receptor potential (TRP) channels (Fig. 1b). The

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\[\text{alpha-solenoid scaffold incorporates five major domains: three SPRY domains} \]
\(\text{(SPRY1–3), and two pairs of RyR repeats (RY12 and RY34, the latter containing a regulatory protein kinase A phosphorylation site at Ser 2843).}\)
\(\text{Several smaller insertions were also identified, including most importantly a previously predicted EF-hand pair}^{17} \text{that constitutes the presumed conserved Ca}^{2+}-\text{-binding domain in RyRs (Fig. 1b). The RyR1 model exhibits well-defined protomer boundaries (Fig. 1c–e).}\)

**The flexible alpha-solenoid scaffold of RyR1**

The \(\alpha\)-solenoid scaffold of RyR1 can be divided into three major segments (Extended Data Fig. 7). The smallest of these is the N-terminal solenoid (N-Sol), consisting of four repeats, linking the central cytosolic vestibule of the channel to the three SPRY domains at the outer corner of the tetramer (Fig. 1b). The first two of these repeats were present in the crystal structure of the N-terminal fragment, in which they were assigned as ‘domain C’ of this fragment.\(^{16}\)

The second and largest of the three major \(\alpha\)-solenoid regions is the bridging solenoid, which curls around the periphery of the cytosolic assembly in a right-handed superhelix towards the SPRY domains of the adjacent protomer. The bridging solenoid may be further subdivided into N-terminal, central and C-terminal subdomains, denoted BrA, BrB and BrC, respectively (Extended Data Fig. 8).

The N-terminal subdomain, BrA, adopts a distinctly different architecture from the rest of the bridging solenoid, in which distorted, interlocking \(\alpha\)-solenoid repeats wrap around a 72-Å-long central helix that projects towards the three SPRY domains. An additional feature of interest in BrA is the calstabin-binding helix, which projects out from the central helix, with its \(N\) terminus buried in calstabin2 (Extended Data Fig. 9).

Midway along BrB, the second RyR-repeat pair (RY34) projects away from the sarcoplasmic reticulum membrane into the cytosol. The connections between RY34 and BrB are not clearly resolved, leading to uncertainty in the numbering of this region. BrC, the C-terminal subdomain of the bridging solenoid, interacts closely with the SPRY3 domain of the neighbouring subunit, before terminating in a disordered segment.

The last of the major solenoid regions, which we have termed the core solenoid (Fig. 1b), begins with a linker helix (starting approximately at residue 3679) embedded in BrA, and progresses through eight repeats towards the transmembrane pore (Extended Data Fig. 8b). An EF-hand pair, described in detail below, is inserted in the seventh repeat of the core solenoid (Extended Data Fig. 10). Between the seventh and eighth repeats, the core solenoid is interrupted by an extended loop (4180–4200), which probably adopts a \(\beta\)-hairpin structure. The \(\beta\) hairpin and the eighth repeat of the core solenoid form a ‘vice’ surrounding the C-terminal domain of RyR1, as described in detail below.

Inspection of the initial model suggested that the fold of three major regions of the polypeptide could be categorized into one of the many families of \(\alpha\)-solenoid repeat proteins. We used the polyalanine model of each region to search the Protein Data Bank (PDB; using the DALI web server\(^{15}\)). For all three major solenoid regions, the top hits (Z scores > 15) were Armadillo repeat-containing proteins, including adenomatous polyposis coli (APC) and \(\beta\)-catenin (Extended Data Fig. 7b–d).

Previously characterized Armadillo and HEAT repeats are flexible protein–protein interaction modules\(^{20,21}\). We suggest that the flexible \(\alpha\)-solenoid scaffold of RyR1 could facilitate assembly of regulatory proteins, and allow coupling of conformational changes of the scaffold, induced by either protein binding or post-translational modifications, to RyR1 activity.

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**Figure 1 | The architecture of RyR1 at 4.8 Å.**

- **a.** View from the plane of the sarcoplasmic reticulum membrane of a slab of density (blue mesh) coinciding with the channel axis.
- **b.** Colour-coded schematic representation of the RyR1. B-sol, bridge solenoid; C-sol, core solenoid; N-sol, N-terminus solenoid.
- **c.** View in the plane of the sarcoplasmic reticulum membrane.
- **d.** View from the cytosol.
- **e.** View from the lumen of the density map of skeletal muscle RyR1 at 5.0 Å resolution, with one protomer segmented according to the domains assigned in the model, coloured as follows: blue, N-terminal domain; cyan, SPRY1, SPRY2 and SPRY3; salmon, clamp region (RY12 repeats); yellow, calstabin; green, the bridge solenoid scaffold; red, the core solenoid; and orange, transmembrane and C-terminal domains; purple, putative Ca\(^{2+}\)-binding domain (EF). Dashed lines represent major disordered segments.

