Kazuhiro ABE, Ph. D.
E-mail : kabe@cespi.nagoya-u.ac.jp

Structural and functional aspects of gastric proton pump, H^+,K^+-ATPase

In response to food intake, pH of our stomach reaches around 1. This highly acidic environment is indispensable for digestion, and also acts as the first barrier against bacterial or viral infection. Conversely, too much acidification of the stomach induces gastric ulcer or gastroesophageal-reflux diseases. Gastric proton pump, H^+,K^+-ATPase is a membrane protein responsible for the gastric acid secretion, and therefore a prominent drug target for the acid-related diseases (Fig. 1). Besides its significant interest as a drug target, gastric H^+,K^+-ATPase faces a remarkable task of pumping protons against a million-fold gradient ranging from approximately pH 7 in the parietal cell to 1 in the stomach. Maintaining a potent concentration gradient of six orders of magnitude is hardly met by any other membrane pump in nature. How does the proton pump manage to resist a million-fold H^+ gradient and never go in reverse? By determining medium-resolution structures of gastric H^+,K^+-ATPase employing electron-crystallography of two-dimensional crystals together with conventional biochemical procedures, we would like to address unique molecular mechanisms relevant to its physiological functions.

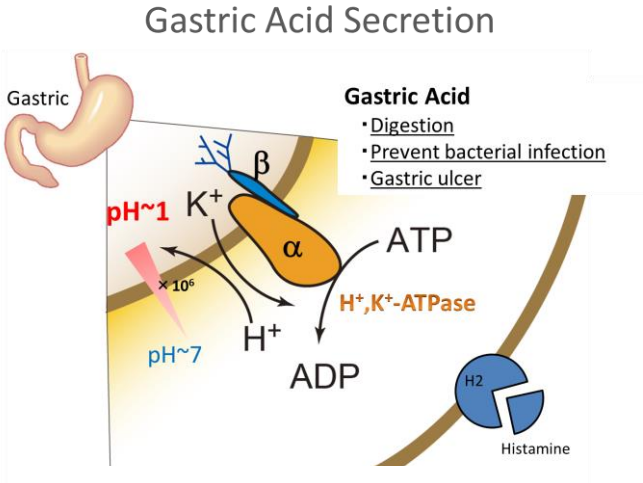

Fig. 1. Gastric proton pump, H^+,K^+-ATPase

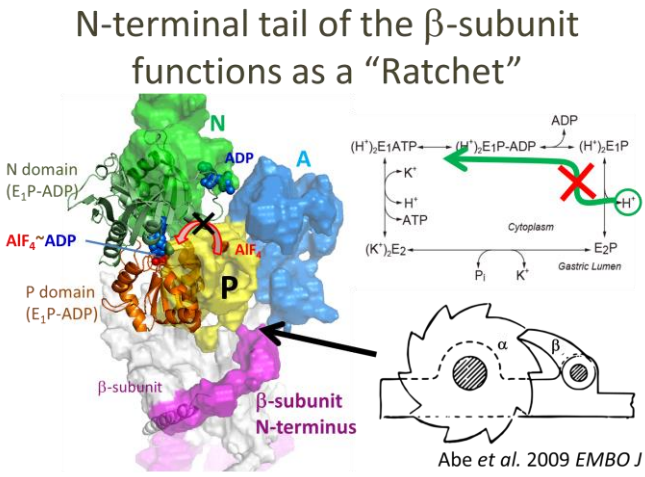

Fig. 2. Ratchet model

By determining 6.5Å structure of H^+,K^+-ATPase at *E2*P conformation, we could find a novel inter-subunit interaction between N-terminal tail of the β-subunit and the P-domain. This inter-subunit interaction stabilizes the *E2*P conformation, and preventing reverse reaction of the transport cycle. Thus, the β-subunit N-terminus

functions as a “ratchet”, to ensure the transport cycle of the H^+,K^+-ATPase can only proceed in the forward direction, therefore resist the massive proton pressure across the parietal cell membrane (Abe et al. 2009 *EMBO J*).

