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Periplasmic Binding Protein-Dependent Transport Systems: The Membrane- Associated Components [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1990 **326**, 353-365
doi: 10.1098/rstb.1990.0017

Phil. Trans. R. Soc. Lond. B **326**, 353–365 (1990)
Printed in Great Britain

Periplasmic binding protein-dependent transport systems: the membrane-associated components

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Periplasmic binding protein-dependent transport systems are multicomponent, consisting of several inner membrane-associated proteins and a periplasmic component. The membrane-associated components of different systems are related in organization and function suggesting that, despite different substrate specificities, each transport system functions by a common mechanism. Current understanding of these components is reviewed. The nature of energy coupling to periplasmic transport systems has long been debated. Recent data now demonstrate that ATP hydrolysis is the primary source of energy for transport. The ATP-binding transport components are the best characterized of a family of closely related ATP-binding proteins believed to couple ATP hydrolysis to a variety of different biological processes. Intriguingly, systems closely related to periplasmic binding protein-dependent transport systems have recently been identified in several Gram-positive organisms (which lack a periplasm) and in eukaryotic cells. This class of transport system appears to be widespread in nature, serving a variety of important and diverse functions.

1. Introduction

Periplasmic binding protein-dependent transport systems were first distinguished from other classes of transport systems over 20 years ago by their susceptibility to cold osmotic stock (Neu & Heppel 1965). Many 'shock-sensitive' transport systems, each specific for a different low molecular mass substrate such as sugars, amino acids, peptides and inorganic ions, have now been identified (reviewed by Furlong (1987)). Although most remain relatively poorly characterized, it is becoming apparent that, regardless of their substrate, the binding protein-dependent transport systems comprise a distinct class of transport system, being structurally, mechanistically and probably evolutionarily related. As a generalization, what is true for one system is also true for all of the others. Nevertheless, specific differences are also apparent between the components of different systems, the reasons for which are often not fully understood.

Osmotic-shock sensitivity of these transport systems is due to the release of an essential protein component located in the periplasm, between the cytoplasmic (inner) and outer membranes. These periplasmic binding proteins have high affinities for their specific substrates and serve as the primary receptors for transport. Because of their high abundance and water solubility they are by far the best characterized components of this class of transport system and many of their properties are well understood (see F. A. Quiocho, this symposium). In addition to the periplasmic binding protein, each transport system requires a complex of several

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Typescript received – 23 *February* 1989 – *Revised* 21 *March* 1989.

cytoplasmic membrane-associated proteins. The periplasmic binding proteins deliver substrate to these protein complexes, which in turn, mediate translocation of the substrate across the membrane. It is these membrane-associated proteins that are the subject of this article.

2. Membrane-associated components

Binding protein-dependent transport systems were first shown to involve at least one membrane-associated protein over a decade ago (Ames & Nikaido 1978). However, characterization of these proteins has proceeded rather slowly. There are several reasons for this. Firstly, they are extremely low in abundance, often as few as 50–100 molecules per cell. Secondly, for reasons not yet understood, they are deleterious to cell growth when overproduced. Thirdly, they are hydrophobic and hence resistant to many conventional techniques of protein purification. Finally, they show no enzymatic activity to assist their purification; they function as a complex in intact cells and cannot be readily assayed once the cell is disrupted as a prelude to purification. Thus much of what we know about these proteins has emerged indirectly, from studies of the genes that encode them. Only recently have we begun to understand the biochemistry of the proteins themselves.

The amino acid sequences of the membrane-associated proteins from several transport systems have now been deduced from the nucleotide sequences of the corresponding genes. Although the overall organization of each system is essentially similar, important differences in the number and organization of the membrane proteins exist. These differences are probably best appreciated by comparison with a 'typical' system, illustrated by the oligopeptide permease, Opp (figure 1*a*). This transport system consists of two highly hydrophobic, integral membrane proteins (OppB and OppC) involved in transporting the substrate across the membrane, and two relatively hydrophilic membrane proteins (OppD and OppF), which bind ATP and are believed to couple ATP hydrolysis to the transport process (Hiles *et al.* 1987). The structure, location and function of the 'hydrophobic' and 'ATP-binding' components of each system are quite distinct and it is most convenient to consider them separately.

