workers (Dalbadie-MacFarland et al., 1982). M13 derivatives have the advantage of ease of preparation of single-stranded substrate for the priming reaction. However, insofar as plasmids may be nicked and gapped under the appropriate conditions, single-stranded regions of genes cloned in plasmids can also be mutagenized using the primer-directed method.

The change we hope to make is a C to A transversion in the third position of the His-95 CAC codon. This will change the active-site histidine to a glutamine. We anticipate that this change will change the activity of the enzyme but not eliminate it if the histidine is used not as a proton donor but as a conduit for the dipole of the D1 z-helix. This is the glutamine amide side chain has the same hydrogen-bonding capability as does the unprotonated imidazole, and the distances between appropriate atoms in the mutant enzyme should not be changed enough to alter the active site geometry significantly. We will test the proper folding of the mutant enzyme by crystallizing it and examining its X-ray structure at high resolution. Other mutants will be made and studied in the same way.

In addition, we are preparing an expression system for the production of a periplasmic ribonuclease A in E. coli. A cDNA clone of the entire coding sequence was the kind gift of Dr. R. MacDonald (MacDonald et al., 1982), and this has been subcloned into the expression vector pPLC28 (Remaut, 1981). We hope to use this cloned gene to test theories of catalysis in pancreatic RNase. We also intend to carry out mutagenesis experiments designed to alter the specificity of the enzyme.

Molecular mechanisms of bacterial periplasmic transport systems

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Bacterial transport systems in which the substrate crosses the membrane in a chemically unaltered form can be divided into two main groups: (i) unicomponent systems such as the lactose permease which are energized by ion-gradients; (ii) multicomponent, periplasmic binding-protein-dependent systems which are energized directly by ATP. The molecular events by which transport is mediated in these systems generally constitute only a small proportion of the total cell protein and, in addition, cease to exhibit any assayable function as soon as the membrane is disrupted. However, recently the application of a combination of genetic and molecular genetic techniques to this problem has begun to shed some light on the molecular events by which transport is mediated.

The high-affinity histidine transport system of Salmonella typhimurium is one of the best characterized of the periplasmic transport systems. Four genes are required for histidine transport, hisJ, hisQ, hisM, and hisP, which are organized as a single operon (Higgins et al., 1982). hisJ encodes a typical periplasmic binding protein of M, 26000, which is located in the periplasm and has a high affinity for l-histidine (K, of approx. 10^-4; Ames & Lever, 1972). The hisQ, hisM and hisP genes encode proteins of M, 24000, 26000 and 29000 respectively, which are located in the cytoplasmic membrane. Genetic complementation data indicate that the Q, M and P proteins interact with each other, forming a complex within the membrane (Higgins et al., 1982). During histidine transport the periplasmic J protein interacts specifically with the P protein in the membrane (Ames & Spudich, 1976). Interestingly, a second periplasmic protein, LAO, also requires the function of the Q, M and P proteins in order to mediate arginine transport (Kustu & Ames, 1973). Thus two independent periplasmic proteins with different substrate specificities funnel their substrates through a common complex of three proteins in the membrane. LAO is encoded by the argT gene which is located on the chromosome immediately upstream from the histidine transport operon (Higgins et al., 1982). We have now cloned the histidine transport operon, together with the argT gene, and have obtained the entire nucleotide sequence of this region of the chromosome (Higgins et al., 1982). This information, together with analysis of many mutations resulting in different phenotypes, now provides a framework for consideration of transport mechanisms.

Periplasmic components

The hisJ and argT genes are 70% homologous and presumably arose by gene duplication and divergence (Higgins & Ames, 1982). Each protein has a typical 22 amino acid signal peptide which is removed during secretion into the periplasmic space. A comparison of these two proteins, together with analysis of a variety of hisJ and argT mutants, has enabled us to assign specific functions to different domains of the binding proteins. A region of 30 amino acids near the C-terminal of J and LAO is completely conserved between the two proteins and would seem to be a likely candidate for the site of interaction with the membrane-bound P protein. This has been confirmed by sequencing hisJ mutations which fail to interact effectively
with the P protein (Higgins & Ames, 1982). Mutations which affect substrate binding by the J and LAO proteins tend to map towards the N-terminal portion of the proteins.

