THE PLASMA MEMBRANE

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SERIES PREFACE

Membrane transport is rapidly becoming one of the best-worked fields of modern cell biology. The Transport in the Life Sciences series deals with this broad subject in monograph form. Each monograph seeks to trace the origin and development of ideas in the subject in such a way as to show its true relation to membrane function. It also seeks to present an up-to-date and readable outline of the main problems in the subject and to guide thought on to new lines of investigation.

The task of writing a monograph is not a light one. My special gratitude is to the various authors for expounding their subjects with scholarly care and force. For the preparation of the indexes I thank Dr. Barbara Littlewood.

E. EDWARD BITTAR

Madison, Wisconsin
June 1980

PREFACE

This monograph is an attempt at an elaboration of the current trends in the study of the structure and function of the plasma membrane. Such a discourse should be of interest to senior undergraduate and graduate students and postdoctoral fellows in biological and basic medical sciences. Teachers of cell biology may find it useful as supplementary reading material. Freshmen in general biology would find it worth their while going through it after their introductory courses in cell biology and biochemistry. Rapid advances are likely to take place in the field of the biology of membranes, particularly in the understanding of the functional organization of specific membrane-related phenomena. Nonetheless, this monograph will be useful as a general guide to the background literature for some years to come.

No attempt has been made to provide information on relevant tools and techniques or historical background and reviews of the vast and rapidly expanding literature available on various aspects of the plasma membrane or biological membranes in general. An attempt has been made to cover such topics on the structure and function of the plasma membrane as are likely to be common to a vast majority of the cells. Even though highly specific plasma membrane-associated structures such as acetylcholine receptors, acetylcholinesterase, and bacteriorhodopsin have been selected, the major features that emerge from the discussion of these topics are likely to be applicable to a general discussion of the structure of comparable membrane proteins. Examples from natural membrane systems have been cited, though a great deal of work on the lipid-water systems (myelin figures), black lipid membranes, and reconstituted membranes provided the fundamental basis for current investigations.

With a view toward limiting the list of references, I have often resorted to citation of the most recent publications and reviews by authors whose previous work is quoted. Therefore, such references have been indicated by "reviewed by or see . . ." in parentheses in the text.

S. K. MALHOTRA

Edmonton, Alberta
June 1983

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I am grateful, also, to the editorial and production staff of John Wiley & Sons for taking pains in editing the manuscript, and its publication.

S.K.M.

ABBREVIATIONS

Ach	Acetylcholine
AchE	Acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7)
α-BGT	α-Bungarotoxin
BTX	Batrachtoxin
BOTX	Botulinum toxin
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CD	Circular dichroism
CNS	Central nervous system
Con A	Concanavalin A
E face	Concave fractured face (exoplasmic half)
ESR	Electron spin resonance
FSH	Follicle-stimulating hormone
GDP; GTP	Guanosine diphosphate; Guanosine triphosphate
IMPs	Intramembranous particles in freeze fracture
IR	Infra red
LDL	Low-density lipoprotein
NAD	Nicotinamide-adenine dinucleotide
NMR	Nuclear magnetic resonance
ORD	Optical rotatory dispersion
P face	Convex fractured face (cytoplasmic half)
PNS	Peripheral nervous system
Protein kinase	(ATP: protein phosphotransferase, EC 2.7.1.37)
rbc	Red blood cells
S values	Sedimentation coefficients

SBA	Soya bean agglutinin
STX	Saxitoxin
TEA	Tetraethylammonium chloride
TTX	Tetrodotoxin
UV	Ultraviolet
WGA	Wheat germ agglutinin

CONTENTS

1

Introduction

The plasma membrane is the essential physiological barrier at the cell surface (Nägeli, 1855) and consists of a now well-established lipid bilayer (Gorter and Grendel, 1925) and associated membrane proteins. Some of these proteins are loosely attached to the membrane, whereas others are more strongly intercalated in the bilayer. Both lipid molecules and membrane proteins may carry carbohydrate moieties that in some cell types extend a good deal out from the lipid bilayer and form a well-defined layer recognizable in electron micrographs. On the cytoplasmic side the membrane proteins interact with other fibrous cytoskeletal elements, which are now known to be relevant to the functional organization of membranes. Thus, the concept of a plasma membrane should incorporate such structural features as may exert a direct effect on its functional parameters.

