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Bacterial Amino Acid Transport Systems

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I. INTRODUCTION

The roles of the cell membrane as both a permeability barrier to foreign substances and as a selective filter which admits nutrient molecules have been recognized as fundamentally important to animal physiology since the first decade of this century. It was not until the 1950's, however, that microbiologists recognized that bacteria possess membrane transport systems, distinct from their metabolic enzymes, that mediate the uptake of amino acids and other nutrients. The work of Cohen and Monod (1957) established modern research on bacterial membrane transport. While much of this early work focused on transport of sugars, Gale provided the first evidence for accumulation of amino acids by bacteria during this era (Gale, 1947, 1954).

Today, amino acid transport systems for almost all of the amino acids have been recognized and studied. This review will tabulate information on these systems, but it is not intended to be a comprehensive treatment of all amino acid transport systems. For additional information on other systems, the reader is referred to recent reviews on the subject (Oxender, 1972, 1974, 1975; Slayman, 1973; Boos, 1974; Halpern, 1974; Simoni and Postma, 1975; Wilson, 1978; Iaccarino *et al.*, 1980; Anraku, 1980; Furlong and Schellenberg, 1980).

The existence of active amino acid transport systems has been deduced or inferred from a variety of biochemical and genetic approaches. In some instances, the existence

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and role of specific amino acid transport proteins is well documented while, in other cases, the existence of a given amino acid transport system has been inferred from kinetic analysis of amino acid uptake measurements. The two best characterized amino acid transport systems are the high-affinity periplasmic binding protein-(BP) dependent transport system for leucine, isoleucine, and valine (LIV-I) system in *E. coli* (Piperno and Oxender, 1966, 1968; Nakane *et al.*, 1968; Penrose *et al.*, 1970; Rahmanian and Oxender, 1972; Rahmanian *et al.*, 1973; Harrison *et al.*, 1975; Quay *et al.*, 1975a,b, 1977, 1978; Anderson *et al.*, 1976; Oxender and Quay, 1976a–c; Quay and Oxender, 1976, 1977, 1979, 1980a,b; Anderson and Oxender, 1977, 1978; Oxender *et al.*, 1977, 1980a,b; Landick *et al.*, 1980, 1983; Daniels *et al.*, 1980, 1981; Landick and Oxender, 1981; Wood, 1975; Guardiola *et al.*, 1974a,b; Templeton and Savageau, 1974a,b; Yamato and Anraku, 1977, 1980); and the high-affinity histidine transport system in *S. typhimurium* (Ames, 1964, 1972; Ames and Roth, 1968; Ames and Lever, 1970, 1972; Rosen and Vasington, 1971; Lever, 1972; Kustu and Ames, 1973; Shaltiel *et al.*, 1973; Ames and Spudich, 1976; Ames *et al.*, 1977; Robertson *et al.*, 1977; Ames and Nikaido, 1978; Kustu *et al.*, 1979; Noel *et al.*, 1979; Manuck and Ho, 1979; Ho *et al.*, 1980; Ardeshir and Ames, 1981; Ardeshir *et al.*, 1981; Higgins and Ames, 1981, 1982; Higgins *et al.*, 1982a,b; Gilson *et al.*, 1982b). These systems both fall in the class of high-affinity, periplasmic, BP-dependent systems and possess remarkable similarities. This review will focus on these two examples of amino acid transport systems. While this approach will necessarily limit treatment of the various mechanisms and components that are found in different amino acid transport systems, we hope it will give a comprehensive and focused picture of the chosen model systems.

We begin by considering definitions of amino acid transport and the different types of systems which have been recognized and then proceed to a tabulation of the multiple amino acid transport systems found in bacteria and a detailed examination of the protein components and genetic organization of the histidine and LIV-I systems. The sequence of events which leads from the synthesis of transport system components to their final assembly in the inner membrane and periplasm is discussed. This is followed by a review of the energization and reconstitution of amino acid transport systems. We conclude with discussions of how amino acid transport systems are regulated and how amino acid transport systems may have evolved.

II. CLASSES OF TRANSPORT SYSTEMS

The terms *transport system* and *permease* will be considered synonymous and taken to mean the entire assembly of protein components which are required for a given transport process. The individual components will be referred to as *transport proteins* whether they are membrane-bound or soluble periplasmic proteins.

Transport of solutes across membranes is usually divided into three classes: (1) *passive diffusion,* meaning diffusion through the lipid–protein bilayer without specific interaction, (2) *facilitated diffusion,* meaning passage through the membrane by specific binding to and release from a carrier protein without requiring energy, and (3) *active transport,* meaning passage of a solute through the membrane by binding to and release

from a carrier protein in a process coupled to metabolic energy. While passive diffusion and facilitated diffusion require that the solute always move to dissipate a concentration gradient, active transport is usually taken to mean that the solute is accumulated against a concentration gradient. This review will be limited to amino acid transport systems that fulfill the criteria of active transport.

At least four distinct mechanisms of bacterial active transport have been demonstrated: (1) sodium-independent, membrane-bound transport systems which require only membrane components, (2) sodium-dependent, membrane-bound transport systems which require only membrane components and sodium ions, (3) periplasmic BP-dependent transport systems which require both periplasmic and membrane-bound components, and (4) cation-stimulated ATPase systems which are multicomponent assemblies and are thought to be responsible for creating a transmembrane proton gradient (Mitchell, 1970). Of these mechanisms, only numbers (1)–(3) have been shown to function for amino acid transport and will be discussed here. A fifth, somewhat distinct mechanism of transport is *group translocation* which is best exemplified by the phosphoenolpyruvate-utilizing phosphotransferase systems that transport various carbohydrates (Postma and Roseman, 1976; Dills *et al.*, 1980). There are no known examples of group translocation involving amino acids in bacteria.

A. Multiplicity of Amino Acid Transport Systems

Table 1 lists the various amino acid transport systems that have been described in the literature. In compiling this list, we have given consideration to the criteria used in identifying systems and the arguments presented above. Rather than compile a comprehensive list, we have attempted to present those systems which are based on the strongest evidence. Where conflicts exist in reports about a given system or class of systems, we have used the nomenclature or values from the best documented evidence. References to other interpretations are included. The table contains the information available on *E. coli* and *S. typhimurium;* these two organisms are extremely similar from the biological and genetic points of view and, in many cases, the results obtained with one species also apply to the other. Limited information is also given on *P. aeruginosa* where amino acid transport systems similar to those found in *E. coli* and *S. typhimurium* have been characterized.

It is obvious from this table that each amino acid may be concentrated from the extracellular medium by several different permeases or transport systems with overlapping specificities. Leucine enters by at least three systems: LIV-I, LS, and low-affinity, membrane-bound transport system for leucine, isoleucine, and valine (LIV-II; Piperno and Oxender, 1966, 1968; Nakane *et al.*, 1968; Penrose *et al.*, 1970; Rahmanian and Oxender, 1972; Rahmanian *et al.*, 1973; Harrison *et al.*, 1975; Quay *et al.*, 1975a,b, 1977, 1978; Anderson *et al.*, 1976; Oxender and Quay, 1976a–c; Quay and Oxender, 1976, 1977, 1979, 1980a,b; Anderson and Oxender, 1977, 1978; Oxender *et al.*, 1977, 1980a,b; Landick *et al.*, 1980, 1983; Daniels *et al.*, 1980, 1981; Landick and Oxender, 1981; Wood, 1975; Guardiola *et al.*, 1974a,b; Templeton and Savageau, 1974a,b; Yamato and Anraku, 1977, 1980); histidine, by five systems: aromatic, high-affinity histidine, LAO, and two kinetically distinct residual systems

Table 1. Bacterial Amino Acid Transport Systems

System name	Comments	Substrates	K(μM)	Organism characterized in	Genes	References[a]
Arginine-specific	Uses the Arg-BP, repressed by L-arginine or L-ornithine	L-Arginine, L-ornithine, D-arginine	0.12	*E. coli*	*abpS*, Arg-BP, gene, min 50	1–7
					abpR, Regulatory gene, min 50, mutation allows growth on D-arginine	
				S. typhimurium		8
General aromatic	General aromatic amino acid transport system	L-Phenylalanine,	0.1–0.5	*S. typhimurium*	*aroP*, Min 3	9,10
		L-tyrosine,	0.1–0.5	*E. coli*	*aroP*, Min 2	11,12
		L-tryptophan, L-histidine	0.1–0.5	*P. aeruginosa*		13
Asparagine-I	Repressed by asparagine	L-Asparagine, L-aspartic acid	3.5	*E. coli*		14
Asparagine-II	Necessary for utilization of asparagine as a nitrogen source	L-Asparagine	80	*E. coli*		14
Aspartate-specific	A membrane-bound transport system	L-Aspartic acid	3.7	*E. coli*		15,16
Cystine	A binding protein-dependent system that uses Cys-BP	L-Cystine,	0.3	*E. coli*		17–20
		diamino-pimelic acid	14	*S. typhimurium*		21
DAG	A membrane-bound system for small amino acids; mutation renders cells resistant to D-serine and D-cycloserine	Glycine,	3–5	*E. coli*	*dag*, Min 83	22–27,11,93
		L-alanine,	2			
		D-alanine,	2			
		L-serine, D-serine				
	D-cycloserine	D-serine				

