

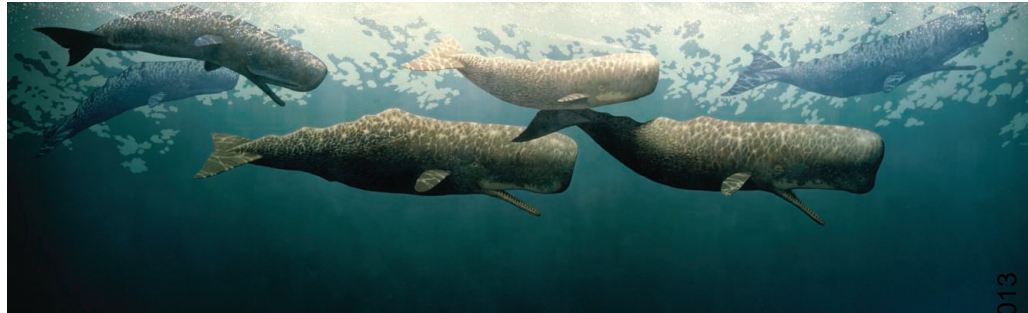
EVOLUTION

Better Oxygen Delivery

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The ability to use oxygen as the terminal acceptor of electrons in the cellular respiratory chain—a process that is substantially more efficient in generating energy from organic molecules than anaerobic alternatives—was a major evolutionary innovation that may have opened the doors for multicellular complexity (1, 2). At a given time in evolution, aerobic metabolism took the stage. Three studies in this issue, by Mirceta *et al.* (3) on page 1303, Natarajan *et al.* (4) on page 1324, and Rummer *et al.* (5) on page 1327, discuss how myoglobin and hemoglobin, respiratory pigments responsible for oxygen uptake, transport, and storage have evolved in vertebrates to meet the demands of different lifestyles.

Mirceta *et al.* traced how myoglobin, an iron- and oxygen-binding protein found in the muscles of vertebrates, has evolved across different mammalian lineages. The authors show that diving mammals (see the figure) acquired increasingly charged myoglobin to accommodate larger amounts of protein in their muscles. This change optimized myoglobin as an oxygen storage facility—the electrostatically charged proteins repel each other and are thereby less prone to aggregate. By becoming more soluble in the cellular milieu, charged myoglobin and the oxygen it carries is more abundant and readily available. By contrast, Natarajan *et al.* studied the oxygen affinity of hemoglobin variants in deer mice. Hemoglobin is another iron-containing protein that binds to oxygen and is responsible for its transport in the blood. In most vertebrates, hemoglobin consists of four subunits (two alpha and two beta). The authors demonstrate that the forms of hemoglobin prevalent in mice that inhabit high and low altitudes constitute two out of many possible combinations of these polymorphic subunits. The remaining combinations are poor oxygen carriers, likely removed from populations by natural selection. Hence, the adaptive value of any given biochemical substitution depends on the altitude and the genetic background of the local population. Rummer *et al.* describe how a highly acid-sensitive type of



Extreme physiology. Elite mammalian divers, such as the whale, can forage under water for long periods of time because of their enhanced oxygen storage capacity, acquired through evolved forms of myoglobin with increased solubility. This maximizes myoglobin concentration and oxygen reserves in muscles.

hemoglobin, present only in ray-finned fishes, and responsible for the so-called Root effect, can unload oxygen to peripheral tissues without compromising its uptake in the gills during blood acidosis. The Root effect describes highly specialized situations in which lower pH causes a decrease in hemoglobin's carrying capacity for oxygen. In fish, the effect allows hemoglobin to release oxygen into the swim bladder, which provides buoyancy and reduces the necessity to expend energy on swimming. The circumstances under which this response might have evolved have puzzled physiologists for decades.