3. The hydrophobic integral membrane proteins

Two highly hydrophobic membrane proteins are essential components of each transport system. Comparison of the proteins from each system reveals little sequence similarity, although all are highly hydrophobic and seem to be structurally related (Hiles *et al.* 1987). Hydropathy plots show that each protein consists of a core structure of five or six potential membrane-spanning α-helices (Hiles *et al.* 1987) separated by short stretches of hydrophilic sequence (figure 2); one of these hydrophilic stretches appears to be conserved in many of these proteins (Dassa & Hofnung, 1985). This short, conserved sequence is probably exposed to the cytoplasmic face of the membrane and may interact with the peripherally located ATP-binding components. Although there is no direct evidence to confirm the structural models predicted from the sequence, the fact that these components are responsible for mediating transport across the bilayer implies that they span the membrane. The model is further supported by studies that indicate that these proteins interact both with the binding proteins at the periplasmic face of the membrane (Treptow & Shuman 1985; Prossnitz *et al.* 1988) and the ATP-binding proteins at the cytoplasmic face of the membrane (Shuman & Silhavy 1981),

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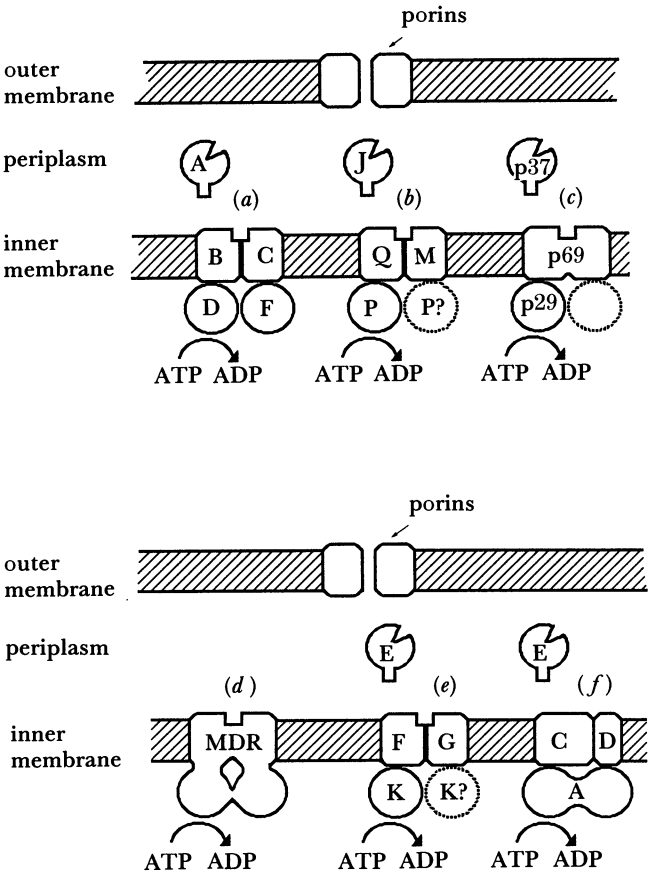

FIGURE 1. Schematic representation of the components of several binding-protein dependent transport systems. (*a*) The oligopeptide permease, Opp, of *S. typhimurium* (Hiles *et al.* 1987). (*b*) The histidine transport system of *S. typhimurium* (Higgins *et al.* 1982). (*c*) A mycoplasma system associated with invasiveness of mammalian tissue culture cells (Dudler *et al.* 1988). (*d*) The Mdr multidrug resistance protein of mammalian tumours (Gros *et al.* 1986). (*e*) The maltose transport system of *E. coli* (Shuman 1982). (*f*) The ribose transport system of *E. coli* (Buckel *et al.* 1986). The 'proteins' indicated by dashed lines illustrate the suggestion that two identical ATP-binding components might operate as a homodimer in these systems (see text for discussion).

as well as by results obtained with Tn*phoA*, which imply that the MalF protein spans the membrane several times and adopts the organization predicted from hydropathy profiles (Boyd *et al.* 1987).

A comparison of the sequences of the two integral membrane components from a single system (e.g. OppB with OppC, HisQ with HisM; see figure 1*a*, *b*) does, however, reveal a significant sequence similarity and, even where sequences substantially diverge, a structural similarity appears to be maintained. Thus the two hydrophobic membrane proteins from each system are thought to function as a pseudodimer (Ames 1985; Hiles *et al.* 1987). This view is borne out by the recent finding that in a putative transport system from *Mycoplasma*, the equivalents of these two proteins are fused into a single larger polypeptide (p69, figure 1*c*) (Dudler *et al.* 1988). Similarly, in the Mdr transport system from eukaryotes (figure 1*d*) the equivalents of these two hydrophobic proteins are encoded as part of a single polypeptide. The archetypal transport system therefore appears to require two similar hydrophobic domains, encoded either as two single polypeptides (e.g. OppB and OppC) or as one larger, two-domain protein (e.g. p69).

