Watch Water Flow

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The association between enhanced oxygen delivery and increased performance seems to be a recurrent theme, and illustrates how a mechanistic understanding of extant organisms can provide insight into the past. Nonetheless, sloths and koalas remind us that selection often favors slower lifestyles, and that highly derived forms may be inadequate to understand the factors that have shaped the evolution of their ancestors (*12*). In the absence of direct physiological and ecological evidence from the fossil record, establishing associations between different lifestyles and various aspects of performance in ancestral lineages remains a major challenge. Only by integrating multiple lines of evidence, from broad comparative studies to detailed molecular analyses, may we understand how and why increased aerobic capacity has evolved repeatedly, and how it might have contributed to the evolutionary diversification of very disparate lineages.

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BIOCHEMISTRY

Watch Water Flow

A high-resolution structure elucidates how water flows through aquaporin into and out of biological cells.

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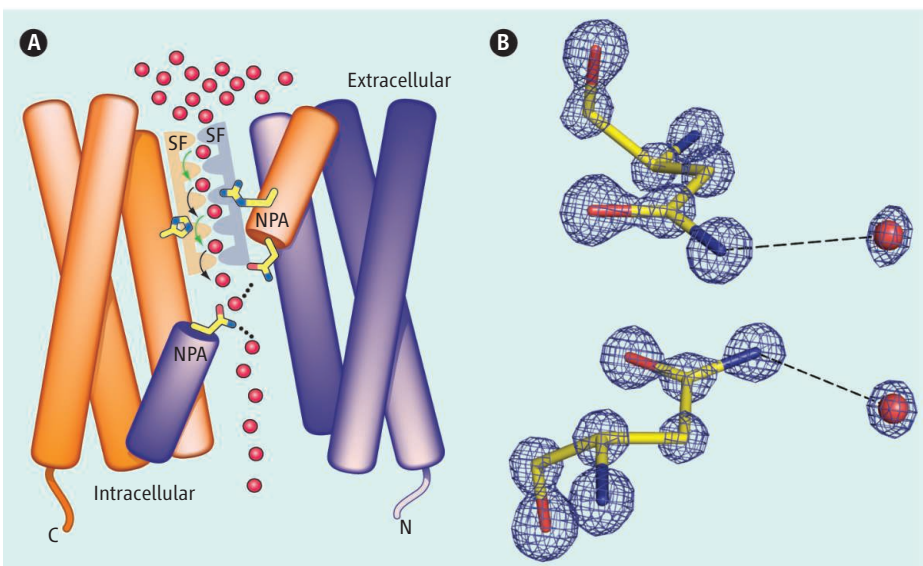
Cells have numerous channels and transporters that facilitate movement of specific molecules and ions across biological membranes. However, it is unclear how these proteins facilitate the rapid passage of specific ions while impeding other, often very similar, substrates. The mechanism by which potassium ions (K^+) are transported through K^+ channels was revealed more than a decade ago (*1*–*4*). Computer simulations mirrored the x-ray data, demonstrating the complementary nature of the two techniques. On page 1346 of this issue, Kosinska Eriksson *et al*. (*5*) report the crystal structure of yeast aquaporin1 at subangstrom resolution in conjunction with molecular dynamics simulations, revealing that, much like K^+ in K^+ channels, water flows through the aquaporin channel in a pairwise manner.

Aquaporins selectively and rapidly conduct water across biological membranes in response to osmotic pressure changes. Crystal structures have been determined for many aquaporins from a wide range of organisms (*6*–*9*). All structures show a conserved fold composed of six transmembrane α helices arranged in a homotetramer, with each monomer acting as an independent water channel. Aquaporins have an inverted repeat structure, where the amino- and carboxyl-terminal halves of the protein share a similar structural fold and are related by a twofold symmetry (see the figure, panel A) (*10*, *11*).

In addition to the six transmembrane α helices, a seventh pseudo-transmembrane α helix is formed by reentrant loops from opposite sides of the membrane. Two conserved regions are responsible for selectivity (see the figure, panel A): the NPA-signature motif (asparagine-proline-alanine) in the center of the channel and the selectivity filter (SF) on the extracellular side, directly above the NPA-signature motif. These two elements orient the water molecules through a narrow pore, where water selectivity is conferred by electrostatic and steric factors (*12*, *13*). Together, these segments allow rapid water transport while excluding protons and other ions, thereby preserving the cell's electrochemical membrane potential.

A wealth of structural, mutational, and simulation data have driven various hypotheses regarding water translocation and the



High-precision water transport. (**A**) Aquaporins contain two conserved segments responsible for selectivity: the NPA signature motif (Asn^{112} and Asn^{224}, shown as ball-and-stick in the center of the channel), and the selectivity filter (SF) on the extracellular side of the channel. These two elements guide the water molecules (red circles) through a narrow pore, where selectivity for water is conferred by electrostatic and steric factors. Kosinska Eriksson *et al*. (*5*) show that water permeates through the pore in a choreographed pairwise fashion (black and green arrows), and that SF residues His^{212} and Arg^{227} (shown as ball-and-stick on the extracellular side) exclude OH^- and H_3O^+, respectively. (**B**) High-resolution electron density maps (*5*) show the precise hydrogen-bonding network between the NPA residues Asn^{112} and Asn^{224} and two independent water molecules. Electron density is seen to be delocalized across the carbon-oxygen double bond.

