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Bacterial Transport

edited by Barry P. Rosen

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edited by

BARRY P. ROSEN

Department of Biological Chemistry
University of Maryland School of Medicine
Baltimore, Maryland

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PREFACE

This volume, because it is part of the "Microbiology Series," deals exclusively with procaryotic microorganisms. However, there is a more fundamental reason to limit it thusly. At the risk of publicizing a competing text, I would have to admit that Christensen is correct in his "plea for eclecticism" [H. N. Christensen, Biological Transport (2nd ed.), W. A. Benjamin, Reading, Mass., 1975, 514pp.]. There has been too little cross-fertilization between the various subspecialties of biological transport: the mitochondrial, eucaryotic cytoplasmic, plant, and bacterial areas. Sadly, this book cannot correct that defect. The field of bacterial transport encompasses too much information to allow for coverage of the other subspecialties; yet this text is designed for the same audiences, namely graduate and advanced undergraduate students. It should also provide a useful summary for investigators, but it is not designed to be utterly comprehensive.

Knowing that the time lag in publication is too great to allow for a current summary, I have encouraged each contributor to briefly summarize their areas and to provide an intensive look at specific points. In addition, I have requested speculation where possible. Most of that will turn out to be incorrect; by the time publication is achieved our collective score may be known. That type of divination, however, is something to which our students should be exposed.

Finally, I would like to thank Loretta Leive for her advice during the initiation of this project. I must also acknowledge gratefully the gentle pressure to push on to completion applied by Simon Silver and Eva Kashket. Thanks are also due the members of my laboratory for reading and criticizing the various chapters: Lawrence Adler, Jeanne Beck, Robert Brey, Syed Hasan, Tomio Ichikawa, and Erik Sorensen, and especially to Lisa Schwender for much-needed assistance in proofreading and compilation of the Subject Index.

Barry P. Rosen
Baltimore, Maryland

CONTRIBUTORS

Yasuhiro Anraku, Department of Botany, Faculty of Science, University of Tokyo, Hongo, Bunkyo-Ku, Tokyo, Japan

Philip J. Bassford, Jr., [1] Department of Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia

Winfried Boos, Department of Biology, University of Konstanz, Konstanz, Federal Republic of Germany

Thomas Ferenci, Department of Biology, University of Konstanz, Konstanz, Federal Republic of Germany

Masamitsu Futai, [2] Department of Botany, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan

Charles Gilvarg, Department of Biochemical Sciences, Frick Chemical Laboratory, Princeton University, Princeton, New Jersey

John B. Hays, Department of Chemistry, University of Maryland, Baltimore County, Catonsville, Maryland

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Present Address

[1] Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts

[2] Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan

Robert J. Kadner, Department of Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia

Eva R. Kashket, Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts

William W. Kay, [3] Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Edward G. Moczydlowski, Department of Biology, The John Muir College, University of California, San Diego, La Jolla, California

John W. Payne, Department of Botany, University of Durham, Durham, DH1 3LE, England

Barry P. Rosen, Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland

Milton H. Saier, Jr., Department of Biology, The John Muir College, University of California, San Diego, La Jolla, California

Thomas J. Silhavy, [4] Department of Biology, University of Konstanz, Konstanz, Federal Republic of Germany

Simon Silver, Department of Biology, Washington University, St. Louis, Missouri

Jeffrey B. Smith, [5] Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York

David B. Wilson, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York

---

Present Address

[3] Department of Biochemistry, University of Victoria, Victoria, British Columbia, Canada

[4] Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts

[5] Imperial Cancer Research Fund, London, WC2A 3PX, England

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CONTENTS

Chapter 12

INTRODUCTION

Barry P. Rosen

Why was this book written? A standard question for which there is more-or-less standard answer: the field has progressed sufficiently rapidly to justify the appearance of a new text. That is a true and valid answer, but, rather than simply leaving it at that, I would like to justify the statement with a brief review of the history of the field. I have divided the development of the thought in the field of bacterial transport into four periods: (1) discovery (to 1955), (2) physiology (1955 to 1960), (3) biochemistry (1960 to 1970), and (4) molecular biology (1970 onwards). The time periods are, of course, approximate, and overlap each other.

