New and Notable

Does the Sodium Pump have Secret Levels?

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Animal life without sodium pumps, or to be more precise, without the Na,K-ATPase, is not possible. Animal cells need to maintain a high K^+ and a low Na^+ concentration in their cytoplasm as opposed to high Na^+ and low K^+ concentrations of their extracellular environment. The Na,K-ATPase creates electrochemical potential gradients for both ion species that are essential for cellular functions such as secondary active transport, excitability, and volume regulation. In 1957 the Na,K-ATPase was discovered by J. C. Skou (1), and since then there has been a wealth of studies published, providing increasing knowledge and insight into the structure and function of this active ion transporter of the plasma membrane at many different levels. Quite early the pump cycle was elucidated, the stoichiometry of 3 Na^+/2 K^+/1 ATP established, a consecutive or Ping-Pong-type mechanism (first Na^+ out then K^+ in) was demonstrated, and all these results were compiled into the famous Post-Albers cycle (2) that became the prototype of the reaction scheme for all P-type ATPases.

However, as it often happens, those perfect and simple concepts were challenged in many different ways and had to be revisited and supplemented as experimental investigations dug deeper—particularly as investigators began to probe pump function in a wider range of conditions, including nonphysiological conditions. First, noncanonical flux modes were revealed (3) when Na^+ and/or K^+ ions were removed from one side of the membrane or the other. Then, in 1995 Wang and Horisberger reported the perturbing finding that in the absence of external Na^+ and K^+ ions, protons are able to sneak through the Na,K-ATPase (4). They flow down their electrochemical potential gradient into the cytoplasm when the pump is arrested in the E2P state, before binding of K^+ ions from the extracellular side and continuation through the pump cycle. Furthermore, the steeper the electrochemical potential gradient is across the membrane, the larger the proton flux. This finding was not too alarming at the time because cells in a living organism will not face a situation in which monovalent cations are absent from their surrounding fluid.

Recently it has been shown, however, that such proton-leak currents may be observed with near-physiological ion concentrations when mutated Na,K-ATPase species were expressed in oocytes. It is of interest that the same mutations in the human proteins are known to be involved in the pathology of hyperaldosteronism-induced hypertension (5) and familial hemiplegic migraine type II (6). Now, many mutations may damage the function of an ion pump, and one could still assume that the sodium pump would function normally enough, so as not to endanger the health of the cell.

But most recently, Vedovato and Gadsby provided evidence (7) that normal Na,K-ATPase expressed in oocyte allows a proton influx through the plasma membrane at physiological Na^+ and K^+ concentrations. They studied the α1 isoform of the Na,K-ATPase that is common to most cells and is the predominant isoform in the brain, heart, and kidney. Their somewhat disconcerting findings are that a reduction of the extracellular pH to a value of 6 causes a considerable influx of protons via the pump at negative potentials. They call our attention to the fact that such pH drops are observed during ischemia in the brain and even during vigorous exercise. They are able to deduce from their findings that even at a (physiological) pH of 7.6 "one proton enters every 25–30 pump cycles at −80 mV" (7) and at room temperature. Presumably, this is a condition cells can cope with, and they qualify their findings by indicating that it remains to be established whether this proton influx plays a physiological role.

Nevertheless, there is an intriguing question whether the pump has secret levels of activity that have remained hidden in the Na,K-ATPase and that contain regulatory switches, or if this phenomenon is only another example of the knowledge that "nobody is perfect!" Based on the above-mentioned findings, Don Hilgemann has offered a commentary (8) in which he pondered on mechanistic explanations and possible implications of those proton leaks through the sodium pump, and he has called for further work on this subject. Happily, in this issue of the *Biophysical Journal* we will find a next tranche advancing the understanding of proton transport through the Na,K-ATPase.

Mitchell et al. (9) present what is, to our knowledge, the first study investigating proton influx through native Na,K-ATPase in unmodified Guinea-pig myocytes. This is important because it refutes the argument that the expression of the Na,K-ATPase in oocytes could modify its properties by some kind of environmental mismatch. The proton influx is indeed an intrinsic property of the completely native enzyme. They confirm also the conclusion in all previous studies that one specific binding site of the three Na^+-binding sites of the pump is paving the way for proton passage into the cytoplasm. This so-called site III is a specific one because it can be occupied only by a sodium ion (10,11), while the other two sites are

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used alternatingly also by the transported potassium ions. Proton-leak currents can be observed only when site III is empty. A Na^+ ion in this site blocks any passage of protons, perhaps sterically and definitely by electrostatic repulsion.

An important and interesting new finding is a dual effect of protons that are able to activate and inactivate the proton flux depending on their concentration, similar to the effect of Na^+, which reduces the proton flux at low and high concentrations, and promotes it at intermediate concentrations of ~10 mM Na^+ as reported here, and as was found before (4). Mitchell et al. (9) offer an elegant explanation for the complex pH and Na^+-concentration dependence of the proton flux: In the E2P conformation of the Na, K-ATPase, when the unspecific ion-binding sites, I and II, are accessible from the extracellular side of the membrane, occupation by two identical ions, 2 K^+, 2 Na^+, or 2 H^+ inhibits the proton leak through the sodium pump. In contrast, mixed occupation, such as 1 Na^+ and 1 H^+, does permit the leak current. From all that we know, it can be taken for granted that in this condition the Na^+-specific site III will be empty when sites I and II are not occupied by two Na^+ ions. Therefore, such a mixed occupation state provides the condition that is mandatory for proton translocation through the protein.

