Journal of Bioenergetics and Biomembranes, Vol. 22, No. 4, 1990

MINI-REVIEW

Binding Protein-Dependent Transport Systems

C. F. Higgins[1,3], S. C. Hyde[1], M. M. Mimmack[1], U. Gileadi[1], D. R. Gill[1], and M. P. Gallagher[2]

Received February 26, 1990

Abstract

Bacterial binding protein-dependent transport systems are the best characterized members of a superfamily of transporters which are structurally, functionally, and evolutionary related to each other. These transporters are not only found in bacteria but also in yeasts, plants, and animals including man, and include both import and export systems. Although any single system is relatively specific, different systems handle very different substrates which can be inorganic ions, amino acids, sugars, large polysaccharides, or even proteins. Some are of considerable medical importance, including Mdr, the protein responsible for multidrug resistance in human tumors, and the product of the cystic fibrosis locus. In this article we review the current state of knowledge on the structure and function of the protein components of these transporters, the mechanism by which transport is mediated, and the role of ATP in the transport process.

Key Words: ATP; periplasm; binding protein; cystic fibrosis; multidrug resistance; P-glycoprotein; import; export; transport; membrane protein.

Introduction

The distinction between binding protein-dependent transport systems and other bacterial transporters was made some fifteen years ago, based on two criteria: sensitivity to cold osmotic shock, and differential sensitivity to metabolic inhibitors (Berger, 1973; Berger and Heppel, 1974). The sensitivity of binding protein-dependent transport systems to osmotic shock is due to

[1]ICRF Laboratories, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, England.
[2]Department of Microbiology, University of Edinburgh, King's Buildings, West Main Road, Edinburgh EH9 3JG, Scotland.
[3]To whom correspondence should be addressed.

0145-479X/90/0800-0571$06.00/0 © 1990 Plenum Publishing Corporation

the loss of an essential protein component of the transport system, normally located in the periplasm between the cytoplasmic (inner) and outer membranes. In addition to the periplasmic substrate-binding protein, each transport system requires a distinct complex of proteins associated with the cytoplasmic-membrane. The periplasmic binding protein delivers substrate to this protein complex, which in turn, mediates its translocation across the membrane.

Many binding protein-dependent transport systems have now been identified in Gram-negative bacteria, each specific for a different substrate such as a sugar, amino acid, peptide, or an inorganic ion (reviewed by Ames, 1986; Higgins *et al.*, 1990; Hyde *et al.*, 1990). Although most remain relatively poorly characterized, it is becoming apparent that regardless of the type of substrate recognized, the binding protein-dependent transport systems are closely related in terms of sequence, organization, structure, mechanism, and probably evolutionary origin. Furthermore, other members of this transport superfamily, including bacterial export systems and transporters from a variety of eukaryotic species, have now been identified and are all related at the sequence level and share a similar domain organization. In global terms, what is true for one system appears to be true for each of the others although, of course, specific differences are also apparent. Those transporters in this superfamily which have been identified to date are listed in Table I. Because this class of transport system was first characterized in Gram-negative bacteria, they have, historically, been designated binding protein-dependent or periplasmic transport systems. This name is now inappropriate as exporters or the eukaryotic equivalents do not require a periplasmic component (see below). The frequent use of Mdr to describe this family is also a misnomer as it incorrectly implies that related proteins from other species serve the same function as Mdr. The description "ATP-dependent transport systems" cannot be used as other ATP-dependent transport systems are known which are otherwise unrelated to this superfamily (e.g., for arsenate; Chen *et al.*, 1986a). The distinguishing feature of these transport systems is the ATP-binding cassette, a domain of about 200 amino acids which is highly conserved (see below). We have suggested (Hyde *et al.*, 1990) that this superfamily of transport systems be designed "ABC (ATP-binding cassette) transporters."

In terms of the molecular mechanisms by which active transport is mediated, it is essential to understand the structure and function of the protein components of these transporters. We now have a substantial understanding of the number and nature of the protein components and what they do. The general organization of a "typical" transport system in this superfamily is illustrated in Fig. 1 using the oligopeptide permease as an example. Each system requires four distinct membrane-associated domains. Two of these domains are highly hydrophobic, integral membrane proteins

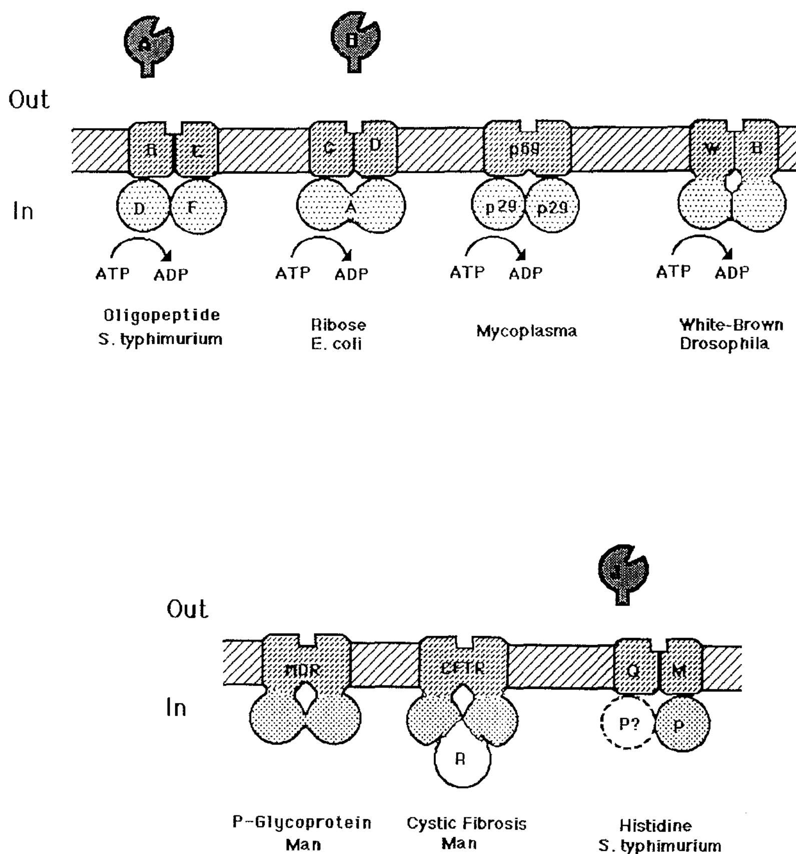

Fig. 1 Diagrammatic representation of different organizations of ABC transporters Periplasmic binding proteins are shaded (▩); the hydrophobic membrane proteins/domains ▩); the ATP-binding domains (▩). References to the systems are given in Table I.

consisting (usually) of six putative membrane-spanning α-helices, while the other two domains are periphically located on the cytoplasmic face of the membrane and couple ATP hydrolysis to the transport process. In most bacterial transporters (e.g., the oligopeptide permease) these four domains are present as separate polypeptide chains. However, domains are frequently fused into larger, multifunctional polypeptides. For example, in the ribose system of *E. coli* the two ATP-binding subunits are fused into a single polypeptide, while in the *Mycoplasma* p69 system the two hydrophobic domains are fused into a single polypeptide. The *Drosphila* white/brown gene products each consist of one hydrophobic and one ATP-binding domain fused together, while in all of the other eukaryotic systems characterized to date (e.g., MDR and CFTR) all four domains into a single polypeptide.