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RyR1 is a 6TM ion channel

The transmembrane domain is one of the best-resolved regions of the map (Fig. 2a, b), allowing us to identify all secondary structural elements. The architecture of the pore clearly identifies it as a member of the 6TM ion channel superfamily22, with six transmembrane helices per protomer surrounding a central pore. An extended peptide (the P-segment), structurally analogous to the selectivity filter of potassium and sodium channels, delineates the luminal aperture (Fig. 2c). The transmembrane region contains two domains: the pore domain, formed by S5, S6, the pore helix and the P-segment; and a pseudo voltage-sensor domain (pVSD), formed by S1–S4 that interfaces with the pore domain of the adjacent subunit (Fig. 2b). The RyR1 pore domain most closely resembles that of the voltage-gated sodium channel, NavAb (Fig. 2d), although the P-segment is more similar to the equivalent region of the TRPV1 cation channel23,24 (Fig. 3a). Moreover, the luminal aperture is comparable to that of the highly Ca2+-permeable25 TRPV1 channel. The brevity of the constriction suggests that it may accommodate only one Ca2+-binding site.

The P-segment contains many acidic residues that are expected to be anionic under physiological conditions, probably contributing to the high conductance of RyR1 (for example, 110 pS for Ca2+, >700 pS for K+)26. Seven additional negatively charged residues are located along the cytosolic extension of S6, presumably serving a similar function (Fig. 3b).

The transmembrane region includes three apparently amphipathic helices (JM1, the first helix of the S2–S3 insertion and the S4–S5 linker helix) that are expected to lie in the plane of the cytosolic surface of the membrane.

There is a single cytosolic constriction in the ion conduction pathway, at the S6 bundle crossing. The Ca2+-Ca2+ distance at this point is 11 Å, similar to the minimal Ca2+-Ca2+ distance observed in the structure of NavAb (10.41 Å at Cys 217). On the basis of structural alignment with the closed NavAb pore (Fig. 2d), and the diameter of hydrated Ca2+ (8.2 Å; ref. 27), we conclude that the pore is in a non-conducting state.

The S6 helix has a 24º kink in the middle, centred on Gly 4934 near the cytosolic face of the membrane, which orients the cytosolic extension of S6 roughly parallel to the channel axis (Fig. 3c). Conserved glycine residues in the pore-lining helices of other 6TM ion channels may operate as ‘glycine-hinges’, facilitating reorientation of the pore-lining helix and consequent alteration in the cytosolic aperture of the channel28. Gly 4934 is conserved in all RyR isoforms and in a closely related channel, the inositol-1,4,5-trisphosphate receptor.

The S6 helix extends ~30 Å into the cytosol, terminating in the C-terminal domain (CTD), a small α-helical domain that extends laterally from the channel axis into the core solenoid (Fig. 1b). The CTD is secured by an extended loop and a pair of helices that are reminiscent of a thumb and forefingers (Fig. 4).

An additional helix, in weaker density, may correspond to half of the predicted transmembrane hairpin located between amino acids 4325 and 4370 (Extended Data Fig. 6).

A cytosolic Ca2+ sensor contacts the pore

Unlike other 6TM channels, RyR1 possesses a compact, folded cytosolic domain inserted within the pVSD, between helices S2 and S3 (residues 4675–4790). A curved, cytosolic extension of S2 leads into a bundle of four short helices, which are separated by an unresolved segment spanning 31 amino acids from an amphipathic helix leading into S3 (Fig. 2c). The S2–S3 helical bundle lies in close proximity to the putative Ca2+-binding domain and the CTD (Fig. 4), suggesting that this domain may be involved in transmitting conformational changes in the cytosolic assembly to the pore.

RyR1 is activated by increased cytosolic Ca2+ concentration29. A calmodulin-like domain inserted within the core solenoid was previously suggested to be a Ca2+ sensor30. This region is highly conserved
in all RyRs, and shares 26% sequence identity with the C-lobe of human calmodulin (Extended Data Fig. 10a). Six of the eight residues that coordinate Ca\(^{2+}\) in calmodulin are conserved in the putative Ca\(^{2+}\)-binding domain of RyR1.

