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A missing link in membrane protein evolution

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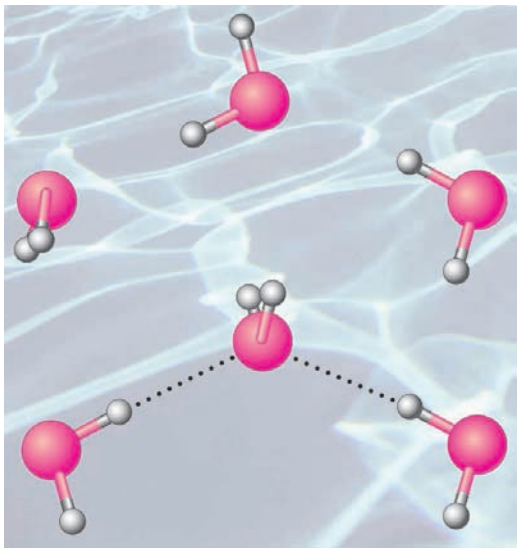

Three-body effects. The two hydrogen bonds may act cooperatively, each reinforcing the other (**top**), or they may oppose each other (**bottom**). The top configuration is bound more strongly than the bottom one. Three-body effects of this kind play an important role in water clusters and liquid water. Bukowski *et al.* (*1*) describe a potential derived entirely from first principles that captures these and other properties of water.

tal properties are averages over the potential surface, and it is difficult or impossible to extract the details of the potential surface unambiguously from such properties. Alternatively, the intermolecular potential can be calculated from first principles. The most accurate method in current use is coupled-cluster theory with inclusion of single, double, and triple excitations, the triples noniteratively. Unfortunately, this CCSD(T) method gives only the total pair potential energy, and only at isolated points. It provides no information about the functional form of the pair potential, which is needed for most applications.

Perturbation theory, on the other hand, does give information about the functional form, but is very complex and computationally demanding, and cannot yet achieve the same accuracy. Recent practice has been to refine the details of the potential by fitting to experimental data.

Fitting is a well-established technique in many fields of science, but it has pitfalls: It may improve some properties at the expense of others. For example, in the case of the simple potentials mentioned above, enhancement of the molecular dipole moment improves the calculated properties of the liquid but ruins the description of the dimer. In that case, however, the form of the potential is known to be inadequate. Careful use of perturbation theory can give the right functional form, and the numerical parameters can be refined by fitting to the very accurate experimental data that have been obtained from high-resolution spectroscopy on the dimer and small clusters. This approach can lead to potentials that give a good account of both small clusters and the bulk liquid (*7*).

Nevertheless, the challenge to obtain a good intermolecular potential energy function entirely by calculation from first principles, using experimental data only as a test of its quality, has not been met. This is what Bukowski *et al.* have been able to do. They have used perturbation theory to determine the form of the potential function, and fitted the parameters in it using data points for the water dimer calculated by the CCSD(T) method.

An advantage of this approach is that the CCSD(T) data points cover the energy surface much more completely than the spectroscopic data, which describe only the region in the neighborhood of the energy minimum and the barriers to neighboring minima. The CCSD(T) calculations were carried out only for the dimer, but with a good functional form the resulting potential is able to give a good account of the many-body interactions and hence of the liquid properties as well as the dimer spectrum.

This work is not the end of the story. The calculated properties are good, but leave room for improvement. The form of the potential function omits some of the smaller terms. Ideally, one would wish to obtain accurate numbers as well as the functional form from perturbation theory. Nevertheless, Bukowski *et al*. have been able to show that a good description of water from first principles is becoming feasible.

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BIOCHEMISTRY

A Missing Link in Membrane Protein Evolution

Bert Poolman, Eric R. Geertsma, Dirk-Jan Slotboom

Discerning the orientation of subunits of an unusual bacterial membrane protein suggests how the particular topology of other membrane proteins may have evolved.

Most proteins embedded in biological membranes have vectorial functions, such as transporting molecules into or out of cells or transducing signals. It is thus essential that these membrane proteins have unique orientations in the lipid bilayer. To achieve a unique orientation, membrane proteins carry signals in their amino acid sequences that are recognized during the membrane insertion process. Intriguingly, some membrane proteins have structurally similar, homologous regions with opposite orientations in the membrane, raising questions about their evolution. On page 1282 of this issue, Rapp *et al.* (*1*) offer a compelling explanation for how such proteins may have evolved.

