The Structure and Chemistry of Mammalian Cell Membranes

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A GREAT DEAL MORE IS KNOWN about the functions of cell membranes than is known about their detailed structure. It is a truism of molecular biology, however, that before a satisfactory understanding of the functions of any biologic system can be achieved, the structure of that system must be known. A satisfactory understanding of antigenic markers and receptors on lymphocytes, the subject of this Symposium, must ultimately depend on a knowledge of the composition and the structure of lymphocyte and other cell membranes. By the structure of cell membranes, we mean the detailed arrangement and conformations of the individual proteins, lipids, oligosaccharides and other components of membranes. While we are a very long way from such knowledge at the present time, significant progress has been made in the study of membrane structure in recent years, largely because of advances in biophysical technics and chemical methodology, and their application to membrane systems.

One of us [1] has recently examined in some detail the present status of theoretic and experimental studies on membranes. The first part of the present paper is a brief summary of this review; for further details the reader is referred to the original. In the following discussion, we will confine our attention to plasma membranes of cells, although much of the discussion, with appropriate modification, is equally applicable to other types of membranes. Studies of plasma membranes that have been carefully prepared and adequately freed of other cellular constituents have revealed certain features that may be accepted as characteristic of these membranes. These features, and the interpretation we place on them, are enumerated below.

1. *Proteins Constitute the Major Class of Constituents of Plasma Membranes.* The ratio by weight of proteins to lipids ranges from 1.5

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to about 4 for different plasma membranes (*cf* the review by Korn[2]).

This fact suggests that at least some portion of this protein must play an important role in determining and maintaining the structure of plasma membranes; *ie*, membrane structure is not likely to be exclusively determined by lipids.

2. *The Proteins of Plasma Membranes are Grossly Heterogeneous.* This is particularly revealed by the powerful method of acrylamide-gel electrophoresis in detergents.[3–5] Initial reports to the contrary notwithstanding,[6] it is now generally accepted that there is no convincing evidence that there exists one type or class of membrane proteins that is specifically and exclusively "structural protein." In this respect, plasma membranes differ from certain special membrane systems, such as retinal rod membranes,[7] which appear to contain only one or a few proteins.

It follows from features 1 and 2 that probably many different kinds of membrane proteins play important structural roles in plasma membranes.

3. *The Proteins of Membranes are Generally Hydrophobic in Character.* The importance of this fact to the structure of membranes was first emphasized by Green and his coworkers.[6,8] If the lipids of plasma membranes are extracted, the residual proteins are largely insoluble in neutral aqueous buffers, and require the addition of lipids or detergents to solubilize them.

This fact suggests that (a) membrane proteins interact with lipids predominantly by hydrophobic interactions and only secondarily by electrostatic interactions, and (b) the proteins of membranes are by and large unique to membranes (and are not present in substantial concentrations elsewhere) because of their unusual solubility properties.

4. *A Large Fraction of the Protein of Intact Plasma Membranes Is in the α-Helical Conformation.*[9–12] In red blood cell membranes, 40% of the protein is α-helical.[13] This is a larger fraction of α-helix than is exhibited by most of the *globular* soluble proteins that have been examined.[14]

This fact suggests that, by and large, the proteins in intact plasma membranes are globular in their conformation rather than spread out as monolayers.

5. *The Bulk of the Phospholipid of Plasma Membranes is in the Form of Bilayer.* It has been demonstrated by a variety of physical

methods, including differential thermal analysis,[15] x-ray diffraction [16] and spin labeling,[17] that the fatty-acid chains of the phospholipids of intact plasma membranes are in a "liquid" (disordered) state at 37 C in aqueous media, but upon lowering the temperature sufficiently, they may undergo a reversible phase transition to a "crystalline" (ordered) state. In both liquid and crystalline states, the phospholipid is arranged as a bilayer, as is directly demonstrated by x-ray diffraction.[16]

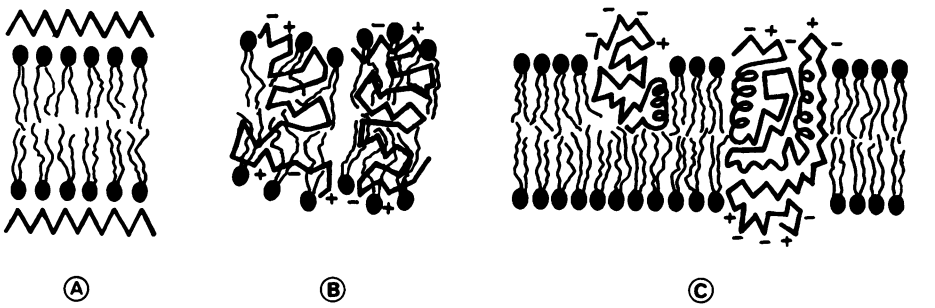
It is important to note two qualifications to these conclusions: (a) some of the lipid may not be in the bulk bilayer phase, but may be more strongly interacting with proteins in the membrane and (b) the experimental data do not distinguish between whether the phospholipid is an infinitely continuous, or is an interrupted, bilayer. In physicochemical terms, the data that show that the phospholipid bilayer of the intact membrane can undergo a change of phase do not establish the number of lipid molecules in the cooperative unit undergoing the phase transition; this number may be as small as 100–1000.