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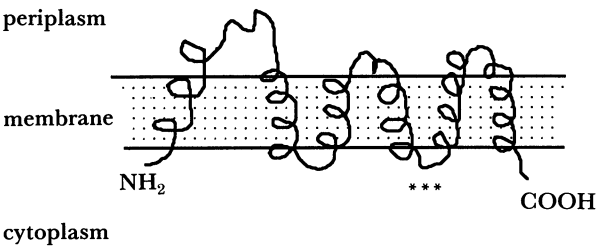

FIGURE 2. Diagram illustrating the possible folding of a 'typical' hydrophobic integral membrane proteins determined for OppB (unpublished data); (***) indicates the location of the short hydrophilic sequence conserved in many of these proteins (Dassa & Hofnung 1985).

There are, of course, apparent departures from this generalization although they may not be as different as appears at first sight. Thus, although the MalF protein is considerably larger than the 'typical' integral membrane component (Froshauer & Beckwith 1984), it can really be considered as a 'typical' component with an additional N-terminal domain; the function of this domain is not known. Similarly, although the arabinose transport system apparently involves just a single hydrophobic component, AraH (Scripture *et al.* 1987), this protein is sufficiently large, and possesses sufficient potential membrane-spanning helices, to be equivalent to a dimer of two 'typical' subunits. For the ribose system the two hydrophobic components are of very different sizes, one large (33 kDa; RbsC) and one small (15 kDa; RbsD) (Bell *et al.* 1986); again it is possible to consider the aggregate of these two dissimilar subunits as equivalent to the more typical situation of two similarly sized subunits. We suggest that the differences in size and number of the membrane components between systems may simply represent flexibility within a general theme, providing slightly different means of obtaining a similar, core, trans-membrane structure.

The hydrophobic membrane-associated components are generally believed to be responsible for mediating translocation of substrate across the lipid bilayer. However, the mechanisms by which this is achieved are obscure and await biochemical and structural analysis of the proteins. Attempts to purify these proteins have so far met with little success and those clues that we have come principally from indirect, although elegant, genetic studies. One of the key considerations is the suggestion that the membrane-associated proteins possess a specific substrate-binding site. This, of course, makes teliological sense. If specificity were solely conferred by the periplasmic component then one could imagine that the various periplasmic proteins could pass substrate to a single complex of membrane proteins; one would not necessarily expect a unique complex of membrane-associated components for each substrate. One line of evidence in support of this view comes from the isolation of mutations that alter substrate specificity. Although most such mutations, as expected, alter the periplasmic protein, mutations that alter the hydrophobic membrane components can also be isolated (Higgins *et al.* 1982; Payne *et al.* 1985). The clearest evidence, however, comes from studies on the maltose transport system (Shuman 1982; Treptow & Shuman 1985; Reyes *et al.* 1986). Normally, transport will not occur in the absence of the periplasmic MalE protein. However, in *malE* deletions, it has been possible to select mutants that transport maltose (albeit inefficiently), independently of any periplasmic protein. These mutations map to the *malF* and *malG* genes. As transport in these binding-protein independent mutants appears to be specific, the implication is that the MalF and MalG proteins (separately or together) must themselves

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possess a specific substrate-binding site. Nevertheless, the location of the substrate-binding site(s) in these proteins, and an understanding of its role, awaits detailed structural analysis.

One final point needs to be made. Although periplasmic binding proteins are essential components of this class of transport system, mutants that function in the absence of such proteins can be isolated (Shuman 1982). In addition, the Mdr system (figure 1*d*) from eukaryotic cells appears to lack any periplasmic component (perhaps not suprisingly as the cells in which they function do not have a periplasm). Thus the periplasmic protein cannot be an absolute requirement for the membrane transport process itself. Perhaps binding-protein-dependent transport systems should really be considered as a membrane complex that mediates transport, with the periplasmic component not being integral to the basic mechanism by which these complexes mediate translocation of substrate across the bilayer and by which energy is coupled to the process. The periplasmic component might best be considered as a 'bolt-on' component, serving specific aims such as an adaptation to the existence of a periplasm in Gram-negative species (Brass *et al.* 1986), to achieve higher substrate affinities, or add directionality to the transport process.

