Journal of Bioenergetics and Biomembranes, Vol. 20, No. 1, 1988

MINI-REVIEW

Structure and Mechanism of Bacterial Periplasmic Transport Systems

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Received March 26, 1987

Abstract

Bacterial periplasmic transport systems are complex, multicomponent permeases, present in Gram-negative bacteria. Many such permeases have been analyzed to various levels of detail. A generalized picture has emerged indicating that their overall structure consists of four proteins, one of which is a soluble periplasmic protein that binds the substrate and the other three are membrane bound. The liganded periplasmic protein interacts with the membrane components, which presumably form a complex, and which by a series of conformational changes allow the formation of an entry pathway for the substrate. The two extreme alternatives for such pathway involve either the formation of a nonspecific hydrophilic pore or the development of a ligand-binding site(s) on the membrane-bound complex. One of the membrane-bound components from each system constitutes a family of highly homologous proteins containing sequence domains characteristic of nucleotide-binding sites. Indeed, in several cases, they have been shown to bind ATP, which is thus postulated to be involved in the energy-coupling mechanism. Interestingly, eukaryotic proteins homologous to this family of proteins have been identified (mammalian *mdr* genes and Drosophila *white* locus), thus indicating that they perform a universal function, presumably related to energy coupling in membrane-related processes. The mechanism of energy coupling in periplasmic permeases is discussed.

Key Words: Periplasm; periplasmic permeases; membranes; binding proteins; energy coupling.

Introduction

Gram-negative bacteria have a complex cell surface, consisting of three layers: an outer membrane, the cell wall proper or peptidoglycan, and a

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0145-479X/88/0200-0001$06.00/0

cytoplasmic membrane (Nikaido and Vaara, 1985). Nutrients, therefore, have to pass through this rather formidable architectural and protective structure. Small solutes cross the outer membrane by way of proteinaceous channels, which can be substrate-nonspecific (the proteins in this case are named *porins*) or substrate-specific (Nikaido and Vaara, 1985). The cell wall proper is commonly regarded as a widely open, entirely permeable layer, which confers rigidity and through which nutrients diffuse readily. The cytoplasmic membrane, on the other hand, is impermeable to almost every solute unless a special transport system is provided. These systems can be categorized according to their response to a physical treatment, osmotic shock (Neu and Heppel, 1965), into shock-sensitive and shock-resistant permeases. During osmotic shock a special class of proteins is released into the medium, the *periplasmic proteins*, so called because they are thought to reside in a special cell compartment, the *periplasm*, located between the inner and the outer membranes. Shock-sensitive permeases, also referred to as *periplasmic permeases*, then are those systems that are inactivated during osmotic shock because of the loss of an essential protein component which is referred to as the periplasmic component. In all cases studied, the periplasmic component is a protein that binds the transported solute with high affinity. Numerous permeases have been shown to belong to this class and several have been extensively characterized (Ames, 1986a; Furlong, 1987). Shock-resistant permeases, on the other hand, are those that retain all of their activity [and therefore, their component(s)] upon osmotic shock. This class of permeases is thought to be composed of a single protein that is tightly membrane-bound. A classic representative of this class is the β-galactoside permease which has been intensively studied (Kaback, 1983; Overath and Wright, 1983).

An additional characteristic subsequently utilized to distinguish between these two classes of permeases has been the nature of the mechanism of energy coupling. Shock-resistant permeases are powered by the protonmotive force (Kaback, 1983; Overath and Wright, 1983), while energy coupling in shock-sensitive permeases has been postulated to be dependent on substrate-level phosphorylation energy (Berger, 1973; Berger and Heppel, 1974); however, this mechanism is presently controversial, as will be discussed later.

It is important to realize that all transport assays involve whole cells or, at best, membrane vesicles. Thus, in the past, the use of such complex assay systems did not achieve an understanding of the composition of the individual transport systems. It was only when careful genetic analysis was introduced into this field that it became possible to unravel the complexities of these permeases. With the availability in recent years of recombinant DNA technology, a number of periplasmic permeases have been analyzed in great detail and a general picture is starting to emerge. At the moment, the most