Table I. Members of the Superfamily of ABC Proteins

Organism	Protein(s)	Function	Transported substrate	Reference
Prokaryotes				
S. typhimurium	OppD/OppF	Import	Oligopeptides	Hiles *et al.*, 1987
Streptococcus pneumoniae	AmiE/AmiF	Import[a]	Oligopeptides	Alloing *et al.*, 1990
B. subtilis	OppD/OppF	Import[b]	Oligopeptides	Perego, in preparation
S. typhimurium	HisP	Import	Histidine	Higgins *et al.*, 1982
E. coli	MalK	Import	Maltose	Gilson *et al.*, 1982
E. coli	RbsA	Import	Ribose	Bell *et al.*, 1986
E. coli	AraG	Import	Arabinose	Scripture *et al.*, 1987
E. coli	LivM/LivG	Import	Leucine/Isoleucine/Valine	D. Oxender, personal communication
Pseudomonas aeruginosa	BraF/BraG	Import	Leucine/Isoleucine/Valine	Hoshino, T., and Kono, 1989
S. typhimurium	ProV	Import	Glycine betaine	Stirling *et al.*, 1989
E. coli	ProV	Import	Glycine betaine	Gowrishankar, 1989
E. coli	PstB	Import	Phosphate	Surin *et al.*, 1985
E. coli	ChlD	Import	Molybdenum	Johann and Hinton, 1987
E. coli	NosF	Import	Copper?	W. G. Zumft, personal communication
E. coli	UgpC	Import	Glycerol-3-phosphate	Overduin *et al.*, 1988
E. coli	BtuD	Import	Vitamin B12	Friedrich *et al.*, 1986
E. coli	FecE	Import	Ironcitrate	Staudenmaier *et al.*, 1989
E. coli	FhuC	Import	Iron-ferrichrome	Coulton *et al.*, 1987
E. coli	FtsE	Cell division	?	Gill *et al.*, 1986
R. leguminosum	NodI	Nodulation	?	Evans and Downie, 1986
Mycoplasma hyorhinis	p29	?	?	Dudler *et al.*, 1988
E. coli.	UvrA	DNA repair	None	Husain *et al.*, 1986
Rhizobium meliloti	ORFI	?	?	Albright *et al.*, 1989
Staphylococcus	MsrA	Export	Antibiotics	Ross *et al.*, 1990
Haemophilus influenzae	BexA	Export	Capsular polysaccharide	Kroll *et al.*, 1988
E. coli	HlyB	Export	Protein (hemolysin A)	Felmlee *et al.*, 1985
Pasturella haemolytica	LtkB	Export	Protein (leukotoxin A)	Strathdee and Lo, 1989
Rhizobium melilotti	NdvA	Export	β-(1 $\rightarrow$ 2) Glucan	Stanfield *et al.*, 1988
Agrobacterium tumofaciens	ChrA	Export	β-(1 $\rightarrow$ 2) Glucan	Cangelosi *et al.*, 1989
Bordatella pertussis	CyaB	Export	Protein (cyclolysin)	Glaser, 1988

Yeast				
Saccharomyces cerevisiae	STE6	Export	*a*-Factor polypeptide	McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989
Saccharomyces cerevisiae	Elongation factor 3	?	?	M. Tuite, personal communication
Protozoa				
Plasmodium falciparum	pfmdr	Export	Chloroquine (?)	Foote *et al.*, 1989
Insects				
Drosophilia	white[c]	Import	Eye pigment	O'Hare *et al.*, 1984
	brown[c]	Import	Eye pigment	Dreesen *et al.*, 1988
Plants				
Marchantia chloroplast	MbpX	?	?	Ohyama *et al.*, 1986
Mammals				
Man	Mdr-1[d]	Export	Lipophilic drugs	Chen *et al.*, 1986b
Hamster	Mdr-1[d]	Export	Lipophilic drugs	Gerlach *et al.*, 1986
Mouse	Mdr-1[d]	Export	Lipophilic drugs	Gros *et al.*, 1986
Man	CFTR	?	?	Riordan *et al.*, 1989

[a] *ami* mutants have other unexplained phenotypes including sensitivity to branched chain amino acids, methotrexate resistance, and altered transmembrane electric potential

[b] *opp* mutants of *Bacillus* are also sporulation defective.

[c] white and brown mutations confer different phenotypes, but the two proteins are thought to interact.

[d] the mdr-2 and mdr-3 genes, present in some mammalian cells, are very closely related to mdr-1; they do not confer multidrug resistance, and their functions are unknown.

In addition to these membrane-associated domains, the bacterial "binding protein-dependent systems" require the function of a periplasmic substrate-binding protein (Fig. 1). Although required by the transport systems with which they are associated, these components are not essential to the mechanism by which solute is transported across the membrane and should, perhaps, be considered as accessory components (discussed below). An equivalent component will not necessarily be associated with all members of this superfamily of transport systems.

Certain transport proteins have also acquired domains apparently connected with the transport process itself. For example, the CTFR protein (the product of the cystic fibrosis locus; Fig. 1) contains a distinct and highly charged domain (the R-domain; Riordan *et al.*, 1989) which is believed to serve a regulatory role. The MalK protein from the maltose transport system of *E. coli* possesses a C-terminal extension which is thought to serve an enzymatic role unconnected with the transport process (Reidl *et al.*, 1989). The LamB maltoporin, which is co-expressed with the maltose transport system, plays no role in uptake across the cytoplasmic membrane and is simply an aid to the passage of maltodextrins across the outer membrane (reviewed in Shuman, 1982b). Similarly, exporters such as the HlyB system are often associated with a second component (HlyD) which is required to get substrate (hemolysin) out of the cell (reviewed in Blight and Holland, 1990). However, the extra protein is probably required only to transport substrate across the outer membrane and plays little or no role in transport across the cytoplasmic membrane.

Finally, some bacterial systems appear to lack a domain. Thus, the histidine and maltose transporters of *S. typhimurium* and *E. coli*, respectively, each possess only one gene encoding an ATP-binding subunit (Higgins *et al.*, 1982; Gilson *et al.*, 1982, Fig. 1); although there is no direct evidence, it seems likely that two of these subunits are associated with each transport complex as a homodimer. Minimalist systems such as the ProU glycine betaine transport system (Stirling *et al.*, 1989; Gowrishankar, 1989) or the HlyB hemolysin secretory protein possess only one hydrophobic subunit and one ATP-binding subunit (which in the case of HlyB are fused into a single two-domain polypeptide); two molecules of each of these proteins may comprise the four domains of a functional transport system. Because the characteristics of the different domains of each transport system are quite distinct, even when they form part of a single multifunctional polypeptide, it is most convenient to consider them separately.

The Periplasmic Binding Protein

The release of a periplasmic substrate-binding protein from Gram-negative bacteria by osmotic shock was the initial diagnostic characteristic of this class

of transporter (Berger and Heppel, 1974). These periplasmic proteins have high affinities for their specific substrates and serve as the primary receptors for transport (reviewed by Furlong, 1987; Adams and Oxender, 1989; Quiocho, 1990). A number of binding proteins also function as chemotactic receptors, interacting with the membrane-bound methyl-accepting chemotaxis proteins as the first step in responding to chemical gradients. Because of their relative abundance (they are present in considerable excess over the membrane-associated proteins) and water solubility, they are by far the best characterized components of the transport systems and many of their properties are now well understood.

About 20 different periplasmic substrate-binding proteins have now been identified in *E. coli* and *S. typhimurium* (Furlong, 1987). The primary sequences of many of these proteins are known, mostly via the sequences of the corresponding genes. The proteins are very different in size, ranging from 25 kDa (histidine; Higgins and Ames, 1981) to 59 kDa (oligopeptide; Hiles and Higgins, 1986). Furthermore, there is little or no primary sequence conservation between the various binding proteins. The only exceptions to this rule are the pairs of periplasmic proteins which interact with the same complex of membrane transport proteins (e.g., the histidine and lysine–arginine–ornithine binding proteins, Higgins and Ames, 1981; the leucine and isoleucine–leucine–valine binding proteins, Landick and Oxender, 1985). The various sugar binding proteins also possess a short sequence motif characteristic of sugar binding sites, but are otherwise entirely unrelated in sequence (Argos *et al.*, 1981; Muller-Hill, 1983).