4. The ATP-binding components

In addition to the hydrophobic, integral membrane components, each binding protein-dependent transport system possesses either one or two hydrophilic membrane proteins. Unlike the periplasmic proteins and the hydrophobic membrane components, these proteins share extensive sequence similarity, regardless of the system with which they are associated, (*ca.* 30% sequence identity over their entire length) and almost certainly share a common evolutionary origin (Higgins *et al.* 1985, 1986, 1988). These proteins bind ATP and are believed to couple ATP hydrolysis to the transport process (see below). Two such proteins, each possessing an ATP-binding site, are required for the oligopeptide permease (OppD and OppF; figure 1*a*). In the ribose system the equivalents of these two polypeptides are fused into a single, two-domain protein (figure 1*f*; Buckel *et al.* 1986). Similarly, in the Mdr protein two ATP-binding domains are encoded as a single, large polypeptide (figure 1*d*). Thus two ATP-binding domains, either as two separate proteins or as one fused polypeptide, appear to be required for many members of this class of transport system. In contrast, several transport systems (e.g. histidine, HisP; maltose, MalK; figure 1*b*, *e*) require only one such polypeptide; it seems reasonable to suppose that in these systems two separate but identical polypeptide chains function together as a homodimer (Higgins *et al.* 1986). The concept of two ATP-binding domains as part of each transport system is also consistent with evidence suggesting that two ATP molecules may be hydrolysed per transport event (see below).

The ATP-binding proteins, from several different transport systems (e.g. histidine, maltose, ribose, phosphate, arabinose, galactose and vitamin B12; see Higgins *et al.* (1988); Gallagher *et al.* (1989) for a more detailed discussion) have been shown to be associated with the cytoplasmic membrane. As the proteins are hydrophilic and contain no potential membrane-spanning helices, they have generally been assumed to be peripherally associated with the cytoplasmic face of the membrane, a location compatible with their proposed role in coupling ATP hydrolysis to transport. However, the only direct evidence in support of this supposition comes from recent studies on the OppF protein, showing it to be accessible to proteases only from the cytoplasmic face of the membrane (Gallagher *et al.* 1989). Although such a location

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is generally accepted, two anomalies remain. First, the OppF and HisP proteins are tightly associated with the membrane, even in the absence of the other transport components (Hobson *et al.* 1984; Gallagher *et al.* 1989). How such a hydrophilic protein has affinity for the membrane remains a mystery. In contrast, the MalK protein is released to the cytoplasm in the absence of MalF and MalG, implying attachment to the membrane via an interaction with these other components (Shuman & Silhavy 1981). Secondly, genetic data, based on suppressor mutations, indicate an interaction between HisP and the periplasmic HisJ protein (Ames & Spudich 1976), implying that HisP spans the membrane and that at least part of the protein is exposed to the periplasm. Although other interpretations of this genetic data are possible, this apparent anomaly has not been resolved.

5. Energy coupling

The use of metabolic inhibitors first provided evidence that the mechanism of energy-coupling to binding protein-dependent transport systems differs significantly from that of membrane-bound systems that are driven by the proton motive force (PMF). Furthermore, these studies indicated a requirement for ATP hydrolysis (Berger 1973; Berger & Heppel 1974). Subsequently, there has been a very considerable debate as to the role of ATP; many alternative energy sources have been suggested. These include acetyl phosphate (Hong *et al.* 1979), NADPH (Gilson *et al.* 1982), lipoic acid (Richarme 1985; Richarme & Heine 1986), and succinate (Hunt & Hong 1983). Arguments against a direct role for ATP have included the demonstration that, under certain conditions, a reduction in the cellular ATP pool does not lead to a corresponding reduction in transport (Plate *et al.* 1974; Lieberman & Hong 1976; Ferenci *et al.* 1977). Further confusion has arisen from findings that perturbation of the PMF can affect binding protein-dependent transport without necessarily having a major effect on ATP pools (Plate 1979; Singh & Bragg, 1979; Hunt & Hong, 1983; Ames 1986). However, recent studies from our laboratory, and that of Ames, now leave little doubt that ATP hydrolysis provides the energy for solute transport via this class of transport system.