One of the best-understood signals for membrane protein topology is the "positive-inside rule": Positively charged residues such as lysine (K) and arginine (R) tend to be most abundant ("K+R bias") in loops located at the cytoplasmic side of plasma and endoplasmic reticulum membranes (*2*). Crystallography has shown that many membrane proteins contain homologous domains with opposite (antiparallel) membrane orientation, leading to proteins with a quasi–two-fold axis in the plane of the membrane. Well-known examples are the members of the aquaporin family, in which the first three transmembrane segments are homologous to the last three but with opposite membrane orientation. Exactly how such quasi-symmetrical proteins have come about has been puzzling. Rapp *et al.*

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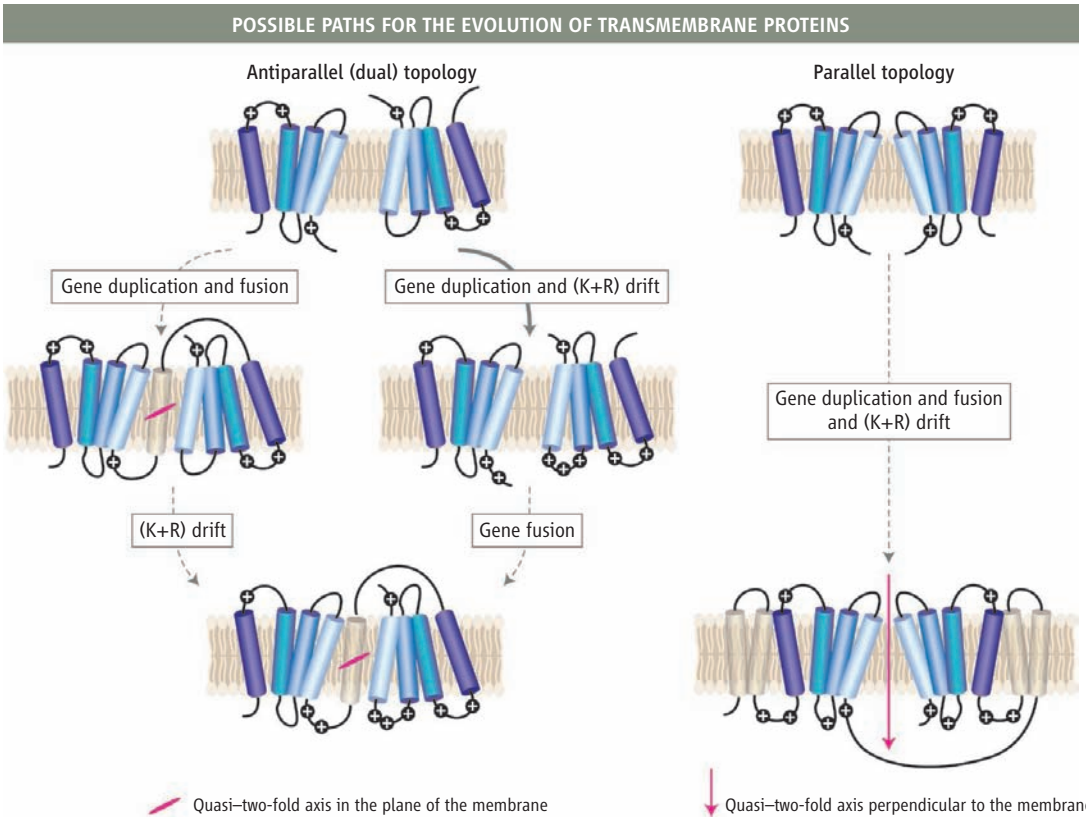

Plausible evolutionary paths. Membrane proteins with multiple homologous domains may have evolved through gene duplication, gene fusion, and drift events [bias to lysine (K) and arginine (R) residues in cytoplasmic regions of the protein]. The resulting proteins have similar domains with either antiparallel topologies (**bottom left**), or parallel topology (**bottom right**). Shaded cylinders depict additionally inserted transmembrane segments. Bold arrow indicates the evolutionary path simulated by Rapp *et al.* (*1*); dashed arrows indicate hypothetical events.

reinforce their earlier proposal that rare "dual-topology" proteins form a missing link in the evolution of membrane proteins with antiparallel domains. They trace the evolutionary path by examining the multidrug transporter EmrE from *Escherichia coli* and demonstrate that antiparallel dual topology of the transporter's subunits is required for its functioning.