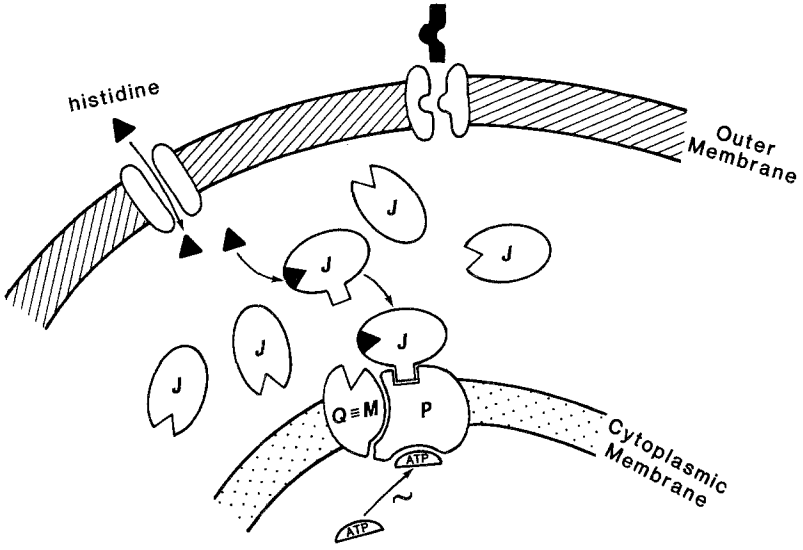

Fig. 1. Schematic representation of the histidine periplasmic permease. The three membrane-bound proteins are represented as forming a complex within the cytoplasmic membrane. The periplasmic binding protein is shown in two different conformations, with and without bound substrate (▲) respectively. The liganded binding protein and the membrane-bound complex are represented as interacting directly. The histidine molecule can penetrate the outer membrane through nonspecific hydrophilic pores. The squiggle (~) suggests an involvement of ATP in energy coupling in an unknown way. The J protein is shown as interacting with the P protein on the basis of genetic evidence. Possibly, the binding protein interacts with all three of the membrane-bound components. See text for detail.

thoroughly characterized systems are those for histidine, maltose, branched-chain amino acids, oligopeptides, β-methyl galactoside, ribose, arabinose, phosphate, and glutamine. Several others are in various stages of development.

Accumulated evidence shows that periplasmic permeases are typically composed of one periplasmic substrate-binding protein and three membrane-bound components. This has been found to be true for seven permeases: histidine (Higgins *et al.*, 1982), maltose (Hengge and Boos, 1983), branched-chain amino acids (Landick *et al.*, 1985), oligopeptides (Hiles and Higgins, 1987), ribose (Bell *et al.*, 1986), β-methylgalactoside (Harayama *et al.*, 1983; Robbins *et al.*, 1976; Muller *et al.*, 1985), and phosphate (Surin *et al.*, 1985). This organization is schematically represented in Fig. 1 for the histidine permease. The outer membrane is represented as containing pores which allow entrance of the substrate into the periplasm. The membrane-bound components of periplasmic permeases are represented as forming a complex within the cytoplasmic membrane. As will be discussed later, indirect evidence suggests an interaction between the periplasmic binding protein and

the membrane-bound proteins. The genetic structure of the most extensively characterized permeases is very similar (Ames, 1986a). In all cases a single operon contains all the genes coding for the known transport components, except for the maltose permease which is composed of two divergent operons. In at least two cases (histidine and the branched-chain amino acids) there is an additional, separately regulated gene coding for a second periplasmic binding protein of different specificity (*argT* and *livJ*, respectively), which will be discussed later. In some cases the region contains additional genes whose function is either unknown at present (e.g., *livL* in the branched-chain amino acid permease, Landick *et al.*, 1985) or is involved in further catabolism of the transported substrates (e.g., *rbsK* codes for a ribose kinase, Hope *et al.*, 1985).

It seems, then, that complexity is the norm for this particular mode of transport. These systems typically concentrate substrates inside the cell against a very large gradient (e.g., 10^5-fold in the case of maltose (Szmelcman *et al.*, 1976): perhaps achieving and maintaining such large concentration gradients requires a complex mechanism, possibly in relation to energy coupling. Alternatively, the high efficiency of transport which these systems usually display requires a complex structure. They are in fact able to scavenge solutes off very low concentrations: the apparent K_m's for uptake range from 0.01 to 1 μM (Ames and Lever, 1970; Szmelcman *et al.*, 1976; Rosenberg *et al.*, 1977). By comparison, the apparent K_m for the transport of lactose through the shock-resistant, monocomponent, β-galactoside permease (*lacY*) is 190 μM (Winkler and Wilson, 1966). The very high efficiency of these permeases may be a necessity for the cells, at least where amino acid transport is concerned, since biosynthetically produced amino acids can leak out of the cell and these high-affinity permeases recapture and concentrate the lost amino acid (Ames, 1972). This recapture may constitute an important evolutionary advantage, thus justifying the existence of complex, multicomponent permeases, since amino acid biosynthesis is expensive: e.g., it has been calculated that 41 ATP molecules are being sacrificed for each histidine molecule made (Brenner and Ames, 1971).