Now two more questions remain unanswered: How does the proton get to the cytoplasm from its location in site III which is accessible in the E2P state from the extracellular aqueous phase? And, what is so special about those states in which two identical ions occupy sites I and II?

Possible answers to the first question have been suggested. Vedovato and Gadsby (7) considered transitions between phosphorylated (sub)states that are involved in the proton import. Based on this concept, Hilgemann formulated a working model (8) that proposes a conformational rearrangement of the pump's membrane domain after Na^+ site III is cleared of its normal incumbent. Such a rearrangement would open a narrow pathway for protons through the barrier separating site III from the cytoplasmic aqueous phase. An alternative was raised by Wang and Horisberger (4), who considered a likely explanation to be that "the barrier closing the intracellular access in the E2 conformation … is not tight for protons." An experimental finding supporting this idea is the observation that protons are able to escape from their occluded state in a bacterial P-type H-ATPase (12). Also, bacteriorhodopsin teaches us how protons brachiate through the protein without need for an explicit ion-channel structure (13). It seems that we have to wait for additional experimental details to respond to this question with a refined explanation, and surely, a crystal structure of the E2P conformation with an empty site III would be highly desirable to increase our understanding.

To address the second question about the role of the occupation of sites I and II, we have to remember that in the E2P state the exchange of ions between these binding sites in the membrane domain and the extracellular aqueous phase is a continuous, diffusion-controlled coming and going of ions through a wide, water-filled access channel. The only directionality in these stochastic processes is the so-called binding affinity of the ion sites that makes some ion species fit tighter (and therefore longer) than other species. As a consequence, one will find a continuously changing occupation of both sites with Na^+, K^+, and H^+ ions, and the probability at which an ion is bound will be controlled by the ion concentrations in the aqueous bulk phase. When two K^+ ions happen to fill the sites (and they have the highest binding affinity to those sites), the protein is able to undergo the next step forward in the pump cycle, i.e., dephosphorylation and occlusion of the binding sites, and then proceeds onwards. This reaction sequence is also possible with two bound H^+ ions that are known to act as congeners of K^+, but with much lower efficiency. When two Na^+ ions occupy the binding sites, which is probable at high Na^+ concentrations, a step backward in the pump cycle may occur, i.e., one of the Na^+ will slip into site III, and a third Na^+ may bind.

With these processes in mind, one can offer a working model that explains why states occupied by two identical ions are special: They promote progress to the next or to the previous state in the Post-Albers cycle in which the door is closed for the proton influx, either by occlusion of all sites or by occupation of site III. In contrast, when a proton is present in one of these mixed E2P states, it may sneak into the empty site III and carry on to the cytoplasm.

If this model turns out to be true, there would be no secret level, the standard model would do, and one could allude to the motto "nobody is perfect." Then another question remains to be asked: How does the pump know that there are two identical ions in its sites? But that is another story.

REFERENCES

1. Skou, J. C. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta.* 23:394–401.
2. Post, R. L., C. Hegyvary, and S. Kume. 1972. Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. *J. Biol. Chem.* 247:6530–6540.
3. Glynn, I. M. 1985. The Na^+,K^+-transporting adenosine triphosphatase. *In* Membrane Transport. A. N. Martonosi, editor. Plenum Press, New York, pp. 35–114.
4. Wang, X., and J. D. Horisberger. 1995. A conformation of Na^+-K^+ pump is permeable to proton. *Am. J. Physiol.* 268:C590–C595.
5. Azizan, E. A., H. Poulsen, …, M. J. Brown. 2013. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat. Genet.* 45:1055–1060.
6. Tavraz, N. N., T. Friedrich, …, M. Dichgans. 2008. Diverse functional consequences of mutations in the Na^+/K^+-ATPase α2-subunit causing familial hemiplegic migraine type 2. *J. Biol. Chem.* 283:31097–31106.

7. Vedovato, N., and D. C. Gadsby. 2014. Route, mechanism, and implications of proton import during Na^+/K^+ exchange by native Na^+/K^+-ATPase pumps. *J. Gen. Physiol.* 143:449–464.

8. Hilgemann, D. W. 2014. Fishing for holes in transporters: how protons breach the Na/K pump security gates. *J. Gen. Physiol.* 143:437–441.

9. Mitchell, T. J., C. Zugarramurdi, …, P. Artigas. 2014. Sodium and proton effects on inward proton transport through Na/K pumps. *Biophys. J.* 106: 2555–2565.

10. Kanai, R., H. Ogawa, …, C. Toyoshima. 2013. Crystal structure of a Na^+-bound Na^+,K^+-ATPase preceding the E1P state. *Nature.* 502:201–206.

11. Schneeberger, A., and H. -J. Apell. 1999. Ion selectivity of the cytoplasmic binding sites of the Na,K-ATPase: I. Sodium binding is associated with a conformational rearrangement. *J. Membr. Biol.* 168:221–228.

12. Apell, H. -J., and M. Solioz. 1990. Electrogenic transport by the *Enterococcus hirae* ATPase. *Biochim. Biophys. Acta.* 1017:221–228.

13. Lanyi, J. K. 2006. Proton transfers in the bacteriorhodopsin photocycle. *Biochim. Biophys. Acta.* 1757:1012–1018.