Another key requirement for the generation of the steep proton gradient is the transport stoichiometry. Because of the limited free energy available for ATP hydrolysis, the stoichiometry of transport cation must vary from $2H^+/2K^+$ to $1H^+/1K^+$ per hydrolysis of one ATP molecule as the luminal pH decreases. A strong density located in the transmembrane cation bonding site of the Rb^+-bound structure (Fig. 3) highly likely represents a single bound Rb^+ ion, which is clearly different from Rb^+-free or K^+-bound structures. Measurement of radioactive $^{86}Rb^+$ binding suggests that binding stoichiometry varies depending on pH, and approximately half the amount of Rb^+ is bound under acidic crystallized conditions compared with the neutral pH. These data represent structural evidence of $1H^+/1K^+/1ATP$ transport mode of H^+,K^+-ATPase, which is prerequisite for the 10^6-fold proton gradient across the membrane (Abe et al. 2012 PNAS).

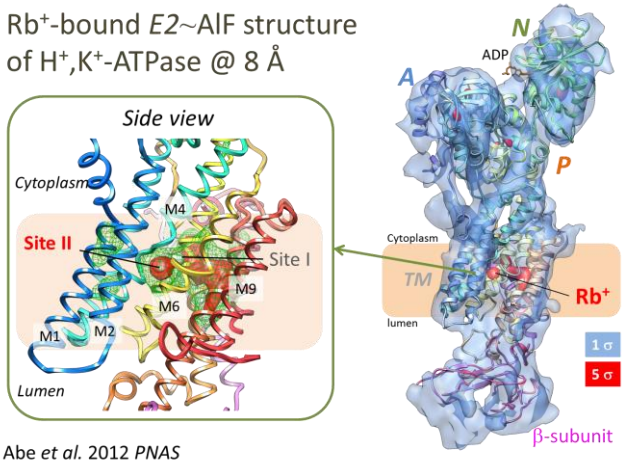

Fig. 3. Rb^+-bound structure

In notable contrast to Rb^+-free *E2*AlF, the β-subunit N-terminus is not in direct contact with the P domain in the (Rb^+)*E2*~AlF structure, indicating that the *E2*P-stabilizing structural interaction is abolished by Rb^+-binding, which drives the transport cycle in the forward direction. Upon binding of the second transported cation(s) K^+ (or Rb^+) to the *E2*P conformation, the βNt is dissociated from the P domain, as substantiated by our present

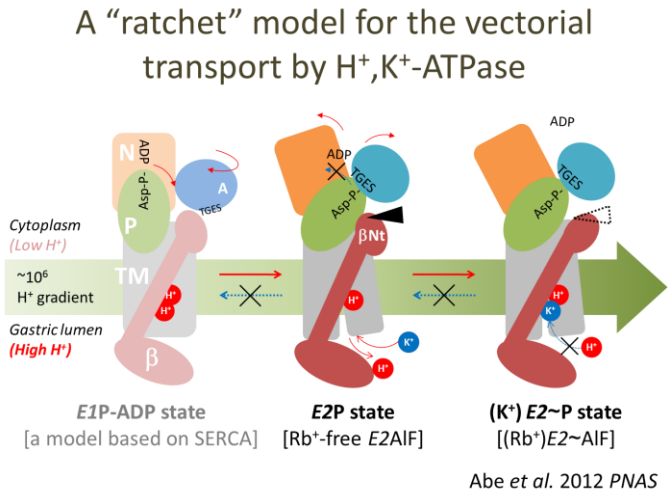

Fig. 4. Vectorial transport model

"ratchet released" structure, thus providing a mechanistic rationale for the directional transport achieved by H^+,K^+-ATPase. Because the risk for proton re-binding and subsequent reversal of the transport cycle might be considerably reduced after the cation binding site is occupied by K^+, the finely timed dissociation of this inter-subunit interaction is feasible. The proposed vectorial transport model (Fig. 4) describes how gastric H^+,K^+-ATPase can generate the highly acidic condition in the gastric lumen (Abe et al. 2012 *PNAS*).

