BL-1A, BL-5A, BL-17A, AR-NW12A, AR-NE3A/ 2016G015, 2014G179

Crystal structures of eukaryotic and prokaryotic TRIC channels

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1 Introduction

Ca^{2+} signaling underlies a variety of fundamental cellular activities including muscle contraction, neurotransmitter release, cell growth and apoptosis[1]. Release of Ca^{2+} from the intracellular stores is supported by a series of ion channels in sarcoplasma or endoplasma reticulum (SR/ER). Among them, two isoforms of the TRimeric Intracellular Cation (TRIC) channel family, named TRIC-A and B, modulate the release of Ca^{2+} through ryanodine receptor (RyR) or inositol triphosphate receptor (IP_3R), and maintain the homeostasis of ions within SR/ER lumen[2]. As a SR/ER ion channel permeable to monovalent cations, TRIC channels may account for the activities of long-sought SR K^+ channel[3]. TRIC-A channel may regulate the Ca^{2+}-release process through RyR, and contributes to the maintenance of normal blood pressure[4]. TRIC-B channel is believed to be involved in modulating the Ca^{2+}-release activity of IP_3R and is essential for the perinatal lung development[5], collagen synthesis and bone mineralization[6]. Mutations of the gene encoding TRIC-B (*TMEM38B*) in human are associated with a hereditary brittle bone disease (osteogenesis imperfecta)[7,8]. According to previous studies, TRIC channels are crucial components for the Ca^{2+} signaling and excitation-contraction coupling in eukaryotes, and the orthologs found in bacteria and archaea are likely involved in K^+ uptake in prokaryotes[3,9].

Despite the important physiological functions of TRIC channels, the lack of high-resolution structural information has hindered elucidation of their pore architectures and gating mechanisms. Here we present structural studies on two eukaryotic TRIC-B channels (with/without Ca^{2+}) from *Caenorhabditis elegans* (*Ce*TRIC-B1/B2)[10], as well as a prokaryotic TRIC channel from *Sulfolobus solfataricus* (*Ss*TRIC)[11].

2 Experiment

Protein expression and purification

The genes encoding *Ce*TRIC-B1/B2 were synthesized (Genscript) with optimized codon usage for protein expression in *Pichia pastoris.* The target cDNA was inserted into the pPICZ-A (or pPICZ-C for CeTRIC-B2) vector. To improve stability of protein, 48 and 61 amino acid residues at the flexible C-terminal regions of *Ce*TRIC-B1 and B2 were truncated, respectively. *Ce*TRIC-B1/B2 was expressed in *P. pastoris* GS115 strain cells with 0.05% methanol for 48 h at 24℃. The cells were harvested and the protein was extracted from the membrane by 1.5% Triton X-100.

The gene encoding *Ss*TRIC protein was synthesized (GenScript) with optimized codon usage for protein expression in *Escherichia coli* and inserted into pET21b vector. Expression of the *Ss*TRIC protein was carried out in C41(DE3) cells with 1 mM IPTG for 2 h at 37℃. The cells were harvested and the membrane protein was solubilized by 1.5% dodecyl-β-D-maltoside (β-DDM).

Purification of the target proteins was achieved through nickel/cobalt-affinity chromatography in solutions with n-decyl-β-D-maltopyranoside(DM). The eluted protein from affinity-column was concentrated and further purified by passing through the gel filtration column Superdex 200 10/300 GL. The protein was concentrated to 10-20 mg/ml for crystallization screening and optimization.

Crystallization

The *Ce*TRIC-B1 protein was initially crystallized through hanging-drop vapor diffusion by mixing the protein sample with a well solution containing 22% PEG550 MME, 0.2 M NaCl, 0.1 M HEPES pH7.0, and the CH_3HgCl-derivated crystal in this plate form(in $C222_1$ space group) was used for phasing. Another kind of tetragonal-bipyramid crystals (in $P4_12_12$ space group) from *Ce*TRIC-B1 was grown with the reservoir solution containing 20-24% PEG400, 10% glycerol, 50 mM ADA buffer pH6.5, and the Ca^{2+}/Rb^+/Cs^+- derivatized crystals in this form were used for structure refinement and analyses.

The *Ce*TRIC-B2 protein was crystallized via hanging-drop vapor diffusion by mixing 1μl protein with 1 μl well solution containing 20% PEG400, 50 mM NaAc pH 4.4, 50 mM $MgAc_2$ and 10 mM betaine hydrochloride.

The crystals of native and CH_3HgCl/Tl^+ derivated *Ss*TRIC were obtained through hanging-drops vapor diffusion method by mixing 10 mg/ml protein with a reservoir solution containing 22%-26% PEG 3000, 0.1 M Tris-HCl pH 8.0, 0.2 M NaAc, 0.2 M KCl and 30% ethylene glycol at 1:1:0.2 (v:v:v) ratio.

