Microbiol Monogr (5)
V. F. Wendisch: Amino Acid Biosynthesis
DOI 10.1007/7171_2006_069/Published online: 22 February 2007

Amino Acid Transport Systems in Biotechnologically Relevant Bacteria

Kay Marin · Reinhard Krämer (✉)

Institut für Biochemie, Universität zu Köln, Zülpicher Str. 47, 50674 Cologne, Germany
r.kraemer@uni-koeln.de

Electronic Supplementary Material
The electronic reference of this chapter is http://dx.doi.org/10.1007/7171_7171_2006_069. The online version of this chapter includes electronic supplementary material. This material is available to authorised users and can be accessed by means of the ESM button beneath the abstract. To cite or link to this chapter you can use the above reference.

Abstract Besides metabolic pathways and regulatory networks, transport reactions are also pivotal for understanding amino acid metabolism and production in bacteria. Apart from substrate uptake, this refers to product (amino acid) excretion as well as product re-uptake. Both the mechanistic (kinetic and energetic) as well as structural properties of these transport systems are relevant for understanding their significance and for providing a basis for rational metabolic design. Transport systems have been classified into numerous different carrier families, according to their structural properties and their putative evolutionary relation. The diversity of amino acid uptake and excretion systems in two biotechnologically relevant organisms, namely *Escherichia coli* K12 and *Corynebacterium glutamicum* ATCC 13032 is described in this review on the basis of their relation to these different transporter families. Particular functional and molecular properties of specific amino acid excretion systems in these two organisms, in particular those responsible for efflux of lysine (and arginine), threonine, branched chain amino acids, cysteine (and cysteine derivatives) and glutamate are described. A complete list of all secondary and primary transport systems in *C. glutamicum* putatively related to amino acid transport is provided.

1 Introduction

Cells exchange matter, energy and information with their surroundings and membrane-bound solute transport systems are essential for these processes. Bacteria are equipped with a broad variety of transport systems, with some of them, e.g., phosphotransferase systems and binding protein dependent ABC transporters, being exclusively present in prokaryotic organisms. Amino acid transport systems, which are found in bacteria like in all other living cells, include members of many different transporter families and are thus in principle not distinct from other substrate categories of transport systems, e.g., organic acids or carbohydrates. From a historic point of view, however, they are remarkable in a sense that the significance of specific solute excretion mechanisms was recognized earlier in the case of amino acids than for

most other solutes except cytotoxic compounds. This refers both to the physiological and biochemical description of export systems as well as to their molecular definition, for reviews see: (Krämer 1994; Burkovski and Krämer 2002; Eggeling and Sahm 2003; Eggeling 2005). Beside amino acid uptake, however, amino acid excretion was discovered already early in the history of modern biotechnology (Kinoshita et al. 1957). At that time, solute transport in general and solute export in particular was not acknowledged to be a central step within the network of biochemical pathways. In the meantime it is obvious that, besides the sum of metabolic and regulatory events within the bacterial cell, transport reactions are also of core importance.

In this contribution, due to space restrictions, we will focus on a few central aspects of this broad topic. Based on their importance in biotechnology as well as on the availability of detailed knowledge of genomic and biochemical data, amino acid transport systems of *E. coli* K12 and *C. glutamicum* ATCC 13032 will be reviewed mainly. An inventory of amino acid transport systems of these two organisms will be presented, organized in those being proven by both genetic and biochemical data, and those being suggested only on the basis of gene annotation and/or similarity screen. Where available, they will be combined with physiological and biochemical data on amino acid uptake and excretion.

2
Basic Properties of Bacterial Amino Acid Transport Systems

Before listing particular protein families of amino acid transport systems as well as properties of individual transport systems, some basic characteristics of transport systems in general and amino acid transport systems in particular will be summarized. This includes a brief outline of available structural and functional data of this type of carrier systems and mechanisms.

2.1
Structure of Transport Systems

Most amino acid transporters belong to the class of secondary carriers (see below) and are thus polytopic integral membrane proteins, comprising one or several subunits with different numbers of α-helical transmembrane segments. Although still few in number, several high resolution 3D structures are now available for a small number of integral membrane transport proteins (www.tcdb.org, www.membranetransport.org, www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct) also including a few amino acid transporters, e.g., the glutamate carrier of *Pyrococcus horikoshii* (Yernool et al. 2004) or the leucine carrier from *Aquifex aeolicus* (Yamashita et al. 2005). Several representatives of amino acid transport proteins belong to primary ac-

tive ABC-type carriers (see below), where a number of structures (Chang and Roth 2001; Locher et al. 2002; Dawson and Locher 2006) are available, however, not from entire amino acid transporting ABC carrier systems.

2.2 Transport Mechanisms: Kinetics and Energetics

The formal treatment of transport kinetics follows the well-established formulation of classical enzyme kinetics and will not be discussed here. In this context, V_{max} and K_t (equivalent to an apparent K_m value for transport reactions) and K_i values are used to describe the biochemical properties of transport systems with respect to activity, specificity and inhibitory action. For practical considerations, transport energetics is of high significance for the description of the diversity of amino acid transport systems in one single organism in the presence of a multitude of systems for one particular substrate (amino acid). In addition, knowledge on transport energetics is helpful for a mechanistic description of efflux systems (see below and Fig. 1) as well as for a combination of uptake and efflux mechanisms for the same substrate (Sect. 2.3).

In bacteria, the majority of amino acid transport systems functions as secondary systems, i.e., their driving force is an electrochemical ion potential across the plasma membrane, which, in general, means the electrochemical H^+ or Na^+ potential (Fig. 1). Alternatively, bacterial amino acid uptake systems may follow a primary transport mechanism, i.e., depending on ATP as the energy source and involving an external binding protein (ABC transport systems). So far, with the exception of the CydDC system in *E. coli* (Sect. 3.20), amino acid export systems exclusively follow secondary mechanisms. However, a first report on the involvement of a channel type of transport mechanism in the case of glutamate export has appeared recently (Sect. 4.2.5).

2.3 Uptake versus Excretion

Besides the variety of sophisticated mechanisms for protein excretion, a significant number of export systems for solutes has been described in bacteria, mainly falling into the group of MDR (multidrug resistance) proteins. These systems are in general responsible for the excretion of cytotoxic compounds or waste products. *C. glutamicum* is the paradigm for which the simultaneous presence of both uptake and export systems specific for the same solute (amino acid) has been described first, later also followed by similar combinations mainly in *E. coli*. Typical examples are the amino acids lysine, leucine/isoleucine, threonine and glutamate, which will be described below in more detail. It is obvious that the presence of both uptake and efflux systems for the same amino acid poses an extra problem for the cell, both in terms

of energetics and regulation, because of the possible occurrence of energy wasting futile cycling (Krämer 1994, 1996; Krämer and Hoischen 1994).

3
Repertoire of Amino Acid Transporters in *C. glutamicum* and *E. coli*

Within this chapter we will present data illustrating the equipment of the biotechnological important bacteria *C. glutamicum* and *E. coli* with carriers for amino acids. We included known and characterized transport proteins as well as predicted candidates for particular transport functions of different transporter families.

3.1
Carrier Identification, Nomenclature and Occurrence

Although the unequivocal identification of a transport protein requires the biochemical characterization of its transport function, carrier proteins are frequently predicted based on sequence data. The overall amino acid sequence similarity, the occurrence of domains specific for a certain transporter class, and the topology derived from the number and position of predicted transmembrane helical segments (TMS) provide useful hints on the putative carrier function of a membrane protein. The increasing number of known bacterial genome sequences provides a large pool of putative transporters to be used for development of prediction methods and identification of specific domains or motif sequences. However, in view of the fact that the prediction of substrate specificity from sequence data is not possible for the majority of transport systems, a detailed characterization by biochemical methods is still indispensable for the correct assignment of a carrier. Furthermore, only by biochemical studies so far unknown carriers of new transporter classes can be discovered.

A large variety of transport systems was classified by several systematic approaches. The most common classification system, the TC nomenclature, developed by the Saier group, is similar to the enzyme nomenclature (Busch and Saier 2004). The system is accessible online at http://www.tcdb.org (Saier et al. 2006) and was applied for the genome-wide prediction of transporter repertoires of prokaryotic and eukaryotic organisms (Ren and Paulsen 2005) accessible at http://www.membranetransport.org. In this chapter functionally characterized as well as predicted amino acid carriers from *C. glutamicum* and *E. coli* are presented. Detailed information for *E. coli* K12 is available online in public databases like EchoBase (http://www.biolws1.york.ac.uk/echobase), ecogene (http://www.ecogene.org) or NCBI (http://www.ncbi.nlm.nih.gov). For *C. glutamicum*, however, open data sources of this quality are missing. A general comparison of the transporter equipment of *C. glutamicum* and

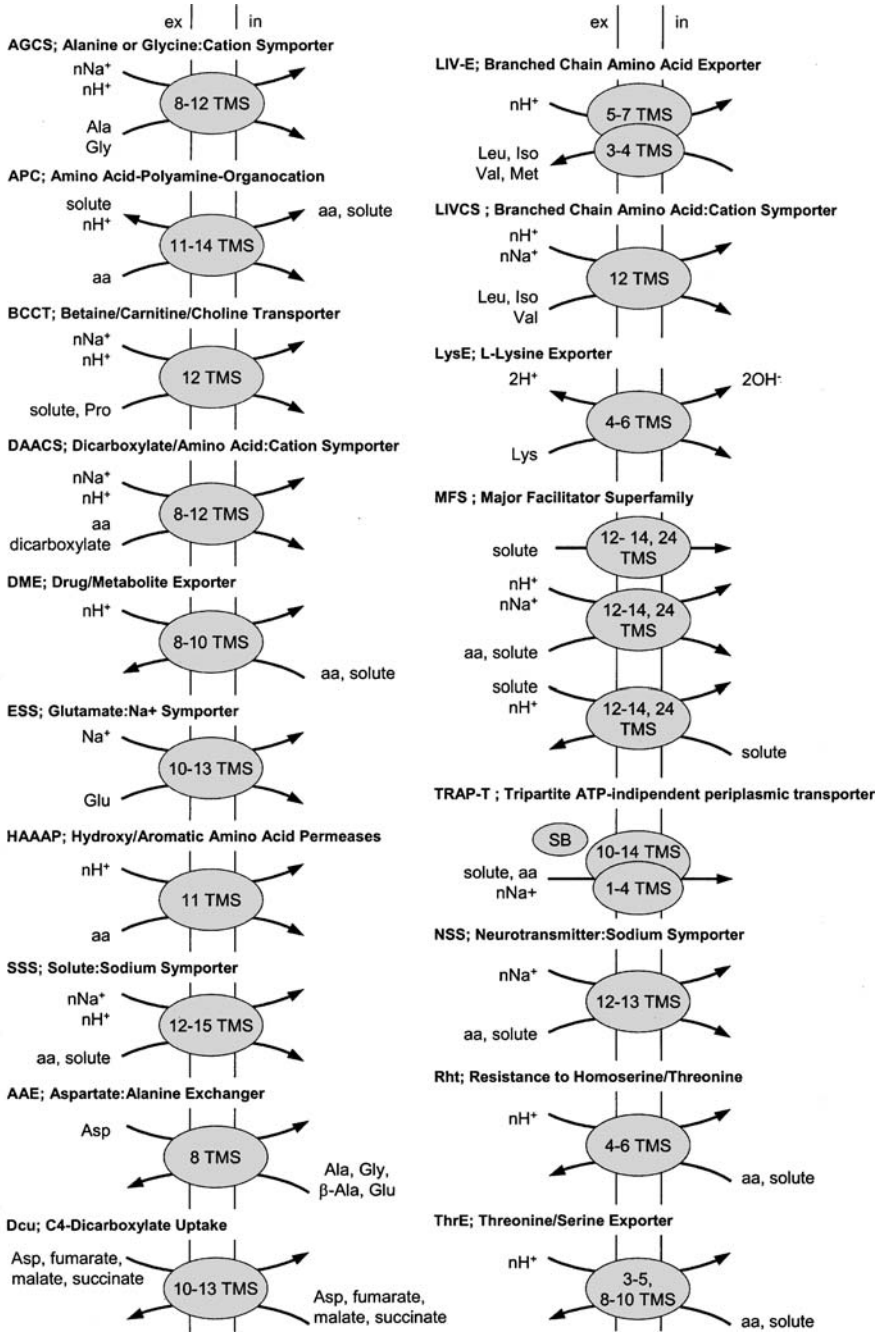

Fig. 1 Membrane topology as well as transport mechanism of secondary carriers (putatively) involved in amino acid transport. (ex: external, in: internal, aa: amino acid, TMS: transmembrane segments)

C. efficiens is available (Winnen et al. 2005). We provide for *C. glutamicum* detailed information on members of all transporter classes putatively involved in amino acid transport plus information on protein structure and function as well as experimental evidence for carrier function (see electronic supplementary material online Table S1 and S2).