The putative Ca\(^{2+}\)-binding domain of RyR1 is adjacent to the S2–S3 insertion from a neighbouring protomer and the CTD of the originating protomer (Fig. 4a), suggesting a mechanism for Ca\(^{2+}\)-mediated gating in which Ca\(^{2+}\)-dependent changes in the conformation of the Ca\(^{2+}\)-binding domain are transmitted to the pore via contacts with the S2–S3 loop and/or the CTD, inducing a conformational change that alters the cytosolic aperture of the channel.

NTDs form the cytosolic vestibule

A prominent feature of the RyR1 structure, already evident at lower resolution\(^{30,31}\), is a large cytosolic vestibule around the four-fold axis above the pore in the cytosolic domain (Fig. 5). We docked the crystal structure of the three-domain N-terminal fragment of RyR1 (ref. 18) into the density map in the same location as previously proposed. Several loops that were unresolved in the crystal structure are now clearly visible and were added into the model, as were residues 533–603 of NTD-C, which were not resolved in the previous structure. This addition completes the N-terminal solenoid, which connects to the beginning of SPRY1 through a short linker that is well resolved in the density map (Fig. 5a).

The NTDs make key interactions stabilizing the cytosolic assembly, both with other domains in the same protomer, and with domains contributed by neighbouring protomers. NTD-A contacts the fifth repeat in the core solenoid of the adjacent protomer via interactions mediated by Arg 76 and Glu 177 (Fig. 5b), and interacts with NTD-B of the neighbouring protomer via Gln 156 (NTD-A) and Asp 385 (NTD-B) (Fig. 5a). NTD-A interfaces with the sixth repeat of BrB in the bridging solenoid of the adjacent protomer, in an interaction involving the 121–134 loop on NTD-C, as well as Phe 195, Met 196 and Ser 175 (Fig. 5a). Arg 392 and Gln 465 in NTD-C contribute to an interface with the core solenoid of the same protomer (Fig. 5b). NTD-C forms a structurally contiguous segment of α-solenoid repeats with the first set of repeats in the
bridging solenoid region following SPRY3 (Fig. 5a). A major feature of the cytosolic domain are four prominent openings in the structure (one in each protomer) that are bounded by outer edges of the NTDs, the inner edges of the SPRY domains, and the bridging solenoid from the adjacent protomer (Fig. 5a).

**RYR repeats, SPRY and calstabin interactions**

RYR1 contains two pairs of RY repeats, RY12 (residues 850–1055) and RY34 (residues 2734–2941). RY12 is located between SPRY1 and SPRY2, whereas RY34 is inserted in BrB (Fig. 1b). RY12 and RY34 are expected to adopt the same overall fold, as they share 29% sequence identity. The structure of RY34 (which contains a regulatory PKA phosphorylation site) protrudes from BrB on top of the cytosolic assembly projecting into the cytosol (Fig. 5c, d). This assignment agrees with the observation that RY34, which seems to be the most exposed and flexible of the RYR repeat pairs, is also the most susceptible to proteolysis.

In our 5.0 Å map, we see two densities that share this distinctive shape. One is located in the previously identified location, abutting the SPRY domains, and another, less well-resolved density with the same overall shape protrudes upwards from the bridging solenoid. In the primary sequence, RY12 is located between SPRY1 and SPRY2. Therefore, we place RY12 at the corner of the channel, and RY34 (containing the PKA phosphorylation site) protrudes from BrB on top of the cytosolic assembly projecting into the cytosol (Fig. 5c, d). This assignment agrees with the observation that RY34, which seems to be the most exposed and flexible of the RY repeat pairs, is also the most susceptible to proteolysis.

Interestingly, the location of RY12 (Fig. 5c), projecting laterally from the corners of the cytosolic assembly, places it in an appropriate position to serve as a contact site between neighbouring channels in the paracrystalline RyR arrays that have been shown to assemble on the sarcoplasmic reticulum membrane.

