Cellular Membranes in Development

Edited by

MICHAEL LOCKE

22nd SYMPOSIUM OF THE
SOCIETY FOR THE STUDY
OF DEVELOPMENT AND GROWTH

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Michael Locke

Developmental Biology Center
Western Reserve University
Cleveland, Ohio

1964

ACADEMIC PRESS, New York and London

ACADEMIC PRESS INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 63-14493

PRINTED IN THE UNITED STATES OF AMERICA

Contributors and Presiding Chairmen

Numbers in parentheses indicate the page on which the authors' contributions begin.

H. W. BEAMS, Department of Zoology, State University of Iowa, Iowa City, Iowa. (175)

Chairman: WILLIAM H. TELFER, University of Pennsylvania

P. R. BELL, Botany Department, University College, London and Institut für Allgemeine Botanik, Eidgenössische Technische Hochschule, Zurich. (221)

Chairman: IAN SUSSEX, Yale University

ARTHUR L. COLWIN and LAURA HUNTER COLWIN, Department of Biology, Queens College, Flushing, New York. (233)

Chairman: CHARLES B. METZ, Florida State University

GEORGE G. LATIES, Department of Botany and Plant Biochemistry, University of California, Los Angeles, California. (299)

Chairman: ARMIN C. BRAUN, Rockefeller Institute

YVONNE MOULÉ, Centre de Recherches sur la Cellule Normale et Cancereuse, Villejuif, (Seine), France. (97)

Chairman: ALEX B. NOVIKOFF, Yeshiva University

WALTER J, NICKERSON, Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey. (281)

Chairman: MELVIN S. FULLER, Brown University

J. DAVID ROBERTSON, Harvard Medical School, Research Laboratory, McLean Hospital, Belmont, Massachusetts. (1)

Chairman: MICHAEL LOCKE, Western Reserve University

MALCOLM S. STEINBERG, Department of Biology, The Johns Hopkins University, Baltimore, Maryland. (321)

Chairman: EDGAR ZWILLING, Brandeis University

THOMAS E. THOMPSON, Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland. (83)

Chairman: PETER F. CURRAN, Harvard Medical School

W. GORDON WHALEY, JOYCE E. KEPHART, and HILTON H. MOLLENHAUER, The Plant Research Institute, The University of Texas, Austin, Texas. (135)

Chairman: PETER ALBERSHEIM, Harvard University

Preface

In the recent revolution in our knowledge of the organization of living systems the discovery of the ubiquity of membranes, all with a remarkably constant structure, ranks in importance with the finding that nucleic acids are the hereditary material. Vitalism has at last been buried with the realization that protoplasm has a morphology clearly related to its function. Anatomy has come into its own again, and at the ultrastructural level it has achieved a new significance as physiology and biochemistry studied at a point in time. The biochemist has ceased to think of cells as bags of enzymes which he must isolate and study; his analytical approach has had its successes, but he knows now that cells are three-dimensional systems whose integrity is destroyed in the test tube. The anatomist, for his part, has realized that structure at the molecular and supramolecular level only become meaningful in relation to function. The disciplines static in time have recognized mobility; the disciplines with time as a variable have recognized the need for a structural basis to account for the ordered complexity within a cell. Both meet in this volume in the study of membranes.

The structural similarity of all membranes has led to the unit membrane concept elaborated here by Robertson. With the electron microscope, cell surfaces, membranous organelles, and even the envelopes of viruses, are seen as twin dense layers about 20 Å thick separated by an interzone of about 35 Å. Electron micrograph and X-ray diffraction studies of smectic lipid systems with varying degrees of hydration confirm interpretations of the unit membrane as a bimolecular leaflet with the outer polar ends covered by protein films. This constancy of structure only means that membranes are alike in a general way. Local differences in chemical composition and the physical state of the constituent molecules probably account for, among other things, the fairly consistent variations in thickness of different membrane species.