High-resolution X-ray crystal structures of six periplasmic binding proteins have now been determined (L-arbinase, D-galactose, maltose, sulfate, isoleucine/leucine/valine, and leucine) and, despite the different substrates handled, all are structurally related (reviewed in Adams and Oxender, 1989; Quiocho, 1990). The proteins are ellipsiodal with an axial ratio of about 2 : 1, consisting of two similiar, globular domains with a cleft between which forms the substrate binding site. Each domain contains residues from the N- and C-terminal segments of the polypeptide chain, with the chain crossing three times between domains. The domains are composed of three α-helices surrounding an array of five β-sheets, somewhat reminiscent of the Rossmann nucleotide binding fold (Rossmann, 1975). Upon binding the substrate, the cleft closes around it, removing the substrate from the solvent. This displacement of ordered water of hydration is entropically favorable, perhaps facilitating interaction between the substrate and the transport protein complex within the membrane. Remarkably, whatever the type of substrate (i.e., charged, hydrophobic, etc.), it is primarily bound by the protein via hydrogen bonds (Quiocho, 1986; Pflugrath and Quiocho, 1988). Although unexpected, this finding is consistent with the fact that all the proteins bind substrates with similar, high affinities (K_d around 0.1–1.0 μM).

Importantly, the *in vitro* binding specifities and affinities measured for the purified proteins correspond well with *in vivo* characteristics of the transport process, implying that the binding provides the rate-limiting step for transport (Miller *et al.*, 1983).

Although the overall structures of the various binding proteins are similar, some of the proteins exhibit unique features. For example, the galactose-binding protein has a bound calcium ion, although the physiological role of calcium, if any, is obscure (Vyas *et al.*, 1987). Some of the binding proteins bind large substrates, presumably recognizing only a portion of them with the rest of the substrate "hanging out" of the binding cleft. Thus, the maltose binding protein can bind oligosaccharides of up to seven sugar residues, while the oligopeptide binding protein (OppA) will bind more or less any peptide from two to six amino acid residues with little regard to the constituent amino acids. There is evidence from *in vivo* (Goodell and Higgins, 1987) and *in vitro* studies (Guyer *et al.*, 1986) that the OppA protein has two substrate-binding sites with somewhat different specificities. Coupled with the fact that OppA is considerably larger than any of the other periplasmic binding proteins, it may turn out to be different in structure from the other periplasmic proteins. Although crystals of OppA have been obtained (Tolley *et al.*, 1988), a structure is not yet available.

Upon binding substrate, the periplasmic binding protein undergoes a conformational change, trapping the substrate in the cleft (Mao *et al.*, 1982; Sach *et al.*, 1989). This conformational change enables the binding protein to interact with the complex of membrane proteins; it is generally believed, though not formally proven, that this interaction cannot occur in the absence of bound substrate. The interaction of the binding protein with the membrane complex appears to involve both of the hydrophobic membrane proteins and both domains of the periplasmic protein (Treptow and Shuman. 1985, 1988; Kossman *et al.*, 1988).

What is the role of the periplasmic components? While they are normally required for the transport systems with which they are associated, binding proteins are not integral to the process of solute translocation across the membrane. Thus, mutants can be selected which allow solute transport in the absence of the periplasmic protein (Shuman, 1982a); in these cases specificity is imposed by the membrane components. The eukaryotic equivalents of this class of transporter also lack a periplasmic equivalent, as do those bacterial systems which export rather than import. Thus, the periplasmic protein is best considered as an accessory component, a rather specific adaptation required by this group of bacterial uptake systems. The reason these proteins are required is not yet known although many possibilities have been suggested. Hengge and Boos (1983) have presented persuasive arguments against two of the most usual explanations, that the binding proteins increase the effective

concentration of substrate in the periplasm or that they can enhance the affinity of otherwise binding protein-independent transport systems. It is conceivable that binding proteins may facilitate passage of substrate across the periplasm (Brass *et al.*, 1986). The periplasm is a gel-like matrix and proteins diffuse slowly within this compartment (Hobot *et al.*, 1984; Brass *et al.*, 1986; Foley *et al.*, 1989). As most of the water in the periplasm may be sequestered as water of hydration, diffusion through the periplasm at low substrate concentrations could become rate limiting, and the binding proteins may facilitate movement across the periplasm by passing substrate from protein to protein until it reaches the membrane complex (Brass *et al.*, 1986). There is some evidence that binding proteins do interact with each other, and that this may affect substrate binding affinities (Rasched *et al.*, 1976; Richarme, 1982; Mowbray and Petsko, 1983).

An alternative possibility is that the binding proteins may be an adaptation required for capturing and retaining substrate; bacteria experience major fluctuations in substrate concentrations and often have to scavenge from very dilute solutions. This is different from export systems or eukaryotic cells where such fluctuations in substrate concentrations are unlikely. As one of the major roles of the binding protein systems is to recapture substrates which leak from the cell (Stirling *et al.*, 1989), a mechanism for retaining substrate in the vicinity of the cell and preventing it from diffusing into the environment may be available.

The unexpected identification of substrate-binding protein equivalents in Gram-positive bacteria (see below), which lack an outer membrane and therefore a periplasm, does not immediately fit with these models. However, as a dense matrix equivalent to a periplasm exists around Gram-positive bacteria, the same requirement for a periplasm protein might exist. The sequences of the amino termini of the Gram-positive binding proteins suggest that they may be lipoproteins, and hence be anchored to the membrane. Thus, their movement will be limited to two dimensions (even in Gram-negative bacteria the dimensions of the periplasm are such that most movement is principally lateral and therefore effectively in two dimensions, Hobot *et al.*, 1984); it may simply be the means of preventing their loss from the cell which differs between Gram-positive and Gram-negative species.

The Hydrophobic Integral Membrane Domains

Two highly hydrophobic, integral membrane proteins are essential components of each transport system. A comparison of the sequences of the two integral membrane components from a single system (e.g., OppB with OppC; HisQ with HisM), or the two domain of Mdr, reveals significant

sequence similarity. 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, Fig. 1) (Dudler *et al.*, 1988). Similarly, in the Mdr transport system from eukaryotes (Fig. 1) the equivalents of these two hydrophobic proteins are encoded as separate, but closely related domains of a single polypeptide. The archetypal transport system, therefore, appears to require two similar hydrophobic domains encoded either as two separate polypeptides or as part of a larger, multidomain protein.

Comparison of these proteins between systems reveals little sequence similarity, although all are highly hydrophobic and seem to be structurally related. Each protein consists of a core structure of six potential membrane-spanning α-helices (Hiles *et al.*, 1987) separated by short stretches of hydrophilic sequence. Intriguingly, a total of twelve membrane-spanning helices seems to be a recurring feature of active transporters (Maloney, 1990). One of these hydrophilic stretches is conserved in many, if not all, of these proteins (Dassa and Hofnung, 1985). This sequence is probably exposed to the cytoplasmic face of the membrane and may interact with the peripherally located ATP-binding components (unpublished results). There are, of course, apparent departures from this generalization. For example, the MalF protein is considerably larger than the "typical" integral membrane component (Froshauer and Beckwith, 1984) although it can be considered as a "typical" component with an additional N-terminal domain of unknown function. Similarly, although the arabinose transport system 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. 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 of twelve (two pairs of six) trans-membrane helices.

