REVIEW ARTICLE

Canonical and ECF-type ATP-binding cassette importers in prokaryotes: diversity in modular organization and cellular functions

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Abstract

Since their discovery in the 1960s as 'osmotic shock-sensitive' transporters, a plethora of so-called binding protein-dependent (canonical) ATP-binding cassette (ABC) importers has been identified in bacteria and archaea. Their cellular functions go far beyond the uptake of nutrients. Canonical ABC importers play important roles in the maintenance of cell integrity, responses to environmental stresses, cell-to-cell communication and cell differentiation and in pathogenicity. A new class of abundant micronutrient importers, the 'energy-coupling factor' (ECF) transporters, was originally identified by functional genomics. ABC ATPases are an integral part of both canonical ABC and ECF importers. Fundamental differences include the modular architecture and the independence of ECF systems of extracytoplasmic solute-binding proteins. This review describes the roles of both types of transporters in diverse physiological processes including pathogenesis, points to the differences in modular assembly and depicts their common traits.

Introduction and historical background

Transport across biological membranes is fundamental to any form of life. Referring to the source of energy used by transport proteins, one can distinguish channels, primary and secondary transporters (Saier, 2000). Whereas secondary transporters couple transport to electrochemical gradients across the membrane, for example a proton or a sodium potential, primary transporters such as proton-pumping ATPases or ATP-binding cassette (ABC)-transporters harness the free energy of ATP hydrolysis.

What is known today as binding protein-dependent ABC transporters were historically distinguished from other classes of transport systems by their susceptibility to cold osmotic shock (Neu & Heppel, 1965). The sensitivity of these transport systems against osmotic shock was soon found to be due to the release of an essential protein component located in the periplasm of *Escherichia coli* and *Salmonella typhimurium* (official designation, *Salmonella enterica* serovar Typhimurium), between the inner and the outer membrane – the periplasmic solute-binding protein (SBP) (Pardee, 1968; Berger, 1973; Berger & Heppel, 1974). Because *E. coli* and *S. typhimurium* were the preferred bacterial model organisms at that time, it was assumed that this type of transporter must be confined to gram-negative bacteria. This (first) dogma was overthrown in the late 1980s when similar transport systems were discovered in gram-positive bacteria encompassing a solute-binding lipoprotein

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that is anchored to the extracellular side of the cytoplasmic membrane by fatty acids covalently linked to the N-terminal cysteine residue of these proteins (reviewed in Sutcliffe & Russell, 1995).

With the first nucleotide sequences of the histidine and maltose transporters of *S. typhimurium* and *E. coli*, respectively, at hand in the early 1980s, it became obvious that despite their chemically different substrates, both systems contained a homologous component (Gilson *et al.*, 1982). Moreover, in 1986, a mammalian protein (P-glycoprotein or multidrug resistance protein) involved in resistance of certain cancer cells to chemotherapeutic agents was recognized to share significant similarity to the bacterial transport proteins (reviewed in Ames, 1986). These findings gave rise to the notion that a common transport principle might be present in all organisms from bacteria to humans. Sequence motifs were identified that are required for nucleotide binding and this led to the designation of 'ABC' transport proteins by Higgins and colleagues (Hyde *et al.*, 1990). Using this information as a template, subsequent genome sequence projects led to the discovery of a plethora of ABC transporters in organisms belonging to all three kingdoms of life that made them one of the largest protein families, including both ABC importers (the originally described 'osmotic shock-sensitive' systems) and export systems. The occurrence of a solute-binding component was taken as an earmark of ABC-type importers.

This (second) dogma was subsequently proven wrong by two pioneering studies. First, in 1993, a new class of secondary transporters – the TRAP family (tripartite ATP-independent periplasmic transporter) – was discovered, which includes a solute receptor for the capture and delivery of their substrates (Forward *et al.*, 1993). In the accompanying article, Mulligan and Thomas summarize the current knowledge on these transport proteins. Second, in 2006, bioinformatic analysis of whole-genome sequences from bacteria and archaea led to the discovery of the new and abundant subclass of energy-coupling factor (ECF)-type ABC import systems that lack an extracytoplasmic SBP (Rodionov *et al.*, 2006, 2009; Hebbeln *et al.*, 2007). The designation 'ECF transporters' was chosen to honor the work of Henderson *et al.* (1979), who published already in 1979 that the folate, thiamine and biotin transport systems of *Lactobacillus casei* each function via a specific 'binding protein' (later designated as 'S components', i.e. transmembrane proteins that are unrelated to SBPs of canonical ABC importers), and that they require, in addition, a common, but unknown component present in limiting amounts per cell. The latter was hypothesized to couple energy to these transport processes and named the 'ECF'. More than 25 years later, 'ECFs' were identified as protein modules consisting of two ABC ATPases and a moderately conserved transmembrane protein. These ECF modules form stable complexes even in the absence of an S unit (Rodionov *et al.*, 2006, 2009; Hebbeln *et al.*, 2007).

Numerous excellent review articles and two books focusing in great detail on the crystal structures and the mechanistical aspects of ABC transporters have been published in recent years (e.g. Schmitt & Tampe, 2002; van der Heide & Poolman, 2002; Holland *et al.*, 2003; Albers *et al.*, 2004; Biemans-Oldehinkel *et al.*, 2006a; Dawson *et al.*, 2007; Linton, 2007; Davidson *et al.*, 2008; Moussatova *et al.*, 2008; Locher, 2009; Ponte-Sucre, 2009; Rees *et al.*, 2009). In contrast, the genetics, molecular biology, biochemistry and physiology of ABC transporters mediating the uptake of diverse solutes in prokaryotes were last summarized in 2001 (Dassa & Schneider, 2001). Thus, it is the aim of this article to provide the reader with an up-to-date and comparative overview on canonical (binding protein-dependent) and ECF-type ABC importers in the context of their physiological significance. While in the case of canonical systems, we have focused on summarizing available experimental data, bioinformatic and experimental results are reviewed for ECF-type transporters since bioinformatics provided major clues to these systems.

ABC transporters

General description and classification

ABC systems are found in all three domains of life and form one of the largest protein superfamilies of paralogous sequences (Davidson *et al.*, 2008). The human genome, for example, includes 48 genes for ABC transporters, while in the genome of *E. coli* serotype K-12, between 52 and 80 genes were reported to encode discrete ABC transport systems (reviewed in Moussatova *et al.*, 2008). Plant genomes code for a high number of ABC systems, for example >120 have been identified in *Arabidopsis thaliana* and *Oryza sativa* (rice) (Rea, 2007). Yet, in relation to genome size, the highest number of ABC systems is found in bacteria (Davidson *et al.*, 2008).

Canonical ABC transporters share a common structural organization comprising two transmembrane domains (TMDs) that form the translocation pore and two nucleotide-binding domains (NBDs) that hydrolyze ATP. The ABC superfamily includes exporters and importers, the latter being, until recently, confined to prokaryotes (but see Acknowledgements), and nontransport ABC systems that do not possess TMDs. A new mode of classification for both eukaryotic and prokaryotic ABCs has been proposed by Davidson *et al.* (2008). On the basis of sequence comparison, the authors postulate three classes of ABC systems that were already present in the last common ancestor of archaea, bacteria and eukarya. Class I comprises transporters with fused TMDs and NBDs, class II includes nontransport ABCs Q2

lacking TMDs and class III includes mainly transporters with NBDs and TMDs formed by separate polypeptide chains. Interestingly, class III is absent in eukaryotes.

Substrates transported by ABC transporters are diverse, such as sugars, amino acids, peptides, vitamins, ions, xenobiotics and even polypeptides, linking these proteins to various cellular functions that range from energy supply to osmoregulation, detoxification and virulence.

Prokaryotic canonical ABC importers that are dependent on high-affinity substrate-binding proteins, mediate the uptake of nutrients, osmoprotectants, various growth factors or trace elements (Holland *et al.*, 2003). Canonical ABC importers, such as the enterobacterial maltose transporter $MalFGK_2$-E (Ehrmann *et al.*, 1998), the histidine transporter $HisQMP_2$-J (Ames *et al.*, 2001) or the glycine betaine (OpuA) transporter of *Lactococcus lactis* (Patzlaff *et al.*, 2003), are model systems for the study of ABC transporters' structure and function. Crystal structures of two archaeal and four bacterial ABC importers have been resolved.

ECF transporters comprise an abundant class of importers for micronutrients in bacteria and archaea. ECF transporters consist of pairs of ABC ATPase domains (A components), a conserved transmembrane protein (T component) and a transmembrane substrate-capture protein (S component) in an unknown stoichiometry, but lack an SBP (Rodionov *et al.*, 2009).

The transporter classification (TC) system currently includes a total of 33 subfamilies of ABC import systems within family 3.A.1.1 (http://www.tcdb.org). It is analogous to the Enzyme Commission system for classification of enzymes, except that it incorporates both functional and phylogenetic information. In an alternative classification system, Dassa & Bouige (2001) subdivided the ABC transporters into three uptake and six export classes. For a most recent description, see Dassa (2007). Here, the TC system will be used for the classification of canonical ABC importers (22 subfamilies) (Table 1), but will not be applied to the ECF transporters. Currently, the TC system does not contain the category of ECF transporters, but rather classifies certain members or components of ECF systems as ABC systems or as secondary active transporters.

Canonical ABC importers

Modular organization

The architecture of canonical ABC importers reflects their universal mechanism. All members consist of two TMDs that form the translocation pore and an NBD dimer at the cytoplasmic face of the membrane. The NBDs bind and hydrolyze ATP, thereby generating conformational changes that are coupled to the TMDs and ultimately lead to substrate translocation. These domains can be arranged as a single fused polypeptide chain, as half-transporters or – in prokaryotic ABC importers only – as separate proteins. Importers require an additional component, the SBP, for function (Fig. 1).

NBDs

The NBDs can be considered as the 'motor domains' of ABC transporters. The tertiary structure and catalytic cycle of these domains are relatively well understood (Schmitt & Tampe, 2002; Davidson & Chen, 2004; Dawson *et al.*, 2007; Davidson *et al.*, 2008). NBDs share a conserved architecture among im- and exporters. They consist of a RecA-like and an α-helical subdomain, interconnected by two flexible loop regions, one of which contains a conserved glutamine residue (Q-loop). The RecA-like subdomain is also found in other ATPases, whereas the helical subdomain is specific for the NBDs of ABC systems (Davidson *et al.*, 2008).

In the assembled transporter, NBDs are present as a dimer, with the nucleotide-binding sites of each monomer facing the other. Binding and hydrolysis of ATP are catalyzed by residues from several conserved motifs (see Davidson & Chen, 2004 as a general reference). Both of the so-called 'Walker' motifs map to the RecA-like subdomain. The 'Walker A' motif forms a loop structure that binds to the β- and γ-phosphate groups of ADP or ATP, while the 'Walker B' motif is arranged as a β-strand, whose terminal aspartate coordinates a Mg^{2+} ion through water needed for ATP hydrolysis. The 'Q-loop' is part of the loop region connecting RecA-like and helical subdomains. It is characterized by a conserved glutamine that binds to the Mg^{2+} cofactor. Located at the dimer interface as well as at the interface to the TMDs, this region is probably involved in interdomain signaling (Dawson *et al.*, 2007). Mobility of the Q-loop is essential to complete the catalytic cycle of the transporter (Daus *et al.*, 2007b). Moreover, NBDs contain the 'H-loop' or 'switch' with a conserved histidine residue that contacts the γ-phosphate of ATP and is required for hydrolysis, and finally the 'signature' or the 'LSGGQ' motif (C-loop) (Schneider & Hunke, 1998).