Firstly, OppD, HisP, MalK, and the equivalent proteins from other transport systems, all possess consensus ATP-binding sites (Higgins *et al.* 1985). Furthermore, OppD and HisP have been shown to bind ATP or ATP analogous (Higgins *et al.* 1985; Hobson *et al.* 1984). This does not, of course, preclude the possibility that ATP-binding only plays a regulatory or structural role. However, we have recently demonstrated ATP hydrolysis during transport (Mimmack *et al.* 1989; figure 3). In *unc* mutants, ATP generation can only occur as a result of glucose fermentation. If this process is blocked with iodoacetate, ATP can no longer be synthesized and the intracellular pool falls slowly (figure 3). When a transported substrate such as maltose is added (to a *malPQ* strain, which cannot metabolize maltose) this results in a rapid and specific fall in the intracellular ATP pool, which by the judicious use of appropriate mutants, can be shown to be a specific consequence of transport. Moreover, the number of ATP molecules hydrolysed per molecule of substrate transported can be calculated as between one and two. The data suggest a stoichiometry of two although inherent experimental difficulties make it impossible to be certain that the correct value is not actually one. A stoichiometry of two would be consistent with the notion that each transport system requires two ATP-binding domains. Interestingly, a very indirect estimation of the stoichiometry, based upon growth yields on different substrates, yielded a similar figure (Muir *et al.* 1985). It should be pointed out that

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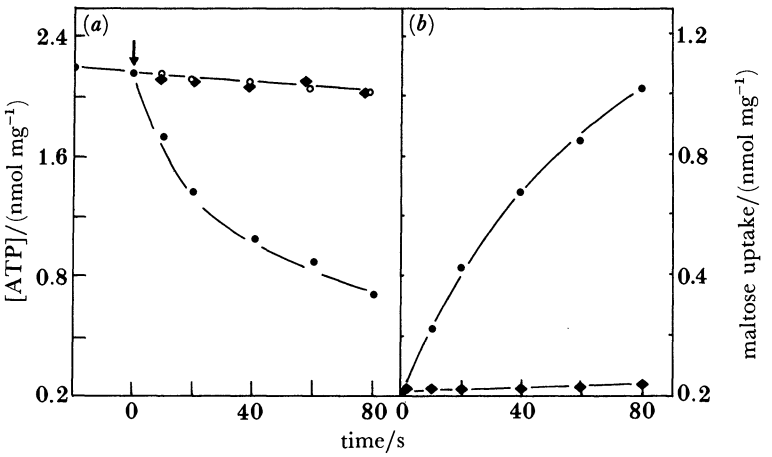

FIGURE 3. Demonstration of ATP hydrolysis during transport. (*a*) *E. coli* cells (*unc*, *malT*^{c}, *malPQ*) were grown to exponential phase, washed and preincubated with iodoacetate. At time zero, indicated by arrow, maltose was added (●) and the fall in intracellular ATP pools monitored. No fall in intracellular ATP was seen if maltose was omitted (○) or if maltose was added to a maltose transport-deficient mutant (◆). (*b*) Maltose uptake measured under the same conditions for the parental strain (●) and a maltose transport-deficient derivative (◆). Calculations from these, and other similar data suggest that two ATP molecules are consumed per molecule of maltose transported. See Mimmack *et al.* (1989) for further details.

this stoichiometry appears to be less efficient than the 0.5 ATP equivalents required to take up a molecule of substrate via PMF-linked transporters. Presumably this loss in energy efficiency is a necessary consequence of increased substrate affinities and the imposed directionality of periplasmic transport systems.

It is necessary to explain data that are apparently in contradiction with the conclusion that direct hydrolysis of ATP energizes these transport systems. Data showing that transport rates do not fall as ATP pools decline presumably reflect a relatively high affinity of the transport components for ATP compared with intracellular pools. Indeed, HisP is estimated to have an affinity for ATP of about 100 μM (Ames *et al.* 1989) compared with cytoplasmic ATP levels which are in the millimolar range. Furthermore, the Ames laboratory has recently obtained evidence in vesicle systems (Joshi *et al.* 1989) that PMF is neither essential nor sufficient to drive histidine transport in *Salmonella typhimurium*. It seems probable that perturbation of PMF inhibits binding-protein dependent transport systems indirectly by affecting cytoplasmic pH, a factor known to regulate amino acid transport systems in *Streptococcus* (Poolman *et al.* 1987; Dreissner *et al.* 1987). It is also worth pointing out that unless inordinate stoichiometries are imposed, the degree of accumulation that can be achieved by these systems (up to 10^5-fold against the concentration gradient) cannot, thermodynamically, be driven by the PMF.

Although there now seems no doubt that hydrolysis of ATP provides the driving force for transport, it is not possible to eliminate the possibility that the high energy phosphate of ATP is transferred first to an intermediate compound, which then interacts with the transport proteins. The fact that OppD and HisP bind ATP argues strongly that ATP hydrolysis is mediated directly by the transport components but, as yet, hydrolysis by the purified proteins has not been detected. It is also relevant that the closely related UvrA protein (see below) has been shown to hydrolyse ATP (Seeberg & Steinum 1982). Final confirmation of ATP hydrolysis by the transport components may have to await reconstitution of transport from individually purified components; this remains an aim for the future.

