24. P. J. Schatz and J. Beckwith, *Annu. Rev. Genet.* **24**, 215 (1990).
25. T. A. Rapoport, *Nature* **349**, 107 (1991).
26. S. M. Simon and G. Blobel, *Cell* **65**, 371 (1991).
27. ———, *ibid.* **69**, 677 (1992).
28. A. E. Johnson, personal communication.
29. R. J. Deshaies and R. Schekman, *J. Cell Biol.* **105**, 633 (1987).
30. C. J. Stirling, J. Rothblatt, M. Hosobuchi, R. Deshaies, R. Schekman, *Mol. Biol. Cell* **3**, 129 (1992).
31. D. Görlich, S. Prehn, E. Hartmann, K.-U. Kalies, T. A. Rapoport, *Cell*, in press.
32. S. L. Sanders, K. M. Whitfield, J. P. Vogel, M. D. Rose, R. Schekman, *ibid.* **69**, 353 (1992).
33. A. Müsch, M. Wiedmann, T. A. Rapoport, *ibid.*, p. 343.
34. S. High and B. Dobberstein, personal communication.
35. S. High, D. Görlich, M. Wiedmann, T. A. Rapoport, B. Dobberstein, *J. Cell Biol.* **113**, 35 (1991).
36. L. Brundage, J. P. Hendrick, E. Shievel, A. J. M. Driessen, W. Wickner, *Cell* **62**, 649 (1990); J. Akimaru, S. Matsuyama, H. Tokuda, S. Mizushima, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6545 (1991). It has also been reported, however, that SecYp is not involved in translocation into proteoliposomes [M. Watanabe, C. V. Nicchitta, G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1960 (1990)].
37. N. Borgese, W. Mok, G. Kreibich, D. Sabatini, *J. Mol. Biol.* **88**, 559 (1974).
38. A. J. Savitz and D. I. Meyer, *Nature* **346**, 540 (1990).
39. S. Tazawa *et al.*, *J. Biochem.* **109**, 89 (1991).
40. J. M. Nunnari, D. L. Zimmerman, S. C. Ogg, P. Walter, *Nature* **352**, 638 (1991), P. G. Collins and R. Gilmore, *J. Cell Biol.* **114**, 639 (1991).
41. G. Blobel and B. Dobberstein, *J. Cell Biol.* **67**, 852 (1975).
42. L. L. Randall, *Cell* **33**, 231 (1983).
43. D. Görlich, E. Hartmann, S. Prehn, T. A. Rapoport, *Nature* **357**, 47 (1992).
44. C. Nicchitta and G. Blobel, *Cell* **60**, 259 (1990).
45. M. Wiedmann, T. V. Kurzchalia, E. Hartmann, T. A. Rapoport, *Nature* **328**, 830 (1987).
46. U. C. Krieg, A. E. Johnson, P. Walter, *J. Cell Biol.* **109**, 2033 (1989); M. Wiedmann, D. Görlich, E. Hartmann, T. V. Kurzchalia, T. A. Rapoport, *FEBS Lett.* **257**, 263 (1989).
47. N. Green, H. Fang, P. Walter, *J. Cell Biol.* **116**, 597 (1992).
48. Different degrees of glycoprotein depletion are possible because cholate (*44*), in contrast to *N,N*-bis(3-D-gluconamidopropyl) cholamide (BigCHAP) (*43*), does not inhibit the binding of glycoproteins to concanavalin A–Sepharose.
49. S. High, B. Dobberstein, J. Brunner, personal communication.
50. R. Deshaies and R. Schekman, *Mol. Cell. Biol.* **10**, 6024 (1990).
51. D. Feldheim, J. Rothblatt, R. Schekman, *ibid.* **12**, 3288 (1992).
52. R. J. Deshaies, S. L. Sanders, D. A. Feldheim, R. Schekman, *Nature* **349**, 806 (1991).
53. I. Sadler *et al.*, *J. Cell Biol.* **109**, 2665 (1989).
54. J. Brodsky and R. Schekman, personal communication.
55. J. P. Vogel, L. M. Misra, M. D. Rose, *J. Cell Biol.* **110**, 1885 (1990).
56. E. Evans, R. Gilmore, G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 581 (1986); J. T. YaDeau and G. Blobel, *J. Biol. Chem.* **264**, 2928 (1989).
57. G. S. Shelness and G. Blobel, *J. Biol. Chem.* **265**, 9512 (1990); P. C. Boehni, R. J. Deshaies, R. Schekman, *J. Cell Biol.* **106**, 1035 (1988).
58. D. J. Kelleher, G. Kreibich, R. Gilmore, *Cell* **69**, 55 (1992).
59. E. Hartmann, M. Wiedmann, T. A. Rapoport, *EMBO J.* **8**, 2225 (1989).
60. G. Migliaccio, C. V. Nicchitta, G. Blobel, *J. Cell Biol.* **117**, 12 (1992).
61. D. Görlich *et al.*, *ibid.* **111**, 2283 (1990). The sequences of the γ and δ SSR subunits are unpublished (S. Prehn, E. Hartmann, D. Görlich, T. A. Rapoport).
62. I. Wada *et al.*, *J. Biol. Chem.* **266**, 19599 (1991).
63. P. Klappa, P. Mayinger, R. Pipkorn, M. Zimmermann, R. Zimmermann, *EMBO J.* **10**, 2795 (1991); D. L. Zimmerman and P. Walter, *Cell Regul.* **2**, 851 (1991).
64. R. N. Thrift, D. W. Andrews, P. Walter, A. E. Johnson, *J. Cell Biol.* **113**, 809 (1991); K. V. Kellaris, S. Bowen, R. Gilmore, *ibid.* **114**, 21 (1991), A. Robinson, M. A. Kaderbhai, B. M. Austen, *Biochem. J.* **242**, 767 (1987); E. Hartmann and T. A. Rapoport, in *Membrane Biogenesis and Protein Targeting*, W. Neupert and R. Lill, Eds. (Elsevier, New York, in press).
65. Y. Akiyama and K. Ito, *EMBO J.* **6**, 3465 (1987).
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Transport Proteins in Bacteria: Common Themes in Their Design

Hiroshi Nikaido and Milton H. Saier, Jr.

Bacterial transport proteins mediate passive and active transport of small solutes across membranes. Comparison of amino acid sequences shows strong conservation not only among bacterial transporters, but also between them and many transporters of animal cells; thus the study of bacterial transporters is expected to contribute to our understanding of transporters in more complex cells. During the last few years, structures of three bacterial outer membrane transporters were solved by x-ray crystallography. Much progress has also occurred in the biochemical and molecular genetic studies of transporters in the cytoplasmic membranes of bacteria, and a unifying design among membrane transporters is gradually emerging. Common structural motives and evolutionary origins among transporters with diverse energy-coupling mechanisms suggest that many transporters contain a central module forming a transmembrane channel through which the solute may pass. Energy-coupling mechanisms can be viewed as secondary features added on to these fundamental translocation units.