The general application of the unit membrane description has made attractive the controversial hypothesis that all membrane systems are topologically continuous, either permanently or at least during their genesis. There is now evidence for thinking of cells as three-phase systems—the nuclear contents and cytoplasm forming one system linked through the nuclear pores; the membranes, for the most part in topological continuity, forming a second; and the contents of the membrane-bounded cavities making up a third which connects with the environ-

ment. A corollary of this hypothesis has important consequences for heredity. Membranes are perhaps never formed *de novo,* but always by some kind of autosynthetic process, leading to the possibility that local specificity of structure may be carried over from one generation to the next with a degree of independence from the nucleus. Another sort of local specificity of structure in the unit membrane reviewed by Robertson concerns the nature of the connections between cell surfaces. Some of these connections, for example, those at synaptic discs, may perhaps be due to a phase change of the lipids from a layered phase to one with a hexagonal array of short cylinders normal to the surface. There is a parallel between the structure of some phases described in lipid–water systems by Luzzati and Husson (in 1962) and membrane structures. Lipid–water systems may pass from a neat (a lamellar phase of bimolecular leaflets comparable to a stack of unit membranes) through a complex hexagonal (hexagonal arrays of bilayered cylinders structurally similar to septate desmosomes) to the middle phase (hexagonal arrays of monolayered cylinders similar to the hexagonal arrays described by Robertson in synaptic discs and elsewhere). There is the fascinating possibility that a cell controls its membrane structure locally, with profound consequences to permeability and adhesion, by altering the ratio of lipid to water and inducing the appropriate phase change.

With Thompson's work on the properties of bimolecular phospholipid membranes, we can say that the synthetic approach to the study of living systems has at last begun. The first phase in the creation of a living system may well be the formation of unit membranes to act as surfaces for ordered sequences of reactions. Thompson has found it possible to form and study a lipid bilayer comparable in structure to the lipid component of a unit membrane. Some of its properties could not have been predicted. For example, the electrical resistance has marked temperature-dependent discontinuities, perhaps due to phase changes comparable to those already discussed. If changes are controlled within a cell by metabolically linked alterations of membrane composition, they could play the role of biological amplifiers and switches. This hypothesis deserves special study in view of the characteristic nature of the membrane at nerve junctions described by Robertson. It is surely more than coincidence that we should suspect phase changes to be responsible for permeability changes and that we should observe the appropriate structure in a position on a cell where variation in permeability is most likely to be found. Perhaps the most unexpected of Thompson's findings is that the surface tension of the lipid bilayers is about as low as that of a cell membrane, so that the

existence of naked areas of lipid, not covered by protein, would not be revealed by surface tension experiments.

Thompson's phospholipid bilayer appears to be a suitable framework on which to build systems with biological activity. It may be that problems of active transport, permeability, co-ordination of synthesis, and the molecular architecture of membranes will be more readily studied on these synthetic membranes whose composition is under the control of the experimenter.

Moulé's account of the endoplasmic reticulum takes the analytical approach. Given the techniques of centrifugation and electron microscopy, what have they shown us of the structure and function of the endoplasmic reticulum of rat liver cells, and what is the relation between membrane and RNA? RNA may well be in membranes, as well as in the ribosomes.

Whaley and his collaborators draw attention to the virtues of the corn root tip for obtaining cells in various stages of development from the same preparation. They confirm the continuity between the membranes of the Golgi vesicles, the endoplasmic reticulum (ER), and the plasma membrane. Their most remarkable finding is that ER or ER-like membranes may arise from the ground substance of cytoplasm within seconds after the application of some stimuli. It seems that there must be a reserve of membrane precursors present in the cytoplasm which is invisible by electron microscopy. The speed at which the membranes may appear suggests that the reserve is perhaps transformed by a phase change of the sort described by Luzzati and Husson. These experiments emphasize again the importance of fundamental studies on the physical chemistry of lipids for the future of cell biology.