Conceptually, these three studies represent nuances of a common theme—an association between aerobic capacity and high performance. Diving mammals rely on oxygen stores to sustain the activity of muscles while hunting prey under water. Increased oxygen stores would enable these lineages to exploit new food resources and avoid competition, accounting for the general pattern described by Mirceta *et al.* Similarly, efficient oxygenation of peripheral tissues allows deer mice to remain warm and active during harsh winters (6); therefore, their hemoglobin variants adapted to different altitudes might partly explain their abundance and widespread distribution, as suggested by Natarajan *et al.* The finding by Rummer *et al.* that oxygen delivery to the muscles is enhanced by the Root effect in the rainbow trout could imply that selection on swimming performance may have contributed to the evolution of this response in ray-finned fishes. Well-oxygenated muscles allow for sustained swimming at lower speeds and for the oxidation of lactate produced during sprinting, which would

The evolution of proficient oxygen delivery is associated with the rise of lifestyles that require enhanced performance.

prove advantageous for lineages that actively search and capture prey (7).

As proposed for birds and mammals (8), selection for enhanced oxygen delivery may have been associated with the adoption of increasingly active lifestyles in fishes. By relying on aerobic metabolism to maintain elevated activity levels, vertebrate lineages have seemingly employed the same general solution across land and water to deal with similar ecological problems. Furthermore, efficient oxygen-delivery systems have paved the way for the origin of evolutionary novelties considered essential for the success of these lineages: the ability to regulate, by physiological means, buoyancy in ray-finned fishes (9) and body temperature in birds and mammals (10).

These commonalities provide a general idea of how highly integrated complex physiological systems evolve. Ecological processes, such as competition or predator avoidance, drive the evolution of physiological attributes that enhance performance, which, in turn, may give rise to new ecological opportunities. The transition from land to water or across different altitudes has altered the selective regimes underlying oxygen usage and, as illustrated by Mirceta *et al.* and Natarajan *et al.*, the molecular structure of relevant respiratory pigments. From the opposite perspective, the function ascribed by Rummer *et al.* to the Root effect might clarify which selective pressures may have shaped this response in ancestral ray-finned fishes. Interestingly, selection for locomotor capacity may be particularly strong among visual fish predators (11); hence, the concomitant role of the Root effect for retina and muscle oxygenation might be more than a coincidence.

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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

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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 Eriksen *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.

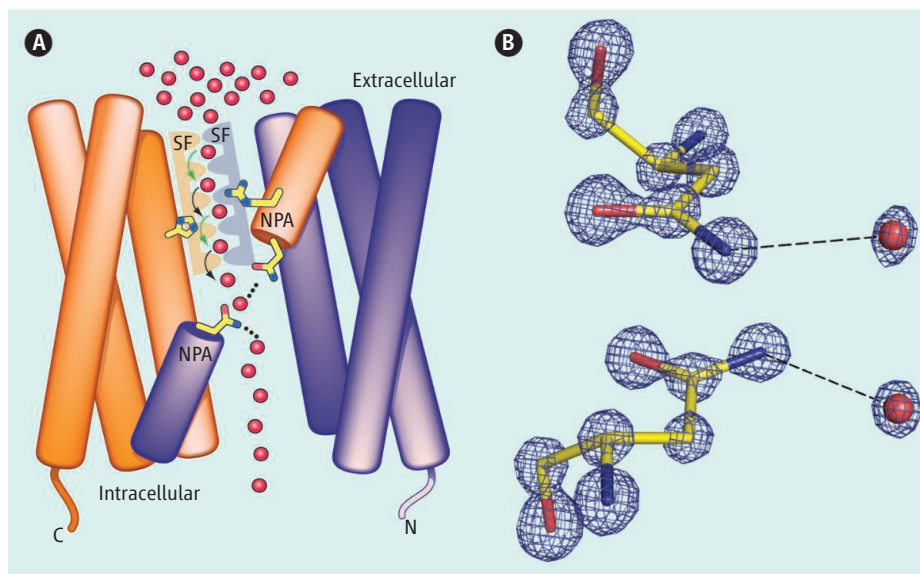
Aquaporins selectively and rapidly conduct water across biological membranes in response to osmotic pressure changes. Crystal structures have been determined for many aquaporins from a wide range of organisms (6–9). All structures show a conserved fold composed of six transmembrane α helices arranged 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

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

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 (Asn¹¹² and Asn²²⁴, 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 Eriksen *et al.* (5) show that water permeates through the pore in a choreographed pairwise fashion (black and green arrows), and that SF residues His²¹² and Arg²²⁷ (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 Asn¹¹² and Asn²²⁴ and two independent water molecules. Electron density is seen to be delocalized across the carbon-oxygen double bond.

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