The genome of *E. coli* comprises more than 4,200 protein encoding genes. For more than 1,000 of the respective gene products, the localization within the cytoplasmic membrane is predicted thus making the function as a carrier (-subunit) possible. Using bioinformatic tools, researchers identified 66 different transporter classes (Ren and Paulsen 2005). They comprise 52 different secondary transporter families, more than 68 ABC-type transporters and six PTS-type carriers. In total, more than 530 genes are predicted to encode for components of transport systems. In *E. coli* K12, members of 16 different transporter classes are shown or are predicted to be involved in amino acid transport (Table 1). At least one carrier system was found or predicted for the uptake of each amino acid, whereby the import of a number of amino acids is accomplished by several carriers. Beside a few systems involved in the exchange of amino acids, several systems have been found capable for the export of cysteine (and derivatives), threonine, homoserine, leucine, and arginine.

The genome of *C. glutamicum* carries more than 2,900 protein encoding genes. For more than 750 proteins, a membrane localization and function as a transporter (-subunit) is possible. Up to now 57 different transporter families have been predicted to exist. Among them are more than 130 secondary transporter (units), at least 57 primary transport systems of the ABC-type and four PTS-type carriers. The components of these carrier systems are encoded by 344 genes. Members of 17 transporter classes are shown or predicted to be involved in amino acid transport (Table 2). As in *E. coli*, the import of a number of amino acids is accomplished be several carriers of different transporter types. For some amino acids, the presence of carriers has only been derived from biochemical data, but until now the proteins are unknown. In particular, uptake carriers for glycine, cysteine, serine, threonine and other polar amino acids are not (yet) identified. In contrast to *E. coli*, *C. glutamicum* is characterized by a limited catabolism of several amino acids e.g., lysine (Nakayama 1985), isoleucine (Kennerknecht et al. 2002), threonine (Simic et al. 2001) or methionine (Trötschel et al. 2005), which may be related to a more pronounced capacity for amino acid export (see below).

In the following the variety of transporter families putatively comprising amino acid carriers are described and functionally characterized or predicted members of these families in *C. glutamicum* and *E. coli* are mentioned, using the TC nomenclature. For each carrier family, the general topology of TMS and a scheme of the transport reaction mechanism are listed in Figs. 1 and 2. For genes in *E. coli*, gene names as well as the locus tag (*b* number) for the MG1655 genome (Blattner et al. 1997) are listed. For genes in *C. glutamicum*, the *cg* nomenclature is used (Kalinowski et al. 2003). As supplementary mate-

Table 1 Transporter families (putatively) involved in amino acid transport in *E. coli* [a]

Transporter class		members in E.c.	Substrate: Gly	Ala	Val	Leu	Ile	Cys	Met	Phe	Tyr	Trp	Pro	Ser	Thr	Asn	Gln	Asp	Glu	His	Lys	Arg	derivatives
MFS	TC 2.A.1	71						○ 1					○ 2										○ 1
APC	TC 2.A.3	22	○ 3	○ 3						○ 4	○ 4		× 5	○ 6				× 5	○ 7		○○ 8	○ 9	○○ 10
DME	TC 2.A.7	16										× 11			○ 12								
Dcu	TC 2.A.13	2																○ ?					
BCCT	TC 2.A.15	3																					○ 14
SSS	TC 2.A.21	4											○ 15										
NSS	TC 2.A.22	0																					
DAACS	TC 2.A.23	3												○ 16					○ 17				
AGCS	TC 2.A.25	1		× 5																			
LIVCS	TC 2.A.26	1			○ 18	○ 18	○ 18																
ESS	TC 2.A.27	1																	○ 19				
HAAAP	TC 2.A.42	8									○ 20	○ 21		○ 22	○ 23								
TRAP-T	TC 2.A.56	1																	× 5				
LysE	TC 2.A.75	1?																			×? 24	○ 24	
RhtB	TC 2.A.76	5				○ 25		○ 26							○ 27								○ 27
LIV-E	TC 2.A.78	1?			× 5	× 5	× 5																
ThrE	TC 2.A.79	1																					
AAE	TC 2.A.81	2		× 5														× 5					
ABC	TC 3.A.1	>41			○ 28	○ 28	○ 28	○ 29	○ 30				○ 31			× 32	○ 33	○ 34	○ 35	○ 36	○ 37	○ 38	○ 39

(a) Beside the number of particular members of the transporter families (for abbreviations see text) the carrier identification by experimental characterization (○) or bioinformatic tools (×) is indicated. The presence of different carriers of the same transporter family for particular amino acids predicted either experimentally or by bioinformatic methods is also indicated (⊗). The function as importer (red), exporter (blue) or antiporter (green) is specified by the color and the particular references are: [1] (Yamada et al. 2006), (Dassler et al. 2000); [2] (Culham et al. 1998); [3] (Lee et al. 1975), (Steffes et al. 1992); [4] (Chye et al. 1986); [5] (Ren and Paulsen 2005); [6] (Pi et al. 1991); [7] (Metzer and Halpern 1990); [8] (Steffes et al. 1992), (Meng and Bennett 1992); [9] (Gong et al. 2003); [10] (Kashiwagi et al. 1992), (Meng and Bennett 1992), (Metzer and Halpern 1990), (Gong et al. 2003); [11] Ryback et al. 2006; [12] (Livshits et al. 2003); [13] (Andresen et al. 1988), (Jung et al. 2002); [14] (Jackowski and Alix 1990); [15] (Jung 1998); [16] (Ogawa et al. 1997), (Kim et al. 2002); [17] (Deguchi et al. 1989); [18] (Guardiola et al. 1974); [19] (Essenberg 1984); [20] (Whipp et al. 1980); [21] (Heatwole and Somerville 1991); (Sarsero et al. 1991); [22] (Kayahara et al. 1992); [23] (Sumantran et al. 1990); [24] (Nandineni and Gowrishankar 2004); [25] (Kutukova et al. 2005); [26] (Franke et al. 2003); [27] (Zakataeva et al. 1999); (Eggeling and Sahm 2003); [28] (Oxender et al. 1980), (Ovchinnikov et al. 1977), (Adams et al. 1990), (Sack et al. 1989); [29] (Pittman et al. 2002); [30] (Merlin et al. 2002); [31] (Checroun and Gutierrez 2004), (Dattananda and Gowrishankar 1989); [32] (Willis and Woolfolk 1975), (Ren and Paulsen 2005); [33] (Nohno et al. 1986); [34] (Willis and Furlong 1975); [35] (Liu and Ames 1997); [36] (Oh et al. 1993); [37] (Wissenbach et al. 1995); [38] (van der Ploeg et al. 1996), (Berger and Heppel 1972), (Leive and Davis 1965).

Table 2 Transporter families (putatively) involved in amino acid transport in *C. glutamicum* [a]

Transporter class		members in C.g.	Substrate																					
			Gly	Ala	Val	Leu	Ile	Cys	Met	Phe	Tyr	Trp	Pro	Ser	Thr	Asn	Gln	Asp	Glu	His	Lys	Arg	derivatives
MFS	TC 2.A.1	49						× 1					⊗ 2										
APC	TC 2.A.3	4		○ 3	○ 3	○ 3				○ 4	○ 4	○ 4									○ 3		
DME	TC 2.A.7	4																					
Dcu	TC 2.A.13	0																					
BCCT	TC 2.A.15	4											○ 5										○ 6
SSS	TC 2.A.21	2											○ 7										
NSS	TC 2.A.22	1																					
DAACS	TC 2.A.23	3												× 8									
AGCS	TC 2.A.25	1		× 1																			
LIVCS	TC 2.A.26	1			○ 9	○ 9	○ 10																
ESS	TC 2.A.27	1																	○ 11				
HAAAP	TC 2.A.42	1								× 1	× 1	× 1											
TRAP-T	TC 2.A.56	1?																	× 1				
LysE	TC 2.A.75	2													× 1						○ 12	○ 12	
RhtB	TC 2.A.76	3				×		×															
LIV-E	TC 2.A.78	2			⊗	⊗	⊗		○														
ThrE	TC 2.A.79	1												○	○								
AAE	TC 2.A.81	2		×														×					
ABC	TC 3.A.1	>57			×	×	×	×	×								×	×	○	×	×	×	×
YggB	???	1																	○				

(a) Beside the number of particular members of the transporter families (for abbreviations see text) the carrier identification by experimental characterization (○) or bioinformatic tools (×) is indicated. The presence of different carriers of the same transporter family for particular amino acids predicted either experimentally or by bioinformatic methods is also indicated (⊗).The function as importer (red), exporter (blue) or antiporter (green) is specified by the color and the particular references are: [1] (Ren and Paulsen 2005); [2] (Peter et al. 1998); (Ren and Paulsen 2005); [3]; (Bröer and Krämer 1991a); [4]; (Wehrmann et al. 1995); [5] (Peter et al. 1998), (Steger et al. 2004); [6] (Peter et al. 1998); [7] (Peter et al. 1997); [8] This work; [9] This work, (Ebbighausen et al. 1989); [10] (Tauch et al. 1998); [11] (Trötschel et al. 2003); [12] (Bellmann et al. 2001); [13] This work; [14] (Kennerknecht et al. 2002), This work; [15] (Trötschel et al. 2005); [16] (Simic et al. 2001); [17] (Ren and Paulsen 2005), This work; [18] (Kronemeyer et al. 1995); [19] (Nakamura et al. 2006).

rial a listing of all genes of *C. glutamicum* encoding known or putative, amino acid transporters is given according to the transport mechanism (secondary transporters in supplementary Table S1, primary transporters in supplementary Table S2) according to the particular transporter class. The tables are accessible at: http://www.springer.com.

3.2
The Major Facilitator Superfamily (MFS) (TC 2.A.1)

The MFS is a large and diverse superfamily including more than 6,900 sequenced members found in all kingdoms of live. They catalyze uniport, solute:cation (H^+ or Na^+) symport and/or solute:H^+ or solute:solute antiport and possess either 12, 14 or 24 TMS, whereby the proteins with 24 TMS are most likely fusion proteins of two homologous but distinct MFS permeases (Fig. 1). MFS permeases transport sugars, polyols, drugs, neurotransmitters, Krebs cycle metabolites, phosphorylated glycolytic intermediates, amino acids, peptides, osmolytes, siderophores (efflux), iron-siderophores (uptake), nucleosides, organic anions, inorganic anions, etc. (Busch and Saier 2004). For the MFS carriers GlpT, LacY (*E. coli*) and OxlT (*Oxalobacter formigenes*) high resolution structures are available (Abramson et al. 2003; Hirai et al. 2003; Huang et al. 2003). As the only amino acid substrate of an MFS uptake carrier proline was found (Culham et al. 1993; Peter et al. 1998). In addition, the Bcr protein was shown to be involved in efflux of cysteine and other amino acids or derivatives (Yamada et al. 2006).

In *C. glutamicum*, 49 MFS systems are predicted to exist. Among them, the ProP protein encoded by *cg3395* was shown to be capable of proline uptake (Peter et al. 1998). The gene *cg3403* encodes a carrier of high similarity but smaller size and proline was proposed as substrate (Ren and Paulsen 2005). In *E. coli*, 71 MFS systems are found, among them are the proline uptake carrier ProP, encoded by the gene *b*4111 (Culham et al. 1993), as well as the proteins YhjE and YdfJ encoded by the genes *b*3523 and *b*1543, respectively, for which proline was proposed as putative substrate. The efflux carrier Bcr (*b*2182) has already been mentioned above. Because of the variety of substrates known for MFS transporters in *E. coli* and *C. glutamicum*, the potential participation in uptake as well as export of further amino acids has to be considered.

3.3
The Amino Acid-Polyamine-Organocation (APC) Superfamily (TC 2.A.3)

The APC superfamily of transport proteins currently includes more than 1400 members, which occur in bacteria, archaea, and eukaryotes. They function as solute:cation symporters and solute:solute antiporters and vary in length and topology (Saier 2000), (Fig. 1). Some members possess only 10 TMS and rather function as amino acid receptors (Jack et al. 2000; Cabrera–Martinez

et al. 2003). Some animal homologs associate with a small membrane chaperone, which is essential for insertion and/or activity of the permease and linked by a disulfide bridge. In general, APC carriers were found or proposed to be involved in the transport of a variety of amino acids, namely glycine, alanine, leucine, isoleucine, valine, tyrosine, tryptophan, histidine, S-methylmethionine, phenylalanine, proline, serine, lysine and asparagine.