The oocyte is a specialized cell containing all the membrane systems found in other types of cells. The role of the different organelles in development is discussed by Beams. Although membrane organelles may be clearly defined morphologically, they may overlap in function. Yolk, for example, may arise from mitochondria, multivesicular bodies, Golgi, or the endoplasmic reticulum.

The work discussed by both Whaley and Beams generally supports the idea of topological continuity between the membranes of all the organelles except the mitochondria. In Bell's paper, we have evidence that the mitochondria also share the same membrane lineage. In the egg of bracken, *Pteridium aquilinum,* the nuclear membrane buds off hooded protrusions which develop into the peculiar umbo-shaped mitochondria

of the mature egg. Smaller and simpler evaginations may give rise to the proplastids. A striking feature of the fern egg is the degeneration of the old generation of mitochondria and plastids. It may be that the elimination and replacement of cell organelles is the basic mechanism which prevents the inheritance of acquired characters. It will be most interesting if this death and rebirth of organelles should take place in all cells in which rejuvenation occurs, indicating that it may play a part in the elimination of the effects of aging. If there is a hereditary continuity of membranes, with the genesis of new organelles in each generation, then the nuclear membrane would be well situated to ensure the early incorporation of information relevant to membrane structure from the genes.

Nowhere is the union and compatibility of different membrane species more strikingly displayed than during the fertilization of an egg. The Colwins show most clearly that the fertilized egg has a plasma membrane which is a mosaic derived from the plasma membrane of the sperm, the acrosomal membrane, and the plasma membrane of the unfertilized egg. One wonders how long it will be before eggs can be made with a mosaic of synthetic membranes similar to those prepared by Thompson. A direct approach of this sort may be the only way to determine how membranes grow and age.

An outstanding problem posed by unit membranes concerns the factors which control their shape. What controls the form of mitochondria or the ER, for example? Related to this is the degree to which the form of a cell and an organism is determined by the plasma membrane. A solution may come from Nickerson's study of dimorphism in the yeast *Trigonopsis variabilis* in which greater synthesis of phospholipid is related to the change from ellipsoidal to triangular form.

Although the tonoplast membrane surrounding the vacuole of plant cells appears indistinguishable from the plasma membrane and readily unites with it experimentally, it has very different permeability properties. Laties discusses the permeability of both membranes, the metabolic control of permeability, and how permeability varies with the degree of maturation.

In the concluding chapter, Steinberg advances a hypothesis to account for the reactions of cells of different types when mixed in tissue culture. In aggregates containing cells of two types, one always becomes peripheral to another. A hierarchy of adhesiveness can be constructed for different tissues, and it is possible to account for all the observed behavior of mutually adhesive cells by supposing that there is only a quantitative difference in the number of adhesive sites.

The Symposium was held at Storrs, Connecticut, June 17–19, 1963. The Society is thankful for the hospitality of the University of Connecticut and the efficiency of the local committee. The meeting was made possible only by a grant from the National Science Foundation.

MICHAEL LOCKE

Developmental Biology Center
Western Reserve University
November, 1963

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Unit Membranes: A Review with Recent New Studies of Experimental Alterations and a New Subunit Structure in Synaptic Membranes

J. DAVID ROBERTSON

Harvard Medical School, Research Laboratory, McLean Hospital, Belmont, Mass.

The Unit Membrane Concept

The unit membrane concept originated from studies of the origin of peripheral nerve myelin. It would be inappropriate here to review again the evolution of the concept. The reader is referred to previous review articles by the author (Robertson, 1959, 1960a, b, 1961a, b, 1962, 1963). However, in order to place the new material in perspective, it seems worthwhile stating very briefly, the findings on which the concept is based, using diagrams for illustration.