True bacteria are divided into Gram-positive and Gram-negative organisms, depending on their reaction to a staining protocol. In the former group, the plasma membrane is surrounded only by a mechanically rigid and rather porous cell wall (peptidoglycan). In contrast, the latter group (which includes *Escherichia coli*) produces a second membrane, the outer membrane, that is located outside the plasma (cytoplasmic) membrane and the thin peptidoglycan layer. A fundamental function of biological membranes is to serve as a selective permeability barrier. Consonant with its location, the outer membrane serves as an efficient permeability barrier that protects Gram-negative bacteria from a number of harmful compounds, such as some antibiotics, disinfectants, and detergents (*1*). Both the outer and inner membranes contain transport proteins that mediate the passage of a limited range of solutes. These prokaryotic cell membranes have proven to be excellent experimental systems for studying transporters because of the ease of biochemical and genetic manipulation in bacteria. The study of bacterial transporters is significant also because their amino acid sequences are now known to be strikingly similar to those many transporters of cells of higher animals (see below).

This review will summarize recent data obtained from the study of a variety of bacterial transporters. These data suggest a common theme in the design of many transporters. These transport proteins contain similar transmembrane domains encompassing a membrane-spanning channel (*2*). These domains are made up of transmembrane β strands in outer membrane proteins, whereas transmembrane α helices, frequently a pair of domains each containing six helices, are found in plasma membrane transporters. The diversity of the transporters often seems to originate from the specificity of these channels and peripheral domains employed to couple energy to active transport processes. Because of limited space, many transport systems are not discussed; these include primary ion pumps such as bacteriorhodopsin and P-type adenosine triphosphatases (ATPases).

Channels in the Outer Membrane

The outer membrane contains three types of channels (Fig. 1) (*1*). (i) Proteins known as porins (Fig. 1A) contain large, open,

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water-filled channels that nonspecifically mediate the passive penetration of ions and small, hydrophilic nutrient molecules. (ii) Porin-like proteins (Fig. 1B) containing specific binding sites carry out the facilitated diffusion of some nutrients that penetrate only slowly through the nonspecific porin channels. (iii) At least in *E. coli* and its relatives, large nutrient molecules that exist in very low concentrations outside (vitamin B_{12}, Fe^{3+}-chelator complexes) are actively accumulated across the outer membrane by a system (Fig. 1C) composed of a high affinity outer membrane receptor and the TonB protein, which is anchored in the cytoplasmic membrane and is thought to couple solute translocation to energy input (*3*).

Porins of enteric bacteria, including *E. coli*, were identified by searching for outer membrane proteins that produced nonspecific diffusion channels in reconstituted proteoliposomes (*4*). The archetypal porins, such as OmpF (outer membrane protein F), OmpC, and PhoE of *E. coli* K12, are proteins of 36,000 to 38,000 daltons, and in terms of mass they are usually the most abundant proteins in *E. coli*. They allow the diffusional influx of small nutrient molecules but exclude toxic compounds that usually are larger. Measurement of the diffusion rates of sugars of various sizes led to the estimated pore diameter of about 11 to 12 Å. This small size, however, means that even small nutrient molecules frequently fail to enter the pore when they are deflected by the rim of the channel. For this reason, every *E. coli* cell has nearly 100,000 copies of these proteins.

The porins are not hydrophobic proteins and do not contain any of the long stretches of hydrophobic amino acids typically found in integral proteins of the plasma membrane. These puzzling observations were explained by the x-ray crystallographic structure of porin from *Rhodobacter capsulatus* at 1.8 Å resolution (*5*). Like other classical porins, this porin exists as a stable trimer. The polypeptide chain of each subunit traverses the membrane 16 times as antiparallel β strands, forming a β-barrel structure surrounding a large channel. Each subunit produces a channel, and the trimer therefore contains three channels. Because every other amino acid side chain faces into the hydrophilic channel, a β barrel spans the membrane without stretches of consecutive hydrophobic residues.

The following observations have been made regarding the channel. (i) The pore is clearly open, in agreement with the observation that porin channels are usually open under physiological conditions (*4*). (ii) The pore at its narrowest point has dimensions of 8 by 10 Å. (iii) Because the distance between neighboring β strands is 4.5 Å, 16 β strands should produce a pore with a backbone-to-backbone diameter of $(4.5 \times 16)/\pi = 23$ Å. That the actual diameter is much less is due to one of the "extramembranous" loops folding back into the channel and producing a narrowing of limited depth. [This is reminiscent of the structure of potassium channels of animals, where a loop is thought to fold back into the interior of the channel (here composed of α helices) to create a narrowing that defines the critical selective behavior of the channel (*6*).] This design, with a wide entrance and exit and a short central constriction, produces a channel that is effective in excluding larger solutes while minimizing the frictional interactions between the solutes and the walls of the pore. (iv) At the narrowest point, one side of the channel is lined with negatively charged residues and the opposite side with positively charged residues. This configuration may utilize electrostatic forces to produce a constriction of well-defined and rigid shape.

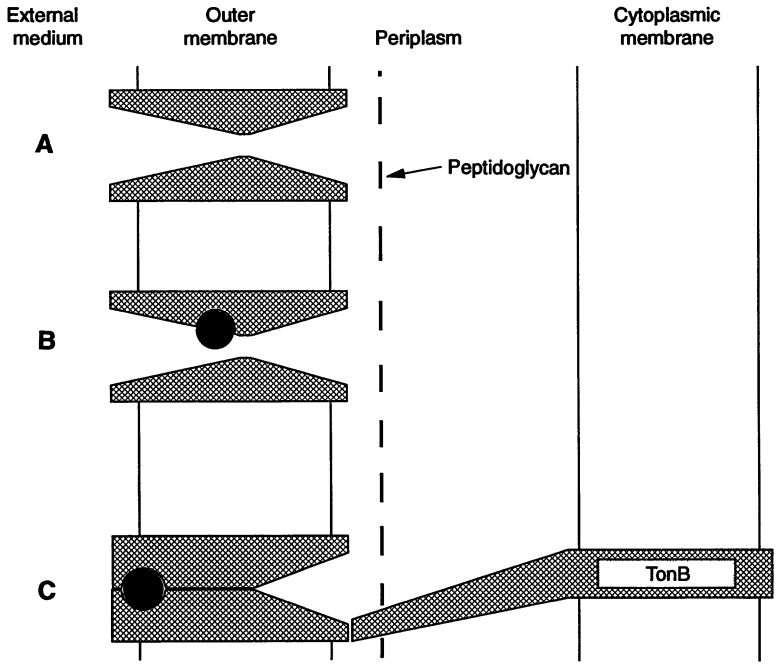

Fig. 1. Models of outer membrane transporters. (**A**) Porin. (**B**) Open, solute-specific channels. (**C**) TonB-dependent receptors. Black circles indicate ligands that are specifically recognized and transported.