6. *The Average Thickness of Different Plasma Membranes is 75–90 Å According to Electron Microscopic Studies.* The thickness of a phospholipid bilayer is about 45 Å. The arrangement of the proteins and lipids of membranes must be consistent with these dimensions.

In particular, an arrangement in which a continuous lipid bilayer would be coated on both surfaces with a layer of *globular* proteins would result in an average membrane thickness that was substantially larger than is observed.

These facts, together with the thermodynamic properties of macromolecular systems in aqueous solution, must be accommodated by any model for the organization of the proteins and lipids of membranes.[1] For the purposes of this discussion, three schematic models for this organization may be considered (Text-fig 1). These models, it should be emphasized, are very schematic; each is intended to indicate a different predominant way for the lipids and those proteins that play a structural role in membranes (called *integral* proteins by Singer [1]) to be arranged. As such, the three models represent different ways of thinking about membrane organization, rather than detailed descriptions of that organization.

As is shown in detail elsewhere,[1] only the lipid-globular protein mosaic model (Text-fig 1C) is consistent with all the facts and restrictions mentioned above. The classical Davson-Danielli-Robertson model (Text-fig 1A) is not consistent with the large α-helical content of the membrane proteins, nor does it rationalize the hydrophobic



TEXT-FIG 1—Three schematic models for the organization of the proteins and lipids of plasma membranes. The phospholipids are depicted with their polar heads represented by filled circles and their fatty acid tails by wavy lines. The polypeptide chains of the proteins are represented by the thick lines, and the positions of the ionic residues of the proteins are shown by plus and minus signs.

A—The Davson-Danielli Robertson model. The lipids are arranged in a continuous bilayer with their polar heads covered by protein largely spread as monolayers (pleated sheet, or β-conformation). The proteins and lipids are presumably bound together primarily by electrostatic interactions between the ionic heads of the lipids and the ionic residues of the proteins.

B—The Benson model. The fatty acid chains of the lipids and the hydrophobic portions of the proteins are intertwined to form lipoprotein subunits. These subunits are stacked in two dimensions to form the membrane.

C—The lipid-globular protein mosaic model. The integral proteins (or lipoproteins) of the membrane are largely globular and are intercalated into a matrix consisting of a discontinuous lipid bilayer. The ionic and polar heads of the lipids and the ionic residues of the protein are all in contact with the aqueous phase. The fatty acid chains of the lipids and the hydrophobic residues of the proteins provide an interior hydrophobic core to the membrane.

character of membrane proteins. It is also thermodynamically less stable than the other two models.[1] The so-called Benson model (Text-fig 1B) is not consistent with the fact that the bulk of the lipid of a membrane is in a bilayer form.

In the lipid-globular protein mosaic model, the integral proteins of the membrane are globular and alternate with patches of lipid bilayer to form a mosaic structure. The hydrophobic regions of the proteins are intercalated into the interior of the membrane along with the non-polar fatty acid chains. The ionic groups of the proteins, along with the ionic and polar groups of the phospholipids, are essentially all at the exterior surfaces of the membrane where they are in contact with the bulk aqueous phase.

In order to test this mosaic model, Lenard and Singer [18] investigated, and Glaser *et al* [19] made more extensive studies of, the effects of the enzyme phospholipase C on red blood cell membranes. This enzyme cleaves the phosphorylated amines from phospholipids, leaving behind a diglyceride residue. These investigators found that some 70% of the phosphorus of the intact membrane could be removed by phospholipase

C, and that while the surface area of the membrane decreased, the membrane remained intact after this treatment. Furthermore, not only was there no protein released from the membrane, but the average conformation of the protein as measured by circular dichroism in the region of peptide-bond absorption region, did not change detectably after the enzyme action. These results suggested that (a) the polar heads of the phospholipids in the intact membranes are able to come into atomic contact with the active site of the phospholipase C molecule; (b) electrostatic interactions between the phospholipid ionic groups and the protein ionic groups cannot be the major source of stability of the membranes since removal of 70% of the phospholipid ionic groups has no marked structural effect and (c) the bulk of the phospholipids in the intact membrane cannot be interacting strongly with the proteins. Of the models shown in Text-fig 1, the mosaic one is clearly the most consistent with these three conclusions.