Confirmation that substrate specificity is determined solely by the 130 N-terminal amino acids of the protein was obtained by analysis of the substrate-binding properties of the chimeric proteins resulting from a fusion of the arg T and hisD genes. These data, together with X-ray crystallographic and n.m.r. studies of the histidine and other binding proteins (for example Gilliland & Quiocho, 1981), indicate that periplasmic binding proteins consist of two physically and functionally distinct domains. One, consisting primarily of amino acids from the N-terminal of the protein, is responsible for substrate binding. The second, comprising the C-terminal amino acids of the protein, is responsible for interactions with the membrane-bound components of the transport system. The structural similarity observed between binding proteins for a variety of different substrates would suggest that this separation of functional domains is a property of most, if not all, binding proteins.

**Membrane components**

The amino acid sequences of the membrane components Q, M and P, derived from the corresponding nucleotide sequences, show several interesting features. The proteins are relatively basic and, unlike the very hydrophobic lactose permease (Buchel et al., 1980), are not particularly hydrophobic and do not contain long regions of α-helix which could span the membrane. In addition, Q, M and P do not possess signal peptides. Thus insertion of proteins into the inner membrane of bacteria does not require a typical signal peptide, despite the fact that at least part of one of the proteins, P, must be exposed on the outer face of the membrane to facilitate the interaction with the periplasmic components.

Two alternative models can be imagined for the role of the membrane proteins of periplasmic transport systems. They could function as relatively non-specific pores, opening up to allow the substrate to pass through the membrane upon interaction with the substrate-binding protein complex. Alternatively, the membrane proteins could themselves impart specificity, possessing substrate-binding sites. It now seems that the latter alternative is correct. A number of mutations have been isolated and sequenced which alter the specificity of transport and yet fall in the hisQ or hisM genes. Thus by altering the membrane proteins one can alter the specificity of transport. A comprehensive demonstration that the membrane components of the maltose transport system impart specificity has also been presented (Shuman, 1982)

**Model for transport**

A model for histidine transport, compatible with all available data, is proposed. The periplasmic protein J binds histidine inducing a conformational change in the binding protein, such a change has been observed (Ho et al., 1980). This change allows the J-histidine complex to interact with the P protein in the inner membrane. P, Q and M are present as a complex in the membrane. A conformational change is induced in these membrane proteins by the interaction of the J-histidine complex, histidine being passed to substrate binding sites on M and Q and so through the membrane. Techniques are now available to permit this model to be tested and refined.

While this model is mainly based upon data obtained from the histidine transport system, other periplasmic transport systems seem to be very similar. Thus this model can be considered as a general model for periplasmic transport systems. Other periplasmic systems which have been analysed in detail seem to have similar numbers of components. The maltose system of *Escherichia coli* has three inner-membrane-bound components in addition to the periplasmic protein (Boos, 1982) and, while less well characterized, the peptide, galactose and branched-chain amino acid systems all have at least two (and probably three) components in addition to the binding protein. Furthermore, it has recently been shown that a membrane-bound component of the maltose system of *E. coli*, malK, is 30%, homologous to the hisP protein (Gilson et al., 1982). Thus the two systems must be functionally closely related and probably share a common evolutionary origin.

**Energy coupling**

While it is known that energy is coupled to transport directly by the hydrolysis of ATP (or possibly acetyl phosphate; Hong et al., 1979) no phosphorylated transport component has yet been identified (Ames & Nikaido, 1981). Presumably ATP is hydrolysed by one of the transport components, yet a covalent intermediate is either not formed or is highly unstable. Circumstantial evidence suggests that the malK protein of the maltose system, and by analogy the hisP protein of the histidine system, is involved in regulation and energy coupling. Inspection of the sequence of the histidine transport proteins for the consensus nucleotide-binding site of Walker (Walker et al., 1982) shows that a very similar sequence is present on the hisP protein (centred around residues 43 and 175). The possibility that this sequence represents an ATP binding/hydrolysis site is supported by the existence of a similar sequence in the malK protein. Indeed, this putative ATP-binding site is located in the regions of hisP and malK which show greatest sequence similarity. While direct experimental evidence is required to support this hypothesis, the identification of a potential ATP-binding site provides a basis for detailed experimental investigation of energy coupling to transport.

**Conclusions**

The isolation and characterization of mutations, both in vivo and in vitro, has enabled the function of transport components to be studied in detail. In addition, it should not be long before overproduction of some of these components by various cloning techniques will permit their purification and biochemical characterization and thus allow us to directly test and confirm the model for transport presented above.

Giovanna Ames has been primarily responsible for the development of the histidine system as a useful model for the study of transport. In addition, I am very grateful for the facilities and the stimulating environment she provided during the period in which most of this work was carried out.