of volcanism's far-reaching effects. It is now clear that total SO_2 is at least as important to climatic impact as eruptive volume, the traditional volcanological measure of eruption size and the only one that can be conveniently estimated for ancient events. Furthermore, these two measures are not always correlated, and several high-SO_2 eruptions of modest size have put up significant stratospheric aerosols (5). These findings complicate attempts to evaluate climatic and other impacts of ancient eruptions.

But all these complications and weaknesses in the record help to underscore the importance of alternative approaches from other disciplines to the building of a reliable chronology of global volcanism. The H_2SO_4 aerosols eventually settle to Earth, and pioneering work by Danish glaciologists in the 1970s (6) showed that the resulting acidity layers in deep ice cores from Greenland provide a volcanic chronology. American and French groups found the same evidence in Antarctica and, by correlating several layers and confirming the common composition of their (rare and tiny) volcanic glass fragments, showed that some eruptions have a truly global distribution of products (7). These results have continued, through painstaking work on ever more cores, and the report by Zielinski *et al.* discusses results from the newest and deepest Greenland core (1). The authors suggest more accurate dates for several large eruptions and provide many new dates (particularly before 0 B.C.) from unknown sources. The largest signal in the last 7000 years, also detected in Antarctic cores (7), was from an unknown source around 1258 A.D. However, four larger signals were found in the 7th millennium B.C., marking this as easily the most volcanically active part of postglacial time.

The new results are exciting to all scientists interested in the volcanological record. The principal problem of this approach, however, is that aerosols move swiftly eastward around the globe but their latitudinal spread is relatively slow. This means that an eruption from high north latitudes (Iceland, Alaska and Kamchatka) leaves a relatively large volcanic deposit on Greenland, whereas a comparable one from low latitudes leaves a much smaller record, and one from the Southern Hemisphere may leave none at all. Until more cores are obtained from mid- and low-latitude sites (not famous for their stable glaciers), substantial uncertainty will surround the identification and calibration of eruptive sources. Added to this problem is the danger of misinterpreting the completeness of volcanism's recent historical record. Very large eruptions may well have been missed only a few hundred years ago in some parts of the world, so the matching of sulfate spikes with poorly constrained dates from the volcanic record needs caution. Nobody should be surprised to learn tomorrow of a previously unreported larger eruption around that same time from another part of the world.

The ice core approach is enormously exciting, however, and holds great promise. The pieces of a large puzzle seem to be falling into place. The linking of these results to proxy records of past climate, such as tree-ring chronologies (8), offers the opportunity to refine both volcanic and climatic chronologies while gaining a more profound understanding of the relation between volcanism and climate change.

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Folding Pattern Diversity of Integral Membrane Proteins

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Membranes consist of phospholipid bilayers that are highly insulating and confine cells and subcellular compartments. Solute transport, signal transduction, and energy conversion across these barriers are catalyzed by proteins that traverse the hydrophobic core of the membrane (a width of about 30 Å). The surface of these proteins that interacts with the membrane core is also hydrophobic, a property that distinguishes these proteins from globular ones and causes them to aggregate in aqueous solution (unless amphiphiles such as detergents are used to replace the lipids). At the boundaries between the lipidic and aqueous compartments, these proteins contact the polar head groups of the phospholipids. Beyond, they may exhibit domains of various sizes, which are exposed to the aqueous phase.

The number of times a polypeptide spans a membrane varies from one to perhaps two dozen times. Of the few proteins for which structures have been determined at high resolution, the domains within the membrane exhibit rather simple topologies, suggesting that structure prediction of the integral membrane domains of these proteins should be straightforward. There are fundamental limitations to the applicability of the methods currently in use, however, and these, together with ways to overcome them, are discussed here.

Bacteriorhodopsin provided the first glimpse into the organization of a polypeptide in a membrane (1) (panel A in the figure). Its seven transmembrane α-helical segments each consists of ~25 hydrophobic residues. They expose hydrophobic surfaces in the membrane core and allow the hydrogen bonding potential of the backbone to be saturated *within* each segment (panel B). The structure suggested a prediction algorithm (2) that is now applied routinely to obtain structural models of transmembrane proteins and that identifies potential membrane-spanning segments on the basis of the existence of hydrophobic stretches, allowing protein sequence data banks to be scrutinized rapidly. When the structure of a photosynthetic reaction center was solved to atomic resolution, the prediction fitted the structure well (3). As a consequence, membrane-spanning proteins are now generally conceived as containing long, hydrophobic α helices. This concept also suggests an attractive mechanism for membrane insertion and secretion by partitioning (4). This algorithm has been diversified (5) and complemented by the finding of asymmetrical distributions of residues (for example, positive charges inside) in α-helical membrane-spanning segments (6). This rule helps to define the ends of such segments