The dimeric arrangement of the NBDs corresponds well with the finding that ATPase activity is highly cooperative (Davidson *et al.*, 1996; Liu *et al.*, 1997; Moody *et al.*, 2002). According to several crystal structures of dimeric NBDs (Hopfner *et al.*, 2000; Smith *et al.*, 2002; Chen *et al.*, 2003) and a full transporter (Oldham *et al.*, 2007), the NBD monomers form a 'nucleotide sandwich' (Fig. 2). In this arrangement, ATP is bound along the dimer interface by Walker A residues of one monomer and residues from the LSGGQ motif of the other monomer. Upon binding of two ATP molecules, the NBD dimer closes in a 'tweezer-like' fashion (Chen *et al.*, 2003). Hydrolysis of ATP occurs in the tightly dimerized state, which then reopens to release phosphate. The ADP-bound conformer of the NBD dimer resides in an intermediary, semi-open state, which

Table 1. Subfamilies of canonical ABC importers

Carbohydrate uptake transporter-1 (CUT1)	3.A.1.1.1–3.A.1.1.33	Mono-, di- and oligosaccharides, glycerolphosphates, polyols	Maltose/maltodextrin transporter MalE-FGK$_2$ (*E. coli*/*Salmonella*)
Carbohydrate uptake transporter-2 (CUT2)	3.A.1.2.1–3.A.1.2.13	Monosaccharides (tetraoses, pentoses, hexoses, purine nucleosides), autoinducer AI-2	Ribose porter RbsABCD, arabinose porter AraFGH, galactose/glucose porter MglABC (all from *E. coli*)
Polar amino acid uptake transporter (PAAT)	3.A.1.3.1–3.A.1.3.20	Polar amino acids, octopine, nopaline, diaminopimelate, cystine, cystathionine, ectoine/hydroxyectoine	Histidine/lysine, arginine, ornithine porter HisJ/LAO-HisQMP$_2$ (*S. typhimurium*); Glutamine porter GlnHPQ, arginine porter ArtI/ArtJ-ArtMQP (both from *E. coli*); Arginine, lysine, histidine, ornithine porter ArtJ-MP (*Geobacillus stearothermophilus*)
Hydrophobic amino acid uptake transporter (HAAT)	3.A.1.4.1–3.A.1.4.6	Hydrophobic amino acids, general amino acids, urea/thiourea/hydroxyl-urea	Leucine, isoleucine, valine porter (*E. coli*)
Peptide/opine/nickel uptake transporter (PepT)	3.A.1.5.1–3.A.1.5.24	Oligopeptides, mono-, di- and oligosaccharides, β-glucosides, glutathione, proline/betaine, microcins, EDTA	Oligopeptide porters OppABCDF (*S. typhimurium*, *Lactobacillus lactis*); Ni porter NikABCDE (*E. coli*)
Sulfate/tungstate uptake transporter (SulT)	3.A.1.6.1–3.A.1.6.6	Sulfate, thiosulfate, tungstate, vanadate, molybdate	Sulfate/thiosulfate porter CysP-CysTWA (*E. coli*)
Phosphate uptake transporter (PhoT)	3.A.1.7.1–3.A.1.7.2	Phosphate	PhoS-PstACB (*E. coli*)
Molybdate uptake transporter (MolT)	3.A.1.8.1–3.A.1.8.2	Molybdate, tungstate	Molybdate porter ModABC (*E. coli*, *Methanosarcina acetivorans*)
Phosphonate uptake transporter (PhnT)	3.A.1.9.1–3.A.1.9.2	Phosphonate, organophosphate ester, phosphate	Phosphonate/phosphate porter PhnCDE (*Mycobacterium smegmatis*)
Ferric iron uptake transporter (FeT)	3.A.1.10.1–3.A.1.10.3	Fe^{3+} (Ga^{3+}, Al^{3+})	Ferric iron porter SfuABC (*Serratia marcescens*)
Polyamine/opine/phosphonate uptake porter (POPT)	3.A.1.11.1–3.A.1.11.6	Polyamines, mannopine, chrysopine, aminoethyl-phosphonate, γ-aminobutyrate	Polyamine porter PotABCD (*E. coli*)
Quarternary amine uptake transporter (QAT)	3.A.1.12.1–3.A.1.12.11	Glycine betaine, proline, choline, carnitine, ectoine	Glycine betaine/proline porter ProVWX (*E. coli*), OpuAA-OpuA porter (*L. lactis*)
Vitamin B_{12} uptake transporter (B12T)	3.A.1.13.1	Vitamin B_{12}	BtuCDF (*E. coli*)
Iron chelate uptake transporter (FeCT)	3.A.1.14.1–3.A.1.14.20	Fe^{3+}, Fe^{3+}-citrate, Fe^{3+}-enterobactin, Fe^{3+}-hydroxamate, iron-chrysobactin, heme, iron-vibriobactin, iron-vibrioferrin, corrinoid, desferrioxamine, coelichelin, bacillibactin	Fe^{3+}, Fe^{3+}-citrate porter FecBCDE (*E. coli*); Fe^{3+}-enterobactin porter FepBCDG (*E. coli*); Corrinoid porter BtuCDE (*Halobacterium* sp.) heme porter Shp/HtsABC (*Streptococcus pyogenes*)
Manganese/zinc/iron chelate uptake transporter (MZT)	3.A.1.15.1–3.A.1.15.11	Mn^{2+}, Zn^{2+}, Fe^{2+}	Mn^{2+}/Zn^{2+} porter MntABC (*Neisseria meningitides*)
Nitrate/nitrite/cyanate uptake transporter	3.A.1.16.1–3.A.1.16.3	Nitrate, nitrite, cyanate, bicarbonate	Nitrate/nitrite porter NrtABCD (*Synechococcus* sp.)
Taurine uptake transporter (TauT)	3.A.1.17.1–3.A.1.17.5	Taurine (2-aminoethane sulfonate), aromatic sulfonate, (hydroxyl-methylpyrimidine), phthalate	Taurine porter TauABC (*E. coli*), phthalate porter OphFGH (*Burkholderia capacia*)
Thiamin uptake transporter (ThiT)	3.A.1.19.1	Thiamin, thiamin monophosphate, thiamine pyrophosphate	ThiBPQ (*S. typhimurium*)

Table 1. Continued.

Brachyspira iron uptake transporter (BIT)	3.A.1.20.1	Iron	BitABCDEF (*B. hyodysenteriae*)
Siderophore-Fe^{3+} uptake transporter (SIUT)	3.A.1.21.1–3.A.1.21.2	Fe^{3+}-yersiniabactin, Fe^{3+}-carboxymycobactin	Fe^{3+}-yersiniabactin porter YbtP (*Yersinia pestis*)
Methionine uptake transporter (MUT)	3.A.1.24.1–3.A.1.24.4	L- and D-methionine, formyl-L-methionine	Methionine porter MetNIQ (*E. coli*)
γ-Hexachlorocyclohexane (HCH) family	3.A.1.27.1–3.A.1.27.2	γ-Hexachlorocyclohexane	LinKLMN porter (*Sphingobium japonicum*)

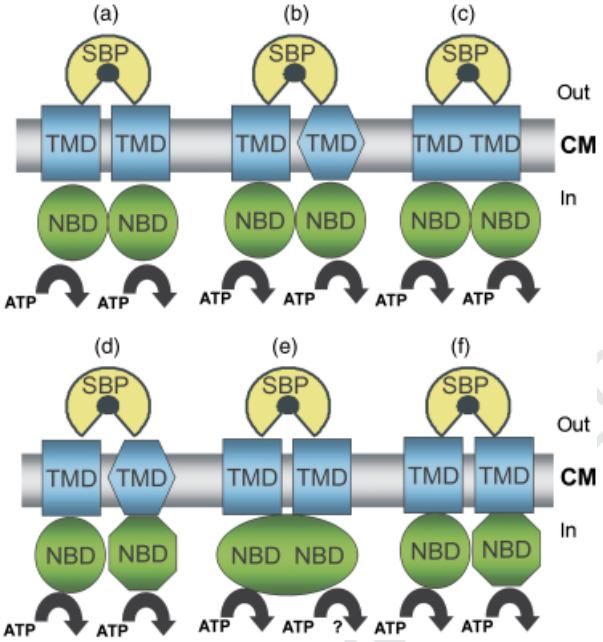

Fig. 1. Diversity in the domain architecture of canonical ABC importers. Examples: (a) vitamin B_{12} transporter, $Btu(CD)_2$; (b) maltose/maltodextrin transporter, $MalFGK_2$; (c) Fe^{3+}-hydroxamate transporter, $FhuBC_2$; (d) oligopeptide transporter, OppBCDF; (e) arabinose transporter, $AraH_2G$ (ATPase activity of the C-terminal NBD is unclear as denoted by a question mark); (f) nitrate/nitrite transporter, $NrtM_2CD$ (the second NBD-NrtC- is a C-terminal fusion with a solute-binding domain). Examples (a)–(e) are from *Escherichia coli*, (f) is from *Synechococcus*. SBP, (extracytoplasmic) solute-binding protein; TMD, transmembrane domain; NBD, nucleotide-binding domain; CM, cytoplasmic membrane. See text for details.

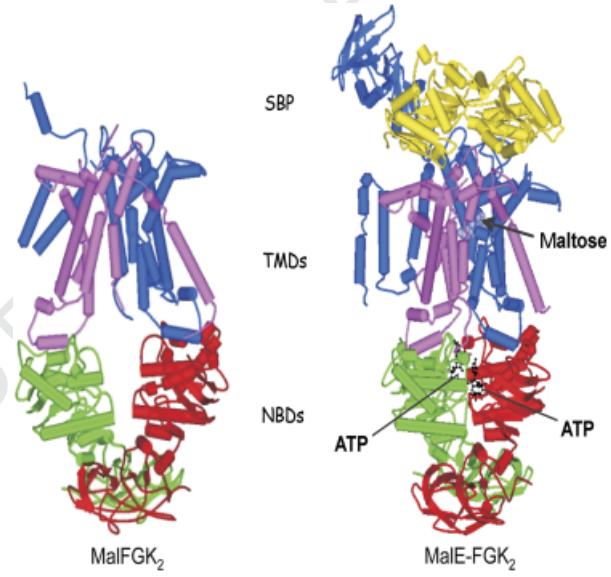

Fig. 2. Structures of the maltose/maltodextrin transporter of *Escherichia coli*. The transporter consists of MalE (SBP, yellow), MalF (TMD, blue), MalG (TMD, magenta) and two copies of the NBD MalK (shown in green and red for clarity). $MalFGK_2$ represents the inward-facing (apo-) state and $MalE\text{-}FGK_2$ represents the outward-facing (intermediate) state of the transporter. In the latter, MalE, in its open conformation, is tightly associated with MalFG, maltose resides within the pore by interaction with residues from MalF only and two ATP molecules are sandwiched between both MalK monomers. The large periplasmic P2 loop of MalF, which is not resolved in the apo-state, is in close contact to MalE. See text for details. The figure was drawn with DISCOVERY STUDIO VISUALIZER 2.5 (Accelrys, Cambridge, UK) using the coordinates from entry 3FH6 and 2R6G, respectively, in the Brookhaven Protein Data Bank.

presumably returns to the open apo-state after the dissociation of ADP (Lu *et al.*, 2005). The observed positive cooperativity of ATP hydrolysis makes it highly likely that two molecules of ATP are needed for NBD dimer closure (Chen *et al.*, 2003; Davidson *et al.*, 2008).

TMDs

The TMDs mainly consist of four to 10 membrane-spanning α-helices. As compared with the NBDs, the sequence of the TMDs is less conserved. A substrate-binding site has thus far been clearly identified only in the MalF subunit of the *E. coli* maltose importer $MalFGK_2$ (Oldham *et al.*, 2007) (Fig. 2). One maltose molecule is interacting with 10 surrounding residues through H-bonds, van der Waals interactions and aromatic ring stacking. Six of these residues were already known to reduce transporter activity when mutated *in vivo* (Oldham *et al.*, 2007). Intra- and extracellular loops connecting the transmembrane segments are of varying lengths and possess functional significance for contacting the NBDs

or extracellular interaction partners. The 'coupling helices' are architecturally conserved elements forming the NBD–TMD interface. In the crystal structures, they are located in grooves on the NBDs' surfaces (Locher, 2009).

Nucleotide-induced conformational changes are thought to be transmitted via noncovalent interactions from the Q-loop region of the NBDs to these short helices. In prokaryotic importers, the coupling helices are characterized by a conserved sequence of amino acids [consensus EAA-X3-G-X9-I-X-LP (Saurin *et al.*, 1994)]. These 'EAA' or 'L' loops map to the last cytoplasmic loop of the respective TMDs (Mourez *et al.*, 1997). Certain loops on the extracellular side are possibly involved in the recognition or the stabilization of interacting proteins or subsequent signal transduction. Large loops are found, for example in MalFG of the enterobacterial maltose transporter (comprising 180 amino acids) (Oldham *et al.*, 2007).

SBPs

Extracellular SBPs (also called receptors) are essential components of canonical ABC importers. In gram-negative bacteria, the binding proteins diffuse freely in the periplasm between the inner and the outer membrane. Gram-positive bacteria and archaea anchor the binding proteins to the outer surface of the cell membrane via an N-terminal lipid moiety (Sutcliffe & Russell, 1995) or, as observed for some archaea, an N-terminal hydrophobic helix. Moreover, solute-binding domains can also be fused to the TMDs, resulting in two or four substrate-binding sites (van der Heide & Poolman, 2002; Biemans-Oldehinkel *et al.*, 2006a) (Fig. 3).

Substrates bound by SBPs with a high specificity are highly diverse, ranging from inorganic or organic ions and sugars to peptides or vitamins (Wilkinson & Verschueren, 2003). Because of their high substrate affinities (K_d values typically range from 0.01 to 1 μM), capture and accumulation of substrate in proximity to the transporter can be regarded as the main *raison d'être* of SBPs. However, as these proteins are also indispensible at very high substrate concentrations, they probably also play an important functional role in the catalytic cycle of the transporter (Shilton, 2008) (discussed below).