If a mosaic model best fits the data obtained from plasma membranes, we may then ask whether the matrix of the mosaic is formed by the lipids or the proteins. Using the analogy of bricks and mortar, is the lipid or the protein the mortar of the membrane? If the lipid provides the matrix of the mosaic and no other constraints are present, then *a prediction of such a mosaic model is that there is no long-range order in plasma membranes.* In other words, if the lipid is the matrix into which proteins are intercalated, either as individual molecules or as aggregates, there is nothing to impose any *long-range order* upon the arrangement of the membrane proteins. In particular, if a specific membrane component was represented by, say, 10,000 molecules per cell membrane, one would predict, from the model of a lipid-globular protein mosaic with a lipid matrix, that the 10,000 molecules would *not* be distributed in a regular, periodic array over the membrane surface, but rather, either as single molecules or aggregates, would be distributed in a random aperiodic array in two dimensions.

In order to test this prediction and to obtain new and useful information about membranes in general, we have devised methods for visualizing with the electron microscope the two-dimensional distribution of specific cell-surface antigens. Visualization of different antigens is achieved through staining with ferritin-conjugated antibodies specific for the antigens. The two-dimensional distribution of the stain may be observed by the following procedure: cells are lysed at an air-water interface; upon lysis, the surface forces at the interface often cause the cell membrane to spread completely flat; such membranes can then be picked up from the interface on carbon-coated collodion

films on electron microscope grids and then stained with ferritin-conjugated antibodies.[20–22] In this manner, we have studied the distribution of the $Rh_0(D)$ antigen on human red blood cell membranes.[22] After first attaching a saturating amount of ^{125}I-labeled purified human anti-$Rh_0(D)$ antibodies to O, Rh-positive cells, the sensitized cells were lysed at the interface and the flattened membranes picked up on the grid and indirectly stained with ferritin-conjugated goat antibodies directed against human γ-globulin. As shown in Fig 1, the ferritin was found distributed in discrete clusters each containing two to eight ferritin particles on the membrane surface. The numbers of such ferritin clusters per cell were found to correspond closely to the numbers of ^{125}I-labeled human anti-$Rh_0(D)$ antibody molecules bound per cell, for cells of different Rh phenotype and $Rh_0(D)$ antigen content. Each ferritin cluster most probably represents a single $Rh_0(D)$ antigenic site [23] since the human γ-gobulin molecule as an *antigen* is capable of binding on the order of seven specific antibody molecules.[24] The conclusion from results such as those shown in Fig 1 is that the $Rh_0(D)$ antigen, which is probably protein in nature (*cf Green* [25,26]), is apparently molecularly dispersed in the human red blood cell membrane and is distributed in an aperiodic, random two-dimensional array on the membrane surface.

The distribution of H-2 alloantigens on mouse red blood cell membranes has been studied by similar technics.[22] In this case, the ferritin particles of the indirect stain are again found in clusters, but the clusters occur in patches rather than in isolation as in the case of the $Rh_0(D)$ antigen. The patches contain variable numbers of clusters, but are apparently arranged in an irregular, more-or-less random, two-dimensional array on the membrane surface.

Many other systems should be investigated by these technics, and we are continuing such studies with red blood cells, lymphocytes, plasmacytes, and other cell types. The results so far obtained, however, are consistent with the predictions of the lipid-globular protein mosaic model whose matrix is formed by lipid: there appears to be no long-range order in the distribution of the $Rh_0(D)$ antigen on human red blood cell membranes or the H-2 alloantigens on mouse red blood cell membranes.

There are two ways of interpreting random distributions such as those observed in Fig 1. Either (a) the structure is a mosaic, but is essentially *static;* or (b) the structure is a fluid and *dynamic* one which has been instantaneously fixed in the specimen under examination. If the lipid forms the matrix of the mosaic, and the lipid is itself fluid under

physiologic conditions (see above), the mosaic may truly be *a two-dimensional solution,* albeit a viscous one. At least some of the protein components of the membrane may therefore undergo translational diffusion in the plane of the membrane. That such diffusional motion may occur is strongly suggested by the experiments of Frye and Edidin[27] with cell fusion heterokaryons. In such heterokaryons, intermingling of antigenic determinants from two different plasma membranes was found to occur at a rate that was not affected by metabolic inhibitors, energy uncouplers, or protein synthesis inhibitors. If indeed a plasma membrane is a two-dimensional solution of proteins in a viscous but fluid lipid solvent, then proteins and other membrane constituents not otherwise immobilized may be redistributed in the membrane when the membrane is subjected to any of a variety of chemical and physical perturbations. It is clear that such effects could have profound biochemical and physiologic consequences.