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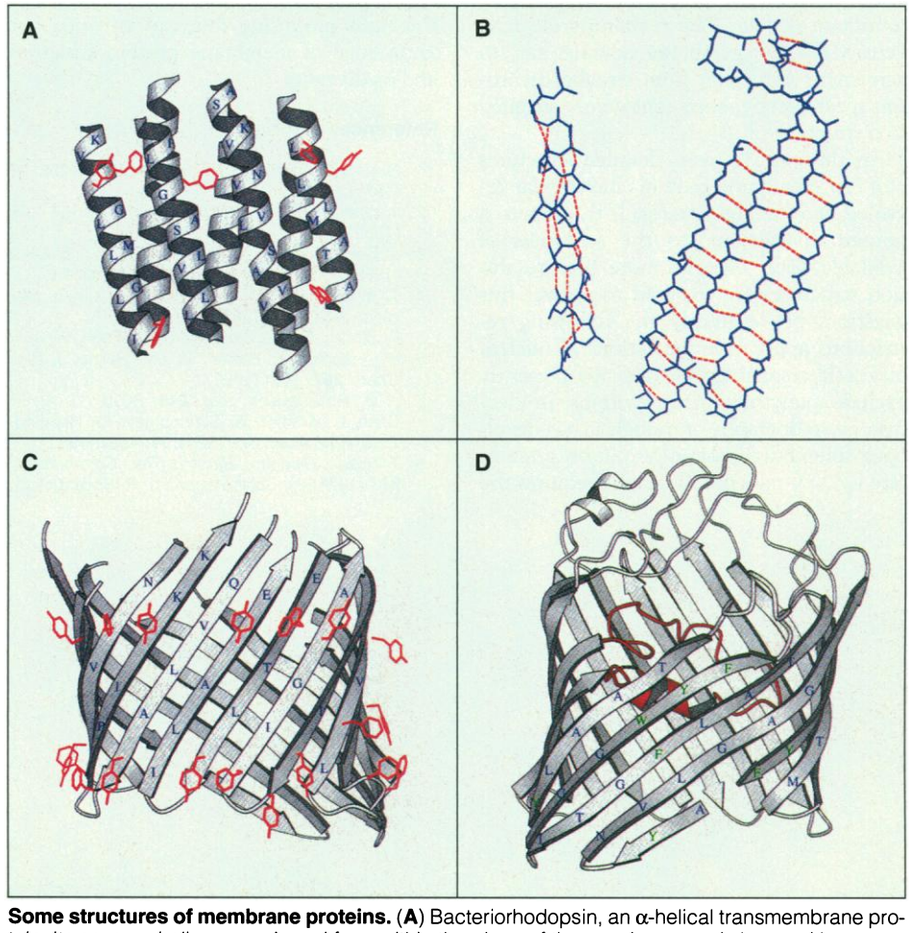
and their orientation in the membrane.

The universal validity of this algorithm has been challenged by the existence of an integral membrane protein that is highly polar, lacks hydrophobic segments, and consists predominantly of β structure (*7*). This trimeric protein forms water-filled, voltage-gated channels across bacterial outer membranes and facilitates the diffusion of small polar solutes (less than 600 daltons) across this barrier, hence its name porin (*8*). In membranes or in the isotropic state (after detergent solubilization), it exhibits an unusually high stability, a characteristic that is well explained by the x-ray structures of three members of the porin family (*9*). As seen in panel C, a simple β barrel spans the hydrophobic membrane core, with small, aliphatic residues exposed to the lipidic phase. Donor and acceptor groups of the polypeptide backbone are fully saturated by hydrogen bonds *between* adjacent antiparallel β strands. Relatively simple algorithms have been devised to predict the structure of such proteins: One relies on alternation of hydrophobic and polar residues exposed to the membrane core and the water-filled channels, respectively, another β turn exposed to the aqueous phase, and a third on hydrophilicity profiles (*10*). However, in an extended polypeptide, eight to nine residues suffice to span the hydrophobic core of the membrane, and of these, only four to five are exposed to the lipids and need to be hydrophobic. Moreover, the intervening residues need not be fully accessible to water in the transmembrane channels and hence are not necessarily polar. The constraints are thus minimal, and searches identify many such short segments in nonmembrane proteins. As is shown in panel C, porin exhibits belts of aromatic residues at the two hydrophobic-hydrophilic boundaries that presumably anchor the protein in the membrane. Similar, although less striking, distributions are observed in bacteriorhodopsin (panel A) and in reaction centers. This property can be exploited as an additional constraint in prediction algorithms. Attaching arbitrarily high hydrophobicity values to aromatic residues has been used in a structure prediction of maltoporin (*11*). This protein shares several functional and structural properties, but no sequence homology, with other porins, and the result of the prediction is in agreement with topological studies (*12*).