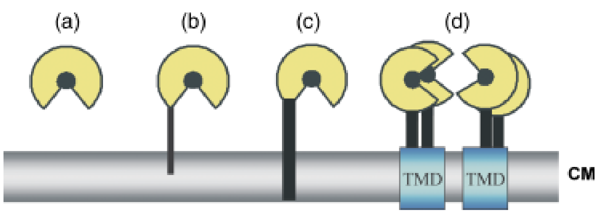

Fig. 3. Structural organization of SBPs. In gram-negative bacteria, SBPs reside in the periplasm (a) while in gram-positive bacteria and archaea, lacking an outer membrane, they are attached to the extracellular side of the cytoplasmic membrane via an N-terminal lipid anchor (solute-binding lipoproteins are also found in cyanobacteria) (b). In some archaea, the SBP is associated with the membrane by an N-terminal peptide (c). In some bacteria and archaea, including *Lactococcus lactis*, *Helicobacter pylori* and *Sulfolobus solfataricus*, transporters exist that comprise two or four SBPs fused to the TMDs (d). NBDs and TMDS [except in (d)] are omitted for clarity. CM, cytoplasmic membrane.

Generally, SBPs consist of two symmetrical lobes or domains (termed N- and C-lobe according to the protein's termini), both of which display an α-/β-fold, connected by a hinge region (Quiocho & Ledvina, 1996; Davidson *et al.*, 2008). The amino acid sequences of SBPs were originally grouped into eight clusters or subfamilies based on their similarities (Tam & Saier, 1993). Alternatively, SBPs have been classified by the topology of their globular domains (Fukami-Kobayashi *et al.*, 1999; Wilkinson & Verschueren, 2003; Dwyer & Hellinga, 2004) or by the number of interdomain connections (Quiocho & Ledvina, 1996). In all three families or groups identified so far, the substrate-binding site is located in a cleft between the two lobes. Substrate specificity is achieved mostly by differential H-bonding. In type I and II SBPs, the lobes differ in the number and order of β-strands and are connected by a flexible hinge region. They are represented, for example by the *E. coli* galactose/glucose (MglB) – and maltose-binding proteins (MalE), respectively. Substrate binding causes a rotation of these lobes toward each other, rendering the central cleft inaccessible from the aqueous surrounding ('Venus flytrap' model, Quiocho & Ledvina, 1996).

In type III SBPs (constituting the new cluster 9 based on sequence similarities), as represented, for example by the *E. coli* vitamin B_{12}-binding protein, BtuF, the two ligand-binding domains are connected by a rigid α-helix, suggesting a rather small conformational change upon ligand binding (Lawrence *et al.*, 1998; Lee *et al.*, 1999; Karpowich *et al.*, 2003). However, a molecular dynamics simulation indicated that BtuF appears to be more flexible than implied by the X-ray structures, displaying clear opening and closing motions that are compatible with the Venus fly trap model (Kandt *et al.*, 2006).

Interaction patterns of SBPs with the transmembrane domains of the transporter could be system specific, as indicated by the different affinities of SBPs for their cognate membrane components (Chen *et al.*, 2001; Hollenstein *et al.*, 2007; Oldham *et al.*, 2007). Positive cooperativity between two substrate-binding domains fused to the TMDs exists in the glycine betaine transporter OpuA from *L. lactis* (Biemans-Oldehinkel *et al.*, 2006b).

Binding protein-independent (BPI) mutants have been described for the maltose and the histidine transporters of *E. coli* and *S. typhimurium*, respectively. In case of the maltose transporter, the mutations map to transmembrane

regions of the TMDs, MalF and MalG. The phenotype of these mutants is characterized by a spontaneous ATPase activity and a decline in affinity for maltose by three orders of magnitude. Moreover, maltodextrins are no longer recognized as substrates (Covitz *et al.*, 1994). Maltose-binding protein actually inhibits the activity of the mutants, likely by preventing the access of free maltose to the substrate-binding site within the pore (Dean *et al.*, 1992). BPI mutants are thought to reside in a transition state-like conformation (Mannering *et al.*, 2001; Daus *et al.*, 2006, 2007b, 2009). As inferred from the known crystal structures of the transporter, all mutations lie at sites of interaction that are altered in the inward-to-outward transition (Khare *et al.*, 2009).

In contrast, mutations causing a BPI phenotype of the histidine transporter all map to a region in HisP, the NBD of the system, immediately preceeding the Walker B motif and in between the D and the H loops (Speiser & Ames, 1991). These mutants exhibit high spontaneous ATPase activity, but rather low transport rates *in vitro* that can be stimulated substantially by the binding protein, HisJ (Liu *et al.*, 1999). The latter finding is consistent with the notion that the phenotype is caused by an altered nucleotide-binding site (Speiser & Ames, 1991).

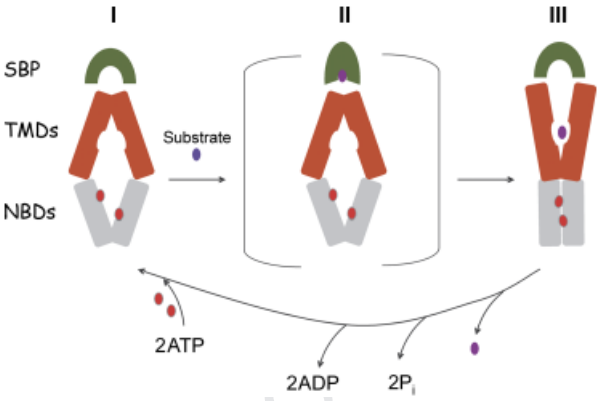

Fig. 4. Simplified model of the translocation mechanism of an SBP-dependent ABC transporter. In the resting state (I), SBP and NBDs are in their open conformations while the TMDs expose the substrate-binding site within the pore to the cytoplasm (inward-facing). Under physiological conditions, ATP is likely to bind to the NBDs. When a substrate becomes available, the SBP is shifted to the closed state (II), thereby triggering a conformational change that results in closing of the NBD dimer, opening of the TMDs to the extracellular side (outward-facing) and release of the substrate into the pore (III). ATP hydrolysis enables the TMDs to revert to the inward-facing conformation, thereby completing translocation of the substrate to the cytoplasm. Subsequent dissociation of phosphate and ADP and reloading of the NBDs with ATP restore the resting state. See text for further details.

Colour

The 'alternating access' model of transport

X-ray structures of isolated NBDs and complete transporters as well as biophysical evidence support an alternating access mechanism for substrate transfer (Borbat *et al.*, 2007; Hollenstein *et al.*, 2007; Oldham *et al.*, 2007; Khare *et al.*, 2009): conformational changes generated in the NBDs by ATP binding and hydrolysis cause an alternation between an outward-facing (open to the exterior) and an inward-facing (open to the cytoplasm) structure of the pore (Figs 2 and 4). In the following, we will present a short transport scenario that is largely based on structural, biochemical and biophysical data accumulated for the maltose transporter of *E. coli*/*Salmonella*, by far the best-studied ABC importer to date, but also includes evidence from other systems (Boos & Shuman, 1998; Davidson & Chen, 2004; Daus *et al.*, 2006, 2007a, b; Oldham *et al.*, 2007; Grote *et al.*, 2008, 2009; Orelle *et al.*, 2008; Khare *et al.*, 2009). The model might not be applicable in all aspects to larger ABC importers, such as the vitamin B_{12} transporter BtuCD-F from *E. coli* (Lewinson *et al.*, 2010). For a more detailed discussion of current transport models, the reader is referred to (Davidson *et al.*, 2008; Locher, 2009; Rees *et al.*, 2009).

The maltose transporter consists of the membrane-integral subunits, MalF and MalG, a homodimer of the ATPase, MalK and the periplasmic maltose-binding protein, MalE. In the absence of substrate, the resting state is thought to be represented by the ATP-bound form of the transporter (Daus *et al.*, 2007a; Oldham *et al.*, 2008) (Fig. 4, state I). Although it is usually considered that MalE docks onto the $MalFGK_2$ complex in the substrate-loaded, closed conformation, several lines of experimental evidence suggest a permanent association of a copy of MalE with the transport complex (Daus *et al.*, 2007b, 2009; Grote *et al.*, 2009) (see Daus *et al.*, 2007a, for a detailed discussion) (Fig. 4, state II).

As a result of substrate binding, MalE is shifted to its closed conformation, which in turn promotes a tight association of the MalK monomers, thereby resulting in opening of the transport channel to the periplasmic side. Concomitantly, MalE is pushed toward its open conformation (Orelle *et al.*, 2008), the substrate becomes released and enters the pore. At this stage, MalE forms a stable complex with the transporter (Oldham *et al.*, 2007) (Fig. 4, state III). The unusually large periplasmic P2 loop of the MalF subunit plays a pivotal role in communicating substrate availability to the NBDs (Grote *et al.*, 2009). The chemical nature of the substrate appears not to be important for initiating the transport cycle as demonstrated using a MalE mutant that binds either maltose or sucrose (Gould & Shilton, 2010). The scenario is complicated by the observation that unliganded binding proteins can also have a stimulatory effect on the ATPase activity of the transporter. In case of the maltose transporter, this effect was contributed to open, unliganded MalE, which might interact with a yet to be identified small population of transporter molecules in the required conformation (Gould *et al.*, 2009).

The following steps are still highly speculative. Hydrolysis of one ATP, as can be experimentally achieved by preventing dissociation of ADP with vanadate, results in no or little opening of the MalK dimer (Daus *et al.*, 2007b; Grote *et al.*, 2008), and thus might push the substrate only further into the pore. However, release of the substrate to the cytoplasm at this stage cannot yet be fully excluded (Chen *et al.*, 2001). Hydrolysis of the second ATP would then switch the transporter back to the inward-facing conformation, thereby lowering the affinity of the binding protein for the transporter and allowing release of the substrate in the case of the first scenario. Dissociation of phosphate and ADP and subsequent rebinding of ATP would restore the resting state.

The model implies that two molecules of ATP are consumed per substrate molecule transported, which is consistent with a study on the glycine-betaine importer, OpuA, of *L. lactis* (Patzlaff *et al.*, 2003). However, the numbers of ATP molecules hydrolyzed and substrate molecules translocated per catalytic cycle as well as the mechanistic role of each hydrolysis step are still a matter of debate. For a detailed discussion, see for example Davidson *et al.* (2008).

Table 2. Known and predicted substrate specificities of S components of ECF transporters Q34

Vitamins, cofactors and precursors	
BioY	Biotin
RibU	Riboflavin, FMN
FolT	Folate, 5-formyltetrahydrofolate
ThiT	Thiamine
PanT	Pantothenate
YkoE	Hydroxymethylpyrimidine (HMP)
ThiW	Thiazole?
PdxU	Pyridoxine?
NiaX	Niacin?
LipT	Lipoate?
CbrT	Cobalamin?
CblT	Dimethylbenzimidazole (DMB)?
MtsT	*S*-adenosylmethionine (SAM)?
MtaT	Methylthioadenosine?
Transition metal ions	
NikM	Nickel (Ni^{2+})
CbiM	Cobalt (Co^{2+})
Amino acids	
TrpP	Tryptophan
Queuosine and its precursors	
QrtT	Queuosine?
QueT	7-Aminomethyl-7-deazaguanine ($preQ_1$)

ECF transporter

Classification and modular organization

ECF transporters were shown to be an abundant class of importers for micronutrients in bacteria and archaea. The evolving list of substrates contains a set of water-soluble vitamins, cofactors and their metabolic precursors, the transition-metal ions Ni^{2+} and Co^{2+}, the amino acid tryptophan and queuosine and its metabolic precursors (Table 2). Many bacteria with restricted cofactor biosynthetic capacities rely on vitamin uptake by ECF import systems that are encoded by essential genes in several gram-positive pathogens including *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Mycoplasma genitalium* (Rodionov *et al.*, 2009).

Like the aforementioned canonical ABC importers, ECF systems contain ABC ATPase subunits and are thus considered to couple ATP hydrolysis to substrate uptake. Fundamental differences between classical ABC importers and ECF transporters lie in the modular architecture and in the absence of extracytoplasmic substrate-binding proteins among the ECF systems.

Q4 ECF transporters consist of pairs of ABC ATPase domains (A components), a conserved transmembrane protein (T component) and a transmembrane substrate-capture protein (S component, ≈ 21 kDa in many cases) in an unknown stoichiometry. As depicted in Fig. 5, the systems contain two different A components (A1, A2), two copies of the same A component (A1, A1) or two ABC ATPase domains fused to yield a single polypeptide (A1, A2, indicated by encircling in the very left panel in Fig. 5). Based on the utilization of a dedicated (subclass I) or a shared (subclass II) AAT module, ECF systems fall into two groups. Subclass I ECF systems are encoded in operons containing one or two genes for A units, a T-unit and an S-unit gene. About half of the ECF transporters – subclass II, found in most *Firmicutes*, *Thermotogales* and some archaeal species – share the same EcfA1A2T module, i.e. various and highly diverse S components in a cell compete for the same module in order to form the holotransporters. These EcfA1A2T modules are encoded by operons, whereas the S-unit genes are scattered around the genome. In *Firmicutes*, *ecfA1A2T* genes are part of larger operons encoding essential cellular functions (ribosomal proteins, RNA polymerase subunit, pseudouridine synthase, etc.), suggestive of their robust and constitutive expression.

Recent bioinformatic analyses identified > 20 highly diverse families of S components, each with a distinct predicted or an experimentally established substrate specificity (Rodionov *et al.*, 2009). Most of these S-unit genes are individually expressed under control of corresponding metabolite-responsive riboswitches or transcription factors (Rodionov *et al.*, 2009). The presence of *ecfA1A2T* operons in *Methanosarcina* sp. that do not contain any of the previously identified S components points to the existence of S components of ECF transporters with as yet unrecognized specificities.