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ability of aquaporins to exclude protons (*13*). Water molecules move in single file past the NPA and SF segments, where they are guided by hydrogen bonds from essential residues that mediate their movement while at the same time preventing the transport of hydroxide (OH^-) and hydronium (H_3O^+) ions.

Kosinska Eriksson *et al*. resolved the structure of yeast aquaporin 1 at ultrahigh resolution (0.88 Å, a record for membrane proteins) and in doing so could experimentally observe previously unresolved aspects of water transport. Many aquaporin structures have been solved at high resolution (<2 Å) (*7*–*9*), but the precise hydrogen bonding between key amino acids and the water molecules emerges only at subangstrom resolution. This unprecedented level of detail for a membrane protein provides a foundation for future studies to investigate the interplay between protein structure and water flux.

Aquaporins are highly selective for water transport while restricting proton flux. It has been proposed that a central water molecule simultaneously receives hydrogen bonds from both asparagine residues of the NPA, thereby preventing proton transmission via the Grotthuss mechanism, in which protons hop along a hydrogen-bond network of water molecules (*13*). In contrast, the new structure (*5*) reveals that the NPA asparagines donate hydrogen bonds to two independent water molecules (see the figure, panel B) while the bipolar distribution on the two halves of the channel is maintained. The study provides a molecular mechanism for strongly constrained water conformational dynamics in both the NPA and SF regions, with the SF segment providing a more effective barrier for disrupting Grotthuss proton transport.

The new structure also helps to explain how the SF excludes OH^- and H_3O^+ ions. Both the side chain of His^{212} and the neighboring backbone hydroxyl of Ala^{221} simultaneously accept hydrogen bonds from a water molecule, enabling its passage. The passage of OH^-, which can form only one hydrogen bond at this site, is thus precluded. Similarly, a localized positive charge on the side chain of Arg^{227} is concentrated in close proximity to the water pathway. This enacts a repulsive electrostatic effect upon any H_3O^+ ions that may attempt passage. These observations explain why mutations to His^{212} and Arg^{227} impair the molecule's ion exclusion abilities.

Perhaps the most rewarding structural observation is the electron density for four water molecules located between His^{212} and Arg^{227} within the SF. Because of their close proximity to one another, all four water molecules cannot be bound simultaneously. Proposing a mechanism analogous to the pairwise movement of K^+ ions through the SF of the K^+ channel (*3*–*5*), Kosinska Eriksson *et al*. suggest that a pair of water molecules—separated by a vacant position—shift in choreographed fashion between two configurations (see the figure, panel A). As in K^+ channels, the four water positions maintain almost equal crystallographic occupancy, implying that the four sites are equally favorable, which allows for rapid transport through the channel.

The Kosinska Eriksson *et al*. study reveals an extremely specific and previously unknown water structure within the NPA and the SF segments of aquaporin. The similarities between the SF mechanisms in aquaporins and K^+ channels may be indicative of convergent evolution, whereby these two protein families have ensured the efficient exclusion of nonsubstrate molecules while maintaining rapid flux across cell membranes. These critical experimental insights will bolster new theoretical developments and further drive mechanistic probing at an extraordinary level of accuracy.

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NEUROSCIENCE

Circuit Logic of Avoidance and Attraction

The intensities and context of sensory stimuli are encoded by specific neural circuits that instruct behavioral responses.

Chih-Ying Su and John R. Carlson

How an animal responds to a sensory stimulus depends on its intensity. Animals prefer food with moderate concentrations of salt and avoid food high in salt content. Responses may also depend on the context. Carbon dioxide (CO_2) emitted by stressed fruit flies elicits an avoidance response (*1*). However, CO_2 emitted by fermenting fruits is tolerated by flies in the context of food odors (*2*). How is a sensory stimulus encoded when presented at different intensities or in different contexts? On pages 1338 and 1334 in this issue, Lin *et al*. (*3*) and Zhang *et al*. (*4*) provide new insight into this fascinating problem by investigating how different concentrations of CO_2 and salt are encoded by the olfactory and gustatory systems, respectively, in the fruit fly *Drosophila melanogaster*.

In fruit flies, the ab1C class of olfactory receptor neurons detects CO_2 (*5*). Activation of the ab1C neural circuit can drive robust aversion responses (*1*, *6*). Yet CO_2 emitted by fruit does not cause aversion. Certain food odorants may directly inhibit the CO_2 receptor of ab1C neurons (*7*). Alternatively, strong activation of a neighboring olfactory receptor neuron (ab1A) by food odorants may attenuate the response of an ab1C neuron through nonsynaptic inhibition (*8*). Both of these mechanisms operate in the antennae. Lin *et al*. provide another explanation for how low concentrations of CO_2 can be tolerated in the context of food.

From antennae, ab1C neurons send axons to a spheroidal module of neuropil (density of neuronal and glial processes) called the V glomerulus in the antennal lobe of the brain (see the figure). In the V glomerulus, the ab1C axon terminals form synapses with projection neurons. Lin *et al*. found that multi-

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