Although there is no structural evidence to confirm the existence of the predicted membrane-spanning helices, 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 showing that these proteins interact with the binding proteins at the periplasmic face of the membrane (Treprow and Schuman, 1985; Prossnitz *et al.*, 1988) and with the ATP-binding proteins at the cytoplasmic face of the membrane (Shuman and Silhavy, 1981). Genetic data obtained with Tn*phoA* fusions also support the model that the MalF protein spans the membrane several times and adopts the organization predicted from hydropathy profiles (Boyd *et al.*, 1987), and

protease accessibility studies (S. R. Pearce and C. F. Higgins, unpublished data) are entirely consistent with this model.

The hydrophobic membrane-associated components are assumed to be responsible for mediating translocation of substrate across the lipid bilayer. However, the mechanisms by which this is achieved are obscure. We have no idea how putative the helices are arranged in three dimensions within the membrane. Attempts to purify these proteins have 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 makes sense since, if specificity were conferred by the periplasmic component alone, one would inagine that the different periplasmic proteins could deliver substrate to a single complex of membrane proteins; there would not need to be a unique complex of membrane-associated components for each substrate. The first line of evidence to support this view was the isolation of mutations that alter substrate specificity, and which map to the genes encoding the hydrophobic membrane components (Higgins *et al.*, 1982; Payne *et al.*, 1985). The clearest evidence, however, comes from studies on the maltose transport system (Schuman, 1982a; Treptow and Schuman, 1985; Reyes *et al.*, 1986). Normally, transport cannot 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 is relatively specific, the implication is that the MalF and MalG proteins (separately or together) must themselves possess a specific substrate-binding site. Nevertheless, the location of the substrate-binding sites on these proteins, and an understanding of their role, awaits detailed structural analysis of the proteins.

The ATP-Binding Domains

Besides the hydrophobic, integral membrane components, each binding protein-dependent transport system requires the function of one or two hydrophilic membrane proteins. Unlike the periplasmic proteins and the hydrophobic membrane components, these proteins share extensive sequence similarity (ca. 30% sequence identity over their entire length), regardless of the system with which they are associated, and presumably share a common evolutionary origin (Higgins *et al.*, 1985, 1986, 1988). These proteins bind ATP and couple ATP hydrolysis to the transport process (see below). For the oligopeptide permease, two such proteins (OppD and OppF) are required, each possessing an ATP-binding site (Hiles *et al.*, 1987). In the ribose system

the equivalents of these two polypeptides are fused into a single, two-domain protein (Buckel *et al.*, 1986). Similarly, for eukaryotic systems such as Mdr and CFTR two ATP-binding domains are encoded as a single large polypeptide (Gros *et al.*, 1986; Riordan *et al.*, 1989). Thus, two ATP-binding domains appear to be required for this class of transport system either as separate proteins or as one multidomain polypeptide. As discussed above, several transport systems require one gene encoding an ATP-binding polypeptide; it seems reasonable to suppose that in these systems two molecules of the polypeptide 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 hydrolyzed per transport event (Mimmack *et al.*, 1989; see below).

The ATP-binding proteins from several different transport systems are associated with the cytoplasmic membrane (see Ames, 1986; Gallagher *et al.*, 1989; Higgins *et al.*, 1990 for more detailed discussions). As the proteins are hydrophilic and contain no potential membrane-spanning helices, they are assumed to be peripherally associated with the cytoplasmic face of the membrane, compatible with their proposed role in coupling ATP hydrolysis to transport. However, the only direct evidence for this comes from recent studies on the OppF protein which show it to be accessible to proteases only from the cytoplasmic face of the membrane (Gallagher *et al.*, 1989). Although such a location is generally accepted, two anomalies remain. First, the hydrophilic 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). 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 and Silhavy, 1981). Second, genetic data based on suppressor mutations indicate an interaction between HisP and the periplasmic HisJ protein (Ames and Spudich, 1976), implying that HisP spans the membrane and that at least part of the protein is exposed to the periplasm. Although it is now considered unlikely that HisP spans the membrane, this anomaly has not been resolved.

Energetics of Transport

Structure of ATP-Binding Cassette

The ATP-binding proteins of periplasmic transport systems have, like other membrane proteins, proved difficult to analyze biochemically although some progress has been made (Hobson *et al.*, 1984; Higgins *et al.*, 1985; Gallagher *et al.*, 1989). Some of these proteins can now be overproduced and purified to homogeneity (unpublished data) although no crystallographic

data have yet been obtained. One important point is that the ATP-binding components of the ABC transporters share considerable sequence similarity besides the short ATP-binding motif itself, extending over an entire domain of about 200 amino acids residues (Higgins *et al.*, 1986). Furthermore, closely related ABC proteins (e.g., FtsE, NodI, UvrA) are not necessarily associated with transport processes at all. This extended sequence similarity must reflect function over and above the binding and hydrolysis of ATP, presumably involving domain–domain interactions transmitting conformational changes resulting from ATP hydrolysis (see below) to whichever biological process ABC protein is associated with. A deeper understanding of the molecular mechanisms involved is unlikely to be forthcoming until three-dimensional structures of one or more of the proteins are available. In the absence of crystallographic data, we have constructed a tertiary structure model for the ATP-binding cassette based on multiple sequence alignments and a "knowledge-based" approach (Hyde *et al.*, 1990). The structure is based on

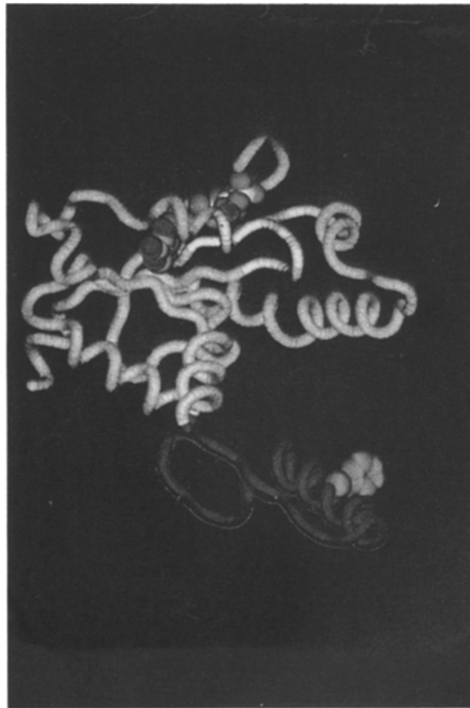

Fig. 2. Tertiary structure model of the ATP-binding cassette. For details see Hyde *et al.* (1990). Loop 2 (see text) is shaded. The location of ATP is shown with space-filling atoms. The space-filling atoms in loop 2 show the phenylalanine residue deleted in 70% of cystic fibrosis patients.

that of adenylate kinase which has been determined crystallographically. While only a model, there are many reasons for believing that this structure is correct in its essence, providing a solid basis for further experiments (Fig. 2). One of the most important features of the model is the finding that the ABC protein can be modelled closely on adenylate kinase with the exception of loop 2. This loop protrudes from the core ATP binding/hydrolysis structure and is inappropriately positioned to play any role in the binding or hydrolysis of ATP. We have proposed (Hyde *et al.*, 1990) that this loop interacts with the membrane-associated transport components/domains and serves to couple conformational changes to the other subunits, facilitating transport. Various aspects of the model structure are consistent with this view. Intriguingly, most cystic fibrosis patients are deleted for one amino acid in this loop Phe508 (Riordan *et al.*, 1989). This mutation may alter the interaction between domains resulting in the cystic fibrosis phenotype. This is consistent with the observation that cystic fibrosis requires specific mutations rather than complete loss of function of the CFTR protein (a mutation preventing ATP-binding would presumably completely inactive the protein). Other CF mutations have yet to be identified although, on the basis of data presented here, we predict they will either alter loop 2 of an ABC domain, sequences playing a structural role linking loop 2 to the ATP-binding site, or the cytoplasmic loop(s) of the membrane domains with which the ABC cassette interacts.