An interesting aspect of some periplasmic systems is the fact that the membrane-bound components are multifunctional, i.e., they are needed for transport of additional substrates and for that purpose are utilized by more than one binding protein. For example, in the case of the histidine permease, the membrane-bound proteins are also essential for transport of arginine under conditions of nitrogen starvation, via the lysine-arginine-ornithine-binding protein (LAO protein, coded for by the *argT* gene (Ames, 1986a; Kustu and Ames, 1973). This means that whatever mechanism is deduced from the available data, it will have to include the alternating interaction with and removal of each of the different periplasmic components from contact

with the same set of membrane-bound components. The existence of alternative periplasmic binding proteins utilizing the same set of membrane-bound components has been shown also for the branched-chain amino acid permease (Landick *et al.*, 1985; Landick and Oxender, 1985).

In some cases, large-size periplasmic permease substrates may be unable to cross the outer membrane: e.g., the maltose permease transports also higher polymers of glucose (maltodextrins), up to six or seven glucose residues long (Wandersman *et al.*, 1979). Permeation of these large molecules through the outer membrane occurs through a substrate-specific channel-forming protein, the product of the *lamB* gene (which also functions as receptor for phage λ) which is part of the maltose transport operon. Since permeability through the outer membrane usually seems to be unencumbered for substrates of periplasmic permeases and since the properties of the LamB protein have been reviewed recently (Nikaido and Vaara, 1985; Hengge and Boos, 1983; Shuman and Treptow, 1985), this subject will not be discussed further.

Characteristics of Individual Components

The Periplasmic Substrate-Binding Protein

The binding proteins are the most thoroughly analyzed of these transport components for obvious reasons. Their purification is easy (being soluble and easily assayable proteins), they can be obtained in large quantities, and most of them are remarkably stable. A summary of the properties they share as a group is as follows (reviewed in Furlong, 1987; see references for individual cases therein). They are monomeric proteins with molecular weights varying from about 25,000 to about 56,000; several are stable to heat; they have high affinity for their substrates; they undergo a conformational change upon binding of substrate; and they have two functionally and genetically separable active domains. The latter three properties, discussed below, are the most interesting with respect to the function of these proteins.

The binding affinity (K_D) is between 0.1 and 1 μM for sugar substrates and around 0.1 μM for amino acids. Presumably because of this high affinity, some of these proteins have been purified with tightly bound substrates (Miller *et al.*, 1980, 1983). The substrate, which can be easily removed by reversible denaturation with guanidine-HCl (Miller *et al.*, 1983), causes no problem for kinetic measurements if these are performed by equilibrium dialysis against a large volume of ligand rather than against a volume comparable to that occupied by the protein (Richarme and Kepes, 1974; Lever, 1972). A thorough analysis by stopped-flow rapid-mixing techniques

of the kinetics of ligand binding to the arabinose-, galactose- and maltose-binding proteins revealed that in all cases the variation in the affinity constants for several different substrates is due primarily to differences in the dissociation rate constants (which vary by 100-fold) while the association rate constants are similar for all substrates (Miller *et al.*, 1983).

Binding proteins undergo a conformational change upon binding of substrate, as has been measured in the case of the histidine-, maltose-, arabinose-, ribose-, galactose-, glutamine-, and leucine-isoleucine-valine-binding proteins by a variety of methods (see Ames, 1986a and Furlong, 1987 for references): fluorescence spectroscopy, nuclear magnetic resonance, scanning calorimetry, and immunology. The use of a distant reporter group method, which was applied to the histidine-, galactose-, and maltose-binding proteins, has the particular advantage that it can distinguish between movement of a residue right at the ligand-binding site from changes reflecting movement in regions of the molecule removed from the actual ligand-binding site. The demonstration that a substrate-induced conformational change occurs in all binding proteins analyzed indicates that this is an essential aspect of their mechanism of action.

