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MINI-REVIEW

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Systems and mechanisms of amino acid uptake and excretion in prokaryotes

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The plasma membrane of bacterial cells functions as a permeability barrier for most solutes. Except for some small molecules (gases, water), only apolar (hydrophobic) compounds show significant permeability, e.g. tryptophan or glycerol. The translocation of the majority of solutes is catalyzed by carrier proteins, which thus provide a central function indispensable for every living cell. The significance of membrane transport, however, is not simply to supply the cell with certain compounds but also to remove metabolic waste products. In order to understand the metabolic network of the cell, not only the knowledge of the pathways and their regulation, but also detailed information concerning both the uptake of substrates and excretion of products is essential. Amino acids are transport solutes of particular interest for the cell, and this holds for both transport directions, i.e. uptake and excretion. Fig. 1 indicates the essential membrane transfer reactions involved in amino acid metabolism. If externally available, amino acids are taken up by corresponding uptake systems. In general, the major part of the cytosolic amino acids is synthesized by the cell using anabolic pathways via conversion of appropriate substrates which themselves must be taken up. On the other hand, amino acids can be excreted in many bacteria. Finally, if an amino acid is sufficiently membrane-permeable, diffusion may play a role both in the influx and efflux direction. Since the amino acid concentration is in general higher in the cytosol as compared to the medium, this process is normally only relevant in the direction of excretion.

A large number of primary sequences of prokaryotic and eukaryotic carriers in general and amino acid transporters in particular are available. This minireview, however, will focus on the functional aspects of this kind of systems, describing the mechanisms by which amino acids cross the bacterial membrane in both directions. Furthermore, attention is concentrated on the possible interactions of transport systems both in terms of regulation and with respect to the presence of futile cycles (uptake and excretion).

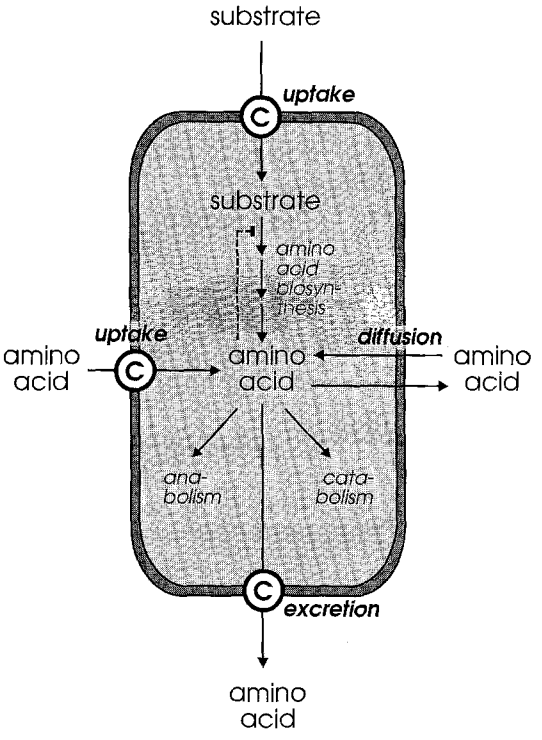

Fig. 1 Transport processes related to amino acid metabolism

1. Transport mechanism

The reaction catalyzed by a carrier protein includes at least three steps, (i) binding of the solute to the binding site, (ii) translocation, (iii) dissociation of solute from the carrier protein. The translocation step involves a major conformational change (reorientation) of the carrier protein (or its binding sites), thus representing the essential "switch" in the transport reaction. Since models involving

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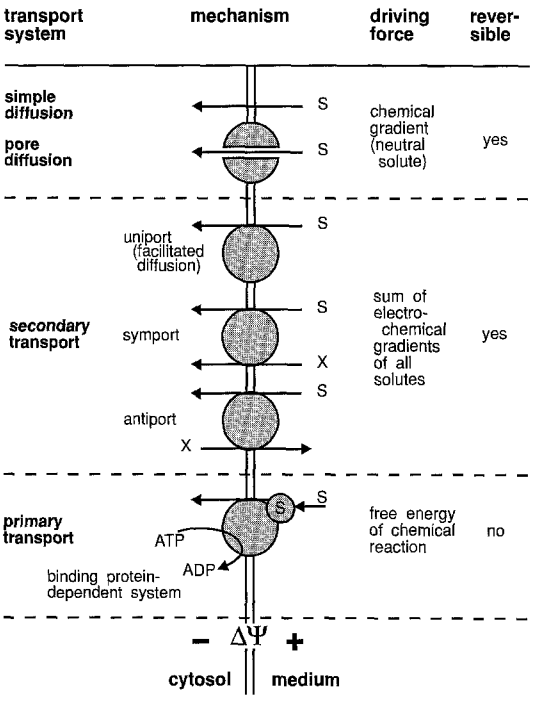