We also determined the 3D structure of H^+,K^+-ATPase with bound SCH28080, a representative of K^+-competitive acid blockers. The drug binding to the enzyme is not accountable by a simple "lock and key" model. The binding induces the widely-opened luminal cavity in the transmembrane domain, which is in turn transmitted to the cytoplasmic domains via A-M2 linker. The observed conformational change suggest that the mechanism, which generates the luminal-open conformation, is surprisingly conserved among H^+,K^+-ATPase and SERCA, and most likely such tightly coupled motion between two highly separated part of the enzyme (that is, cytoplasmic domains and transmembrane helices) provide a framework for all of P-type ATPases (Abe et al. 2011 *Nat Commun*) .

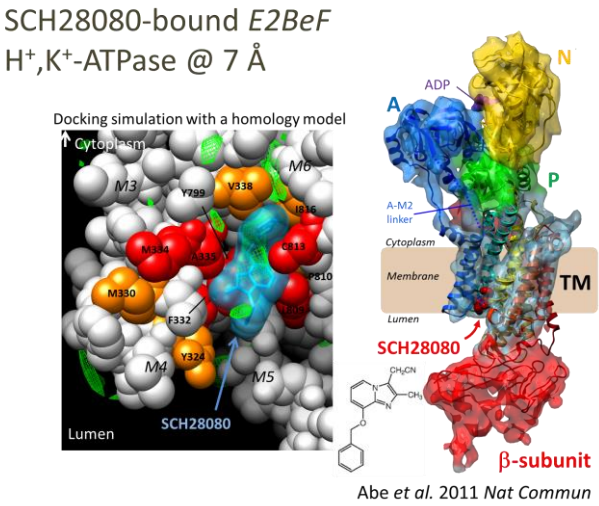

Fig. 5. Inhibitor-bound structure

History

2003 JSPS Research Fellow (DC2)
2004 PhD, Graduate School of Science, Hokkaido University
2004 JSPS Research Fellow (PD), Kyoto University
2008 JBiC Research Associate
2011 Assistant Professor, Cellular and Structural Physiology Institute (CeSPI) and Graduate School of Pharmaceutical Science, Nagoya University

2003 *FASEB* Summer Research Conference Young Scientist Award

2008 Margrethe Møller Award

2011 *JBC* Herbert Tabor Award

Publications (ResearcherID : D-7662-2013)

Papers

1. Cryo-EM structure of gastric H^+,K^+-ATPase with a single occupied cation-binding site.
   Abe, K., Tani, K., Friedrich, T. & Fujiyoshi, Y.
   Proc. Natl. Acad. Sci. USA., 109 (45), 18401-18406 (2012)

2. Conformational rearrangement of gastric H^+,K^+-ATPase with an acid suppressant.
   Abe, K., Tani, K. & Fujiyoshi, Y.
   Nat. Commun., 2, 155 (2011).

3. Structural and functional characterization of H^+,K^+-ATPase with bound fluorinated phosphate analogs.
   Abe, K., Tani, K. & Fujiyoshi, Y.
   J. Struct. Biol., 170 (1), 60-68 (2010).

4. Epigallocatechin-3-gallate is an inhibitor of Na^+,K^+-ATPase by favoring the E_1 conformation.
   Ochiai, H., Takeda, K., Soeda, S., Tahara, Y., Takenaka, H., Abe, K., Hayashi, Y., Noguchi, S., Inoue, M., Schwarz, S., Schwarz, W. & Kawamura, M.
   Biochem. Pharmacol., 78 (8), 1069-1074 (2009).