RYR1 contains three SPRY domains, SPRY1 (residues 643–794), SPRY2 (residues 1073–1205) and SPRY3 (residues 1419–1567). Structures of SPRY domains from other proteins have shown that the domain adopts a distinctive β-sheet sandwich structure consisting of two antiparallel 7- or 8-stranded β-sheets. In our 5.0 Å map of dephosphorylated RyR1, we identify the three SPRY domains on the basis of the two curved sheets that form the β-sandwich (Fig. 5c, d). The plane of the triangle is oriented roughly perpendicular to the channel axis, and parallel to the edge of the cytoplasmic assembly.

High-affinity binding of calstabin1 and 2 has a critical role in stabilizing the closed states of RyR1 and RyR2, respectively, and in preventing pathological intracellular calcium leak. Calstabin2 binds with high affinity to both RyR1 and RyR2 (but is not expressed in skeletal muscle), and calstabin1 binds preferentially to RyR1 (ref. 38). Calstabin1 and calstabin2 differ at only 18 positions out of 108 residues. The calstabin-binding site is located at the mutual interface of the bridging solenoid, SPRY1 and SPRY2 (Extended Data Fig. 9). The N-terminal end of a helix originating from subdomain BrA is located in the hydrophobic binding pocket of calstabin2. Calstabin binding may stabilize the connection between the cytoplasmic regulatory domains and the pore by rigidifying the BrA–SPRY1–SPRY2 interface and possibly altering the relative orientation of these domains.

**Concluding remarks**

The model and density maps presented here provide, to our knowledge, a first glimpse of the transmembrane architecture of ryanodine receptors. Our analysis confirms that RyR1 is a 6TM ion channel, reveals a flexible cytosolic α-solenoid scaffold of remarkable size, and places every previously known domain, including a putative calcium-binding domain. This structure is of RyR1 in the closed state, but its features provide a foundation for understanding the open state and the process of Ca2+-activation for all members of the RyR family.

*Note added in proof:* While this paper was in press, high-resolution structures of the SPRY2 domains from RyR1 and RyR2 were published. These authors placed the SPRY2 domain in the location where we have assigned the SPRY3 domain. Now, having the authentic SPRY2 structure...
instead of a homology model, we find equally good fittings to our map for either alternative, leaving some ambiguity about the connectivity in this region.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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methods

RyR1 purification from rabbit skeletal muscle. Detergent-solubilized RyR1 was purified from *Oryctolagus cuniculus* skeletal muscle by calstabin-affinity chromatography using a procedure modified from ref. 41. The protein was eluted using calstabin2 (also known as FKB12.6), rather than the physiological binding partner calstabin1 (FKB12.12), owing to the higher affinity of the former for the RyR1 channel. Importantly, calstabin1 and calstabin2 share the same binding site on RyRs, and both have the same effects on RyR1 function. Addition of EGTA reduced the concentration of free Ca\(^{2+}\) in the sample to the low nanomolar range, such that the channel is closed according to functional studies using purified RyR1 reconstituted into planar lipid bilayers\(^{8}\).

Rabbit skeletal muscle (100 g) flash-frozen in liquid nitrogen was blended for 90 s in 500 ml of ice-cold containing 10 mM Tris-maleate, pH 6.8, 1 mM diithiothreitol, 1 mM EDTA, 150 μM PMSF (phenylmethylsulfonyl fluoride) and 1 mM benzamidine. The mixture was then centrifuged for 10 min at 8,000g. The supernatant was centrifuged for 20 min at 40,000g. Pellets were solubilized in 50 mM buffer containing 10 mM HEPES, pH 7.5, 1% (w/v) CHAPS, 1 M NaCl, 2 mM EGTA, 2 mM Tris-(2-carboxyethyl)phosphine (TCEP) and a protease inhibitor cocktail (Roche). The solubilized membranes were then diluted 1:1 in the same buffer without NaCl and centrifuged for 30 min at 100,000g. The supernatant was then filtered using a 0.2-μm filter and allowed to bind overnight at 4°C to a pre-equilibrated 5 ml GST-fusion (Novagen healthcare) column with bound glutathione-S-transferase (GST)-calstabin1 (derived from 0.5 lB-21 cells overexpressing GST–calstabin1). The column was then washed with 10 column volumes of cold solubilization buffer (with the following alterations: 0.5% (w/v) CHAPS and 0.5 M NaCl) and RyR1 was eluted with two column volumes of 10 μM calstabin2 in the same buffer. The eluent was pre-cleared with 0.5 ml glutathione beads and treated with calf intestine alkaline phosphatase (CIP, NEB, 100 U ml\(^{-1}\)) for 4 h at room temperature. The CIP-treated RyR1 was concentrated in a 100-kDa cut-off centrifugal filter unit (Millipore) to less than 500 μl, loaded on a size-exclusion column (Tosoh G50WXL) and eluted in solubilization buffer with the following alterations and substitutions: 5 mM EGTA, 0.25% (w/v) CHAPS and 0.001% (v/v) DOPC (Avanti). The fractions corresponding to the RyR1 peak (Extended Data Fig. 3b) were then combined and concentrated in a 100-kDa cut-off centrifugal filter unit (Millipore) to a concentration of ~10 mg ml\(^{-1}\) as determined using spectroscopy (A\(_{280\text{nm}}\) at 1 mg ml\(^{-1}\) = 1.018, measured using a Nanodrop spectrophotometer).