It is commonly postulated that binding proteins interact with the membrane-bound components. However, *direct* evidence for this hypothesis is lacking. In the case of the histidine-binding protein J, such a hypothesis is based on four separate sets of data. The first consists of the characterization of a mutant J protein which cannot function in transport despite the fact that it has an intact histidine-binding site (Kustu and Ames, 1974). This suggests the existence of a region of the protein that is essential for transport but not necessary for binding histidine. By using the distant reporter group method (Zukin *et al.*, 1986) and by nuclear magnetic resonance investigations (Manuck and Ho, 1979) it was shown that this mutant J protein is unable to undergo a normal ligand-induced conformational change, thus adding further evidence that the specific conformational change is intimately involved in the functioning of binding proteins in transport. The second piece of evidence depends on an entirely genetic argument (Ames and Spudich, 1976): a *hisP* mutation has been characterized which suppresses the described mutation in the second domain of the J protein; this can be interpreted most easily, but not only, as a result of an interaction between different components of a structure, in which the J defect is corrected by a corresponding alteration in the interacting P protein. The possibility that the mutated P protein might be functioning altogether without the aid of the J protein was discarded by isolating a derivative strain which carried the mutated P protein but lacked entirely the J protein: this strain was unable to transport histidine. The third piece of evidence is deduced from comparing the sequence of the J protein with that of the closely related lysine-arginine-ornithine-binding

protein (LAO, the *argT* gene product): these two proteins have an overall homology of 70%, but two portions of the molecules are better than 90% homologous (Higgins and Ames, 1981). Since both the J and the LAO proteins require the Q, M, and P proteins for function and since the mutation in the P-interaction site of the J protein is located in one of these two highly homologous stretches, this suggests that the two proteins are involved in an identical function, presumably the interaction with a common membrane component (Higgins and Ames, 1981; Ames and Higgins, 1983), besides each of them having a specific substrate-binding site. The fourth piece of evidence comes from the preliminary characterization of *hisJ* mutants which interefere with the proper functioning of the membrane components, such as would be expected from a mutant binding protein that binds irreversibly to one of them (Ames Ferro-Luzzi, unpublished results). Genetic evidence was also obtained for an interaction occurring between the maltose-binding protein and the *malF* and *malG* gene products. Mutants are available in these genes which allow transport of maltose in the absence of the binding protein (see below); the introduction into these mutants of a wild-type *malE* gene completely inhibits maltose uptake (Treptow and Shuman, 1985). These data have been explained by postulating an interaction between the MalF and MalG proteins and the maltose-binding protein: presumably the function of the mutated membrane components is inhibited by the presence of wild-type binding protein because of a nonproductive and interfering interaction with the altered membrane components. Evidence that the maltose-binding protein has separate domains, for binding maltose and for interacting with the MalF and MalG proteins, has not yet been obtained. It has been suggested that the maltose-binding protein interacts also with the outer membrane protein, LamB (Bavoil and Nikaido, 1981). Indirect evidence for the existence of two sites was also obtained for the glutamine permease by chemically altering the glutamine-binding protein in such a way that its binding activity was unaffected while its ability to participate in transport was lost (Hunt and Hong, 1983b). Even though all of these data can be explained in terms of an interaction between the periplasmic and the membrane-bound components, this hypothesis will have to be supported finally by biochemical data.

A great deal of important structural information has been derived from the X-ray crystallographic studies of several binding proteins, mostly from Quiocho's laboratory. The most advanced structural analysis has been achieved on the arabinose-binding protein. This protein molecule is arranged in two globular domains (lobes) forming a cleft and connected by a flexible hinge: the overall shape resembles a kidney bean (Gilliland and Quiocho, 1981). The binding site for the substrate is located in the concave region between the two lobes. The molecule is flexible, the cleft closing down somewhat upon binding of substrate (Newcomer *et al.*, 1981; Mao *et al.*, 1982),

thus "trapping" the substrate deep within the protein (hence the name "Venus's flytrap" for this type of binding mechanism). These data agree nicely with the evidence that binding proteins in general undergo a conformational change upon binding of substrate. Since there is a direct relationship between higher affinity and lower rate of dissociation of a substrate from the *closed* form of the protein, it is likely that this closed, more stable, liganded form of the protein is "encouraged" to release the substrate by an external stimulus, presumably by interaction with the membrane-bound transport components (see below for model). Other binding proteins have been analyzed by X-ray crystallography, giving results that are entirely compatible with the above general picture (Saper and Quiocho, 1983; Vyas *et al.*, 1983; Mowbray and Petsko, 1983; Pflugrath and Quiocho, 1985). The formation of hydrogen bonds is involved in the protein–ligand interaction in the case of the glutamine-binding protein, in particular involving the amide bond of glutamine (Shen *et al.*, 1985); and in the case of the sulfate-binding protein of *S. typhimurium* the charged oxygen atoms of the sulfate molecule are stabilized by hydrogen bonds with the protein, rather than by salt-bridges (Pflugrath and Quiocho, 1985). There has been no evidence that binding proteins undergo covalent modification except for a report that an arginine-binding protein can be phosphorylated by ATP while transporting arginine (Celis, 1984). No evidence of phosphorylation of the histidine-binding protein was obtainable (Ames Ferro-Luzzi, unpublished results; Ames and Nikaido, 1981).