EmrE is a well-characterized protein with four transmembrane segments. Its functional unit is a homodimer, but the membrane orientation of the two subunits of EmrE is a matter of fierce debate. The protein does not have strong topological signals (weak K+R bias), and evidence has been presented for both an antiparallel (dual topology) and parallel orientation of the subunits (see the figure) (*3*–*7*). The possibility of oppositely oriented subunits in EmrE was first proposed by Tate and colleagues (*7*) on the basis of a cryo–electron microscopy analysis of two-dimensional (2D) crystals. A model consistent with most of the available biochemical and biophysical data was proposed in which EmrE could be arranged as an antiparallel homodimer (*8*). On the other hand, a rigorous cross-linking study pointed toward a parallel orientation of the EmrE subunits (*5*). Regrettably, some of the assumptions in the latter work were based on a structural model that has recently become obsolete (*9*).

Rapp *et al.* now present strong evidence for dual topology of the EmrE subunits as a requirement for its function. They forced the subunits to insert into the membrane in a single orientation [with the carboxyl terminus either inside, EmrE(C_{in}), or outside, EmrE(C_{out})] by manipulating the number of positive charges in the loops connecting the transmembrane segments, resulting in a stronger K+R bias. EmrE(C_{in}) and EmrE(C_{out}) were inactive when expressed independently in bacteria. However, expression of both subunits simultaneously restored drug resistance to the level observed with the wild-type EmrE, which is indicative of a functional transporter. Thus, oppositely oriented subunits of EmrE are required for its drug efflux activity.

This work explains the occurrence of antiparallel domains observed in the 3D structures of many membrane proteins by providing a plausible path for the evolution of such transmembrane proteins: After gene duplication, a dual-topology protein could evolve via genetic drift toward a K+R bias, whereby the subunits obtain a fixed orientation (see the figure). A subsequent gene fusion event would allow a single polypeptide to accommodate all functionalities. For proteins with an even number of transmembrane segments, this requires the insertion or deletion of a transmembrane segment. In principle, the order of these events could be reversed. Either pathway leads to a membrane protein with a quasi–two-fold axis in the plane of the membrane (see the figure).

For comparison, a protein with a quasi–two-fold axis perpendicular to the membrane plane could evolve from the fusion of proteins with a parallel topology (see the figure). Prototypic of this class are the members of the major facilitator superfamily. The currently available 3D structures of channels and transporters indicate that proteins with quasi-symmetry, resulting from the duplication and fusion of ancestral proteins with either parallel or anti-parallel topologies, are the rule rather than the exception.

In a proteome-wide screen of the topology of transmembrane proteins in *E. coli*, von Heijne and colleagues previously showed that the vast majority of the proteins exhibit a unique topology (*10*). Obviously, for many membrane proteins, a unique orientation is required. For instance, domains that bind to ligands, possess certain enzymatic activities, or are chemically modified (such as by phosphorylation) need to be located on the physiologically relevant side of the membrane. However, the EmrE case shows that in principle, transporters could have dual topology.

So why are dual-topology proteins so rare? EmrE is a dimeric protein, and ideally, the subunits for such a dual-topology dimer should insert exactly 50% EmrE(C_{in}) and 50% EmrE(C_{out}). A large excess of either orientation would be a waste of cellular resources and might exert a detrimental effect if "unpaired" subunits are toxic to the cell. The realization of equal amounts of oppositely oriented subunits may well be beyond the control of the membrane insertion machinery and, in

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addition, would put strong constraints on the evolution of such proteins. Any mutation that would alter the optimal insertion ratio would be a selective disadvantage, even though it could improve the catalytic activity. The dual-topology organization of EmrE likely represents an evolutionary transitional form. The work by Rapp *et al*. tips the balance in the controversy about one protein's unusual orientation in the membrane. A broader consequence of this observation may be a plausible evolutionary path for membrane proteins with antiparallel domains.

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ECOLOGY

How the Wood Moves

Recent studies show that the movement of plant genes across the landscape involves a complex web of pollinators and fruit-eating animals.

Katriona Shea

At first glance, an obvious difference between animals and plants is movement: Elephants move, trees don't. This is in part why Tolkien's ents (*1*), Wyndham's triffids (*2*), and the march of Birnam Wood in Shakespeare's *Macbeth* (*3*) elicit such a strong response. But in fact plants do move, although only at certain life stages and usually with outside help. For example, dandelion seeds blow and sycamore samaras helicopter in the wind, acorns and berries are moved by mammals and birds, and pollen is spread by wind and insects. These movements spread plant genes across the landscape, generating the spatial patterns of distribution and abundance of species that we observe in nature. Recent work demonstrates just how complex the web of pollinators and fruit-eating animals that determine tree gene flow can be (see the figure).