A severe limitation to structure prediction of any membrane protein is the existence of segments that lie within membrane boundaries without spanning the bilayer: A long loop in porin, which forms the essential constriction site in the channel (panel D), folds into the lumen of the pore and lies parallel to the membrane plane without contacting lipids. An analogous structure may exist in highly specific ion channels (*13*).

On the basis of energetic considerations, it is clear that periodic structures must be involved in the saturation of the hydrogen-bonding potential of transmembrane segments in contact with lipids. It would seem that there is no a priori limitation to α helices and β barrels, however, and that other periodic structures, such as 3_{10} or π helices, might do just as well. Search methods for any periodicity have been devised and applied (*14*), but the results remain unconfirmed. In globular proteins, very short segments of such periodicities are found at the ends of α helices. In membrane proteins, such structures may be stabilized by electrostatic interactions which, in an environment with a low dielectric constant, are considerably stronger than in aqueous solution (*15*).

But must we rely on predictions alone? Experimental methods to address membrane topology were actually the first to provide clues concerning the organization of proteins in membranes (*16*). These have been refined over the past 20 years, and complemented by immunochemical and genetic approaches, as illustrated with porins (*12*, *17*). Such methods cannot cope, however, with the ever increasing rate at which membrane protein sequences emerge: They are slow, laborious, require considerable amounts of protein, and their interpretation, although at times highly provocative (*18*), is not always unequivocal. Secondary structure, which is critical



Some structures of membrane proteins. (**A**) Bacteriorhodopsin, an α-helical transmembrane protein. Its seven α helices are viewed from within the plane of the membrane and shown without connecting loops. Residues facing the viewer are labeled in the single-letter code, with aromatic groups shown in full. (**B**) Saturation of the hydrogen-bonding potential in peptides within membrane boundaries. At left is an α helix from bacteriorhodopsin. The hydrogen-bonding potential is saturated by *intra*segmental bonds (dotted lines). On the right, four β strands from porin show how all hydrogen bonds are saturated *inter*segmentally. (**C** and **D**) Two views of the porin monomer. Each monomer is a highly regular β barrel with 16 antiparallel β strands. Panel C highlights the segregation of nonpolar and polar residues. Individual strands are connected to their nearest neighbors by short turns on the periplasmic side (bottom) and by longer loops (truncated here for clarity) on the extracellular face of the protein (top). The surface exposed to lipids consists of short, aliphatic residues. Panel D shows the opposite face of the barrel where residues are involved in interactions with neighboring subunits (near the threefold molecular axis). In this representation, the external loops forming the channel entrance are shown. One loop (in red) folds into the channel and forms the constriction. It lies within the membrane boundaries but is not in contact with lipids. Diagrams were produced with the programs "Molscript" and "O" (*23*).

to an evaluation of structure predictions, can be examined readily by spectroscopy. At the present time, the most informative method is Fourier-transformed infrared spectroscopy, which is rapid and requires little material. This technique is useful when proteins contain a single type of secondary structure, but various helical structures, such as 3_{10} and α helices, are difficult to distinguish (*19*). The assignment of mixed secondary structure motifs to single or multiple domains, within or without membrane boundaries, is, moreover, complex and difficult. An illustrative example is the structure of the acetylcholine receptor protein, curently resolved to 9 Å by electron crystallography. It reveals a single membrane-spanning α helix per subunit, whereas all previous structural predictions suggested a minimum of four. The remaining electron density in the membrane domain has, in agreement with data from circular dichroism spectroscopy, been tentatively assigned to β structure (*20*).

In the past 10 years detailed structures of a few membrane proteins have so far revealed two folding patterns: Is this due to a limited diversity or to the scantness of available data? Clearly, more high-resolution structures are required to answer this question, but currently the following restrictions apply. Size limitations of nuclear magnetic resonance spectroscopy presently exclude many membrane proteins. In electron crystallography, although it has developed splendidly, atomic resolution remains rare (*21*). X-ray crystallography requires the growing of crystals, and membrane proteins are often unstable once removed from their native quasi-solid state Their intrinsic characteristic, the coexistence of hydrophobic and hydrophilic surfaces, complicates the selection of crystallization conditions, and the colloidal chemistry of detergent solutions is not simple. Yet, combining different methods has proved rewarding: A recent illustrative example is the toxin aerolysin. The high resolution x-ray structure (2.8 Å) of its soluble form reveals β sheets in two domains which, according to electron microscopy at a resolution of 25 Å, suggests association to a β barrel upon oligomerization (*22*). Thus, an imaginative, open-minded, and critical assessment of structure prediction in combination with results from physical methods should prove the most promising route to advance our knowledge of membrane protein structure and its diversity.

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