The *E. coli* OmpF porin was one of the earliest membrane proteins to be crystallized (*7*), but the x-ray crystallographic studies proved problematic. Consequently the structures of OmpF and PhoE were solved only recently (*8*). The structures are remarkably similar to that of *R. capsulatus* porin in that a monomer spans the membrane 16 times as a β barrel and that the channel is narrowed by the folding back of one of the extramembranous loops. The constricted opening has a size of 7 by 11 Å in remarkable agreement with the size determined from the diffusion rates of sugars, 11 to 12 Å (*1*). PhoE porin, which is produced in *E. coli* under phosphate starvation conditions, has a preference for anions in contrast to the cation preference of OmpF porin; this alteration in charge preference was traced to the Lys^{125} of PhoE, which replaces a glycine residue of OmpF (*9*). These residues are located at the narrowest portion of the channel, where a strong influence on ion selectivity is expected (*8*).

In addition to these classical trimeric porins, there are also monomeric proteins that exhibit pore-forming activity. The OmpA protein of *E. coli* and the major porin OprF of *Pseudomonas aeruginosa* belong to this class. They both allow only very slow diffusion of solutes in spite of the fact that fairly large solute molecules are able to pass through these channels (*10*).

Like the porins, almost all of the outer membrane proteins that have been sequenced appear to have β-sheet structures (*4*), in contrast to the integral proteins of the plasma membrane, which contain multiple transmembrane segments of hydrophobic α helices (see below). It seems likely that the β-barrel construction is related to the mechanism of export. Thus the outer membrane proteins, which have a low overall hydrophobicity, may be exported easily into the periplasm (the space between the outer and the cytoplasmic membranes) (*11*). Upon insertion into the outer membrane, they refold into their stable β-barrel conformations through an interaction with

lipopolysaccharide, a lipid unique to the outer membrane (*11*). If these proteins were to contain sequences that could form transmembrane α helices, they probably would be retained by the plasma membrane and would not be exported into the periplasm. Interestingly, the β-barrel design appears to be present in at least one eukaryotic protein forming large, open channels, the mitochondrial porin that is found in the mitochondrial outer membrane (*12*); perhaps this is a legacy of the bacterial symbiont origin of this organelle.

The LamB protein (lambda receptor, so named because it is also the receptor for phage λ) of *E. coli* is the best studied outer membrane transporter catalyzing a specific diffusion process. Because disaccharides such as maltose penetrate the porin channels rather slowly, these cells need a mechanism for facilitating the transport of maltose and larger maltodextrins across the outer membrane. The LamB protein is a porin-like trimeric protein replete with β structure and contains open channels that additionally allow the diffusion of small solutes structurally unrelated to maltose (*13*). The structural similarity between LamB and classical porins suggests that the solute diffusion occurs by an essentially similar mechanism. However, there is a specific binding site for maltose within the channel (*13*). Theory predicts that such a channel would produce Michaelis-Menten– or saturation-type kinetics of diffusion (*14*). Thus the downhill diffusion of specific ligands through such a channel would become accelerated at low external concentrations. This saturation phenomenon was experimentally demonstrated for LamB as well as for the D2 channel of *P. aeruginosa*, which is specific for basic amino acids (*15*). These specific channels thus show kinetics that are characteristic of facilitated diffusion. We believe that the principle of first building a hydrophilic diffusion channel and then adding a specific ligand-binding site within it has been followed many times during the evolutionary development of specific transporters of the plasma membrane.

We know much less about the mechanism of action of the third type of outer membrane transporters, involved in the apparently active uptake of iron-chelator complexes and vitamin B_{12}. These receptors bind their ligands with much higher affinity than the specific channels described above, and the receptors from wild-type strains have not been reported to function as open channels. However, internal deletions within FepA, which belongs to this receptor family, were shown to convert the protein into nonspecific, TonB-independent, diffusion channels (*16*), and this suggests that these receptors may also be constructed as channels.

Plasma Membrane Transporters

Many transport systems in the plasma membrane are coupled to the input of energy. Thus they are usually classified according to the nature of their energy source. We shall discuss here the following classes (Fig. 2): (i) facilitated diffusion systems (Fig. 2A); (ii) secondary transporters (Fig. 2B) (*13*) that typically couple the uphill transport of nutrients with the downhill influx of H^+ or Na^+; (iii) group translocation systems (Fig. 2C) (*13*) as exemplified by the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) which phosphorylates sugars by using PEP concomitant with their translocation; and (iv) active transport systems (Fig. 2D) that use periplasmic binding proteins and that are energized by adenosine triphosphate (ATP) hydrolysis ["primary translocation systems" in Mitchell's terminology (*13*)]. Recent studies have revealed a surprising unity of design as well as some evolutionary relationships among these diverse systems.

Facilitated diffusion system. The glycerol facilitator (GlpF) is the only known example, in *E. coli*, of a plasma membrane transporter believed to catalyze facilitated diffusion (*17*). The sequence of this 281-residue protein suggests that it contains six transmembrane α helices (*18*). The sequence shows homology with several eukaryotic proteins (*18*), including the major intrinsic protein of the bovine lens junction, a protein that produces large, open, aqueous channels presumably at the interface between four subunits (*19*). Some properties of GlpF are those expected for channel-type transporters (*17*). However, the channel cannot be always be open because this would produce leakage of various ions and metabolic intermediates from the cytoplasm. As is true with other transporters of the plasma membrane, the channel is likely to be gated so that it opens only when the proper ligand binds to its binding site (Fig. 3A) (*2*).

Secondary transporters with a 6 + 6 helix construction. Many bacterial transporters couple the transport of ligands to the downhill flux of H^+, Na^+, or phosphate. Evolutionary relation among some of these proteins as well as between this group and transporters of higher animals first attracted attention when Henderson's group discovered that the arabinose:H^+ symporter and the xylose:H^+ symporter from *E. coli* share strong sequence similarity with the glucose facilitator of human hepatoma cells and of rat brain (*20*). Amino acid identity was about 30%. Subsequently, many more transporters were added to this family. One striking feature of this group is the predicted folding pattern, which incudes 12 hydrophobic transmembrane α helices. The similarity between the NH_2-terminal domain containing the first six helices and the COOH-terminal domain containing the last six helices indicates that the genes for these proteins may have arisen by duplication of an ancestral gene coding for six helices. This folding pattern is often referred to as the 6 + 6 transmembrane helix.