5 *E. coli* 16,28

Cystine	A binding protein-dependent system that uses Cys-BP	L-Cystine, diamino-pimelic acid	0.3 14	*E. coli* *S. typhimurium*		17–20 21
DAG	A membrane-bound system for small amino acids; mutation renders cells resistant to D-serine and D-cycloserine	Glycine, L-alanine, D-alanine, L-serine, D-serine	3–5 2 2	*E. coli*	*dag*, Min 83	22–27,11,93
Low-affinity glutamate, aspartate	A membrane-bound system which is inhibited by β-hydroxyaspartate	L-Glutamic acid, L-aspartic acid, β-hydroxy-aspartic acid	5 5	*E. coli*		16,28
High-affinity glutamate, aspartate	A binding protein-dependent system which uses glutamate, aspartate BP	L-Glutamic acid, L-aspartic acid	0.5 0.5	*E. coli*		16,29–35
Glutamate-specific transport system	A membrane-bound, sodium-dependent transport system	L-Glutamate L-methyl-glutamate	1.5	*E. coli*		16,36–40
Glutamine	Inhibited by glutamylhydrazide and glutamylhydrazone, repressed by glutamine, derepressed by nitrogen starvation	L-Glutamine	0.05	*E. coli* *S. typhimurium*		40–45 46,47
Histidine	A binding protein-dependent system which uses the His-BP	L-Histidine, D-histidine	0.01	*S. typhimurium*	*hisJ*, Min 48.5, *hisP*, min 48.5, *hisQ*, min 48.5, *hisM*, min 48.5, *duaH*, min 48.5; mutation allows cells to grow on D-histidine as a nitrogen source	48–53
				E. coli		54
LAO	A binding protein-dependent system which uses the LAO-BP and the *hisPQM* components	L-Lysine, L-arginine, L-ornithine, L-histidine		*S. typhimurium*	*argT*, Min 48.5, *hisP*, min 48.5, *hisQ*, min 48.5, *hisM*, min 48.5	46,53–57

(continued)

Table 1. (Continued)

System name	Comments	Substrates	K(μM)	Organism characterized in	Genes	References[a]
LIV-I	A binding protein-dependent system which uses the *livGH* components, repressed by L-leucine	L-Alanine, L-leucine, L-isoleucine, L-valine, L-threonine	30 0.2–0.6	*E. coli*	*livJ*, Min 74.5, *livH*, min 74.5, *livG*, min 74.5, *livR*, Min 20, a regulatory gene, mutation derepresses LIV-I transport	58–63
				S. typhimurium		64
				P. aeruginosa		65,66
Leucine-specific (LS)	A binding protein-dependent system that uses the LS-BP and *livGH* components, repressed by L-leucine	L-Leucine, D-leucine	0.2–0.6	*E. coli*	*livK*, Min 74.5, *livG*, min 74.5, *livH*, min 74.5, *lstR*, min 20, a regulatory gene; mutation allows cells to use D-leucine as a source of L-leucine	58,59,63,67
LIV-II	A membrane-bound system that is only weakly repressed by leucine	L-Leucine, L-isoleucine, L-valine	10–20 5 7	*E. coli*	*livP*, Min 75	58,59,68–71
				P. aeruginosa		66
Methionine-I		L-Methionine, D-methionine	0.075	*E. coli*	*metD*, Min 5	72–74
				S. typhimurium	*metD*, Min 6	47,75
Methionine-II		L-Methionine, D-methionine		*E. coli*		72–74
Phenylalanine-specific		L-Phenylalanine	1	*S. typhimurium*	*pheP*, Min 15–35	48,49
				E. coli		12,79

	that is only weakly repressed by leucine	L-isoleucine, L-valine	5 7			
Methionine-I		L-Methionine, D-methionine	0.075	*P. aeruginosa* *E. coli*	*metD*, Min 5	66 72–74
Methionine-II		L-Methionine, D-methionine		*S. typhimurium* *E. coli*	*metD*, Min 6	47,75 72–74
Phenylalanine-specific		L-Phenylalanine	1	*S. typhimurium*	*pheP*, Min 15–35	48,49
				E. coli		12,79
Proline PP-I	A membrane-bound, high-affinity system, induced by proline, catabolite repressed, required for growth on proline as sole carbon or nitrogen source	L-Proline, L-hydroxy-proline	2	*S. typhimurium*	*putP*, Min 22 *putA*, Min 22, gene for a PP-I system repressor	77–80
				E. coli		81–87
				P. aeruginosa		13
Proline PP-II	Low-affinity, membrane-bound system, induced by amino acid starvation	L-Proline	300	*S. typhimurium*	*proP*, Min 92	77,88,89
				E. coli		83
Proline PP-III	Functions only at high osmolarity (0.3 M NaCl)	L-Proline		*S. typhimurium*	*proU*, Min 59	90
Tryptophan-specific I	Inducible by tryptophan	L-Tryptophan	0.1	*E. coli*	*trpP*, Min 69	11,12,95
				S. typhimurium		48,49
Tryptophan-specific II	Constitutively expressed	L-Tryptophan	1	*E. coli*		12
				S. typhimurium		48,49
Tryptophan-specific III (T_3A)	Catabolite repressed, induced by tryptophan	L-Tryptophan	10	*E. coli*	*tnaB*, Min 83	91,92,94,95
Tyrosine-specific		L-Tyrosine	1	*S. typhimurium*		48,49
				E. coli	*tyrP*, Min 42	12,76

[a] (1) Rosen, 1971, (2) Rosen, 1973b, (3) Rosen, 1973a, (4) Cells, 1981, (5) Cells, 1977, (6) Cells, 1982, (7) Cells *et al.*, 1973, (8) Kreischman *et al.*, 1973, (9) Ames, 1964, (10) Ames and Roth, 1968, (11) Piperno and Oxender, 1968, (12) Brown, 1971, (13) Kay and Gronlund, 1969, (14) Willis and Woolfolk, 1975, (15) Kay, 1971, (16) Schellenberg and Furlong, 1977, (17) Leive and Davis, 1965a, (18) Leive and Davis, 1965b, (19) Berger and Heppel, 1972, (20) Oshimaa *et al.*, 1974, (21) Baptist and Kredich, 1977, (22) Lee *et al.*, 1975, (23) Lombardi and Kaback, 1975, (24) Cosloy, 1973, (25) Kaback and Kostellow, 1968, (26) Wargel *et al.*, 1970, (27) Wargel *et al.*, 1971, (28) Barash and Halpern, 1975, (29) Willis and Furlong, 1975, (30) Furlong and Schellenberg, 1980, (31) Schellenberg, 1978, (32) Miner and Frank, 1974, (33) Barash and Halpern, 1975, (34) Schellenberg and Furlong, 1977, (35) Aksamit *et al.*, 1975, (36) Frank and Hopkins, 1969, (37) Kahane *et al.*, 1975, (38) Tsuchiya *et al.*, 1977, (39) Britten and McClure, 1962, (40) MacDonald *et al.*, 1977, (41) Weiner and Heppel, 1971, (42) Weiner *et al.*, 1971, (43) Willis *et al.*, 1975, (44) Masters and Hong, 1981b, (45) Plate, 1979, (46) Kustu *et al.*, 1979, (47) Ayling, 1981, (48) Ames, 1964, (49) Ames and Roth, 1968, (50) Ames and Nikaido, 1978, (51) Ames *et al.*, 1977, (52) Masters and Hong, 1981b, (53) Higgins *et al.*, 1982a, (54) Ardeshir and Ames, 1981, (55) Ardeshir *et al.*, 1981, (56) Higgins and Ames, 1981, (57) Higgins and Ames, 1982, (58) Rahmanian *et al.*, 1973, (59) Anderson and Oxender, 1977, (60) Oxender *et al.*, 1977, (61) Anderson and Oxender, 1978, (62) Oxender *et al.*, 1980a, (63) Landick *et al.*, 1980, (64) Kiritani, 1974, (65) Hoshino and Nishio, 1982, (66) Hoshino and Kageyama, 1982, (67) Oxender *et al.*, 1980b, (68) Wood, 1975, (69) Guardiola *et al.*, 1974b, (70) Yamato and Anraku, 1980, (71) Iaccarino *et al.*, 1978, (72) Kadner, 1974, (73) Kadner and Watson, 1974, (74) Kadner, 1977, (75) Ayling *et al.*, 1979, (76) Whipp *et al.*, 1980, (77) Wood, 1981, (78) Ratzkin *et al.*, 1978, (79) Wood *et al.*, 1979, (80) Menzel, 1980, (81) Kaback and Stadtman, 1966, (82) Kaback and Deuel, 1969, (83) Morikawa *et al.*, 1974, (84) Amanuma *et al.*, 1977, (85) Rowland and Tristam, 1974, (86) Motojima *et al.*, 1978, (87) Condamine, 1971, (88) Anderson *et al.*, 1980, (89) Menzel and Roth, 1980, (90) Csonka, 1982, (91) Boezi and Demoss, 1961, (92) Burrous and Demoss, 1963, (93) Robbins and Oxender, 1973, (94) Edwards and Yudkin, 1982, (95) V. Stewart, personal communication.

(Ames, 1964; Ames and Roth, 1968; Ames and Lever, 1970; Kustu *et al.*, 1979; Higgins *et al.*, 1982a); and proline, by at least three systems: PP-I, PP-II, and PP-III (Kaback and Stadtman, 1966; Kaback and Deuel, 1969; Morikawa *et al.*, 1974; Amanuma *et al.*, 1977; Wood, 1981; Ratzkin *et al.*, 1978; Wood *et al.*, 1979; Menzel, 1980; Rowland and Tristam, 1974; Motojima *et al.*, 1978; Condamine, 1971; Kusaka *et al.*, 1976; Anderson *et al.*, 1980; Menzel and Roth, 1980; Csonka, 1982). Glutamate may enter bacteria by as many as five routes and aspartate and arginine by at least three (Furlong and Schellenberg, 1980; Schellenberg and Furlong, 1977; Ames, 1972; Rosen, 1973a; Celis, 1977, 1981, 1982; Higgins and Ames, 1981).

Determining the existence of multiple amino acid transport systems for a given substrate should involve several approaches (Ames, 1972). The existence of system multiplicity has often been predicated almost entirely on kinetic analysis alone. On occasion, multiple transport systems have been postulated solely on the basis of biphasic transport kinetics. While the demonstration of biphasic Lineweaver–Burk plots is suggestive of multiple transport systems, it is by no means conclusive. It is important to keep in mind that the Michaelis–Menten assumptions are most likely not fulfilled by complex transport processes even if the individual components may obey the equation. It is, therefore, not appropriate to base arguments for multiple routes of entry on this type of analysis alone. A variety of other explanations for two or more apparent K_ms of amino acid uptake are possible. Carrier proteins may be subject to allosteric effects which alter their affinity for substrates as a function of substrate concentration. Other cellular processes may respond to changes in the concentration of a given amino acid and alter the properties of carrier proteins indirectly by changing, for instance, the transmembrane electrochemical potential; and, on occasion, impurities in the radioactively labeled amino acids used in uptake measurements can lead to spurious conclusions about transport kinetics. Given these potential pitfalls, multiphasic kinetics should not be used alone as the only argument for multiple transport systems.