In *C. glutamicum*, four genes code for members of the APC family, including the LysI system (*cg1105*), which catalyzes exchange of lysine against lysine, alanine, valine, or leucine with very low activity (Bröer and Krämer 1991a; Seep-Feldhaus et al. 1991). The gene *cg1257* encodes AroP, the only known uptake system for aromatic amino acids in *C. glutamicum* (Wehrmann et al. 1995) and the genes *cg0555* and *cg1305* code for carriers of unknown substrates. In *E. coli*, 22 members of the APC family were found. The AroP (*b0112*) system is capable of phenylalanine and tyrosine uptake (Chye et al. 1986), the PheP (*b0576*) protein represents an uptake system for phenylalanine (Pi et al. 1991), the CycA (*b4208*) system is the main alanine carrier in *E. coli* but also competent for serine and glycine uptake (Lee et al. 1975; Schneider et al. 2004) and the LysP (*b2156*) protein acts as a lysine importer (Steffes et al. 1992). Moreover, three systems involved in pH regulation, the GabP (*b2663*) transporter responsible for exchange of glutamate and γ-aminobutyrate (Metzer and Halpern 1990), the CadB (*b4132*) system exchanging cadaverine and lysine (Meng and Bennett 1992), and the arginine agmatine antiporter AdiC (YjdE, *b4115*) (Gong et al. 2003) belong to this class. As transporters for amino acid derivatives, PotE (*b0692*) exchanging putrescine and ornithine (Kashiwagi et al. 1992) and MmuP (*b0260*) taking up S-methylmethionine (Thanbichler et al. 1999) are present. Further APC transporter of *E. coli* are YeeF (*b2014*), YifK (*b3795*), YjeH (*b4141*), YjeM (*b4156*), YhfM (*b3370*), YgjI (*b3078*), XasA (*b1492*), ProY (*b0402*), YbaT (*b0486*), YcaM (*b0899*), AnsP (*b1453*), YdgI (*b1605*) and YcjJ (*b1296*), including the putative proline carrier ProY and the possible asparagine uptake system AnsP (Ren and Paulsen 2005). These data indicate that members of this family are capable of transporting a large variety of substrates and further members may be supposed to represent still missing amino acids carriers.

3.4
The Drug/Metabolite Transporter (DMT) Superfamily (TC 2.A.7)

The DMT Superfamily currently consists of more than 1,800 members ordered in 18 recognized families, each with a characteristic function, size and topology. Within these subfamilies amino acid carriers are identified only for the drug/metabolite exporter subgroup (2.A.7.3, DME). Proteins of the DME family range from 287 to 310 amino acyl residues and carry 8–10 putative α-helical TMS (Busch and Saier 2004). In *C. glutamicum*, four members of the DME subfamily are predicted to be encoded by the genes *cg0168*, *cg0701*, *cg2339* and

cg2356. None of them was functionally characterized but they are supposed to be involved in amino acid export. In *E. coli*, 16 members of the DME subfamily were identified, among them, the YddG (*b1473*) carrier is supposed to be involved in tryptophan export (see below). The YdeD carrier (*b1533*, EamD) was shown to represent an exporter of cysteine and cysteine derivatives (Dassler et al. 2000). The RhtA (YbiF, *b0813*) protein has been related to efflux of threonine and homoserine based on an increased resistance against these amino acids after overexpression of the *rhtA* gene (Livshits et al. 2003).

3.5
The C4-Dicarboxylate Uptake (Dcu) Family (TC 2.A.13)

More than 100 members of the Dcu family are found. They consist of approx. 440 amino acyl residues in length and possess 10–12 TMS. In *E. coli*, the DcuA (*b4138*) and DcuB (*b4123*) proteins are known as exchangers of aspartate, malate, fumarate and succinate under anaerobic growth conditions (Engel et al. 1994). Two additional genes encoding the dicarboxylate transporters DcuC (*b0621*) and DcuD (*b3227*) are present showing weak sequence similarity to DcuA and DcuB and they were, therefore, assigned to a distinct family (TC 2.A.61). While in *C. diphtheriae* DcuA and DcuB homologs are found, no member of the Dcu family is known in *C. glutamicum*.

3.6
The Betaine/Carnitine/Choline Transporter (BCCT) Family (TC 2.A.15)

More than 140 proteins of the BCCT family are found in bacteria and archaea. In general, they transport solutes with a quaternary ammonium group $[R-N^{+}(CH_3)_3]$ (Busch and Saier 2004). The only amino acid substrate of BCCT carriers is proline. Some of these transporters exhibit inherent osmosensory and osmoregulatory properties (Rübenhagen et al. 2000). In *C. glutamicum*, BetP encoded by *cg1016*, EctP encoded by *cg2539* and LcoP encoded *cg2563* were characterized (Peter et al. 1998; Steger et al. 2004). All three are capable of uptake of the amino acid derivatives glycine betaine and ectoine, but only EctP and LcoP accept proline. In *E. coli*, three BCCT transporters, BetT, CaiT and YeaV that are encoded by *b0314*, *b0040*, and *b1801*, respectively, are present. None of these systems seems to be competent for the transport of proline or another amino acid (Andresen et al. 1988; Jung et al. 2002).

3.7
The Solute:Sodium Symporter (SSS) Family (TC 2.A.21)

More than 420 members of the SSS family have been identified in bacteria, archaea and animals possessing 12-15 TMS and catalyzing solute: Na^{+} symport

(Fig. 1). A variety of solutes such as sugars, amino acids, organic cations (e.g., choline), nucleosides, inositols, vitamins, urea or anions are transported. For some members regulatory domains are found homologous to histidine kinases (Jung 2001; Busch and Saier 2004). In *E. coli*, four members of the SSS family are present. Besides the PutP (*b1015*) system catalyzing proline uptake (Jung 1998), the PanF protein, encoded by *b3258*, involved in pantothenate uptake, the acetate uptake system ActP (*b4067*), as well as the putative myo-inositol transporter YidK (*b3679*) are members of this family (Jackowski and Alix 1990; Gimenez et al. 2003; Ren and Paulsen 2005). In *C. glutamicum*, two SSS-type carriers are found including a PutP protein (*cg1314*) as the main proline uptake system at low external osmolality (Peter et al. 1997). The gene *cg0953* encodes an additional SSS transporter with high sequence similarity to the acetate transporter ActP of *E. coli*, but the substrate is still unknown.

3.8
The Neurotransmitter:Sodium Symporter (NSS) Family (TC 2.A.22)

Members of the NSS family catalyze uptake of a variety of neurotransmitters, amino acids, osmolytes and related nitrogenous substances by a solute:Na^+ symport mechanism (Fig. 1). More than 190 members are mostly found in animals, but bacterial and archaeal homologues have also been identified. TnaT of *Symbiobacterium thermophilum* has been shown to be a Na^+-dependent tryptophan uptake permease (Androutsellis-Theotokis et al. 2003). For the LeuT protein from *Aquifex aeolicus* facilitating leucine sodium co-transport, the crystal structure has been determined (Yamashita et al. 2005). Glycine and tyrosine were shown or proposed as additional substrates of eukaryotic NSS carriers (Morrow et al. 1998; Ren and Paulsen 2005). Whereas for *C. glutamicum* one member of the NSS family with unknown substrate specificity encoded by *cg1169* was found, in *E. coli* no transporter of this family is present.

3.9
The Dicarboxylate/Amino Acid : Cation (Na^+ or H^+) Symporter (DAACS) Family (TC 2.A.23)

The more than 340 members of the DAACS family catalyze Na^+ and/or H^+ symport together with a Krebs cycle dicarboxylate or with an amino acid as indicated in Fig. 1. The 3D structure of a member of the DAACS family, the glutamate carrier from *Pyrococcus horikoshi*, has been determined (Yernool et al. 2004). DAACS carriers were found or proposed to be involved in the transport of acidic, small zwitterionic, as well as basic amino acids. In *C. glutamicum*, three members were predicted to be encoded by the genes *cg2810*, *cg2870* and *cg3356*. The gene *cg2870* encodes a DctA homolog, making a function as dicarboxylate transporter likely. Furthermore, all three

proteins display a weak similarity to the serine uptake system SstT of *E. coli*. In *E. coli*, three members of the DAACS family were found, the dicarboxylate transporter DctA (Lo and Bewick 1978), the glutamate carrier GltP (Deguchi et al. 1989) and the serine and threonine transporter SstT (YgjU) (Ogawa et al. 1997; Kim et al. 2002) encoded by *b3528*, *b4077* and *b3089*, respectively.

3.10
The Alanine or Glycine:Cation Symporter (AGCS) Family (TC 2.A.25)

Members of the AGCS family in general transport alanine and/or glycine in symport with Na^+ and or H^+ and comprise 8–12 TMS (Fig. 1). They are found in bacteria and archaea and similarity with the APC family (TC 2.A.3) has been established (Busch and Saier 2004). Three members of the AGCS family have been functionally characterized as alanine uptake systems in different organisms (MacLeod and MacLeod 1992; Kanamori et al. 1999; Moore and Leigh 2005). The single member of this family in *C. glutamicum* is encoded by *cg0254* and alanine was proposed as substrate. In *E. coli*, also a single member of the AGCS family is present, the YaaJ protein encoded by *b0007*. Also in this case, alanine was proposed as substrate; however, CycA (DagA, *b4208*), a member of the APC carrier family, has been identified as the main alanine uptake system (see above).

3.11
The Branched Chain Amino Acid:Cation Symporter (LIVCS) Family (TC 2.A.26)

Characterized members of this family import all three branched chain amino acids. More than 110 members were found in bacteria; they function by a Na^+ or H^+ symport mechanism. In *E. coli* as well as in *C. glutamicum*, a single LIVCS system was found named BrnQ encoded by *b0401* and *cg3537*, respectively (Guardiola et al. 1974; Tauch et al. 1998).

3.12
The Glutamate : Na^+ Symporter (ESS) Family (TC 2.A.27)

This family comprises more than 50 members only known in bacteria and proteins from *E. coli*, *Salmonella*, and *C. glutamicum* have been functionally characterized (Essenberg 1984; Alvarez-Jacobs et al. 1986; Trötschel et al. 2005). In *C. glutamicum*, the GltS system is encoded by *cg3080* and was characterized at the molecular and biochemical level (Burkovski and Krämer 1995; Trötschel et al. 2005). In *E. coli*, the single member of this family was also named GltS and is encoded by *b3653* (Marcus and Halpern 1969). In both organisms, other glutamate uptake systems are present besides the GltS permease and belong to the ABC or DAACS family, respectively (see Sects. 3.20 and 3.9).

3.13
The Hydroxy/Aromatic Amino Acid Permease (HAAAP) Family (TC 2.A.42)

More than 150 homologues are present in bacteria and most of them were found or proposed to be involved in the transport of tyrosine, tryptophan, serine and threonine. In *E. coli*, the HAAAP family comprises eight proteins including the high affinity tryptophan permease, Mtr (*b3161*), the low affinity tryptophan carrier TnaB (*b3709*), the tyrosine-specific permease TyrP (*b1907*), the serine permease SdaC (*b2796*) as well as the threonine permease TdcC (*b3116*) (Whipp et al. 1980; Sumantran et al. 1990; Heatwole and Somerville 1991; Sarsero et al. 1991; Kayahara et al. 1992). Further members are encoded by *b3539* (YhjV), *b3110* (YhaO) and *b2845* (YgeG), but their transport substrates are unknown. In *C. glutamicum*, a single member of the HAAAP family is encoded by *cg0568*. Based on sequence similarity, aromatic amino acids are supposed as substrates of this carrier.

3.14
The Tripartite ATP-Independent Periplasmic (TRAP-T) Transporter Family (TC 2.A.56)

TRAP-T family carrier consists of three components, two integral membrane proteins of 12 and four TMS, respectively, as well as a periplasmic substrate binding protein, therefore representing the only known binding protein dependent secondary carriers. The substrate binding protein can be anchored in the membrane in gram negative and gram positive bacteria. As observed for ABC-type transporters, subunit/domain fusions and splicing occurred during evolution (Busch and Saier 2004). More than 300 members of this family are known in bacteria and archaea. The only report on amino acid transport by a TRAP-T carrier concerns glutamate uptake in *Rhodobacter sphaeroides* (Jacobs et al. 1996). In *E. coli*, a single TRAP-T-type carrier is known comprising the membrane bound subunits YiaM (*b3577*) and YiaN (*b3578*) as well as the substrate binding component YiaO (*b3579*). In *C. glutamicum*, the genes *cg2568* and *cg2569* encode the large and small membrane bound subunits, respectively and *cg2570* the substrate binding protein of a TRAP-T system of unknown function. Additionally, the orphan gene *cg2546* encodes a large membrane component.

3.15
The Lysine Exporter (LysE) Family (TC 2.A.75)

More than 70 LysE family members are found widely distributed in bacteria. Together with the cadmium resistance family (CadC) and the resistance to homoserine/threonine family (RhtB) the LysE family forms the LysE superfamily. The energy source for transport is proton motive force (H^+ antiport

or OH^- symport, (Bröer and Krämer 1991b). Two members of the LysE family were functionally characterized, LysE of *C. glutamicum* encoded by *cg1424* and ArgO of *E. coli* encoded by the gene *b2923* (Vrljic et al. 1996; Nandineni and Gowrishankar 2004). Whereas for LysE of *C. glutamicum*, the specific export of lysine and arginine was demonstrated experimentally (Bellmann et al. 2001), for ArgO (YggA) of *E. coli* the specific efflux of arginine was proposed (Nandineni and Gowrishankar 2004), (Sect. 4.2.1). In *C. glutamicum*, an additional LysE-type carrier is encoded by *cg0183*, which is annotated as putative threonine carrier, but probably responsible for the export of so far unknown substrates.