For both plants and animals, studying movement is just as difficult as it seems. Animal ecologists have radio-collared cheetahs and lions, attached satellite-linked tracking devices to seals, and painted marks on beetles to track their movements. Plant ecologists, however, are usually faced with a problem of scale. How can we follow the movement of smaller diaspores (dispersal units), such as seeds and pollen grains? Approaches broadly fall into two categories—tracking of diaspores from a source, or relocation of diaspores at different distances from parent plants (*4*)—and scientists have become very creative in their quest (*5*). Notable approaches include observational studies of dispersers at the parent plant for animal-dispersed species; studies of the environmental conditions promoting seed release of wind-dispersed species; trapping of seeds at different distances from possible parents; marking of seeds on the parent plant with ink, fluorescent powder, or radioactive markers to allow later relocation and identification of seeds; genetic methods to link seeds or pollen to possible parents; and even chasing individual seeds as they blow across the landscape.

Over the past decade, Jordano and collaborators have been patiently disentangling the gene flow story for a key tree species in southeastern Spain by combining several of these methods (*6*). Mahaleb cherry, *Prunus mahaleb*, is a tree with delicate white flowers and black fruits. Some trees are hermaphrodites (with both male and female functions); others are functionally female. Thus, both types of trees are potential seed sources, but only the hermaphrodites can provide pollen. Jordano *et al.* have studied nine distinct populations of these trees and determined the genotypes of all reproductive individuals. Their observational studies show that mahaleb cherry fruits (each contain-

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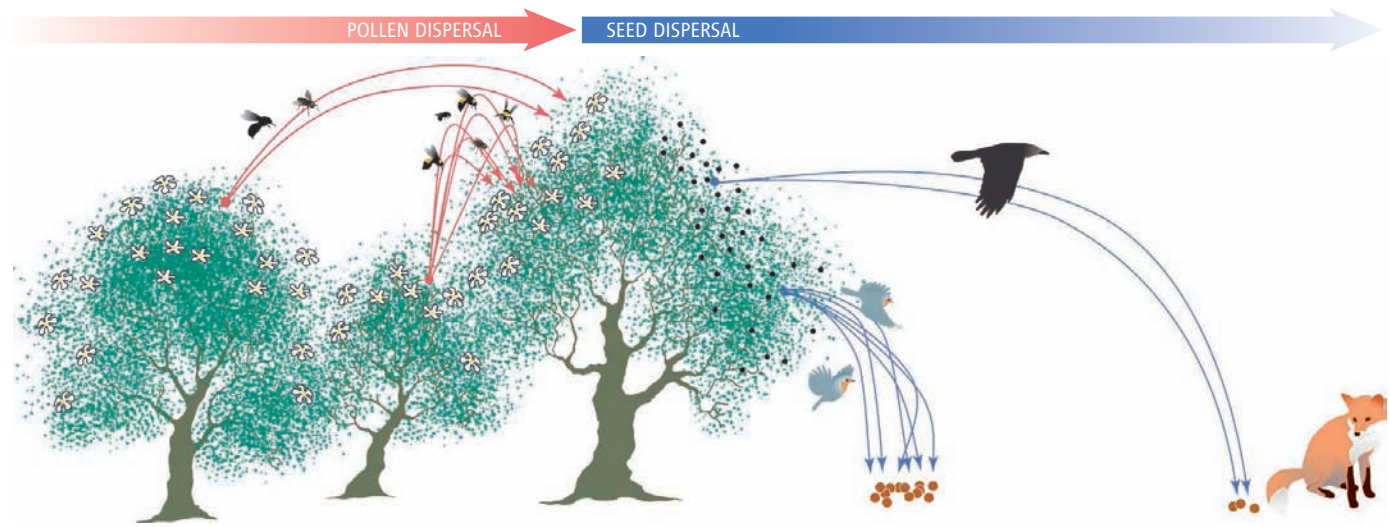

Gene flow in trees. Mahaleb cherry pollen and seeds are moved over short and long distances by strikingly different assemblages of pollinators and fruit-eating animals. This has important implications for gene flow within and between populations and for the establishment of new populations.

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