Summary

A schematic model for the organization of the lipids and the integral proteins of plasma membranes, the lipid-globular protein mosaic model, is discussed. In this model, the proteins are largely globular and are intercalated into lipid bilayer to form a mosaic structure with the lipid as the matrix. This model is consistent with the best recent physical and chemical information about plasma membranes, as well as with the thermodynamics of macromolecular systems in water. A prediction of the model is that there is no *long-range* order in plasma membranes: if many molecules of a specific component are present in a plasma membrane, they would be expected to be distributed in a random aperiodic two-dimensional arrangement in the membrane. We have devised methods to localize and to determine the two-dimensional distribution of macromolecular antigens on membrane surfaces by electron microscopy, and have applied them to the $Rh_0(D)$ antigen on human erythrocyte membranes. The distribution of this antigen was indeed found to be random and aperiodic on the membrane surface.

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[*Illustrations Follow*]

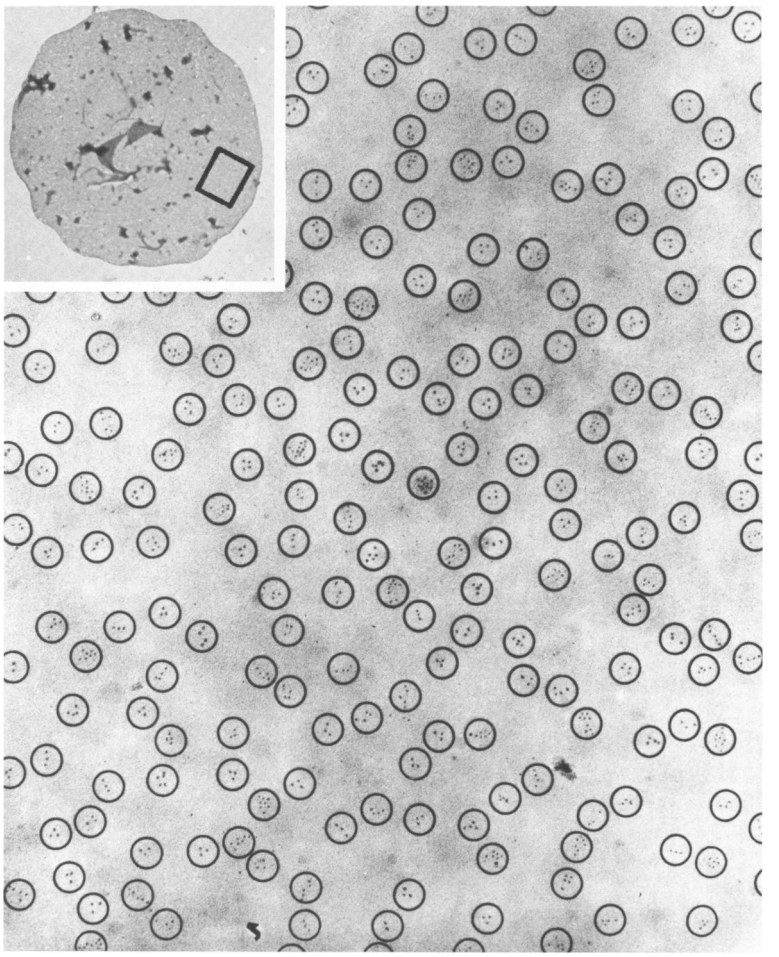

Fig 1—The outer surface of the membrane of an Rh-positive human red blood cell indirectly stained with ferritin-labeled antibody. The cells were first maximally labeled with purified ^{125}I-labeled human anti-Rh_o (D) antibodies. The cells were then lysed at an air-water interface, and the flattened membranes (**inset,** low magnification) were picked up on a coated electron microscope grid. Under suitable conditions, the sensitized membranes were then indirectly stained with ferritin-labeled goat anti-human γ-globulin. The ferritin appears in discrete clusters of two to eight particles; each cluster is circumscribed by a circle of 300 Å radius. The numbers of such clusters per cell (16,100) is equal to the number of ^{125}I-labeled human anti-Rh_o (D) molecules bound per cell (16,700). Each cluster therefore corresponds to an individual Rh_o (D) antigenic site. The discreteness of the clusters indicates that the Rh_o (D) antigenic sites are dispersed singly in the membrane, and the arrangement of the clusters indicates that the Rh_o (D) antigen is randomly distributed on the membrane surface (× 60,000; insert, × 3500).

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