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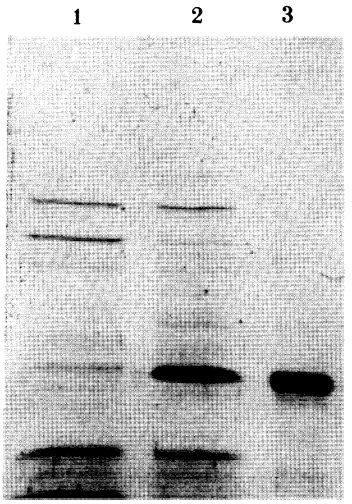

FIGURE 4. Coomassie-stained SDS-polyacrylamide gel showing overproduction and purification of the ATP-binding OppF protein. The *oppF* gene was cloned under control of the inducible λ P_L promoter and the strong *atpE* ribosome-binding site. Lane 1; total protein from uninduced cells; lane 2; total proteins from cells induced for 60 min at 42 °C; lane 3; OppF protein after a one-step purification from inclusion bodies.

The ATP-binding proteins have, like the integral membrane protein components, proved difficult to analyse biochemically, although some progress has been made (Hobson *et al.* 1984; Higgins *et al.* 1985; Gallagher *et al.* 1989). We have now overproduced the OppF protein to about 30% of total cell protein and purified it to homogeneity. This required site-directed mutagenesis to place the gene under control of a heterologous strong promoter and ribosome binding site (figure 4) and protease (*lon htpR*)-deficient strains. Although the protein forms inclusion bodies it can be solubilized and purified by FPLC in sufficient quantities for biochemical and structural studies. Such studies are now in progress.

6. Related ATP-binding proteins

As pointed out above, the ATP-binding components from the different transport systems share extensive sequence similarity. This similarity is far more extensive than is simply required to facilitate ATP-binding; indeed, other than a short consensus ATP-binding sequence, little similarity is seen when a comparison is made with a wide variety of other ATP-binding proteins. Intriguingly, this high degree of sequence relatedness is also found with a variety of other proteins apparently unconnected with binding-protein-dependent transport processes. These include the HlyB protein, which is involved in haemolysin secretion; NodI, a *Rhizobium* protein implicated in nodulation; UvrA, which mediates DNA repair; FtsE a component of cell division; the *Drosophila white* locus and a chloroplast protein from liverworts (reviewed in Higgins *et al.* (1986, 1988)). The ATP-binding, transport proteins appear to be archetypes of a family of structurally and probably evolutionarily related proteins that serve to couple ATP hydrolysis to a wide range of biological processes in both prokaryotes and eukaryotes. The reason for such extensive sequence similarity, in excess of that required to mediate ATP-binding, remains unclear.

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7. Related transport systems in Gram-positive bacteria and eukaryotic cells

Characterization of binding protein-dependent transport systems has been restricted to the Gram-negative bacteria *E. coli* and *S. typhimurium*. Because of the nature of the cell wall of Gram-negative bacteria, enclosing a periplasmic space, it was generally considered that the requirement for a periplasmic protein might be restricted to such species. However, it is now becoming clear that equivalent systems are present in both Gram-positive bacteria and in eukaryotic cells. Sequences highly homologous to the MalE and OppA proteins of Gram-negative bacteria have been identified in *Streptococcus pneumoniae* (named MalX and AmiA) and, at least in the case of the Ami system, components equivalent to OppBCDF are also apparent (Gilson *et al.* 1988). Although no transport activity has been demonstrated with these systems, transport seems their most likely function. An operon of genes encoding proteins characteristic of the several components of a binding-protein dependent transport system has also been identified in *Mycoplasma* (Dudler *et al.* 1988) and a homologous system to Opp has been identified in *Bacillus subtilis* (J. Hoch, personal communication). We have recently demonstrated that this *Bacillus* system is indeed a transport system (unpublished results). Finally, a homologue of the periplasmic binding protein-dependent phosphate transport system has been identified in *Mycobacteria* (D. Young, personal communication). The existence of periplasmic binding proteins in species lacking a periplasm is puzzling. It has been suggested that the proteins may be attached to the outer face of the cytoplasmic membrane via a lipid moiety (Gilson *et al.* 1988). However, at least in the case of the *Bacillus* Opp system the 'periplasmic' protein is released freely into the growth medium (C. F. Higgins *et al.* unpublished results) and is presumably serving a communal function within a population of cells. In the natural growth state such proteins may perhaps be retained within a colony, rather than lost to the external environs, by capsular material, which is secreted around growing cells.

