Journal of Bioenergetics and Biomembranes, Vol. 25, No. 6, 1993

Introduction

Giovanna Ferro-Luzzi Ames[1]

Received June 30, 1993; accepted July 15, 1993

Substrate transport through the cell envelope is a major and challenging problem in biology. It also has been a notoriously difficult one because of the lack of simple biochemical assays in *in vitro* situations. The difficulties are somewhat smaller when the research utilizes bacterial organisms, mainly because simple genetic techniques for the isolation and characterization of transport mutants can be used and because of the ease with which recombinant DNA research can be applied. It is a relevant question whether findings obtained in a prokaryotic organism can be extrapolated to eukaryotic ones; this question has been recently answered with a resounding yes. Indeed, the case of the newly described superfamily of traffic ATPases, which includes medically important proteins such as the multidrug resistance Pglycoprotein (MDR) and the cystic fibrosis gene product, CFTR, has shown that prokaryotic research can provide important guidelines to guide the experimentation and for the drawing of conclusions in several eukaryotic fields of research.

This issue of the Journal deals exclusively with transport into bacterial cells and, because of shortage of space, it does not even cover all the possible areas. The contributions have been chosen so as to provide an overview of three entirely different mechanisms of bacterial transport: periplasmic permeases, covalently modifying transport systems, and a classic proton symport system. The closely related problems of translocation through the outer membrane, of transfer of energy from the energy-generating inner (cytoplasmic) membrane through the periplasmic space, of membrane-bound mechanisms for internal pH maintenance, and for transport regulation are also discussed.

The first cell envelope barrier encountered by external molecules in Gram-negative bacteria is the outer membrane. Permeability through this membrane is relatively well understood. Nonspecific passive diffusion has been known as the basis of this permeability for a long time; however, it appears now that specificity and channel structure are also part of the picture. An extremely useful and elegant advance has been the solution of the crystal structure of porins (see cover illustration), which form water-filled diffusion channels in the outer membrane of these bacteria. This particular piece of information has provided a solid basis for studying the mechanism of diffusion of substrates through outer membranes. This area of research has special implications for the understanding of antibiotics uptake and for devising therapeutic approaches.

The outer membrane is separated from the inner membrane by the peptidoglycan layer (a passive, permeable, rigid network which maintains the cell shape) and by an empirically defined, protein-filled space, the periplasm. Therefore, after crossing the outer membrane, substrates are exposed to this periplasmic environment, where some of them use periplasmic permeases to enter the cytoplasm. Periplasmic permeases have become the paradigm for the superfamily of traffic ATPases (Ames *et al.*, 1992), translocating complexes that share strong similarities, both structurally and with respect to the primary sequence. Progress in this area is moving at a rapid pace in many laboratories studying independently many different bacterial permeases. The availability of numerous related systems is a considerable advantage because it has allowed the field to extract basic principles and to define the unique characteristics of

[1] Department of Molecular and Cell Biology, University of California, Berkeley, California, 94720.

0145-479X/93/1200-0577$07.00/0

this very large family of transporters. The recent advances in the analysis of the maltose permease are presented here by the Shuman laboratory. Other permeases under intensive study and extensively characterized are the oligopeptide permease, the branched amino acid permease, and the histidine permease; several others are in various levels of development. For a coverage of the status of the field in all of the permeases the reader is referred to several reviews (Ames, 1986; Shuman, 1987). Genetics and biochemistry have provided important advances with respect to energy coupling (reviewed in Ames, 1990) and structural analysis (Kerppola *et al.*, 1991, 1992). In bacteria substrates are bound tightly by specialized periplasmic substrate-binding proteins and delivered in concentrated form to a membrane-bound complex that performs the actual translocation step. A particularly important finding has been the discovery that periplasmic permeases are not irrevocably dependent on the presence of the periplasmic component, but can be mutated to function in its total absence [reviewed here for the maltose permease; see also Petronilli and Ames (1991) and Speiser and Ames (1991) for similar findings for the histidine permease]. This notion has led to the elimination of one of the controversial differences between eukaryotic and prokaryotic traffic ATPases and to the suggestion that they may all function as channels, with the periplasmic permeases having developed the substrate-binding protein for trapping and concentrating the substrate at the mouth of the channel formed by the membrane-bound complex (Ames, 1992). How a channel-like mechanism can operate in systems that have been traditionally thought of as "active" (i.e., concentrating) transport systems has been discussed (Ames and Lecar, 1992). Therefore, it is possible to think in terms of a single molecular mechanism of action for both the eukaryotic and the prokaryotic transporters. Indeed, recently the suggestion has been made that periplasmic components, when loaded with substrate, signal their presence to the membrane-bound complex, so that the channel is opened with the expenditure of ATP energy and allows the passage of the substrate (Shuman in this issue; Petronilli and Ames, 1991 for the histidine permease).