Analogue inhibition analysis can provide alternative criteria for multiple transport systems (Ames, 1964, 1974) and has been used extensively in studies of animal cell transport (Oxender and Christensen, 1963; Christensen, 1975, 1982). With this method, multiple systems are revealed when some component of uptake remains after inhibition with a large concentration of a structural analogue. For example, only 80–90% of L-leucine uptake at 0.5 μM substrate can be inhibited by 200 μM L-threonine. The 10–20% remaining is uptake by the LS transport system while the threonine inhibitable fraction is due to uptake by the LIV-I system. Since it avoids relying on the validity of kinetics assumptions, the analogue inhibition strategy is a valuable technique for identifying multiple transport systems.

The most definitive method for defining bacterial amino acid transport systems is mutational analysis. If mutants in a given transport system can be isolated by selection or identified by screening, other systems with overlapping specificities can be readily identified. Furthermore, once mutants are obtained, identification of the protein components affected by the mutation and molecular cloning of the gene(s) for the defective component(s) are possible. This type of genetic approach has been exploited in our analysis of the leucine and histidine transport systems and has led to rapid advances in our understanding of how these systems are assembled and how they function.

Although a precise definition of all the amino acid transport systems in *E. coli* or *S. typhimurium* is not yet possible, a general picture has emerged. Bacterial amino acid transport systems usually transport a class of amino acids, such as basic amino acids or branched-chain amino acids, but this specificity is not as broad as is found for amino acid transport systems in eukaryotic microorganisms such as yeast or *Neurospora* or in higher eukaryotes. *Neurospora* and yeast, for instance, each possess a transport system which transports all amino acids, a so-called general amino acid permease (Pall, 1969; Grenson and Hon, 1972; Seaston *et al.*, 1973, 1976; Rao *et al.*, 1975; DeBusk and DeBusk, 1980; Ogilvie-Villa *et al.*, 1981).

In addition to systems transporting multiple amino acids, most amino acids are also transported by systems which are specific for a single amino acid. No such systems have been found, so far, in eukaryotes. However, unlike eukaryotic cells, bacteria are able to synthesize all 20 amino acids and therefore adapt to a wide variety of environments which may be deficient in one or more amino acids. Any one amino acid may utilize either membrane-bound systems or periplasmic BP-dependent systems, or both.

B. Membrane-Bound Systems

The best-characterized example of a sodium-independent membrane-bound system is the proline transport system (PP-I in Table 1) in *E. coli* and *S. typhimurium* (Kaback and Stadtman, 1966; Kaback and Deuel, 1969; Morikawa *et al.*, 1974; Amanuma *et al.*, 1977; Wood, 1981). The best-characterized example of a sodium-dependent, membrane-bound system is the glutamate transport system of *E. coli* (Britten and McClure, 1962; Frank and Hopkins, 1969; Schellenberg and Furlong, 1977; Kahane *et al.*, 1975; MacDonald *et al.*, 1977; Tsuchiya *et al.*, 1977). Sodium-dependent transport of glutamate has also been observed in the halophilic genus *Halobacter* (Stevenson, 1966) and in the marine genus *Pseudomonad* (Wong *et al.*, 1980). The sodium-dependent, membrane-bound glutamate transport system is one of three distinct *E. coli* systems which transport glutamate. It is distinguished from the other systems by its resistance to osmotic shock and inhibition by the amino acid analog α-methylglutamate. Sodium-dependent amino acid transport is relatively rare in bacteria, unlike mammalian cells where transport of many solutes is linked with the movement of sodium ions. Early in the study of amino acid transport, some systems were erroneously thought to be sodium-dependent because inclusion of sodium ion in uptake assay and wash buffers partially blocked dumping of amino acid pools when cells were treated with cold wash buffer (Piperno and Oxender, 1968; Britten and McClure, 1962). Once the phenomenon of cold-shock-induced amino acid pool loss was widely appreciated and experimental protocols were modified to include room temperature washes, most of these effects disappeared.

C. Periplasmic BP-Dependent Systems

In addition to uptake by membrane-bound systems, some amino acids, as well as some ions and sugars, are transported by more complicated systems which include

both periplasmic and membrane-bound components. The periplasmic components are solute BPs which bind specific substrates or sets of substrates with very high affinity. When the periplasmic BPs are removed from the cell by the osmotic shock procedure of Neu and Heppel (1965) or by lysozyme–ethylenediamine tetraacetic acid (EDTA) treatment, transport of amino acids by these high-affinity systems is no longer observed. Hence, these systems have been termed periplasmic BP-dependent transport systems or shock-sensitive systems. Bacterial periplasmic BPs that bind arginine [lysine, arginine, ornithine-binding protein (LAO-BP) and Arg-BP] (Neu and Heppel, 1965; Wilson and Holden, 1969a,b; Rosen, 1971, 1973a,b; Celis *et al.*, 1973; Celis, 1977, 1981, 1982; Kustu *et al.*, 1979; Wong *et al.*, 1980; Ardeshir *et al.*, 1981; Higgins *et al.*, 1982a), aspartic acid [glutamate, aspartate-BP] (Miner and Frank, 1974; Willis and Furlong, 1975; Aksamit *et al.*, 1975; Schellenberg, 1978), cystine [cystine-BP] (Berger and Heppel, 1972; Oshimaa *et al.*, 1974), glutamic acid [glutamate, aspartate-BP] (Miner and Frank, 1974; Willis and Furlong, 1975; Schellenberg, 1978), glutamine [glutamine-BP] (Weiner and Heppel, 1971; Weiner *et al.*, 1971; Kreischman *et al.*, 1973; Schellenberg and Furlong, 1977; Schellenberg, 1978; Kustu *et al.*, 1979; Celis, 1982), histidine [histidine-BP] (Rosen and Vasington, 1971; Ames and Lever, 1970, 1972; Lever, 1972; Shaltiel *et al.*, 1973; Ames and Spudich, 1976; Robertson *et al.*, 1977; Ames and Nikaido, 1978; Manuck and Ho, 1979; Ho *et al.*, 1980), isoleucine [leucine, isoleucine, valine-binding protein (LIV-BP), also referred to in other publications as leucine, isoleucine, valine, threonine-binding protein (LIVT-BP) and leucine, isoleucine, valine, alanine, threonine-binding protein (LIVAT-BP) and isoleucine-preferring, leucine, valine-binding protein (ILV-BP), also referred to in other publications as isoleucine-preferring, leucine, valine, threonine-binding protein (ILVT-BP] (Piperno and Oxender, 1966; Anraku and Heppel, 1967; Nakane *et al.*, 1968; Anraku, 1968a,b; Penrose *et al.*, 1970; Furlong *et al.*, 1973; Amanuma and Anraku, 1974; Amanuma *et al.*, 1976; Willis *et al.*, 1974; Antonov *et al.*, 1974, 1976; Oxender and Quay, 1976c; Quay *et al.*, 1977; Anderson and Oxender, 1977; Ovchinnikov *et al.*, 1977; Oxender *et al.*, 1977; Hoshino and Kageyama, 1980; Daniels *et al.*, 1980; Landick and Oxender, 1981; Hoshino and Nishio, 1982; Landick *et al.*, 1983), leucine [LIV-BP leucine-specific binding protein (LS-BP), also referred to in other publications as leucine-binding protein (LBP), and ILV-BP] (Piperno and Oxender, 1966; Anraku and Heppel, 1967; Nakane *et al.*, 1968; Anraku, 1968a,b; Penrose *et al.*, 1970; Furlong and Weiner, 1970; Weiner and Heppel, 1971; Amanuma and Anraku, 1974; Amanuma *et al.*, 1976; Willis *et al.*, 1974; Antonov *et al.*, 1974, 1976; Oxender and Quay, 1976c; Quay *et al.*, 1977; Anderson and Oxender, 1977; Ovchinnikov *et al.*, 1977; Oxender *et al.*, 1977, 1980b; Hoshino and Kageyama, 1980; Daniels *et al.*, 1980, 1981; Landick and Oxender, 1981; Hoshino and Nishio, 1982; Landick *et al.*, 1983), lysine [LAO-BP] (Wilson and Holden, 1969a,b; Kustu *et al.*, 1979), phenylalanine [phenylalanine-BP] (Klein *et al.*, 1970; Kuzaya *et al.*, 1971), and valine [LIV-BP and ILV-BP] (Piperno and Oxender, 1966; Anraku and Heppel, 1967; Nakane *et al.*, 1968; Anraku, 1968a,b; Penrose *et al.*, 1970; Furlong *et al.*, 1973; Amanuma and Anraku, 1974; Amanuma *et al.*, 1976; Willis *et al.*, 1974; Antonov *et al.*, 1974, 1976; Oxender and Quay, 1976c; Quay *et al.*, 1977; Anderson and Oxender, 1977; Ovchinnikov *et al.*, 1977; Oxender *et al.*, 1977; Hoshino and Kageyama, 1980; Daniels *et al.*, 1980;

Landick and Oxender, 1981; Hoshino and Nishio, 1982; Landick *et al.*, 1983) have been identified and characterized. In addition to BPs, periplasmic BP-dependent transport systems require the presence of proteins in the inner membrane to interact with the BPs and accomplish the actual transport event.