The concept developed from studies of vertebrate peripheral nerve myelin. Figure 1 illustrates the stages in the formation of myelin. Early myelinating fibers are recognizable as axons associated with single Schwann cells, as indicated in Fig. 1a. The diagram shows the surface membrane of the Schwann cell, as well as the membrane of the axon, to be a triple-layered structure consisting of two dense strata bordering a light central zone. This is essentially the appearance that unit membranes give in electron micrographs. One sees two dense strata, each about 20 Å thick, separated by a light interzone about 35 Å wide, making a unit which is about 75 Å thick. While the thickness may vary within certain limits, this triple-layered unit pattern is demonstrable at all cell surfaces and in all membranous cell organelles. For example, Fig. 2 is an electron micrograph of a unit membrane at the surface of a red blood cell, showing the typical appearance of the triple-layered structure.

One of the first steps in the formation of myelin is the obliteration of the gap between the two unit membranes of the mesaxons with intimate apposition of the outside dense strata to make the future intraperiod line of compact myelin, as in Fig. 1b. The mesaxon elongates in

a simple spiral around the axon as development proceeds. Eventually, the material between the cytoplasmic surfaces of the mesaxon loops is obliterated and the two dense strata of the apposed unit membranes unite to make the major dense line of compact myelin, as indicated in Fig. 1c. As the diagram shows, there is also a partial and somewhat irregular obliteration of the gap between the axon membrane and the Schwann cell membrane as myelination proceeds. Figure 3 is an electron micrograph that shows a developing myelin sheath at a fairly late stage, in which the various light and dense strata within the compact myelin

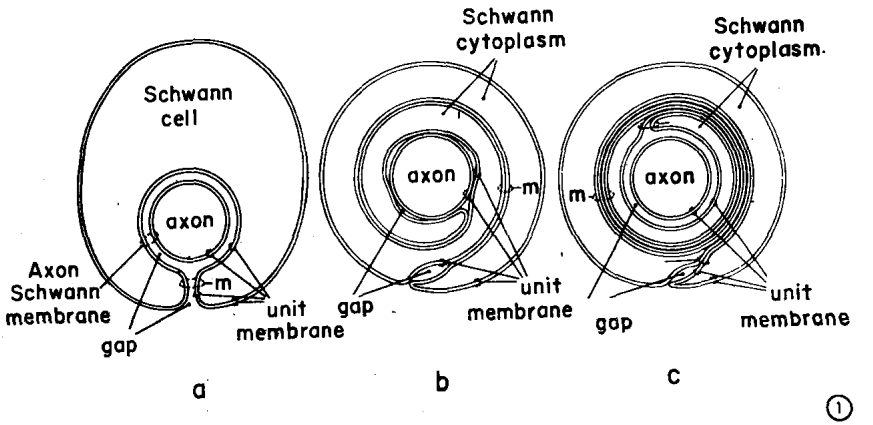

FIG. 1. Diagrams showing the Mechanism of formation of peripheral nerve myelin. *a*. The earliest stage in which one Schwann cell is associated with one axon with a short mesaxon (m). In *b* the mesaxon is elongated in a spiral. This is the intermediate fiber stage. *c*. Another stage in the formation of compact myelin.

structure can be traced directly into the unit membranes of the mesaxon and shown to correspond with similar strata in the surface membrane of the Schwann cell, as indicated in Fig. 1c.

In the past biophysical studies of myelin structure in fresh unfixed tissues using the techniques of X-ray diffraction and polarization optical analysis had led to a general conception of the molecular organization of the repeating unit in peripheral nerve myelin before the electron microscope was applied. Figure 4 is a diagram showing a possible molecular pattern for the repeating unit in myelin based on the polarized light studies by W. J. Schmidt in 1936 and the X-ray diffraction studies by Schmitt, Bear, and Clark (1935), Schmitt, Bear, and Palmer (1942), and Finean (1956). Figure 4 is taken from Finean (1956) and based mainly on X-ray diffraction analysis. However, it takes into account certain

FIG. 2. Portion of a human red blood cell, fixed with permanganate and sectioned, showing the unit membrane structure bounding the cell. 280,000 ×.