

Imperial Prize and Japan Academy Prize to:

Chikashi TOYOSHIMA
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for “Elucidation of the Molecular Mechanism of
ATP-driven Ion-Transport”

***Outline of the work:***

The concentrations of ions such as Na^+ , K^+ , and Ca^{2+} largely differ between the interiors and exteriors of cells. Living organisms utilize these concentration differences for energy and cell signaling. Intracellular ion concentrations are regulated by channel and pump proteins. Channels transport ions without spending energy, as they naturally follow their concentration gradients. In contrast, pumps translocate specific ions against their concentration gradients using the chemical energy of ATP. Na^+, K^+ -ATPase and Ca^{2+} -ATPase have been known as membrane pumps of Na^+ and Ca^{2+} , respectively. Nevertheless, how these molecules rigorously select their target ions and efficiently translocate them across the membrane remains enigmatic.

In 2000, Prof. Chikashi Toyoshima purified and crystallized Ca^{2+} -ATPase from rabbit skeletal muscle and determined the first crystal structure at 2.6 Å resolution by X-ray diffraction at the SPring-8 facility, a synchrotron light source. He thereby demonstrated that two Ca^{2+} ions are co-ordinated in the transmembrane region by seven oxygen atoms, including the oxygens of several carboxyl groups, which are arranged for selective binding of Ca^{2+} . He subsequently crystallized and determined the atomic structures of as many as 11 reaction intermediates that almost completely cover the pumping cycle. These crystal structures revealed that the Ca^{2+} pump translocates Ca^{2+} ions by sequentially opening and closing the two gates flanking the bound Ca^{2+} , synchronizing with chemical events at the ATP binding site against a very large (18,000 times) concentration gradient across the membrane. Moreover, in an article published in 2017, he resolved the phospholipids surrounding the transmembrane region of Ca^{2+} -ATPase. He thereby showed that the lipid bilayer, thought to be a mere environment of membrane proteins, actively participates in ion translocation and the structural changes of Ca^{2+} -ATPase.

Prof. Toyoshima has also been elucidating the ion-pumping mechanism of the sodium pump (Na^+, K^+ -ATPase). He purified and crystallized the ATPases from pig kidney and shark rectal gland, and determined their atomic structures by X-ray crystallography at SPring-8. He revealed that the transmembrane cation-binding sites of Na^+ -ATPase substantially differ from those of Ca^{2+} -ATPase; specifically, they accommodate three Na^+ (rather than two Ca^{2+}) while rejecting K^+ , which is only slightly larger than Na^+ (ionic radius 1.33 Å vs. 0.95 Å for Na^+). He also showed that three Na^+ ions must bind before the ATPase conformation admits the forward chemical reaction. Based on the atomic structures, these findings clearly explain why the Na^+ -pump can select and rapidly translocate Na^+ ions exclusively.

In summary, Prof. Toyoshima determined the atomic structures of many intermediates in the reaction cycles of ion pumps such as Ca^{2+} - and Na^+, K^+ -ATPases, and thereby elucidated their marvelously intricate and

intelligent mechanisms. These pump proteins are responsible for generating ion concentration gradients across cell membranes, and their malfunctions are strongly implicated in many diseases.

List of Main Publications

Prof. Toyoshima has published over 90 original papers and 30 reviews. Here are some of his main publications.