III. NATURE OF PROTEIN COMPONENTS OF BP-DEPENDENT TRANSPORT SYSTEMS

A. Periplasmic Components

The properties of periplasmic BPs are remarkably similar. Periplasmic amino acid BPs bind amino acids with high affinity. Equilibrium dialysis measurements usually give K_d values between 10^{-6} and 10^{-7}. Stopped-flow binding studies on the arabinose-BP have shown that the pseudo-first-order rate constant for arabinose binding decreases as the fraction of arabinose-BP containing bound arabinose increases (Miller *et al.*, 1980). This variable affinity, which has also been observed with the LIV-BP (Amanuma *et al.*, 1976), may explain why removal of substrate from BPs usually requires dialysis or gel filtration in the presence of 2–3 M guanidinium HCl or urea. The physiological significance and biochemical explanation of this variable binding are not yet understood. Most amino acid BPs are quite stable and can withstand treatment at 100°C for several minutes or with detergents such as sodium dodecyl sulfate (SDS) without losing binding activity. This stability may reflect a structural organization which resists denaturation and/or easily refolds to active form. BPs must resist degradation in the periplasmic space where proteases readily degrade many proteins and where pH cannot be readily controlled by the cell. Significant conformational differences, as monitored by proton-NMR or quenching of tryptophan fluorescence, between BPs freed of substrate and their bound forms have been demonstrated (glutamine-BP, Weiner and Heppel, 1971; Krieschman *et al.*, 1973; histidine-BP, Robertson *et al.*, 1977; Manuck and Ho, 1979; Ho *et al.*, 1980; S. Zukin, personal communication; LIV-BP, R. Landick, unpublished results). Early attempts to demonstrate this conformational shift with the LIV-BP (Penrose *et al.*, 1970) were unsuccessful because it was not appreciated that simple equilibrium dialysis against substrate-free buffer will not remove substrate bound by some bacterial periplasmic BPs.

Complete amino acid sequences are now available for the histidine-binding protein (his-BP), LAO-BP, LS-BP, and LIV-BP (Ovchinnikov *et al.*, 1977; Higgins and Ames, 1981; Hogg, 1981; R. Landick and D. L. Oxender, manuscript in preparation) as well as for the non-amino acid BPs: ara-BP, gal-BP, and sulfate-BP (Hogg and Hermondson, 1977; Isihara and Hogg, 1980; Mahoney *et al.*, 1981). Comparison of these sequences has not revealed significant conservations except between BPs coded by closely linked genetic loci, e.g., LAO-BP and his-BP. The conservation between the LAO-BP and his-BP is about 70% while the conservation between the LS-BP and LIV-BP is approximately 80%. In the case of the LAO-BP and his-BP pair the pattern of conservation suggests clearly which portions of the BPs are involved in substrate binding and which ones in transport (Higgins and Ames, 1981).

X-ray crystal structure determination has revealed that typical BPs which bind carbohydrate (arabinose-BP), ions (sulfate-BP), and amino acids (LIV-BP) all crystallize in the $P2_12_12_1$ space group with four molecules per unit cell (Quiocho *et al.*, 1979). Like the arabinose-BP (Quiocho *et al.*, 1977), the LIV-BP folds into a bilobate structure in which the cleft between the two domains contains the substrate-binding site (Saper and Quiocho, 1983). It is likely that the other amino acid-BPs share this bilobate structure. For the arabinose-BP and LIV-BP, the N-terminal region of the protein folds to form one domain while the C-terminal region folds to form the other. One possible model for the two domains is that one is important for substrate binding and the second, for interaction with the membrane component(s). This idea is consistent with the finding of two genetically distinct sites, one for substrate binding and one for membrane protein interaction, on the histidine-BP (Kustu and Ames, 1974; Higgins and Ames, 1981). Thus, we envisage that the BP is triggered to release its substrate from its binding site upon interaction of the other site with the membrane component(s). This type of model will be discussed further in a later section of this review.

B. Membrane Components

Isolation and characterization of the membrane components of these systems have not progressed as far as has the study of the BPs, but a tentative picture of their roles is now emerging. The best-characterized membrane components are those of the histidine transport systems of which there are three, P protein, Q protein, and M protein (Higgins *et al.*, 1982a). Three potential proteins have also been identified for the LIV-I transport system (*livG, livH, livM;* Anderson and Oxender, 1977; R. Landick, T. Su, P. Nazos, and D. L. Oxender, manuscripts in preparation). A carbohydrate transport system, the maltose transport system, also appears to have three membrane-bound components: *malF, malG,* and *malK* (Shuman *et al.*, 1980; Bavoil *et al.*, 1980; Shuman and Silhavy, 1981; Shuman, 1982). While it has not yet been demonstrated that all of these proteins are, in fact, membrane-bound or membrane-associated components, it has been suggested that two components may be required to form a transport channel or binding site in the membrane, and that the third component may function either to act as a link between the periplasmic component, bound substrate, and the membrane components (Kustu and Ames, 1974; Ames and Spudich, 1976; Higgins *et al.*, 1982a), or in coupling transport to a source of energy (Shuman, 1982). The complete nucleotide sequences of all three of these genes for the histidine genes have been determined (Higgins *et al.*, 1982a).

IV. GENETIC AND PHYSICAL MAPS OF THE LIV-I AND HISTIDINETRANSPORT GENES

Both leucine and histidine are transported into *Salmonella* and *E. coli* by more than one route, but in each case, the high-affinity, periplasmic BP-dependent system has been characterized most extensively. Each system is composed of both soluble,

periplasmic proteins and membrane-bound proteins which are coded for by genes that are situated together in a cluster on the chromosome. These two gene clusters, *liv* at min 74.5 in *E. coli* and *his* at min 47 in *S. typhimurium*, are remarkably similar. Figure 1 shows a schematic representation of the *his* and *liv* gene clusters.

The four genes responsible for the high-affinity histidine permease, together with a regulatory locus, *dhuA*, constitute an operon located at min 47 on the *S. typhimurium* chromosome (Higgins *et al.*, 1982a; see Figure 1). The *hisJ* gene encodes the his-BP (J) which has been purified and characterized (Ames and Lever, 1972; Lever, 1972). The *hisQ* and *hisM* genes code for very hydrophobic, basic proteins (Q and M) which are probably integral membrane proteins (Higgins *et al.*, 1982a). The *hisP* gene codes for an inner membrane-bound protein (P) which is basic and very hydrophilic (Ames and Nikaido, 1978; Higgins *et al.*, 1982a). The P protein is presumed, on the basis of genetic evidence, to interact directly with the periplasmic J protein during histidine transport (Kustu and Ames, 1974; Ames and Nikaido, 1978). The Q, M, and P proteins are also thought to interact with each other, on the basis of complementation studies and, thus, possibly form a multicomponent membranous complex. Models suggesting mechanisms of action for this system are presented in a later section.

Four genes are also responsible for the high-affinity leucine permease and constitute an operon at minute 74.5 on the *E. coli* chromosome which is surprisingly similar to the *S. typhimurium* histidine transport operon. The *livK* gene, which codes for the LS-BP, is the first gene in the operon and is followed by three genes which code for proteins synthesized in relatively lower amounts. The first of these genes, *livH*, encodes a basic hydrophobic protein (H). The last two genes in the operon, *livM* and *livG*, code for the M and G protein. The H, M, and G proteins are thought to be membrane-associated proteins or integral membrane proteins. It is quite likely that the H, M, and G leucine transport proteins function in a membrane complex mechanistically identical to the Q, M, P histidine transport protein complex.

Of particular interest are the findings (1) that the Q, M, and P histidine transport proteins are also essential for an arginine transport system which requires the LAO-

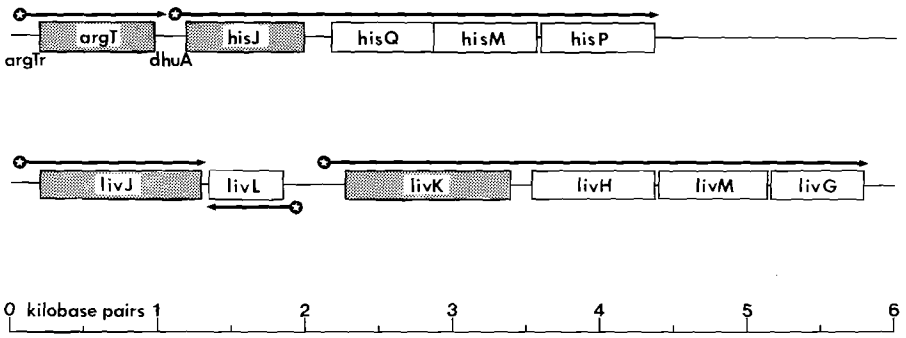

Figure 1. Organization of the histidine and LIV-I transport genes. Stars indicate the positions at which transcription of the genes originates. The arrows indicate the length of each transcript. Transcription of the *livL* gene occurs from the opposite strand in and in opposite direction from the other transport genes.

BP (Kustu and Ames, 1973; Kustu *et al.*, 1979; Higgins and Ames, 1981) and (2) that the H, M, and G leucine transport proteins are also required for a branched-chain amino acid transport system which requires the LIV-BP. The gene coding for the LAO protein (*argT*) and its regulatory locus are adjacent to the histidine transport operon and form a separate monocistronic operon (Higgins and Ames, 1982). Likewise, the gene coding for the LIV-BP (*livJ*) and its regulatory region form a monocistronic operon which is near the leucine-specific transport operon (R. Landick and D. L. Oxender, manuscript in preparation). The LAO-BP is believed to interact with the P protein during arginine transport in a manner analogous to that of the J protein (his-BP) during histidine transport. The his-BP and LAO-BP are 70% homologous while the LIV-BP and LS-BP are 80% homologous. Each pair of genes is believed to have arisen by duplication of an ancestral gene coding for an amino acid BP.

The only striking difference between these two transport gene clusters is the *livL* gene which is located between *livJ* and *livK* in the *liv* cluster. *livL* has been characterized on the basis of DNA-sequencing studies and by gene fusion to *lacZ* to create a hybrid protein. These experiments have revealed that *livL* is transcribed in the opposite orientation to the other *liv* genes and that it can be translated into a basic, hydrophilic protein with a mass of 18.7 K. There is no known function for the *livL* protein, but it has been identified as an *in vitro* transcription–translation product of several plasmids containing the *livL* gene. It is possible that the *livL* protein may play a role in the regulation of *liv* gene expression.