Fig. 5. Modular architecture of ECF transporters. ECF transporters consist of (1) two different ABC ATPases (A1, A2) that are fused to a single protein in certain subclass I importers or copies of the same ABC protein (A1, A1), (2) a conserved transmembrane protein (T) and (3) a substrate-specific integral membrane protein (S). Cobalt- and nickel-transporting ECF systems contain bipartite (or rarely tripartite, not shown) S units (green contours). Shared use of the same A1A2T module by many highly diverse S proteins is a hallmark of subclass II systems. Subclass I ECF transporters have dedicated A and T units.

Properties

Analyses of bacterial Co^{2+} and biotin transporters provided an initial indication of the role of individual components of ECF transporters. Heterologous production of the CbiMNQO system from *S. enterica* serovar Typhimurium and of the α-purple bacterium *Rhodobacter capsulatus* in *E. coli* resulted in recombinants with Co^{2+}-uptake activity (Rodionov *et al.*, 2006). Whereas the role of an NBD that needs to dimerize for function was immediately obvious from the amino-acid sequence of CbiO (S unit), clues to the roles of the three transmembrane proteins CbiM, CbiN and CbiQ were not provided by sequence analysis. Mature CbiM proteins have seven predicted TMDs and an invariant His residue at position 2. CbiNs are unique small integral membrane proteins with two TMDs and a large extracellular loop in between. CbiQ proteins belong to the family of T proteins. Sequential deletion of genes from the 3′-end of *cbiMNQO* operons and assays of Co^{2+}-transport activity in recombinant *E. coli* yielded the surprising result that significant basal activity was detectable for CbiMNQ and CbiMN. This finding suggests a secondary active mode of transport in the absence of the NBD (CbiO) and identified CbiMN as the minimal module required for basal uptake activity (Rodionov *et al.*, 2006; P. Hebbeln & T. Eitinger, unpublished data). The Co^{2+} (and Ni^{2+}) transporters are an exception among the ECF systems inasmuch as their S components (e.g. CbiMN) are heterooligomeric.

More detailed analyses were reported for the *R. capsulatus* biotin transporter BioMNY. The sequence similarities between CbiO and BioM (A components) and between CbiQ and BioN (T components) in conjunction with the strong diverseness between CbiMN and BioY (S components) suggested that BioMN and BioY represent the energy-coupling module and the S unit, respectively (Hebbeln *et al.*, 2007). Biochemical analyses of the BioMNY system confirmed its modular organization and the function of BioY as the S unit. Notably, this protein can function as a high-capacity transporter in its solitary state. High-affinity transport reflecting the requirements in the natural environment, however, depends on the presence of a functional BioM ATPase. Copurification studies revealed that BioM and BioN form stable bipartite complexes and tripartite complexes together with BioY. Because the expression of *bioMY* and *bioMNY* resulted in comparable high-affinity biotin-uptake rates of recombinant *E. coli*, compared with low-affinity uptake caused by the solitary *bioY*, the existence of BioMY complexes in the absence of BioN (the T component) is conceivable. Such complexes, however, could not be purified, suggesting that BioN is required as a stabilizing factor. ATPase assays with purified components in a detergent solution as well as experiments with proteoliposomes found that neither the solitary BioM nor the bipartite BioMN complex, but only the tripartite BioMNY complex is capable of hydrolyzing ATP (Hebbeln *et al.*, 2007; A. Alfandega & T. Eitinger, unpublished data).

Shared use of the same EcfA1A2T module by many diverse S units is an amazing feature of subclass II ECF transporters and was experimentally demonstrated for lactobacterial folate, pantothenate, riboflavin and thiamine importers (Neubauer *et al.*, 2009; Rodionov *et al.*, 2009). The molecular patterns that allow efficient recognition between the energy-coupling module and various highly diverse S components remain elusive. The T units among the various ECF transporters differ in the number of TMDs and membrane topology (Fig. 6). A function of T units beyond that of a molecular adhesive that holds together the S and A components was recently shown for the BioMNY transporter, and likewise, for the promiscuous vitamin (folate, pantothenate and riboflavin) transporters of the lactobacterium *Leuconostoc mesenteroides* (Neubauer *et al.*, 2009). Two short motifs with an arginine residue in the center,

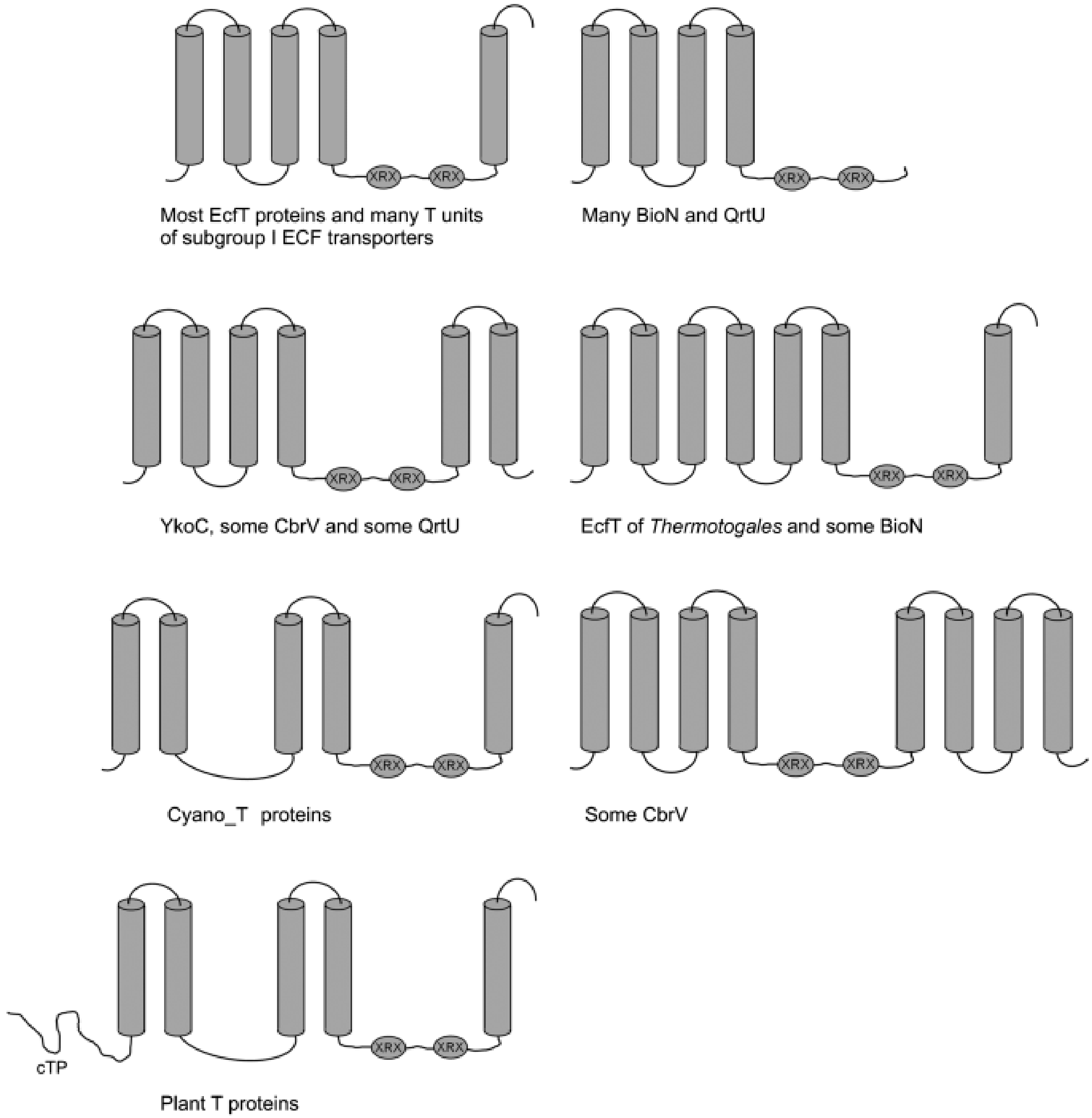

Fig. 6. Topology of T components. XRX, conserved three-amino-acid motifs, mainly found as ARG, SRG, LRG, VRG, MRG or ARS. cTP, predicted plastid-specific transit peptide in a couple of plant T components (see Perspectives for a description of 'Cyano_T' and T components in plants). Classification of T units: EcfT, promiscuous systems; BioN, biotin transporter; CbrV, transporter for cobalamin precursor; QrtU, transporter for queuosine precursor; YkoC, transporter for thiamine precursor.

frequently occurring as Ala-Arg-Gly and located on the cytoplasmic face in the C-terminal region, are the most conserved feature of T units (Fig. 6). Individual replacements of the Arg residue in motif II introduced into BioN and EcfT led to diminished activity of the biotin transporter and inactivated the *L. mesenteroides* vitamin transporters, respectively. The double mutations strongly destabilized the transporter complexes. Single replacements of the Arg residue in motif I affected the activity of both the biotin and the subclass II vitamin transporters, but did not destabilize the complexes. The latter finding suggests that T units are essential for intramolecular signaling and effective coupling of ATP hydrolysis to substrate translocation.

Substrate recognition

The substrate specificity of the S components CbiMN (Co^{2+}), NikMN (Ni^{2+}) (Rodionov *et al.*, 2006), BioY (biotin) (Hebbeln *et al.*, 2007), FolT (folates) (Eudes *et al.*, 2008; Rodionov *et al.*, 2009), PanT (pantothenate) (Neubauer *et al.*, 2009), RibU (riboflavin) (Burgess *et al.*, 2006;

Duurkens *et al.*, 2007; Vogl *et al.*, 2007) and ThiT (thiamine) (Eudes *et al.*, 2008; Rodionov *et al.*, 2009; Schauer *et al.*, 2009) has been analyzed experimentally. The metal specificity of CbiMNQO and NikMNQO systems was addressed by uptake assays with recombinant *E. coli* cells expressing the *cbiMNQO* operons of *R. capsulatus* or *S. enterica* serovar Typhimurium, and the *nikMNQO* operons of *R. capsulatus* or *Methanothermobacter thermoautotrophicus* (Rodionov *et al.*, 2006; Hebbeln, 2009). These studies showed a strong preference for Co^{2+} over Ni^{2+} for the Cbi systems and the opposite for the Nik systems. PanT of *L. mesenteroides* was shown to transport pantothenate, provided that the EcfA1A2T components were coproduced (Neubauer *et al.*, 2009). BioMNY mediates high-affinity biotin transport into recombinant *E. coli* cells; the biotin concentration resulting in half-maximal transport velocity was estimated to 5 nM (Hebbeln *et al.*, 2007). High-affinity, but substoichiometric binding of biotin was shown for the purified BioMNY complex and the solitary BioY in detergent solution by equilibrium dialysis (A. Alfandega & T. Eitinger, unpublished data). Analysis of riboflavin binding to the *L. lactis* S unit RibU, purified from cells grown in the absence of riboflavin, by isothermal titration calorimetry, flow dialysis and fluorescence titration, revealed a 1 : 1 stoichiometry (riboflavin : RibU) and a dissociation constant (K_d) of 0.6 nM. FMN and the toxic flavin analog roseoflavin also bind to RibU with a high affinity, but FAD does not (Duurkens *et al.*, 2007). Growth experiments and riboflavin-uptake experiments with *Bacillus subtilis* suggested that the RibU-containing transporter accepts riboflavin and its analogs in the order riboflavin ≈ roseoflavin > FMN ≫ FAD (Vogl *et al.*, 2007). Dissociation constants in the nanomolar range were also determined via quenching of intrinsic tryptophan fluorescence upon addition of vitamins to the purified *L. casei* FolT and ThiT. The reported K_d values are 9 nM folate and 5 nM 5-formyltetrahydrofolate for FolT, and 0.5 nM thiamine for ThiT. Binding stoichiometries are as low as about 1 : 10 and this finding was explained by copurification of vitamins that are tightly bound to the S units and occupy binding sites in the 'as-isolated' state (Eudes *et al.*, 2008). Thus, the available data point to extremely high affinity as a characteristic trait of ECF transporters that renders them well suited for their role in micronutrient uptake.

Genomic and phylogenetic analysis

The comparative analysis of ~400 microbial genomes using the SEED platform (Overbeek *et al.*, 2005) provided the basis for the identification of a variety of families of ECF transporters and the genomics-based prediction of their specificities (Rodionov *et al.*, 2009). Similarity searches with the *cbiQO* and *bioMN* genes identified multiple homologs of A- and T-component genes unevenly distributed in prokaryotic genomes. About one half of these genes were found in potential operons with genes encoding unrelated hypothetical transmembrane proteins (S components). The S components were classified into ~20 families. A combination of genome context analysis (gene clustering, coregulation, co-occurrence) and metabolic reconstruction was successfully utilized for the prediction of substrate specificities for the majority of S-component families; many of these were later validated in experiments (Table 2 and ECF-type transporters).