Berger and Heppel (1974), using metabolic inhibitors, first distinguished the energetic requirements or binding protein-dependent transport systems from those of other classes of transporter. The inference drawn from these seminal studies was that binding protein-dependent transport systems are most probably energized directly by ATP hydrolysis. There is now little doubt that Berger and Heppel were correct, although until recently there has been considerable controversy over the energetic requirements of these transporters (reviewed by Higgins, 1990b). Two principal arguments have brought into question the role of ATP as the energy source for binding protein-dependent transport systems. First, experimentally induced reductions in the cytoplasmic ATP pools did not necessarily affect the rate of transport (Plate *et al.*, 1974; Lieberman and Hong, 1976; Ferenci *et al.*, 1977). Second, perturbation of the electrochemical gradient inhibited transport without necessarily altering ATP pools (Plate, 1979; Singh and Bragg, 1979; Hunt and Hong, 1983; Ames, 1986). Although these studies implicate the electrochemical gradient in the energization of transport (Ames, 1986), purely thermodynamic consideration preclude the electrochemical gradient from supporting the high degree of substrate accumulation observed for this class of transport system (Hengge and Boos, 1983). Furthermore, proton movement cannot be detected during substrate transport by binding protein systems (Darawalla *et al.*,

(1981). Thus if the electrochemical gradient plays any role, it must be indirect, and recent vesicle experiments (Bishop *et al.*, 1989) provide strong evidence that the electrochemical gradient plays no role at all in binding protein-dependent transport. Consequently, a variety of alternative energy sources have been proposed, including acetyl phosphate (Hong *et al.*, 1979), NADPH (Gilson *et al.*, 1982), lipoic acid (Richarme, 1985; Richarme and Heine, 1986), and succinate (Hunt and Hong, 1983). No convincing evidence in favor of one or other of these alternatives has been obtained, and many of the results which are apparently incompatible with a role for ATP can now be adequately explained (see below).

The first direct evidence of a role for ATP came from the identification of consensus ATP-binding motifs on subunits of the oligopeptide, histidine, and maltose transporters (Higgins *et al.*, 1985). This motif is found in many nucleotide binding proteins, forming part of the ATP-binding pocket (Walker *et al.*, 1982), and is conversed on the equivalent components of all other transport systems in the ABC superfamily, prokaryotic or eukaryotic, which have subsequently been characterized. Biochemical evidence for ATP-binding was subsequently demonstrated for the OppD, HisP, and MalK proteins of the oligopeptide, histidine, and maltose transport systems, respectively, using affinity columns and a variety of ATP analogues (Hobson *et al.*, 1984; Higgins *et al.*, 1985). More recently, the eukaryotic Mdr protein has also been shown to bind ATP affinity analogues, and mutations in the ATP-binding site inhibit its function (Azzoria *et al.*, 1989). Besides binding ATP, ATP is essential for the function for these transporters. In vesicle systems an absolute ATP requirement for maltose and histidine transport in *E. coli*, and drug transport by the human Mdr protein, has been demonstrated (Dean *et al.*, 1989; Prossnitz *et al.*, 1989; Horio *et al.*, 1988). Nevertheless, the demonstration that these transport proteins bind and require ATP does not necessarily mean that ATP is hydrolyzed or that ATP provides the energy source for transport. There are many precedents for bound nucleotides serving a purely structural or regulatory role. To overcome such objections unambiguously, ATP hydrolysis by the purified transport proteins must be demonstrated. This has not yet been possible for the ATP-binding components of the bacterial transporters, presumably because ATP hydrolysis requires the presence of the other subunits. However, the fact that the closely related UvrA protein hydrolyzes ATP (Seeberg and Steinum, 1982; Doolittle *et al.*, 1986) suggests that these subunits do have the potential to catalyze ATP hydrolysis. There is also some evidence that purified Mdr protein can hydrolyze ATP (Hamada and Tsuruo, 1988). Further support for a role for ATP hydrolysis comes from evidence that nonhydrolyzable ATP analogues inhibit transport (Ames *et al.*, 1989).

The most compelling evidence comes from two recent studies which provide direct evidence that ATP is hydrolyzed during transport (Bishop *et al.*, 1989; Mimmack *et al.*, 1989). In the first study Ames, Maloney, and their co-workers developed an *in vitro* procedure for reconstituting partially purified histidine transport complexes into liposomes (Bishop *et al.*, 1989). In these vesicles, ATP hydrolysis could be measured and shown to be totally dependent upon the transport of histidine across the vesicle membrane. In the other study, Mimmack *et al.* (1989) demonstrated ATP hydrolysis *in vivo* during the transport of maltose, peptides, and glycine betaine. The use of appropriate mutations allowed an unambiguous demonstration that ATP consumption is dependent on transport and not metabolism. Such ATP consumption was not observed for transport systems (e.g., the major proline transporter, PutP) which is linked to the electrochemical gradient. Furthermore, when cells were depleted of ATP, transport could immediately be restored by addition of phosphoenol pyruvate which allowed regeneration of ATP. Thus, there is no doubt that ATP is hydrolyzed and can provide the primary source of energy for these transporters. It is still a formal possibility that the high-energy phosphate bond from ATP is transferred to an intermediate compound which then interacts with the transport proteins, although this seems highly unlikely in view of studies (described above) in which direct ATP binding has been demonstrated.

Stoichiometry of ATP Hydrolysis

The stoichiometry of ATP hydrolysis has been estimated, somewhat indirectly from growth yields on different substrates, to be 1.0–1.2 molecules of ATP hydrolyzed per molecule of substrate transported (Muir *et al.*, 1985). More direct measurements in membrane vesicles gave a stoichiometry of five ATP molecules hydrolyzed per histidine molecule transported (Bishop *et al.*, 1989). However, this cannot represent the true stoichiometry *in vivo*; at a stoichiometry of 5 : 1 more ATP would be consumed transporting maltose into the cell than could be generated from it by anaerobic metabolism (yet *E. coli* can grow anaerobically on maltose!). Presumably there is a degree of uncoupling or leakiness in the reconstituted vesicles. The stoichiometry of ATP hydrolysis determined *in vivo*, in experiments in which substrate transport was measured simultaneously with the decrease in cytoplasmic ATP pools (Mimmack *et al.*, 1989), gave a value of close to two molecules of ATP hydrolyzed per molecule of substrate transported (for the maltose and glycine betaine transport systems). While the experiments did not allow a rigorous demonstration that the stoichiometry is not actually 1 : 1, a stoichiometry of 2 : 1 is consistent with the fact that many, if not all, binding protein-dependent transport systems require the function of two ATP-binding domains (Higgins *et al.*, 1986). Two ATP-binding sites are also present on

other classes of carrier, such as the arsenate transporter (Chen *et al.*, 1986), and may be a general mechanistic requirement. A stoichiometry of 2 appears inefficient but may be the penalty to be paid for the ability to concentrate substrate against very large gradients.