Discovery

The third and final edition of Marjory Stephenson's classic text Bacterial Metabolism [1] appeared in 1949. This text represented a major contribution to the field of microbiology since it demonstrated that the physiology and metabolism of microorganisms were areas which could be studied in more than a descriptive fashion. Yet, the subject index contains no entries on transport or other membrane-related phenomena. There is one short section (pp. 142-143) on the assimilation of amino acids by bacteria in which some of the pioneering work on transport is cited, but the point of permeation as a phenomenon separate from metabolism is missed. As Gale relates Stephenson's attitude, "...permeability is the last resort of the biochemist who cannot find any better explanation" [2]. Stephenson's feelings were not unusual. The first edition of the report of the Carnegie Institute of Washington on Studies of Biosynthesis in Escherichia coli concluded "...it does not appear that any mechanism other than simple diffusion through a highly permeable membrane is involved in the transport of material across the membrane...the protoplasm may be likened to a sponge, the cell membrane to a surrounding hair net unable to exclude the entrance or emergence of small molecules" [3].

But within half a decade following the publication of Stephenson's third edition, review articles on the nature of active transport were written by Gale [4] and Mitchell [5]. Further, the conclusions of the Carnegie group were modified in the second edition of their report, a scant 2 years after the first: "Accordingly the simple concept of a freely permeable cell was no longer tenable. The other simple concept of a cell with an impermeable cell wall was equally incompatible with the data ...since it now appears that the cells are neither completely permeable nor completely impermeable, the whole situation must be reconsidered" [3]. By that time there were few who doubted the existence of active transport.

Physiology

The early part second stage is summarized in the reviews by Cohen and Monod [6] and Kepes and Cohen [7]. That period included the work of the Paris school on lactose transport and metabolism. Upon this framework was built a wealth of information about what bacteria transport, including the recognition that a large number of discrete systems exist, each with its own specificity and kinetic characteristics. Within this period also was the conceptual advance of recognizing the genetic basis of transport systems: on the one hand, the isolation of mutants with transport defects, and on the other hand, the ability to induce certain systems cryptic in the absence of inducer. These observations led to the idea that specific proteins, "permeases", were involved in the transport reaction [7].

Biochemistry

Once the thought surfaced that proteins might be components of transport systems, the field expanded rapidly from microbiology to biochemistry. Microbial physiology utilized the intact organism; biochemical characterization required in vitro systems. The major cell-free system for the study of bacterial transport is that devised by Kaback [8]. Without a doubt, the use of isolated bacterial membrane vesicles resulted in a quantum jump in our ability to gather knowledge of bacterial transport. Cross-fertilization has in this case occurred between this area and that of the mammalian transport physiologist: more and more, cytoplasmic membrane vesicle preparations are being utilized for biochemical investigations of mammalian transport systems [9,10].

Again, with the image of proteins in mind, investigators turned to the isolation of protein components of transport systems. The first major advance was the identification of the M protein as a component of the lactose transport system by Fox et al. [11]. Although the inability to isolate that protein in any sort of active form has been a disappointment, it did provide an impetus to other investigators to search for transport-related proteins.

At about the same time Neu and Heppel demonstrated that an osmotic shock procedure caused the loss of periplasmic proteins [12]. Since such proteins are associated with the cell envelope of Escherichia coli, it was a reasonable next step to look for transport-related proteins in the supernatant fluid from osmotically shocked cells. Piperno and Oxender [13] did just that, discovering that leucine transport activity was reduced by osmotic shock treatment with the simultaneous appearance of a leucine binding protein in the shock fluid. Subsequently, numerous binding proteins related to numerous transport systems have been reported [14]. Even though the exact function of these proteins is still unknown, they were the first proteins definitely known to be components of transport systems to be isolated in an active form. The use of active should be qualified, since it is not possible to assay for an unknown function. As the name applies, however, the binding proteins are active in forming complexes with the substrates of their associated transport systems. None have been found to have associated enzymatic activity, though, and no convincing demonstration of a binding protein functioning in a reconstituted transport system has appeared.