The distribution of ECF-transporter components across prokaryotic genomes was captured in the 'ECF class transporters' subsystem in the SEED comparative genomics resource (http://theseed.uchicago.edu/FIG/index.cgi). This subsystem provides an easy access to information about the sequences, genomic locations, functional annotations and distribution of ECF component proteins in a periodically updated genome collection. Table 3 presents an overview of the distribution of transport systems per ECF family in major taxonomic classes of prokaryotes. The tabulated subclass I and subclass II ECF systems include ~700 S components and ~400 AT (or AAT) modules in 35 archaeal and 168 bacterial genomes. Of these, the majority of these S components were detected in *Firmicutes* (68%), *Actinobacteria* (8%), *Proteobacteria* (8%), *Archaea* (8%), and *Thermotogae* (3%). Overall, nearly one half of the analyzed prokaryotic species have at least one ECF transporter.

The relative abundance of various S components of subclass I and subclass II ECF transporters in the analyzed prokaryotic genomes is presented in Fig. 7. BioY is the most abundant S unit and belongs to either subclass I (48 cases) or subclass II (65 cases) ECF systems. Another 54 *bioY* genes occur in organisms that do not contain any AAT module genes. Five S component families (NikM, CbiM, YkoE, MtsT and MtaT) always belong to subclass I ECF systems. Three families (CbrT, QrtT and HtsT) are mostly represented by subclass I systems with exceptions when some of their homologs (found in *Firmicutes*) appear to belong to subclass II. The remaining 11 S component families mainly belong to the subclass II ECF systems and were predominantly found in *Firmicutes* and *Thermotogae*. Among seven such families, a small proportion of S-component genes (26 cases, found mainly in *Archaea* and *Actinobacteria*) are accompanied by AAT module genes and were classified into subclass II systems.

Major families of S components as well as all A and T components were analyzed by generating phylogenetic trees [illustrated with DENDROSCOPE (Huson *et al.*, 2007) in the supplemental figures] using protein sequences collected from the 'ECF class transporters' subsystem in the SEED database. Preliminary similarity searches using the position-specific iterated (PSI-) BLAST program did not reveal any significant cross-identification between different S-component

Table 3. Occurrence of subclass I and subclass II ECF transporters in prokaryotic genomes

		Archaea			Bacteria															
		Crenarchaeota	*Euryarchaeota*	*Korarchaeota*	*Thermotogae*	*Firmicutes/ Bacillales*	*Firmicutes/ Clostridia*	*Firmicutes/ Lactobacillales*	*Firmicutes/ Mollicutes*	*Actinobacteria*	*Proteobacteria*	*Chloroflexi*	*Deinococcus-Thermus*	*Fusobacteria*	*Cyanobacteria*	*Spirochaetes*	*Chlamydiae*	*Bacteroidetes Chlorobi*	*Aquificae-Planctomycetes*	**Total**
	# genomes	12	29	1	7	15	17	25	17	24	180	4	3	1	19	5	5	16	3	**384**
Subclass I	*BioMNY	1	12	–	–	–	1	–	–	12	17	–	2	1	–	2	–	–	–	48
	NikMNQO	2	19	1	–	–	9	2	–	4	16	–	–	–	6	1	–	1	–	61
	CbiMNQO	1	16	–	1	3	15	1	–	2	9	–	–	–	3	–	–	1	–	52
	YkoCDE	3	–	–	–	11	1	13	–	14	–	–	–	–	–	–	–	–	–	42
	MtsTUV	1	–	1	–	4	1	17	3	–	6	–	–	–	–	–	–	–	–	33
	*HtsTUV	–	1	–	–	1	5	6	–	5	–	–	–	–	–	1	–	–	–	19
	*QrtTUVW	–	–	–	1	–	1	3	–	5	6	–	–	–	–	–	–	–	–	16
	*CbrTUV	–	1	–	–	3	5	2	–	6	–	–	–	–	–	–	–	–	–	17
	MtaTUV	1	–	–	–	–	2	–	–	1	–	–	–	–	–	–	–	–	–	4
	*RibU +AAT	1	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	3
	*PanT +AAT	–	–	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	2
	*TrpP +AAT	–	7	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	9
	*QueT +AT	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
	*PdxU +AAT	2	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	4
	*ThiW +AAT	3	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	7
	*CblT +AAT	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1
Subclass II	EcfA1–A2–EcfT	1	7	1	7	15	16	25	17	1	–	–	–	–	–	–	–	–	–	90
	*BioY	–	4	–	6	15	16	22	1	1	–	–	–	–	–	–	–	–	–	65
	*RibU	1	–	–	6	13	11	25	5	–	–	–	–	–	–	–	–	–	–	61
	*PanT	–	–	–	1	–	8	23	4	–	–	–	–	–	–	–	–	–	–	36
	ThiT	–	–	–	–	8	11	11	3	–	–	–	–	–	–	–	–	–	–	33
	FolT	–	–	–	1	–	8	14	14	–	–	–	–	–	–	–	–	–	–	37
	*TrpP	–	–	–	–	5	9	–	–	–	–	–	–	–	–	–	–	–	–	14
	*QueT	1	–	1	1	7	11	18	1	1	–	–	–	–	–	–	–	–	–	41
	*PdxU	1	4	–	3	–	11	19	–	–	–	–	–	–	–	–	–	–	–	38
	*ThiW	–	–	1	–	5	5	9	–	1	–	–	–	–	–	–	–	–	–	21
	NiaX	–	–	–	–	–	7	11	–	–	–	–	–	–	–	–	–	–	–	18
	*CblT	–	–	–	–	3	8	–	–	–	–	–	–	–	–	–	–	–	–	11
	*HtsT	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	2
	*QrtT	–	–	–	–	–	2	5	–	–	–	–	–	–	–	–	–	–	–	7
	*CbrT	–	–	–	–	1	–	11	–	–	–	–	–	–	–	–	–	–	–	12
	LipT	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	3
	BioY alone	–	3	–	–	–	–	–	–	4	22	–	1	–	19	–	5	–	–	54

The number of genomes possessing a particular type of ECF transporter is indicated. Families of S components that occur in both subclasses are indicated by an asterisk. 'BioY alone' shows the distribution of BioY transporters that are neither accompanied by a dedicated nor a shared 'AAT' module.

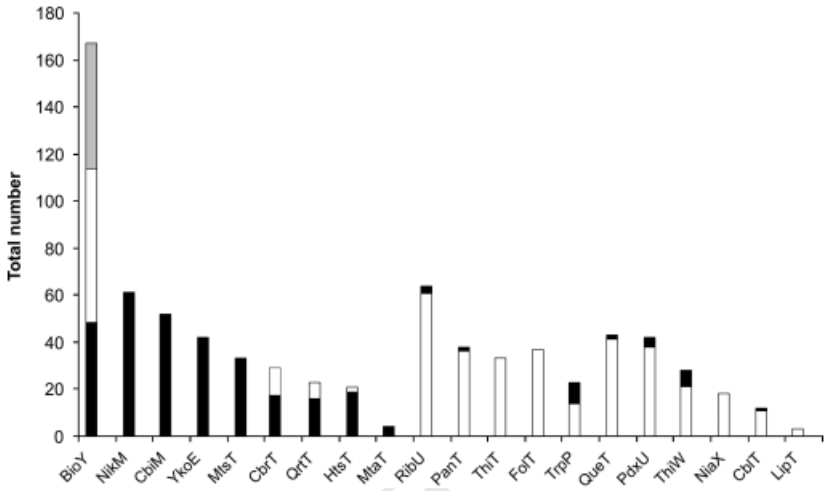

Fig. 7. Relative distribution of ECF transporters in prokaryotic genomes. The occurrence of 20 families of S components was analyzed in 341 bacterial and 42 archaeal genomes. The total numbers of organisms that encode members of different families classified to either subclass I (black bars) or subclass II (white bars) of ECF transporters are presented. The gray bar for the BioY family indicates the number of organisms that encode a solitary BioY protein, but neither a dedicated (BioMN) nor a shared (EcfA1A2T) module.

families, with the only exception being the homologous CbiM and NikM families. These results agree with the apparently weak (if any) similarity detected between S proteins from different families (average 11%, maximum 21% identity) (Rodionov *et al.*, 2009). Therefore, with the exception of the CbiM/NikM proteins, separate phylogenetic trees of other S components were generated (Supporting Information, Fig. S1). The CbiM/NikM tree is subdivided into two large branches corresponding to nickel and cobalt transporters (Rodionov *et al.*, 2006). BioY proteins are most diverse; their phylogenetic tree contains multiple separate branches corresponding to the BioMN-associated homologs (subclass I transporters), the EcfA1A2T-dependent homologs (subclass II transporters) and the solitary BioY proteins (independent of any AAT module). For instance, the BioY proteins from various *Alphaproteobacteria* are not clustered together, but dispersed between two very diverged branches of the tree corresponding to the BioMNY systems and the solitary BioY proteins, respectively. This tree suggests the complex evolutionary history of the BioY family including multiple duplication and horizontal transfer events, and different rates of sequence divergence in the different organisms caused by different (if any) AAT module partners. In addition to BioY, some other S-component families (e.g. FolT, PdxU and QueT) also include multiple diverse paralogs in the same genome, suggesting a likely divergence in their substrate specificities.

The amino acid sequence identity between individual T components of subclass I and subclass II transporters and among T units of different subclass I systems varies considerably and lies between ~15% and 60%. The phylogenetic tree of T components (Fig. S2a) was constructed using the representative subset of ~320 prokaryotic proteins and 10 additional homologs encoded in genomes of the various plants. The tree shows an overall consistent clustering of the major functional groups of ECF transporters with several exceptions including multiple distinct branches representing the BioM and EcfT proteins from *Archaea*, and a separate branch of QrtU proteins from enterobacteria. Interestingly, EcfT homologs from plants form a separate branch together with homologs from *Cyanobacteria* (those that are not encoded by operons for subclass I systems), suggesting their common evolutionary origin. These cyanobacterial and plant EcfT homologs are not accompanied by any recognizable EcfA homolog and their interaction partners and function remain elusive (Acknowledgements). Q2

A phylogenetic tree of A components of ECF systems (Fig. S2b) was constructed using a joint collection of ~260 single-domain ATPase proteins and ~230 domains of duplicated ATPases (MtsU, YkoD, HtsV, CbrU and MtaU) from prokaryotes. The tree shows a clear separation of duplicated A domains into diverse branches, suggesting that the duplication of ATPase domains occurred early in their evolution. The only exception from this observation is a subgroup of YkoD proteins from *Lactobacillales*, whose N- and C-terminal ATPase domains are most closely related to each other, suggesting that they probably originated from a relatively recent duplication.

The NBDs from canonical ABC and ECF importers form two distinct branches in a phylogenetic tree. A more detailed comparison is presented in Common features of canonical ABC and ECF importers, and perspectives.

Cellular functions of ABC import systems

Binding protein-dependent ABC importers

Nutrient supply and cell growth

This chapter summarizes the current knowledge on canonical ABC import systems involved in the uptake of macronutrients, trace elements, vitamins and polyamines.

Carbon and energy sources

SBP-dependent ABC transporters mediating the uptake of a diverse range of carbohydrates for carbon and energy metabolism cluster primarily in the CUT1 and CUT2 families of the TC classification system (Saier, 2000; Schneider, 2001). The 'earmark' of CUT2 family members is the primary structure of their NBDs, which appear to be a 'natural' fusion of two ABC domains (Schneider, 2001). Some carbohydrate transporters of hyperthermophilic *Archaea*, for example *Pyrococcus furiosus* (Koning *et al.*, 2001) and *Sulfolobus solfataricus* (Elferink *et al.*, 2001), *Thermotoga maritima* (a hyperthermophilic bacterium) (Conners *et al.*, 2005; Nanavati *et al.*, 2006) and of the root nodule-forming bacterium *Rhizobium meliloti* are also found within the PepT subfamily, otherwise comprising transporters for peptides, opines and nickel ions.

While the *per se* existence of mono- and disaccharides in nature is rather limited (e.g. fruits, milk, sugar-rich diet of animals, including humans), the major sources of sugars that can be utilized by prokaryotes are plant- and fungi-derived polysaccharides, including starch, cellulose, xylan, pectin and chitin. Thus, most prokaryotes that thrive in soil, aquatic environments, sewage digesters or the digestive tracts of animals secrete hydrolases for the degradation of biopolymers. The resulting mono-, di- and oligosaccharides are then internalized primarily due to the action of specific ABC transporters.

Maltose, maltodextrins, cyclodextrins. The maltose/maltodextrin transporter MalEFGK$_2$ of *E. coli*/*S. typhimurium*, by far the best-characterized member of the CUT1 family that also serves as a model for ABC transporters in general (see The 'alternating access'-model of transport), enables the facultative anaerobes at the entry of the large intestine to feed on sugars formed by the cleavage of starch from the animals' diet in the stomach and that escaped adsorption in the small intestine (Flint *et al.*, 2008). Quantitatively predominant bacteria in the large intestine such as *Bifidobacterium* have multiple gene clusters encoding putative oligosaccharide ABC transporters in their genomes (Schell *et al.*, 2002).

