A proton-controlled check valve for sodium ion transport

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Transmembrane electrochemical ion gradients are the thermodynamic forces exploited by living cells to drive specific substances across the membrane. A new study begins to reveal the molecular mechanisms by which a transporter protein harnesses these driving forces.

The molecular mechanism by which energy is converted to perform useful work in living cells raises many fascinating questions. In the case of membrane transporters, through which some species passively diffuse in the direction of their downhill gradients while others move uphill against their gradients, one would like to elucidate the nature of the coupling between the various fluxes. In molecular terms, one would like to understand how the protein succeeds to enforce such coupling. In a recent article, Arkin et al.1 present extensive molecular dynamics simulations and experiments that help unravel the mechanism of NhaA, a Na+/H+ transmembrane antiporter.

At a conceptual level, membrane transporters are widely thought to function according the classic “alternate accessibility mechanism” suggested by Jardetzky in 1966 (ref. 2). In its simplest terms, this mechanism requires that the transporter protein, like a check valve, ‘toggles’ between inward- and outward-facing conformations, thereby controlling the accessible solution with which the bound species are allowed to partition and equilibrate. In cartoon representations, this mechanism is typically pictured as a series of doors, opening and closing sequentially. But at the molecular level, what those doors are and what controls them still remains a mystery.

The simulations of Arkin et al.1 provide a structural explanation for the alternate accessibility mechanism in NhaA, a Na+/H+ antiporter from Escherichia coli. The only available atomic-resolution structure of NhaA was determined using X-ray diffraction from protein crystals obtained at pH 4, a state in which the transporter is inactive3. With the atomic structure of critical functional states still missing—namely the active outward-facing and inward-facing conformations—the mechanisms of transport remained poorly understood despite the great crystallographic achievement. Using a combinatorial approach, Arkin et al. simulated the protein in various states of protonation for the residues previously identified by experiments as being critical for the function of NhaA1. Because the function of NhaA is abolished when two aspartic acid residues (Asp163 and Asp164) are mutated, these residues were known to be particularly important. A number of potentially functional conformations of the transporter were then discovered using moderately short molecular dynamics trajectories (less than 100 ns) started from the inactive X-ray structure. The conditions for which the latter was stable revealed the residues possibly responsible for the pH-driven inactivation of the transporter.

The results of the simulations by Arkin et al.1 outline the central mechanistic role of Asp163 and Asp164 (Fig. 1). The protonation state of Asp164 controls binding and release of the translocated Na+. The protonation state of Asp163 acts as a conformational

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**Figure 1** Representation of the alternating accessibility mechanism in the Na+/H+ antiporter NhaA. One clockwise cycle results in the inward movement of two H+ and the outward movement of one Na+. Initially, the transporter is in the inward-facing conformation (upper left), in which Asp164 is allowed to equilibrate with the intracellular solution (the bound H+ is replaced by a Na+). Then, a H+ diffuses from the extracellular solution to bind to Asp163 (upper right). This switches the conformation of the transporter to the outward-facing state (lower right), in which Asp164 is now allowed to equilibrate with the extracellular solution (the bound Na+ is replaced by a H+). Finally, the proton bound at Asp163 diffuses and leaves on the intracellular side (lower left), and the transporter returns to its initial state.

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switch controlling the alternate accessibility through a global, yet subtle, reorganization of inward- and outward-facing water-filled crevices. When Asp163 is protonated, the cytoplasmic entrance is closed while the periplasmic exit is open. Deprotonation of Asp163 leads to the closure of the periplasmic Na⁺ exit and the opening of the cytoplasmic entrance. At no moment is there a continuous water density across the protein (which could lead to proton leakage), which shows that the protein maintains tight control over the alternating accessibility. The mechanism accounts beautifully for the known stoichiometry of NhaA: one Na⁺ is excreted from the cytoplasm by using the energy from the cotransport of two protons down their electrochemical gradient into the cell. Lastly, free energy computations show that the binding site presented by Asp164 is highly selective for Na⁺ over K⁺, a prerequisite for this transporter to function normally.

It is difficult to imagine how one could have reached such a deep level of insight on the mechanism of Na⁺/H⁺ antiporting in NhaA without the aid of the current simulations, and they should significantly contribute to establishing a rich paradigm for antiporters. As in the case of the KcsA channel, aquaporin and F1 ATPase, to give a few examples, it is clear that molecular dynamics simulations based on atomic models can be a powerful tool to further our understanding of membrane transport proteins, particularly when high-resolution X-ray structures are available and the mechanistically relevant degrees of freedom can be adequately sampled. In retrospect, this study may owe part of its success to the fact that the transported substrates are small (Na⁺ and H⁺) and that the alternate accessibility mechanism in NhaA requires relatively modest conformational changes (on the order of ~2 Å r.m.s.). It is currently believed that larger conformational changes occur during the function of other membrane transporters—for example, on the order of ~10 Å for GltPh (ref. 9) and LacY (ref. 10).

Undoubtedly, many challenges remain to fully understand NhaA. For example, it would be very interesting to determine the energetics of H⁺ binding with and without Na⁺ (that is, the pKₐ of Asp163 and Asp164). Furthermore, though the alternating accessibility mechanism pictures the transport process as a sequence of near-equilibrium situations, it will be important to better characterize the transitions (rates and reaction pathways) between the various stable states.

The total amount of simulations of an atomic model of NhaA embedded in an explicit membrane performed by Arkin et al. is ~3 μs (ref. 1). Although these are certainly extensive, continued improvement in computational methods suggests that we will be able to increasingly look to simulations to provide unprecedented insight, rational hypotheses, and accurate prediction. With the advance of new algorithms for the high-speed, parallel execution of molecular dynamics simulations, and the development of accurate atomic force fields, structural biology shall have at its disposal a reliable investigative tool to better understand molecular reality.

As structural biology of membrane transport proteins and ion channels is becoming a reality, it is increasingly important to go beyond arguments and considerations based solely on static structures. After all, conformational changes are an essential part of how these proteins work. To paraphrase Richard Feynman, it is necessary to visualize the “jigglings and wigglings” of all the atoms to understand how these complex molecular devices carry out their functions. Arkin et al. made this possible for NhaH with the aid of the ‘virtual reality’ provided by molecular dynamics simulations.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.