A simplistic view of the plasma membrane would be that it is a conglomeration of functional domains that may interact with each other and thus govern the activities of the entire membrane. Such an approach is implicit in this monograph since certain functional do-

mains have been selected for consideration. It is also recognized that such an approach may give an impression of the existence of discrete regions in the plasma membrane. In some cases such functional regions are clearly identifiable, for example, the synapse or the neuromuscular junction, regions of cell-to-cell contacts, coated pits, nodes of *Ranvier* in myelinated axons, the apical region of the sperm head, and the purple membrane of the halobacterium. In other cases, for instance, red blood cells, the plasma membrane has a uniform structure, though there may be an underlying microheterogeneity in the distribution of functional domains. Such a simple view of a multifunctional membrane can be reconciled with the current widely held view of the mobility of membrane lipids and proteins by either restricting fluidity to within the functional domains or having no fluidity, as with the Ach receptors in the sarcolemma in the region of the neuromuscular junction (Section 7.5) or in the normal adult human rbc (Kehry et al., 1977). However, such a notion of restricted mobility within the functional domains is likely to be weakened when one considers that the sites of exocytosis and endocytosis of synaptic vesicles, for example, appear to be in different regions of the presynaptic endings (Heuser and Reese, 1973). Also, in the biosynthesis of membrane proteins (Section 15.5), the site(s) of insertion into membranes may be different from the site(s) of assembly into functional units. In the absence of an as yet complete understanding of the process of biosynthesis and the interactions between various seemingly apparent functional domains, the molecular organization of plasma membranes remains poorly understood.

The plasma membrane serves various functions in a cell, so many, served by such a variety of cell types in an organism, that the scope of this monograph must be restricted to consideration of a few selected topics. These topics represent the bias of the author, but some of them are areas of recent advances and others are currently being investigated. Although most are taken from animal cells, particularly mammalian, their general features should be universally applicable to biological membranes, and the rationale for their inclusion appears in the respective section of each chapter. The structure of the purple membrane of the halobacterium represents the best-

understood membrane protein system and is therefore included here. Emphasis is laid on the correlation of structure with function and, in particular, advances that have been made since the unit membrane concept was proposed by Robertson in the late 1950s (see Robertson, 1960). The unit membrane concept evolved after the introduction of electron microscopy into biological research and includes advances made since the classical Danielli-Davson (Danielli and Davson, 1935) model was published. More recent advances in membrane biology have been in the elucidation of lipid-protein interactions, in the structure of membrane proteins that traverse the lipid bilayer (transmembrane proteins), and in the fluid and highly dynamic nature of the membranes. These conceptual advances have gone hand in hand with technological advances in membrane biology. One technique is freeze-fracture and freeze-etching for electron microscopy (Bullivant, 1974; Steere, 1957), which has provided perhaps the best available evidence in favor of the lipid bilayer structure of biological membranes (Bretscher and Raff, 1975). During freeze-fracturing the membranes split in half, revealing the two internal fractured faces and the fracture plane follows the hydrophobic interior of the membrane (Branton, 1966; Branton et al., 1975). In addition, the true surfaces of the membranes can be also visualized by etching. This provides the means to detect membranous components (e.g., receptors and antigens) that extend from the bilayer by using appropriate ligands (Tipnis and Malhotra, 1979).

High-resolution micrographs of crystalline arrays of membrane proteins, taken at a low dose of electrons to minimize radiation damage, have been exploited to determine the three-dimensional structure by Fourier transform (Henderson and Unwin, 1975; Unwin and Zampighi, 1980). Improvements in isolation procedures by using various kinds of detergents and protein analysis have added a new dimension to the understanding.

Spectroscopy (IR, UV, CD, ORD, NMR, ESR), fluorescence photobleaching, electrophysiology, and immunology have contributed to advances in membrane biology in a large variety of natural and model membrane systems. The models include lipid-water systems (myelin figures; Luzzati and Husson, 1962; Stoeckenius, 1962), lipid bilayers (thin lipid membranes or black membranes; Mueller et

al., 1962) and liposomes (see Kell, 1981), and reconstituted natural membranes. Furthermore, the availability of affinity agents and toxins such as α-BGT (Chang and Lee, 1963) and TTX (Narahashi, 1974) has provided valuable tools for combining morphological and physiological investigations. Recent developments in hybridoma technology to produce monoclonal antibodies (Köhler and Milstein, 1975; Milstein, 1981) provide additional valuable means to probe the structure and functions of membranes.

The plasma membrane can be readily recognized by its location in intact cells by electron microscopy, and biochemical analysis of characteristic properties (enzymatic activity, receptors) of particular cells facilitates identification in isolated fractions. However, there appears to be no single universal marker for the plasma membrane (reviewed by Glick and Flowers, 1978). The cholesterol-to-phospholipid ratio is often used for this purpose for animal cells but the reported values range from 0.3 to 1.4. A commonly used marker for the plasma membrane is 5′-nucleotidase activity, but the extent of activity is not always reported. Adenylate cyclase seems universally associated with the plasma membrane but has also been reported in intracellular membranes (Section 11.3). Biochemical characterization of the plasma membrane is not further discussed in this monograph, though an attempt has been made to include such features as can be clearly identified with it.