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Review

Structural aspects of ion pumping by Ca^{2+}-ATPase of sarcoplasmic reticulum

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ABSTRACT

Ca^{2+}-ATPase of muscle sarcoplasmic reticulum is an ATP-powered Ca^{2+}-pump that establishes a >10,000-fold concentration gradient across the membrane. Its crystal structures have been determined for nine different states that cover nearly the entire reaction cycle. Presented here is a brief structural account of the ion pumping process, which is achieved by a series of very large domain rearrangements.

Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum (SERCA1a), an integral membrane protein of M_r 110 K, is an ATP-powered Ca^{2+}-pump and comprises a single polypeptide chain of 994 amino acid residues. It was first identified in the 'relaxing factor' of muscle contraction and gave rise to the calcium theory that Ca^{2+} is a fundamental and ubiquitous factor in the regulation of intracellular processes [1]. In muscle contraction, Ca^{2+} is released from sarcoplasmic reticulum (SR)[1] into muscle cells via Ca^{2+}-release channel. Ca^{2+}-ATPase then pumps back the released Ca^{2+} into the SR to cause relaxation. This pump runs as long as ATP and Ca^{2+} are present in the cytoplasm, and establishes a $>10^4$-fold concentration gradient across membranes. SERCA1 is both structurally and functionally the best characterised member of the P-type (or E1/E2-type) ion translocating ATPases. According to the classical E1/E2 theory [2,3], transmembrane Ca^{2+}-binding sites have high affinity and face the cytoplasm in E1, and have low affinity and face the lumen of SR (or extracellular side) in E2. Actual transfer of bound Ca^{2+} is thought to take place between two phosphorylated intermediates, E1P and E2P, in exchange of H^+ from the lumenal side (inset in Fig. 1 for a simplified reaction scheme).

Several types of Ca^{2+}-ATPases exist in different tissues [3]. Although all transfer Ca^{2+} from the cytoplasm to the opposite side of the membrane and countertransport H^+, they appear to have different stoichiometry of Ca^{2+}:H^+:ATP. It is well established that SERCA1a can transfer two Ca^{2+} per ATP hydrolysed in the forward direction [4] and 2–3 H^+ in the opposite direction [5]. Therefore, active transport of Ca^{2+} is an electrogenic process, although no H^+ gradient is built up across the SR membrane because it is leaky to H^+. This Ca^{2+}/H^+ exchange may cause pathological pH effects with plasma membrane Ca^{2+}-ATPase [6].

Because Ca^{2+} is critically important in regulation of biological processes, malfunctioning of Ca^{2+}-pumps is likely to be deleterious to living organisms. Therefore, although Darier and Hailey–Hailey diseases are well-known [7], human diseases related to Ca^{2+}-ATPases are not many. Conversely Ca^{2+}-ATPases can be utilised for killing cells. For instance, cancer cells may be killed by delivering a potent inhibitor of this pump, such as thapsigargin (TG) [8]. Artemisinin, a well-known anti-malarial drug inhibits a malarial Ca^{2+}-ATPase [9]. In cardiac muscles, phospholamban and sarcolipin work as regulators of Ca^{2+}-ATPase and are becoming therapeutic targets [10].

We crystallised Ca^{2+}-ATPase from rabbit white skeletal muscle (SERCA1a) in phospholipid bilayer and solved the first atomic structure (E1 · $2Ca^{2+}$ form) in 2000 [11]. By now more than 20 crystal structures have been reported from three laboratories for this ATPase in nine different states that approximately cover the entire reaction cycle (Table 1; Fig. 1) [11–18]. We do not yet have an exact analogue of E1P. However, as shown by limited proteolysis [19,20], domain organisations of E1 · AMPPCP and E1 · AlF_4^- · ADP crystal structures are virtually the same [13,16] and expected to be very similar to that of E1P. Thus, we can describe the entire reaction cycle with four principal structures presented in Figs. 1 and 2. We also carried out all-atom molecular dynamics simulations for wild-type and some mutants to understand the functional roles of critical residues [21]. As a result, we can now propose a fairly detailed scenario of ion pumping and

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[1] *Abbreviations used:* SR, sarcoplasmic reticulum; TG, thapsigargin.