The receptor, MalE, binds maltose and maltooligosaccharides up to seven glucose units (Boos & Shuman, 1998), which are delivered to the MalFGK$_2$ complex for translocation to the cytoplasm. In contrast, reduced or oxidized maltodextrins or β-cyclodextrin bind to MalE, but the resulting liganded receptor fails to initiate the transport cycle, due to a different binding mode (Hall *et al.*, 1997a, b). The NBD MalK and MalFGK$_2$ were purified and extensively characterized in liposomes (reviewed in Schneider, 2003; Davidson & Chen, 2004). Crystal structures were resolved of MalE complexed with various substrates (Quiocho *et al.*, 1997), of the MalK dimer (reviewed in Davidson & Chen, 2004) and the complete transporter in the apo form in the absence of MalE (Khare *et al.*, 2009) and in an ATP-bound form with tightly associated MalE (Oldham *et al.*, 2007). The C-terminal extension of the MalK, the NBD of the maltose/maltodextrin transporter from *E. coli*/*Salmonella*, binds MalT, the positive transcriptional regulator of the *mal* regulon, thereby preventing its activation through binding of ATP and maltotriose and subsequent oligomerization (Joly *et al.*, 2004; Richet *et al.*, 2005). It is proposed that substrate availability is sensed through the transporter, which, in the idling mode, binds MalT and thereby represses *mal* gene transcription. In the presence of a substrate, however, transport activity is switched on, i.e. ATP is hydrolyzed at the MalK subunits, thus causing the release of MalT and subsequent induction of maltose-regulated gene expression (Böhm & Boos, 2000). The maltose transporter is also involved in a second regulatory process called 'inducer exclusion', which is part of the global carbon regulation in enteric bacteria. Here, in the presence of the preferred carbon source, glucose, the transport of inducer molecules for alternative metabolic pathways is prevented. This is achieved by inhibition of the respective transport systems via a component of the glucose transporter, the dephosphorylated enzyme IIA^{Glc} of the phosphoenolpyruvate phosphotransferase system (PTS) (Postma *et al.*, 1996). In the case of the maltose transporter, enzyme IIA^{Glc} binds predominantly to the C-terminal extension of the MalK subunits, thereby inhibiting ATP hydrolysis (reviewed in Schneider, 2003). Although the precise mechanism of inhibition is still unknown, the structure of the MalK dimer suggests binding sites for enzyme IIA^{Glc} on the C-terminal domain of one monomer and the N-terminal domain of the opposing monomer (Samanta *et al.*, 2003). Although an additional C-terminal domain of their NBDs is shared by all members of the CUT1 family, in the majority of cases, nothing is known on the possible regulatory processes involved.

Other related transporters discriminate between maltose and maltooligosaccharides by specific properties of their cognate receptors. Thus, the maltotriose porter of *Thermus thermophilus* does not recognize the disaccharide maltose, while the MalEFGK$_2$ transport system of *Thermococcus litoralis* accepts maltose and trehalose, but no longer dextrins. On the other hand, trehalose is not a substrate of the *E. coli* maltose/maltodextrin porter (Cuneo *et al.*, 2009b). Comparison of crystal structures of these proteins in complex with the cognate substrates revealed the presence of four subsites that bind individual glucose rings (Samanta *et al.*, 2003; Cuneo *et al.*, 2009b).

An ABC transporter homologous to maltose/maltodextrin porters of the CUT1 family also plays a key role in the proposed 'carbophor' function of the pseudo-maltotetraose acarbose produced by bacteria from the genera *Actinoplanes*

and *Streptomyces* (reviewed in (Wehmeier & Piepersberg, 2004). Acarbose, which is also a substrate of the *E. coli* maltose transporter (Brunkhorst *et al*., 1999), consists of an unsaturated C7 cyclitol bound via an imino bridge to 4-amino-4,6-dideoxyglucose (together named acarviosine) to which a maltose moiety is attached. According to this hypothesis, acarbose is synthesized and secreted into the environment, where, due to the action of a transferase, the acarviosyl group is hooked to maltotriose or other oligosaccharides. These are taken up by the GacHFG-MsiK ABC transporter, thereby resulting in a net gain in carbon and energy. Crystal structures and biochemical characterization of the binding protein GacH have demonstrated that longer acarbose homologs are in fact ligands of the protein, thereby corroborating this notion (Vahedi-Faridi *et al*., 2010).

Two CUT1 family members with strong sequence similarities to the *E. coli* maltose transport proteins, but distinctive substrate specificities, have been described in *Streptococcus mutans*, which is central to dental caries in humans. While the MsmEFGK system transports raffinose, melibiose and stachyose, the MalXFGK transporter is specific for maltodextrins. Maltose is a poor substrate for both systems. Mutations affecting the binding proteins, MsmE and MalX, respectively, were shown to cause the respective defects in sugar utilization (Webb *et al*., 2008). These results somewhat contradict a study by Kilic *et al*. (2007), who reported overlapping substrate specificities for both systems. However, mutating either one of the ATPase subunits, MsmK and MalK, had no phenotype, suggesting that the remaining NBD is shared by both systems (Webb *et al*., 2008).

Similar conclusions were drawn previously in the case of the MsiK protein, which assists in the uptake of several oligosaccharides through distinct transporters in *Streptomyces* (Hurtubise *et al*., 1995).

Bacteria such as *Klebsiella oxytoca* secrete cyclodextrin glycosyltransferases, which can cyclize linear maltodextrins first to α-cyclodextrins that are converted mainly to β-cyclodextrins. Utilization of cyclodextrins as carbon and energy source involves a cyclodextrin-ABC transport system of the CUT1 family (Fiedler *et al*., 1996). Crystal structures of a cyclodextrin-binding protein from *Thermoactinomyces vulgaris* (*Tvu*CMBP) have been reported in complex with α-, β- and γ-cyclodextrins as well as with maltotetraose (Matsumoto *et al*., 2009). The structures revealed that *Tvu*CMBP, in contrast to *E. coli* MalE, adopts the closed conformation with α-and β-cyclodextrins, but the open conformation with maltotetraose.

Cellooligodextrins. Extracellular degradation of cellulose results mainly in the production of cellotriose and cellobiose, which are internalized by ABC transport systems. In species of the genus *Streptomyces* that thrive in soil, an operon (*cebEFG*) was identified, encoding a solute-binding lipoprotein, CebE, and two membrane proteins: CebF and CebG (Schlösser *et al*., 1999). Transcription of the operon is induced by cellobiose. The gene for an NBD, as often observed for sugar ABC transporters in gram-positive bacteria, is not linked, but the MsiK protein was demonstrated to assist in cellobiose/cellotriose and maltose transport (Schlösser *et al*., 1997).

While the Ceb system of *Streptomyces* is a member of the CUT1 family, the hyperthermophilic archaea *P. furiosus* and *S. solfataricus* contain high-affinity cellobiose-uptake systems, homologous to oligopeptide transporters. This holds for quite a few sugar transporters of archaea and of the bacterium *T. maritima*, whereas the majority of systems belong to the CUT family (Albers *et al*., 2004). The *P. furiosus* Cbt transporter exhibits a K_m for uptake of 175 nM and a K_d for solute binding of 45 nM. The binding protein, CbtA, which is anchored to the cytoplasmic membrane via an N-terminal transmembrane helix, has a broad substrate specificity, accepting cellodextrins up to five glucose units and laminaribiose, a degradation product of laminarin (1,3-β-D-glucan) (Koning *et al*., 2001). The crystal structure of a cellobiose-binding protein homologous to oligopeptide-binding proteins from *T. maritima* revealed a semi-specific recognition of the substrates. While the disaccharide cellobiose binds specifically at its nonreducing end, additional rings up to five (cellopentaose) are located in a solvent-filled groove. Interactions of the reducing end with the protein define the acceptable length of the substrate (Cuneo *et al*., 2009a).

The anaerobic thermophilic bacterium *Clostridium thermocellum* grows very efficiently on cellulose due to a multienzyme complex, the cellulosome. Five sugar ABC transporters were identified in the organism, which, by analysis of the binding properties of their cognate solute-binding lipoproteins, were demonstrated to display specificities for cellodextrins (CbpB-D), cellotriose (CbpA) and laminaribiose (Lbp), respectively (Nataf *et al*., 2009).

Hemicelluloses. Because of the tight association of hemicelluloses with cellulose fibrils in the plant cell wall, cellulose degraders also require hemicellulolytic activity. Hemicelluloses are a mixture of branched and linear polysaccharides. Endo-1,4-β-xylanases release short, modified oligoxylose units of two or more sugars from the polymer's backbone.

In *Streptomyces thermoviolaceus*, an ABC transporter, BxlEFG (a gene for an ATPase subunit is not linked), operates that takes up xylodextrins. The recombinant binding protein, BxlE, shows the highest affinity for xylobiose ($K_d \sim 10^{-8}$ M) and xylotriose ($K_d \sim 10^{-7}$ M). Repression of transcription by the regulator protein BxlR is relieved in the presence of xylobiose (Tsujibo *et al*., 2004). A similar system, XynEFG, was found in *Geobacillus stearothermophilus*. The purified binding protein, XynE, binds the substrates up to

six xylose units with K_d values in the low μM range. Transcription of the operon is repressed by a specific regulator and activated in the presence of xylose by the regulator component of a two-component system (Shulami *et al.*, 2007).

A putative ABC transporter proposed to mediate the uptake of methyl-α-D-glucuronosyl-xylotriose, a degradation product of side groups of xylan, was identified in *Bacillus stearothermophilus* T-6, but not characterized further (Shulami *et al.*, 1999).

Pectin. The plant cell wall component pectin, the major matrix polysaccharide, consists of α-1,4-linked galacturonate residues forming the backbone of the molecule. Attached to it are neutral sugars such as rhamnose, arabinose, galactose or xylose. Pectin can be depolymerized by pectinases, leading to maceration.

In the plant pathogen *Erwinia chrysanthemi*, which causes soft rot disease on various plants, an ABC transporter, TogMNAB, besides others, internalizes oligogalacturonides and is involved in the chemotactic response toward these compounds (Hugouvieux-Cotte-Pattat *et al.*, 2001). The structure of the binding protein component, TogB, of the homologous transporter from *Yersinia enterocolitica* was resolved and revealed selectivity for digalacturonic acid, especially for the 4,5-unsaturated form of the sugar (Abbott & Boraston, 2007).

Ramified regions of pectin contain galactan (β-1,4- or β-1,3-linked D-galactopyranose residues), which can also be utilized by *E. chrysanthemi*. The *ganEFGK* operon, encoding a transporter displaying a high similarity to the maltose transporter of *E. coli*, is required for growth on galactan as shown by mutational analysis (Delangle *et al.*, 2007).

Rhamnose, a methyl-pentose sugar that is also found in the mucilage of legume plants, is taken up by the root–nodule symbiont *Rhizobium leguminosarum* bv. *trifolii* via the activity of the RhaSTPQ system, representing a binding protein, an ATPase and two membrane proteins, respectively (Richardson *et al.*, 2004). Transport experiments revealed strict specificity for rhamnose as other pentoses, such as L-arabinose or L-fucose, failed to inhibit the uptake of radiolabeled rhamnose. The capability to internalize rhamnose is required for the bacteria to successfully compete for nodule occupancy. As an interesting yet to be unraveled feature, the transport activity is dependent on an active rhamnose kinase (Richardson & Oresnik, 2007). The rhamnose ABC transporter, like those for other pentoses found in numerous bacteria, for example arabinose or xylose, is a member of the CUT2 family (Schneider, 2001).

Chitin. Chitin, the major cell wall component of fungi, is a polymer of *N*-acetyl-D-glucosamine. Soil bacteria of the genus *Streptomyces* secrete chitinases and internalize the chitooligomers produced, chitobiose (*N*, *N*′-diacetylchitobiose) and *N*-acetylglucosamine (NAG), which can be utilized as carbon and energy sources.

The Ngc transporter, comprising the solute-binding lipoprotein, NgcE, and two membrane-integral subunits, NgcF and NgcG, of *Streptomyces olivaceoviridis*, is the only known uptake system for NAG besides a phosphotransferase system. NgcE specifically binds NAG and chitobiose (Saito & Schrempf, 2004). Analogous ABC transporters were found by bioinformatics in *Silicibacter* and *Rhizobiales* (Yang *et al.*, 2006). In addition, *Streptomyces* contain a gene cluster, *dasABC*, that encodes a transporter for the uptake of chitobiose, which also plays a role in cell differentiation (Saito *et al.*, 2007). The binding protein, DasA, exhibits the highest affinity for chitobiose (32 nM), but also accepts chitooligomers up to five NAG units with a reasonable affinity. Low-affinity binding was found for NAG (25 μM). The transporter is completed by the MsiK-ATPase as demonstrated by mutational analysis (Saito *et al.*, 2008).

Chitin is also produced in massive amounts in marine environments by crustaceans. Gene clusters for chitin utilization including genes that encode an ABC importer were found in *Vibrio chlolerae* and *Vibrio furnissii* (Li & Roseman, 2004).