Fig. 2 Diversity of mechanisms in amino acid uptake

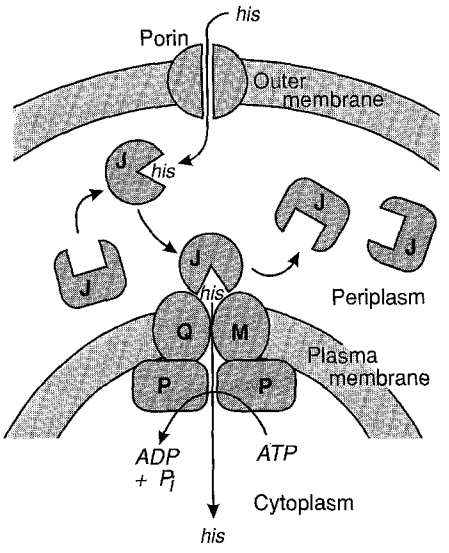


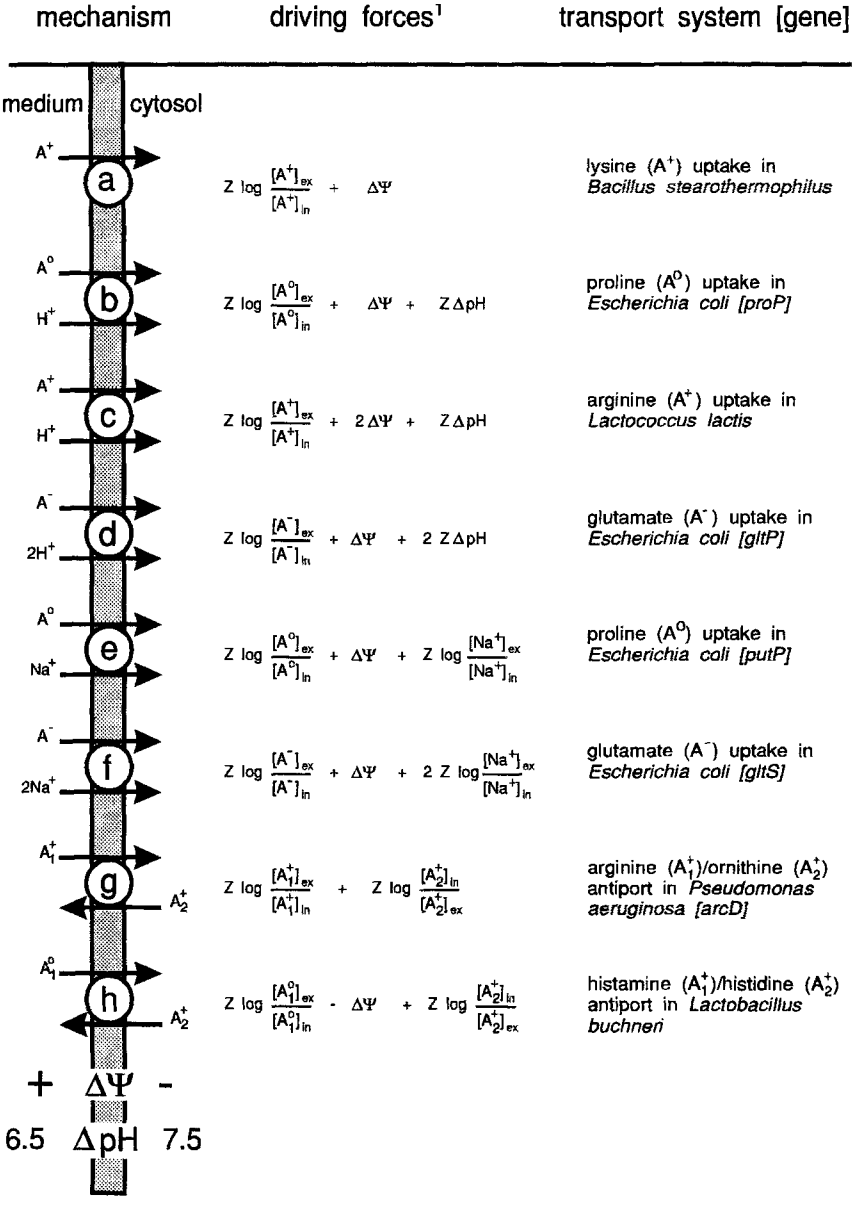
Fig. 3 Schematic representation of the binding protein-dependent histidine uptake system of *Salmonella typhimurium*. The abbreviations mean: J, hisJ, histidine binding protein; Q, M, hisQ and hisM, integral membrane proteins; P, hisP, ATP binding subunit at the cytosolic side

rotational movement of the carrier have been discarded long ago for basic energetic reasons, the most simple rationale for this conformational change is a model based on alternating access of the solute to the binding site (Jardetzky 1966, Tanford 1983). The understanding of transport mechanism was greatly stimulated by comparing the functional concept of enzymes and carriers. Carrier systems can be regarded as membrane-bound enzymes. Instead of catalyzing the conversion of substrate to product, as "true" enzymes do, they mediate the vectorial reaction of solute transfer from one compartment to the other. Because of the various metabolic purposes of solute transport, the widely differing nature of transported solutes, and different membranes across which transport occurs, there is a great diversity in transport mechanisms and systems. This diversity is most extreme in the bacterial kingdom. Some systems in fact only occur in prokaryotes, e.g. binding protein-dependent systems as well as phosphotransferase systems. The observed diversity in transport could be explained by the different functional and physiological requirements which have to be met. Among these are (i) the need for specificity with respect to structure of particular substrates, (ii) particular energetic requirements (e.g. high accumulation ratios) under which a certain solute is transported, and (iii) the need for mechanisms to couple various kinds of energy input to solute translocation.

Transport processes can be classified according to structural, kinetic, or energetic aspects. The concept introduced by Mitchell (Mitchell 1967), based on the utilization of energy sources for transport, is now widely accepted. Figure 2 shows representative examples with respect to amino acid transport.

(I) In primary transport the vectorial reaction of solute translocation is directly coupled to a chemical or a photochemical reaction. Primary transport systems thus directly convert light or chemical energy into electrochemical energy, i.e. the electrochemical potential of a given solute. With respect to amino acid transport, only the ATP-driven, binding protein-dependent systems for amino acid uptake have to be mentioned here (Fig. 3).

(II) The driving force in secondary transport is the electrochemical energy of a given solute. This energy can be utilized to drive the uphill transport of another solute, i.e. against its own concentration gradient. This is achieved, depending on the direction of the gradients, either by cotransport (symport) or by countertransport (antiport) of the driving and the driven ion. Because of the similarity in energy dependence and mechanism, also carrier-mediated unidirectional transport simply driven by its own electrochemical gradient is classified as secondary transport (uniport). Symport and antiport may lead to uphill transport of the driven ion and have thus been classified as "secondary active". They are, however, not intrinsically active. On the other hand, secondary uniport is often named "facilitated diffusion". This designation is also misleading because carrier-catalyzed transport is in principle different from the mechanism of diffusion. Another classification refers to the net movement of charges. Electroneutral transport does not involve net charge transfer. This may result from transport of uncharged solutes, from symport of an equal number of positive and negative charges, or from antiport of two ions with equal charge (see Fig. 4). Electrical transport involves net charge transfer and is influenced by the membrane potential. The term "electrical" includes the cases of "electrogenic" transport (creating a potential, e.g. H^+ extrusion by the respiratory



1 For reasons of simplification, the sign of the different driving forces was adjusted in the following way: positive sign, driving in the direction of the indicated transport system; negative sign, opposing force (Z = 2.3 RT/F)

Fig. 4 Different driving forces of some representative secondary amino acid uptake systems. The letters in the carrier symbols refer to the explanations in the text

chain) and that of "electrophoretic" transport (solute transfer driven by the membrane potential, e.g. Na^+-coupled proline uptake).

(III) Group translocation differs from all other mechanisms due to the fact that the transported solute becomes chemically modified during translocation. The only systems of this class are the phosphoenolpyruvate: sugar phosphotransferase systems (PTS) in bacteria. Except for substrate uptake (Fig. 1), these systems are not relevant for amino acid transport.