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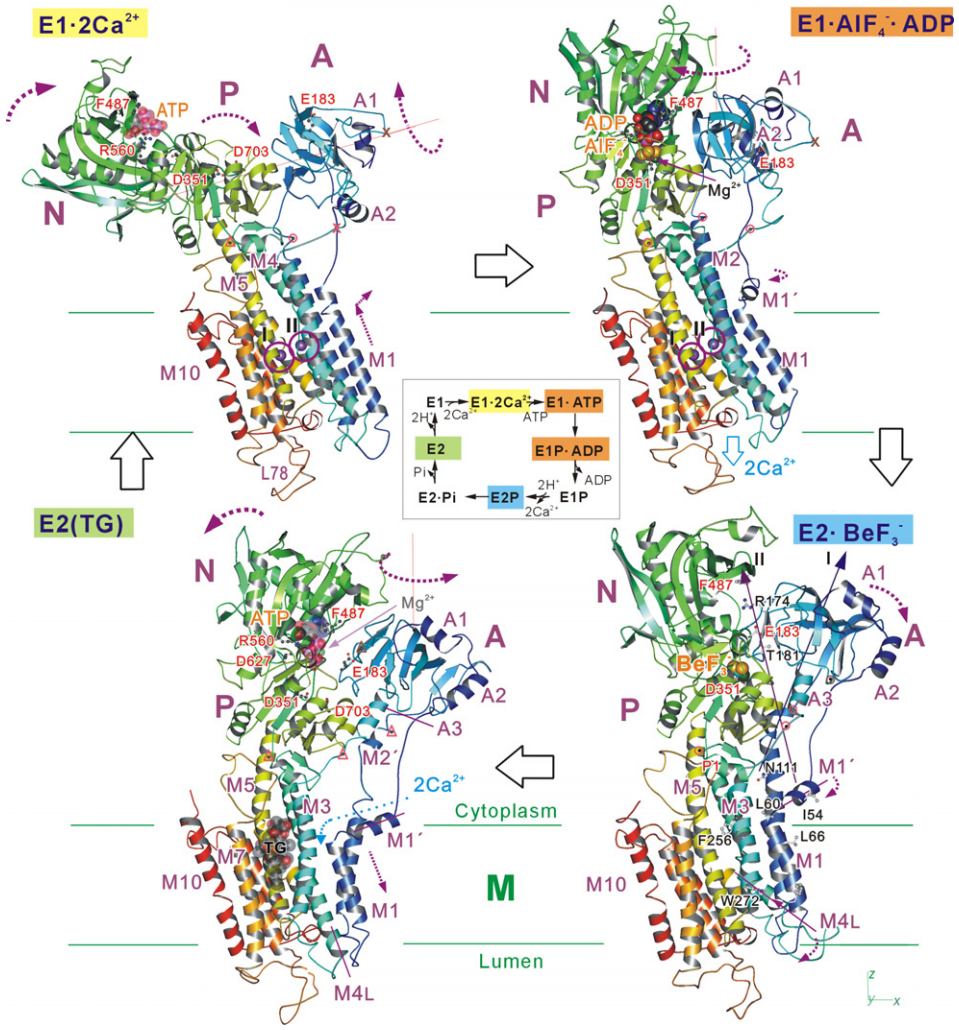

Fig. 1. Front views (parallel to the membrane ($x - y$) plane) of Ca^{2+}-ATPase in four different states and a simplified reaction scheme (showing only the forward direction), in which different colours correspond to the respective structures presented here. Colours change gradually from the amino terminus (blue) to the carboxy terminus (red). Purple spheres (numbered and circled) represent bound Ca^{2+}. Three cytoplasmic domains (A, N and P), the α-helices in the A-domain (A1–A3) and those in the transmembrane domain (M1–M10) are indicated. M1′ is an amphipathic part of the M1 helix lying on the bilayer surface. Digestion sites are shown in small circles (protected), triangles (partially protected) and crosses (not protected) for proteinase K (orange) and trypsin (T2 site, brown). Docked ATP and TG (thapsigargin) are shown in transparent space fill. Several key residues—E183 (A), F487 and R560 (N, ATP binding), D351 (phosphorylation site), D627 and D703 (P) are shown in ball-and-stick. Axis of rotation (or tilt) of the A-domain is indicated with thin orange line except for E2 · BeF_3^-, in which two long arrows indicate the axis for E1P → E2P (I) and that for E2P → E2 · Pi (II) [24]. PDB accession codes are 1SU4 (E1 · $2Ca^{2+}$), 2ZBD (E1 · AlF_4^- · ADP), 2ZBE (E2 · BeF_3^-) and 2AGV(E2 (TG)). Atomic co-ordinates of the aligned models are available at the author's web site (http://www.iam.u-tokyo.ac.jp/StrBiol/resource/res.html).

describe how the affinity of the transmembrane Ca^{2+} binding sites is altered, and how the lumenal gate is opened and closed by events that occur around the phosphorylation. They also allow us to approach more fundamental questions like (i) what are the roles of ATP [13] and phosphorylation [14], (ii) why such large domain movements are necessary [14], and (iii) why H^+ countertransport is necessary despite that the SR membrane is leaky to H^+ [15].

An earlier overview of Ca^{2+}-ATPase structures is found in [22], and more general ones on P-type ATPases in [3] and [23]. Atomic co-ordinates of the aligned structures, movies on the structural changes during the reaction cycle and some results of molecular dynamics simulations are available in the author's homepage (http://www.iam.u-tokyo.ac.jp/StrBiol/resource/res.html). A very extensive database for mutations (http://www.fi.au.dk/jpa/smd/) is now made available by J.P. Andersen.

Architecture of Ca^{2+}-ATPase

SERCA1a consists of three cytoplasmic domains (A, actuator; N, nucleotide binding; P, phosphorylation), 10 transmembrane (M1–M10) helices and small lumenal loops (Fig. 1). As described below, the A-domain, connected to the M1–M3 helices with rather long linkers, works as the actuator of the transmembrane gating mechanism that regulates Ca^{2+} binding and release (Fig. 2) [14,24]. Crystal structures show that the linkers are flexible [25], yet the mutagenesis studies demonstrate that the length of the A-domain—M1 linker, at least, is critically important in gating of the ion pathway [26,27]. The A-domain contains one of the signature sequences ^{181}TGES motif [3], which plays an important role in dephosphorylation [28–30]. The P-domain contains the phosphorylation residue Asp351, magnesium co-ordinating residue Asp703 and many other critical residues that characterise the P-type ATPase as a member of the haloacid dehalogenase superfamily [31]. These critical residues are also shared by bacterial two-component regulators [32,33], which have a different folding pattern. The P-domain is wedge-shaped and has a flat top surface to allow a large rotation of the A-domain on the top surface of the P-domain (Fig. 3) [24]. The Rossmann fold is particularly suited for this purpose. The N-domain, a long insertion between two parts forming the P-domain, contains the residues (e.g. Phe487) for adenosine binding