Structural and mechanistic studies on a human calcium ATPase SERCA2b by cryo-EM single-particle analysis

Title of Ph.D Dissertation:

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(クライオ電子顕微鏡単粒子解析によるヒトカルシウム ATPase SERCA2b の構造および機構に関する研究)

Graduate School of Life Science
Tohoku University
ZHANG YUXIA

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分子化学生物学専攻

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Introduction

The calcium ion (Ca^{2+}) is one of the most common and important second messengers, and it regulates lots of physiological functions, including muscle contraction, neuronal excitability, organelle communication, cellular motility, fertilization, cell migration and growth. Considering versatile and critical roles of Ca^{2+}, the cellular systems to maintain the Ca^{2+} homeostasis have drawn lots of attention, and their defects have been reported to cause some fatal diseases including diabetes, cardiovascular disease and cancer. Generally, the concentration of intracellular free Ca^{2+} varies widely depending on its location. The concentration of Ca^{2+} in the cytoplasmic under steady conditions is about 100nM, which is 10,000-fold lower than that in the extracellular space (*1*). Furthermore, Ca^{2+} concentration is largely different inside and outside of some organelles, such as the endoplasmic/sarcoplasmic reticulum (ER/SR) and the Golgi apparatus. The most well-known calcium storage organelle is the ER, which also catalyzes post-translational processes such as N-linked glycosylation and disulfide bond formation (*2*). Importantly, calcium depletion inside the ER impairs structure and function of many intracellular proteins and hence generates unfolded or misfolded proteins. The accumulation of misfolded proteins triggers unfolded protein response (UPR)(*3*), which could eventually lead to cell death. Notably, there is a large gradient of Ca^{2+} concentration between the cytosol and the ER lumen, which reaches as high as 10,000-times difference(*4*). The gradient is maintained by the ER-resident Ca^{2+} pumps, Ca^{2+} binding proteins and Ca^{2+} channels. Ca^{2+} channels release Ca^{2+} from the ER via IP3R and RyRs (Fig. 1) (*4*). Ca^{2+} binding proteins such as stromal interaction molecule (STIM) sense the decrease of intracellular Ca^{2+} concentration via the EF hand motifs, and thereby activate the Ca^{2+} influx to increase the Ca^{2+} concentration in the ER lumen(*5*). The most famous and important Ca^{2+} pump located in the ER is the endoplasmic/sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) family members, which transport large amount of Ca^{2+} into the ER lumen utilizing energy released from the ATP hydrolysis (Fig. 1). Here, we mainly focus on the structure and molecular mechanisms of SERCA2b since this isoform is ubiquitously expressed and has unique structural and mechanistic features due to the presence of the extra 11th transmembrane helix (TM11) and 12-residue luminal extension tail (LE) at the C-terminal region.

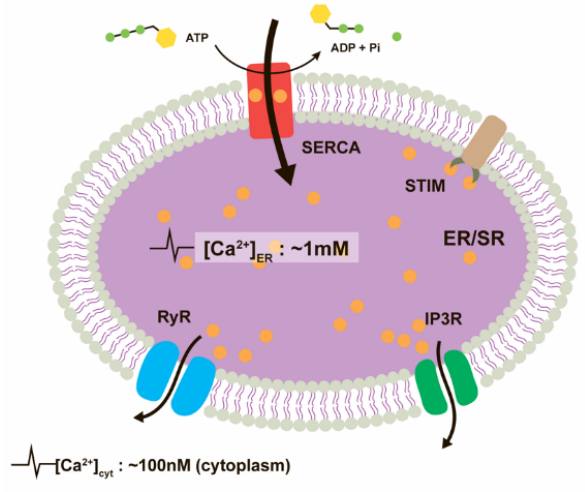

Fig. 1 Ca^{2+} pump (SERCA) and channels (RyR & IP3R) involved in the Ca^{2+} homeostasis in the ER

Results

To investigate the mechanism underlying the structural and functional regulation of SERCA2b by the C-terminal extension, we determined cryo-EM structures of human SERCA2b in E1·2Ca^{2+}-AMPPCP and E2-BeF_3^- states at resolutions of 2.9 Å and 2.8 Å, respectively. The high-resolution structures of SERCA2b illuminated both the backbone and side-chain conformations over almost the entire part of this Ca^{2+} pump, including the three cytosolic domains, 11 transmembrane helices, and the luminal extension (LE) in the cryo-EM maps of both the E1·2Ca^{2+}-AMPPCP and E2-BeF_3^- states. The cryo-EM structures show that SERCA2b undergoes a significant rearrangement in both the cytosolic and TM domains upon ATP hydrolysis and subsequent ADP dissociation, leading to the facilitated release of Ca^{2+} (Fig. 2).

Furthermore, to define the location of the LE and gain deep insight into its regulatory roles, we determined cryo-EM structures of SERCA2b T1032stop, a truncated construct that spans the protein chain up to TM11 but lacks the LE. According to the structural comparisons between SERCA2b WT and T1032stop, SERCA2b undergoes a significant relocation of both the cytosolic and TM domains accompanied by the generation of multiple overall conformations, allowing the unregulated transition from E1·2Ca^{2+}-ATP to E2P states.

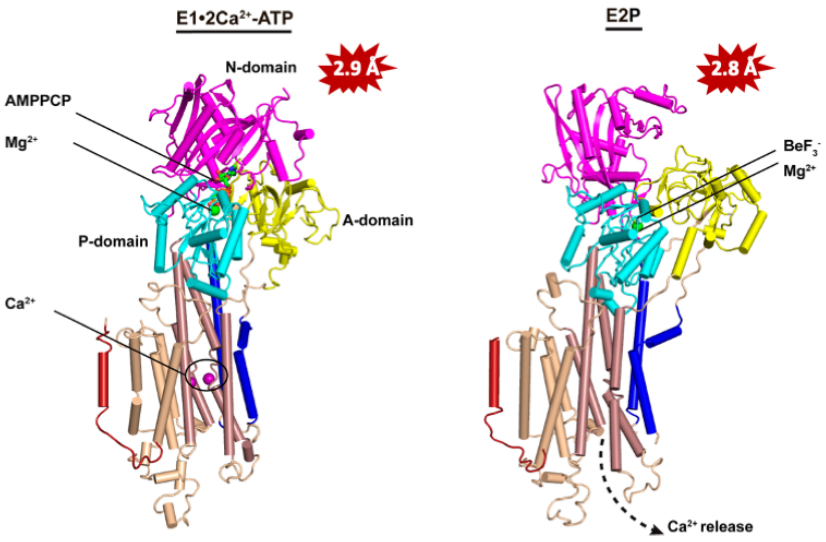

Fig. 2 cryo-EM structures of SERCA2b in E1·2Ca^{2+}-ATP and E2P states

Consequently, our present findings of the wild-type (WT) SERCA2b and the T1032stop variant reveals the exact location of the LE and the mechanism of the LE-mediated structural regulation in SERCA2b.

Conclusions

The experimental methods for the high-yield production of human SERCA2b and the preparation of its different reaction intermediates toward extensive structure analyses have been established. Furthermore, a series of high-resolution cryo-EM structures of SERCA2b revealed functional roles of TM11 and the LE characteristic of SERCA2b, and tempted us to propose new mechanistic models of this Ca^{2+} ATPase. The present structural studies have greatly advanced our understanding of molecular mechanisms of the regulated catalytic cycle of SERCA2b.