Introduction to Biological Membranes

MAHENDRA KUMAR JAIN
ROGER CURTIS WAGNER

Department of Chemistry and
Institute of Neurosciences
School of Life and Health Sciences
University of Delaware,
Newark

A Wiley-Interscience Publication
JOHN WILEY AND SONS
New York • Chichester • Brisbane • Toronto

Library of Congress Catalog in Publication Data

Jain, Mahendra K 1938-
Introduction to biological membranes.

"A Wiley-Interscience publication."
Includes index.
1. Membranes (Biology) I. Wagner, Roger Curtis, 1943- joint author. II. Title. [DNLM: 1. Cell membrane. WH601 J25i]
QH601.J36 574.8'75 79-16690
ISBN 0-471-03471-1

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Contents

1

What Godel proved was that . . . any consistent axiomatization is incomplete . . . . One has, at this stage, simply to admit that mechanism is not a solution to the problem of the universe but a strategy, just as induction and all previous methods of doing science were.

JACOB BRONOWSKI
in *The Origins of Knowledge and Imagination*

Introduction

A membrane surrounds all living cells. It is not easy to conceive how life in all its manifestations could have evolved without a membrane that separates components of vital metabolic processes from the external milieu; that is, the membrane provides an identity to a cell. Since a cell depends upon and communicates with the external environment, its membrane must allow the passage of certain molecules while preventing the passage of others. Thus cell membranes are sites of a large variety of cellular processes (Fig. 1-1) ranging from permeability, transport and excitability to intercellular interaction, morphological differentiation, and fusion. In this text we discuss the structural characteristics and the functional consequences of biological membranes.

Numerous models have been proposed to articulate the organization of lipids and proteins in biomembranes (see Jain and White, 1977, for a review of these models). The consensus emerging out of such studies may be summarized in the following generalizations:

1. Biological membranes are essentially a two-dimensional matrix—a predominantly phospholipid bilayer structure interrupted by proteins. Thus the