The Membrane-Bound Components

These are the hardest to study. They are often present in very small amounts, they need to be solubilized before they can be subjected to biochemical studies, and no activity assay is as yet available for any of them. Their number has been determined for several permeases by genetic or recombinant DNA techniques, and in all cases in which exhaustive data are available they are usually present in the number of three (Ames, 1986a). This uniformity indicates that something in the basic makeup of these permeases requires such architectural and functional composition. Two of the components are highly hydrophobic (Higgins *et al.*, 1982; Hiles and Higgins, 1987; Surin *et al.*, 1985; Hope *et al.*, 1985; Gilson *et al.*, 1982; Froshauer and Beckwith, 1984; Dassa and Hofnung, 1985). The third one has an amino acid sequence which is not recognizably hydrophobic (Bell *et al.*, 1986; Surin *et al.*, 1985; Gilson *et al.*, 1982; Higgins *et al.*, 1985), despite the fact that it is membrane-bound; however, the strength of the membrane attachment might vary from one system to the other. For example, the *MalK* protein is thought to be a peripheral membrane protein, while the *hisP* protein is more tightly

membrane-bound (Shuman and Silhavy, 1981; Ames and Nikaido, 1978). The HisP, HisQ and HisM proteins have been shown to be located in the cytoplasmic membrane (Ames and Nikaido, 1978; Jaskot and Ames Ferro-Luzzi, unpublished results). Similarly, the MalF and MalK proteins have been localized in the cytoplasmic membrane (Shuman *et al.*, 1980; Bavoil *et al.*, 1980).

It has recently become clear that each periplasmic permease contains a member of a family of homologous proteins. I will refer to each member of this family as the "conserved" component. These include *hisP*, *pstB*, *oppD*, *oppF*, *malK*, *araG*, *rbsA*, *glnQ*, and *chlD*. An alignment of most of the sequences of these conserved proteins is shown in Fig. 3 of a previous review (Ames, 1986a). Additional proteins not shown in that figure are *glnQ* and *chlD*, the sequences of which appeared recently (Nohno *et al.*, 1986; Johann and Hinton, 1987). The similarity among these proteins is extensive, including several long stretches of amino acid sequences: presumably these protein regions are involved in the performance of functions which are common to the entire family. In particular, two regions, named A and B, share homology with several proteins known to have ATP-binding sites such as the α and β-subunits of the proton-translocating ATPase, myosin, adenylate kinase, RecA protein, and others (Walker *et al.*, 1982; Higgins *et al.*, 1985). Recently it has been found that both HisP and MalK carry a nucleotide-binding site by obtaining covalent derivatives between these proteins and the photoaffinity label 8-azido-ATP (Hobson *et al.*, 1984). Competition of 8-azido-ATP labelling with a variety of nucleotide-containing compounds suggested that ATP and/or GTP are the substrates of the HisP protein (Hobson *et al.*, 1984). A fusion product between the *oppD* gene product and β-galactosidase was also shown to react with the ATP analog 5′-*p*-fluorosulfonylbenzoyladenosine (Higgins *et al.*, 1985). Since nucleotide binding has been demonstrated for several of these proteins, it is reasonable to generalize that all conserved membrane components bind a nucleotide and that such function is essential for all periplasmic systems, perhaps involving the energy-coupling mechanism (Higgins *et al.*, 1985; Hobson *et al.*, 1984). However, no evidence of ATP hydrolysis by those proteins has yet been obtained. Possibly the bound nucleotide does not undergo hydrolysis and serves an entirely regulatory function.

None of the other membrane-bound components from any other system has been purified, or shown to have a functional site. The HisQ and HisM proteins bear strong homology to each other, indicating that they originated by a gene duplication. A hydropathicity plot of HisQ and HisM indicates very similar patterns which, together with their sequence homology, suggested the possibility that these two proteins form a pseudodimer in the membrane (Ames, 1985). A clear homology is also evident between proteins PstC and

PstA (Ames Ferro-Luzzi and Doolittle, unpublished results). Homology has also been detected between MalF and MalG (Dassa and Hofnung, 1985). However, none was evident between OppB and OppC.