1. C. Toyoshima and N. Unwin (1988) Ion channel of acetylcholine receptor reconstructed from images of postsynaptic membranes. *Nature* **336**, 247–250.
2. C. Toyoshima, H. Sasabe and D. L. Stokes (1993) Three-dimensional cryo-electron microscopy of the calcium ion pump in the sarcoplasmic reticulum membrane. *Nature* **362**, 469–471.
3. P. Zhang, C. Toyoshima, K. Yonekura, N. M. Green and D. L. Stokes (1998) Structure of the calcium pump from sarcoplasmic reticulum at 8-Å resolution. *Nature* **392**, 835–839.
4. C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa (2000) Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* **405**, 647–655.
5. C. Toyoshima and H. Nomura (2002) Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* **418**, 605–611.
6. C. Toyoshima and G. Inesi (2004) Structural basis of ion pumping by Ca²⁺-ATPase of the sarcoplasmic reticulum. *Ann. Rev. Biochem.* **73**, 269–292.
7. C. Toyoshima and T. Mizutani (2004) Crystal structure of the calcium pump with a bound ATP analogue. *Nature* **430**, 529–535.
8. C. Toyoshima, H. Nomura and T. Tsuda (2004) Lumenal gating mechanism revealed in calcium pump crystal structures with phosphate analogues. *Nature* **432**, 361–368.
9. Y. Sugita, N. Miyashita, M. Ikeguchi, A. Kidera and C. Toyoshima (2005) Protonation of the acidic residues in the transmembrane cation-binding sites of the Ca²⁺ pump. *J. Am. Chem. Soc.* **127**, 6150–6151.
10. K. Obara, N. Miyashita, C. Xu, I. Toyoshima, Y. Sugita, G. Inesi and C. Toyoshima (2005) Structural role of countertransport revealed in Ca²⁺ pump crystal structure in the absence of Ca²⁺. *Proc. Natl. Acad. Sci. USA* **102**, 14489–14496.
11. C. Toyoshima, Y. Norimatsu, S. Iwasawa, T. Tsuda and H. Ogawa (2007) How processing of aspartylphosphate is coupled to lumenal gating of the ion pathway in the calcium pump. *Proc. Natl. Acad. Sci. USA* **104**, 19831–19836.
12. T. Shinoda, H. Ogawa, F. Cornelius and C. Toyoshima (2009) Crystal structure of the sodium-potassium pump at 2.4 Å resolution. *Nature* **459**, 446–450.
13. H. Ogawa, T. Shinoda, F. Cornelius and C. Toyoshima (2009) Crystal structure of the sodium-potassium pump (Na⁺,K⁺-ATPase) with bound potassium and ouabain. *Proc. Natl. Acad. Sci. USA* **106**, 13742–13747.
14. Y. Sugita, M. Ikeguchi and C. Toyoshima (2010) Relationship between Ca²⁺-affinity and shielding of bulk water in the Ca²⁺-pump from molecular dynamics simulations. *Proc. Natl. Acad. Sci. USA* **107**, 21465–21469.
15. C. Toyoshima, S. Yonekura, J. Tsueda and S. Iwasawa (2011) Trinitrophenyl derivatives bind differently from parent adenine nucleotides to Ca²⁺-ATPase in the absence of Ca²⁺. *Proc. Natl. Acad. Sci. USA* **108**, 1833–1838.

16. C. Toyoshima, S. Iwasawa, H. Ogawa, A. Hirata, J. Tsueda and G. Inesi (2013) Crystal structures of the calcium pump and sarcolipin in the Mg^{2+} -bound E1 state. *Nature* **495**, 260–264.
17. R. Kanai, H. Ogawa, B. Vilsen, F. Cornelius and C. Toyoshima (2013) Crystal structure of a Na^+ -bound Na^+,K^+ -ATPase preceding the E1P state. *Nature* **502**, 201–206.
18. K. Yonekura, K. Kato, M. Ogasawara, M. Tomita and C. Toyoshima (2015) Electron crystallography of ultrathin 3D protein crystals: Atomic model with charges. *Proc. Natl. Acad. Sci. USA* **112**, 3368–3373.
19. H. Ogawa, F. Cornelius, A. Hirata and C. Toyoshima (2015) Sequential substitution of K^+ bound to Na^+,K^+ -ATPase visualised by X-ray crystallography. *Nat. Commun.* **6**, 8004.
20. Y. Norimatsu, K. Hasegawa, N. Shimizu and C. Toyoshima (2017) Protein-phospholipid interplay revealed with crystals of a calcium pump. *Nature* **545**, 193–198.