On the other hand, a group of proteins found to comprise an enzymatic complex was subsequently shown to function in a transport capacity. That refers, of course, to the phosphoenolpyruvate-sugar phosphotransferase system discovered by Kundig et. al. [15]. Since the components of the phosphotransferase system could be investigated as enzymes, the system provided a unique opportunity to study a translocating mechanism with biochemical tools. Chapter 2 by Hays discusses the phosphotransferase system in such terms, while Chapter 3 by Saier and Moczydlowski considers it in more physiological terms.

No scientific field is complete without its major guiding light. Thomas Kuhn in his commentary on the evolution of scientific thought [16] suggests that normal science progresses by the accretion of knowledge, leading to the formulation and subsequent acceptance of a paradigm. According to Kuhn, there are two criteria which a hypothesis must meet to be a paradigm: it must be sufficiently unprecedented to attract a following and must be open-ended enough to allow for extensive experimentation, modification, and/or confirmation. Kuhn points out that "...acquisition of a paradigm and of the more esoteric type of research it permits is a sign of maturity in the development of any given scientific field" [16]. The chemiomotic hypothesis proposed by Peter Mitchell [17] is the only major paradigm to have originated in the area of bacterial transport. Little space will be given here to an exposition of the hypothesis, since it is covered in detail in the final chapter of this book.

There are a few points which should be made, though. First, the paradigm was certainly unprecedented as far as the biochemists and microbial physiologists were concerned; so unprecendented, in fact, that it was not taken seriously for a number of years. The idea of forces

acting at a distance was vaguely disquieting to the biochemist, who thought in terms of intermolecular interactions. But to the biophysicist and mammalian physiologist, membrane potentials and ion fluxes were everyday events. So from that point of view, Mitchell's formulation was not unprecedented. But the mammalian physiologists were unconcerned with molecules and mechanisms. Mitchell can be credited with a true integration of the purity of thought of the physiologist with the practicality of the biochemist. Although acceptance was slow, especially among the mitochondriologists, Mitchell's ideas were eminently testable and had the tremendous advantage of providing correct predictions. Much of the most recent work in the area of bacterial transport has centered around the ramification of Mitchell's postulates. Even though the hypothesis in itself does not predict molecular mechanisms, the acquisition of the paradigm is right now permitting a more esoteric type of research to be performed. It is certainly a sign of maturity in the development of the field!

Molecular Biology

There are those who would quibble about the use of the term "molecular biology" for an area which does not deal with nucleic acids, protein synthesis, or related matters. But the direction in which current ideas in biological transport are pointing appear to be the understanding of permeation in terms of the molecular mechanisms of the individual components and of the way in which they form supramolecular structures. So, assembly and mechanism will be the major concern in years to come. Already there are numerous laboratories active in the isolation of the intrinsic membrane proteins which are most likely the carriers themselves. The ability to incorporate those proteins into artificial membrane systems provides the means to study both assembly and mechanism. From another point of view, investigations of the energetics in intact cells and membrane vesicles have progressed to the point where meaningful statements can be made in molecular terms.

On the other hand, there is a whole class of transport systems which is not accounted for in Mitchell's terms, namely those linked more intimately with phosphate bond energy, as first described by Berger [18]. It is much too early to think about this type of system in molecular terms; basic biochemical studies are yet to be performed. But it is quite apparent that the future must include a detailed examination of that type of transport system, perhaps leading to the acquisition of a new paradigm of importance equal to that of Mitchell.

In conclusion, this abbreviated guided tour through the development of the basic tenets of the bacterial transport domain was meant to demonstrate the necessity for a text of this sort. Of necessity I have had to pass over many important contributions by many respected colleagues: May they not take offense!

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