(IV) In simple passive mechanisms, solutes cross the membrane without the involvement of a carrier protein ("simple diffusion"). In particular cases, translocation of a solute may be facilitated by the presence of a pore protein. Movement of a solute through the water-filled channel of such a protein may include properties of channel-type and carrier-type mechanisms.

Before discussing particular carrier systems, we will briefly consider their kinetic and energetic principles. Most simply, a solute can cross the membrane by diffusion. The rate of unidirectional transfer (v_I) from one compartment (I) to the other (II) is directly proportional to the concentration of the solute (S) in compartment I.

$$v_I = P\ A\ [S]_I \quad (1)$$

P is the permeability coefficient and A the area of the membrane. A corresponding equation holds for the reverse flux from side II to side I. The net flux v of a solute (S) from side I to side II is then described by

$$v = P\ A\ ([S]_I - [S]_{II}) \quad (2)$$

Net transport by diffusion occurs only from the compartment of higher to that of lower concentration and is not specific.

Carrier transport involves the participation of a proteinaceous component, i.e. the carrier protein, and shows many properties known from enzyme kinetics. The major arguments indicating the action of a carrier are mainly of a kinetic type. These are, similar to enzymes, (i) saturation kinetics, (ii) substrate specificity, (iii) inhibition by specific agents, and (iv) the particular kinetic observation of counterflow. In order to characterize carrier processes, it is convenient to start with secondary uniport (facilitated diffusion). This passive process is carrier-mediated, the net flux is thus not simply proportional to the concentration difference of the solute. Many transport systems, not only those of secondary uniport, can be described by Michaelis-Menten kinetics.

$$v = V_{max} \cdot [S] / (K_m + [S]) \quad (3)$$

K_m is used by analogy to enzyme kinetics, it means the solute concentration at which the transport rate v reaches half its maximum (V_{max}), and is also called K_t (for *t*ransport).

In contrast to diffusion processes, transport reactions in biological systems frequently lead to the accumulation of a given solute in the cytosol. This "uphill" transport is characterized by a positive free energy (ΔG), i.e. this reaction will never occur spontaneously. In order to take place, it must be "coupled" to another reaction with a (high) negative ΔG, leading to an overall negative change in free energy of the coupled reactions. Only two main mechanisms are known: chemical coupling (primary transport) and coupling by ion currents (secondary transport). Consequently, these two alternatives are connected to two types of energy "currency": ATP and a few other high energy compounds on the one hand (chemical coupling), and electrochemical ion potentials on the other (coupling by ion currents).

In secondary coupling, a primary transport system pumps ions (H^+ or Na^+) across the membrane, thus establishing an electrochemical potential. This is then used as the source of free energy for systems which convert a downhill H^+ or Na^+ flux into useful work for the cell. The chemiosmotic description of energy coupling requires closed membrane structures. If a short cut is introduced, e.g. by the action of "uncouplers", coupling is abolished. The electrochemical potential $\Delta\tilde{\mu}_S$ of a particular ion S (in the two compartments I and II) is

$$\Delta\tilde{\mu}_S/F = n\Delta\Psi + RT/F \cdot 1n\,[S_{II}]/[S_I] \quad (4)$$

When expressed for protons ($S = H^+$, $n = 1$), in general Mitchell's term protonmotive force (Δp) is used. At 25° C, Δp becomes (in millivolts)

$$\Delta p = \Delta\Psi - 59\ \Delta pH \quad (5)$$

Δp can easily be converted into a change in free energy.

$$\Delta G = F \cdot \Delta p \quad (6)$$

The energy stored in the electrochemical proton potential may be used by other transport systems to drive the vectorial movement of a solute against its concentration gradient.

The principle of secondary transport is now to couple the fluxes of driving and driven ion in such a way that one cannot take place without the other. In thermodynamic terms, a carrier system (primary or secondary), under steady-state conditions equilibrates the chemical (ATP) or electrochemical potential (proton or sodium motive force) of the driving force with that of the driven solute. Figure 4 exemplifies this for a number of secondary amino acid transport systems. The different chemical and electrical driving forces must be multiplied according to the transport stoichiometry and the number of individual charges. The list of possible contributions to driving forces in secondary transport systems is immense (Poolman et al. 1992; Poolman and Konings 1993).

If a transport system is tightly coupled, a negative ΔG of the energy source (especially in primary systems) should lead to high electrochemical potentials (gradients) of the transported solute. Because of deviations from tight coupling, explained as "leaks" and "slips", the driving forces are often far from being in equilibrium with the potential of the driven ion. The reason for this is at least twofold. For most solutes the lipid membrane has a measurable permeability, which leads to unspecific (back)-flow of solutes by diffusion ("leakage"). Furthermore, the construction of carrier proteins involves a certain probability of kinetically "forbidden" reactions within the catalytic cycle, which leads to a deviation from tight coupling ("slippage"). Thus "tight" coupling of an uptake system is only observed in the presence of low external substrate concentrations, whereas the accumulation ratio diminishes as the external concentration increases. In summary, the final accumulation of an amino acid taken up depends on several parameters (Driessen et al. 1987; Driessen 1989), namely (i) the activity of the uptake system, (ii) the hydrophobicity of the amino acid, (iii) the permeability properties of the plasma membrane of a given bacterium, and (iv) the external amino acid concentration, because of "slippage" effects in the carrier protein.

The simplest mechanism of solute transfer is passive diffusion. Consequently, this was held to be responsible for amino acid excretion in many cases. Nevertheless, relatively few data are available for evaluating the significance of this process. Amino acids differ widely in the hydrophobicity of their side chains and thus show significantly different membrane permeability (Driessen 1989; Milner et al. 1987). Permeability coefficients of particular amino acids for transfer across bacterial membranes are in general not available. Based on partition coefficients between lipid/water phases, however, it seems reasonable that some hydrophobic amino acids, e.g. aromatic and branched chain amino acids, may diffuse through the plasma membrane at significant rates. Some authors have determined the rates of diffusion of particular amino acids across bacterial membranes, major examples being proline, alanine, and branched chain amino acids (Driessen 1989; Krämer et al. 1994; Milner et al. 1987). Transmem-

Table 1 Permeability of various amino acids across lipid bilayer membranes. Diffusion rates measured in liposomes cannot be directly compared with that from bacteria, due to different size. Therefore relative rates are listed, which have been normalized to give maximum overlap of values for identical amino acids. The absolute values for permeabilities have been measured in the indicated bacterial species. By multiplication with the internal amino acid concentrations, the efflux rates can be obtained (in μmol · (mg dw · min)$^{-1}$). EYPC, egg yolk phosphatidylcholine; PA, phosphatidic acid. The data for *C. glutamicum* and *Z. mobilis* are from (Krämer et al. 1994) and (Krämer R, unpublished). Other data are from (a): (Driessen et al. 1987), (b): (Chakrabarti and Deamer 1992), (c): (Wilson and Wheeler 1973), (d): (Wookey et al. 1984), (e): (Milner et al. 1987)