3.16
The Resistance to Homoserine/Threonine (RhtB) Family (TC 2.A.76)

More than 420 proteins, derived from bacteria and archaea, comprise the RhtB family as subfamily of the LysE superfamily. The characterization of a few of them revealed a topology and function as amino acid exporters (Fig. 1). *E. coli* possesses five paralogues of about the same size and apparent topology. The genes *b3823*, *b3824*, *b1798*, *b0328*, and *b2578* encode the RhtC, RhtB, YeaS, YahN, and YfiK proteins, respectively. The overexpression of *yeaS*, *yfiK*, *rhtC* and *rhtB* provides resistance to threonine whereas an additional resistance to homoserine and homoserine lactone was observed by overexpression of *yeaS* and *rhtB* (Zakataeva et al. 1999; Eggeling and Sahm 2003). The overexpression of *yahN* had no positive effect on growth of *E. coli* cells exposed to higher concentrations of threonine, homoserine, and homoserine lactone. For the YfiK protein, the export of cysteine, O-acetylserine and azaserine was demonstrated as well (Franke et al. 2003), whereas YeaS was recently identified as leucine exporter and consequently named LeuE (Kutukova et al. 2005). In *C. glutamicum*, three members of the RhtB family are encoded by the genes *cg0183*, *cg2574* and *cg2941*, respectively, but for none of them a transport substrate could be identified and ThrE of the threonine/serine exporter family was found as the major threonine export system (Sect. 3.18). Based on sequence similarity, the *cg0183* and *cg2574* gene products could be assigned to homoserine export while *cg2941* might encode a leucine exporter.

3.17
The Branched Chain Amino Acid Exporter (LIV-E) Family (TC 2.A.78)

The LIV-E family consists of more than 104 members in a diverse group of bacteria and archaea. Pairs of integral membrane proteins comprise an efflux pump for branched chain amino acids. In *C. glutamicum*, two homologous systems are found. The BrnFE system (*cg0315*, *cg0314*) has been functionally characterized to catalyze export of methionine, isoleucine, leucine and valine (Kennerknecht et al. 2002; Trötschel et al. 2005). The second system was

named AzlCD (*cg3412* and *cg3413*), but the substrate specificity is unknown. In *E. coli*, a similarity to the large subunits of LIV-E systems was found for YgaZ encoded by *b2682*; however, no counterpart of a small subunit is known until now. It remains unclear, therefore, whether a complete LIV-E system is present in *E. coli*, since efflux of leucine and other amino acids can probably be accomplished by the LeuE system (Sect. 3.16).

3.18
The Threonine/Serine Exporter (ThrE) Family (TC 2.A.79)

Around 60 members of the ThrE family are diverse in sequence and are ubiquitous in bacteria, archaea and eukaryotes. It is interesting to note that most of these transporters carry an extended N-terminal domain showing a weak sequence similarity to hydrolases, which has been supposed to be responsible for additional functions of this type of carriers (Eggeling and Sahm 2003). In *C. glutamicum*, ThrE (*cg2905*), the only member of this family, has been functionally characterized as proton motive force-dependent exporter of threonine and serine (Simic et al. 2001). In *E. coli*, the gene *b4363* was predicted to encode a small ThrE carrier, the YjjP protein, for which the substrate is still unknown.

3.19
The Aspartate:Alanine Exchanger (AAE) Family (TC 2.A.81)

A single functionally characterized protein, the aspartate:alanine exchanger AspT of the Gram-positive lactic acid bacterium *Tetragenococcus halophila* D10 served to define the AAE family (Abe et al. 2002; Busch and Saier 2004). Until now at least 40 further members of the AAE family have been predicted in many bacteria. In *C. glutamicum*, the genes *cg0683* and *cg2425* encode proteins that display similarity to the AspT protein sequence, whereas in *E. coli* the proteins encoded by *b3685* and *b0847* belong to the AAE family. In both organisms, however, the proteins have not been functionally characterized.

3.20
The ATP-binding Cassette (ABC) Superfamily (TC 3.A.1)

The ABC superfamily contains both uptake and efflux transport systems, and genes for subunits of individual members of these two groups frequently form gene clusters. According to their substrate specificity, numerous families within the ABC superfamily were classified. The members of the ABC superfamily consist of two integral membrane domains/proteins and two cytoplasmic domains/proteins that constitute a homo- or heterodimer each. In addition, the uptake systems possess extracytoplasmic solute-binding receptors (one or more per system), which in Gram-negative bacteria are found

in the periplasm, whereas in Gram-positive bacteria they are in general tethered to the external surface of the cytoplasmic membrane by lipid anchors (Fig. 2). Some members possess receptor domains fused to either the N- or C-terminus of the translocating membrane protein (van der Heide and Poolman 2002). ABC-type uptake systems have not been identified in eukaryotes, but ABC-type efflux systems are widely found both in pro- and eukaryotes. Efflux systems frequently have four domains (two cytoplasmic and two membrane domains) fused into either one or two polypeptide chains. The three-dimensional structures of several entire bacterial drug export proteins (MsbA, BtuCD, and Sav1866) were solved (Chang and Roth 2001; Locher et al. 2002; Dawson and Locher 2006). Moreover, several substrate binding proteins were crystallized, see Table 3. According to the transporter classification system by Saier (Busch and Saier 2002), amino acid transporter subfamilies are the polar amino acid uptake transporter (PAAT, TC 3.A.1.3) family, the hydrophobic amino acid uptake transporter (HAAT, TC 3.A.1.4) family, the quaternary amine uptake transporter (QAT, TC 3.A.1.12) family, the methionine uptake transporter (MUT, TC 3.A.1.24) family and the cydDC cysteine exporter (CydDC-E, TC 3.A.1.129.1) family. In general, ABC-type carriers have been shown or proposed to be involved in the transport of glycine, valine, leucine, isoleucine, cysteine, methionine, proline, asparagine, glutamine, aspartate, glutamate, histidine, lysine, and arginine.

In *E. coli*, more than 68 ABC-type transport systems are present. Among them are 13 supposed to be involved in amino acid transport (Table 3). Members of the PAAT family are the lysine, arginine and ornithine uptake system ArgTHisPQM (Wissenbach et al. 1995) and the histidine uptake system HisJMPQ, which comprise the same permease and ATP binding proteins, but a different substrate binding protein (Liu and Ames 1997). ArtIMPQ catalyzes uptake of arginine (Wissenbach et al. 1993, 1995), GlnHPQ of glutamine (Nohno et al. 1986), GltIJKL is responsible for the uptake of glutamate and aspartate (Willis and Furlong 1975; Deguchi et al. 1989) and CysXYZ catalyzes

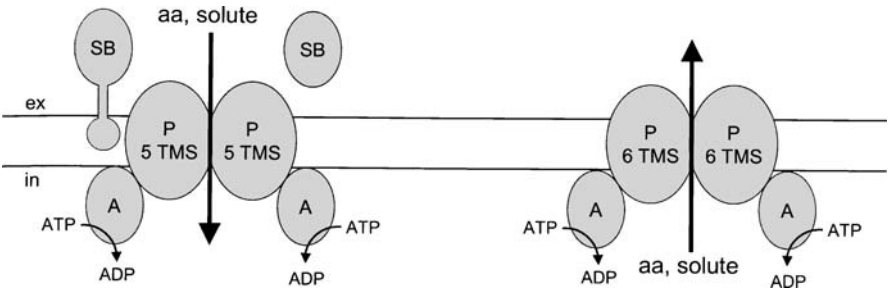

Fig. 2 Membrane topology as well as transport mechanism of primary active carriers (putatively) involved in amino acid transport (ex: external, in: internal, aa: amino acid, TMS: transmembrane segments)

Table 3 Known ABC-type amino acid transporters in *E. coli*[a]

ABC-system	TC class	Permease subunits	Substrate binding protein	ATP binding proteins	Substrate	Refs.
ArgTHisPQM	3.A.1.3.1	HisM (*b2307*) HisQ (*b2308*)	ArgT (*b2310*) S	HisP (*b2306*)	lysine arginine ornithine	1
HisJMPQ	3.A.1.3.1	HisM (*b2307*) HisQ (*b2308*)	HisJ (*b2309*) S	HisP (*b2306*)	histidine	2
GlnHPQ	3.A.1.3.2	GlnP (*b0810*)	GlnH (*b0811*) S	GlnQ (*b0809*)	glutamine	3
ArtIMPQ	3.A.1.3.3	ArtM (*b0861*) ArtQ (*b0862*)	ArtI (*b0863*)	ArtP (*b0864*)	arginine	4
GltIJKL	3.A.1.3.4	GltJ (*b0654*) GltK (*b0653*)	GltI (*b0655*)	GltL (*b0652*)	glutamate aspartate	5
CysXYZ	3.A.1.3.10	YecS (*b1917*)	FliY (*b1920*)	YecC (*b1918*)	cystine diamino-pimelic acid	6
LivFGHJM	3.A.1.4.1	LivM (*b3456*) LivH (*b3457*)	LivJ (*b3460*) S	LivF (*b3454*) LivG (*b3455*)	leucine iso-leucine valine	7
ProU	3.A.1.12.1	ProW (*b2678*)	ProX (*b2679*)	ProV (*b2677*)	glycine betaine proline	8
YehWXYZ	3.A.1.12.?	YehW (*b2128*) YehY (*b2130*)	YehZ (*b2131*)	YehX (*b2129*)	proline?	9
TauABC	3.A.1.17.1	TauC (*b0367*)	TauA (*b0365*)	TauB (*b0366*)	taurine	10
MetD	3.A.1.24.1	MetI (*b0198*)	MetQ (*b0197*)	MetN (*b0199*)	D-methio-nine	11
CydDC	3.A.1.129.1	CydD (*b0887*) CydC (*b0886*)			cysteine glutathione	12
YhdWXYZ	3.A.1.?	YhdY (*b3270*) YhdX (*b3269*)	YhdW (*b3268*)	YhdZ (*b3271*)	amino acid?	13

[a] ABC-type carriers and the particular subunits as well as identified substrates in *E. coli*. The 3D structure of several binding proteins was solved (S). The particular references are: [1] (Wissenbach et al. 1995), (Oh et al. 1993); [2]; (Liu and Ames 1997), (Yao et al. 1994); [3] (Nohno et al. 1986), (Sun et al. 1998); [4] (Wissenbach et al. 1993), (Wissenbach et al. 1995); [5] (Willis and Furlong 1975), (Deguchi et al. 1989); [6] (Berger and Heppel 1972), (Leive and Davis 1965); [7] (Oxender et al. 1980), (Ovchinnikov et al. 1977), (Adams et al. 1990), (Sack et al. 1989); [8] (May et al. 1986), (Dattananda and Gowrishankar 1989); [9] (Checroun and Gutierrez 2004); [10] (van der Ploeg et al. 1996); [11] (Merlin et al. 2002); [12] (Pittman et al. 2002); [13] (Ren and Paulsen 2005).

uptake of cystine and diaminopimelic acid (Leive and Davis 1965; Berger and Heppel 1972). The LivFGHJM system was described as an importer for hydrophobic amino acids (Ovchinnikov et al. 1977; Oxender et al. 1980; Adams et al. 1990). The QAT family consists of three members in *E. coli*, the ProU system, which is a proline carrier and a component of the osmotic stress response, (May et al. 1986, Dattananda and Gowrishankar 1989), the TauABC transporter as taurine uptake system (van der Ploeg et al. 1996) and the putative proline transporter YehWXYZ (Checroun and Gutierrez 2004). Uptake of D-methionine in *E. coli* is catalyzed by the MetD (MetINQ) system (Merlin et al. 2002), and YhdWXYZ was proposed as a further transporter for amino acids (Ren and Paulsen 2005). The only known primary active export system for amino acids in *E. coli* is the CydDC transporter, an ABC-type cysteine and glutathione exporter required for cytochrome assembly (Pittman et al. 2002). The dimeric protein is comprised of two subunits with a fused permease and ATP binding domain each. In this family of transporters, the structure of four substrate binding proteins of amino acid carriers (ArgT, HisJ, GlnH and LivJ) was solved (Sack et al. 1989; Oh et al. 1993; Yao et al. 1994; Sun et al. 1998).

In *C. glutamicum*, at least 57 ABC-type uptake systems are present and several orphan genes encoding single components of ABC-carriers are found. Until now, only one ABC-type uptake system for amino acids has been characterized, the GluABCD transporter (Kronemeyer et al. 1995), catalyzing uptake of glutamate. The substrate binding protein GluB shows similarity to the GltI protein of the Glt system in *E. coli*. An uptake system for polar amino acids is supposed to be encoded by the gene cluster *cg1502-04* because of the high sequence similarity of the binding protein (*cg1504*) to the GlnH, ArgT and HisJ proteins of *E. coli*. A high degree of sequence similarity also exists between the components MetI, MetN, and MetQ of the methionine uptake system MetD in *E. coli* and the corresponding *C. glutamicum* proteins encoded by the genes *cg0735*, *cg0736*, and *cg0737*. Furthermore, sequence similarity also suggests a taurine transporter binding protein, encoded by *cg1441*, however, a permease gene is not found in the adjacent gene cluster *cg1438* and *cg1440*. Because of sequence similarity, the protein encoded by the orphan gene *cg3045* was proposed to function as permease of an uptake system for glutamine or arginine (Ren and Paulsen 2005). A high sequence similarity to the CydDC system of *E. coli* is found for the proteins encoded by the genes *cg1298* and *cg1299* of *C. glutamicum*, indicating that these proteins may constitute an efflux carrier for cysteine and glutathione. As transport system for branched chain amino acids the ABC-type system encoded by the genes *cg1061-66* (Ren and Paulsen 2005) was proposed. It was recently shown, however, that these components constitute the urea uptake system of *C. glutamicum* (Beckers et al. 2004). Interestingly, no primary uptake system for proline is present in *C. glutamicum*, since proline uptake is facilitated by BCCT transporters (Sect. 3.6) only (Morbach and Krämer 2003).