5. E_2P state stabilization by the N-terminal tail of the H,K-ATPase beta-subunit is critical for efficient proton pumping under *in vivo* conditions.
   Dürr, K., Abe, K., Tavraz, N.N. & Friedrich, T.
   J. Biol. Chem., 284 (30), 20147-20154 (2009)

6. Inter-subunit interaction of gastric H^+,K^+-ATPase prevents reverse reaction of the transport cycle.
   Abe, K., Tani, K., Nishizawa, T. & Fujiyoshi, Y.
   EMBO J., 28 (11), 1637-1643 (2009)

7. Structural analysis of 2D crystals of gastric H^+,K^+-ATPase in different states of the transport cycle.
   Nishizawa, T.†, Abe, K.†, Tani, K. & Fujiyoshi, Y. (†co-first authors)
   J. Struct. Biol., 162 (2), 219-228 (2008)

8. New evidence for ATP binding induced catalytic subunit interactions in pig kidney Na/K-ATPase.
   Tanoue, K., Kaya, S., Hayashi, Y., Abe, K., Imagawa, T., Taniguchi, K. & Sakaguchi, K.
   J. Biochem., 140 (4), 599-607 (2006)

9. Evidence for a relationship between activity and the tetraprotomeric assembly of solubilized pig gastric H/K-ATPase.
   Abe, K., Kaya, S., Taniguchi, K., Hayashi, Y., Imagawa, T., Kikumoto, M., Oiwa, K. & Sakaguchi, K.
   J. Biochem., 138 (3), 293-301 (2005)

10. Correlation between the activities and the oligomeric forms of pig gastric H/K-ATPase.
    Abe, K., Kaya, S., Hayashi, Y., Imagawa, T., Kikumoto, M., Oiwa, K., Katoh, T., Yazawa, M. & Taniguchi, K.
    Biochemistry, 42 (51), 15132-15138 (2003)

11. K^+-induced simultaneous liberation of two moles of Pi, one from one mole of EP and the other from EATP, of oligomeric H/K-ATPase from pig stomach.
    Abe, K., Kaya, S., Imagawa, T. & Taniguchi, K.
    Ann. N. Y. Acad. Sci., 986, 281-282 (2003)

12. Gastric H/K-ATPase liberates two moles of Pi from one mole of phosphoenzyme formed from a high-affinity ATP binding site and one mole of enzyme-bound ATP at the low-affinity site during cross-talk between catalytic subunits.
    Abe, K., Kaya, S., Imagawa, T. & Taniguchi, K.
    Biochemistry, 41 (7), 2438-2445 (2002)

13. Acid-labile ATP and/or ADP/Pi binding to the tetraprotoemeric form of Na/K-ATPase accompanying catalytic phosphorylation-dephosphorylation cycle.
    Yokoyama, T., Kaya, S., Abe, K., Taniguchi, K., Katoh, T., Yazawa, M., Hayashi, Y. & Mårdh, S.
    J. Biol. Chem., 274 (45), 31792-31796 (1999)

Reviews

1. Unique properties of gastric H^+,K^+-ATPase and conserved conformational changes among P-type ATPases
   Abe, K
   SEIKAGAKU, 84 (2), 115-119 (2012)

2. Structural analysis of gastric proton pump by cryo-electron microscopy.
   Abe, K.
   Microscopy (Tokyo), 45 (4), 264-267 (2010)

3. A novel ratchet mechanism of gastric H^+,K^+-ATPase revealed by electron crystallography of two-dimensional crystals.
   Abe, K., Tani, K., Nishizawa, T. & Fujiyoshi, Y.
   Yakugakuzassi, 130 (2), 205-210 (2010)

4. Relationship between activity and tetraprotomeric structure of ion-transporting ATPases.
   Abe, K., & Kaya, S.
   Seikagaku, 79 (6), 527-534 (2007)

5. The oligomeric nature of Na/K-transport ATPase.
   Taniguchi, K, Kaya, S., Abe, K. & Mårdh, S.
   J. Biochem., 129 (3), 335-342 (2001)