Grid preparation and data collection. Three microtubes of each sample was applied to holey carbon grids (C-flat CF-1.2/1.3-2C-T, Protochips Inc.) without prior plasma treatment. A-C, the RY12 and RY34 repeat pairs, and the EF-hand pair, we were able to place the majority of the transmembrane region. The final model has been presented as a C\(_{4}\)-symmetric particle. Although the overall resolution did not improve, the local resolution improved substantially in some regions compared to the previous protein preparation (Extended Data Fig. 4c, d). This map has been used for most of the map interpretation and model building described here. It is important to note that the observed improvements in the map may relate to dephosphorylation of the protein, but may also be due to the larger size of the data set.

Notably, the local resolution in the cytosolic assembly was greatly improved in a reconstruction obtained from the CIP-treated sample (Extended Data Fig. 4b, c), and this map was used for completion of the model, as described below. The local resolution, as measured using the program ResMap, varies between 4.5 and 6 Å across most of the map (Extended Data Fig. 4b–d).

Reported resolutions are based on the gold-standard FSC = 0.143 criterion, and were corrected for the effects of a soft mask on the FSC curve using high-resolution noise substitution\(^{42}\). Before visualization, all density maps were sharpened by applying a negative B-factor that was estimated using automated procedures\(^{43}\).

Density maps segmentation and display. Cryo-EM reconstructions were segmented using the SEGGER module\(^{45}\) implemented in UCSF Chimera\(^{46}\). Segments were manually combined to generate segmented densities corresponding to individual structural domains, as represented in Fig. 1c–e and Extended Data Fig. 5. All other maps were left unsegmented. All density figures were rendered and displayed using UCSF Chimera.

Model building. The 5.0 Å map of dephosphorylated RyR1 (Fig. 1a) has allowed us to place all domains in RyR1 (Fig. 1b), as well as to determine the boundaries of the four identical protomers that comprise the channel (Fig. 1c–e), and to build a largely complete C\(_{4}\)-symmetric particle. Although the overall resolution did not improve, the local resolution improved substantially in some regions compared to the previous protein preparation, comprising 3.757 of the 5.027 residues that constitute the full-length polypeptide chain, in addition to the 105 residues comprising the co-purified subunit, calstabin2. Most of the unassigned residues are located in regions of the sequence that are predicted to be disordered or to lack regular secondary structure. In addition, several of the regions that are either not present in the model or are less well ordered (for example RY34) have previously been shown to be highly accessible to proteolysis consistent with their surface exposure\(^{44}\).

Given the absence of homologous structures for most domains in RyR1 it is important to address the limitations of our model. In some regions, specifically NTD A–C, the RY12 and RY34 repeat pairs, and the EF-hand pair, we were able to place a crystal structure or high sequence identity homology model. For the SPRY1–3 domains, we placed homology models, and the numbering within the SPRY domains is consequently uncertain because of limited sequence identity with template structures. In all other areas, we built the model de novo as a polyalanine trace, and did not attempt to model side chains, even where visible. Helices are clearly defined in the density in most regions of the map; in the best areas, the pitch and curvature of the helices is clearly defined and bulky side chains are visible, although not identifiable (Extended Data Fig. 5). Features of the map correspond well to the local resolution measured using the program ResMap (Extended Data Fig. 4b–d). The model is of good quality in most regions, as demonstrated by analyses well to the local resolution measured using the program ResMap (Extended Data Fig. 4b–d). The model is of good quality in most regions, as demonstrated by analyses well to the local resolution measured using the program ResMap (Extended Data Fig. 4b–d).
These sequence assignments should be considered provisional owing to the absence of identifiable side chains and the presence of multiple disordered regions (Extended Data Fig. 5).

