The Enzymes of Biological Membranes

Volume 3
Membrane Transport

Edited by

Anthony Martonosi
Department of Biochemistry
School of Medicine
Saint Louis University

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Contributors

ADOLPH ABRAMS, Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado.

R. W. ALBERS, Laboratory of Neurochemistry, National Institutes of Health, Bethesda, Maryland.

RANDALL S. ALBERTE, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California

ARNOLD F. BRODIE, Department of Biochemistry, University of Southern California School of Medicine, Los Angeles, California

ERNESTO CARAFOLI, Department of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland

MARTIN CROMPTON, Department of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland

PATRICIO J. GARRAHAN, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

RICHARD W. HENDLER, Laboratory of Cell Biology, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland

THOMAS R. HINDS, Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington

PAUL C. HOLLAND, Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Ontario, Canada

ADAM KEPES, Centre National de la Recherche Scientifique, Institut de Biologie Moléculaire, Laboratoire des Biomembranes, Université Paris VII, Paris, France

MARTIN KLINGENBERG, Institut für Physiologische Chemie und Physikalische Biochemie, Universität München, Munich, Germany

DAVID W. KROGMANN, Department of Biochemistry, Purdue University, West Lafayette, Indiana

WERNER KUNDIG, Department of Biology and The McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland

DAVID H. MACLENNAN, Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, Toronto, Ontario, Canada

ALTON MEISTER, Department of Biochemistry, Cornell University Medical College, New York, New York

RAJENDRA PRASAD, Department of Biochemistry, University of Southern California School of Medicine, Los Angeles, California

ALCIDES F. REGA, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

LEONARD L. ROSS, Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, Pennsylvania

GIORGIO SEMENZA, Laboratorium für Biochemie der ETH Zurich, Zurich, Switzerland

SURESH S. TATE, Department of Biochemistry, Cornell University Medical College, New York, New York

J. PHILIP THORNBER, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California

FRANK F. VINCENZI, Department of Pharmaceutical Sciences, School of Pharmacy and Department of Pharmacology, School of Medicine, University of Washington, Seattle, Washington

Preface

Much of the information currently available on the transport systems of bacterial and animal cell membranes and their mode of coupling to metabolic supply of energy can be found in this volume. Consideration of the participating enzymes dictated the choice of topics: Several transport systems where little information is available on the enzymology of the process are not included, while separate chapters deal with γ-glutamyl transpeptidase and intestinal disaccharidases which meet many of the requirements of transport enzymes. The volume also includes two chapters on photosynthetic membranes as a general introduction to the topic. Other aspects of biological transport and photosynthesis will be developed in detail in a forthcoming volume now in preparation.

These chapters reveal the excitement and rapid advance of the field, the daily reports of new concepts, new techniques, and new experimental findings which instantly interact to generate further progress. Our aim was to provide a starting point for those who are just beginning, and an opportunity for others to stop, take stock, and start in a new direction.

My warmest thanks to all who contributed to this volume.

St. Louis, Missouri
January, 1976

ANTHONY N. MARTONOSI

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Bacterial Membrane Transport Proteins

ADAM KEPES

I. Introduction

Bacterial transport systems are historically associated with the acceptance of the idea that the crossing of the cell membrane by a physiologically significant solute was mediated by the specialized operation of a protein or an array of molecules including specific proteins. This idea was in opposition to the predominant-view of permeability, a membrane property, as the principal factor governing the passage of solutes.

The concept of the specialized transport protein gained general recognition primarily because of the possibility of varying the amount of transport protein separately from that of surface area of the membrane through specific genetic change and through inductive or repressive regulation of its biosynthesis (Cohen and Monod, 1957). The word "permease" (Rickenberg *et al.*, 1956) stirred up considerable discussion which served to emphasize the transport proteins. It suggested in a condensed form the involvement of a protein with enzyme-like specificity and catalytic (i.e., cyclic) activity. Unfortunately, it failed to stress distinction between permeability and active transport which later was recognized to be a nearly universal feature of all transport systems to which the term has ever been applied (Kepes, 1964). The enzyme-like denomination "-ase" can be justified on the ground that a typically enzymatic event, the breakdown of chemical (or electrochemical) energy, is partly or totally dependent on the transport protein. The phosphoenolpyruvate sugar phosphotransferase system (Kundig *et al.*, 1964) (see also Kundig, this volume) is an illustration of this statement, although since the discovery of its chemical

ADAM KEPES · Centre National de la Recherche Scientifique, Université Paris VII, Institut de Biologie Moléculaire, Laboratoire des Biomembranes, Tour 43-2, place Jussieu, 75221 Paris cedex 05, France.

mechanism it has seldom been included among permeases. Presumably, once similarly well-defined biochemical mechanisms have been established for all transport systems, the term permease will have completely lost its usefulness.

In this chapter both old and recent evidence concerning the membrane location of transport proteins will be reviewed. This view is supported by their limited freedom of movement along the membrane surface. Some facts will be reported which permit speculation about the insertion of membrane protein into membrane during biosynthesis; other facts and speculations will be relevant to the possibility of transmembrane movements.

The likelihood of multiple configurational changes during the transport cycle will be documented by several lines of indirect evidence. The energy coupling to active transport will be discussed briefly on the basis of experiments with inhibitors (Kepes, 1974) and isolated membrane vesicles (Kaback, 1971, 1972). The chemiosmotic theory and its methodology (Harold, 1972; West, 1970; West and Mitchell, 1972, 1973; Rosen, 1973) and the genetic analysis of the energy pathways in bacterial transport will be briefly reviewed (Haddock and Schairer, 1973; Devor *et al.*, 1974; Simoni and Shallenberger, 1972). For a large class of transport systems the results can be tentatively summarized as the utilization of an energized state of the membrane to fuel active transport. Finally, recent evidence for transport systems utilizing chemical-bond energy independent from the *energized state of the membrane* will be briefly described (Berger, 1973).

II. Transport Proteins as Membrane-Bound Proteins

The plasma membrane is the main diffusion barrier to penetration of hydrophilic solutes from the medium and to the escape of many hydrophilic metabolites from the cell. This basic impermeability is the cause of the inaccessibility of intracellular metabolic enzymes to their substrates dissolved in the medium (Deere *et al.*, 1939). This inaccessibility (termed crypticity) helped establish the generalized requirement for transport systems to carry out the uptake of all exogenously furnished nutrients (Cohen and Monod, 1957).

The logical counterpart of the virtual impermeability of the plasma membrane is the necessary location within its hydrophobic fabric of essential parts of the transport machinery. Some of these must either encompass the thickness of the barrier permanently or be able to shuttle occasionally or periodically from one face to another. Such logic does not unambiguously designate a membrane protein. The best examples of nonprotein carriers are the polyisoprenoid molecules which help the hydrophilic building blocks of murein or lipopolysaccharide to cross the hydrophobic membrane. The logic is somewhat tightened when a transport process is performed in isolated membranes in the absence of cytoplasmic factors, since only structures permanently linked to the membrane can participate in the translocation process from the recognition of the solute to its release on the other side. Such a sequence implies a stereospecific site for the transport substrate and strongly supports the presence of the key protein.