3.21
So far Unidentified Carrier Systems

As a result of current efforts both on *C. glutamicum* and *E. coli* by using bioinformatics as well as molecular, biochemical and physiological tools the identification and functional characterization of still missing or even unknown carrier systems is expected. These proteins may belong to known transporter families or to new classes of transporters. For a number of substrates the presence of carrier systems can be predicted based on biochemical and physiological results combined with the fact that for many of the related putative substrates a limited rate of diffusion was proven. Recently, the 3D structure of the *Campylobacter jejuni* CjaA periplasmic substrate binding protein with a bound cysteine was solved, indicating the existence of ABC-type carriers for this particular amino acid (Müller et al. 2005). Examples for further expected carrier systems of this kind are mentioned in the chapters on specific amino acid uptake and excretion systems.

4
Particular Amino Acid Transport Systems in *C. glutamicum* and *E. coli*

For the same reasons as described above, we will restrict ourselves to the two biotechnologically highly relevant bacteria *E. coli* K12 and *C. glutamicum* in this chapter. This seems a bit arbitrary in the case of uptake systems; however, it is fully appropriate for amino acid export systems, since they have only been explored in these two organisms to a significant extent.

4.1
Amino Acid Uptake Systems

A list of amino acid uptake systems in *C. glutamicum* and *E. coli* K12 is shown in Table 1, and the corresponding mechanisms are listed in Fig. 1. A multiplicity of transport systems are observed for several amino acids both in the case of *E. coli* and *C. glutamicum*, at least when also predicted transport systems are included, and an even higher degree of multiplicity may turn out to be true if further putative carriers will be assigned in the future. Frequently, the observed multiplicity has a particular meaning in terms of regulation. This is true, for example, for proline in both organisms or for aromatic, as well as branched chain amino acids in *E. coli*. "Housekeeping systems" are constitutively expressed and frequently show a broad specificity and low affinity. On the other hand, additional transport systems with confined specificity and higher affinity are regulated on the level of transcription and are only synthesized, when a particular amino acid substrate is present extracellularly, or in case of special needs, e.g., under hyperos-

motic conditions, where proline and amino acid derivatives are required for osmoprotection.

As mentioned in the first part of this review, the majority of amino acid uptake carriers function according to a secondary mechanism, i.e., they are driven by electrochemical ion potentials; however, in particular for *E. coli*, also a significant number of primary ABC-type systems that depend on ATP as driving force were found. The transport affinities are in general in the μm range for specific substrates and up to mM for the uptake of alternative substrates, the definition of which is of course subject to interpretation.

Because of the opposing direction of substrate flux, amino acid uptake systems may be of significance for amino acid production by bacteria, too. At least in the case of tryptophan and threonine production it has been shown that modification of the respective amino acid uptake activity by deletion or overexpression of genes coding for uptake systems may affect amino acid production (Ikeda and Katsumata 1995; Okamoto et al. 1997). Overexpression of the *aroP* gene, coding for an aromatic amino acid uptake system resulted in a drastic decrease of tryptophan production, whereas mutants impaired in tryptophan uptake were shown to be more effective in tryptophan production than the corresponding parent strains (Ikeda and Katsumata 1995).

Interestingly, a number of amino acid transport systems are still missing, at least with respect to their molecular definition, although many of them have been shown to be present by functional tests such as growth dependence or biochemical uptake measurements. This is true in *C. glutamicum* for alanine, for glutamine and asparagine, for the basic amino acids histidine and arginine, as well as for the polar amino acids serine, threonine, and cysteine. Uptake of glycine seems to be absent in *C. glutamicum* (Krämer, unpublished observation). For *E. coli*, the list of carrier systems seems to be much more complete and only uptake system(s) for cysteine have not yet been assigned to particular gene products.

4.2 Amino Acid Excretion Systems

In contrast to amino acid uptake, which is well studied in many bacterial species and for which numerous examples of uptake systems are described in terms of physiology, biochemistry and molecular biology, this is not true for amino acid excretion. There are a number of reviews available on this particular topic (Krämer 1994; Burkovski and Krämer 2002; Eggeling and Sahm 2003; Eggeling 2005), but these reviews only refer to the biotechnologically relevant organisms *E. coli* and *C. glutamicum*. It is an interesting and yet unsolved question, both in terms of basic microbial physiology as well as in terms of biotechnological application, whether amino acid excretion systems are wide spread among bacterial organisms or whether they are more restricted to particular species, like *E. coli* and *C. glutamicum*. Basically, the occurrence of

members of carrier families, which are known to function as amino acid excretion systems in many organisms (e.g., members of the LysE, ThrE, and RhtB family are found in genomes of 72, 50, and 104 different species, respectively), clearly argues for a broad distribution. Moreover, the fact that for the production of other amino acids, e.g., glutamine, serine and alanine, besides the major "work horses" *C. glutamicum* and *E. coli*, also other bacteria are used, e.g., *Serratia marcescens* and *Bacillus subtilis*, indeed argues for a broader distribution of this kind of systems.

Another valid argument for this view is based on the physiological explanation for most of the observed amino acid excretion processes (Krämer 1994; Burkovski and Krämer 2002). Besides glutamate (see below), the presence of bacterial amino acid export systems can, in general, be explained by a putative function as emergency valves for situations of metabolic imbalance. An example is the uptake of peptides as sole or at least major source of carbon and energy and possibly also nitrogen. Since in *C. glutamicum*, pathways of amino acid catabolism are relatively limited (Nakayama 1985), a fact which is also the basis for application of the peptide feeding method for inducing amino acid efflux (Bröer and Krämer 1991a,b; Simic et al. 2001), particular amino acids may accumulate in the cytoplasm and are excreted in order to guarantee a high overall metabolic flux under these conditions. Although *E. coli* is better equipped with amino acid degradation pathways, they may still be kinetically limiting under particular metabolic conditions thus creating an evolutionary advantage of the presence of excretion systems.

All transporters that are known to catalyze amino acid export in *E. coli* and *C. glutamicum* are included in Tables 1–3. The first amino acid excretion systems have been described in physiological and biochemical terms about 15 years ago (Hoischen and Krämer 1989; Bröer and Krämer 1991a), at least in *C. glutamicum*, and the majority of them has been defined in molecular terms to a large extent by the work of L. Eggeling and his group (Eggeling and Sahm 2003; Eggeling 2005). Since a number of instructive reviews (see above) are available for the transport systems known so far, besides a general overview, details on these systems will not be given here.

4.2.1 Lysine and Arginine Export (LysE of *C. glutamicum* and ArgO of *E. coli*)

Lysine is the amino acid with the second largest biotechnological production capacity (Kelle et al. 2005). Furthermore, lysine export in *C. glutamicum* was the first amino acid excretion system to be described in mechanistic terms (Bröer and Krämer 1991b) and to be identified on the molecular level (Vrljic et al. 1996). In view of the impermeability of this amino acid by passive diffusion through the plasma membrane, on the one hand, and because of the energetically unfavorable charge movement (a cation would be moved to the positive side of the membrane), on the other, the requirement of a regulated

and energy driven extrusion system for this amino acid seems to be obvious. The lysine export carrier is an integral membrane protein with five (Vrljic et al. 1996) to six (Haier and Krämer, unpublished) transmembrane segments, which most probably functions in the membrane as a dimer. Besides lysine, it also accepts arginine as a substrate, and it is energetically driven by antiport with hydroxyl ions, or cotransport with protons, respectively (Bröer and Krämer 1991b). Expression of the *lysE* gene is under the control of the LysR-type transcription factor LysG which, in the presence of the co-inducers lysine or arginine (or citrulline or histidine), increases expression of *lysE* (Bellmann et al. 2001). The ArgO transporter of *E. coli*, which has a relatively high similarity to LysE of *C. glutamicum*, accepts arginine as a substrate and the expression of *argO* is under control of the LysR-type transcriptional activator ArgP (Nandineni and Gowrishankar 2004).

It has been shown convincingly that loss of the lysine export carrier creates a serious problem in *C. glutamicum* by accumulation of excessive internal lysine concentrations when growing on peptide substrates (Vrljic et al. 1996). The direct consequence of this observation, namely that overexpression of the *lysE* gene should lead to enhanced excretion of lysine, has in fact been shown (Vrljic et al. 1996; Kelle et al. 2005), however, a successful application to lysine producing *C. glutamicum* strains is not known. It may, thus, be assumed that in these lysine production strains lysine export, although being carrier mediated, is most probably not rate limiting in the overall process. On the other hand, the presence of LysE may increase lysine production in other organisms (Gunji and Yasueda 2006).

4.2.2 Threonine and Homoserine Export (ThrE of *C. glutamicum* and the RhtB Family Transporter of *E. coli*)

Threonine export was biochemically characterized in *C. glutamicum* (Palmieri et al. 1996) and the prototypical ThrE exporter was identified in the same organism (Simic et al. 2001). At elevated internal threonine concentrations, ThrE was shown to be responsible for the majority of threonine efflux. The remaining export activity is due to other, yet unidentified excretion systems as well as to passive diffusion (Simic et al. 2001). For threonine export, it has been shown that overexpression of the *thrE* gene in fact may lead to a significantly increased external threonine accumulation (Simic et al. 2002).

For biotechnological threonine production, however, *E. coli* rather than *C. glutamicum* strains are used (Leuchtenberger et al. 2005), but the situation concerning threonine export systems is not as clear. Carriers of the RhtB family of transporters, comprising five members in *E. coli*, namely *yahN*, *yeaS*, *yfiK*, *rhtB*, and *rhtC*, have been made responsible for export of this amino acid (Aleshin et al. 1999; Zakataeva et al. 1999). Later on, a major contribution of RhtB and RhtC to threonine efflux has been excluded (Kruse et al.

2002), although an influence of these proteins on resistance to threonine, homoserine, and homoserine lactone has been shown (Eggeling and Sahm 2003). Moreover, RhtA, a transporter belonging to the DME family (DMT superfamily, see Sect. 3.4) has recently been made responsible for increasing the resistance to inhibitory concentrations of threonine and homoserine, which was taken as an indication for being involved in threonine efflux (Livshits et al. 2003).

4.2.3 Export of Branched Chain Amino Acids and Methionine (BrnFE of *C. glutamicum*)

Isoleucine was one of the first amino acids for which active export has been demonstrated in *C. glutamicum*, in addition to a basic flux due to passive diffusion (Zittrich and Krämer 1994; Hermann and Krämer 1996). When the exporter was identified, it turned out to be a novel two-component carrier encoded by the *brnFE* genes (Kennerknecht et al. 2002). Previously, a similar transport system has been found in *B. subtilis* to be related to 4-azaleucine resistance (Belitsky et al. 1997). The originally identified substrates for this proton motive force-driven secondary system were the three branched-chain amino acids. Recently, however, methionine was identified to be most probably the major export substrate of BrnFE in *C. glutamicum* (Trötschel et al. 2005). In this publication, it was also shown that *C. glutamicum* carries at least one further methionine export system in addition to BrnFE. In *E. coli*, the existence of an entire LIV-E system is unclear, but the *yeaS* gene product has recently been related to the efflux of leucine (Kutukova et al. 2005).

4.2.4 Export of Cysteine and Cysteine Derivatives in *E. coli*

In *E. coli*, three proteins from very different transporter families have previously been identified to catalyze export of cysteine or cysteine derivatives, YdeD from the DME family (Dassler et al. 2000), YfiK from the RhtB family (Franke et al. 2003), as well as the ABC-type system CydDC (Pittman et al. 2002). Additionally, a recent systematic study on the effect of transporter gene overexpression revealed altogether eight different transporters as putative cysteine exporters (Yamada et al. 2006). The sensitivity of an *E. coli* strain carrying a disrupted cysteine desulfhydrase gene, which leads to a block of cysteine degradation, was reversed by overexpression of these genes. Further transport assays indicated a major contribution of the Bcr protein, a member of the MFS family. One of the reasons for the difficulty in unequivocally assigning the correct export system in this case may be due to the fact that it is not yet clear whether cysteine itself or derivatives of it are transported across the

plasma membrane, such as cystine or the condensation product with pyruvate, 2-methyl-2,4-thiazolidinecarboxylic acid (Dassler et al. 2000; Yamada et al. 2006).