Explanation of Data Which Apparently Contradict the ATP Model

Two lines of evidence have been used as arguments against a role for ATP. Explanations of these data can now be provided. First, the finding that transport rates do not necessarily correlate with the size of the cytoplasmic ATP pool is not, of course surprising if the affinity of the transport proteins for ATP is sufficiently high that they are normaly saturated. This appears to be the case as the apparent K_m of the histidine transport system for ATP is about 100 μM (Ames *et al.*, 1989) while the cytoplasmic ATP pools of growing cells are around 5 mM (Kashket, 1982). Second, perturbation of the electrochemical gradient has been found to inhibit binding protein-dependent transport systems. While this was originally thought to imply a specific role for the electrochemical gradient, it is now clear that the effect was probably an indirect effect of changes in intracellular pH; this class of transporter is highly sensitive to intracellular pH (Driessen *et al.*, 1987; Poolman *et al.*, 1987; Joshi *et al.*, 1989).

One key remaining question is the mechanism by which ATP hydrolysis drives substrate accumulation. There is no evidence that any of the transport proteins are phosphorylated (Ames and Nikaido, 1981; unpublished results) although these are, of course, negative results. Nevertheless, it is unlikely that phosphorylation would have been missed. It therefore seems probable that ATP hydrolysis induces a conformational change in the ATP-binding subunit which is transmitted, via protein–protein interactions, to the trans-membrane subunits which mediates passage across the membrane. Whether hydrolysis occurs concomitant with transport, occurs after transport to reset the system, or is involved in some other step has never been addressed experimentally.

Related Transport Systems in Gram-Positive Bacteria and Eukaryotic Cells

Binding protein-dependent transport systems were, until recently considered to be restricted to Gram-negative bacteria and principally studied *E. coli* and *S. typhimurium*. However, closely related systems are known in other Gram-negative species (e.g., a branched-chain amino acid system of *Pseudomonas* closely related to that of *E. coli*, Hoshino and Kato, 1989). However, it is now clear that binding protein-dependent transport systems are also present in Gram-positive bacteria. Sequences highly homologous to the periplasmic maltose (MalE) and oligopeptide (OppA) binding proteins of

E. coli have been identified in *Streptococcus pneumoniae* (named MalX and AmiA; Gilson *et al.*, 1988). In the case of the Ami system, equivalents of all the other membrane components are also present (Alloing *et al.*, 1990). An entire Opp operon, which has been shown to transport peptides, including a periplasmic protein, is also present in *Bacillus subtilis* (Perego *et al.*, in preparation). An operon of genes encoding proteins characteristic of components of a binding protein-dependent transport system has also been identified in *Mycoplasma* (Dudler *et al.*, 1988) although, in this case, function is unknown. 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. The N-terminal sequences of the protein have a signal peptide, implying export from the cell, and for the *Bacillus* system this has been demonstrated (Perego *et al.*, in preparation). Once the signal peptide is removed, the amino terminus is typical of a lipoprotein and it seems likely that the proteins are attached to the outer face of the cytoplasmic membrane via a lipid moiety.

For many years the binding protein-dependent transport systems of Gram-negative bacteria were generally considered to play a straightforward 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. In *E. coli* the Opp system is required for recycling of cell-wall components as well as serving a normal nutritional role (Goodell and 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, resistance to various antibiotics, and a decrease in membrane potential (Alloing *et al.*, 1990). Mutations in the Opp system of *B. subtilis* result in a sporulation-defective phenotype (J. Hoch, personal communication). In *Mycoplasma*, a binding protein-dependent transport system of unknown substrate specificity enhances invasivity of mouse sarcoma cells in culture (Dudler *et al.*, 1988), although the processes by which *Mycoplasma* proteins interact with and influence mammalian cells is completely obscure.

Transport systems similar to the periplasmic binding protein system have also been identified in several eukaryotic cells (Table I; see Ames, 1987; Higgins, 1989, 1990a). The first example identified was the P-glycoprotein from mammalian cells (Endicott and Ling, 1989). Over-expression of this protein is responsible for conferring multiple drug resistance upon tumors, responsible for a serious clinical problem. The P-glycoprotein is a large polypeptide consisting of four domains equivalent to the two hydrophobic and two ATP-binding components of a bacterial periplasmic transport system. In the ATP-binding domains the sequence similarities between the

eukaryotic and prokaryotic proteins is as great as between any two prokaryotic proteins. The Mdr protein has been demonstrated to be an ATP-dependent transport system, expelling drugs from the cell (Horio *et al.*, 1988) and, as discussed above, there is no evidence for an equivalent of a periplasmic protein associated with Mdr. Although Mdr pumps drugs out of cells, its normal, physiological substrate remains unknown. Furthermore, one or two (depending on species) other highly homologous *mdr* genes are present in mammalian cells; amplification of these genes has no observable phenotype, and their roles remain obscure. An Mdr homologue of Mdr has been identified in *Plasmodium* (the malaria parasite) and a similar export mechanism and may play a role in chloroquine resistance (Foote *et al.*, 1989). Other ABC transporters from eukaryotes now include the STE-6 protein of yeast (responsible for a-factor mating phenomenon export; McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989), the *Drosophilia white* and *brown* loci which deposit eye pigments, and the product of the cystic fibrosis locus whose cellular role or transported substrate (if any) remains unknown (Riordan *et al.*, 1989).

An increasing number of ABC transporters are associated with export, including HlyB and closely related proteins which have been shown to mediate specific export of a variety of proteins from the cell (Blight and Holland, 1990). The Bex system of *Haemophilus* (Kroll *et al.*, 1988) exports polysaccharides from the cytoplasm, and the MsrA system of *Staphylococcus* exports various antibiotics and is responsible for conferring resistance (Ross *et al.*, 1990). This is an especially intriguing system as no hydrophobic domains are associated with the two ATP-binding domains of the protein; it presumably confers antibiotic resistance by interacting with membrane components of a system present for "other purposes." Such sequestration of subunits has intriguing implications for the determination of substrate specificity. Thus, the ABC transporters can import (although any one system appears to function in only one direction) and can be specific for any of a wide range of substrates ranging from inorganic ions to proteins. Although it appears that some exporters may be more closely related to each other than to importers (Gerlach *et al.*, 1986; Blight and Holland, 1990), it is still not possible to predict from sequence alone whether a particular system is an importer or an exporter or what its substrate specificity will be. Given the widespread occurrence and diverse functions of the ABC transporters as well as the critical medical importance of some (multidrug resistance and cystic fibrosis), they promise much excitement for many years to come.

Acknowledgments

We are grateful to the Imperial Cancer Research Fund for financial support.