Amino acid	Permeability[1] μl · (mg dw · min)$^{-1}$	Relative permeability (%)			Membrane
		(a)	(b)	(c)	
Tryptophan			210		EYPC liposomes
Phenylalanine			133		EYPC liposomes
				133	PC/PA liposomes
Tyrosine	0.14 (d)				E. coli
Isoleucine	0.13				C. glutamicum
		100			L. lactis vesicles
Leucine		100			L. lactis vesicles
				100	PC/PA liposomes
Proline	0.026 (e)				E. coli
			8.5		L. lactis vesicles
Alanine	0.03 (e)				E. coli
	0.12				C. glutamicum
	0.6				Z. mobilis
				20	PC/PA liposomes
		4.5			L. lactis vesicles
Glycine			4.5		L. lactis vesicles
				3	EYPC liposomes
Threonine	0.01				C. glutamicum
		7			L. lactis vesicles
				6.5	PC/PA liposomes
Lysine	≤ 0.01				C. glutamicum
			2.5		EYPC liposomes[2]
Glutamate	≤ 0.002				C. glutamicum

brane diffusion rates were also found to be influenced by the properties of the particular membrane (In't Veld et al. 1993). Some data with respect to the passive flux of several amino acids are collected in Table 1. They show (i) that diffusion of hydrophobic amino acids out of the bacterial cell may be significant, and (ii) that the rate of diffusion varies considerably with respect to the type of membrane studied. On the other hand, hydrophilic amino acids will not cross the membrane at significant rates.

2. Uptake of amino acids

Bacteria possess two mechanisms for amino acid uptake, namely primary, binding protein-dependent systems (BPDS) as well as all kinds of secondary systems (Fig. 2). In terms of kinetics, there is no basic difference between these two groups, although BPDS have in general higher affinity for their ligands (see Table 2). Also an experimental discrimination in energetic terms is difficult, since the dependence on ATP is hard to prove in intact cells, and variation of the electrochemical gradient frequently also affects the ATP content of the cell. A diagnostic property of BPDS in Gram-negative bacteria is their sensitivity towards osmotic shock by means of which the binding proteins are released from the periplasm and transport is abolished. Secondary systems, on the other hand, are composed of a single membrane-inserted subunit and are not significantly influenced by this treatment. Since only Gram-negative bacteria possess a periplasmic space, BPDS were considered to be unique for these bacteria until recently. A number of BPDS have now been identified also in Gram-positive bacteria (Poolman et al. 1992; Saier et al. 1993). These include amino acid and oligopeptide transport systems in *Lactococcus lactis* (Tynkkynen et al. 1993), and the glutamate uptake system in *Corynebacterium glutamicum* (Krämer and Lambert 1990b). In these cases, the binding proteins are anchored to the plasma membrane by a lipid moiety.

The pattern of solute transport systems is complicated by the fact that many organisms show a multiplicity of uptake systems. At least a twofold diversity in bacterial transport systems is observed. First, different mechanisms for uptake exist, as described above. Second, in one organism frequently more than one type of uptake system for a particular solute is found. In *E. coli*, for example, there are at least five uptake systems for glutamate and aspartate (see Table 2). The diversity in uptake mechanisms mainly depends on three aspects, (i) on the availability of a particular amino acid (ii) on its importance for the cell, (iii) as well as on the energetic demands of metabolism and transport. In general, multiple uptake systems are present if the solute is an important substrate in a given habitat, and if it is present in widely varying concentrations. Primary transport systems normally have a high substrate affinity and are often practically unidirectional, thus mak-

Table 2 Properties of some selected amino acid uptake systems in *E. coli*. Data are taken from (Antonucci and Oxender 1986; Celis 1990; Higgins and Ames 1982; Landick et al. 1984; Milner et al. 1987; Sarsero et al. 1991; Schellenberg and Furlong 1977; Stirling et al. 1989; Wood 1989)

System	Transport solute	Mechanism	K_m (μM)	Regulation
Branched-chain amino acids				
1 LIV-I	leu, val, ile[a]	BPDS[b]	0.01–0.2	I:[c] leucine
2 LIV-II	leu, val, ile	Na^+-coupled	2–4	Constitutive
Proline (data from *E. coli* and *S. typhimurium*)				
1 PutP	pro	Na^+-coupled	2	I: proline
2 ProP	betaine/pro	H^+-coupled	40/300	I: hypoosmotic
3 ProU	betaine/pro	BPDS	1/ 35	conditions[d]
Anionic amino acids				
1	glu, asp	BPDS	0.5	
2 GltP	glu, asp	H^+-coupled	5	
3 GltS	glu	Na^+-coupled	1.5	
4	asp	Secondary	?	
5 Dct	dicarboxylates, asp	Secondary	30	
Aromatic amino acids				
1 AroP	tyr, phe, trp, his	Secondary	0.5	R:[c] tyr, phe, trp
2 TyrP	tyr	Secondary	2	R: tyr, I: phe
3 PheP	phe	Secondary	2	
4 Mtr	trp	?	3	R: trp, I: tyr, phe
5 TrpP	trp	?	70	
Basic amino acids				
1 LAO	lys, arg, orn	BPDS	0.005–0.1	R: lys I: N lim[e]
2 ABP	arg	BPDS	0.125	R: arg, orn
3	lys	Secondary	5	R: lys
4	orn	?	3	R: orn, arg
5	arg, orn	BPDS	0.2	
6 HisJPQM	his	BPDS	0.01	I: N limitation[e]

[a] The LIV-I system also transports ala, ser and thr with lower affinity
[b] Binding protein-dependent transport system
[c] I: induced by; R: repressed by
[d] This type of regulation is valid for ProP and ProU
[e] Limitation of nitrogen source

ing high accumulation ratios possible. In contrast, secondary systems are in general reversible, they may thus lead to leakage of the transport substrate in situations of low external substrate or low energy. Accumulation ratios of secondary uptake systems are in general not higher than 10^2–10^3. Thus, if a solute is present in the medium at very low concentration, the driving force of secondary carriers is often not high enough for sufficiently great solute accumulation. The driving force of primary systems, on the other hand, provides enough free energy for high accumulation ratios. ATP-driven binding protein-dependent uptake of glutamate in *C. glutamicum*, for example, leads to $2 \cdot 10^5$-fold accumulation (Krämer et al. 1990).