4.2.5 Glutamate Export in *C. glutamicum*

Glutamate was the first amino acid to be produced by bacteria (Kinoshita et al. 1957), and it is by far the amino acid with the highest production capacity (Kimura 2005). It was also one of the first amino acids for which carrier-mediated export was demonstrated (Hoischen and Krämer 1989, 1990; Gutmann et al. 1992). Nevertheless, significant progress in defining the molecular basis for glutamate excretion has been achieved only very recently. In contrast to the excretion of other amino acids, such as lysine or isoleucine, glutamate excretion always seemed to be connected with a particular physiological situation called "overflow metabolism" (Tempest and Neijssel 1992), which describes surplus of energy, carbon and nitrogen in the presence of a particular limiting factor, leading in general to cessation of growth. A number of treatments have been developed in the course of the years, which ultimately lead to massive export of glutamate. It has to be taken into account that, even under normal physiological conditions, the cytoplasm of *C. glutamicum* shows a high steady-state concentration of glutamate, in the range of up to several 100 mM (Gutmann et al. 1992). Nevertheless, under these conditions, glutamate transport functions only in one direction, namely uptake, with extremely high efficiency of accumulation (Krämer and Lambert 1990), due to the presence of the GluABC uptake system (Kronemeyer et al. 1995).

The original concept explaining the physiological basis of glutamate excretion was the combination of two major aspects. On one hand, a strongly decreased or even missing activity of the Krebs cycle enzyme oxoglutarate dehydrogenase (OGDH) was assumed and the significance of this enzyme for glutamate production was shown by inactivation (Shiio et al. 1961; Kawahara et al. 1997). Later on, it turned out that this enzyme activity is rather difficult to measure in vitro because of its inherent instability (Kimura 2005). Nevertheless, in production strains it was found that in fact the OGDH was severely impaired (Shingu and Terui 1972). The second aspect obviously related to glutamate excretion was an altered state of the cell envelope. Upon application of different, highly diverse kinds of treatment, which include, among others, (1) biotin limitation, (2) addition of particular detergents, (3) addition of antibiotic substances with different modes of action, such as penicillin, ethambutol and cerulenin, and (4) use of fatty acid or glycerol auxotrophs, changed metabolic conditions in *C. glutamicum* cause continuous and efficient efflux of glutamate without a loss of basic viability (Kimura 2005).

Obviously, all these treatments are, in one way or the other, connected to the integrity of the cell envelope, either affecting directly the state of the plasma membrane and/or that of the cell wall. This has led to the suggestion that primarily the permeability properties of the cell wall may be responsible for glutamate efflux (Eggeling et al. 2001; reviewed in Kimura 2005), which, in the case of Corynebacteria, in fact, represents a second permeability barrier in addition to that of the plasma membrane. This concept, however, was challenged by the observation that at the same time, uptake of glutamate, which has to cross the same permeability barrier, is not increased after treatments leading to an increased efflux (Burkovski and Krämer 2002).

Interestingly, in a recent publication, the first hint to the molecular basis of a possible connection between OGDH activity and glutamate excretion was found (Niebisch et al. 2006). A novel mechanism for the regulation of this enzyme was identified, including a 15 kDa protein OdhI, which inhibits the OGDH when it is present in its unphosphorylated form, as well as the action of a soluble Ser/Thr protein kinase PknG on the OdhI protein. Thus, OdhI and PknG are interesting putative candidates for a regulatory linkage between treatments leading to glutamate excretion and metabolic responses.

According to results described in a recently published patent, it seems that the central question on the mechanism of glutamate excretion in *C. glutamicum* has been solved (Nakamura et al. 2006; Nakamura et al., submitted for publication). Based on the observation that glutamate producing strains of *C. glutamicum* in general carry a defective OGDH, it has been demonstrated that strains in which solely this enzyme was mutated did in fact excrete glutamate, however, at relatively low level. These strains now turned out to be genetically unstable, and secondary mutations were found triggering much higher glutamate excretion. When these mutations were analyzed, repeatedly a mutated *yggB* gene was identified, which could be interpreted in terms of an altered activity regulation or an altered specificity. The YggB protein in *C. glutamicum* has previously been described as a putative mechanosensitive channel (Nottebrock et al. 2003), similar to the *E. coli* MscS system (Levina et al. 1999). It was shown that point mutations in the *yggB* gene for modification of particular domains as well as overexpression led, even in the absence of an altered OGDH, to significantly enhanced glutamate excretion, which could further be stimulated by the well known membrane triggers, e.g., addition of tween or biotin limitation. Deletion of the *yggB* gene, on the other hand, reduced glutamate efflux both in the presence and absence of the triggers mentioned above. A connection between osmotic stress and glutamate excretion has in fact been noted earlier (Lambert et al. 1995). It has to be elucidated in the future (1) whether the main function of the *C. glutamicum* YggB protein is, in fact, glutamate excretion or rather unspecific solute export in case of hypoosmotic stress, and (2) how the known mechanisms triggering glutamate excretion are related to YggB function.

4.2.6
Further Amino Acid Excretion Systems

On the one hand, it is known that those amino acids, which are currently produced in *C. glutamicum*, can in general be produced in *E. coli*, too, and the other way round. Threonine, for example, is currently produced in *E. coli* strains, and methionine can also be produced in the same organism, (Bestel–Core et al. 2005), but, in contrast to *C. glutamicum*, the responsible export carriers are not clearly defined (threonine) or not known (methionine). *E. coli* is also able to excrete glutamate under particular conditions (Broda 1968), however, a glutamate export system has not been identified so far. Tryptophan can be produced by both organisms (Berry 1996; Ikeda 2005), but there is no clear evidence for a carrier-mediated export so far, although the *yddG* gene product has been related to tryptophan excretion in *E. coli* (Ryback et al. 2006). Whereas it seems plausible that phenylalanine may cross the membrane barrier effectively by passive diffusion, due to its hydrophobic nature, this is not very probable for the more polar tyrosine and tryptophan thus asking for the presence of efflux systems in these cases.

There are further observations of amino acid efflux, which are not related to known amino acid export systems. A general observation under a number of metabolic conditions is export of alanine, which is frequently found in significant concentrations in the medium, and the same is true for glycine. At least the former amino acid is supposed to have a significant rate of passive, diffusion-controlled permeability (Ruhrmann et al. 1994). Another example is proline, which is found to be excreted in *C. glutamicum* under situations when mechanosensitive efflux channels are not supposed to be active (Krämer, unpublished observations). Finally, in view of the rather unexpected mechanism suggested to be valid for glutamate efflux in *C. glutamicum* and in view of a significant residual efflux activity even in the absence of YggB, further mechanisms and/or carriers responsible for this glutamate efflux may be present.

5
Conclusions and Perspectives

Bacterial amino acid transport is a potentially important factor in biotechnological amino acid production. At least three different transport reactions, namely carbon and nitrogen substrate uptake, product excretion and product re-uptake, contribute to the overall net flux starting with the substrate removed from the surrounding medium by the production organism and ending with the product accumulating in the external medium. Even in the best studied bacterial organisms of biotechnological relevance, namely *E. coli* and *C. glutamicum*, a significant part of putative amino acid transport sys-

tems has not yet been identified and characterized. Detailed biochemical characterization is of particular significance in the case of transport systems because of the notoriously unreliable assignment of transport substrate specificity based on sequence similarity. These tasks, e.g., identification of substrate specificity, transport mechanism, as well as integration into regulatory networks, will still require extensive effort in terms of biochemical analysis in the future. The final goal for biotechnological application will be the availability of various kinds of transport systems with various kinds of mechanisms (e.g., energy dependence) and specificity (e.g., discrimination of major and minor substrates), in order to improve the number of valuable tools for rational strain design.

Acknowledgements We are grateful for information and help from Lothar Eggeling and Jörn Kalinowski during preparation of this review. Thanks to Jun Nakamura for information prior to publication. The contribution of Muhammad Ashfaq in establishing our in house data bank on *C. glutamicum* genes and proteins is gratefully acknowledged.

References

Abe K, Ohnishi F, Yagi K, Nakajima T, Higuchi T, Sano M, Machida M, Sarker RI, Maloney PC (2002) Plasmid-encoded asp operon confers a proton motive metabolic cycle catalyzed by an aspartate-alanine exchange reaction. J Bacteriol 184:2906–2913

Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, Iwata S (2003) Structure and mechanism of the lactose permease of *Escherichia coli*. Science 301:610–615

Adams MD, Wagner LM, Graddis TJ, Landick R, Antonucci TK, Gibson AL, Oxender DL (1990) Nucleotide sequence and genetic characterization reveal six essential genes for the LIV-I and LS transport systems of *Escherichia coli*. J Biol Chem 265:11436–11443

Aleshin VV, Zakataeva NP, Livshits VA (1999) A new family of amino-acid-efflux proteins. Trends Biochem Sci 24:133–135

Alvarez-Jacobs J, de la Garza M, Ortega MV (1986) Biochemical and genetic characterization of L-glutamate transport and utilization in *Salmonella typhimurium* LT-2 mutants. Biochem Genet 24:195–205

Andresen PA, Kaasen I, Styrvold OB, Boulnois G, Strom AR (1988) Molecular cloning, physical mapping and expression of the bet genes governing the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. J Gen Microbiol 134:1737–1746

Androutsellis-Theotokis A, Goldberg NR, Ueda K, Beppu T, Beckman ML, Das S, Javitch JA, Rudnick G (2003) Characterization of a functional bacterial homologue of sodium-dependent neurotransmitter transporters. J Biol Chem 278:12703–12709

Beckers G, Bendt AK, Krämer R, Burkovski A (2004) Molecular identification of the urea uptake system and transcriptional analysis of urea transporter- and urease-encoding genes in *Corynebacterium glutamicum*. J Bacteriol 186:7645–7652

Belitsky BR, Gustafsson MC, Sonenshein AL, Von Wachenfeldt C (1997) An *lrp*-like gene of *Bacillus subtilis* involved in branched-chain amino acid transport. J Bacteriol 179:5448–5457

Bellmann A, Vrljic M, Patek M, Sahm H, Krämer R, Eggeling L (2001) Expression control and specificity of the basic amino acid exporter LysE of *Corynebacterium glutamicum*. Microbiology 147:1765–1774

Berger EA, Heppel LA (1972) A binding protein involved in the transport of cystine and diaminopimelic acid in *Escherichia coli*. J Biol Chem 247:7684–7694

Berry A (1996) Improving production of aromatic compounds in *Escherichia coli* by metabolic engineering. Trends Biotechnol 14:250–256

Bestel-Core G, Chateau M, Figge RM, Raynaud C, Soucaille PNP (2005) WO 2005-108561

Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (1997) The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1474

Broda P (1968) Ribonucleic acid synthesis and glutamate excretion in *Escherichia coli*. J Bacteriol 96:1528–1534

Bröer S, Krämer R (1991a) Lysine excretion by *Corynebacterium glutamicum*. 1. Identification of a specific secretion carrier system. Eur J Biochem 202:131–135

Bröer S, Krämer R (1991b) Lysine excretion by *Corynebacterium glutamicum*. 2. Energetics and mechanism of the transport system. Eur J Biochem 202:137–143

Burkovski A, Krämer R (1995) Functional expression of the glutamate uptake system from *Corynebacterium glutamicum* in *Escherichia coli*. FEMS Microbiol Lett 127:263–266

Burkovski A, Krämer R (2002) Bacterial amino acid transport proteins: occurrence, functions, and significance for biotechnological applications. Appl Microbiol Biotechnol 58:265–274

Busch W, Saier MH Jr (2002) The transporter classification (TC) system, 2002. Crit Rev Biochem Mol Biol 37:287–337

Busch W, Saier MH Jr (2004) The IUBMB-endorsed transporter classification system. Mol Biotechnol 27:253–262

Cabrera-Martinez RM, Tovar-Rojo F, Vepachedu VR, Setlow P (2003) Effects of overexpression of nutrient receptors on germination of spores of *Bacillus subtilis*. J Bacteriol 185:2457–2464

Chang G, Roth CB (2001) Structure of MsbA from *E. coli*: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. Science 293:1793–1800

Checroun C, Gutierrez C (2004) Sigma(s)-dependent regulation of *yehZYXW*, which encodes a putative osmoprotectant ABC transporter of *Escherichia coli*. FEMS Microbiol Lett 236:221–226

Chye ML, Guest JR, Pittard J (1986) Cloning of the *aroP* gene and identification of its product in *Escherichia coli* K-12. J Bacteriol 167:749–753

Culham DE, Dalgado C, Gyles CL, Mamelak D, MacLellan S, Wood JM (1998) Osmoregulatory transporter ProP influences colonization of the urinary tract by *Escherichia coli*. Microbiology 144:91–102

Culham DE, Lasby B, Marangoni AG, Milner JL, Steer BA, van Nues RW, Wood JM (1993) Isolation and sequencing of *Escherichia coli* gene *proP* reveals unusual structural features of the osmoregulatory proline/betaine transporter, ProP. J Mol Biol 229:268–276

Dassler T, Maier T, Winterhalter C, Böck A (2000) Identification of a major facilitator protein from *Escherichia coli* involved in efflux of metabolites of the cysteine pathway. Mol Microbiol 36:1101–1112

Dattananda CS, Gowrishankar J (1989) Osmoregulation in *Escherichia coli*: complementation analysis and gene-protein relationships in the *proU* locus. J Bacteriol 171:1915–1922

Dawson RJ, Locher KP (2006) Structure of a bacterial multidrug ABC transporter. Nature 443:180–185