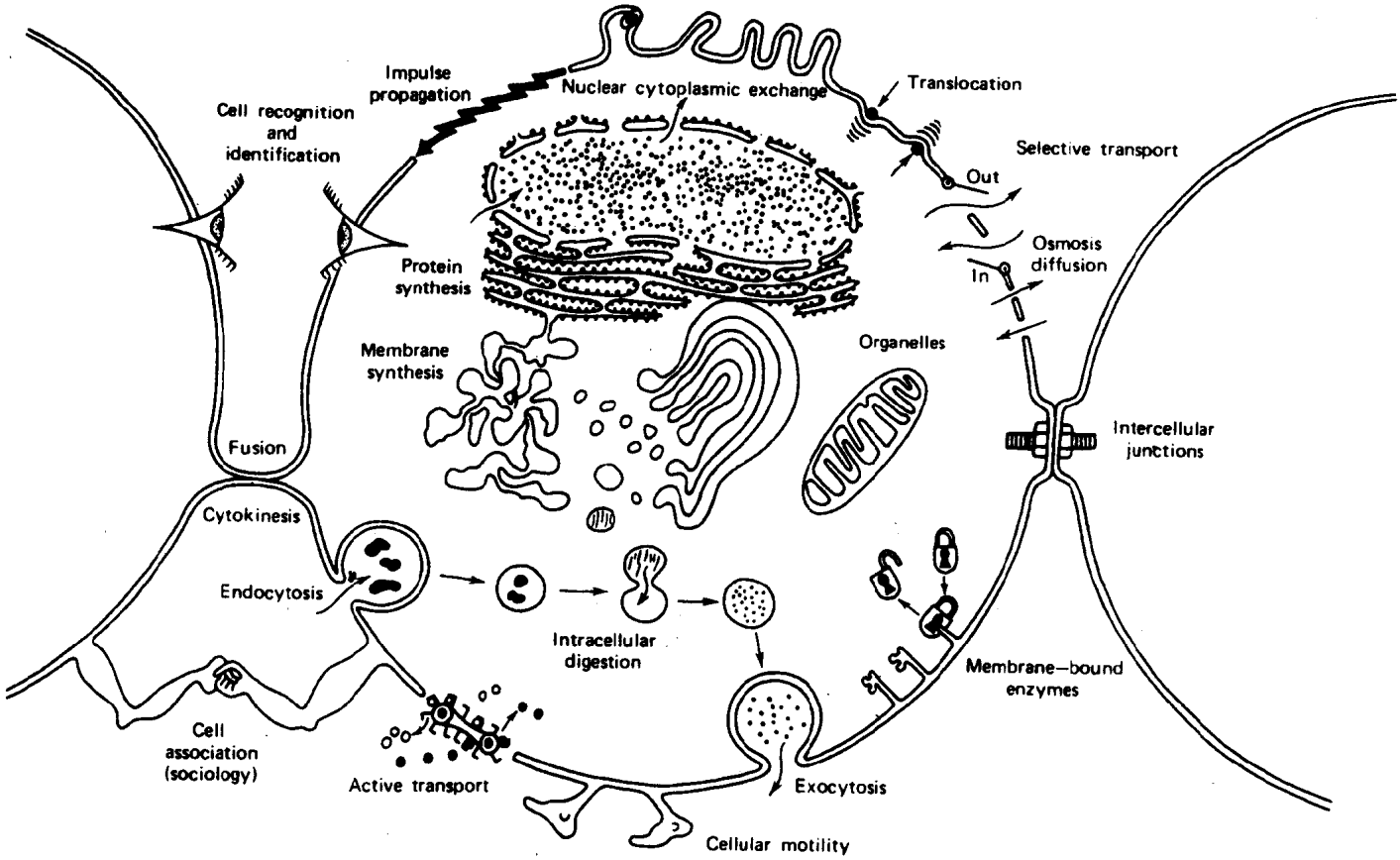


Fig. 1-1. Cartoon of a typical cell. It emphasizes the various processes modulated by the cell membrane.

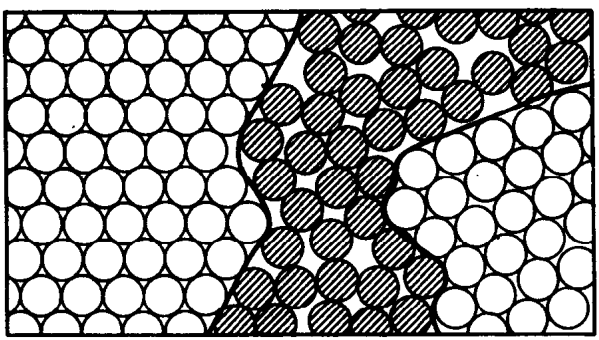
interior of the membrane is much less polar than the interfacial region. Such an arrangement is a direct consequence of the "hydrophobic effect."

2. The components of the membrane matrix are held together largely by noncovalent forces. For example, the acyl chains of phospholipids interact with each other and with proteins by van der Waals interactions, and the interactions of the polar groups at the interface are expected to be largely of the coulombic and hydrogen-bonding types.

3. The uncatalyzed exchange of components from one interface of the membrane to the other is slow. This gives rise to a morphological and functional asymmetry between the two interfaces.

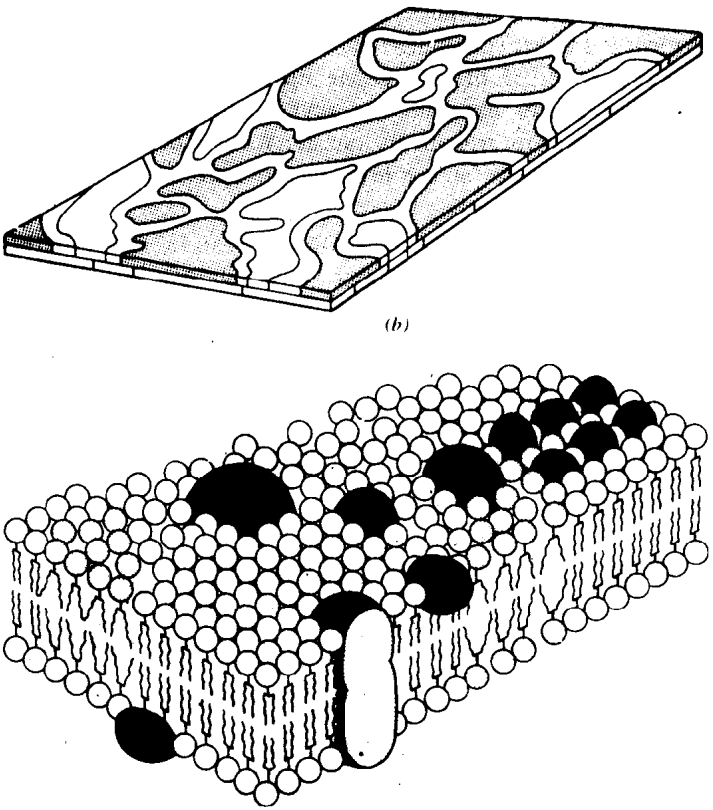
4. Specific interactions between the membrane components lead to a selective ordering and segregation of the components in the plane of the membrane.