Genetic data obtained in the maltose and histidine systems have indicated that some of the membrane components might carry a substrate-binding site. The best evidence comes from mutants in *malF* and *malG* which are able to transport maltose in the absence of the maltose-binding protein (Treptow and Shuman, 1985; Shuman, 1982). The apparent affinity for maltose in these mutants is about 2000-fold poorer than in the wild type (in presence of maltose-binding protein). Other evidence is derived from the existence of *hisQ* and *hisM* mutants in which an altered *specificity* of transport can be best explained as an alteration in a substrate-binding site (Higgins *et al.*, 1983; Payne *et al.*, 1985).

Transport Models

From all the available information a model can be drawn representing the histidine permease, but the model fits the findings obtained with all other periplasmic permeases to date (Fig. 1). The substrate (histidine) crosses the outer membrane (through specific or unspecific channels) and encounters the binding protein (J), by which it is bound reversibly. The concentration of *free* substrate is the same inside and outside the periplasm, but the *total* concentration (bound plus free) is higher inside and dependent on the binding protein concentration (fully induced maltose-binding protein has been estimated to be 1 mM in the periplasm (Dietzel *et al.*, 1978)) and on its affinity for the substrate. The liganded binding protein undergoes a conformational change that allows its interaction with one of the membrane-bound components. This interaction triggers conformational changes in the membrane-bound apparatus (composed of proteins Q, M, and P), thus eliciting both the release of substrate from the binding proteins and the appearance of binding site(s) on the membrane-bound component(s), which allow passage of the substrate from one binding site to the other, into the inside of the cell. It is also necessary to postulate that the membrane components do not normally have an accessible (or active) binding site unless the loaded binding protein has interacted with the membrane, because no transport has been detected in spheroplasts or membrane vesicles deprived of the binding protein, or in mutants lacking the binding protein. The loaded site of the binding protein must be very close (juxtaposed) to the next site, thus not allowing release of the substrate molecule into the periplasm, which otherwise would negate any special function to the binding protein itself. Energy coupling might occur, in an unspecified fashion, via the nucleotide-binding site. While the initial

steps—binding of substrate to the periplasmic component and conformational change of the latter—are well established, all of the rest is based on indirect evidence. Interaction might be with one of the hydrophobic membrane-bound components and/or with the "conserved" protein element. The existence of a substrate-binding site on the hydrophobic component(s) is likely, but not proven. The newly discovered nucleotide-binding site might be involved in energy coupling, presumably by allowing ATP hydrolysis concomitantly with transport. It is, however, possible that the nucleotide-binding site has a regulatory function.

An analogous, but alternative model utilizes the opening and closing of a pore through the membrane-bound permease components, instead of binding sites (Higgins *et al.*, 1982). In this case the liganded periplasmic component triggers opening of the pore and the substrate diffuses through the pore, which closes up again once the free binding protein is released from the membrane. This ensures one-way entry into the cytoplasm. It is hard to reconcile with this model the results obtained with mutants in the membrane-bound components which cause a change in specificity or eliminate the need for the binding protein.

In order to prove the correctness of various aspects of the model of Fig. 1, it will be necessary to isolate, purify, and characterize each of the membrane-bound components, and to reconstitute the entire system in liposome vesicles. This is the hardest area of investigation. However, it should at least be possible to confirm biochemically in the near future the interaction of the periplasmic component with one or more of the membrane-bound components, since overproduction of these proteins by genetic engineering means has been achieved in several systems.

Energy Coupling

It has been postulated that ATP or some form of phosphate bond energy is responsible for powering periplasmic systems, as opposed to shock-resistant systems which would be powered directly by the protonmotive force (Berger, 1973; Berger and Heppel, 1974). Data were obtained with cells which had been starved to eliminate endogenous energy sources and which, in addition, were either treated with a variety of metabolic poisons or were defective in the proton-translocating ATPase. The treatments were intended to affect differentially and specifically various metabolic steps, either lowering the ATP level or the protonmotive force. The results can be summarized as follows: arsenate inhibits periplasmic systems much more strongly than shock-resistant ones; in an ATPase mutant, periplasmic systems cannot be energized by D-lactate or phenazine methosulfate/ascorbate, while