V. ASSEMBLY OF TRANSPORT COMPONENTS

After synthesis, the components of the LIV-I and histidine transport systems must be assembled into their proper locations in either the periplasm or cytoplasmic membrane. Like all other known periplasmic proteins, LAO-BP, his-BP, LIV-BP, and LS-BP are synthesized as precursor molecules with N-terminal signal peptides of 22 (*his*) or 23 (*liv*) amino acids (Oxender *et al.*, 1980b; Daniels *et al.*, 1980; Higgins and Ames, 1981; Higgins *et al.*, 1982a). These sequences are shown in Table 2. A comparison between these two pairs of evolutionarily related signal sequences reveals that they have diverged from each other faster than the mature portion of the BPs. Thus, his-BP and LAO-BP signal sequences are 41% conserved vs. 70% conservation in the mature sequences, and the LIV-BP and LS-BP signal sequences are 47% conserved vs. 80% conservation in the mature sequences. This difference indicates less selective pressure on the signal sequence composition which is in agreement with the widely held belief that the hydrophobic nature of the signal rather than a precise sequence of amino acids is required for protein secretion. This view is further supported by a comparison of the his-BP and LAO-BP signal sequences with the LIV-BP and LS-BP signal sequences where 23% is the maximum conservation of sequence which can be produced by various alignments.

Secretion of the LS-BP has been studied in considerable detail (Oxender *et al.*, 1980b; Daniels *et al.*, 1980, 1981; Landick *et al.*, 1983). The intact precursor of the

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Table 2. N-*terminal Sequences of LIV-I and Histidine-Transport Proteins*

N-terminal sequence																									Transport protein
														Processing site[a] ↓											
	Met	Lys	Lys	Leu	Ala	Leu	Ser	Leu	Ser	Leu	Val	Leu	Ala	Phe	Ser	Ser	Ala	Thr	Ala	Ala	Phe	Ala	Ala	Ile	Pro	His-BP
	Met	Lys	Lys	Thr	Val	Leu	Ala	Leu	Ser	Leu	Leu	Ile	Gly	Leu	Gly	Ala	Thr	Ala	Ala	Ser	Tyr	Ala	Ala	Leu	Pro	LAO-BP
Met	Asn	Ile	Lys	Gly	Lys	Ala	Leu	Leu	Ala	Gly	Leu	Ile	Ala	Leu	Ala	Phe	Ser	Asn	Met	Ala	Leu	Ala	Glu	Asp	Ile	LIV-BP
Met	Lys	Arg	Asn	Ala	Lys	Thr	Ile	Ile	Ala	Gly	Met	Ile	Ala	Leu	Ala	Ile	Ser	His	Thr	Ala	Met	Ala	Asp	Asp	Ile	LS-BP
Met	Leu	Tyr	Gly	Phe	Ser	Gly	Val	Ile	Leu	Gln	Gly	Ala	Ile	Val	Thr	Leu	Glu	Leu	Ala	Leu	Ser	Ser	Val	Val	Leu	*hisQ* Protein
Met	Ile	Glu	Ile	Ile	Gln	Glu	Tyr	Trp	Lys	Ser	Leu	Leu	Trp	Thr	Asp	Gly	Tyr	Arg	Phe	Thr	Gly	Val	Ala	Ile	Thr	*hisM* Protein
Met	Met	Ser	Glu	Asn	Lys	Leu	His	Val	Ile	Asp	Leu	His	Lys	Arg	Tyr	Gly	Gly	His	Glu	Val	Leu	Lys	Gly	Val	Ser	*hisP* Protein
Met	Ser	Glu	Gln	Phe	Leu	Tyr	Phe	Leu	Gln	Gln	Met	Phe	Asn	Gly	Val	Thr	Leu	Gly	Ser	Thr	Tyr	Ala	Leu	Ile	Ala	*livH* Protein
Met	Trp	Ser	His	Ser	Pro	Cys	Asp	Ser	Gly	Ala	Ala	Gly	Asp	Ala	Asp	Arg	Tyr	Ser	Gly	Ser	Pro	Gly	Gly	Gly	Glu	*livM* Protein
Met	Lys	Leu	Thr	Ile	Ile	Arg	Leu	Glu	Lys	Phe	Ser	Asp	Gln	Asp	Arg	Ile	Asp	Leu	Ala	Lys	Asp	Leu	Gly	Arg	Glu	*livL* Protein

[a] The processing site refers only to the first four sequences, the periplasmic His-BP, LAO-BP, LIV-BP, and LS-BP. The remaining N-terminal sequences are shown to illustrate their lack of a consensus signal sequence for secretion.

LS-BP has been synthesized *in vitro* and is processed to the mature form (Daniels *et al.*, 1980) by the leader peptidase enzyme characterized by Zwizinski and Wickner (1980). *In vivo,* the LS-BP can also be detected as a precursor during pulse-chase labeling experiments with ^{35}Smethionine (Daniels, 1981; Landick *et al.*, 1984). This result suggests that the LS-BP precursor is synthesized as a complete polypeptide chain prior to being processed by the leader peptidase and is at odds with the cotranslational secretion model as postulated by Blobel and Dobberstein (1975a,b) and extended to bacterial periplasmic proteins by Davis and co-workers (Ron *et al.*, 1966; Smith *et al.*, 1977, 1978). Randall and co-workers have recently shown that maltose-BP may be processed either posttranslationally or cotranslationally, but never before the polypeptide chain is 80% complete (Josefsson and Randall, 1981). Secretion of some proteins, such as the maltose-BP, appears to be primarily cotranslational while secretion of others, presumably including the LS-BP, is primarily posttranslational. Secretion of periplasmic BPs has been found to be dependent on the inner membrane electrochemical potential (Daniels *et al.*, 1981; Enequist *et al.*, 1981; Landick *et al.*, 1983). Processing of the LS-BP can be blocked by membrane potential dissipating agents such as carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) or valinomycin (Daniels *et al.*, 1981). When secretion is blocked by treating spheroplasts with valinomycin, the LS-BP precursor builds up in the cytoplasmic membrane. The trapped precursor can be processed and released into the periplasm in a completely posttranslational fashion by resuspending valinomycin-treated spheroplasts in the absence of KCl (Daniels *et al.*, 1981). These conditions create a transient membrane potential which is apparently sufficient to stimulate processing and secretion of the LS-BP. Based on these data, we envisage a secretory process that takes advantage of the transmembrane electrochemical field to align the signal sequence across the membrane and allow the signal peptidase, which is located on the outside of the cytoplasmic membrane, to cleave off the signal sequence at the correct location. The inserted signal peptide loop appears to be a structural requirement for initiation of secretion. This conformation may be achieved by an incomplete nascent chain on the ribosome or by a freely soluble protein which would account for the observation of both posttranslational and cotranslational processing of periplasmic BPs. This secretory model is shown in Figure 2.

In addition to the secretory process, periplasmic BPs must fold into the proper conformation once they are released into the periplasm. This conformation is conserved among periplasmic nutrient BPs which have been examined (Quiocho *et al.*, 1979). This conformation has two domains, one of which includes primarily the N-terminal portion of the protein and one of which includes primarily the C-terminal portion. The two domains are connected by a hinge region. In the case of the LS-BP, this conformation is resistant to trypsin, even though the protein contains 27 lysines and nine arginines (Oxender *et al.*, 1980b). This trypsin-resistant conformation is specific to the mature form of the protein; the LS-BP precursor is rapidly degraded by trypsin. We believe that the trypsin resistance of the LS-BP reflects a conformation which may be generally resistant to protease degradation because the periplasmic space contains several proteolytic activities (Sveedhara-Swamy and Goldberg, 1982), and no degradation of the LS-BP occurs in the periplasm. Small deletions in the C-terminal region of the LS-BP render it susceptible to periplasmic protease degradation so that none of

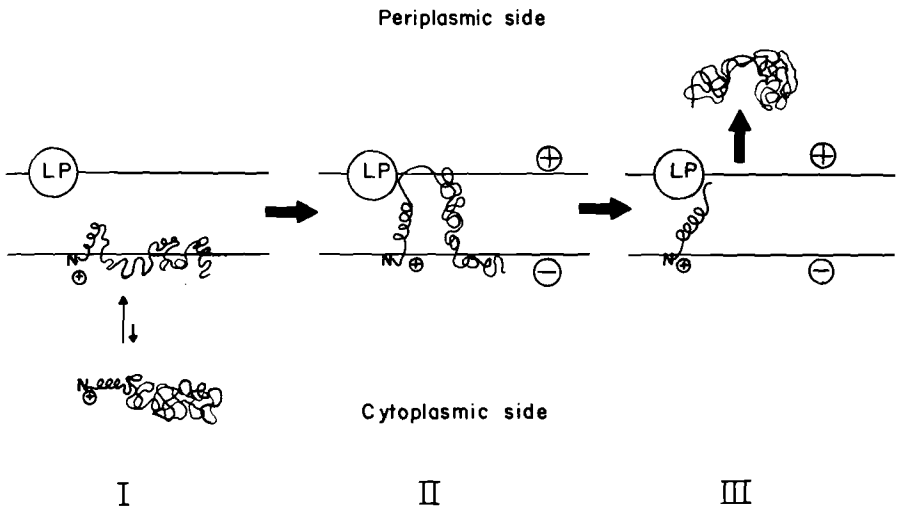

Figure 2. Model for the secretion of the LS-BP.

the mutated protein can be detected in the periplasm, even though it is processed and secreted normally (Landick *et al.*, 1984).

The three membrane proteins of the histidine transport system, two potential membrane proteins of the LIV-I system, and the *livL* protein do not have typical signal sequences at their N-termini (see Table 2). The signal sequence is apparently important for transport of the proteins into the periplasm or into the outer membrane, but a cleavable signal is not required for proteins to be inserted into the cytoplasmic membrane. This is also the case for other membrane proteins (Gay and Walker, 1981a,b; Young *et al.*, 1981; Gilson *et al.*, 1982a; Higgins *et al.*, 1982a). The lactose permease gene codes for what might possibly be a signal peptide, but no processing occurs (Ehring *et al.*, 1980; Buchel *et al.*, 1980). Whether the *liv* and *his* membrane transport proteins are inserted cotranslationally, individually inserted posttranslationally, or assembled in the cytoplasm and then inserted as a complex has not been determined. Because these proteins are highly basic, hydrophobic, and produced in exceedingly small quantities, experiments to answer these questions will be difficult. We anticipate that overproduction of the membrane components by recombinant DNA methods will facilitate these studies.