Binding protein-dependent uptake systems

Bacterial binding protein-dependent transport systems (BPDS) belong to a large superfamily of carriers present both in prokaryotes and eukaryotes (Tam and Saier 1993), which is called "ABC transporters", based on the presence of a conserved structural motif of an *A*TP-*b*inding *c*assette (Higgins 1992). Members of this superfamily are related to a multitude of biological processes both in bacteria (e.g. solute uptake, antibiotic resistance, cell development) and in eukaryotes (e.g. multidrug resistance, peptide and Cl^--transport) (Higgins 1992). In *E. coli* and *S. typhimurium*, more than 20 BPDS are known so far (Table 2). The most extensively characterized examples are the uptake systems for maltose, histidine, and oligopeptides. BPDS of Gram-negative bacteria are typically composed of four distinct membrane-associated domains (Fig. 3), two being highly hydrophobic, whereas the two other domains are presumably only peripherally associated at the cytoplasmic face and carry two sequence motifs indicative of ATP binding. The characteristic feature is the presence of a soluble substrate-binding protein in the periplasm. Some systems additionally involve a specific porin protein which mediates the passive permeation of the substrate through the outer membrane.

A functional scheme of the histidine BPDS in *S. typhimurium* is shown in Fig. 3. After permeation through outer membrane porins, the substrate is tightly bound by HisJ. The bilobed structure of HisJ thereby switches from an "open" to a "closed" state (Quiocho 1986). The HisJ-histidine complex interacts with the membrane-bound subunits, this interaction causes the substrate to be released from HisJ and translocated along an unidentified pathway through the membrane domains. Recently, it has been clearly shown in reconstituted systems that translocation is coupled to ATP hydrolysis by the HisP component (Ames and Joshi 1990). Both the mechanism of translocation and its coupling to ATP hydrolysis, however, are still unknown. Transport reactions are in general reversible processes, e.g. most secondary carriers or many

ATPases. BPDS show unprecedented behavior in that the vectorial reaction is practically irreversible and that some typical carrier properties like countertransport are not observed.

The membranous domains of BPD systems catalyze substrate translocation only if substrate-loaded binding protein is available externally. On the other hand, mutants have been obtained which mediate (slow) translocation even in the absence of binding protein. These and other findings have been interpreted to indicate that interaction of the liganded binding protein at the periplasmic side transmits a signal to the nucleotide binding domain (HisP) by conformational coupling, thus controlling ATP hydrolysis (Davidson et al. 1992). Evidence has even been presented that the substrate-triggered interaction of the subunits involves a direct contact between the soluble binding protein and a membrane-spanning loop of the cytoplasmic domain (Petronilli and Ames 1991). The necessary putative conformational changes have, however, not been proven so far.

In summary, the peculiar properties of BPDS are (i) high substrate specificity and affinity, (ii) the unidirectionality and (iii) the direct coupling to the free energy of ATP. The last two properties are the reason for the extraordinarily high accumulation ratios. They are furthermore the basis for the typical metabolic role of BPDS. These transporters are in general "scavenger" systems, designed to function under conditions of low external substrate concentration and thus in the demand of high affinity and high accumulation ratios. Consequently, they are in general inducible and repressed under conditions of ample amino acid availability. Thus, in this respect they are the complete opposite of secondary systems (see below).

Secondary amino acid uptake systems

In comparison to BPDS, secondary carriers have usually a lower affinity (Table 2) with K_m values from about 1 μM up to 1 mM. Widely varying V_{max} values from 0.1 to about 50 nmol $\cdot$ min^{-1} $\cdot$ mg dw^{-1} are found. The systems with lower affinity are in general constitutively expressed and designed for conditions of "normal" availability of amino acids. Due to differences in the ligand charge, in the type of coupling (uniport, symport or antiport) and in the coupling ion (H^+ or Na^+), there are many possible mechanisms. The energetic parameters contribute to a different extent to the driving forces. As explained above, three forces are available, (i) the chemical gradient of the transported amino acid, (ii) the chemical gradient of the coupling ion, if present, and (iii) the electrical gradient, if net transfer of charges is involved. Figure 4 exemplifies this for some representative transport systems. (a) Electrogenic amino acid uniport. An example is lysine and arginine uptake in *Bacillus stearothermophilus* (Heyne et al. 1991). (b–f) Electrogenic amino acid/cation symport. This is the most frequent secondary mechanism, either coupled to H^+ (b–d) or to Na^+ (e, f). The substrate may be cationic (c), as in the case of arginine and lysine in a number of bacteria (Poolman and Konings 1993), neutral (b, e), like proline or branched chain amino acids (Konings 1993; Konings et al. 1989; Poolman and Milner et al. 1987; Wood 1989), or anionic (d, f), like glutamate and aspartate (Deguchi et al. 1990; Milner et al. 1987; Poolman and Konings 1993; Schellenberg and Furlong 1977; Tolner et al. 1992). Antiport systems are found relatively seldom. Among these are (g) arginine/ornithine exchange in *Pseudomonas aeroginosa* (Verhoogt et al. 1992), (h) histidine/histamine exchange in *Lactobacillus buchneri* (Poolman and Konings 1993), and lysine/alanine exchange in *Corynebacterium glutamicum* (Bröer and Krämer 1990, Seep-Feldhaus et al. 1991). The former two examples are among the precursor/product antiport systems (Poolman 1990). In these systems one carrier catalyzes both the uptake of the substrate of an enzymatic (fermentative) reaction as well as the extrusion of the product of this reaction. Since the substrate gradient is directed inwards and that of the product outwards, no metabolic energy is needed for this reaction, which makes it attractive for organisms with a limited energy gain. The antiport reaction may even contribute to the generation of metabolic energy, e.g. in the histidine/histamine exchange mentioned above (example h in Fig. 4). In this electrogenic transport, antiport of the two solutes results in net outward movement of positive charge, leading to the generation of an electrical potential (outside positive).