Deguchi Y, Yamato I, Anraku Y (1989) Molecular cloning of *gltS* and *gltP*, which encode glutamate carriers of *Escherichia coli* B. J Bacteriol 171:1314–1319

Ebbighausen H, Weil B, Krämer R (1989) Transport of branched-chain amino acids in *Corynebacterium glutamicum*. Arch Microbiol 151:238–244

Eggeling L (2005) Export of amino acids and other solutes. In: Eggeling L, Bott M (eds) Handbook of *Corynebacterium glutamicum*. Taylor & Francis, Boca Raton, pp 187–214

Eggeling L, Krumbach K, Sahm H (2001) L-glutamate efflux with *Corynebacterium glutamicum*: why is penicillin treatment or Tween addition doing the same? J Mol Microbiol Biotechnol 3:67–68

Eggeling L, Sahm H (2003) New ubiquitous translocators: amino acid export by *Corynebacterium glutamicum* and *Escherichia coli*. Arch Microbiol 180:155–160

Engel P, Krämer R, Unden G (1994) Transport of C4-dicarboxylates by anaerobically grown *Escherichia coli*. Energetics and mechanism of exchange, uptake and efflux. Eur J Biochem 222:605–614

Essenberg RC (1984) Use of homocysteic acid for selecting mutants at the *gltS* locus of *Escherichia coli* K12. J Gen Microbiol 130:1311–1314

Franke I, Resch A, Dassler T, Maier T, Böck A (2003) YfiK from *Escherichia coli* promotes export of O-acetylserine and cysteine. J Bacteriol 185:1161–1166

Gimenez R, Nunez MF, Badia J, Aguilar J, Baldoma L (2003) The gene *yjcG*, cotranscribed with the gene *acs*, encodes an acetate permease in *Escherichia coli*. J Bacteriol 185:6448–6455

Gong S, Richard H, Foster JW (2003) YjdE (AdiC) is the arginine:agmatine antiporter essential for arginine-dependent acid resistance in *Escherichia coli*. J Bacteriol 185:4402–4409

Guardiola J, De Felice M, Klopotowski T, Iaccarino M (1974) Mutations affecting the different transport systems for isoleucine, leucine, and valine in *Escherichia coli* K-12. J Bacteriol 117:393–405

Gunji Y, Yasueda H (2006) Enhancement of l-lysine production in methylotroph *Methylophilus methylotrophus* by introducing a mutant LysE exporter. J Biotechnol 127:1–13

Gutmann M, Hoischen C, Krämer R (1992) Carrier-mediated glutamate secretion by *Corynebacterium glutamicum* under biotin limitation. Biochim Biophys Acta 1112:115–123

Heatwole VM, Somerville RL (1991) Cloning, nucleotide sequence, and characterization of *mtr*, the structural gene for a tryptophan-specific permease of *Escherichia coli* K-12. J Bacteriol 173:108–115

Hermann T, Krämer R (1996) Mechanism and Regulation of Isoleucine Excretion in *Corynebacterium glutamicum*. Appl Environ Microbiol 62:3238–3244

Hirai T, Heymann JA, Maloney PC, Subramaniam S (2003) Structural model for 12-helix transporters belonging to the major facilitator superfamily. J Bacteriol 185:1712–1718

Hirokawa T, Boon-Chieng S, Mitaku S (1998) SOSUI: classification and secondary structure prediction system for membrane proteins. Bioinformatics 14:378–379

Hoischen C, Krämer R (1989) Evidence for an efflux carrier system involved in the secretion of glutamate by *Corynebacterium glutamicum*. Arch Microbiol 151:342–347

Hoischen C, Krämer R (1990) Membrane alteration is necessary but not sufficient for effective glutamate secretion in *Corynebacterium glutamicum*. J Bacteriol 172:3409–3416

Huang Y, Lemieux MJ, Song J, Auer M, Wang DN (2003) Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. Science 301:616–620

Ikeda M (2005) L-Tryptophan production. In: Eggeling L, Bott M (eds) Handbook of *Corynebacterium glutamicum*. Taylor & Francis, Boca Raton, pp 489–510

Ikeda M, Katsumata R (1995) Tryptophan production by transport mutants of *Corynebacterium glutamicum*. Biosci Biotechnol Biochem 59:1600–1602

Itou H, Okada U, Suzuki H, Yao M, Wachi M, Watanabe N, Tanaka I (2005) The CGL2612 protein from Corynebacterium glutamicum is a drug resistance-related transcriptional repressor: structural and functional analysis of a newly identified transcription factor from genomic DNA analysis. J Biol Chem 280:38711–38719

Jack DL, Paulsen IT, Saier MH (2000) The amino acid/polyamine/organocation (APC) superfamily of transporters specific for amino acids, polyamines and organocations. Microbiology 146:1797–814

Jackowski S, Alix JH (1990) Cloning, sequence, and expression of the pantothenate permease (*panF*) gene of *Escherichia coli*. J Bacteriol 172:3842–3848

Jacobs MH, van der Heide T, Driessen AJ, Konings WN (1996) Glutamate transport in *Rhodobacter sphaeroides* is mediated by a novel binding protein-dependent secondary transport system. Proc Natl Acad Sci USA 93:12786–12790

Jager W, Kalinowski J, Pühler A (1997) A *Corynebacterium glutamicum* gene conferring multidrug resistance in the heterologous host *Escherichia coli*. J Bacteriol 179:2449–2451

Jung H (1998) Topology and function of the Na+/proline transporter of *Escherichia coli*, a member of the Na+/solute cotransporter family. Biochim Biophys Acta 1365:60–64

Jung H (2001) Towards the molecular mechanism of Na(+)/solute symport in prokaryotes. Biochim Biophys Acta 1505:131–143

Jung H, Buchholz M, Clausen J, Nietschke M, Revermann A, Schmid R, Jung K (2002) CaiT of *Escherichia coli*, a new transporter catalyzing L-carnitine/gamma -butyrobetaine exchange. J Biol Chem 277:39251–39258

Kalinowski J, Bathe B, Bartels D, Bischoff N, Bott M, Burkovski A, Dusch N, Eggeling L, Eikmanns BJ, Gaigalat L, Goesmann A, Hartmann M, Huthmacher K, Krämer R, Linke B, McHardy AC, Meyer F, Mockel B, Pfefferle W, Pühler A, Rey DA, Rückert C, Rupp O, Sahm H, Wendisch VF, Wiegrabe I, Tauch A (2003) The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. J Biotechnol 104:5–25

Käll L, Krogh A, Sonnhammer EL (2004) A combined transmembrane topology and signal peptide prediction method. J Mol Biol 338:1027–1036

Kanamori M, Kamata H, Yagisawa H, Hirata H (1999) Overexpression of the alanine carrier protein gene from thermophilic bacterium PS3 in *Escherichia coli*. J Biochem (Tokyo) 125:454–9

Kashiwagi K, Miyamoto S, Suzuki F, Kobayashi H, Igarashi K (1992) Excretion of putrescine by the putrescine-ornithine antiporter encoded by the *potE* gene of *Escherichia coli*. Proc Natl Acad Sci USA 89:4529–4533

Kawahara Y, Takahashi-Fuke K, Shimizu E, Nakamatsu T, Nakamori S (1997) Relationship between the glutamate production and the activity of 2-oxoglutarate dehydrogenase in *Brevibacterium lactofermentum*. Biosci Biotechnol Biochem 61:1109–1112

Kayahara T, Thelen P, Ogawa W, Inaba K, Tsuda M, Goldberg EB, Tsuchiya T (1992) Properties of recombinant cells capable of growing on serine without NhaB Na+/H+ antiporter in *Escherichia coli*. J Bacteriol 174:7482–7485

Kelle R, Herrmann T, Bathe B (2005) L-Lysine Production. In: Eggeling L, Bott M (eds) Handbook of *Corynebacterium glutamicum*. Taylor & Francis, Heidelberg, pp 465–488

Kennerknecht N, Sahm H, Yen MR, Patek M, Saier MH Jr, Eggeling L (2002) Export of L-isoleucine from *Corynebacterium glutamicum*: a two-gene-encoded member of a new translocator family. J Bacteriol 184:3947–3956

Kim YM, Ogawa W, Tamai E, Kuroda T, Mizushima T, Tsuchiya T (2002) Purification, reconstitution, and characterization of Na(+)/serine symporter, SstT, of *Escherichia coli*. J Biochem (Tokyo) 132:71–76

Kimura E (2005) L-Glutamate production. In: Eggeling L, Bott M (eds) Handbook of *Corynebacterium glutamicum*. Taylor & Francis, Boca Raton, pp 439–464

Kinoshita K, Udata S, Shimono M (1957) Studies on the amino acid fermentation. I. Production of L-glutamic acid by various microorganisms. J Gen Appl Microbiol 7:193–205

Koch DJ, Rückert C, Rey DA, Mix A, Pühler A, Kalinowski J (2005) Role of the *ssu* and *seu* genes of *Corynebacterium glutamicum* ATCC 13032 in utilization of sulfonates and sulfonate esters as sulfur sources. Appl Environ Microbiol 71:6104–6114

Krämer R (1994) Secretion of amino acids by bacteria: Physiology and mechanism. FEMS Microbiol Rev 13:75–94

Krämer R (1996) Genetic and physiological approaches for the production of amino acids. J Biotechnol 45:1–21

Krämer R, Hoischen C (1994) Futile cycling caused by the simultaneous presence of separate transport systems for uptake and secretion of amino acids in *Corynebacterium glutamicum*. In: Westerhoff H (eds) Biothermokinetics. Intercept Publ., Amsterdam, pp 19–26

Krämer R, Lambert C (1990) Uptake of glutamate in *Corynebacterium glutamicum*. 2. Evidence for a primary active transport system. Eur J Biochem 194:937–944

Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580

Kronemeyer W, Peekhaus N, Krämer R, Sahm H, Eggeling L (1995) Structure of the *gluABCD* cluster encoding the glutamate uptake system of *Corynebacterium glutamicum*. J Bacteriol 177:1152–1158

Kruse D, Krämer R, Eggeling L, Rieping M, Pfefferle W, Tchieu JH, Chung YJ, Saier MH Jr, Burkovski A (2002) Influence of threonine exporters on threonine production in *Escherichia coli*. Appl Microbiol Biotechnol 59:205–210

Kutukova EA, Livshits VA, Altman IP, Ptitsyn LR, Zyiatdinov MH, Tokmakova IL, Zakataeva NP (2005) The *yeaS* (*leuE*) gene of *Escherichia coli* encodes an exporter of leucine, and the Lrp protein regulates its expression. FEBS Lett 579:4629–4634

Lambert C, Erdmann A, Eikmanns M, Krämer R (1995) Triggering Glutamate Excretion in *Corynebacterium glutamicum* by Modulating the Membrane State with Local Anesthetics and Osmotic Gradients. Appl Environ Microbiol 61:4334–4342

Lee M, Robbins JC, Oxender DL (1975) Transport properties of merodiploids covering the *dagA* locus in *Escherichia coli* K-12. J Bacteriol 122:1001–1005

Leive L, Davis BD (1965) The transport of diaminopimelate and cystine in *Escherichia coli*. J Biol Chem 240:4362–4369

Leuchtenberger W, Huthmacher K, Drauz K (2005) Biotechnological production of amino acids and derivatives: current status and prospects. Appl Microbiol Biotechnol 69:1–8

Levina N, Totemeyer S, Stokes NR, Louis P, Jones MA, Booth IR (1999) Protection of *Escherichia coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. Embo J 18:1730–1737

Liu CE, Ames GF (1997) Characterization of transport through the periplasmic histidine permease using proteoliposomes reconstituted by dialysis. J Biol Chem 272:859–866

Livshits VA, Zakataeva NP, Aleshin VV, Vitushkina MV (2003) Identification and characterization of the new gene *rhtA* involved in threonine and homoserine efflux in *Escherichia coli*. Res Microbiol 154:123–135

Lo TC, Bewick MA (1978) The molecular mechanisms of dicarboxylic acid transport in *Escherichia coli* K12. The role and orientation of the two membrane-bound dicarboxylate binding proteins. J Biol Chem 253:7826–7831

Locher KP, Lee AT, Rees DC (2002) The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. Science 296:1091–1098

MacLeod PR, MacLeod RA (1992) Identification and sequence of a Na(+)-linked gene from the marine bacterium *Alteromonas haloplanktis* which functionally complements the *dagA* gene of *Escherichia coli*. Mol Microbiol 6:2673–2681

Marcus M, Halpern YS (1969) Genetic analysis of the glutamate permease in *Escherichia coli* K-12. J Bacteriol 97:1118–1128

May G, Faatz E, Villarejo M, Bremer E (1986) Binding protein dependent transport of glycine betaine and its osmotic regulation in *Escherichia coli* K12. Mol Gen Genet 205:225–233

Meng SY, Bennett GN (1992) Nucleotide sequence of the *Escherichia coli cad* operon: a system for neutralization of low extracellular pH. J Bacteriol 174:2659–2669

Merkens H, Beckers G, Wirtz A, Burkovski A (2005) Vanillate metabolism in *Corynebacterium glutamicum*. Curr Microbiol 51:59–65

