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One way for the gastric proton pump

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We are occasionally reminded of the gastric proton pump (H^+,K^+-ATPase) acidifying the gastric juice as an unpleasant symptom of heartburn. A paper by Abe *et al* in this issue of the *EMBO Journal* (Abe *et al* (2009)) provides new structural insight into this important ion pump and drug target. Combining a docking model obtained from electron microscopy with mutational studies, the paper addresses the underlying mechanism of resistance of H^+,K^+-ATPase to a million-fold pH gradient.

The painful conditions of dyspepsia, such as gastric ulcer and gastroesophageal-reflux disease, cause a significantly reduced health and well-being for those affected. Fortunately, proton-pump inhibitors (PPI drugs) targeting the gastric H^+,K^+-ATPase provide for very efficient cures, making this pump one of the most prominent drug targets of all in terms of annual sales.

From a molecular point of view the gastric proton pump manifests a remarkable example of the challenges that membrane pumps are confronted with—in this case pumping protons against a million-fold proton gradient ranging from approximately pH 7 in the epithelial cell to 1 in the stomach. Maintaining a potent concentration gradient of six orders of magnitude is hardly met by any other pump in nature. A key requirement is to ensure a tight membrane that keeps the gradient intact, and as a consequence the gastric proton pump should go one way pumping protons out of the cell and absorbing potassium ions—and certainly not the other way.

As a cousin to Na^+,K^+-ATPase (see Figure 1), the gastric H+, K+-ATPase belongs to the P-type ATPase family, including also the sarcoplasmic reticulum Ca^{2+}-ATPase. From these closely related pumps, a wealth of structural and functional data has shown how P-type ATPase pumps work through large conformational changes in a functional cycle coupled to the formation and breakdown of phosphoenzyme intermediates (E1P and E2P—ADP sensitive and insensitive, respectively). These reactions also define the direction of transport—from the cell and out through E1P and by counter-transport into the cell through E2P. The Ca^{2+}-ATPase is stimulated at the physiological level by Ca^{2+} and ATP, which accelerate all partial reactions of the cycle in the forward direction (e.g. Guillain *et al*, 1981; Jensen *et al*, 2006); similarly, the Na^+,K^+-ATPase is pushed forward by cytoplasmic Na^+ and ATP, and otherwise (when Na^+ is limiting) kept shut in a potassium-occluded E2 state (Post *et al*, 1972). However, both Ca^{2+}-ATPase and Na^+, K^+-ATPase can roll backwards if the gradients get high enough—unlike the gastric proton pump (Helmich-de