Alginate. Sphingomonas sp. A1 can grow on alginate, a linear polymer composed of α-L-guluronate β-D-mannuronate (polymerization grade > 100), and produced by brown seaweed and certain bacteria. Strikingly, and unlike other biopolymers, alginate is not degraded by secreted hydrolases before internalization. This is achieved by the formation of a large pit on the cell surface, which facilitates the transport of alginate to the periplasm, where it is captured by two binding proteins, AlgQ1 and AlgQ2, and delivered to an ABC transporter, consisting of two membrane-integral subunits, AlgM1 and AlgM2, and an ATPase, AlgS. Sequence analysis of the latter groups the transporter in the CUT1 family (Schneider, 2001). Both receptors bind alginate with K_d values around 0.1 μM. X-ray structures of AlgQ1 and AlgQ2, in their open form and in complex with an alginate tetrasaccharide, have been resolved and revealed a larger cleft in between the N- and the C-terminal lobes as observed for other solute receptors. Many positively charged residues in the cleft enable both proteins to bind preferentially to alginate (reviewed in Murata *et al.*, 2008, original references therein).

Other sugars. In bacteria, glucose is often internalized as glucose-6-phosphate via the PTS. In the hyperthermoacidophile *S. solfataricus*, belonging to the phylum *Archaea* that apparently lack PTS, a glucose ABC transporter was identified. The SBP binds glucose with a K_d of 0.4 μM at low pH (Albers *et al.*, 2004). Crystal structures of the NBD, GlcV,

with bound nucleotide and in the ligand-free state, were solved (Verdon *et al*., 2003).

A transporter specific for maltose and trehalose (disaccharide of α-1,1-linked glucose residues, originating from plants and fungi) that also accepts sucrose and palatinose (Silva *et al*., 2005) was characterized in the archaeon *T. litoralis* (Xavier *et al*., 1996). The structure of the cognate-binding protein complexed with trehalose is known (Diez *et al*., 2001) and the biochemical properties of the complete transporter (Greller *et al*., 2001) and the isolated NBD (MalK) (Greller *et al*., 1999) were investigated. The crystal structure of MalK was resolved, but the conformation of the dimer is likely to be an artifact because it differs from almost all other solved NBD dimers (Diederichs *et al*., 2000).

A transporter, AglEFGK, allowing growth on a variety of α-glucosides, including sucrose, maltose and trehalose, was identified in the root–nodule symbiont *Sinorhizobium meliloti* (Willis & Walker, 1999). The sucrose isomer palatinose (6-*O*-α-D-glucopyranosyl-D-fructofuranose) is produced from sucrose by some bacteria as a means of carbon storage. The plant tumorigenic bacterium *Agrobacterium tumefaciens* utilizes palatinose as a carbon source, which is internalized by the PalEFGK transporter displaying homology to the putative maltose/trehalose transporter of *S. meliloti*. Trehalose, which is an osmoprotectant in *S. meliloti*, is not transported by the Pal system as demonstrated by analysis of a *palK* mutant. In addition, the mutation only affected growth on palatinose, but not on glucose, maltose, sucrose or galactose (de Costa *et al*., 2003).

Besides ABC transporters for the uptake of glucose, galactose and xylose, the gram-negative bacterium *Agrobacterium radiobacter* also possesses a binding protein-dependent transport system for lactose, LacEFGK, which is usually transported by pmf-driven porters or PTS (Greenwood *et al*., 1990). The system displays considerable sequence similarity to the MalEFGK transporter of *E. coli*, which is underscored by the observation that the ATPase subunit LacK can replace MalK in maltose transport (Wilken *et al*., 1997).

Ribose, a degradation product of nucleosides, is transported in *E. coli* by the RbsBCA system, the best-characterized member of the CUT2 family (Schneider, 2001).

The hyperthermophilic bacterium *T. maritima* has multiple archaeal homologs of ABC transporters from the PepT subfamily characterized as sugar transporters of various specificities (Nanavati *et al*., 2006). Among 15 SBP components of these ABC transporters tested for sugar binding using fluorescence spectroscopy, 11 were characterized as sugar transporters with their own specific profiles of substrate specificities. Among the substrates bound by these *T. maritima* SBP proteins were several monosaccharides (xylose, ribose), disaccharides (cellobiose, laminaribiose, xylobiose, mannobiose, maltose, trehalose), various oligosaccharides, as well as *myo*-inositol and α-1,4-digalacturonic acid.

Polyols. Sugar alcohols (polyols) are utilized by a variety of bacteria and often internalized by PTS. The existence of an ABC transporter with a specificity for D-mannitol and D-glucitol (sorbitol) and homology to the maltose ABC transporter was deduced from sequence analysis in the purple, nonsulfur bacterium *Rhodobacter sphaeroides* Si4 (Stein *et al*., 1997). An operon, *mtlEFGK*, encoding genes for a transporter with a similar specificity (mannitol, araitol, glucitol) was identified in *Pseudomonas fluorescens* (Brünker *et al*., 1998).

Erythritol is a likely carbon source of root–nodule bacteria from the species *R. leguminosarum*. An operon encoding an ABC transporter, EryABCD, was identified on a plasmid of *R. leguminosarum* bv. *viciae*, which is induced by erythritol. A mutant lacking an intact *eryA* gene that encodes the putative binding protein was impaired in its capability to compete for nodulation against the wild type (Yost *et al*., 2006). Orthologs were found in the genomes of *Brucella* sp..

γ-Hexachlorocyclohexane (γ-HCH). *Sphingomonas japonicum* strain UT26 can grow aerobically on γ-HCH, an insecticide, as the sole source of carbon and energy. The compound is degraded to β-ketoadipate (Lal *et al*., 2010). Analysis of mutants identified a gene cluster, *linKLMN*, encoding a putative ABC transporter to be involved in γ-HCH metabolism (Endo *et al*., 2007). While LinL is a typical nucleotide-binding protein, LinK has features of a membrane-integral subunit, but lacks the EAA motif of canonical ABC importers. LinM has an N-terminal periplasmic signal peptide and is similar to Mce proteins of *Mycobacteria* (see Cholesterol) and might thus be the SBP of the putative transporter. LinN has the features of a lipoprotein. Mutational analysis suggested to the authors that the LinKLMN transporter might contribute to a controlled access of the hydrophobic and thus a potentially toxic substrate to the membrane by slowing down γ-HCH diffusion into the cell (Endo *et al*., 2007).

Phthalate. The capability of bacteria from the genus *Burkholderia* to utilize phthalate as the sole source of carbon and energy by degradation via protocatechuate is well documented (Chang & Zylstra, 1998 and references therein). Phthalates, or phthalate esters, are mainly used in the chemical industry as plasticizers to soften polyvinyl chloride. Besides a secondary transporter, uptake is achieved by the action of an ABC transporter, OphFGH. The encoding genes are part of an operon, together with the gene for a specific outer membrane porin, OphP (Chang *et al*., 2009).

The putative SBP, OphF, does not show high levels of similarity to other SBPs in the database. Phthalate uptake is strongly inhibited by 4-chlorophthalate and salicylate, suggesting that both compounds are accepted as substrates.

Cholesterol. In actinobacteria, including *Mycobacterium tuberculosis* and *Rhodococcus jostii*, evidence for a novel type of ABC transporter for the uptake of cholesterol that is used as a carbon source was provided. *Mycobacterium tuberculosis*, which is the causative agent of tuberculosis, requires host-derived cholesterol for persistence (Pandey & Sassetti, 2008). Genes encoding two ABC-type membrane-integral proteins are part of an operon (*mce*) containing between nine and 13 genes of an otherwise unknown function. The Mce1A protein is considered a candidate for a solute-binding lipoprotein of the system (Sutcliffe & Harrington, 2004). In fact, *M. tuberculosis* contains four *mce* operons and it was proposed (Pandey & Sassetti, 2008) that each putative ABC transporter is energized by an interaction with a genetically unlinked common nucleotide-binding protein of the Mkl family (Davidson *et al.*, 2008). For the Mce4 system of *R. jostii*, cholesterol uptake was verified by transport assays (Mohn *et al.*, 2008). Related operons were also identified by bioinformatics in gram-negative bacteria. Because mutations were found to affect cell envelope integrity, it was proposed that the substrates might be organic acid precursors of cell envelope biogenesis (Casali & Riley, 2007). How this proposal correlates with the experimental findings for cholesterol transport as cited above remains to be elucidated.

Bicarbonate. In the cyanobacterium *Synechococcus* sp., a gene cluster encoding a bicarbonate ABC transporter was identified by mutational analysis and uptake experiments that is activated under CO_2-limiting growth conditions (Omata *et al.*, 1999). The transporter comprises a solute-binding lipoprotein, CmpA, a membrane-integral subunit, CmpB, and two ATPase subunits, CmpC and CmpD. Binding experiments with recombinant CmpA demonstrated that HCO_3^- ($K_d = 0.5\,\mu M$) rather than CO_2 is the substrate (Maeda *et al.*, 2000). The proteins are homologous to the nitrate ABC transporter (NrtABCD) of *Synechocystis* sp. (Koropatkin *et al.*, 2006; see Nitrogen sources). This is underscored in the crystal structure of CmpA, which was resolved in complex with bicarbonate and carbonic acid and in the absence of ligands. Bicarbonate was found to bind in a nearly identical position as nitrate in NrtA. Bicarbonate binding is accompanied by a Ca^{2+} ion that might act as a cofactor or as a cosubstrate in bicarbonate transport (Koropatkin *et al.*, 2007b). Moreover, the ATPase subunit CmpC, like NrtC of the nitrate transporter, contains a C-terminal solute-binding domain involved in the regulation of the transporter's activity (see Nitrogen sources). Interestingly, the C-terminal domain is about 50% similar to NtrA, the SBP of the nitrate transporter, and it was proposed that nitrate and not bicarbonate is the likely substrate (Koropatkin *et al.*, 2006).

Carbon and nitrogen sources

Peptides. Peptides can serve as sources of nitrogen or amino acids in auxotrophs such as lactic acid bacteria. Thus, ABC transporters mediating the uptake of peptides play an important role in the nutrition of these organisms. In addition, peptide transporters play crucial roles in signaling processes and in virulence (reviewed in Detmers *et al.*, 2001; Doeven *et al.*, 2005), which will be discussed in Oligopeptide transporters. Peptide transporters specific for di- and tripeptides (Dpp) or oligopeptides containing five and more residues) (Opp) are grouped within the PepT family. Generally, like the well-characterized systems of *S. typhimurium* and *L. lactis*, oligopeptide transporters consist of an SBP, OppA, and a heterodimer each of the membrane-integral subunits, OppB and OppC, and the ATP-binding subunits, OppD and OppF (Detmers *et al.*, 2001). The oligopeptide-binding proteins determine the selectivity of the system as was shown in an *in vitro* study using purified and membrane-reconstituted Opp of *L. lactis* (Doeven *et al.*, 2004). Structures of DppA from *E. coli* and three OppA proteins from different organisms revealed that the specificity for peptides is determined by hydrogen bonds with the ligand backbone. The side chains are located in pockets that can accommodate any side chain, which is in line with the observed lack of sequence specificity for the ligands (reviewed in Doeven *et al.*, 2005). Most OppA proteins bind peptides with two to seven residues. By contrast, the OppA protein of *L. lactis* MG 1363 handles peptides up to 35 residues (Doeven *et al.*, 2005). Crystal structures of OppA in the open and closed (liganded) conformations provided a clue for this unusual property (Berntsson *et al.*, 2009). The protein has an enlarged substrate-binding cavity due to the movement of two loops to the surface, which, in other OppA orthologs, confine the binding cleft. Analysis of peptides bound to OppA revealed a preference for peptides between nine and 17 residues, enriched in prolines. Crystal structures in complex with peptides revealed a hydrophobic pocket that accommodates one of the peptide's side chains, which was often an isoleucine. These findings coincide with the organism's requirement for branched-chain amino acids and its preference for proline-rich casein.

The diversity of oligopeptide ABC transporters in *L. lactis* and other lactic acid bacteria is reflected by the observation that most strains contain more than one copy of an Opp system and in some organisms more than one peptide-binding protein is associated with a given transporter (Lamarque *et al.*, 2004; Doeven *et al.*, 2005). Likewise, in

the genome of *Borrelia burgdorferi*, the causative agent of Lyme disease, which is deficient in genes for the biosynthesis of amino acids, a single Opp transporter is encoded that might interact with five different peptide-binding proteins, OppA1–OppA5 (Wang *et al.*, 2004). The expression of the encoding genes seems to be controlled by different transcription factors (Medrano *et al.*, 2007). Similarly, four Opp systems were identified by *in silico* analysis in *S. aureus* and shown to be expressed differently (Hiron *et al.*, 2007). However, only one system (Opp3) was required for the growth of *S. aureus* in a medium deficient in glutamate/glutamine, but supplemented with glutamate/glutamine-containing peptides (4-mers to 8-mers).

The OppBCDE transporter of gram-negative bacteria is involved in recycling of cell wall components that are delivered by the specific peptide-binding protein, MppA. The regular receptor, OppA, is not involved. Amidases release murein peptides, such as L-alanyl-γ-D-glutamyl-*meso*-diaminopimelate, into the periplasm, from which they diffuse out of the cell, or may enter the cytoplasm via the MppA-OppBCDF transporter (Park *et al.*, 1998). MppA, which exhibits sequence similarity to OppA, was also reported to transport heme into *E. coli* cells when combined with the dipeptide ABC transporter, DppBCDF. However, the cognate-binding protein, DppA, could replace MppA (Létoffé *et al.*, 2006).