Merlin C, Gardiner G, Durand S, Masters M (2002) The *Escherichia coli metD* locus encodes an ABC transporter which includes Abc (MetN), YaeE (MetI), and YaeC (MetQ). J Bacteriol 184:5513–5517

Metzer E, Halpern YS (1990) In vivo cloning and characterization of the *gabCTDP* gene cluster of *Escherichia coli* K-12. J Bacteriol 172:3250–3256

Moore BC, Leigh JA (2005) Markerless mutagenesis in *Methanococcus maripaludis* demonstrates roles for alanine dehydrogenase, alanine racemase, and alanine permease. J Bacteriol 187:972–979

Morbach S, Krämer R (2003) Impact of transport processes in the osmotic response of *Corynebacterium glutamicum*. J Biotechnol 104:69–75

Morrow JA, Collie IT, Dunbar DR, Walker GB, Shahid M, Hill DR (1998) Molecular cloning and functional expression of the human glycine transporter GlyT2 and chromosomal localisation of the gene in the human genome. FEBS Lett 439:334–340

Müller A, Thomas GH, Horler R, Brannigan JA, Blagova E, Levdikov VM, Fogg MJ, Wilson KS, Wilkinson AJ (2005) An ATP-binding cassette-type cysteine transporter in *Campylobacter jejuni* inferred from the structure of an extracytoplasmic solute receptor protein. Mol Microbiol 57:143–155

Nakamura J, Hirano S, Ito H (2006) US 2006/0141588

Nakayama K (1985) Lysine. In: Moo-Young M (eds) Comprehensive Biotechnology, vol 3. Pergamon Press, New York, pp 607–620

Nandineni MR, Gowrishankar J (2004) Evidence for an arginine exporter encoded by *yggA* (*argO*) that is regulated by the LysR-type transcriptional regulator ArgP in *Escherichia coli*. J Bacteriol 186:3539–3546

Niebisch A, Kabus A, Schultz C, Weil B, Bott M (2006) Corynebacterial protein kinase G controls 2-oxoglutarate dehydrogenase activity via the phosphorylation status of the OdhI protein. J Biol Chem 281:12300–12307

Nohno T, Saito T, Hong JS (1986) Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (*glnHPQ*). Mol Gen Genet 205:260–269

Nottebrock D, Meyer U, Krämer R, Morbach S (2003) Molecular and biochemical characterization of mechanosensitive channels in *Corynebacterium glutamicum*. FEMS Microbiol Lett 218:305–309

Ogawa W, Kayahara T, Tsuda M, Mizushima T, Tsuchiya T (1997) Isolation and characterization of an *Escherichia coli* mutant lacking the major serine transporter, and cloning of a serine transporter gene. J Biochem (Tokyo) 122:1241–1245

Oh BH, Pandit J, Kang CH, Nikaido K, Gokcen S, Ames GF, Kim SH (1993) Three-dimensional structures of the periplasmic lysine/arginine/ornithine-binding protein with and without a ligand. J Biol Chem 268:11348–11355

Okamoto K, Kino K, Ikeda M (1997) Hyperproduction of L-threonine by an *Escherichia coli* mutant with impaired L-threonine uptake. Biosci Biotechnol Biochem 61:1877–1882

Ovchinnikov YA, Aldanova NA, Grinkevich VA, Arzamazova NM, Moroz IN (1977) The primary structure of a Leu, Ile and Val (LIV)-binding protein from *Escherichia coli*. FEBS Lett 78:313–316

Oxender DL, Anderson JJ, Daniels CJ, Landick R, Gunsalus RP, Zurawski G, Selker E, Yanofsky C (1980) Structural and functional analysis of cloned DNA containing genes responsible for branched-chain amino acid transport in *Escherichia coli*. Proc Natl Acad Sci USA 77:1412–1416

Palmieri L, Berns D, Krämer R, Eikmanns B (1996) Threonine diffusion and threonine transport in *Corynebacterium glutamicum* and their role in threonine production. Arch Microbiol 165:48–54

Peter H, Bader A, Burkovski A, Lambert C, Krämer R (1997) Isolation of the *putP* gene of *Corynebacterium glutamicum* and characterization of a low-affinity uptake system for compatible solutes. Arch Microbiol 168:143–151

Peter H, Burkovski A, Krämer R (1996) Isolation, characterization, and expression of the *Corynebacterium glutamicum betP* gene, encoding the transport system for the compatible solute glycine betaine. J Bacteriol 178:5229–5234

Peter H, Weil B, Burkovski A, Krämer R, Morbach S (1998) *Corynebacterium glutamicum* is equipped with four secondary carriers for compatible solutes: identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EctP. J Bacteriol 180:6005–6012

Pi J, Wookey PJ, Pittard AJ (1991) Cloning and sequencing of the *pheP* gene, which encodes the phenylalanine-specific transport system of *Escherichia coli*. J Bacteriol 173:3622–3629

Pittman MS, Corker H, Wu G, Binet MB, Moir AJ, Poole RK (2002) Cysteine is exported from the *Escherichia coli* cytoplasm by CydDC, an ATP-binding cassette-type transporter required for cytochrome assembly. J Biol Chem 277:49841–49849

Ren Q, Paulsen IT (2005) Comparative analyses of fundamental differences in membrane transport capabilities in prokaryotes and eukaryotes. PLoS Comput Biol 1:e27

Rübenhagen R, Rönsch H, Jung H, Krämer R, Morbach S (2000) Osmosensor and osmoregulator properties of the betaine carrier BetP from *Corynebacterium glutamicum* in proteoliposomes. J Biol Chem 275:735–741

Ruhrmann J, Sprenger GA, Krämer R (1994) Mechanism of alanine excretion in recombinant strains of *Zymomonas mobilis*. Biochim Biophys Acta 1196:14–20

Ryback KV, Slivinskaja EA, Kozlov YI, Suzuki T (2006) WO 2006-068273

Sack JS, Saper MA, Quiocho FA (1989) Periplasmic binding protein structure and function. Refined X-ray structures of the leucine/isoleucine/valine-binding protein and its complex with leucine. J Mol Biol 206:171–191

Saier MH Jr (2000) Families of transmembrane transporters selective for amino acids and their derivatives. Microbiology 146:1775–1795

Saier MH Jr, Tran CV, Barabote RD (2006) TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucleic Acids Res 34:D181–D186

Sarsero JP, Wookey PJ, Gollnick P, Yanofsky C, Pittard AJ (1991) A new family of integral membrane proteins involved in transport of aromatic amino acids in *Escherichia coli*. J Bacteriol 173:3231–3234

Schneider F, Krämer R, Burkovski A (2004) Identification and characterization of the main beta-alanine uptake system in *Escherichia coli*. Appl Microbiol Biotechnol 65:576–582

Seep-Feldhaus AH, Kalinowski J, Pühler A (1991) Molecular analysis of the *Corynebacterium glutamicum lysI* gene involved in lysine uptake. Mol Microbiol 5:2995–3005

Shiio I, Ohtska S, Takahashi M (1961) OGDH Significance of α-ketoglutarate dehydrogenase on the glutamic acid formation in *Brevibacterium flavum*. J Biochem 50:164–165

Shingu H, Terui G (1972) Studies on the process of glutamic acid fermentation at the enzyme level: I. On the changes of a-ketoglutaric acid dehydrogenase in the course of culture. J Ferment Technol 49:400–405

Simic P, Sahm H, Eggeling L (2001) L-threonine export: use of peptides to identify a new translocator from *Corynebacterium glutamicum*. J Bacteriol 183:5317–5324

Simic P, Willuhn J, Sahm H, Eggeling L (2002) Identification of *glyA* (encoding serine hydroxymethyltransferase) and its use together with the exporter ThrE to increase L-threonine accumulation by *Corynebacterium glutamicum*. Appl Environ Microbiol 68:3321–3327

Steffes C, Ellis J, Wu J, Rosen BP (1992) The *lysP* gene encodes the lysine-specific permease. J Bacteriol 174:3242–3249

Steger R, Weinand M, Krämer R, Morbach S (2004) LcoP, an osmoregulated betaine/ectoine uptake system from *Corynebacterium glutamicum*. FEBS Lett 573:155–160

Sumantran VN, Schweizer HP, Datta P (1990) A novel membrane-associated threonine permease encoded by the *tdcC* gene of *Escherichia coli*. J Bacteriol 172:4288–4294

Sun YJ, Rose J, Wang BC, Hsiao CD (1998) The structure of glutamine-binding protein complexed with glutamine at 1.94 A resolution: comparisons with other amino acid binding proteins. J Mol Biol 278:219–229

Tauch A, Hermann T, Burkovski A, Krämer R, Pühler A, Kalinowski J (1998) Isoleucine uptake in *Corynebacterium glutamicum* ATCC 13032 is directed by the *brnQ* gene product. Arch Microbiol 169:303–312

Tempest DW, Neijssel OM (1992) Physiological and energetic aspects of bacterial metabolite overproduction. FEMS Microbiol Lett 79:169–176

Thanbichler M, Neuhierl B, Böck A (1999) S-methylmethionine metabolism in *Escherichia coli*. J Bacteriol 181:662–665

Trötschel C, Deutenberg D, Bathe B, Burkovski A, Krämer R (2005) Characterization of methionine export in *Corynebacterium glutamicum*. J Bacteriol 187:3786–3794

Trötschel C, Kandirali S, Diaz-Achirica P, Meinhardt A, Morbach S, Krämer R, Burkovski A (2003) GltS, the sodium-coupled L-glutamate uptake system of *Corynebacterium glutamicum*: identification of the corresponding gene and impact on L-glutamate production. Appl Microbiol Biotechnol 60:738–742

van der Heide T, Poolman B (2002) ABC transporters: one, two or four extracytoplasmic substrate-binding sites? EMBO Rep 3:938–943

van der Ploeg JR, Weiss MA, Saller E, Nashimoto H, Saito N, Kertesz MA, Leisinger T (1996) Identification of sulfate starvation-regulated genes in *Escherichia coli*: a gene cluster involved in the utilization of taurine as a sulfur source. J Bacteriol 178:5438–5446

Vardy E, Steiner-Mordoch S, Schuldiner S (2005) Characterization of bacterial drug antiporters homologous to mammalian neurotransmitter transporters. J Bacteriol 187:7518–7525

Vrljic M, Sahm H, Eggeling L (1996) A new type of transporter with a new type of cellular function: L-lysine export from *Corynebacterium glutamicum*. Mol Microbiol 22:815–826

Wehrmann A, Morakkabati S, Krämer R, Sahm H, Eggeling L (1995) Functional analysis of sequences adjacent to *dapE* of *Corynebacterium glutamicum* reveals the presence of *aroP*, which encodes the aromatic amino acid transporter. J Bacteriol 177:5991–5993

Whipp MJ, Halsall DM, Pittard AJ (1980) Isolation and characterization of an *Escherichia coli* K-12 mutant defective in tyrosine- and phenylalanine-specific transport systems. J Bacteriol 143:1–7

Willis RC, Furlong CE (1975) Interactions of a glutamate-aspartate binding protein with the glutamate transport system of *Escherichia coli*. J Biol Chem 250:2581–2586

Willis RC, Woolfolk CA (1975) L-asparagine uptake in *Escherichia coli*. J Bacteriol 123:937–945

Winnen B, Felce J, Saier MH Jr (2005) Genome analyses of transporter proteins in *Corynebacterium glutamicum* and *Corynebacterium efficiens*. In: Eggeling L, Bott M (eds) Handbook of *Corynebacterium glutamicum*. Taylor & Francis, Boca Raton, pp 149–186

Wissenbach U, Keck B, Unden G (1993) Physical map location of the new *artPIQMJ* genes of *Escherichia coli*, encoding a periplasmic arginine transport system. J Bacteriol 175:3687–3688

Wissenbach U, Six S, Bongaerts J, Ternes D, Steinwachs S, Unden G (1995) A third periplasmic transport system for L-arginine in *Escherichia coli*: molecular characterization of the *artPIQMJ* genes, arginine binding and transport. Mol Microbiol 17:675–686

Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H (2006) Effect of drug transporter genes on cysteine export and overproduction in *Escherichia coli*. Appl Environ Microbiol 72:4735–4742

Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E (2005) Crystal structure of a bacterial homologue of Na+/Cl–dependent neurotransmitter transporters. Nature 437:215–223

Yao N, Trakhanov S, Quiocho FA (1994) Refined 1.89-A structure of the histidine-binding protein complexed with histidine and its relationship with many other active transport/chemosensory proteins. Biochemistry 33:4769–4779

Yernool D, Boudker O, Jin Y, Gouaux E (2004) Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*. Nature 431:811–818

Zakataeva NP, Aleshin VV, Tokmakova IL, Troshin PV, Livshits VA (1999) The novel transmembrane *Escherichia coli* proteins involved in the amino acid efflux. FEBS Lett 452:228–232

Zittrich S, Krämer R (1994) Quantitative discrimination of carrier-mediated excretion of isoleucine from uptake and diffusion in *Corynebacterium glutamicum*. J Bacteriol 176:6892–6899