An interesting point with respect to coupling mechanisms in secondary transport is the nature of the coupling ion. The former view that bacteria mainly couple to H^+ whereas eukaryotes use Na^+ was largely based on the fact that most bacteria have a H^+-ATPase, whereas eukaryotic plasma membranes carry a Na^+/K^+-ATPase as primary ion pump. It is now known, however, that Na^+-coupled transport, especially for amino acids, is common in bacteria, too. Several examples exist where the use of Na^+ as the coupling ion is obvious. Among these are halophiles, alkalophiles, marine bacteria and rumen bacteria. In each of these cases the use of Na^+ seems to be an adaptation to their unique environment (Krulwich and Ivey 1990; Maloy 1990). Recently, thermophiles have been added to this list (Konings et al. 1992b). The argument for this is the high H^+ permeability (in comparison to Na^+) of phospholipid bilayers under high temperatures. In some cases it is not easy to detect Na^+ as the coupling ion because of a high affinity to its binding site. Contaminating concentrations of Na^+ in "Na^+-free buffers" often exceed 50 μM, thus Na^+-dependence of transport systems with an apparent affinity for Na^+ higher than 10 μM may be overlooked. This, for example, holds true for proline and serine transport in *E. coli*, as well as glutamate transport in *Bacillus stearothermophilus* (Poolman and Konings 1993). The observation that some carriers can use either Na^+ or H^+, e.g. the alanine carrier of the thermophile PS3 (Hirata et al. 1984), led to the proposal that the hydronium ion (H_3O^+), which is a steric analog of Na^+, rather that H^+ is the transported species (Boyer 1988) in H^+-coupled systems. This has important consequences for the concept of transport, which is interpreted differently depending on

whether H^+ conductance or Na^+ translocation is considered.

For an optimum physiological function, amino acid transport has to be tightly regulated. This includes adaptation to different conditions, such as lack or presence of nutrients in widely varying concentrations, or the necessity of making a selection between carbon sources of different metabolic quality. Furthermore, the activity of transport systems has to be adapted to the demand for maintaining homeostatic conditions in the cell. Similar to many enzyme proteins in the cell, the activity of carrier systems is regulated by at least two different mechanisms, i.e. on the level of expression (synthesis) and on the activity level. As a general rule, in case several systems for a given solute are present, at least one of them is a constitutive system with comparably low affinity (high K_m) and high capacity (V_{max}). Additional inducible systems then usually have high affinity but relatively low capacity. The latter systems are provided for "cases of emergency" and are called "scavenger" systems. Frequently, inducible transport systems are induced by their major substrate, which is reflected by a regulatory connection of the carrier genes and the respective catabolic enzymes of this substrate. In addition, sophisticated mechanisms on the activity level for regulation of carrier activity may be present. Carriers can be regulated by the energy parameters, by the intra- and extracellular pH (Poolman et al. 1987), or by the medium osmolarity (turgor) (Csonka and Hanson 1991). Further effectors are internal metabolites (e.g. cyclic AMP), regulatory proteins or components of other transport systems (Postma et al. 1993).

Some properties of particular systems

Several examples of the significance of multiplicity in transport systems in *E. coli* for certain amino acids will be discussed in the following. The basic data of these systems are listed in Table 2.

The uptake systems for branched chain amino acids are a typical example of the "division of labor" between the two different kinds of mechanism (Antonucci and Oxender 1986; Landick et al. 1984; Milner et al. 1987). The secondary LIV-II system has a low substrate affinity and sets the basal level of uptake of these amino acids for the cell. Consequently, it is not significantly regulated. The LIV-I system is a high affinity BPDS and is tightly regulated by the intracellular leucine concentration. Whenever the cytosolic leucine level falls to low levels, this system becomes expressed in order to scavenge branched-chain amino acids.

Another instructive example is the triad of the three proline uptake systems in enteric bacteria (Townsend and Wilkinson 1992; Wood 1989). The first system (putP) is a secondary system with relatively high affinity and strict specificity for proline. It serves as a transport system when proline is used as carbon, nitrogen and energy source, consequently it is induced by proline. Both the second (proP) and the third system (proU) have a different function. They are induced by hyperosmotic conditions and are part of the stress response system, in accordance with the fact that glutamate, proline and glycine betaine are the major osmoprotectant substances in *E. coli*. Consequently, the secondary ProP system has a weaker affinity for proline and also transports glycine betaine. The ProU system is a BPDS and in fact clearly prefers glycine betaine (Wood 1989).

At least five transport systems for uptake of glutamate and aspartate are available in *E. coli* (Deguchi et al. 1990; Schellenberg and Furlong 1977; Tolner et al. 1992). One of them is a BPDS, accepting both glutamate and aspartate. Another (gltP) is a secondary aspartate- or glutamate-H^+ symporter and the third one (gltS) couples glutamate uptake to the concomitant transfer of Na^+ ions. In addition, there is a secondary, aspartate-specific uptake system. The systems are regulated differently. Although many *E. coli* strains cannot grow on glutamate as sole source of carbon, energy and nitrogen, glutamate is an important central metabolite and plays, in addition, a central role in osmoregulation, as seen by the fast reaction of the cytosolic glutamate pool to both hyperosmotic (fast uptake) and hypoosmotic shock (immediate efflux).

The transport systems for aromatic amino acids are interesting in another respect. Although their passive permeability is high (Table 1), in general bacteria have additional uptake systems (Milner et al. 1987; Sarsero et al. 1991; Whipp et al. 1980). These systems presumably serve to scavenge these "precious" molecules (in terms of energy requirement for synthesis) under conditions of low external concentrations, where diffusion rates are extremely low. *E. coli* has a common system which accepts all three aromatic amino acids, as well as several specific systems (Antonucci and Oxender 1986; Sarsero et al. 1991; Wookey et al. 1984). Thus, also in the case of solutes (amino acids) with high membrane permeability, the presence of uptake systems has to be expected.

3. Excretion of amino acids

Metabolite excretion is a common function of bacterial cells. There are a variety of excretion processes, the physiological significance of which is evident. Examples are excretion of fermentation end products, such as mono- and dicarboxylic acids, alcohols and ketones. These products either cross the plasma membrane passively (some alcohols, acetone), or they are excreted by secondary mechanisms (mono- and dicarboxylic acids) (Konings et al. 1992a). Another common kind of end product excretion is the substrate/product antiport (see above). Only a few of them have been functionally characterized. In contrast to these examples, however, the physiological significance of the excretion of other metabolites, including amino acids, is not obvious.