VI. ENERGIZATION AND RECONSTITUTION OF AMINO ACID TRANSPORT

Amino acid transport, as we have defined it here, is accumulation of amino acids against a concentration gradient and, therefore, necessarily requires a source of energy. A precise description of the mechanism of energy coupling to active transport is not

yet possible; instead, we will discuss the current status of the energetics of amino acid transport. Comprehensive reviews on this subject have been recently published (Simoni and Postma, 1975; Booth and Hamilton, 1980; Hunt and Hong, 1981b). We will also briefly describe the current status of the reconstitution of amino acid transport systems.

A. Membrane-Bound, Osmotic Shock-Resistant Systems: Energization and Reconstitution

It is now generally accepted that membrane-bound, osmotic shock-resistant amino acid transport systems, such as the proline PP-I system, utilize primarily electrochemical proton-motive force to energize transport (Berger, 1973; Berger and Heppel, 1974; Booth and Hamilton, 1980). The mechanism of energy coupling for these transport systems has been studied in membrane vesicle preparations by addition of compounds which either stimulate or inhibit generation of a proton-motive force (Kaback and Milner, 1970; Kaback, 1972, 1974; Futai, 1974; Ramos and Kaback, 1977a,b). In general, D-lactate is an efficient energy donor for stimulating amino acid transport in vesicle preparations, perhaps because of its rapid translocation into membrane vesicles (Nichols and Hamilton, 1976). D-Lactate is converted to pyruvate by the membrane-bound lactate dehydrogenase which supplies electrons to the respiratory chain in the form of NADH. To a lesser extent, succinate, L-lactate, D,L-α-hydroxybutyrate, and NADH also stimulate osmotic shock-resistant amino acid transport in vesicle preparations. An artificial electron donor system composed of phenazine methosulfate and ascorbate is capable of stimulating transport even more effectively than D-lactate (Konings *et al.*, 1971). In each case, this stimulation may be explained by generation of a proton-motive force which, in turn, drives membrane-bound, osmotic shock-resistant amino acid transport systems. In membrane vesicles, D-lactate or other energy sources can stimulate transport of alanine, aspartate, cysteine, glutamate, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, tryptophan, tyrosine, serine, and valine (Kaback, 1972).

The precise mechanism that couples the proton-motive force to these transport systems is not yet established. The proton-motive force is generally thought to be composed of two components: (1) the actual proton gradient or ΔpH, and (2) the membrane potential or $\Delta\Psi$. Either or both of these components may be coupled to amino acid transport. Ramos and Kaback (1977b) have suggested that the mechanism may be different depending on the nature of the substrate transported. Negatively charged amino acids such as glutamate may be taken up by electrogenic proton symport (cotransport of protons and glutamate molecules), whereas neutral or positively charged amino acids may be taken up by a membrane potential-driven mechanism. Amino acid transport also may be indirectly coupled to the proton gradient by sodium ion cotransport as in the case of sodium-dependent glutamate transport (Britten and McClure, 1962; Frank and Hopkins, 1969; Kahane *et al.*, 1975; MacDonald *et al.*, 1977; Schellenberg and Furlong, 1977; Tsuchiya *et al.*, 1977).

Reconstitution of membrane-bound, osmotic shock-resistant amino acid transport systems from purified protein components may allow a better definition of the mechanism of energy coupling. The *E. coli* lactose carrier has been reconstituted in this

manner (Foster *et al.*, 1982). In this case, it has been suggested that either the membrane potential ($\Delta\Psi$) or the proton gradient (ΔpH) may energize lactose transport. $\Delta\Psi$ may be coupled to lactose transport through a net negative charge at the carrier protein's active site (Schuldiner *et al.*, 1975; Kaback, 1976). Thus, the membrane potential would increase the rate of outward translocation of the free carrier and decrease its lifetime at the inner surface of the membrane. ΔpH may be coupled to lactose transport by cotransport of lactose and protons (lactose–proton symport). Studies on the reconstituted lactose carrier have provided convincing support for these ideas and have even been extended to suggest a role for histidine residues in energy coupling to the proton gradient (Garcia *et al.*, 1982; Patel *et al.*, 1982). Because purified lactose carrier protein was used, these experiments demonstrate that no other proteins are required for coupling of lactose transport to either $\Delta\Psi$ or ΔpH. Further work will be necessary to extend these proposals (if warranted) to membrane-bound, osmotic shock-resistant amino acid transport systems.

B. Energization of BP-Dependent Systems

The mechanism of energy coupling in BP-dependent, osmotic shock-sensitive amino acid transport systems is even less well understood. Both phosphate bond energy and the membrane potential appear to be required for this class of transport system.

The requirement for phosphate bond energy is most clearly evident from two observations: (1) an *E. coli* mutant that is unable to synthesize ATP via oxidative phosphorylation can energize BP-dependent transport of glutamine by metabolism of glucose but not lactate, while the parent strain can utilize either substrate, and (2) arsenate, which rapidly depletes cellular ATP levels (Klein and Boyer, 1972), blocks BP-dependent transport of glutamine when either glucose or lactate is the carbon source (Berger, 1973). Subsequent investigations have suggested a phosphate bond energy requirement for the BP-dependent transport of arginine, histidine, ornithine (Berger and Heppel, 1974; Gutowski and Rosenberg, 1976), methionine (Kadner and Winkler, 1975), leucine, isoleucine, and valine (Oxender, 1972; Wood, 1975). Initially, it was thought that ATP might directly phosphorylate the BPs of osmotic shock-sensitive systems, but no correlation between ATP levels and the extent of osmotic shock-sensitive transport has been found (Plate *et al.*, 1974; Lieberman and Hong, 1976) and, in disagreement with the earlier view, it is now generally thought that some other high-energy phosphate bond metabolite must act as the primary energy coupling factor (Lieberman and Hong, 1976). Based on the observation that an *E. coli* mutant unable to synthesize acetyl phosphate (*pta*) was unable to use pyruvate to stimulate BP-dependent glutamine transport in the presence of cyanide and fluoride, Hong and co-workers proposed that acetyl phosphate may be the intermediate metabolite (Hong *et al.*, 1979). Attempts to obtain more direct evidence for the role of acetyl phosphate as an intermediate have generally been unsuccessful. No change in acetyl phosphate levels was observed when *E. coli* cells were treated with arsenate (Hong and Hunt, 1980). At this point, the available evidence indicates that it is unlikely that acetyl phosphate is an intermediate in the energization of osmotic shock-sensitive transport systems.

Several lines of evidence suggest that phosphate bond energy, alone, is not sufficient for energization of amino acid transport systems involving BPs. Proton ionophores or anaerobiosis which prevent the generation of a proton-motive force can inhibit BP-dependent transport by as much as 60% (Berger, 1973; Berger and Heppel, 1974; Cowell, 1974). In a proton ATPase-negative mutant, treatment of cells with colicin K results in a significant decline of BP-dependent glutamine uptake even though ATP levels rise (Plate *et al.*, 1974). Temperature-sensitive *E. coli* mutants, which lack a membrane potential but have normal proton gradients and ATP levels, exhibit reduced BP-dependent glutamine uptake (Lieberman and Hong, 1976; Plate, 1976; Lieberman *et al.*, 1977). Thus, it appears that the membrane potential, in addition to high-energy phosphate bond energy, has a function in the activation of BP-dependent amino acid transport. This function might be a true contribution to the energization of transport or it might be simply to orient membrane protein components of the transport systems into their proper conformation.

C. *Reconstitution of BP-Dependent Amino Acid Transport*

Ultimately, reconstitution from purified protein components may play an important role in defining the mechanism and energization of BP-dependent amino acid transport systems. To date, however, reconstitutions of BP-dependent systems, even in spheroplasts or membrane vesicles, have been plagued with problems. The large number of controls necessary to rule out artifactual results has made these experiments difficult. In spite of the difficulties, several investigators have reported the successful reconstitution of BP-dependent amino acid transport. Wilson and Holden (1969b) first reported reconstitution of BP-dependent amino acid transport by addition of partially purified arginine-BP to osmotically shocked cells. Anraku also reported stimulation of leucine transport by addition of partially purified LIV-BP and other protein factors to osmotically shocked cells (Anraku *et al.*, 1973). Successful reconstitutions of BP-dependent phosphate (Gutowski and Rosenberg, 1976) and ribose (Robb and Furlong, 1980) transport in spheroplasts have also been reported. More recently, Hong and co-workers have reported successful reconstitution of BP-dependent glutamine transport by addition of glutamine-BP to both spheroplasts (Masters and Hong, 1981a) and membrane vesicles (Hunt and Hong, 1981a). In the latter work, incorporation of NAD into the membrane vesicles and addition of sodium pyruvate to the assay were required for maximal stimulation. Even under this best situation, however, the reconstitution achieved was more than an order of magnitude lower than that achieved with an equal amount of spheroplasts (based on protein content). Correction for the differences in protein content between spheroplasts and membrane vesicles would result in a much greater difference. Even these low levels of reconstituted uptake, however, have been used for recent studies to define the energy requirements of BP-dependent amino acid transport (Hunt and Hong, 1983a,b). These recent experiments have suggested that two classes for compounds (succinate and those which can be metabolized to pyruvate) are capable of stimulating BP-dependent transport of glutamine in membrane vesicles (Hunt and Hong, 1983a). Evidence implicating a role for glutamine-BP tryptophan and histidine residues in the interaction on glutamine-BP with membrane-bound com-

ponents has also been found with reconstitution experiments (Hunt and Hong, 1983b). Most recently, studies on the reconstitution of BP-dependent transport of glutamine in membrane vesicles have led to the conclusion that redox energy and a membrane potential may both be required to energize BP-dependent active transport (J.-s. Hong, personal communication).