5. A consequence of segregation of components in the plane of a membrane is the establishment of a long-range order and cooperativity within the segregated *domains*. The regions of discontinuity and mismatch would exist at the patch boundaries.



(a)

Fig. 1-2. Structural framework of typical biomembrane in various degrees of schematization. (*a*) Organized lipid molecules (white circles) may form discrete plates that are separated from each other by regions of relatively disorganized lipids (hatched circles). The composition and the resulting system properties of such regions may be quite different. (*b*) The organized and disorganized regions are viewed as plates, each having characteristic system properties specified by its components. Some plates may extend through the bilayer. The size, shape, and lifetime of plates and the mobility, exchange rate, and residence times of components within these plates has not been defined. However these parameters are expected to vary significantly from plate to plate and from membrane to membrane. The size of the plates may, for example, be up to several thousand molecular diameters. (*c*) Various molecules (within the bilayer of biomembrane) interact hydrophobically and are distributed asymmetrically. Moreover there is also a distinct long-range organization (over several hundred molecular diameters) of both lipids and proteins. Thus bilayers with distinct organizational features and composition may coexist within the plane of a biomembrane. These organizational changes may arise from distinct molecular conformations and specificity (or lack of it) of intermolecular interactions among components.



(b)

(c)

Fig. 1-2. Continued.

6. The molecules within the membrane matrix can undergo a variety of motions: *rotational motion* along the axis perpendicular to the interface; *trans–gauche* conformational change in the acyl chains that give rise to an increased segmental mobility of chains toward the center of the bilayer; and *lateral diffusion* of the components in the plane of the membrane. The mobilities of the various components in a membrane appear to differ more than what would be expected on the basis of the size of the components.

A model of membrane structure embodied in these generalizations is schematized in Fig. 1-2. This should serve to illustrate the various phenomena and processes discussed in this book.

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2

Ours is a strange profession
We who trap the cells where life beats
And strike them on a diamond
Transmuting them to flakes of gold and silver
Which sparkle as they float upon the water
And we coarse fishermen
Catch them in our little nets

We speak to them by hurling at them
Elemental particles
And they answer in a language
That we can only vaguely understand

D. L. RINGO

Electron Microscopy of Membranes

Although the presence of a boundary layer surrounding cells had been detected in earlier experiments, it was not until the electron microscope became applicable to the study of biological materials that cellular membranes could be visualized. Electron images of membranes were however only attained after an extensive and drastic regimen of speciman preparation. A clear interpretation of the relation of such images to living membranes could not be readily made since numerous sources of artifact were introduced. The relatively poor penetrating power of electrons dictated the use of extremely thin sections of mem-

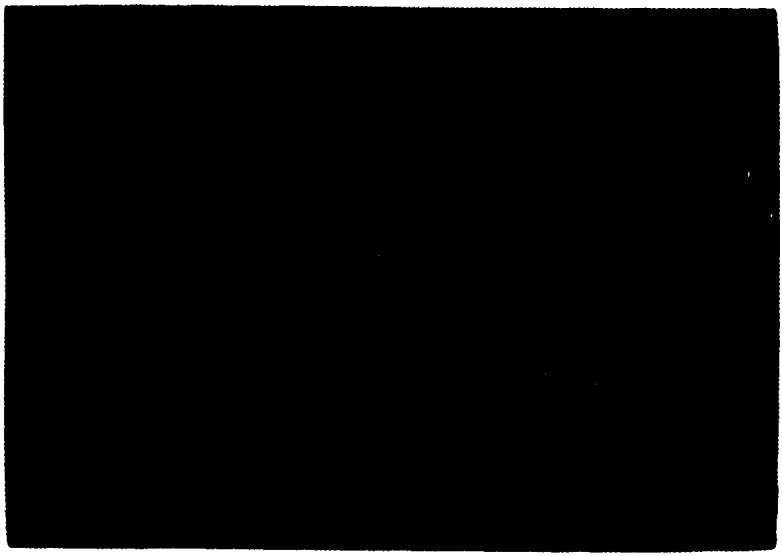

Fig. 2-1. Transmission electron microscopy of a thin section through to opposing liver parenchymal cells. Trilaminar profiles of two adjacent plasma membranes are evident (arrows) as well as those of endoplasmic reticulum (ER) and mitochondria (M). Mag. X 272,500.