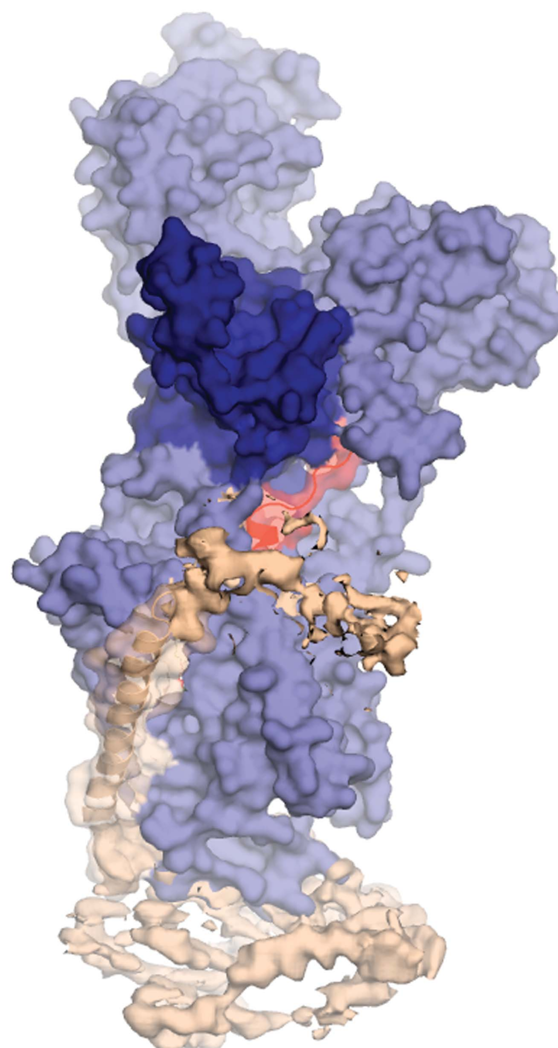

Figure 1 Crystal structure of the Na^+,K^+-ATP—closely related to the H^+,K^+-ATPase. The β subunit is shown in tan colour and is represented by the atomic model for the transmembrane helix, and by plain imaging (the experimental electron density map) for the extracellular domain and the cytoplasmic N-terminal tail. The α subunit is shown in light blue with the P-domain highlighted in dark blue and the linker region between the A-domain and the M3 helix in red. The highlighted regions correspond to the contact areas observed for the β subunit of H^+,K^+-ATPase (Abe *et al* (2009), Figure 1, this issue), whereas for the Na^+,K^+-ATPase it adopts a different configuration. Figure courtesy of J Preben Morth.

Jong *et al*, 1985) and also the plant/fungal plasma membrane H^+-ATPase (also a P-type ATPase, although more remotely related), which is efficiently blocked in reverse reaction, allowing it to build up a very high membrane potential approaching 2–300 mV (Blatt *et al*, 1987; Pedersen *et al*, 2007).

So how does the gastric H^+,K^+-ATPase manage to resist a million-fold pH gradient and never go in reverse? This question may have found at least part of the answer. Abe *et al* (2009) present a three-dimensional model of the H^+,K^+-ATPase derived from electron diffraction studies of two-dimensional crystals, yielding a map at 6.5 Å resolution. The map fulfills important low-resolution validation criteria showing, for example, expected features such as separate cylindrical densities for α helices and distinct density at the phosphorylation site for a bound fluoride complex, and recognisable envelopes of domains and the overall structure. Therefore, the construction and interpretation of a homology model based on the Na^+,K^+-ATPase crystal structure is feasible, and the additional features identified at this level of molecular imaging can be trusted and taken into account. The actual functional state observed by Abe *et al* is, however, somewhat puzzling. Buffer conditions would aim at a protonated E1P state, but the structure is most likely representative of a potassium-bound E2P-like state, although potassium is supposedly absent.

A low-resolution docking model will not allow for detailed discussions of individual residues, but the authors were blessed with an important new finding at an appropriate scale for the resolution of their study: A significant additional density of the β subunit was observed to form a direct contact with the α subunit at a hot spot for conformational changes in the functional cycle, as we know is the case, from Ca^{2+}-ATPase. In comparison, the equivalent part of the Na^+,K^+-ATPase β subunit adopts a far more loose position as shown by X-ray crystallographic imaging (see Figure 1 and Morth *et al*, 2007).

The observation prompted Abe *et al* to carry out mutational studies, where the β subunit of the H^+,K^+-ATPase was truncated from the N-terminus by 4, 8 and 13 residues. Probing these truncated forms by ADP and K^+ sensitivity in dephosphorylation, they found a destabilisation of the E2P state allowing for a reverse E2P-to-E1P transition, unlike that for the wild-type enzyme. Abe *et al* therefore propose that the N-terminal extension of the β subunit represents a determinant of the E2P stabilisation of H^+,K^+-ATPase, preventing the reverse reaction from occurring and thereby keeping the gastric epithelial membrane resistant to the steep pH gradient. The hypothesis is testable and can be further challenged by, for example, mutations at putative sites of interaction between α and β subunits, and by further scrutiny through higher resolution studies.

As mentioned above, the plant/fungal plasma membrane H^+-ATPase is also highly resistant against reverse reaction, but this enzyme contains no equivalent of the β subunit. Instead, its resistance against the reverse mode may be the result of a positively charged arginine residue placed in the transmembrane pathway (Pedersen *et al*, 2007). The H^+,K^+-ATPase also displays a positively charged lysine residue adjacent to the ion-binding sites (Burnay *et al*, 2003)—how this and other features add to functional control remains to be further investigated. Comparative studies on, for example, Na^+,K^+-ATPase function and higher resolution studies will be critical.

Finally, the β subunits of P-type pumps have been implicated in the organisation of the extracellular matrix (e.g. Vagin *et al*, 2006), that is, in effect being 'receptors' in for example cell–cell interactions. As a consequence, they may receive far more attention in the future as we move our focus from the individual enzymes to the higher-order structure and cooperativity of the biomembrane—the transistor between inner and outer environments of the cell.

Conflict of interest

The authors declare that they have no conflict of interest.

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