VII. POSSIBLE MODELS FOR AMINO ACID TRANSPORT

With the availability of the complete nucleotide sequence of all of the genes of the histidine transport operon, with the nucleotide sequence of the LIV-I genes nearing completion, and with the identification of most of the proteins involved in these two permeases, it is now possible to formulate tentative models for the molecular mechanism of transport by these systems (Higgins *et al.*, 1982a). Two possible alternatives are presented here, both using as examples the histidine transport system and taking into account all the knowledge available for that system. The general architecture of the complex of proteins is the same in two models (Figure 3), but in one case, the membrane-bound components bear no specificity for transport, while in the other case, one or more specific substrate-binding sites are present in the membrane-bound components. In the "pore" model (shown in Figure 3A), histidine binds to J (his-BP) causing a conformational change, thus allowing interaction of the J–histidine complex with P. Additional conformational changes in the hypothetical P,Q,M complex allow a pore to be formed within or between the proteins of the complex (or both). Histidine is able to diffuse through such a pore to the cell interior. This model proposes that the specificity of the transport system is dictated by a single specific component, the his-BP, J. The specificity is transmitted to the system as a whole by a specific interaction

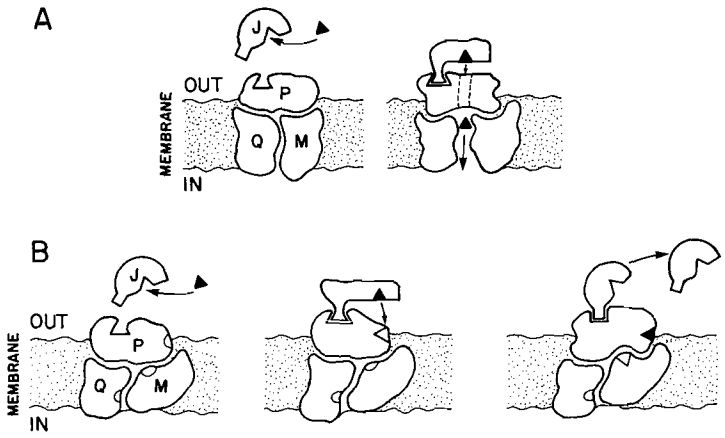

Figure 3. Models for the mechanism of amino acid transport systems. (A) The "pore" model, (B) the "binding-site" model.

between J and P. Presumably, any substrate able to bind to J (and therefore causing the prescribed conformational change) will be transported through an unspecific pore in the membrane. Alternatively, the "binding-site" model (shown in Figure 3B) involves an identical initial step which would be followed, upon interaction of the J–histidine complex with the membrane proteins, by a sequential "activation" of one or more "histidine-binding sites" which would transport histidine in a cascade-like manner through the membrane. This second model postulates a more active role for the membrane-bound proteins, involving a histidine-binding site on each of the membrane components (although, of course, not all of the membrane components need to have such a site). Both models postulate that the interaction of the J–histidine complex with the membrane-bound proteins should be such that histidine is very close to the pore or to the next histidine-binding site. This arrangement is assumed to be necessary to avoid diffusion of the released histidine into the periplasm as soon as it is released from the periplasmic component. In other words, it is possible that the most probable function of the BP is that of "trapping" the substrate in a bound form and delivering it directly to the next site, without allowing it to return to a freely diffusible state. It is also possible to envisage an intermediate situation where a pore is formed which has such a shape and/or disposition of amino acid residues that only histidine or a substrate with a closely related shape can pass through it.

It is too early to distinguish between the two models of Figure 3. However, we can predict that if the binding site model is correct, transport mutants should exist which have a defective binding site in a membrane protein thus resulting in an *altered* spectrum of specificity for transport. Several mutants in membrane components of the histidine permease have been characterized, which indeed present an altered spectrum of specificity, thus tentatively supporting the "binding-site" model (Higgins *et al.*, 1982a). The existence of a substrate-binding site on a membrane component has been postulated also for another, non-amino acid transport system (Shuman, 1982). It should be noted that, although not represented in Figure 3, an energy-coupling mechanism, such as described in the previous section, must be involved in the process of concentrative uptake.

VIII. REGULATION OF AMINO ACID TRANSPORT

Amino acid transport systems are regulated to meet the nutrient requirements of bacteria. Low-affinity transport systems which typically have high V_{max}s may function primarily for concentration of amino acids from the growth medium, while high-affinity systems may function primarily as scavengers to prevent the loss of amino acids which are synthesized intracellularly and leak out through the inner membrane (Ames, 1972; Landick and Oxender, 1981). Since wild type bacteria can synthesize all 20 amino acids, they may have a great need for transport systems to recapture amino acids that they have expended energy to synthesize. For example, it has been calculated (Brenner and Ames, 1971) that, for the synthesis of one molecule of histidine, the potential energy corresponding to 41 ATP molecules is sacrificed. Thus, it should be more desirable from the point of view of cellular economy that the cell be

able to transport one molecule of histidine from the medium or recapture a previously synthesized one rather than synthesize a new histidine molecule *de novo* from glucose. This is achieved through the evolution of high-affinity transport systems which efficiently concentrate any small amounts of environmentally available substrate. Measurement of transport in growing cells, under physiological conditions, reveals an interesting measurable parameter, the *limit concentration*, which is the minimal external concentration required by the permease in order to supply the cell with enough amino acid to carry on protein synthesis without utilizing any of the biosynthetic amino acid (Ames and Roth, 1968; Ames, 1972); the lower this value, the more effective is the uptake mechanism with respect to growth. For example, the limit concentration for histidine is 1.5×10^{-7} M (Ames, 1964). While the histidine and LIV-I transport systems certainly fulfill the role of high-affinity amino acid transport systems, all of their substrate amino acids can also be transported by other, higher K_m and V_{max} systems (see Table 1). The LIV-I system, in particular, may play a central role in fine tuning the intracellular concentration of leucine.

A. Regulation of the Histidine Transport System

The histidine transport system has been shown to be regulated by nitrogen availability: cells respond to nitrogen limitation by increased expression of the histidine transport genes (among others; Kustu *et al.*, 1979; Higgins and Ames, 1982). This system is not affected by the level of histidine in the medium. In addition it is regulated independently from the histidine biosynthetic operon as its expression is unaffected by the presence of regulatory mutations affecting histidine biosynthesis. Thus, it seems that this system must depend on regulatory mechanisms which do not involve a typical transcription attenuation mechanism like those described for biosynthetic operons (Yanofsky, 1981). In agreement with this it was found that the nucleotide sequence of neither regulatory region, *dhuA* or *argT*, displays any of the features typical of the regulatory regions of amino acid biosynthetic operons (Higgins and Ames, 1982). Among the features of interest found in these two regions are several dyad symmetries. Of particular significance may be a dyad symmetry found in each region, the two bearing considerable homology to each other. In both cases, the dyad symmetry overlaps the promoter site (-10 and -35 regions) and retains a stretch of high homology at the "foot" of the hypothetical stem-loop structure which each of them can form. It is not known what, if any, is the function of this structure.

One might expect to find regulatory features which must be involved in the response of these operons to nitrogen availability. A pseudo-mirror symmetry was in fact found in each of these regions, and by model building and computer analysis (cylindrical projection kindly performed by S.-H. Kim) it was possible to determine that the distribution of functional groups in such pseudo-symmetries does indeed correspond to a true twofold symmetry in the minor groove of the DNA double helix and, thus, would be able to respond to a regulatory protein that displays dyad symmetry. Because extensive homology was found between these mirror symmetries and one occurring in the regulatory region of *glnA*, the gene coding for glutamine synthetase and known to be under nitrogen regulation, it was postulated that these structures are

involved in nitrogen regulation (Higgins and Ames, 1982). If this were true, it may be that these sequences are recognition sites for interaction with protein(s) produced by genes involved in responding to nitrogen availability (*ntrA*, *ntrB*, and *ntrC*; McFarland *et al.*, 1981).

B. Regulation of the LIV-I Transport System

LIV-I transport is directly regulated by the concentration of L-leucine in the growth medium (Quay and Oxender, 1976, 1980a; Landick *et al.*, 1980). When cells are grown in the absence of leucine, LIV-I transport is derepressed. Addition of leucine reverses this derepression, but no other amino acid can accomplish this reversal. Thus, the LIV-I transport system, though it functions for uptake of L-leucine, L-isoleucine, L-valine, L-threonine, L-alanine, and D-leucine, is regulated by the single amino acid, L-leucine. This control, however, is mediated by mechanisms which involve several other cellular components. Table 3 shows the various mutations which are known to affect LIV-I gene expression.

Repression by exogenous leucine is a different mode of regulation than that found for other amino acid transport systems, such as the proline PP-II system (see Table 1). Here, amino acid transport activity is induced when the given amino acid is present in the growth medium. Most carbohydrate transport systems are also regulated in this fashion (Dills *et al.*, 1980).

Transcription attenuation of biosynthetic operon expression has been documented for the *trp*, *his*, *ilv*, *leu*, and *phe* biosynthetic operons and the mechanism for this attenuation is reasonably well understood (Yanofsky, 1981). Over the past 7 years, a variety of results have been obtained which suggest that, like the biosynthetic operons, expression of the LIV-I transport gene cluster is controlled by transcription attenuation. Most notable is the derepression of LIV-I transport in strains with mutations in *rho* or *leuS* (Quay *et al.*, 1975a, 1977; Quay and Oxender, 1976, 1977; 1980b; Landick *et al.*, 1980). Recently, this speculation has been strengthened by examination of the DNA sequence preceding the *livJ* gene and studies on the *in vitro* transcription of this gene. We have found that transcription of *livJ* originates 104 bases prior to the beginning of the structural gene, and that the leader region of this transcript potentially codes for a small peptide with tandem leucine codons. On the basis of these and other results we have proposed a model for transcription attenuation of *livJ* (Landick, 1984). In this model, transcription termination by the product of the *rho* gene is coupled to translation of the small leader peptide which is, in turn, controlled by the availability of charged leucyl-tRNA. This model is quite different from the transcription attenuators found in the biosynthetic operons where transcription termination is coupled to translation without the involvement of the *rho* gene product. Further work will be required to verify the model for *livJ* transcription attenuation, but it now appears that this LIV-I transport gene is controlled by a unique transcription attenuator. Similar transcription attenuation of the *livK* gene has not been documented.