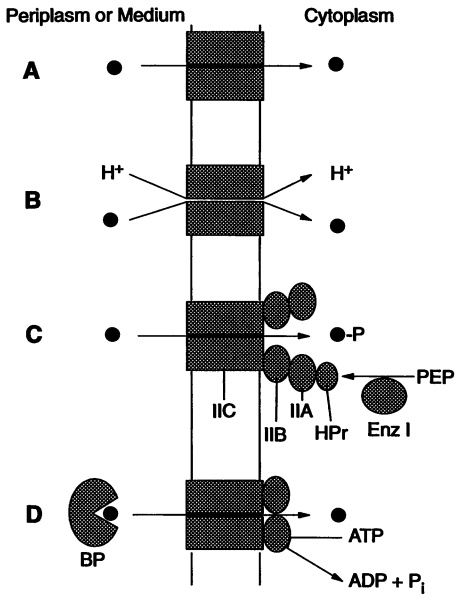

Fig. 2. Models of major classes of transporters in the plasma (cytoplasmic) membrane. (**A**) Facilitator. (**B**) Substrate:H^+ symporter. (**C**) PTS. (**D**) Binding protein–dependent transport system.

The 6 + 6 helix pattern is also seen in other bacterial transporters, but the evolutionary relationship among them has remained obscure (*21*). Most recently, the existence of homology was demonstrated for more than 50 transporters of bacteria and various eukaryotes, which form the major facilitator superfamily (*22*). There are five clusters in this superfamily. (i) Proteins catalyzing either H^+ symport (often in bacteria) or facilitated diffusion (often in animal cells) of sugars such as glucose, galactose, arabinose, or xylose. (ii) Oligosaccharide:H^+ symporters of *E. coli* carrying out the accumulation of lactose (LacY), sucrose (ScsB), and raffinose (RafB). (iii) Phosphate ester (hexose-P, glycerol-P, and P-glycerate): phosphate antiporters of *E. coli* and its close relative, *Salmonella typhimurium*. (iv) Drug (tetracycline, methylenomycin A, quinolones, aminotriazole, and antiseptics) resistance proteins of bacteria and yeast, which are likely to be drug:H^+ antiporter proteins. (v) Citrate:H^+ and α-ketoglutarate:H^+ symporters of *E. coli* and its relatives. We emphasize that there are facilitators such as the brain glucose facilitator, and symporters, such as the arabinose:H^+ symporter of *E. coli*, even within a single cluster of this superfamily, with a very high degree of sequence similarity (see above). This strongly

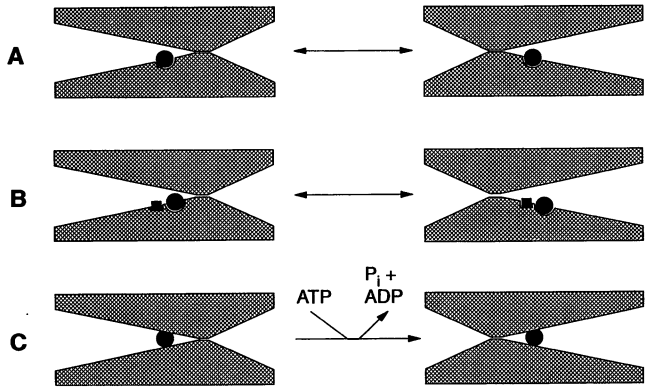

Fig. 3. Hypothetical solute translocation mechanisms. The mobile barrier hypothesis assumes that the critical step is not the translocation of the solute through the channel but is the movement of the barrier within the channel. We assume that this movement is caused by the binding of the solute (black circle) in facilitators (as well as in the IIC channel of PTS) (**A**), by the simultaneous binding of the solute and H^+ (black square) in solute:H^+ symporters (**B**), and by ATP hydrolysis in BP-dependent systems (**C**). The ligand does bind specifically to the channel in the last system (*53*), but this is not specified in the drawing to emphasize the idea that the movement of the barrier is driven primarily by ATP hydrolysis. Although the figure is drawn to indicate that ATP hydrolysis drives the movement of the mobile barrier outward, it is equally possible that it resets the barrier from the external to internal position.

suggests that conversion of a facilitator (uniporter in the terminology of Mitchell) into a symporter or an antiporter does not require a radical redesigning of the protein. This is precisely as predicted by Mitchell in his model of the secondary transporters, which assumes a central binding site(s) and a "mobile barrier" (*14*) (Fig. 3, A and B).

The lactose permease (LacY) was the system that was used by J. Monod's group in 1956 to establish that there are specific, energy-coupled, and saturable transporters in bacteria. Since then, this transporter has been studied in great detail. A decade ago, the LacY protein was purified and was reconstituted into proteoliposomes (*23*) by the octylglucoside dilution method of Racker *et al.* (*24*). This experiment established that this monomeric 46,500-dalton protein was the only protein needed for the 1:1 cotransport of lactose and H^+ (*25*).

The amino acid sequence of LacY suggests that it contains 12 to 14 membrane-spanning α helices. With the plasma membrane proteins of *E. coli*, such predictions can be tested easily and rigorously by creating fusion proteins composed of NH_2-terminal fragments of the membrane protein and *E. coli* alkaline phosphatase from which the signal sequence has been removed. Because alkaline phosphatase folds properly only when the COOH-terminal end of the truncated membrane protein is located within the external (periplasmic) loop and therefore only when the alkaline phosphatase is transported to the periplasm, the folding pattern of integral membrane proteins can be determined by measuring the phosphatase activity in the fusion proteins (*26*). Using this method, LacY was confirmed to contain 12 transmembrane α helices (*27*). Cutting the *lacY* gene into equal halves or even unequal parts and expressing the two parts independently still produced the spontaneous association of the two parts and resulted in active transport (*28*).

Extensive site-directed mutagenesis studies established that many of the residues can be altered without abolishing the transport function. Nevertheless, Arg^{302}, His^{322}, and Glu^{325}, which are all likely to be located very close to each other in helices 9 and 10, were identified as important residues (*29*). Converting Arg^{302} into lysine, or His^{322} into arginine, for example, largely abolished the uphill transport of lactose, which should be coupled to translocation of H^+, while still allowing the downhill flux of the sugar. Because these three residues can take up and release protons, it was suggested that they may act as H^+-translocating network (*29*).

Other results suggested however that the situation may be more complex. First, even when His^{322} was converted into an amino acid residue without a dissociable H^+, such as phenylalanine, the mutant LacY protein still catalyzed the nearly stoichiometric cotransport of H^+ with lactose (*30*). Second, some secondary transporters with the 6 + 6 helix topology use alkali metal cations rather than protons for symport. For example, the melibiose transporter of *E. coli* can use H^+, Li^+, or Na^+ as the cotransported ion, and ion specificity is dependent on which sugar is being transported (*31*). Such versatility would be difficult to explain by the H^+-translocation mechanism.