Model building was performed manually in Coot\textsuperscript{44}. First, the high-resolution structure of the N-terminal three-domain fragment of RyR1 (PDB code 2XOA) was placed in the previously assigned location, and the orientation of the domain was determined using the ’jiggle-fit’ algorithm implemented in Coot, using 1,000 random candidate orientations of the domain followed by rigid body fitting. Homology models for the three SPRY domains were generated using the PHYRE2 web server, and fitted to the density using the same procedure as for the N-terminal fragment, assigned as SPRY1–3 according to the rationale described in the main text. The two pairs of RyR repeats (RY12 and RY34) were modelled based on the structure of RY34 (ref. 18) and fitted to the density in a similar fashion. The two pairs of RyR repeats (RY12 and RY34) were modelled based on the structure of RY34 (ref. 18) and fitted to the density in a similar fashion.

One of the SPRY domains is directly connected to the N-terminal solenoid via an ordered, \(\alpha\)-helical linker, identifying it as SPRY1. Another of the SPRY domains is directly connected to RY12, placing it as SPRY2, and the remaining SPRY domain has been assigned as SPRY3. The three SPRY domains pack side-by-side in a triangular arrangement near the corner of the cytoplasmic assembly.

In the primary sequence, RY12 is located between SPRY1 and SPRY2, whereas RY34 is located –1,000 residues after SPRY3, inserted in the stretch of sequence we have assigned to the bridging solenoid. Two densities of an appropriate size and shape to accommodate RyR repeat pairs are present in the map; one is located at the corner of the tetramer, contacting the SPRY domains, and the other is directly adjacent to the bridging solenoid. On the basis of these observations, we have revised the assignment of the position of RyR repeats such that RY12 is located at the extreme outer corner of the channel (directly adjacent to the SPRY domains), and RY34 (containing the PKA phosphorylation site) projects upward from the bridging solenoid (Fig. 5c, d).

The putative Ca\textsuperscript{2+}–binding domain was placed in a similar fashion to the other domains for which template structures were available, using the structure of the C-lobe of yeast calmodulin (PDB code 1LJ1) as a starting model. The rest of the model was built manually in Coot, using the secondary structure recognition algorithms in Coot to place and extend helices and build the linkages between them. Directionality was determined based on extension of the structure from known fragments, most importantly the N-terminal fragment, and secondary structure predictions\textsuperscript{55} were used to guide alignment of the sequence with the model. After an initial build, segments of the structure were flexibly fit to the density using the real-space refinement algorithms implemented in Coot, with ProSMART restraints (on the basis of high-resolution reference structures where available, otherwise on the assigned secondary structure) applied to allow for deformation and curvature of helices without degrading local geometry. The final model is presented as a C\textsubscript{a} trace, comprising in full 3,757 ordered residues for each of the four RyR protomers, and 105 residues for each of the four bound calstabin2 subunits.

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Extended Data Figure 1 | Skeletal muscle RyR1 purification. a, Coomassie blue staining of SDS–PAGE showing molecular weight standards (MWS), CHAPS-solubilized sarcoplasmic reticulum membrane (SR), RyR1 eluted with calstabin2 from a glutathione S-transferase (GST)–calstabin1 affinity chromatography column (AC), the eluted RyR1 from fast protein liquid chromatography (FPLC) size-exclusion chromatograph (SEC). b, FPLC plot showing the RyR1 peak at ~7 ml elution and the excess calstabin2 (Cs2) peak at ~12 ml elution. c, Immunoblot analysis of CIP-treated RyR1 probed at indicated time points with (from bottom) anti-RyR1 (34C) antibody, anti-phosphotyrosine antibody (pTyr; Abcam ab10321), anti-phosphothreonine antibody (pThr; Abcam ab9851), anti-phosphoserine antibody (pSer; Abcam ab9332) and anti-RyR phospho-specific antibody (RyR1-pS2843) that recognizes the PKA phosphorylated site on RyR1.
Extended Data Figure 2 | Particle picking and two-dimensional class averages. 

**a**, Sample micrograph of the RyR1-CIP-EGTA data set after motion correction, with red boxes around the particles picked by Autopicker. Scale bar, 500 Å.