References

Adams, M. D., and Oxender, D. L. (1989). *J. Biol. Chem.* **264**, 15739–15742.
Albright, L. M., Ronson, C. W., Nixon, B. T., and Ausubel, F. M. (1989). *J. Bacteriol* **171**, 1932–1941.
Alloing, G., Trombe, M-C., and Claverys, J-P. (1990). *Mol. Microbiol.* **4**, 633–644.
Ames, G. F-L. (1985). *Curr. Top. Membr. Transport* **23**, 103–119.
Ames, G. F-L. (1986). *Ann. Rev. Biochem.* **55**, 397–425.
Ames, G. F-L. (1987). *Cell* **47**, 323–324.
Ames, G. F-L., and Nikaido, K. (1981). *Eur. J. Biochem.* **115**, 525–531.
Ames, G. F-L., and Spudich, E. N. (1976). *Proc. Natl. Acad. Sci. USA* **73**, 1877–1881.
Ames, G. F-L., Nikaido, K., Groarke, J., and Petithory, J. (1989). *J. Biol. Chem.* **264**, 3998–4002.
Argos, P., Mahoney, W. C., Hermodson, M. A., and Hanei, M. (1981). *J. Biol. Chem.* **256**, 4357–4361.
Azzaria, M., Schurr, E., and Gros, P. (1989). *Mol. Cell. Biol.* **9**, 5289–5297.
Bell, A. W., Buckel, S. D., Groarke, J. M., Hope, J. N., Kingsley, D. H., and Hermodson, M. A. (1986). *J. Biol. Chem.* **261**, 7652–7658.
Berger, E. A. (1973). *Proc. Natl. Acad. Sci. USA* **70**, 1514–1518.
Berger, E. A., and Heppel, L. A. (1974). *J. Biol. Chem.* **249**, 7747–7755.
Bishop, L., Agbayani, R., Ambudkar, S. V., Maloney, P. C., and Ames, G. F-L. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 6953–6957.
Blight, M. A., and Holland, I. B. (1990). *Mol Microbiol.* **4**, in press.
Boyd, D., Manoil, C., and Beckwith, J. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 8525–8529.
Brass, J. M., Higgins, C. F., Foley, M., Rugman, P. A., Birmingham, J., and Garland, P. B. (1986). *J. Bacteriol.* **165**, 787–794.
Buckel, S. D., Bell, A. W., Rao, J. K. M., and Hermodson, M. A. (1986). *J. Biol. Chem.* **261**, 7659–7662.
Cangelosi, G. A., Martinetti, G., Leigh, J. A., Lee, C. C., Theines, C., and Nester, E. W. (1989). *J. Bacteriol.* **171**, 1609–1615.
Chen, C., Misra, T., Silver, S., and Rosen, B. P. (1986a). *J. Biol. Chem.* **261**, 15030–15038.
Chen, C-J., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M., and Roninson, I. B. (1986b). *Cell* **47**, 381–389.
Coulton, J. W., Mason, P., and Allatt, D. D. (1987). *J. Bacteriol.* **169**, 3844–3849.
Darawalla, K. R., Paxton, T., and Henderson, P. J. F. (1981). *Biochem. J.* **200**, 611–627.
Dassa, E., and Hofnung, M. (1985). *EMBO J.* **4**, 2287–2293.
Dean, D. A., Fikes, J. D., Gehring, K., Bassford, P. J., and Nikaido, H. (1989). *J. Bacteriol.* **171**, 503–510.
Doolittle, R. F., Johnson, M. S., Hussain, I., van Houton, B., Thomas, D. C., and Sancar, A. (1986). *Nature* **323**, 451–453.
Dreesen, T. D., Johnson, D. H., and Henikoff, S. (1988). *Mol. Cell. Biol.* **8**, 5206–5215.
Driessen, A. J. M., Kodde, J., De Jong, S., and Konings, W. N. (1987). *J. Bacteriol.* **169**, 2748–2754.
Dudler, R., Schmidhauser, C., Parish, R. W., Wettenhall, R. E. H., and Schmidt, T. (1988). *EMBO J.* **7**, 3963–3970.
Endicott, J. A., and Ling, V. (1989). *Annu. Rev. Biochem.* **58**, 137–171.
Evans, I. J., and Downie, J. A. (1986). *Gene* **43**, 95–101.
Felmlee, T., Pellett, S., and Welch, R. A. (1985). *J. Bacteriol.* **163**, 94–105.
Ferenci, T., Boos, W., Schwartz, M., and Szmelcman, S. (1977). *Eur. J. Biochem.* **75**, 187–193.
Foley, M., Brass, J. M., Birmingham, J., Cook, W. R., Garland, P. B., Higgins, C. F., and Rothfield, L. E. (1989). *Mol. Microbiol.* **3**, 1329–1336.
Foote, S. J., Thompson, J. K., Conman, A. F., and Kemp, D. J. (1989). *Cell* **57**, 921–930.
Friederich, M. J., de Veaux, L. C., and Kadner, R. J. (1986). *J. Bacteriol.* **167**, 928–934.
Froshauer, S., and Beckwith, J. (1984). *J. Biol. Chem.* **259**, 10896–10903.
Furlong, C. E. (1987). In *Escherichia coli and Salmonella typhimurium* (Neidhart, F. C., ed.), ASM Press, Washington, pp. 768–796.

Gallagher, M. P., Pearce, S. R., and Higgins, C. F. (1989). *Eur. J. Biochem.* **180**, 133–141.
Gerlach, J. H., Endicott, J. A., Juranka, P. F., Henderson, G., Sarangi, F., Deuchars, K. L., and Ling, V. (1986). *Nature (London)* **324**, 425–489.
Gill, D. R., Hatfull, G. F., and Salmond, G. P. C. (1986). *Mol. Gen. Genet.* **205**, 134–145.
Gilson, E., Nikaido, H., and Hofnung, M. (1982). *Nucleic Acids Res.* **10**, 7449–7458.
Gilson, E., Alloing, G., Schmidt, T., Claverys, J-P., Dudler, R., and Hofnung, M. (1988). *EMBO J.* **7**, 3971–394.
Glaser, D. J. (1988). *EMBO J.* **7**, 3997–4004.
Goodell, E. W., and Higgins, C. F. (1987). *J. Bacteriol.* **169**, 3861–3865.
Gowrishankar, J. (1989). *J. Bacteriol.* **171**, 1923–1931.
Gros, P., Croop, J., and Housman, D. (1986). *Cell* **47**, 371–380.
Guyer, C. A., Morgan, D. G., and Staros, J. V. (1986). *J. Bacteriol.* **168**, 775–779.
Hasmada, H. and Tsuruo, T. (1988). *J. Biol. Chem.* **263**, 1454–1458.
Hengge, R. and Boos, W. (1983). *Biochim. Biophys. Acta* **737**, 443–478.
Higgins, C. F. (1989). *Nature (London)* **340**, 342.
Higgins, C. F. (1990a). *Nature (London)* **341**, 103.
Higgins, C. F. (1990b). *Res. Microbiol.*, in press.
Higgins, C. F., and Ames, G. F-L. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 6038–6042.
Higgins, C. F., Haag, P. D., Nikaido, K., Ardeshir, F., Garcia, G., and Ames, G. F-L. (1982). *Nature (London)* **298**, 723–727.
Higgins, C. F., Hiles, I. D., Whalley, K., and Jamieson, D. J. (1985). *EMBO J.* **4**, 1033–1040.
Higgins, C. F., Hiles, I. D., Salmond, G. P. C., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W., and Hermodson, M. A. (1986). *Nature (London)* **323**, 448–450.
Higgins, C. F., Gallagher, M. P., Mimmack, M. L., and Pearce, S. R. (1988). *Bio Essays* **8**, 111–116.
Higgins, C. F., Gallagher, M. P., Hyde, S. C., Mimmack, M. L., and Pearce, S. R. (1990). *Philos. Trans. R. Soc. London B.* **326** 353–365.
Hiles, I. D., and Higgins, C. F. (1986). *Eur. J. Biochem.* **158**, 561–567.
Hiles, I. D., Gallagher, M. P., Jamieson, D. J., and Higgins, C. F. (1987). *J. Mol. Biol.* **195**, 125–142.
Hobot, J. A., Carleman, E., Villiger, W., and Kellenberger, E. (1984). *J. Bacteriol.* **160**, 143–152.
Hobson, A. C., Weatherwax, R., and Ames, G. F-L. (1984). *Proc. Natl. Acad. Sci. USA* **81**, 7333–7337.
Hong, J-S., Hunt, A. G., Masters, P. S., and Lieberman, M. A., 1979). *Proc. Natl. Acad. Sci. USA* **76**, 1213–1217.
Horio, M., Gottesman, M. M., and Pastan, I. (1988). *Proc. Natl. Acad. USA* **85**, 3580–3584.
Hoshino, T., and Kose, K. (1989). *J. Bacteriol.* **171**, 6300–6306.
Hunt, A. G., and Hong, J-S. (1983). *Biochemistry* **22**, 844–850.
Husain, I., Houten, B. V., Thomas, D. C., and Sandcar, A. (1986). *J. Biol. Chem.* **261**, 4895–4901.
Hyde, S. C., Elmsley, P., Hartshorn, M., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Hubbard, R., and Higgins, C. F. (1990). *Nature (London)* , submitted.
Johann, S., and Hinton, S. M. (1987). *J. Bacteriol.* **169**, 1911–1916.
Joshi, A. K., Ahmed, S., and Ames, G. F.-L. (1989). *J. Biol. Chem.* **264**, 2126–2133.
Kashket, E. (1982). *Biochemistry* **21**, 5534–5538.
Kossman, M., Wolff, C., and Manson, M. D. (1988). *J. Bacteriol.* **170**, 4516–4521.
Kroll, J. S., Hopkins, I., and Moxon, E. R. (1988). *Cell* **53**, 347–356.
Kuchler, K., Sterne, R. E., and Thorner, J. (1989). *EMBO J.* **8**, 3973–3984.
Landick, R., and Oxender, D. L. (1985). *J. Biol. Chem.* **260**, 8257.–8261.
Lieberman, M. A., and Hong, J-S. (1976). *Arch Biochem. Biophys.* **172**, 312–315.
Maloney, P. C. (1990). *Res. Microbiol*, in press.
Mao, B., Pear, M. R., McCammon, J. A., and Quiocho, F. A. (1982). *J. Biol. Chem.* **257**, 1131–1133.
McGrath, J. P., and Varshavsky, A. (1989). *Nature (London)* **340**, 400–404.
Miller, D. M., Olson, J. S., Pflugrath, J. W., and Quiocho, F. A. (1983). *J. Biol. Chem.* **258**, 13665–13672.

