STRUCTURAL BIOLOGY

The Atomic Architecture of a Gas Channel

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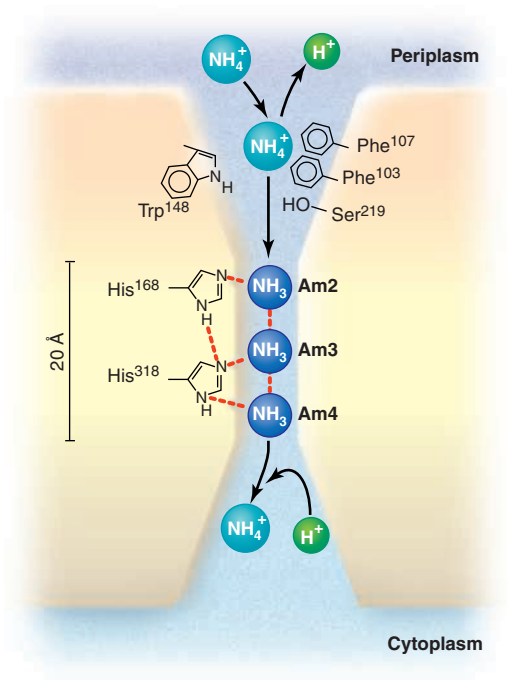
"Form ever follows function." Penned in 1896 by the renowned architect Louis Henri Sullivan in reference to the first tall office buildings, this sentence also applies to the structure of cell membrane proteins. Although high-resolution structures of protein channels that allow passage of ions, uncharged solutes, and even water have been solved, the precise mechanisms by which gases cross biological membranes have remained enigmatic. On page 1587 of this issue, Khademi *et al*. (*1*) provide a quantum leap forward in our understanding of gas transport. They resolve the crystallographic structure of a bacterial ammonia transport channel, AmtB, to 1.35 Å—an unprecedented resolution for an integral membrane protein.

Ammonia (NH_3) is a gas, but when dissolved in water it exists predominantly as the ammonium ion (NH_4^+) with a pK_a of about 9 under physiological conditions. For a bacterium, NH_3 is an important nutrient that must be taken up from the surroundings to provide a source of nitrogen for amino acid synthesis. AmtB is a transport protein present in the bacterial inner membrane between the cytoplasmic and periplasmic spaces that facilitates NH_3 uptake (see the figure). Interestingly, AmtB proteins are genetically related to the structural components of the Rh blood group antigens of mammalian red blood cells. The Rh-related proteins are a family of membrane proteins reported to facilitate the transport of ammonia (*2*) and carbon dioxide across eukaryotic cell membranes (*3*). Human Rh-related proteins are thought to be important in critical physiological processes and, when defective, may result in impairment of systemic pH regulation or central nervous system dysfunction due to ammonium toxicity. The structure of Rh antigens has long been pondered. Now, the trimeric structure of AmtB revealed by Khademi and colleagues suggests a simple explanation for how the three Rh polypeptides of red blood cells—RhAG, RhD, and RhCE—form the Rh antigen complex in the erythrocyte plasma membrane (*4*). In addition, the Khademi *et al*. study reveals a mechanism of ammonia permeation in bacteria that is likely to be similar in eukaryotic cells.



The AmtB ammonia channel of *E. coli*. Resolution of the structure of the bacterial integral protein AmtB reveals a wider vestibule at the top and bottom of the channel. The amino acid residues that line the pore of the outer vestibule—Trp^{148}, Phe^{107}, Phe^{103}, and Ser^{219}—stabilize NH_4^+ (Am1). Midway through the membrane, the channel narrows over a 20 Å span. Here, two pore-lining residues, His^{168} and His^{318}, stabilize three NH_3 molecules (Am2, Am3, and Am4) through hydrogen bonding (red dashed lines). The molecules return to equilibrium as NH_4^+ in the inner vestibule.

Databases of solved protein structures are burgeoning with structural maps of both intracellular and extracellular proteins. However, structures of integral proteins with their many membrane-spanning loops are just now beginning to emerge. A common strategy, and one adopted by Khademi *et al*., is to express paralogous genes from multiple bacterial species, prepare three-dimensional crystals of the proteins they encode, and select the crystal producing the highest resolution x-ray diffraction pattern for analysis. The structures of a few eukaryotic integral proteins have been determined by cryo-electron microscopy of membrane crystals or by molecular modeling using coordinates determined from x-ray analysis of prokaryotic paralogs. Khademi *et al*.'s success with AmtB, an integral membrane protein from *Escherichia coli* with 11 membrane-spanning α helices, foreshadows continued progress with other integral membrane proteins whose structures have been elusive.

Elements of the AmtB structure reveal how this protein channel transports ammonia (see the figure). AmtB has the same structure when crystallized in both the absence and presence of ammonia, leading the authors to conclude that it is a channel rather than a transporter that would be expected to have flexible elements involved in translocation of the substrate. At the two ends of the pore, broader vestibules contain NH_3 in equilibrium with NH_4^+. AmtB has at its center a narrow hydrophobic pore element about 20 Å in length, which allows the passage of NH_3 but not the monovalent ion NH_4^+. This distinction is important because the structure must prevent ions such as K^+ from crossing the inner membrane. Thus, AmtB is an NH_3 channel that does not mediate the net transfer of protons and does not directly alter the membrane potential. Although these conclusions appear contrary to those of prior studies in which biophysical techniques indicated that ammonia translocation is affected by pH and voltage gradients (*5*), Khademi and colleagues argue persuasively that their model (see the figure) is compatible with most of the biophysical data reported so far.