**b**, Sample power spectrum of a twice-decimated micrograph after motion correction.

**c**, Euler angle distribution before symmetry was imposed of the particles that went into the CIP-treated data set final reconstruction. Latitude (radial distance) corresponds to \( \theta \) from 0 to 90°. Longitude (position on the circle) corresponds to \( \phi \), from 0 to 360°. The dots colour and area represent the number of particles in each view.

**d**, Two-dimensional projections of the final CIP-treated map (upper rows; red lines) compared to their respective reference-free two-dimensional class averages (lower rows).

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Extended Data Figure 3 | Classification and protomer boundaries.
a, Classification of the RyR1-EGTA data set. First row, refined volume with all particles. Second row, primary classification with a number of classes ($K = 10$) giving rise to two major classes, one refined to 4.8 Å (blue box) and one refined to 5.0 Å (green box). This class (green box) was subclassified with $K = 10$ (third row), and yielded one class with a missing or disordered cytosolic portion of a protomer (red box). b, Classification of the RyR1-EGTA-CIP-treated data set with $K = 8$. Class 8 (purple box) was refined to 5.0 Å. c, Views from the cytosol, membrane plane and lumen of the RyR1 model superimposed with a difference map between the full tetramer map (blue box) and the map with the cytosolic region of one protomer missing or disordered (red box).
Extended Data Figure 4 | RyR1 cryo-EM local resolution map. a, Gold-standard FSC curve for the three-dimensional reconstructions, marked with resolutions corresponding to FSC = 0.143. b, Cytosolic, membrane plane and luminal views of RyR1 (EGTA- and CIP-treated data set) local resolution distribution from 4 (blue) to 6 (red) Å resolution. c, Local resolution distribution through a slab of density coincident with channel axis. d, Same slab as c for the EGTA-treated without CIP. e, Slices through the volume of the CIP-EGTA data set (top) and EGTA data set (bottom). Slice direction and number are indicated on the images.
Extended Data Figure 5 | Representative densities of RyR1-selected regions.

a–e, Representative density (grey mesh) in selected regions of the map. The protomers are represented as Cα traces, in different colours for clarity, with enlarged views of the following regions: calstabin2 (a), the bridge solenoid (b), NTD (c), the pore region (d) and S6 (e).
Extended Data Figure 6 | RyR1 local model to map correlation. a, Cytosolic, membrane plane and luminal views of the local correlation (calculated in a 5 × 5 voxel sliding window) between a map calculated from the model (filtered to 5 Å) and the density map of dephosphorylated RyR1, depicted in spectral colouring from 0.7 (red) to 1 (blue). b, c, Local model/density map correlation within a slab of density through the plane of the membrane, highlighting the unmodelled rod of density on the periphery of the transmembrane region (b) and a slab coinciding with the channel axis (c).
Extended Data Figure 7 | α-solenoid subdomains. RyR1 density map (grey semitransparent surface) superimposed with the α-solenoid scaffold of RyR1. a, Core of the α-solenoid scaffold (insertions and elaborations not shown). Green, bridging solenoid; blue, NTD solenoid; red, core solenoid. b, Alignment of NTD with an α-solenoid structure (PDB code 3NMX). c, Alignment of core solenoid with an α-solenoid structure (PDB code 1G3J). d, Overlay of bridging solenoid with an α-solenoid structure (PDB code 1WA5). In b–d, RyR1 α-solenoid repeats are depicted in spectral colouring from blue (N terminus) to red (C terminus), and the aligned α-solenoid protein is represented in dark grey.
Extended Data Figure 8 | Architecture of bridging and core solenoids.

a, Density map of RyR1 in dark blue mesh superimposed with the bridging solenoid shown in detail on right, as labelled.

b, Two views of the interaction of the core solenoid (spectral colouring) containing the putative Ca$^{2+}$-binding domain with CTD (grey) as labelled.
Extended Data Figure 9 | Calstabin2-binding site. Views in the membrane plane and cytosol of RyR1 with enlarged views of calstabin2 (yellow) bound to RyR1. SPRY1 is depicted in light blue, SPRY2 in cyan, the bridging solenoid in green and the calstabin binding helix in purple.
Extended Data Figure 10 | Putative Ca\textsuperscript{2+}-binding domain in RyR1.  

a, Sequence alignment of rabbit RyR1–3 with the C-lobe of human calmodulin (hCaM).

b, Structural alignment of the C-lobe of yeast calmodulin with the model of RyR1.