Crystal Structure Analysis of Calcium Pump of Sarcoplasmic Reticulum

Nature uses ion gradients across cell membranes very efficiently. When cell membrane excites, ions come into cytoplasm rapidly following the ion gradients. To restore the original resting state, the ions must be pumped back. P-type ATPase is a family of ion transporting ATPases that are responsible for establishing such ion gradients. They include Na^+K^+-ATPase, sarcoplasmic reticulum (SR) Ca^{2+}-ATPase and gastric H^+K^+-ATPase among others. Of these SR Ca^{2+}-ATPase is the simplest and the best studied member. The transport is thought to be achieved by changing the binding site from high affinity and facing cytoplasm (E1 form) to low affinity and facing the extracellular side (E2 form). The reaction cycle completes by transporting another type of ion (H^+ for Ca^{2+}-ATPase) in the opposite direction. This process is called counter transport.

When muscle contracts, large amounts of Ca^{2+} stored in SR are released into muscle cells. Ca^{2+}-ATPase in the SR membrane pumps Ca^{2+} back into SR to relax muscle cells. Compared to channels, which can transfer millions of ions per second, pumps work much more slowly. Ca^{2+}-ATPase can transfer only 2 Ca^{2+} per ATP-hydrolysis or 60 Ca^{2+} per second. To make the relaxation process efficient, SR membrane is full of Ca^{2+}-ATPase (more than 60% of the proteins in SR membrane). Therefore, Ca^{2+}-ATPase is one of the most suitable membrane proteins for structural studies. Also, because Ca^{2+} is the most ubiquitous factor for regulation of cellular processes, elucidation of the mechanism of Ca^{2+}-ATPase has tremendous importance in both biological and medical aspects.

We have been working on this ATPase using X-ray crystallography and determined its structure to

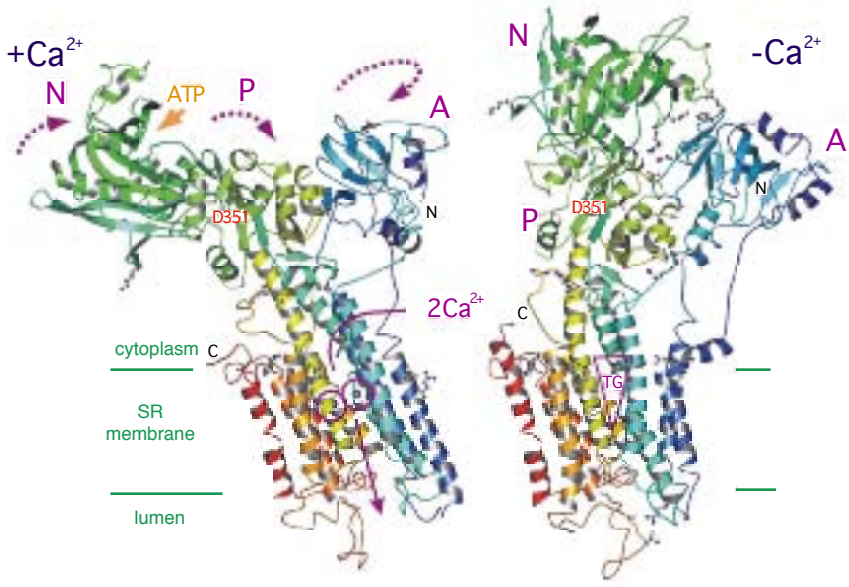

Fig. 1. Structural changes in sarcoplasmic reticulum Ca^{2+}-ATPase accompanying the dissociation of Ca^{2+}. Colour changes gradually from the N terminus (blue) to the C terminus (red). Two purple spheres in the membrane domain represent bound Ca^{2+} ions. Adenosine moiety of ATP binds to the N-domain, whereas the catalytic site (phosphorylation site) Asp351 is located in the P-domain. The binding sites for thapsigargin (TG, a potent inhibitor) is also shown.

2.6 Å resolution for the Ca^{2+}-bound (E1 Ca^{2+}) form [1] and to 3.1 Å resolution for a Ca^{2+}-unbound (E2(TG)) form stabilized by thapsigargin, a very potent inhibitor [2] (Fig. 1). Diffraction data from the E2(TG) crystals were collected at beamline **BL44XU** using imaging plates of 4000 × 4000 pixels. This was essential because one dimension of the unit cell was as large as 600 Å, yet the diffraction spots went out to 3.0 Å resolution.

SR Ca^{2+}-ATPase consists of a single polypeptide chain of 110 kDa. It comprises 3 (A, N and P) cytoplasmic domains and 10 transmembrane α-helices. The differences in structure between the Ca^{2+} -bound and unbound forms are global and very large (Fig. 1). In the transmembrane region, complicated movements of transmembrane helices are observed. In particular, it is surprising to see large (~ 5.5 Å) movements of the M3 and M4 helices normal to the membrane (Fig. 2). Because the M4 helix is a key component of the transmembrane Ca^{2+}-binding sites (Fig. 3), it is clear that such piston-like movements will abolish the binding of Ca^{2+} at site II. Also, the unwound part of M6, another key helix in Ca^{2+} coordination, rotates nearly 90 ° to destroy site I (Fig. 3). It is not obvious why such large movements of the transmembrane helices are necessary. Homology modelling of the cation binding sites of a related pump, Na^+K^+-ATPase, suggests that such movements are required for assuring the release of one type of ion and binding of the other type of ions that are counter-transported at the same time [3].