Attempts to identify substrate-binding residues within the LacY protein were carried out in several laboratories (*32*). Mutants with altered substrate specificity were isolated that transported maltose or sucrose more efficiently, or certain other sugars less efficiently, than the wild-type LacY protein. In these mutants, alterations frequently occurred in Ala^{177}, Tyr^{236}, Ile^{303}, and His^{322}. Although these residues may well be involved in sugar substrate binding, this may not necessarily be the complete explanation because there are often overlapping effects on the transport of sugar and H^+. For example, His^{322} is though to play a central role also in H^+ translocation as described above. On the other hand, the conversion of Ala^{177} into valine, which allows the more efficient transport of maltose and sucrose (*32*), allows protons to leak through LacY protein (*33*). Possibly, in a simple monomeric transporter such as LacY, the sugar and H^+ bind to overlapping sites, and there is no need to propose an independent pathway for H^+ translocation, an idea that easily fits into the scheme of Mitchell (Fig. 3B) (*14*). It has been argued also that in a cotransport system, thermodynamic constraints make it impossible to change the binding behavior of one substrate without altering the behavior of the cosubstrate (*33*). These observations suggest an explanation for the difficulty involved in the functional dissection of domains in a simple transporter such as LacY. Nevertheless, the fact that a single amino acid change can convert LacY into a uniporter facilitator channel favors the idea that these active transporters are simply facilitators that have been modified so that their function [movement of the barrier or of the binding site, according to Mitchell (*14*)] requires the binding of a second ligand (Fig. 3B).

PTS. The PTS appears rather complex in its composition (*34*). The phosphotransfer reaction begins with the phosphorylation of Enzyme I by PEP (Fig. 2C). Enzyme I then transfers its phosphate to a small protein, HPr. The same Enzyme I and HPr are used for the translocation of many sugars, and thus they are the sugar-independent, "general" proteins. Further steps involve sugar-specific proteins (Enzymes II) that are associated with the cytoplasmic membrane. The domain structures in these proteins became clear when the corresponding genes were sequenced. These genes and proteins are the products of extensive domain shuffling (Fig. 4) (*35*). Thus an Enzyme II for a given sugar usually contains three domains, two hydrophilic domains (IIA and IIB) that become phosphorylated successively and a membrane-spanning or channel domain (IIC). The order of these domains is interchanged in various Enzyme II molecules, and domains IIA and IIB may become dissociated from the membrane-associated domains and exist as independent soluble proteins. Even in an Enzyme II molecule that normally contains all three domains in one subunit, such as the mannitol-specific Enzyme II of *E. coli*, the domains can be expressed separately, and they still catalyze the phosphorylation and translocation of the substrate (*36*). In spite of this extensive shuffling, sequence

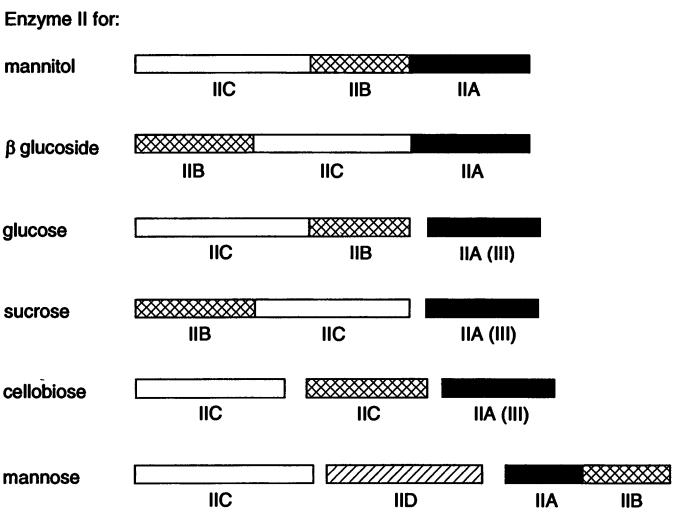

Fig. 4. The domain organization in PTS Enzymes II of *E. coli*. Adjacent rectangles denote individual proteins with the NH_2-terminus at left. The function of the IID domain, present in the mannose-specific Enzyme II, is not known. [Adapted from (*35*)]

similarity exists between corresponding domains of various Enzyme II molecules that transport different sugars (*37*).

The amino acid sequences of the IIC domains suggest that these proteins contain six to eight transmembrane helices. Use of the alkaline phosphatase fusion technique indicated that the IIC domain of the *E. coli* Enzyme II for mannitol contains six membrane-spanning α helices (*38*). Mannitol-specific Enzyme II is known to exist as a tightly associated dimer (*39*), and thus the transmembrane domains of the dimer form the recurring 6 + 6 helix structure.

The separation of an Enzyme II into clearly demarcated phosphorylation domains (IIA and IIB) and a translocation domain (IIC) suggests that the coupling between phosphorylation and translocation of the substrate might not be direct. In fact, it was recently shown that a major effect of the phosphorylation of the IIB domain in the mannitol-specific Enzyme II was to accelerate the diffusion of mannitol, presumably through the IIC domain, by two to three orders of magnitude (*40*). Converting the cysteine residue at the phosphorylation site of IIB into serine produces Enzyme II molecules that are capable of catalyzing facilitated diffusion of sugar in the absence of phosphorylation (*41*). Such uncoupled mutants have also been isolated by starting from a strain deleted for the general components (Enzyme I and HPr) and by selecting for cells able to grow in glucose media (*42*). These mutants, which catalyze rapid facilitated diffusion of glucose via glucose-specific Enzyme IIC, are altered in regions that were originally predicted to be the hydrophilic loops within the IIC domain (*43*) (but see below). Mutants showing the converse phenotype of impaired solute diffusion and nearly normal rates of phosphorylation of sugars in the cytoplasm have also been isolated and map to the IIC domains of mannitol-specific and glucose-specific Enzymes II, again in regions predicted to form loops (*44*). The precise locations of the mutations described in the glucose-specific Enzyme IIC have, however, become less certain because a recent study suggests that the domain spans the membrane eight times rather than six times, as believed earlier (*45*).

More recently, the function of the free-standing IIC domain of the mannitol-specific Enzyme II was studied by deleting the portion of the gene code for IIA and IIB domains (*46*). The IIC domain binds mannitol with high affinity but cannot transport it efficiently. The IIC domain can catalyze the facilitated diffusion of low affinity substrates, such as D-arabinitol, however, a result suggesting that it is indeed a sugar-specific channel. When mutants catalyzing the rapid influx of mannitol were isolated, their IIC domains had a lower affinity for mannitol, and the mutations were located in the same region originally predicted to form loops. These data suggest that some of the segments predicted as loops may actually fold back into the channel and influence the diffusion process in a critical manner. We have already encountered this theme when we considered the porin channels.

The results presented above can be explained most simply by assuming that IIC is a channel catalyzing the facilitated diffusion of the substrates, which upon arriving at the cytoplasmic side of the channel have a high probability of becoming phosphorylated by the IIB domain. Phosphorylation of the IIB domain also increases the diffusion rates of high-affinity substrates (*40*), presumably by lowering their binding affinity to the channel (*46*). Thus there is no obligatory coupling between phosphorylation and solute translocation, and at present there is no need to assume that the phosphorylation-dephosphorylation cycle of Enzyme II is necessary for solute translocation. However, efficient sugar transport in intact cells of the wild type requires the intact Enzyme II complex (*47*).