Simple membrane bilayers have moderate intrinsic NH_3 permeability (*6*), so the necessity of ammonia channels could be questioned. Ammonia channels, however, may serve to accelerate ammonia transport at sites where the diffusion of NH_3 through the lipid bilayer is too slow for physiological needs, or may provide a molecular target for regulating the passage of NH_3. Both functions may be important in the mammalian kidney collecting duct where two

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Rh-related proteins are expressed—RhBG in the basolateral plasma membrane and RhCG in the apical plasma membrane (*5*). Regulated excretion of NH_4^+ by the kidneys is a crucial mechanism for controlling systemic pH (*7*). Synthesized in the proximal tubule, NH_4^+ accumulates in the renal medulla by active transport from the loop of Henle. The final step in NH_4^+ excretion involves rapid NH_3 diffusion across the collecting duct epithelium in parallel with active H^+ secretion. Although it has been assumed that NH_3 diffuses into the collecting duct lumen through the lipid bilayer, the structure of AmtB predicts that the entry of NH_3 is mediated by the Rh-related proteins expressed there. It remains to be seen whether NH_3 penetration through these proteins may be a point where systemic acid-base balance is regulated, or whether Rh-related proteins are involved in clinical disorders such as renal tubule acidosis.

Another site where rapid ammonia transport may be critical to homeostasis is the liver where RhBG is present (*8*). NH_4^+ is produced during the catabolism of amino acids and is also delivered to the portal circulation by intestinal bacteria that break down urea. NH_4^+ is a neurotoxin and must be efficiently cleared from the portal blood by hepatocytes and converted to urea and glutamine to prevent serious systemic consequences. Central nervous system dysfunction occurs if NH_4^+ concentrations are elevated, as seen in hepatic encephalopathy—a common but ominous manifestation of advanced liver failure. RhBG is expressed selectively in the pericentral hepatocytes, just before the portal blood is delivered to the systemic circulation. Thus, RhBG may be important to the process that normally clears the last vestiges of ammonia from the portal blood.

The structural determination reported by Khademi *et al*. provides great insight into the important process of gas transport. As with the transport of water, glycerol, and other uncharged solutes, the phenomenon of gas transport now has a molecular identity and an advanced level of understanding. Thus, physiologists may now be able to ask specific scientific questions about ammonia transport with great precision.

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PHYSICS

Visualizing the Dynamics of the Onset of Turbulence

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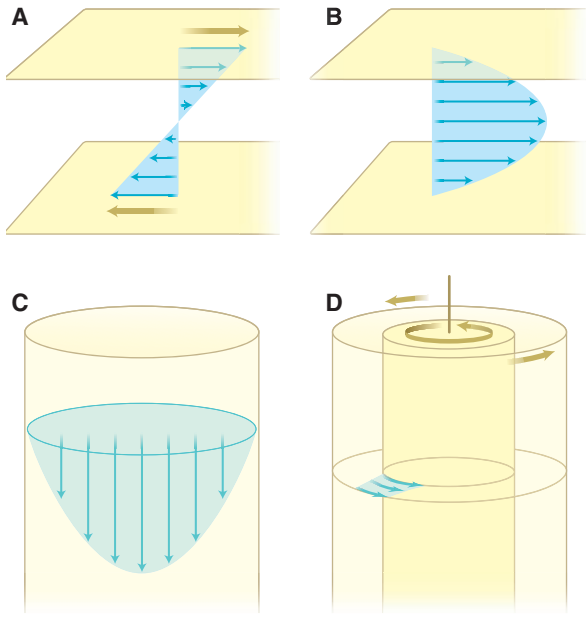
The transition to turbulence in fluid flow is an everyday experience. As a faucet is slowly opened, the initially laminar flow of water changes into an irregular chaotic flow. As a result, friction is much increased and, for the same discharge, a higher pressure head must be applied than in the laminar case. This transition is of fundamental importance in engineering problems dealing with fluid flows. On page 1594 in this issue, Hof *et al.* (*1*) present the first observation of a basic dynamical property of the transition.

The study of the onset of turbulence has a long history. In 1839, Hagen first noted the existence of two distinct flow regimes in the discharge from pipes (*2*). Some 50 years later, Reynolds (*3*) realized that the transition between these regimes only depends on a dimensionless number, $Re = UD/\nu$, where U denotes the mean velocity averaged over the circular cross section of the pipe, D is its diameter, and ν is the kinematic viscosity of the fluid.

In pipe flows, disturbances of finite amplitude are responsible for the transition to turbulence. Reynolds noticed as much when he reported that the transition was delayed to higher values of Re when a particularly smooth entrance region of the pipe was used. However, theoretical studies can treat easily only infinitesimally small disturbances, and this is one reason why theoretical understanding of the transition to turbulence in shear flows has been slow to emerge. For laminar flow in a channel between parallel plates, such analysis suggests that laminar flow should become unstable at $Re = 7696$, but experiments indicate a much lower value of ~1500 for the transition (*4*). For flow between two parallel plates sliding relative to each other with speed U (plane Couette flow) and for flow through a circular pipe (see the figure), the discrepancies are even larger: No growing infinitesimal disturbances could be found theoretically at any Reynolds number.

With today's powerful computers, it is not difficult to simulate turbulent fluid flows at Reynolds numbers of several thousands. Good agreement between statistical properties of turbulence in experiments and in numerical simulations has been found (*5*), but a detailed understanding of the transition process is still lacking.

For configurations other than plane parallel flow, theoretical studies have been more successful. For example, when the circularly symmetric flow between differentially rotating coaxial cylinders becomes unstable, axisymmetric vortices are formed, the amplitude of which increases smoothly with the Reynolds number. This is a typical example of a supercritical bifurcation (*6*), in



Simple laminar shear flows. (**A**) Plane Couette flow; (**B**) channel flow (Poiseuille flow); (**C**) pipe flow; (**D**) circular Couette flow.

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