Energy generated in the cytoplasmic membrane needs to be "carried" to the outer membrane to serve the needs of special translocation mechanisms from the outer membrane through the periplasm and into the cell. Several important nutrients such as iron and vitamin B12, and toxic elements such as colicins and phages, depend on this mechanism to enter the cell. The question of energy transduction is addressed in two of the contributions, where the tantalizing and controversial problem of the mechanism of action of one of the responsible proteins, the TonB protein, is being debated. Models presented by the two authors differ, especially with respect to the organization of TonB and of the interacting TonB Box within the outer membrane. This essentially unique model system for energy transduction between membranes is now well poised for providing important answers.

Transport of carbohydrates has taken a number of routes, one of the best characterized from the biochemical point of view being the large family of phosphotransferases. This class of translocators offers numerous elements for study, also because these systems play an important role in chemotaxis. The biochemical analysis is very advanced and comparative studies aiming at unraveling the evolutionary history add an interesting aspect to this area of research (Postma *et al.*, 1993). The contribution in this issue focuses on the structural and biochemical characterizaton of one of the domains of enzyme II (EII). Progress in this particular area of research is moving steadily forward and we can look forward to a complete elucidation of these systems in the near future.

A well-known transport system such as the β-galactoside permease speaks for itself. Its analysis in excruciating and heroic detail continues in the laboratory of Kaback. It is well known that members of this laboratory are strenuously attempting the enormous task of crystallizing the protein, which is indeed the dream of all transportologists. The final answer on the mechanism of action will come with the resolution of its structure; in the meantime continuous progress provides fascinating insights into its mechanism of action through the use of many sophisticated techniques.

Regulation of transport is among the least explored aspects of transport, although a variety of mechanisms are known, at least in principle, such as substrate induction (for the β-galactoside and maltose permeases (Kühnau *et al.*, 1991)), nitrogen availability for the regulation of the histidine permeases (Kustu *et al.*, 1979), and substrate repression [for the branched chain amino acid permeases (Landick *et al.*, 1984)]. Transport of the phosphate esters of several carbohydrates is regulated by the level of the phosphorylated sugar present in the medium, but not by the intracyto-

plasmic pool. This kind of regulation requires sensing the external substrate concentration and transmittance through the membrane of the appropriate signals to the cytoplasm where transcriptional regulation finally takes place, without interference from the same compound already present in the cell. The contribution from Kadner's laboratory describes one such system, in which a transmembrane protein regulates transport and, interestingly, is itself related in sequence and structure to the transport protein it regulates. The evolutionary history and the mechanism of action of such a system will be interesting.

Finally, a mechanism involved in the maintenance of the internal pH is fundamentally a transport process and is also discussed by Padan and Shuldiner.

What are the future prospects for research in these areas? What is the most promising route to follow? What are the important questions to be asked? This special issue clearly will show the power of the genetic, physiological, and biochemical approaches that have been in use. Their future use will continue to be productive in giving insights into the molecular mechanisms of action. However, the final answers will come from the resolution of the X-ray structure of these transporters, which requires certainly much hard work and faith, but it also requires financial support. This is certainly one of the problems the granting agencies will have to face, since it is a well-known adage that no funds are available until crystals have been obtained. While this is reasonable in the case of soluble proteins, it is not realistic for the much more formidable membrane-bound proteins, for which the crystallization process is the limiting step, rather than the resolution of the structure. Hopefully the near future will bring a realization of this problem and the consequent solution of several transporter structures.

REFERENCES

Ames, G. F.-L. (1986). *Annu. Rev. Biochem.* **55,** 397–425.

Ames, G. F.-L. (1990). In *Bacterial Energetics* (Krulwich, T. A., ed.), Academic Press, New York, pp. 225–245.

Ames, G. F.-L. (1992). *Int. Rev. Cytol.* **137a,** 1–35.

Ames, G. F.-L., and Lecar, H. (1992). *FASEB J.* **6,** 2660–2666.

Ames, G. F.-L., Mimura, C., Holbrook, S., and Shyamala, V. (1992). *Adv. Enzymol.* **65,** 1–47.

Kerppola, R. E., and Ames, G. F.-L. (1992). *J. Biol. Chem.* **267,** 2329–2336.

Kerppola, R. E., Shyamala, V., Klebba, P., and Ames, G. F.-L. (1991). *J. Biol. Chem.* **266,** 9857–9865.

Kühnau, S., Reyes, M., Sievertsen, A., Shuman, H. A., and Boos, W. (1991). *J. Bacteriol.* **173,** 2180–2186.

Kustu, S. G., McFarland, N. C., Hui, S. P., Esmon, B., and Ames, G. F.-L. (1979). *J. Bacteriol.* **138,** 218–234.

Landick, R., Oxender, D., and Ames, G. F.-L. (1984). In. *The Enzymes of Biological Membranes* (Martonosi, A. N., ed.), Plenum Press, New York, pp. 577–615.

Petronilli, V., and Ames, G. F.-L. (1991). *J. Biol. Chem.* **266,** 16293–16296.

Postma, P. W., Lengeler, J. W., and Jacobson, G. R. (1973). *Microbiol. Rev.* **57,** 543–594.

Shuman, H. A. (1987). *Annu. Rev. Genet.* **21,** 155–177.

Speiser, D. M., and Ames, G. F.-L. (1991). *J. Bacteriol.* **173,** 1444–1451.