Three principles emerge from the study of PTS. (i) The system is composed of domains each with clearly defined functions, and the transporter domain is physically separate from the components responsible for phosphoryl transfer. (ii) The transporter is basically a sugar-specific channel, likely to contain 6 + 6 transmembrane helix structure, which in principle is capable of catalyzing facilitated diffusion of its substrates. (iii) The coupling between solute translocation and solute phosphorylation is loose and not obligatory.

Periplasmic binding protein (BP)–dependent systems. Gram-negative bacteria transport sugars, amino acids, and ions by binding these substrates first to periplasmic binding proteins. These transport systems have high affinity for their substrates; the Michaelis constant (K_m) for transport is usually 1 μM or less, in contrast to the solute:H^+ symporters described above, which often show K_m values of 100 μM or greater. The ability to transport substrates with high affinity may be the most important physiological advantage of the BP-dependent systems. Interestingly, BP-dependent systems have also been discovered in Gram-positive bacteria, in which the BP is apparently anchored to the cytoplasmic membrane by lipids covalently linked to its NH_2-terminus (*48*).

In addition to the soluble BP, these systems contain membrane-associated transporter complexes that are composed of two hydrophobic proteins which presumably form the diffusion channel and two hydrophilic subunits which contain sequences characteristic of a nucleotide-binding fold (Fig. 2D). The latter subunits bind ATP and ATP analogues, and function as ATPases. There is strong sequence conservation among the ATPase components, and homologues were found in a large superfamily of transporters. This family, called "ABC (ATP-binding cassette) systems" or "traffic ATPases," also contains transporters of yeast and mammals, and some members are involved in the outward translocation of substrates (*49*). This group includes the human MDR protein, which is involved in the extrusion of anticancer drugs from the cytoplasm of cancer cells; the human cystic fibrosis protein, CFTR; the a-factor export protein of yeast; and Hly complex of *E. coli*, which is involved in the secretion of hemolysin into the medium. In a manner similar to Enzymes II of PTS, there is extensive shuffling of components and domains. In the best studied of the BP-dependent transport systems, the maltose system of *E. coli* (*50*) and the histidine

system of *Salmonella typhimurium* (*51*), all four parts of the membrane-associated complex are made as separate subunits, and the two nucleotide-binding subunits are identical (Fig. 2D). In other systems, the two transmembrane proteins have been fused into a single subunit, or the two ATPase subunits have diverged from each other or have been fused into a single subunit. In extreme cases, such as the MDR protein, all of the components have been joined into a single large protein. Recently, the transmembrane components of many of the BP-dependent systems of bacteria were found to be related to each other and to form four homology clusters (*52*).

Recent progress in the study of BP-dependent systems was ushered in by the demonstration that solute transport occurred in membrane vesicles and reconstituted proteoliposomes, an approach that led to the unequivocal conclusion that ATP, rather than several other potential sources of energy suggested by earlier work with intact cells, is hydrolyzed to energize the solute accumulation process (*50, 51*). Although earlier hypotheses assumed that the input of energy was directly coupled to the solute translocation process, recent evidence points to a somewhat less direct coupling between these processes. In the maltose system, the isolation of mutants able to transport maltose in the total absence of the BP (*53*) was crucial. These strains are altered in the presumed channel subunits, MalF or MalG, and still transport maltose specifically, with a thousandfold greater K_m than the parent strain that contains the BP. When the transporter complex from the parent strain was reconstituted into proteoliposomes with octylglucoside dilution, it hydrolyzed ATP at a high rate only when the BP and maltose were present, as expected (*54*). However, the complex from the BP-independent mutants hydrolyzed ATP constitutively, in the absence of BP and maltose (*40*). These data suggest that (i) solute translocation is not always required for ATP hydrolysis, and that there is no obligate, mechanistic coupling between these processes even though ATP hydrolysis is required for solute translocation (see below); (ii) in the wild type, a major function of the BP is to send a transmembrane signal by binding to the outer surface of the transmembrane subunits and to induce conformational changes in them so that the ATPase subunits, located on the other side of the membrane, will hydrolyze ATP only when the transport substrate is available outside; and (iii) the mutants transport maltose in the absence of BP because the genetically altered transmembrane subunits constantly activate the ATPase and produce cyclic changes of channel structure, leading to solute translocation. A similar role has been proposed for the histidine BP (*55*).

Most of the membrane-spanning subunits of the BP-dependent systems appear to cross the membrane six times, on the basis of the hydrophobicity of the consecutive segments of the amino acid sequence (*49, 56*). Thus the channel portion of these systems appears to form the canonical 6 + 6 helical structure. The channels of the BP-dependent systems, unlike those of PTS Enzymes II, probably cannot catalyze facilitated diffusion because uphill transport demands that solute translocation is coupled to the cyclic conformation changes of the channel [movement of the barrier, according to Mitchell's proposal (*14*)], produced by ATP hydrolysis (Fig. 3C). Indeed, no mutation has been isolated that allows facilitated diffusion of solutes in the absence of the ATPase subunits.

Study of the BP-dependent systems thus suggests principles similar to those we have already encountered with PTS. (i) The system is composed of domains or subunits with separate functions, and the translocation domains are clearly separate from the energy input domains (*57*). (ii) The transporter domain is probably a specific, gated channel which usually has 6 + 6 transmembrane helices. (iii) At least one aspects of the coupling between ATP hydrolysis and solute translocation are indirect.

Perspectives

A decade ago, bacterial transporters appeared to contain many systems of confusingly diverse origins, architectures, and mechanisms. Now we can classify them into several families and propose evolutionary relations within each family. One important conclusion deduced from the comparison of many systems is that the energy coupling mechanism often appears as a secondary, peripheral feature that apparently has been added to a common translocation unit during the evolution of various transport systems. Indeed, the energy coupling components (such as ATPase subunits of BP-dependent systems or the Enzymes IIA and IIB of PTS) show homology to enzymes not involved in transport (*58*), and this confirms the mosaic nature of many of the contemporary transport systems. Although much progress has been made in understanding the structural and functional aspects of energy coupling, the mechanism of solute translocation across the membrane has remained largely hypothetical (Fig. 3). However, the translocator domains of different transporter families often share the same 6 + 6 helix folding pattern, and sequence similarity has sometimes been noted between translocators belonging to different families, for example between the fructose-specific Enzyme IIC of *E. coli* and the human insulin–responsive glucose facilitator, a member of the 6 + 6 helix sugar symporter-facilitator family (*58*). These observations suggest that many translocators are related and provide hope that we may understand the mechanism of translocation in many systems once we obtain clues regarding the mechanism for just one or a few of these systems.

