CRYSTAL STRUCTURE 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. The simplest and a best studied mammalian P-type ATPase is SR Ca^{2+}-ATPase. It consists of a single polypeptide of 110 kDa and transports up to 2 Ca^{2+} per ATP hydrolysis against concentration gradient. When muscle contracts, large amounts of Ca^{2+} stored in SR are released into muscle cells. To relax muscle cells, calcium ions have to be pumped back into SR against concentration gradient. Ca^{2+}-ATPase in SR membrane is responsible for this process. Compared to channels, which can transfer millions of ions per second, pumps work much more slowly. Ca^{2+}-ATPase can transfer only 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, we can easily obtain sufficient amounts of Ca^{2+}-ATPase for crystallization experiments.

We have been working on this ATPase and determined its structure at 2.6 Å resolution by X-ray crystallography [1] with two calcium ions bound in the transmembrane domain consisting of 10 α-helices (Fig. 1). The crystals used were very thin, typically less than 20 μm. Nevertheless, X-rays available in **BL44B2** and **BL41XU** were powerful enough to collect full data sets from individual crystals very efficiently. In this state, the two calcium ions are located side by side surrounded

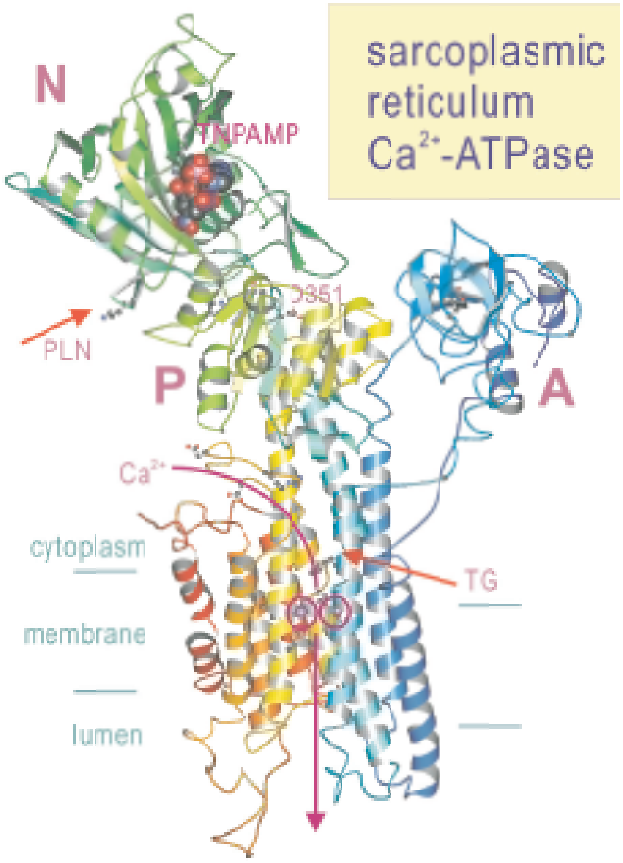
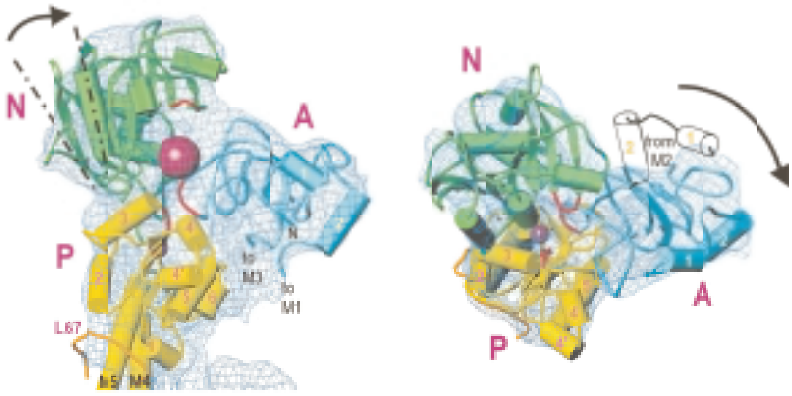


Fig. 1. Architecture of the sarcoplasmic reticulum Ca^{2+}-ATPase. Colour changes gradually from the N terminus (blue) to the C terminus (red). Two purple spheres in the membrane domain represent bound Ca^{2+} ions. Three cytoplasmic domains are well separated and labelled A, N and P. TNP-AMP (an analogue of ATP), in CPK model, is located on domain N and far distant (> 25 Å) from the phosphorylation site (D351), suggesting that domain N will approach domain P when phosphorylation occurs. The binding sites for thapsigargin (TG, a potent inhibitor) and phospholamban (PLN, a regulatory protein in cardiac muscle) are also marked.

by 4 transmembrane helices, two of which are unwound to realize efficient coordination geometry. The cytoplasmic region consists of 3 well-separated domains, with the phosphorylation site (D351) in the central catalytic domain (P) and the adenosine binding site on another domain (N). As a family of ATPases, P-type ATPase has been regarded as a peculiar one, because it lacks the P-loop commonly found in other ATPases and GTPases. The phosphorylation domain has the fold of L-2-haloacid dehalogenase, confirming the proposal by Aravind *et al*. [2]. Thus it is now established that P-type ATPase belongs to a much larger family including enzymes that appear to be totally unrelated to nucleotides.

The atomic model was then fitted to an 8 Å-resolution density map of the enzyme derived by electron microscopy of tubular crystals formed in the absence of Ca^{2+} and the presence of decavanadate [3]. In the tubular crystals the enzyme is considered to be in a state analogous to a phosphorylated (E2P) state. The density map was very well explained by large domain movements in the cytoplasmic region (Fig. 2). By comparing the two models, it has become clear that the enzyme has a mechanism that converts the movements of the cytoplasmic domains to those of the transmembrane helices. This seems to be the mechanism by which calcium ions are transported. Thus, ion transporting ATPases might work like mechanical pumps at the atomic scale.



(a) **(b)**

*Fig. 2. Fitting the atomic model obtained for the Ca^{2+}-bound state (arrows and cylinders) to an 8 Å resolution map (blue net) obtained from tubular crystals [3] formed in the absence of Ca^{2+} and presence of decavanadate (large purple sphere in **a**). Overall orientation of the molecule in a is the same as in Fig 1. In **b**, it is viewed from the cytoplasmic side normal to the membrane. The black arrows show the direction of movements from the Ca^{2+}-bound to unbound state.*

Chikashi Toyoshima, Masayoshi Nakasako and Hiromi Nomura

The University of Tokyo

E-mail: ct@iam.u-tokyo.ac.jp

References

[1] C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa, Nature **405** (2000) 647.

[2] L. Aravind *et al.*, Trends Biochem. Sci. **23** (1998) 127.

[3] P. Zhang *et al.*, Nature **392** (1998) 835.