shock-resistant ones can; finally, in an ATPase mutant, periplasmic systems are slightly more resistant to proton uncouplers than shock-resistant systems. However, since any one of the treatments which were used could have affected multiple metabolic pathways, either directly or indirectly, and since the presumed changes were rarely monitored by direct assay, interpretation of these data should be made with caution. For example, since good assays of the protonmotive force were not available, the energized membrane state was *assumed* to have been low or high under a certain set of conditions and thus to explain a certain set of results. Today we are aware of the complicated interrelationships between protonmotive force and a variety of cell functions; therefore we do not expect that a simple relationship exists between addition of an inhibitor and its unique effect on protonmotive force. In fact, results from two laboratories indicate that the energy-coupling mechanism may be complicated. Plate demonstrated that under conditions where the ATP level of cells is unchanged, but the protonmotive force is decreased, glutamine transport is also decreased, thus indicating (1) that ATP is not sufficient to power transport, and (2) that the protonmotive force plays a role (directly or indirectly) in periplasmic transport (Plate, 1979). In agreement with Plate's results, Singh and Bragg showed that periplasmic systems are functional only under conditions in which a protonmotive force is expected to be generated (Singh and Bragg, 1977). Earlier experiments indicating that ATP is directly involved in energizing both periplasmic and shock-resistant systems should be reinterpreted today taking into consideration the fact that a protonmotive force could have been built up by way of the ATPase activity (Singh and Bragg, 1976). It should be pointed out that whatever the effect of the protonmotive force may be, it probably is not the coupling of transport to movement of protons because it has been impossible to show proton translocation during transport through several periplasmic systems (Darnwalle *et al.*, 1981). An interesting set of experiments implicated acetylphosphate (Hong *et al.*, 1979) or a compound derived from it (Hong and Hunt, 1980) as energy source for periplasmic systems. These suffered from the same sources of ambiguity as the above experiments, since measurements of acetylphosphate were not always performed and the correlation between transport and acetylphosphate was therefore indirect. Critical discussions of theories and experiments concerning energy coupling in shock-sensitive systems have appeared recently (Ames, 1986a; Hengge and Boos, 1983; Hunt and Hong, 1981b) and the reader is referred to these reviews for additional details. Here it suffices to say that it is advisable at this moment to restrict generalizations to a simple statement that the two classes of transport systems *can* behave fundamentally differently with respect to energization, without specifying the primary differences any further.

Reconstituted Membrane Vesicles

An essential step in the understanding of the mechanism of action of periplasmic permeases is the ability to study them in a much simpler system, such as membrane vesicles. Reconstitution of binding protein–dependent active transport in membrane vesicles has been recently obtained (Hunt and Hong 1981a; Hong, 1986; Rotman and Guzman, 1984). Briefly, the method consists of a standard preparation of membrane vesicles from lysozyme-generated spheroplasts. Addition to these vesicles of high concentrations of binding protein and of an energy source allows substrate transport into the vesicles. A crucial factor seems to be that the vesicles be prepared from a mutant strain lacking the binding protein (Hunt and Hong, 1983a); failure to do so yields vesicles with high residual levels of transport which cannot be increased by the addition of binding protein. This result may explain numerous previous failures to obtain reconstitution of transport in membrane vesicles.

One of the important advantages of the much simplified environment of a membrane vesicle is that it allows investigation of individual energy candidates without interference from general metabolism. These hopes have been somewhat frustrated by the finding that vesicles are capable of substantial metabolism (Hunt and Hong, 1983a). Up to now results indicate the importance of the protonmotive force since uncouplers and inhibitors of respiration interfere with reconstituted transport. They also suggest the noninvolvement (at least in a unique way) of either ATP or acetylphosphate as energy-coupling factors since transport can be abolished under conditions where both compounds are still present in the membrane vesicles (Hunt and Hong, 1983a). A serious problem in this kind of reconstitution experiments is the fact that vesicle preparations vary greatly in their ability to utilize external or trapped energy sources, depending on the exact methodology of their preparation.

Evolutionary Aspects

A comparison of the characteristics of all the known periplasmic systems suggests that the underlying mechanism is the same for all of them. All the permeases that have been studied extensively have a similar composition, requiring one (or more, see below) periplasmic component and three membrane-bound components. The genes coding for the components are closely linked on the chromosome, probably forming an operon in all cases (though the maltose permease is coded by two divergent operons). An interaction may occur between the periplasmic binding protein and the membrane-bound components. An additional interesting complexity, which also seems to be

shared by several periplasmic systems, is the duplication and divergent evolution of the gene coding for the periplasmic component. Besides the duplication yielding two related periplasmic proteins, evidence for an additional duplication within the histidine and phosphate transport operons can be drawn from the definite homology existing between *hisQ* and *hisM* (Ames, 1985), and between *pstC* and *pstA*.

