Membranes at EMBO

from M. S. Bretscher

The 2nd EMBO Symposium was held at Hirschhorn-Heidelberg on May 4–8, 1976, and entitled The Structure and Function of Biological Membranes.

THAT the second EMBO Symposium was devoted to "The Structure and Function of Membranes" is a measure of the extraordinary advance made in that area over the past few years. Roughly the first half of the meeting was devoted to transmembrane proteins. These can be divided into two groups: those which extend across the bilayer in a simple manner, such as with a single α-helix—the "fibrous" proteins, and those which have an extensive mass in the plane of the membrane—the "globular" proteins (M. S. Bretscher, MRC Laboratory of Molecular Biology, Cambridge). Examples of the fibrous class include the erythrocyte MN glycoprotein and the spike proteins of Semliki Forest Virus. In the latter case, freeze fracture electron microscopy shows that there are no visible intermembrane particles in the fracture plane, (K. Simons, European Molecular Biological Laboratory, Heidelberg), which fits well with the view that these fibrous proteins have little mass in the plane of the membrane. All the other transmembrane proteins discussed were globular and in freeze fracture are visible as particles.

Had he been present, Walther Stoeckenius would have been pleased to see the progress made on two ordered membrane proteins which he first isolated—the purple protein (bacteriorhodopsin) and gap junctions. The purple protein is induced in a halophilic bacterium when growth conditions become unfavourable. Its assembly can be studied by addition of nicotine to the growth medium which inhibits the synthesis of the cofactor, retinal. Bacterio-opsin is formed which, on subsequent addition of retinal to the culture generates bacteriorhodopsin. In these conditions the bacteria have no purple membranes, however, just a brown membrane in which the bacteriorhodopsin probably exists as single molecules. Formation of the purple membrane seems to be more than just crystallisation of individual molecules of bacteriorhodopsin in the plane of the membrane, as the process requires energy (D. Oesterhelt, University of Wurzburg). The structure of the purple protein, as deduced largely from low dose electron microscope image reconstruction, shows that little of the globular protein extends outside the lipid bilayer. About 70% of the protein can be accounted for by four α-helices which are perpendicular, and three which are tilted, to the plane of the membrane, (R. Henderson, MRC, Cambridge).) So far, X-ray studies of ordered arrays of gap junctions have been hampered by low short-range order. Nevertheless, the electron density profile suggests a rather even concentration of protein across the two bilayers constituting the junction, with about half the intercellular space filled in by protein. A tentative model for this array exists in which each pore is composed of twelve subunits: six in each bilayer (D. Caspar, Brandeis University). These two proteins, then, have taken on a defined shape: they no longer are drawn as wursts (objects with which the meeting became too well-acquainted at meals). Chemical characterisation of the gap junction protein is complicated by proteases, so that the subunit molecular weight of its constituent protein(s) is not yet known (N. Gilula, Rockefeller University).

X-ray or neutron diffraction studies of membranes can be aided by having an oriented specimen. Surprisingly, this can be achieved by applying a magnetic field: some membranes, such as erythrocyte ghosts, orientate parallel to the field, others (purple membranes and rod outer segment disks) perpendicular to it. This may be useful, not only for orientating membranes, but for what it tells us about the overall polarity of α-helices in the membrane. Using this ordering technique, only slight changes occur in the X-ray diffraction pattern of rhodopsin in disk membranes on illumination—changes which are too small to be compatible with the model proposed by Blasie in which rhodopsin sinks a long way into the membrane on bleaching (M. Chabre, University of Grenoble).

A major function of specialised membranes is in energy production—pumping protons and using this gradient to make ATP, as proposed by Peter Mitchell. A beautiful system for studying the relation of proton gradients to electron transport and ATP synthesis is closed vesicles of higher plant chloroplasts, in which a gradient of 4 *p*H units can be generated by light. This *p*H gradient behaves as would be expected if it were an energy-storing device between electron transport and ATP formation. In addition, the pathway is reversible—artificially produced proton gradients drive reverse electron flow, which in turn induces light emission (M. Avron, Weizmann Institute).

The ion channel associated with acetylcholine receptors of denervated frog muscle can be studied with electrodes with exquisite sensitivity by limiting the surface area studied to a patch a few microns in diameter. The opening and closing of individual ion channels can be monitored and their properties, such as mean open time and mean conductance, can be determined. These results agree well with, and thus confirm, previous conclusions on the nature of ion channels obtained from membrane "noise analysis", and show that the current produced by a single channel has a square wave form (B. Sakmann, Max-Planck Institute, Gottingen).

The functional organisation of intracellular organelles is one of the most challenging areas of cell biology. A powerful approach to understanding the biochemical anatomy of higher cells is immunofluorescence microscopy, which has been used to special advantage to study the microfilament system in fibroblasts. This reveals that stress fibres contain, besides actin, myosin, tropomyosin and α-actinin. How the assembly of these structures is controlled, however, or why viral transformation (in SV3T3 cells) should lead to a gross diminution in the amount of fibres seen, is unknown. In addition, this technique has been used successfully to look at the disposition of microtubules and their polymerisation

Fv-1 function

IN the report of April 22, 1976 on the Armand Hammer Symposium at the Salk Institute (*News and Views*, **260**, 669; 1976), it was indicated that our data on the function of the Fv-1 locus of the mouse showed that its product restricts growth of murine leukaemia viruses after integration of the provirus. At the time of the meeting, we had preliminary evidence in that direction which was discussed. More complete investigation, however, has shown that integration is prevented by the Fv-1 gene product although synthesis of proviral DNA is not prevented. Reports of our results and similar results of Sveda and Soeiro are in press in the *Proceedings of the National Academy of Sciences U.S.A.*

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