REFERENCES AND NOTES

1. M. Vaara and H. Nikaido, *Microbiol. Rev.* **49**, 1 (1985).
2. The use of small ionophores, such as valinomycin and gramicidin, as models of transporters led to the classical division of transporters into carriers and channels. Although it was clearly recognized more than 10 years ago [D. B. Wilson, *Annu. Rev. Biochem.* **47**, 933 (1978)] that all translocator proteins must be built as channels, some translocators are still called carriers because they show kinetic features similar to small ionophore carriers. This is a misleading nomenclature, however, because kinetic properties of a transporter can be altered easily [T. Dierks, A. Salentin, R. Kramer, *Biochim. Biophys. Acta,* **1028**, 218 (1990)]. Many workers also draw qualitative distinctions between channels, exemplified by the classical ion channels of nerve cells, and transporters, exemplified perhaps by the lactose permease of *E. coli*. Although there may be transporters operating along the principles quite different from that of the classical channel, we believe that the transporters discussed in this article can be viewed most simply as channels with zero or one binding site per solute. The often cited, quantitative difference between the throughputs of channels and transporters does not prove, in our opinion, that they operate in qualitatively different ways because (i) the generally slower flux through classical transporters may simply reflect the need for conformational change of the transporter protein and (ii) the flux through the classical channel is dependent on the magnitude of the driving force for diffusion, a factor that is not taken into consideration by many workers.
3. K. Postle, *Mol. Microbiol.* 4, 2019 (1990); R. J. Kadner, *ibid.*, p. 2027.
4. For earlier literature on porins see (*1*). More recent data have been reviewed in H. Nikaido, *Mol. Microbiol.* **6**, 435 (1992).
5. M. S. Weiss *et al.*, *Science* **254**, 1627 (1991); E. Schiltz, A. Kreusch, U. Nestel, G. E. Schulz, *Eur. J. Biochem.* **199**, 587 (1991).
6. C. Miller, *Science* **252**, 1092 (1991).
7. R. M. Garavito and J. P. Rosenbusch, *J. Cell Biol.* **86**, 127 (1980).
8. S. W. Cowan *et al.*, *Nature,* **358**, 727 (1992).
9. K. Bauer, M. Struyve, D. Bosch, R. Benz, J. Tommassen, *J. Biol. Chem.* **264**, 16393 (1989).
10. E. Sugawara and H. Nikaido, *ibid.* **267**, 2507 (1992); H. Nikaido, K. Nikaido, S. Harayama, *ibid.* **266**, 770 (1991).
11. K. Sen and H. Nikaido, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 743 (1990); *J. Bacteriol.* **173**, 926 (1991).
12. E. Blachly-Dyson, S. Peng, M. Colombini, M. Forte, *Science* **247**, 1233 (1990).
13. M. Luckey and H. Nikaido, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 167 (1980); *Biochem. Biophys. Res. Commun.* **93**, 166 (1980); R. Benz, A. Schmidt, G. H Vos-Scheperkeuter, *J. Membr. Biol.* **100**, 21 (1987).
14. P. Mitchell, in *Comprehensive Biochemistry*, M. Florkin and E. H. Stotz, Eds. (Elsevier, Amsterdam, 1967), vol. 22, p. 167; *Res. Microbiol.* **141**, 286 (1990).
15. S. Freundlieb, U. Ehmann, W. Boos, *J. Biol. Chem.* **263**, 314 (1988); J. Trias, J. Dufresne, R. C. Levesque, H. Nikaido, *Antimicrob. Agents Chemother.* **33**, 1201 (1989).