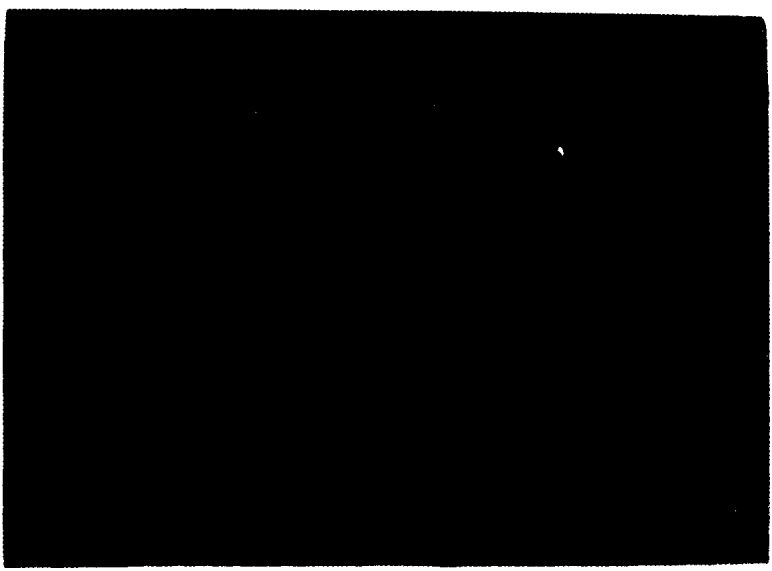

Fig. 2-2. Higher-magnification micrograph through portions of two cytoplasmic vacuoles (V). Mag. X 272,500.

branes, for which plastic resins were the only suitable internal support. Dehydrated specimans were required because an electron beam can be properly focused only under vacuum and the beam itself can be highly damaging to thin specimens. The inherent low contrast in electron density of membranes required contrast enhancement with heavy metal stains. The culmination of these preparative procedures was the typical "railroad track" image that is a constant characteristic of both plasma and intracellular membranes (Figs. 2-1 and 2-2). Although this was sufficient to elucidate the form and contour of membranes, uncertainties persisted as to what the railroad track represents in terms of membrane structure. Many subsequent efforts have been directed at determining the effects of preparative procedures on membranes and identifying artifacts. These efforts in interpretation have recently been aided by innovative techniques and specimen preparation in which treatment of fresh membranes is completely different, thus varying the sources from which artifacts may arise.

MEMBRANES IN ULTRATHIN SECTIONS

Structural Stabilization

Since cells and the membranes comprising them are extremely fragile entities, their dehydration and subsequent embedding in plastic require prior fixation or stabilization of structure. Fixation is accomplished by crosslinking macromolecules, which partially immobilizes them and renders them insoluble. Thus when fixed cells are infiltrated with organic solvents and plastic, cellular macromolecules (primarily proteins) are not extracted or translocated. In some cases fixation is efficient enough to prevent denaturation of proteins, and a significant degree of enzyme activity is retained.

Aldehydes, both formaldehyde and glutaraldehyde, are required for the degree of structural stabilization necessary for electron-microscopic observation. Glutaraldehyde is the most effective in this regard. It forms inter- and intramolecular links between amino acids, yielding rigid heteropolymers of protein. This results in minimal protein conformational changes as confirmed by X-ray diffraction studies and attests to the ability of glutaraldehyde to stabilize without distortion. The tertiary structure of many enzymes is sufficiently preserved so that activity is retained. Glutaraldehyde also increases membrane permeability, which facilitates subsequent infiltration by plastic.

Postfixation with osmium tetroxide further reduces the loss of membrane constituents, primarily phospholipids. In addition to functioning as a fixative, OsO_4 imparts electron density to the membrane owing to its heavy metal component, OsO_4 is believed to form cyclic osmate mono– and diesters involving double bonds of adjacent unsaturated fatty acid chains of phospholipids.