A strong case for the involvement of regulatory proteins can be made for LIV-I transport gene expression. Mutations with repressor-like phenotypes have been found which derepress LIV-I and map at a location far removed from the LIV-I transport

Table 3. LIV-I Transport System Regulatory Factors

Gene	Gene product	Phenotypic effect of the mutation on transport	References
rho	Transcription termination factor Rho	Elevated level of leucine transport	Quay and Oxender (1977), Quay *et al.* (1978), Landick *et al.* (1980)
leuS	Temperature sensitive leucyl-tRNA synthetase	Elevated leucine transport at the nonpermissive temperature	Quay *et al.* (1975a), Quay and Oxender (1976), Quay and Oxender (1980b)
hisT	Enzyme which converts uridine to pseudouridine in tRNA	Unable to derepress leucine transport. Higher concentration of charged leucyl-tRNA	Oxender and Quay (1976a), Quay *et al.* (1978)
relA	A ribosome-associated protein which synthesizes ppGpp from GTP in response to amino acid starvation	Unable to derepress leucine transport	Quay and Oxender (1979)
livR	Unknown	Elevated leucine transport. Primary effect on LIV-BP	Anderson *et al.* (1976), Landick *et al.* (1980)
lstR	Unknown	Elevated leucine transport. Primary effect on the LS-BP	Anderson *et al.* (1976)

genes (Anderson *et al.*, 1976; Landick *et al.*, 1980). These loci, *livR* and *lstR*, map at min 22 on the *E. coli* chromosome and are described in Table 3. Palindromic sequences are located surrounding the origins of transcription for all four transport promoters. Palindromic sequences have been found at the binding sites for other known repressor proteins (Gilbert *et al.*, 1974; Maniatis *et al.*, 1975; Gunsalus and Yanofsky, 1980). In the case of the *livJ* gene transcript, the palindromic symmetry is located directly overlapping the known origin of transcription as determined by sequencing of an *in vitro* synthesized *livJ* transcript.

C. Control of Membrane Protein Synthesis

Present evidence suggests that both the specific amino acid BPs, his-BP and LS-BP, and their respective membrane components are encoded on a single polycistronic mRNA. The membrane components, however, are synthesized in quantities ten-fold lower than the BPs (Ames and Nikaido, 1978). Secondary structure in the mRNA may be responsible for controlling the level of messenger for the membrane component genes to accomplish this lowered synthesis. Some of this reduction may also be due to a weaker ribosome-binding site for the membrane components, but a strong case can be made for regulation of mRNA levels as a mechanism for lowering the expression of these genes. This is best illustrated in the *his* transport regulon where a large stem-loop structure is present in the mRNA between the *hisJ* and *hisQ* genes (Higgins *et al.*, 1982b). Extremely similar structures are also present in the mRNA sequences between the *hisG* and *hisD* biosynthetic genes and between the *lamB* and *molA* genes in the *malK–lamB* operon and others. These structures, which exhibit very high homology may be involved in regulating the differential expression of the individual genes in these operons (Higgins *et al.*, 1982b). The stem-loop structure between *hisJ* and *hisQ* is shown in Figure 4. This structure may function as a recognition site for mRNA processing. Recent data indicate that it is not involved in transcription termination (Stern *et al.*, 1984). A small stem-loop structure is found between *livK* and *livH* in the LIV-I regulon (shown in Figure 4). This structure contains a GC-rich stem and is followed by three Ts, however, it bears no homology to the structure found in the histidine operon. Such a structure may be a typical transcription termination signal. It is unlikely, however, that this termination signal separates the leucine transport genes into two operons both because no promoter sequence is present preceding *livH* and because mutations in *livK* are polar on expression of the downstream *livH* gene. It is possible that regulation in the *his* transport operon utilizes mRNA processing, while the regulation between *livK* and *livH* involves transcriptional attenuation.

IX. EVOLUTIONARY RELATIONSHIPS AMONG PERIPLASMIC SYSTEMS

A comparison of the characteristics of the known periplasmic systems (including non-amino acid ones) suggests that the underlying mechanism of transport is possibly

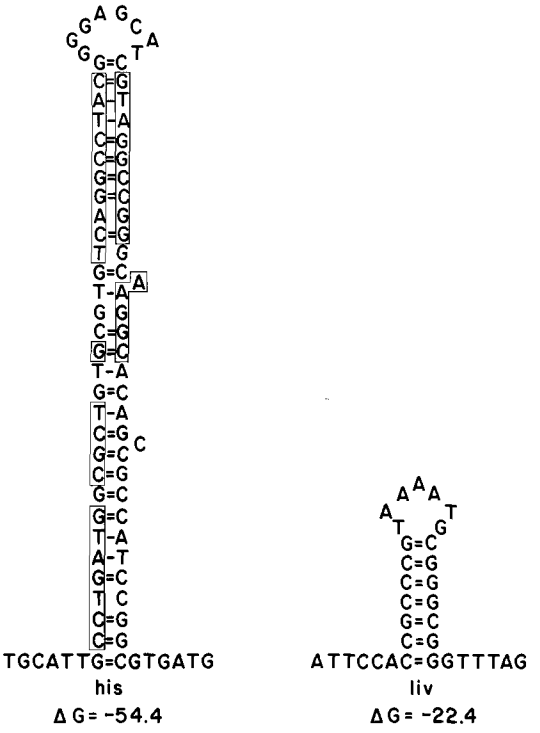

Figure 4. Secondary structures in the mRNA between the *hisJ* and *hisQ* genes and the *livK* and *livH* genes. In this figure the DNA sequence is shown. Thymine residues (T) will appear as uracil residues (U) in mRNA synthesized from the DNA sequence shown here. The free energies of stabilization for the stem loops were computed using the rules of Tinoco *et al.* (1973). Free energies are given in kcal/mole. The regions of sequence conservation with the stem-loop structures found in the *his* biosynthetic and *malK–lamB* operons are enclosed in boxes.

the same for all of them (Ames and Higgins, 1983). The physical composition of all of the permeases which have been extensively studied, i.e., histidine, LIV-I, maltose, and galactose, is similar, involving one or more periplasmic components and two or more membrane components. Where sufficient genetic information is available, it is clear that the genes coding for the components are always closely linked on the chromosome, possibly forming an operon in all cases (two divergent operons are known for the maltose transport system). In at least two cases (histidine and galactose), interaction may occur between the periplasmic BP and a membrane-bound component. In addition, it is interesting that the tandem duplication and divergent evolution of the gene coding for the periplasmic component have occurred in more than one system. This has been shown clearly for the *hisJ* and *argT* genes in the histidine system. A remarkably analogous situation exists for LIV-I where genes *livJ* and *livK* also probably originate by duplication of an ancestral gene. The incomplete characterization of most other systems does not allow a generalization to be formulated as far as duplications of genes for periplasmic components is concerned. However, it is possible that the genes for the galactose- and arabinose-BPs also originated as the result of a duplication. These proteins are antigenically related, they share a small but definite homology, and their genes are located quite close to each other on the chromosome.

Considering the complexity of the organization of the periplasmic systems, it is possible that they have all originated by duplication and divergence from a single ancestral system, perhaps already containing a duplication of the periplasmic component, rather than having arisen independently and then acquired some similarity by convergent evolution. Each system arising by duplication would have evolved different specificity while the same basic architecture would have been retained. If the above were true, it might be possible to uncover sequence homologies between proteins of unrelated systems. In agreement with this hypothesis is the fact that the sequence of one of the membrane-bound components of the histidine transport system, the P protein, has a clearly significant homology with the sequence of a membrane-bound component of the maltose-transport system, the MalK protein (Gilson *et al.*, 1982b). However, comparison of all the available sequences of periplasmic BPs has shown only marginally significant homologies between proteins from several completely unrelated systems (Ferro-Luzzi Ames, Farrah, and Doolittle, unpublished results). A more sophisticated statistical analysis will be necessary before the significance of such homologies can be determined. In favor of a structural and functional relationship between these proteins are also the elegant studies of Quiocho and his collaborators on the X-ray structures of several periplasmic transport proteins, which showed in all cases strongly similar two-domain structures, even though the substrates transported by the proteins analyzed are quite different from each other (leucine-isoleucine-valine, arabinose, and sulfate; Quiocho *et al.*, 1977, 1979; Miller *et al.*, 1980).

A problem arising if we are to conclude that all periplasmic systems arose from an ancestral system which already contained a duplication of the periplasmic components is that homology between the two periplasmic components of one system is greater than the homology between those same components and their hypothetical duplicated versions as they appear in a system of completely different specificity. For example, the LAO-BP resembles the his-BP more than either the LAO-BP or the his-BP resembles the LS-BP or LIV-BP. A reasonable explanation is that the LAO-BP and his-BP are constrained in their evolution by their need to interact with the same membrane component (P), while the LS-BP and LIV-BP presumably need to interact with their own specialized membrane component. In support of this hypothesis is the finding that within the his-BP and LAO-BP are segments which are much more highly conserved (>90% homology) than the overall sequence (70% homology) and which are believed to be involved in forming that domain of the molecule which is responsible for the interaction with the membrane protein P (Figure 3). A similar pattern of sequence homology and divergence is seen for the LIV-BP and LS-BP. These two proteins contain two long stretches (>30 amino acids) of absolute sequence conservation in the N-terminal half of the protein while the C-terminal half of the protein is only about 60% conserved vs. 80% overall conservation. Interestingly, the location of conserved and diverged sequences for the LS-BP and LIV-BP differs significantly from those observed for the his-BP and LAO-BP. Therefore, each would have evolved as a "package" independently from the other periplasmic systems. Regardless of the exact sequence of duplication, it is quite likely that present-day amino acid transport systems have evolved from an ancestral amino acid transport system with much broader specificity but which utilized a similar transport mechanism and a similar arrangement of protein components.

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