A transport system similar to the periplasmic binding protein systems has also been identified in mammalian cells, the Mdr protein. Over-expression of this protein is responsible for conferring multiple drug resistance upon tumour cells, causing a potentially important clinical problem. The Mdr protein is a large polypeptide consisting of four domains equivalent to the two hydrophobic and two ATP-binding components of a bacterial periplasmic transport system (figure 1*d*). In the ATP-binding domains the sequence similarities between the eukaryotic and prokaryotic proteins is as great as between any two prokaryotic proteins. In the Mdr protein, the four separate membrane-associated components of a bacterial transporter are apparently fused into a single, multifunctional polypeptide (see Ames (1987); Higgins *et al.* (1988) for reviews). The Mdr protein has also been shown to function as an ATP-dependent transport system, expelling drugs from the cell (Horio *et al.* 1988). There is no evidence for an equivalent of a periplasmic protein associated with Mdr; the significance of this is discussed above. Nevertheless, it seems certain that the Mdr protein represents a eukaryotic equivalent of the bacterial binding protein-dependent transport systems and will function by a very similar mechanism to achieve solute transport across membranes.

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8. Diverse functions of binding protein-dependent transport systems

It has been established for many years that several periplasmic binding proteins also serve as the primary receptor for chemotaxis. Otherwise, these transport systems were generally considered only to play a role in the uptake of nutrients for cell growth. It is now becoming apparent that many of these transporters have adapted to serve other additional functions. The Mdr protein pumps substrate out of cells, raising the possibility that such transport systems might function in reverse to mediate exit from bacterial cells. Indeed, the Bex system of *Haemophilus*, required for capsulation, may play a role in the export of polysaccharide components from the cytoplasm (Kroll *et al.* 1988). In *Escherichia coli* the Opp system is required for the recycling of cell wall components as well as serving its normal nutritional role (Goodell & Higgins 1987). Mutations at the *ami* locus of *Streptococcus*, apparently an Opp equivalent, cause diverse phenotypes including sensitivity to an imbalance of leucine, isoleucine and valine, and a decrease in membrane potential (Gilson *et al.* 1988). In *Mycoplasma*, a binding-protein dependent transport system of unknown substrate specificity enhances invasivity of mouse sarcoma cells in culture (Dudler *et al.* 1988). How the *Mycoplasma* proteins interact with and influence mammalian cells is completely obscure. Finally, *Bacillus* possesses an entire transport system similar to Opp from *S. typhimurium* (J. Hoch, unpublished results). Synthesis of the 'periplasmic' protein is induced as sporulation proceeds and mutants defective in this system are altered in the initiation of sporulation. This suggests that the *Bacillus* Opp system may serve a regulatory role in this developmental process, possibly as a receptor for peptide signals. A peptide factor has been implicated in the control of *Bacillus* sporulation (Grossman & Losick 1988). These few examples suggest that binding protein-dependent transport systems have evolved to serve a highly diverse set of functions not all of which are associated with nutrient uptake. A more complete understanding of the molecular mechanisms by which they function will have important implications for a variety of biological processes.

We are grateful to Giovanna Ames and Jim Hoch for communicating unpublished results. Work in our laboratory is funded by the C.R.C., M.R.C. and S.E.R.C. We also thank the Imperial Cancer Research Fund for Financial Support. C.F.H. is a Lister Institute Research Fellow.

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Discussion

J. Broome-Smith (*University of Sussex, Brighton, U.K.*). In Gram-positive bacteria, secretion of proteins that one anticipates should be membrane-bound to fufil their biological function is not without precedent. In *Streptomyces* strain R61 one of the peptidoglycan biosynthetic enzymes is found only in the medium and no trace of a membrane-bound form can be detected. However, protein sequencing reveals that it lacks both the signal peptide and the carboxy-terminal 26aa predicted to be present from the gene sequence. So in this case there is thought to be transient carboxy-terminal membrane anchoring followed by proteolytic release, and such a possibility for the Opp binding protein homologue of *Bacillus* should be explored.

C. F. Higgins. The *Streptomyces* protein is an interesting example. However, there is no evidence this is the case for the *Bacillus* OppA protein; it has no hydrophobic C-terminus that could potentially serve as a transient anchor and (although we have not actually sequenced the C-terminus) gel mobilities are not consistent with the proteolytic cleavage. Finally, all (more than 95%) of the *Bacillus* OppA protein is released into the medium; this would not be compatible with the normal transport functions of binding proteins which are thought to concentrate substrate in the vicinity of the membrane transport complexes. We therefore suggest the protein

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may be serving another role, possibly in peptide signalling during sporulation where recapture of a small portion of OppA for the medium might be sufficient to mediate a response.