Considering their similarity despite the complexity of their organization, it has been hypothesized that all the periplasmic systems have originated by duplication and divergence from a single ancestral system, perhaps already containing a duplication both of the periplasmic component and of one of the membrane components (Ames, 1985). Each system would have evolved a different specificity while retaining the same basic architecture. A search for homologies among parallel components of all these systems could answer this question. We have already seen that strong homology exists between one of the membrane-bound components of each of the different permeases. A significant homology exists between RbsC and PstA and PstC and between MalF and PstC, and a weak homology between MalF and HisM (Ames Ferro-Luzzi and Doolittle, unpublished data). Comparison of all the available sequences of periplasmic components has shown that the galactose-, arabinose-, and ribose-binding proteins are significantly homologous to each other (Argos *et al.*, 1981; Groarke *et al.*, 1987) but that no significant homologies exist between several completely unrelated binding proteins (Ames Ferro-Luzzi, Farrah, Johnson, and Doolittle, unpublished data). However, a structural and functional relationship between the binding proteins is shown by the X-ray structure of several of them which yielded in all cases strongly similar two-domain structures (Gilliland and Quiocho, 1981). Thus, there is reasonable evidence that a complex ancestral system would have spawned the present multiplicity of periplasmic permeases (Ames, 1986a).

Universality of the Conserved Component

Interestingly, it has emerged lately that a number of proteins, not obviously related to bacterial periplasmic transport, are homologous with the family of conserved components of these permeases (Ames, 1986b; Higgins *et al.*, 1986; Mount, 1987; Doolittle *et al.*, 1986). These include the bacterial proteins encoded in genes *hlyB*, *ftsE*, *nodI*, *uvrA*. All of these, except for *uvrA*, probably involve membrane-associated functions. Among the others, two eukaryotic proteins are particularly interesting: the *mdr* protein from mammalian cells (Gros *et al.*, 1986; Chen *et al.*, 1986) and the *white* locus of Drosophila. Both are almost certainly involved in some form of transport: *mdr* in the extrusion of toxic drugs from cells, which thus acquire resistance

to those drugs by keeping their intracellular level low; and *white* in the deposit (transfer?) of pigment in the eye and other organs of the fruit fly. It is particularly interesting that these proteins are composed of two domains, one of which is very hydrophobic. This domain may perform a function equivalent to that of the bacterial HisQ and HisM proteins in Fig. 1, being ultimately responsible for the passage of substrates through the membrane. It is not unusual for eukaryotic proteins to be the result of fusion events among proteins that are present individually in simpler organisms. In addition, the *mdr* protein is a fused duplication of two highly conserved moieties, thus containing two ATP-binding sites per molecule. This may be an indication that the function of the conserved component requires it to be at least in a oligomeric state. Support for this hypothesis comes also from the oligopeptide permease system (*opp*) where two separate, highly conserved genes, coding for this component, have been identified (Higgins *et al.*, 1986).

The existence of homologous proteins in organisms so vastly different is an indication of a universality of function which has survived the most extensive evolution. Such function is probably related to an energy-coupling mechanism, though not necessarily for active transport only.

Conclusion

Work on the structure and function of periplasmic systems has reached a stage where essential generalizations can be made because research into numerous systems has started yielding abundant results and, most important, revealing through their similarities the existence of a common composition and organization. The four basic aims in the study of these transport systems are at various levels of development. Firstly, the characterization of the protein composition and genetic organization of a periplasmic permease has been essentially accomplished, since one transport operon (histidine) has been completely sequenced and all gene products have been identified and overproduced and several other permeases are getting very close to complete characterization. Secondly, the investigation of the molecular mechanism of action and the architecture of these systems is beginning to take off, with the first attempts at purifying the membrane protein components and determining their possible enzymatic functions. Antibodies against a number of these proteins have been obtained and more should be available soon. The state of the field is such that very precise questions can now be asked concerning the existence and nature of protein–protein interaction sites by altering specific amino acid residues through the use of recombinant DNA technology and as dictated by the available X-ray structure determination of several of these proteins. By similar techniques the nature of the nucleotide-binding site on

the conserved components and of possible substrate-binding sites on the membrane components can also be explored. With the availability of these tools, in conjunction with the ever essential genetic approach, it should be possible to elucidate the molecular architecture of these permeases in the near future. Thirdly, the least advanced of the three areas of research is the study of the mechanism of energy coupling. Because of the unavailability of a well-characterized *in vitro* system, the complexities arising from doing this research in whole cells have caused the results to be ambiguous. However, the results are tantalizing because they clearly point out differences with the energy-coupling mechanism of shock-resistant permeases. Finally, an intriguing aspect is the evolutionary relationship between several independent permeases. Since we can *guess* that a cell may contain a few dozen periplasmic permeases, it will be an interesting speculative problem to trace their genealogy as more are being discovered and compared to each other. In addition, some important clues concerning their mechanism of action might emerge from such studies. The next few years should bring additional understanding and excitement to the field of periplasmic transport.

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