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Three-Dimensional Structure of Calcium Release Channel from Skeletal Muscle by Electron Cryomicroscopy and Angular Reconstitution

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Electron cryomicroscopy has been advanced to be a unique tool to study macromolecular assemblies which cannot be readily tackled by X-ray crystallography and NMR (Chiu 1993). The reliability of the technique has been illustrated by a number of examples which the X-ray structures of the individual components are known (Jeng et al. 1989, Wang et al. 1992). For the examination of biological structures at different functional or chemical states, advantages of using ice-embedded specimens have been well established (Chiu 1986). For reconstructing the 3-dimensional structures of single particles, the approach of Frank's group is based on the assumption that the particles in the electron images would have the same orientations and their method has been applied to study a variety of specimens (Penczek et al. 1992, Radermacher et al. 1994). An alternative approach of performing three-dimensional reconstruction with particles at random orientations called angular reconstitution has been proposed by van Heel (van Heel 1987, van Heel et al. 1992). This paper reports the first biological application of the angular reconstitution to determine the three-dimensional structure of the Ca^{2+} release channel from rabbit skeletal muscle in a closed state (Serysheva et al. 1995).

The calcium release channel is located in the gap between the transverse tubular system and the junctional membrane of the terminal cisternae of SR. It contains a membrane spanning region and a soluble region with 4 homotetramer of total molecular mass of 2.4 million daltons. The Ca^{2+}-release channel is solubilized and purified from rabbit skeletal muscle (Hawkes et al. 1989). The Ca^{2+}-release channel protein was driven towards its closed state by depleting the Ca^{2+} with 1 mM EGTA in 300 mM KCl, 10 mM MOPS (pH 7.4). Figure 1 shows an electron image of the ice-embedded Ca^{2+}-release channel with a moderate contrast. Although a good number of the channel proteins are oriented with its square shape face perpendicular to the plane of the grid, we have found single channel proteins in all possible orientations.

The 3D structure of the Ca^{2+} release channel seen from two different directions is shown in Figure 2. The view of the channel protein facing the SR lumen side shows an outer square and an inner square, which are rotated with respect to each other over an angle of ~ 40° (top panel). The view of the protein, facing the cytoplasm towards the transverse tubule, shows a central opening of ~ 50 Å in diameter (bottom panel). The rim of the central channel forms a continuous network that extends to the peripheral mass. At the corners of the peripheral mass is a most characteristic domain shaped like a closed laboratory clamp with four fingers. "Handles" or "bridging domains"

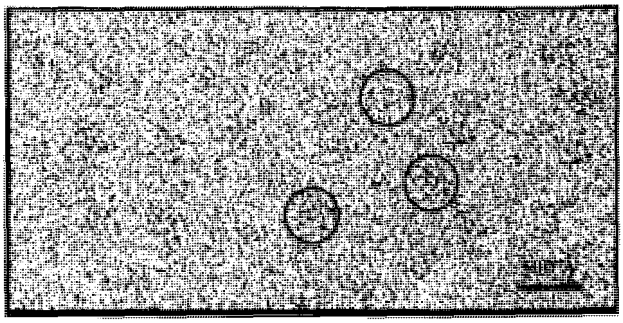

Fig. 1. 100 kV electron image of Ca^{2+} release channel embedded in ice. Several images of the individual channel proteins are circled and appear to have different orientations.

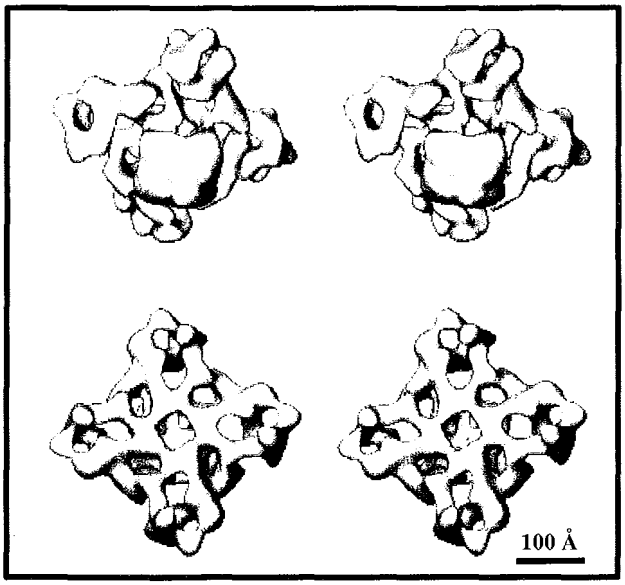

Fig. 2. Stereo views of the 3D structure of ice-embedded Ca^{2+}-release channel in closed state as viewed from the SR lumen side (top panel) and from the cytoplasmic side (bottom panel). The volume of the reconstruction at the chosen density threshold level corresponds to a mass of 2.4 million daltons assuming a protein density of 1.35 g/cm^3. It is a homotetramer with a membrane and a cytoplasmic region. The image reconstruction was carried out with IMAGICS software as following: The selected particle images were aligned by iterative multi-reference alignment techniques, and sorted into homogeneous classes of similar images by automatic multivariate statistical classification procedures. All molecular images belonging to the same class were averaged to produce noise-free class averages or characteristic views. The class averages were assigned to angles by angular reconstitution technique. The 3D reconstruction was computed and used for realignment to improve the image statistics and accuracy in angular determinations. The final 3D map was computed from 150 class averages with a sufficiently uniform distribution of Euler angles to attain 30 Å isotropic resolution. The resolution of the structure was estimated to be ~30 Å based on the Fourier shell correlation coefficients of two independent

interconnect the four clamps. Each of these features may represent distinct domains of the protein. As would be expected for a closed channel, there is no obvious opening of the Ca^{2+}-release channel on the lumenal side of this membrane protein.

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