Membranes thus fixed are very resistant to extraction by organic solvents, and the translational movement of membrane lipids is also greatly restricted. The fact that osmicated membranes appear as an electron-transparent layer sandwiched between two electron-dense layers would seem to be inconsistent with the binding of osmium to acyl chains buried in the hydrophobic interior of membranes. It is possible however that osmium may bind secondarily to the polar regions of phospholipids and consequently would be more tightly packed and collectively more electron dense in those regions. The exact mechanisms of osmication are complex and not well understood. In any event, the railroad track image of membranes does not depend on the presence of osmium, and the electron-dense and electron-transparent lamina of membranes may be better correlated with hydrophilic and hydrophobic regions, respectively.

Embedding

Fixed membranous specimens are dehydrated in organic solvents such as acetone or ethanol. These reagents serve as intermediary solvents that facilitate the replacement of the cell's aqueous components by plastic. They are particularly troublesome in terms of alteration of tissues and membranes since they can extract membrane phospholipids and cholesterol. The amount of extraction can be minimized by prior fixation procedures. It is somewhat surprising that such extraction has a minimal effect on electron images of membranes. It is possible that structural stabilization by fixation is sufficient to allow the extracted components to be replaced with plastic, thereby preventing structural collapse of the bilayer. Proteins are also denatured and extracted to some degree by these solvents. However, other than alterations in contrast and dimension, no particular effect on the railroad track image of membranes can be attributed to these extraction artifacts.

Impregnation with and subsequent polymerization of plastic resins supports tissues and membranes internally and imparts strength for thin sectioning. Low-viscosity methacrylates easily penetrate specimens, but they form linear polymers that are easily etched or sublimed by the electron beam. Etching or crosscut membranes may result in ridge-like elevations that collapse and distort the electron image. Three-dimensional polymers such as the epoxides are superior in this regard since they are more resistant to etching. Epoxides are also capable of extracting lipid, however, and further extraction artifacts may be introduced with embedding. As an alternative, water-soluble extracts of epoxides can be employed to minimize lipid extraction. Also, water-soluble resins such as carbohydrazide can be copolymerized with aldehydes and can be used to directly infiltrate tissues. This eliminates the dehydration procedures and results in tissues that are sufficiently hardened to withstand sectioning.

Sectioning and Staining

For most membrane-related studies sections 500 Å thick or less (gray interference color) are cut with glass or diamond knives. These sections may be observed directly, but the contrast imparted to membranes by osmium alone is very low, especially in thinner sections. Counterstaining the sections with heavy metal salts is usually required in order to obtain a sharp, well-defined image of membranes. Sections are commonly counterstained with aqueous uranyl acetate followed by alkaline lead citrate staining. Uranyl ions react with phosphate and carboxyl groups and possibly with amino acids of proteins. The affinity of embedded membranes for lead salts is not well understood. However lead salts are thought to bind to sites previously occupied by osmium. Contrast enhancement by counterstaining facilitates clear imaging of membranes at the higher resolution provided in very thin sections.

Negative Staining

The membranes of organelles and other isolated membrane systems can be visualized by electron microscopy without the need for dehydration, embedding, and sectioning. In these procedures, heavy metal salts such as phosphotungstic acid, ammonium molydate, and uranyl acetate are employed to impart negative contrast to isolated membrane systems deposited on coated grid supports. These stains, when applied to such preparations, dry and concentrate around the membranes, and their outline thus exhibits a negative or electron-transparent profile (Fig. 2-3). Although this technique may circumvent sources of artifact introduced by preparation for sectioning, new complications arise which dictate care in interpretation of the resulting images. Isolated inner mitochondrial membranes exhibit stalked globules on their surfaces when negatively stained that are not evident in embedded and sectioned mitochondria. Although the globules can be isolated and as such exhibit ATPase activity, their natural form as globules is questionable. They may be an artifact resulting from osmotic shock of the membrane during drying of the negative stain.

Electron-Dense Tracers

In addition to direct staining of membranes, various substances can be utilized to demarcate membrane surface contours, indicate junctional complexes between cells, identify carbohydrate-rich surface coats and antigenic sites, and trace membranes that have been internalized by endocytosis. Some of these substances are intrinsically electron dense or selectively stainable with electron dense stains, and others may act as mordants which enhance the affinity of membranes for heavy metal salts.