Data collection and structure determination

Screening of crystals were mainly performed on BL1A, BL5A, BL17A and NW12A beamlines at PF.

The anomalous diffraction data of Ca^{2+}/Rb^+/Cs^+-derivatized *Ce*TRIC-B1 crystals were collected at BL1A and BL5A. The datasets of CH_3HgCl-derivatized *Ce*TRIC-B1, native *Ce*TRIC-B2 and native *Ss*TRIC were collected at BL17U of Shanghai Synchrotron Radiation Facility (SSRF). The data of Tl^+-containing *Ss*TRIC were

collected at home source. For data processing, iMosflm or HKL2000 programs were used.

The initial experimental phase was solved by using the CH_3HgCl-derivatized *Ce*TRIC-B1 dataset through the single-wavelength anomalous diffraction (SAD) method by using the Autosol program of the Phenix suite. The structural model of *Ce*TRIC-B1 was completed through iterative manual model building in COOT and refinement in CNS program. The structure of *Ce*TRIC-B2 were solved through molecular replacement.

For *Ss*TRIC, the structure was solved by SAD with anomalous diffraction dataset of Hg-labeled A15C crystal.

The structures were refined by using CNS, Phenix and Refmac programs.

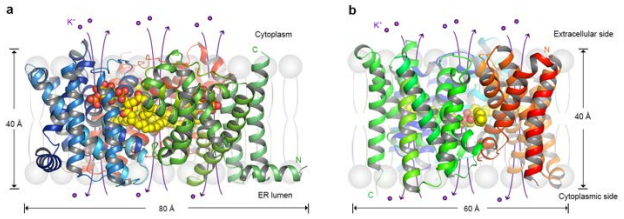

Fig. 1 Structures of TRIC channels. a, The homotrimeric *Ce*TRIC-B1 channel in complex with phosphatidylinositol 4,5-biphosphate (PIP_2) lipid molecules. b, The trimeric *Ss*TRIC channel in complex with triacylglycerol. The protein subunits are presented as ribbon models, the PIP_2 and triacylglycerol molecules are shown as yellow-and-red sphere models. Both structures are viewed along the membrane plane.

3 Results and Discussion

We have solved the structures of *Ce*TRIC-B1 and *Ce*TRIC-B2 channels at two different conformational states (with/without Ca^{2+}-bound) at 3.3 and 2.3 Å resolution respectively, and the structure of *Ss*TRIC channel at 2.2 Å resolution. Our data demonstrated that both eukaryotic and prokaryotic TRIC channels form symmetrical homotrimer with a novel asymmetrical ion-permeating pathway within each monomer. The center of the hourglass-shaped pores are flanked by funnel-like vestibules on cytoplasmic and luminal sides of eukaryotic TRIC channels, or on cytoplasmic and extracellular sides of prokaryotic TRIC channels. The pore center may contain a monovalent cation-binding site likely contributing to the ion selectivity of the TRIC channels.

At the same time, we also discovered several striking features about TRIC channels from structural views. For the *Ce*TRIC-B channels, TRIC-B1/B2 proteins specifically bind an endogenous lipid named phosphatidylinositol 4,5-biphosphate (PIP_2). The PIP_2 molecule mediates trimerization of TRIC channels, contributes directly to the formation of pore architecture, and interacts with the putative voltage-sensing motif as well as a Ca^{2+}-binding loop region. While the finding of a PIP_2 molecule in the intracellular ion channel is unprecedented, it has expanded our current knowledge about the role of PIP_2 in the function of ion channels. Furthermore, by comparing the Ca^{2+}-bound and Ca^{2+}-free conformations, a mechanistic model has been proposed to demonstrate the intriguing gating mechanism of eukaryotic TRIC channels[10].

For SsTRIC, the monomer-monomer interfaces within the trimer were stabilized by hydrophobic interactions between the protein and a putative triacylglycerol molecule instead of a PIP_2 molecule. Meanwhile, the cytoplasmic vestibule of *Ss*TRIC is occluded by a plug-like motif in the loop region between the fifth and sixth transmembrane helices. The plug-like motif binds to the pore lumen in a position resembling that of the PIP_2 head group in CeTRIC-B1/B2. Based on biochemical and electrophysiological data, a mechanistic gating model of *Ss*TRIC channel has been proposed[11].

In conclusion, structural and functional analyses on CeTRIC-B1/B2 and SsTRIC provide a unified framework for understanding the gating mechanism of both prokaryotic and eukaryotic TRIC channels.

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