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# Conformational Change Metrics of the Ryanodine Receptor Studied by CryoEM

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The ryanodine receptor isoform 1 (RyR1) is an intracellular ion channel with an important role in depolarization-induced $Ca^{2+}$ release and skeletal muscle contraction [1]. Several of RyR1's molecular partners are distantly situated from its ion gate, suggesting that long-range conformational pathways play an important role in RyR1's function. DHPR, the "voltage sensor" for RyR1, is situated in the plasma membrane, and fully controls the activity of RyR1 through a stable quaternary arrangement. With a molecular weight of 2.2 MDa, RyR1 is the largest ion channel known; its cytoplasmic domain has a square-prism shape of dimensions 275 x 275 x 100 $Å^3$. Thus, the DHPR must be effecting the ion gate over distances larger than 100 Å. FKBP12, a subunit of RyR1, binds to the cytoplasmic domain 135 Å away from the ion gate, and its removal induces subconductance states in RyR1.

We have compared the following two kinds of conformational changes by cryo electron microscopy, single particle image processing and analysis of the 3D reconstructions: those resulting from the transition of the closed to the open state and those resulting from the removal of FKBP12 under closed-state conditions. The resolutions of the 3D density maps used in this analysis are 10 Å (opening of RyR1) [2] and 13 Å (removal of FKBP12 from RyR1) [3] . The volumes were fragmented into discrete globular domains [4] (see Figure 1A) such that the domains were equivalent in mass and shape between the pair of 3D reconstructions under comparison. Then the coordinates of the center of gravity of each domain were determined in Chimera [5] and the change in these coordinates for each domain was represented as a vector.

The vector representation indicates movement of different magnitudes and directions along the 3D structure of RyR1, with a characteristic trait whereby both the vector's magnitude and the direction are similar amongst neighboring domains. Two regions (per subunit) do not follow the contiguity tendency, forming hinges. The largest magnitude conformational changes, of 10 Å, are found in the vicinity of the putative area of overlap with the DHPR (figure 1B-D), delineating a long-range allosteric pathway that could connect the DHPR and RyR1's ion gate, and suggesting a rationale to understand the tight functional coupling between these two proteins in skeletal muscle. On the other hand, the removal of FKBP12 yielded vectors of similar direction to those determined for the opening, albeit of less magnitude. This is in line with the evidence that the removal of FKBP12 induces subconductance states in RyR1, as it might be expected that subconductance states share conformational characteristics with the open state. The reproducible conformational change found upon both opening and removal of FKBP12 suggests

that the 3D architecture of the RyR1 defines a limited set of long-range conformational pathways.

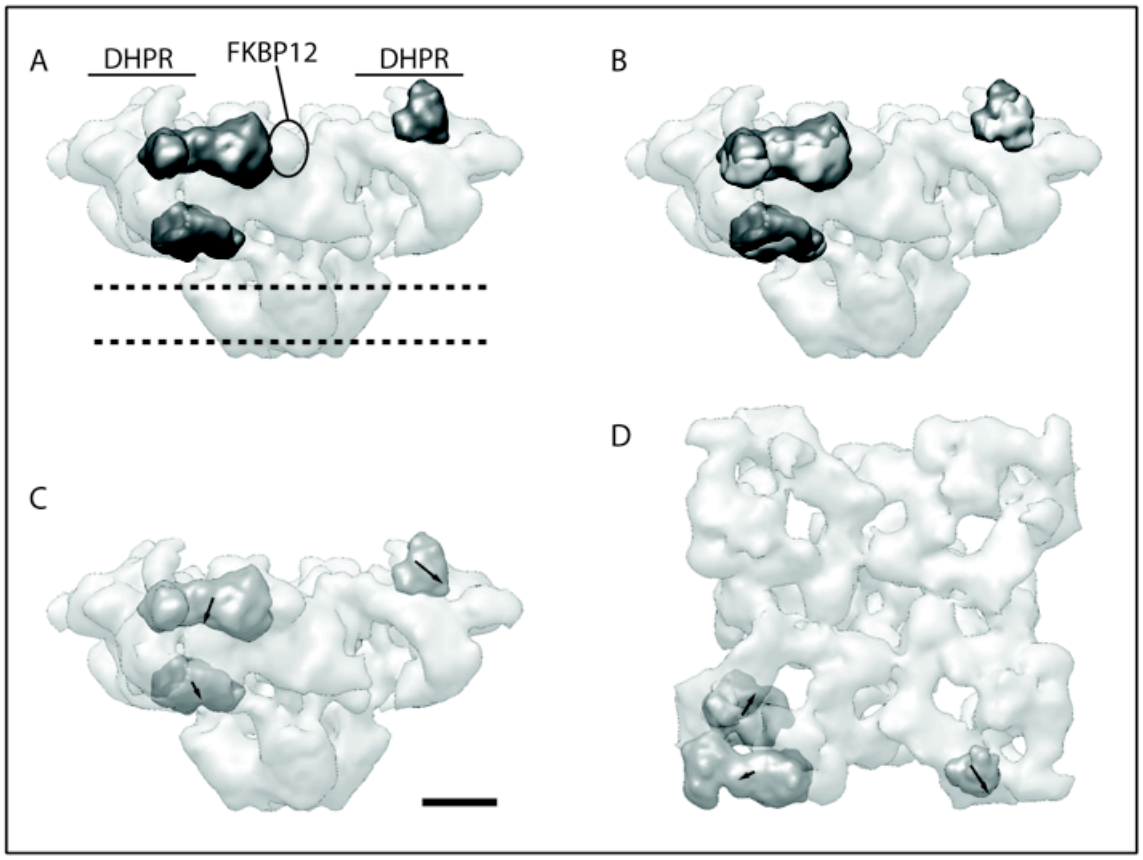


FIG. 1. Volume fragmentation for three domains in the closed and open states, and vector representation of their trajectory. (A) RyR1 in its side view in the closed state, with three fragments highlighted (dark gray). (B) The positions of the same fragments in the open state (lighter gray) are superimposed onto those of the closed state. (C) The vectors represent the movement undergone by the indicated fragments. The length of the vectors has been scaled up 3-fold for better visualization. (D) The same vectors represented on RyR1 in its fourfold view as seen from the cytoplasm. The location of FKPB12, the general area of overlap with the DHPR, and the endoplasmic reticulum membrane are indicated. Scale bar, 50 Å.

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