Mimmack, M. L., Gallagher, M. P., Hyde, S. C., Pearce, S. R., Booth, I. R., and Higgins, C. F. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 8257–8261.
Mowbray, S. L., and Petsko, G. A. (1983). *J. Biol. Chem.* **258**, 7991–7997.
Muir, M., Williams, C., and Ferenci, T. (1985). *J. Bacteriol.* **163**, 1237–1242.
Muller-Hill, B. (1983). *Nature* (*London*) **302** 163–164.
O'Hare, K., Murphy, C., Levis, R., and Rubin, G. M. (1984). *J. Mol. Biol.* **180**, 437–455.
Ohyama, K., Fukuzana, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H., and Ozerki, H. (1986). *Nature* (*London*) **322**, 572–574.
Overduin, P. Boos, W., and Tommassen, J. (1988). *Mol. Microbiol.* **2**, 767–775.
Payne, G., Spudich, E. N., and Ames, G. F.-L. (1985). *Mol. Gen. Genet.* **200**, 493–496.
Pflugrath, J. W., and Quiocho, F. A. (1988). *J. Mol. Biol.* **200**, 163–180.
Plate, C. A. (1979). *J. Bacteriol.* **137**, 221–225.
Plate, C. A., Suit, J. L., Jettern, A. M., and Luria, S. E. (1974). *J. Biol. Chem.* **249**, 6138–6143.
Poolman, B., Hellingwerf, K. J., and Konnings, W. N. (1987). *J. Bacteriol.* **169**, 2272–2276.
Prossnitz, E., Nikaido, K., Ulrich, S., and Ames, G. F.-L. (1988). *J. Biol. Chem.* **324**, 17917–17920.
Prossnitz, E., Gee, A., and Ames, G. F.-L. (1989). *J. Biol. Chem.* **264**, 5006–5014.
Quiocho, F. A. (1986). *Annu. Rev. Biochem.* **55**, 287–316.
Quiocho, F. A. (1990). *Philos. Trans. R. Soc. Landon. B*, **326**, 341–351.
Rasched, J., Schuman, H., and Boos, W. (1976). *Eur. J. Biochem.* **69**, 545–550.
Reidl, J., Romisch, K., Ehrmann, M., and Boos, W. (1989). *J. Bacteriol* **171**, 4888–4899.
Reyes, M., Treptow, N. A., Schuman, H, A. (1986). *J. Bacteriol.* **165**, 918–822.
Richarme, G. (1982). *Biochem. Biophys. Res. Commun.* **105**, 476–481.
Richarme, G. (1985). *J. Bacteriol.* **162**, 286–293.
Richarme, G., and Heine, H-G. (1986). *Eur. J. Biochem.* **156**, 399–405.
Riordan, J. R., *et al.* (1989). *Science* **245**, 1066–1073.
Ross, J. I., Eady, A., Cove, J. H., Cunliffe, W. J., Baumberg, S., and Wootton, J. C. (1990). *Mol. Microbiol.* , in press.
Rossman, M. G. (1975). In *The Enzymes* (Boyer, P. D., ed.), Academic Press, New York, pp. 61–79.
Sack, J. S., Saper, M. A., and Quicho, F. A. (1989). *J. Mol. Biol.* **206**, 171–191.
Schwarz, M., Summers, C., Heptinstall, C., Newton, C., Markham, A., Cain, R., and Super, M. (1990). *Lancet*, (in press).
Scripture, J. B., Voelker, C., Miller, S., O'Donnell, R. T., Polgar, L., Rade, J., Horazdovsky, B. F., and Hogg, R. W. (1987). *J. Mol. Biol.* **197**, 37–64.
Seeberg, E., and Steinum, A.-L. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 988–992.
Schuman, H. A. (1982a). *J. Biol. Chem.* **257**, 5455–5461.
Schuman, H. A. (1982b). *Ann. Microbiol.* (*Inst. Pasteur*) **133A**, 153–159.
Schuman, H. A., and Silhavy, T. J. (1981). *J. Biol. Chem.* **256**, 560–562.
Singh, A. P., and Bragg, P. D. (1979). *Can. J. Biochem.* **57**, 1376–1383.
Stanfield, S. W., Ielpi, L., O'Brochta, D., Helinski, D. R., and Ditta, G. S. (1988). *J. Bacteriol.* **170**, 3523–3530.
Staudenmaier, H., van Hove, B., Yaraghi, Z., and Braun, V. (1989). *J. Bacteriol.* **171**, 2626–2633.
Stirling, D. A., Hulton, C. S. J., Waddell, L., Park, S. F., Stewart, G. S. A. B., Booth, I. R., and Higgins, C. F. (1989). *Mol. Microbiol.* **3**, 1025–1038.
Strathdee, C. A., and Lo, R. Y. C. (1989). *J. Bacteriol.* **171**, 916–828.
Surin, B. P., Rosenberg, H., and Cox, G. B. (1985). *J. Bacteriol.* **161**, 189–198.
Tolley, S. P., Derewenda, Z., Hyde, S. C., Higgins, C. F., and Wilkinson, A. J. (1988). *J. Mol. Biol.* **204**, 493–494.
Treptow, N. A., and Schuman, H. A. (1985). *J. Bacteriol.* **163**, 654–660.
Treptow, N. A., and Schuman, H. A. (1988). *J. Mol. Biol.* **202**, 809–822.
Vyas, N. K., Vyas, M. N., and Quiocho, F. A. (1987). *Nature* (*London*) **327**, 635–638.
Walker, J. E., Saraste, M., Runswick, M. J., and Gay, N. J. (1982). *EMBO J.* **1**, 945–951.