16. J. M. Rutz *et al.*, *Science*, in press.
17. K. B. Heller, E. C. C. Lin, T. H. Wilson, *J. Bacteriol.* **144**, 274 (1980).
18. G. M. Pao *et al.*, *Mol. Microbiol.* **5**, 33 (1991).
19. H. Nikaido and E. Y. Rosenberg, *J. Membr. Biol.* **85**, 87 (1985); T. Aertz, J. Z. Xia, H. Slegers, J. de Block, J. Cauwaert, *J. Biol. Chem.* **265**, 8675 (1990).
20. M. C. J. Maiden, E. O. Davis, S. A. Baldwin, D. C. M. Moore, P. J. F. Henderson, *Nature* **325**, 641 (1987); P. J. F. Henderson, *Res. Microbiol.* **141**, 316 (1990).
21. M. H. Saier, Jr., *Res. Microbiol.* **141**, 281 (1990); P. C. Maloney, *ibid.*, p. 374.
22. M. D. Marger and M. H. Saier, Jr., *Trends Biochem. Sci.* in press.
23. M. J. Newman and T. H. Wilson, *J. Biol. Chem.* **225**, 10583 (1980); M. J. Newman, D. L. Foster, T. H. Wilson, H. R. Kaback, *ibid.* **256**, 11804 (1981).
24. E. Racker, B. Violand, S. O'Neal, M. Alfonzo, J. Telford, *Arch. Biochem. Biophys.* **198**, 470 (1979); this method became a widely applicable method for reconstitution of transporters. For instance, an improved version was used for the reconstitution of sugar phosphate:phosphate antiporter of *E. coli* [S. V. Ambudkar, T. J. Larsen, P. C. Maloney, *J. Biol. Chem.* **261**, 9083 (1986)]. See below concerning the periplasmic binding protein–dependent systems.
25. For earlier literature on LacY permease, see H. R. Kaback, *Ann. N.Y. Acad. Sci.* **456**, 291 (1986).
26. D. Boyd, C. Manoil, J. Beckwith, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8525 (1987); D. Boyd and J. Beckwith, *ibid.* **86**, 9446 (1989).
27. J. Calamia and C. Manoil, *ibid.* **87**, 4937 (1990).
28. E. Bibi and H. R. Kaback, *ibid.*, p. 4325; W. Wrubel, U. Stochaj, U. Sonnewald, U. Theres, R. Ehring, *J. Bacteriol.* **172**, 5374 (1990).
29. P. D. Roepe, T. G. Consler, M. E. Menezes, H. R. Kaback, *Res. Microbiol.* **141**, 290 (1990).
30. S. C. King and T. H. Wilson, *J. Biol. Chem.* **264**, 7390 (1989); R. J. Brooker, *ibid.* **265**, 4155 (1990); P. J. Franco and R. J. Brooker, *ibid.* **266**, 6693 (1991).
31. M. C. Botfield, D. M. Wilson, T. H. Wilson, *Res. Microbiol.* **141**, 328 (1990).
32. R. J. Brooker, *ibid.*, p. 309; S. C. King and T. H. Wilson, *J. Biol. Chem.* **265**, 3153; *ibid.*, p. 9638.
33. S. C. King and T. H. Wilson, *ibid.*, p. 9645; R. J. Brooker, *ibid.* **266**, 4131 (1991).
34. N. D. Meadow, D. K. Fox, S. Roseman, *Annu. Rev. Biochem.* **59**, 497 (1990).
35. M. H. Saier, Jr., and J. Reizer, *J. Bacteriol.* **174**, 1433 (1992).
36. D. W. White and G. R. Jacobson, *ibid.* **172**, 1509 (1990); R. P. van Weeghel *et al.*, *Biochemistry* **30**, 9478 (1991).
37. J. Lengeler, R. Titgemeyer, A. P. Vogler, B. M. Wohrl, *Philos. Trans. R. Soc. London B Biol. Sci.* **326**, 489 (1990); A. Reizer, G. M. Pao, M. H. Saier, Jr., *J. Mol. Evol.* **33**, 179 (1991).
38. J. E. Sugiyama, S. Mahmoodian, G. R. Jacobson, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9603 (1991).
39. F. F. Roossien and G. T. Robillard, *Biochemistry* **23**, 5682 (1984); M. M. Stephen and G. P. Jacobson, *ibid.* **25**, 4046 (1986).
40. J. S. Lolkema, D. S. Dijkstra, R. H. ten Hoeve-Duurkens, G. T. Robbilard, *ibid.* **30**, 6716 (1991).
41. _____, *ibid.*, p. 6721; C. Nuoffer, B. Zanolari, B. Erni, *J. Biol. Chem.* **263**, 6647 (1988); K. Schnetz, S. L. Sutrina, M. H. Saier, Jr., B. Rak, *ibid.* **265**, 13464 (1990).
42. P. W. Postma, *J. Bacteriol.* **147**, 382 (1981).
43. G. J. G. Ruijter *et al.*, *ibid.* **174**, 2843 (1992).
44. R. Manyan *et al.*, *ibid.* **170**, 1290 (1988); A. Buhr, G. A. Daniels, B. Erni, *J. Biol. Chem.* **267**, 3847 (1992).
45. A. Buhr and B. Erni, personal communication.
46. S. Klawitter, J. W. Lengeler, A. Scholle, personal communication.
47. B. Erni, *Biochemistry* **25**, 305 (1986); F. C. Grenier, E. B. Waygood, M. H. Saier, Jr., *J. Cell. Biochem.* **31**, 97 (1986).
48. G. Alloing, M.-C. Trombe, J.-P. Claverys, *Mol. Microbiol.* **4**, 633 (1990); R. R. B. Russell, J. Aduse-Opoku, I. C. Sutcliffe, L. Tao, J. J. Ferretti, *J. Biol. Chem.* **267**, 4631 (1992).
49. C. F. Higgins *et al.*, *J. Bioenerg. Biomembr.* **22**, 571 (1990); M. A. Blight and I. B. Holland, *Mol. Microbiol.* **4**, 873 (1990).
50. D. A. Dean, A. L. Davidson, H. Nikaido, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9134 (1989); A. L. Davidson and H. Nikaido, *J. Biol. Chem.* **265**, 4254 (1990); *ibid.* **266**, 8947 (1991).
51. L. Bishop *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6953 (1989); C. S. Mimura, A. Admon, K. A. Hurt, G. F.-L. Ames, *J. Biol. Chem.* **265**, 19535 (1990).
52. E. Dassa and W. Saurin, personal communication.
53. N. A. Treptow and H. A. Shuman, *J. Bacteriol.* **163**, 654 (1985); *J. Mol. Biol.* **202**, 809 (1988).
54. A. L. Davidson, H. A. Shuman, H. Nikaido, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2360 (1992).
55. V. Petronilli and G. F.-L. Ames, *J. Biol. Chem.* **266**, 16293 (1991).
56. The folding pattern of the MalF protein was studied by the alkaline phosphatase fusion method (*26*) and contains eight transmembrane helices. However, the extreme NH_2-terminal portion of this protein, containing two transmembrane helices, has no homology with other channel proteins of the superfamily and can thus be considered as a special add-on feature of this system.
57. It has not yet been shown experimentally, however, that only the hydrophobic subunits contribute to the formation of the channel.
58. J. Reizer, A. Reizer, M. H. Saier, Jr., G. R. Jacobson, *Protein Sci.* **1**, 722 (1992); L.-F. Wu and M. H. Saier, Jr., *Mol. Microbiol.* **4**, 1219 (1990).
59. We regret that many important studies could not be cited because of the space limitation. H.N. is grateful to A. L. Davidson for insightful criticisms. Studies in our laboratories were supported by U.S. Public Health Service grants AI-9044 (H.N.) and AI-21702 and AI-14176 (M.H.S.).

The Nuclear Membrane

Colin Dingwall and Ronald Laskey

The nuclear membrane forms a major barrier within the cell, permitting levels of regulation not found in prokaryotes. The dynamics and diverse functions of the nuclear membrane and its associated structures are considered in this review. The role of the nuclear pore complex in selective transport across the nuclear membrane has been studied to a considerable degree; however, many crucial questions remain. Components of a signal transduction mechanism are associated with the nucleus, suggesting that nuclear functions may be influenced directly by this system. The involvement of the heat shock cognate protein Hsc70 in nuclear protein import is discussed, and a specific signal-presentation role for this protein is proposed.

The nuclear membrane is the hallmark of eukaryotic cells and a major landmark in evolution. As the most conspicuous boundary inside eukaryotic cells, the nuclear membrane functions to separate the genome from the cytoplasm. This separation permits types of regulation that are not found in prokaryotic cells. In this review, we consider the complex architecture of the nuclear membrane and its associated structures, which are collectively called the nuclear envelope, and we consider the mechanisms by which the nuclear envelope mediates and regulates selective traffic of molecules between the nucleus and cytoplasm. Because many features of the mechanism of nucleocytoplasmic exchange have been reviewed extensively (*1*–*12*), we have chosen to emphasize aspects that have been reviewed less thoroughly. In addition, we propose a specific role for Hsc70, a member of the heat shock class of proteins, in the presentation of the nuclear localization sequence.

Membrane Structure and Dynamics

The nuclear membrane actually consists of two concentric lipid bilayers; the outer lipid bilayer is continuous with the endoplasmic reticulum (ER). Both layers of membrane are perforated by nuclear pore complexes that serve as channels for molecular exchanges between the nucleus and cytoplasm. Nuclear pore complexes also appear to function as rivets that hold the inner and outer layers of membrane together.

The inner nuclear membrane is lined by the nuclear lamina, a layer that is composed of A and B type lamins, a specialized type of intermediate filament protein (*1*, *13*, *14*). The structure of the nuclear lamina varies in different organisms. In *Xenopus* oocytes, the lamina is a dense orthogonal meshwork of fibers (*15*). In contrast, the lamina of interphase cells in *Drosophila* is more disperse, such that areas of the inner nuclear membrane may be exposed directly to the nuclear interior and only a fraction of the chromatin is in contact with the lamins (*16*).

The nuclear envelope disassembles at the onset of mitosis and is reassembled at the end of mitosis. A number of experimental systems have been developed to investigate the mechanisms and to identify the components of these processes. The overall mechanism of assembly involves the attachment of vesicles to the chromatin followed by the fusion of vesicles to produce the

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