Various mechanisms have been proposed for amino acid excretion by bacteria. The most widely accepted hypothesis assumes an efflux of internally accumulated amino acids by diffusion ("leak hypothesis"). This is

thought to be promoted by a physical alteration of the membrane, e.g. in glutamate excretion by *Corynebacterium glutamicum* (Demain and Birnbaum 1968). A variation of this idea was proposed for lysine excretion in *C. glutamicum*, where efflux mediated by osmotically controlled pores was suggested (Luntz et al. 1986). A further hypothesis is based on the fact that carriers, like enzymes, in general catalyze reversible reactions. Since bacteria possess amino acid uptake systems, a functional inversion under altered metabolic conditions (high accumulation, change in energetic parameters) is assumed ("inversion hypothesis") (Clement et al. 1984). A completely different model, assuming the presence of specific efflux carriers ("carrier hypothesis") has recently been described (Krämer 1994 a).

Passive efflux of amino acids has been observed for proline, alanine, aromatic and branched chain amino acids (Driessen et al. 1987; Driessen 1989; Milner et al. 1987; Konings et al. 1992 a; Krämer et al. 1994; Krämer 1994 a). For effective transport by diffusion (leak hypothesis), the respective amino acid must be sufficiently hydrophobic (Table 1). Furthermore, there must be a substantial gradient across the membrane. Then, however, a serious problem arises due to a permanent loss of amino acids, which is obviously disadvantageous for the cell. Thus, if net amino acid excretion by diffusion is observed, some important (metabolic) parameters must have been changed. The three main reasons are (i) a dramatic increase of the internal amino acid concentration, (ii) a change in the permeability properties of the membrane, or (iii) a defect in an uptake system which normally counteracts the amino acid efflux. Explanations (i) and (ii) have frequently been suggested (but usually not measured) to be responsible for excretion. Especially for glutamate, excretion was explained as due to membrane alterations, since glutamate efflux is induced by treatments which may affect the integrity of the membrane. Hypothesis (iii) seems to be correct for proline and presumably for tyrosine in *E. coli* (Konings et al. 1992 a; Milner et al. 1987; Rancourt et al. 1983; Tribe and Pittard 1979; Whipp et al. 1980) and to some extent for isoleucine in *C. glutamicum* (see below). In general, assumptions that a certain solute passes the membrane simply by diffusion must be taken with caution. There may still be a huge number of transport systems which have not yet been described. Even for solutes, which in principle cross the membrane relatively freely, additional transport systems are frequently provided by the cell. Typical examples are glycerol or aromatic amino acids.

It has recently been suggested that carrier proteins are responsible for the excretion of amino acids (Bröer and Krämer 1991 a; Ebbighausen et al. 1989 b; Hoischen and Krämer 1989; Krämer 1994 a). The first conceptual approach in interpreting metabolite efflux as a carrier-mediated process was applied to lactate excretion by *Lactococcus lactis* and *E. coli* (Konings et al. 1992 a; Otto et al. 1980). Although it was clear that solutes are taken up by carriers, this was not generally accepted for amino acid efflux, especially in biotechnolgical processes. It is in fact difficult to assume that the cell should provide carriers for this situation. In the following, three examples will briefly be discussed, i.e. excretion of lysine, isoleucine and glutamate in *C. glutamicum*. These solutes cover a wide spectrum of biochemical properties with respect to charge (anionic, zwitterionic, cationic) and polarity (hydrophilic, hydrophobic).

Glutamic acid is the classic amino acid fermentation product. Although excreted by several bacteria, it has been studied in detail only in *C. glutamicum*. In contrast to other amino acids, glutamate excretion is induced by procedures which possibly affect the physical state or the integrity of the membrane (Krämer 1994 a). Consequently, it was interpreted as being caused by membrane leakage (Demain and Birnbaum 1968). Several findings indicate that this interpretation cannot be valid. (i) The permeability of the membrane to other ions is not significantly increased (Hoischen and Krämer 1989). (ii) Excretion can be induced without changing the composition of the membrane (Krämer 1994 a). (iii) Glutamate can be actively excreted against an existing chemical gradient (Gutmann et al. 1992). (iv) Evidence for the involvement of a specific carrier has been provided (Gutmann et al. 1992; Hoischen and Krämer 1989). It has been suggested earlier that glutamate excretion occurs by inversion of the uptake system (Clement et al. 1984). Recently it was shown that the uptake is mediated by an irreversible BPDS (Krämer et al. 1990; Krämer and Lambert 1990 b). Actually, conditions have been described under which uptake and excretion occur simultaneously, thus creating a futile cycle which leads to a waste of energy (Krämer 1994 a). The actual driving force has not yet been elucidated. Although the membrane potential, the pH-gradient or other ion gradients were excluded, a primary mechanism could not be proven (Gutmann et al. 1992). A possible explanation for the observed dependence on the membrane state is a modulation of the putative carrier by the membrane tension, similar to proline uptake (Culham et al. 1993). Changes in viscosity have, however, not been found in studies using fluorescence methods (Neubeck et al. 1993). On the other hand, high excretion activity can in fact be induced in *C. glutamicum* by adding local anaesthetics under appropriate metabolic conditions, in concentrations known to change the membrane fluidity (Krämer 1994 a).

Several hypotheses for the mechanism of lysine efflux in *C. glutamicum* have been suggested. These include the assumption of increased membrane permeability, as well as the idea of osmotically controlled pores in the plasma membrane (Luntz et al. 1986). Recently lysine efflux in *C. glutamicum* was shown to be mediated by a specific secondary carrier (Bröer and Krämer 1991 a), not related to lysine uptake (Bröer and Krämer 1990, Seep-Feldhaus et al. 1991). It was concluded that lysine is excreted together with two OH^- ions, which is energetically equivalent to antiport against two protons (Bröer and Krämer 1991) (Fig. 5). Due to this mechanism, the excretion activity is regulated by the membrane potential as well as by the pH gradient and the lysine gradient. This carrier seems to be

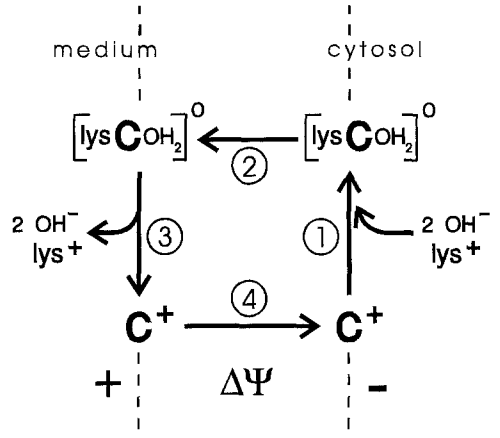


Fig. 5 Suggested mechanism for lysine secretion in *Corynebacterium glutamicum* (Bröer and Krämer 1991). In the binding/dissociation steps (1 and 3) of the catalytic cycle, the carrier (C^+) interacts with the ligands lysine (lys^+) and hydroxyl ions (OH^-). The translocation steps (2 and 4) are modulated by the membrane potential (rate- limiting step 4) as well as the pH-gradient and the lysine gradient (2)

ideally suited as an export system, e.g. because of the high internal K_m (kinetic cis side) and the induction by conditions where high internal lysine concentrations are observed (Erdmann et al. 1993). The latter observation may also explain the physiological significance of this type of excretion system. It was shown that *C. glutamicum*, when using peptides as carbon and energy source, catabolizes only particular amino acids, e.g. glutamate, aspartate and alanine. Others such as lysine or isoleucine are not catabolized and must thus be excreted under these growth conditions.