J. Broome-Smith. Is it known how many OppB and C subunits are present in each functional membrane complex?

C. F. Higgins. No, it is not known. However, there is good, albeit indirect, evidence that the OppB and OppC proteins are present in a 1:1 ration in each function complex.

W. N. Konings (*Department of Microbiology, Biology Centre, University of Groningen, The Netherlands*). Professor Higgins suggested that the role of the proton motive force in binding protein dependent transport can be explained by an effect of the internal pH on the transport activity. Recent studies by Dr T. Abee in my laboratory demonstrated that this explanation is indeed correct for binding protein-dependent transport of alanine (and its analogue α-aminoisobutyrate, AIB) in the phototrophic bacterium *Rhodobacter sphaeroides*. At $pH_{in} = 8.3$ Ala transport activity is maximal and this activity decreases with the internal pH and is completely inhibited at pH 7. This decrease of Ala transport activity is not due to a decrease of the cellular ATP content, which remains at 2.2–2.4 mM. Also, the proton motive force was in this experiment constant and composed only of a $\Delta\Psi$ of -135 mV.

C. F. Higgins. Professor Konings' results are interesting and reassuring. Indeed, Giovanna Ames has direct evidence that internal pH affects the histidine transport system of *Salmonella typhimurium*.

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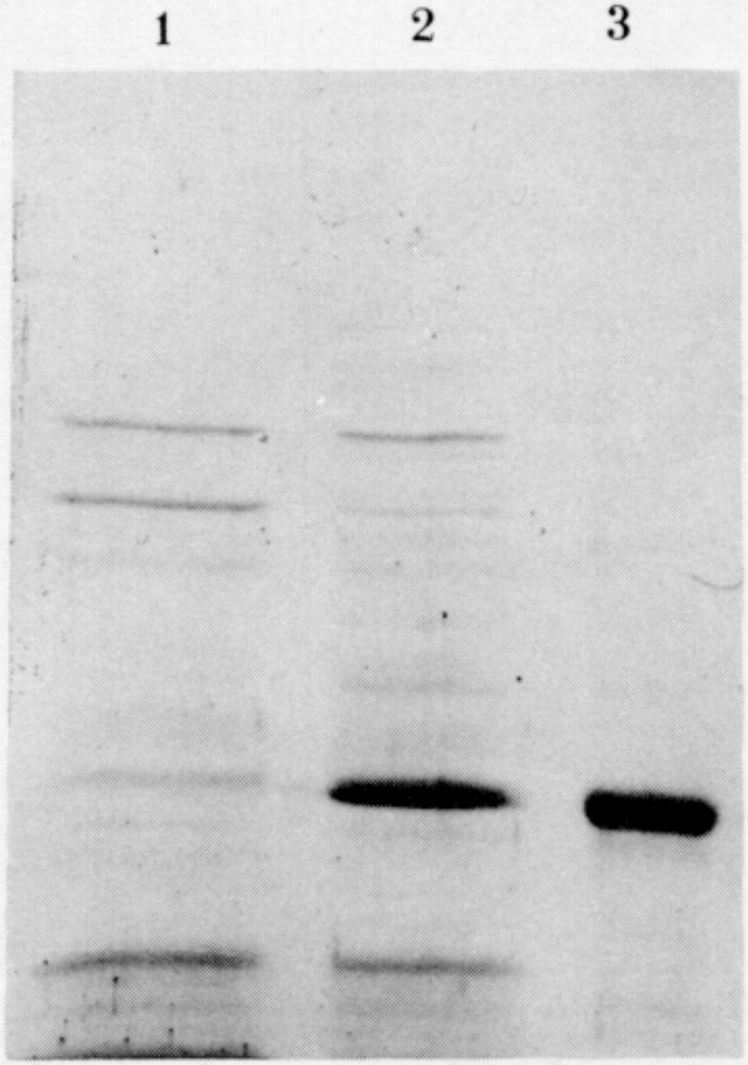

GURE 4. Coomassie-stained SDS-polyacrylamide gel showing overproduction and purification of the ATP-binding OppF protein. The *oppF* gene was cloned under control of the inducible λ P_L promoter and the strong *atpE* ribosome-binding site. Lane 1; total protein from uninduced cells; lane 2; total proteins from cells induced for 60 min at 42 °C; lane 3; OppF protein after a one-step purification from inclusion bodies.