All three cytoplasmic domains show very large domain movements, keeping the structure of each domain virtually unchanged. In the Ca^{2+}-bound form, they are widely separated but gather to form a compact headpiece in the Ca^{2+}-unbound form.

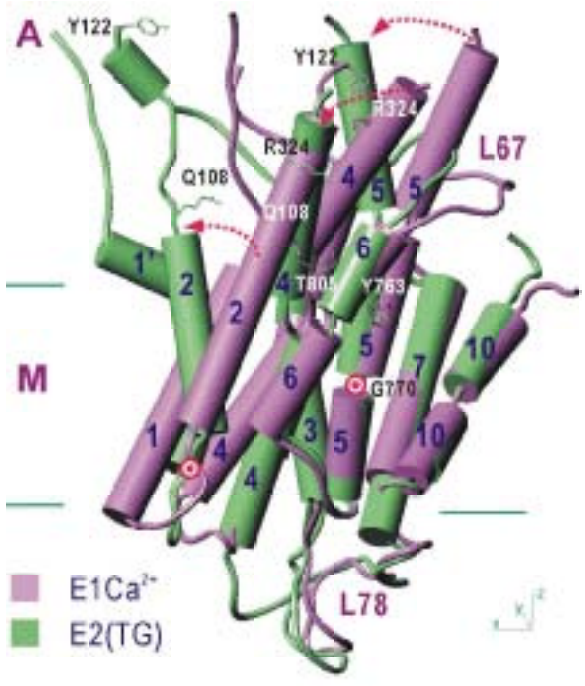

Fig. 2. Rearrangement of transmembrane helices viewed from the side opposite to Fig. 1. Cylinders represent α-helices. Double circles show pivot positions for the M2 and M5 helices. Arrows indicate the directions of movements during the change from Ca^{2+}-bound to unbound form.

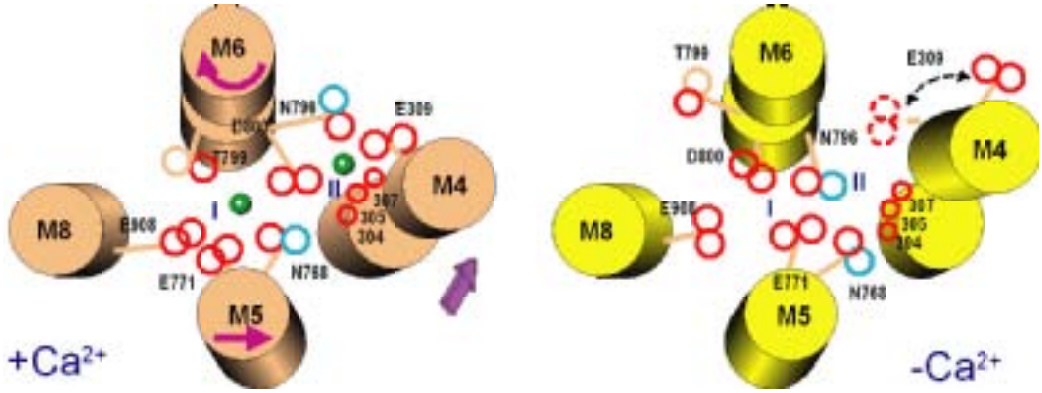

Fig. 3. Schematic diagram of the Ca^{2+}-binding sites of the Ca^{2+}-ATPase in the Ca^{2+}-bound and unbound forms. The arrows in the left panel ($+Ca^{2+}$) show the directions of movements accompanying the dissociation of Ca^{2+}. Oxygen atoms are shown in red, nitrogen in cyan, carbon in orange. Ca^{2+} ions are thought to enter into the binding sites through E309. First Ca^{2+} binds to site I and the second to site II. The binding of the second Ca^{2+} causes conformation changes that result in the hydrolysis of ATP.

Obviously these movements are linked with those of the transmembrane helices. Since no ATP or phosphorylation is required for transition between these two states, thermal energy must be able to cause such large movements. The close association of the 3 cytoplasmic domains will restrict the thermal movements of the transmembrane helices, and will limit the delivery of ATP to the catalytic site in the P-domain (D351 in Fig. 1).

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References

[1] C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa, Nature **405** (2000) 647.
[2] C. Toyoshima and H. Nomura, Nature **418** (2002) 605.
[3] H. Ogawa and C. Toyoshima, Proc. Natl. Acad. Sci. USA. **99** (2002) 15977.