Like lysine, isoleucine belongs to the aspartate family of amino acids. In general, deregulation of the key enzyme threonine dehydratase, which is feedback-inhibited by isoleucine, is the reason for increased isoleucine biosynthesis. Isoleucine excretion is basically different from that of lysine. Isoleucine has no net charge under physiological conditions and is significantly more hydrophobic. Thus, passive diffusion should be relevant in isoleucine excretion. The situation is further complicated by the presence of an uptake system with significant activity (Ebbighausen et al. 1989a). All three possibilities of amino acid efflux (diffusion, uptake inversion and excretion carrier) must therefore be considered. It could be shown that (i) excretion is at least in part due to a carrier-mediated process and (ii) the mechanism of isoleucine uptake is clearly different from that of excretion (Ebbighausen et al. 1989b), which rules out an inversion model. The different components (diffusion, carrier-mediated excretion and uptake) could be independently determined (Krämer 1994a). Obviously, under the conditions in which both excretion and uptake are active, a futile cycle is created. Below a certain cytosolic threshold concentration, however, carrier-mediated excretion was found to be inactive, and diffusion can be counterbalanced by isoleucine uptake. Thus, the peculiar regulation pattern of isoleucine uptake, namely induction by increased internal isoleucine (Boles et al. 1993), was explained according to the "cyclic retention" hypothesis. This assumes that the induction of a particular uptake system saves the solute which is lost by diffusion (Ingraham et al. 1983). The significance of isoleucine excretion then, which is switched on at cytosolic isoleucine concentrations higher than the above mentioned threshold value, is based on the same principles as described for lysine. Again, feeding with isoleucine-containing peptides, leads to massive excretion of this amino acid (Krämer 1994a). Also the mechanism of isoleucine excretion is similar to that of lysine in being secondary transport mainly controlled by the membrane potential (Krämer 1994a).

Besides these three amino acids, many others are also excreted under particular conditions. Some are extruded in response to osmotic downshock. Bacteria react to hypoosmotic stress by releasing various solutes, e.g. K^+, proline, glutamate, as well as glycine betaine (Schleyer et al. 1993). The extremely rapid release of these substances as observed in *E. coli* after an osmotic downshock is currently interpreted as being mediated by stretch-activated channels (Berrier et al. 1992), although the nature of these channels has so far not been revealed. Production mutants of various bacteria (including *E. coli*) excrete a variety of amino acids, e.g. aspartate, threonine, proline, glutamine, serine, alanine, arginine, and aromatic amino acids. These bacteria must necessarily provide some mechanisms for excretion, however, transport has not been studied in these cases. In view of the data discussed above, it can be predicted that several of these amino acids may cross the membrane by passive diffusion, e.g. phenylalanine or tryptophan. Others may possibly use both diffusion- and carrier-mediated processes, e.g. proline and alanine, whereas some will essentially need carrier mechanisms, e.g. threonine, aspartate or arginine.

4. Structural aspects

Similar to other fields of biochemistry, also with respect to carrier proteins, interest is currently focused on structure-function relationships. Only in 1980 was the first primary structure of a carrier protein determined. A large number of amino acid sequences of prokaryotic and eukaryotic carriers is now available, in some cases the transmembrane topology has also been determined. Because of their hydrophobic nature, so far no crystals and thus no 3D-structure of any single carrier has been obtained.

An interesting result of the investigations on structural aspects was the recognition that most of these carriers, whether of prokaryotic or eukaryotic origin, have a common structural design. Irrespective of the wide variety in function and of whether the membranous part of the carrier consists of a dimer (some BPDS) or a monomer (secondary carriers), membrane transport proteins seem to be constructed of an array of about 12 (6 + 6) transmembrane segments. This structural paradigm is best conserved in the large class of secondary carriers, irrespective of

whether they function in uniport, symport, or antiport. This finding led to two conclusions. First, the common design of carrier systems may be related to a common evolutionary origin, and second, a general structural concept may indicate a general principle of function. Both conclusions seem to be more or less true. This is reflected by a series of reviews on structural families and superfamilies of carrier proteins, including amino acid carriers (Tam and Saier 1993; Saier 1994), and by common functional concepts for carrier mechanisms as discussed above (Krämer 1994b; Nikaido and Saier 1992).

5. Perspectives

With the fundamental exception of the 3D-structure, a significant body of knowledge on amino acid uptake systems has been accumulated in the course of the years, both with respect to function and to structure (sequence and topology). This is not true of amino acid efflux systems, where many fundamental questions are still unsolved. (i) In the cases where efflux systems have been studied, the results so far imply that the efflux and uptake routes differ. It is not clear whether this is a general rule. (ii) In a few cases a physiological role for amino acid excretion systems has been indicated, but again it remains unclear whether this can be generalized. There are obvious examples (e.g. glutamate), where this explanation does not hold. (iii) Although indicated by similar mechanisms, it is not clear whether amino acid excretion systems fall into the same structural families as uptake systems. (iv) The regulation of excretion systems both on the expression and the activity level is not yet understood. (v) No metabolite efflux carrier has been isolated and purified so far.

When emphasizing the large number of unsolved questions with respect to amino acid efflux, this should not mean that we fully understand amino acid uptake. Beside the obvious lack of understanding of carrier mechanisms on the molecular level in general, there are still a lot of unsolved problems. Although the basic reasons have been discussed for the observed multiplicity of transport systems with different kinetic, energetic and regulatory properties, the large number of similar uptake systems for particular amino acids is not clear. The same holds true for the complex regulation pattern of these systems, which involves direct regulation by effectors and metabolites but also crosstalk between different transport systems and regulatory networks (regulons and stimulons (Lengeler 1993; Nyström and Neidhart 1993)). Nevertheless, the results discussed in this review clearly indicate that solute transfer reactions, whether in the direction of uptake or efflux, play an important role in the metabolic network in general and in amino acid metabolism in particular.

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