Amino acids. Prokaryotes can utilize amino acids as carbon and/or nitrogen sources or they are required as precursors under auxotrophic growth conditions. Transport systems with overlapping specificities belonging to the pmf-driven major facilitator superfamily and to the ABC transporter superfamily coexist in many organisms. ABC transporters mediating the uptake of amino acids are grouped into three families: the polar amino acid transporters (PAAT), the hydrophobic amino acid transporters (HAAT) and the methionine porters (MUT; discussed under Sources of sulfur) (Hosie & Poole, 2001; Zhang *et al.*, 2003). The histidine-lysine/arginine/ornithine transporter of *S. typhimurium* is the prototype of the PAAT family (reviewed in Hosie & Poole, 2001; Schneider, 2003). Genetic, molecular biological and biochemical studies on the system by G. Ames and colleagues contributed substantially to the current knowledge on ABC transporters. The transporter consists of two SBPs, HisJ and LAO (product of the *argT* gene), two transmembrane subunits, HisQ and HisM, each spanning the membrane only five times, and a homodimer of the ATPase subunit, HisP. HisJ and LAO exhibit high affinities in the nanomolar range for histidine and lysine/arginine/ornithine, respectively, but each binds the preferred substrate(s) of the other with about 10-fold lower affinities. The proteins are 70% identical and may have thus evolved by gene duplication. The crystal structures of HisJ and LAO in complex with their substrates revealed that the ligand preference of both proteins might be the result of a single amino acid exchange (Oh *et al.*, 1994a, b). HisP was the first NBD of any ABC transporter whose structure was resolved, showing the typical nucleotide-binding (RecA-like) and α-helical subdomains (Hung *et al.*, 1998). The reported HisP dimer structure, however, has the NBDs in a different orientation than the vast majority of all other NBD structures and is most likely an artifact. The complete transporter was purified and intensively characterized in proteoliposomes (Ames *et al.*, 2001).

Other PAAT family members closely related to the histidine transporter that have been partially characterized include the *E. coli* glutamine (GlnH-PQ) and arginine-specific (ArtIJ-QMP) transporters (reviewed in Hosie & Poole, 2001) and the arginine/lysine/ornithine/histidine transporter (ArtJ-MP) of the thermophile *G. stearothermophilus* DSMZ 13240 (Fleischer *et al.*, 2005; Vahedi-Faridi *et al.*, 2008). The latter is homologous to the YqiXYZ porter identified in *B. subtilis* (Sekowska *et al.*, 2001). Crystal structures of ArtP of *G. stearothermophilus*, which is 52% identical to HisP, in complex with nucleotides are known and show a dimer organization consistent with that of most other NBDs (PDB code 2OUK, 2OLK, 2OLJ, 2QOH, 3C4J and 3C41).

Two other close relatives of the histidine transporter, the OccT-QMP and NocT-QMP systems, are worth mentioning as they mediate the uptake of the arginine-derived modified amino acids octopine and nopaline as nutritional sources in the plant pathogen *A. tumefaciens*. *Agrobacterium tumefaciens* causes the formation of a tumorous growth on a wide variety of dicotyledonous plants and elicits the synthesis of a number of modified amino acids called opines by the plant. Both transporters were studied by uptake experiments with intact cells only (Zanker *et al.*, 1992). Transporters for other opines are members of the PepT and POPT families, respectively (Table 1).

ABC transporters mediating the uptake of acidic amino acids were characterized from several bacteria. In *Corynebacterium glutamicum*, a high-affinity glutamate transporter, GluBCDA, was identified by mutational analysis and transport assays with intact cells. Whether the transporter is specific for glutamate or also accepts aspartate was not investigated (Kronemeyer *et al.*, 1995). The AatJMQP transporter of *Pseudomonas putida* was shown to transport glutamate and aspartate. The purified SBP, AatJ, binds glutamate and asparagine with an equally high affinity (K_d values of 0.34 and 1.3 μM, respectively), while glutamine and asparagine are recognized with a much lower affinity (Singh & Rohm, 2008). In contrast, transport competition assays revealed that glutamate, aspartate, glutamine and asparagine are substrates of the BztABCD transporter from *R. capsulatus* (Zheng & Haselkorn, 1996).

An interesting ensemble of amino acid transporters was found in the filamentous cyanobacterium *Anabaena* sp. that can fix molecular nitrogen in differentiated cells termed heterocysts. Basically, only three ABC transporters are responsible for amino acid uptake of the organism. While the NatFGH transporter recognizes acidic and neutral amino acids, the BgtAB system takes up basic amino acids. In addition, the NatABCDE porter belonging to the HAAT family prefers proline and hydrophobic amino acids. The BgtAB system is composed of the ATPase subunit, BgtA, and BgtB, a fusion of an N-terminal solute-binding domain and a C-terminal TMD. The *bgtA* gene is not linked to *bgtB*, but included in the gene cluster encoding the NatFGH proteins and shared by both transporters as revealed from uptake assays. Both Nat systems appear to contribute to nitrogen fixation (Picossi *et al*., 2005; Pernil *et al*., 2008).

While most of the transporters are rather specific for chemically closely related amino acids, a few systems accepting a wide range of amino acids are known that seem to be widespread. The best-characterized general amino acid transporter is the AapJQMP porter of the root symbiont *R. leguminosarum*, which prefers basic and acidic amino acids, but also transports aliphatic amino acids (reviewed in Hosie & Poole, 2001). Transport experiments with intact cells revealed that the Aap system, the branched-chain amino acid transporter, Bra, of *R. leguminosarum*, and the histidine transporter of *S. typhimurium* mediate not only the uptake but also the export of amino acids, which might be a means to cope with the accumulation of amino acids as a consequence of metabolism. This finding challenges the concept of the unidirectional transport of ABC import systems depending on an extracellular binding protein (reviewed in Hosie & Poole, 2001). The authors assumed that substrate molecules must gain access to a (low affinity) binding site within a transporter from the cytoplasmic site that does not necessarily have to be identical to the one used for uptake. How this could be achieved was not yet further explored under *in vitro* conditions and whether it is only an intrinsic property of amino acid transporters is not known. The currently available crystal structures do not provide a clue in favor of this notion.

Within the HAAT family, transporters for branched-chain amino acids have been characterized from *E. coli*, *S. typhimurium* and *Pseudomonas aeruginosa* (reviewed in Hosie & Poole, 2001). The LIV-1 system of *E. coli* consists of two SBPs, LivJ and LivK, and a membrane-bound complex comprising two membrane-integral subunits, LivH and LivM, and two ATPase subunits, LivG and LivF. Crystal structures of the LivG homolog from *Methanococcus janaschii* in its monomeric form have been resolved (Karpowich *et al*., 2001). LivK was originally reported to be specific for leucine (K_d~1 μM), while LivJ binds leucine, isoleucine and valine with similar affinities (K_d~0.1–1 μM) and threonine, serine and alanine with a lower affinity. In a more recent study, using an *E. coli* strain deficient of all known transporters for aromatic amino acids, it was demonstrated that the LIV-1 system also transports phenylalanine. Uptake studies further revealed that the transporter, when equipped with LivK, accepts isoleucine and valine similar to leucine (Koyanagi *et al*., 2004).

A similar modular organization is found for the *Salmonella* LIV-1 transporter, whereas the homologous system from *P. aeruginosa* (BraC-DEFG) contains only one SBP (BraC). The Bra system of *P. aeruginosa* is one of the few that have been characterized in proteoliposomes, thereby demonstrating that alanine and threonine are true substrates (Hoshino *et al*., 1992). Other members of the HAAT family display a much broader range of substrate specificity (Hosie & Poole, 2001).

Both broad-specificity amino acid transporters of *R. leguminosarum* (Aap and Bra) are required for effective nitrogen fixation in pea nodules (Lodwig *et al*., 2003). It was shown that bacteroids (differentiated forms of the bacteria within root nodules) become symbiotic auxotrophs for branched-chain amino acids, and depend on the plant for supply (Prell *et al*., 2009).

γ-Aminobutyric acid (GABA) was suggested as a candidate amino acid (beside glutamate) that, in root–nodule symbiosis, is donated by the plant to the bacteroid in exchange for ammonia as a result of nitrogen fixation (Lodwig *et al*., 2003). In mutants of *R. leguminosarum* bv. *viciae* 3841 that grow faster than the wild type on GABA as the sole source of carbon and nitrogen, an ABC transport system, GtsABCD, exhibiting specificity for GABA and related compounds was identified (White *et al*., 2009). The transporter, belonging to the POPT family (Table 1), consists of an SBP, GtsA, two transmembrane subunits, GtsBC, and an ATPase, GtsD. The Gts system was not expressed in pea bacteroids, thereby questioning a role in amino acid cycling between host and symbiont. Rather, the authors consider it likely that this function is achieved by the Bra system, which was also shown to transport GABA (Hosie *et al*., 2002).

Nucleosides. Uptake or scavenging of ribonucleosides from the environment is a means to provide cells with precursors for nucleic acid synthesis and sugars as a source of carbon and energy. In *S. mutans*, the predominant causative agent of dental caries, an ABC transporter for the uptake of ribonucleosides, was identified as the only member of the carbohydrate uptake family CUT2 present in this organism (Webb & Hosie, 2006). The transporter, RnsBACD, consists of an ATPase subunit, RnsA, two membrane-integral subunits, RnsCD, and a solute-binding lipoprotein, RnsB. RnsA, like RbsA and other ATPases of the CUT2 family, is a fused heterodimeric protein, containing two putative NBDs.

Uptake experiments with intact cells using radiolabeled cytidine in combination with competing solutes revealed the acceptance of most ribonucleosides, whereas ribose and nucleobases were not recognized.

An ortholog of the Rns system was found in the genome of *Treponema pallidum*, the causative agent of syphilis (Deka *et al.*, 2006). The putative transporter, PnrABCDE, which could not be studied in intact cells due to the inability to cultivate the organism *in vitro*, was characterized via its cognate-binding protein, PnrA. The protein, which is a lipoprotein despite the dual membrane system of the cell envelope, was demonstrated by isothermal titration calorimetry to bind purine nucleosides with a K_d of ~0.1 μM. The crystal structure of PnrA complexed with inosine (likely procured from the *E. coli* host) was resolved and revealed a polypeptide with structural similarities to family 1-binding proteins, such as ribose- and glucose/galactose-binding proteins of *E. coli*. Because *T. pallidum* lacks the capacity for *de novo* synthesis of purines, the transporter might enable the organism to take up nucleic acid precursors from the human host.

Choline, glycine betaine, proline betaine. Quaternary ammonium compounds are commonly used as osmoprotectants in many bacteria and archaea (Welsh, 2000). In a few bacteria, including the root–nodule symbiont *S. meliloti*, osmoprotectants can be utilized at low osmolarity exclusively as carbon and nitrogen sources or in addition to osmoprotection. Choline (trimethylammonium) is a main constituent of eukaryotic plasma membranes in the form of phosphatidylcholine and is thus readily available in different environments including the soil and the rhizosphere. For its use as a nutrient, it has to be enzymatically converted to glycine betaine, from which pyruvate is subsequently formed (Smith *et al.*, 1988). Uptake of choline in *S. meliloti* is achieved by an ABC transporter composed of the SBP, ChoX, the membrane-integral subunit, ChoW, and the ATP-binding subunit, ChoV. The proteins share the highest similarities to the corresponding subunits from ProU-like transporters involved in glycine betaine and proline uptake for osmoprotection (Dupont *et al.*, 2004). However, the transporter is induced by choline, but not by high salt. The purified ChoX protein binds choline with a high affinity (K_d, 2.7 μM) and acetylcholine with a low affinity, but not glycine betaine or proline betaine. The crystal structures of ChoX, in complex with its ligands and in an unliganded, closed form, were determined (Oswald *et al.*, 2008). Most recently, a crystal structure of ChoX in a semi-open substrate-free form was reported that was attributed to the movement of a subdomain in the N-lobe that might be present in other SBPs as well (Oswald *et al.*, 2009).

In *P. aeruginosa* and *Pseudomonas syringiae*, the CbcXWV system mediates the uptake of choline, betaine and carnitine. The choline-binding protein, CbcX, is an ortholog of ChoX of *S. meliloti*. Two other binding proteins, BetX and CaiX, with specificities for betaine and carnitine, respectively, that are genetically unlinked, deliver their substrates to the CbcWV transporter. Interestingly, transport experiments revealed that CheX and BetX compete with CaiX-betaine for docking to the core transporter in their liganded forms only, thereby suggesting that productive receptor–transporter interactions are dependent on the presence of a substrate (Chen *et al.*, 2009).

Two ABC transporters have been implicated in the transport of proline betaine (*N*,*N*′-dimethylproline) in *S. meliloti*. The Hut transporter displays a high affinity for histidine, proline and proline betaine and is controlled at the transcriptional level by histidine, but not by salt stress. This is different from the ProU system of *E. coli* with which Hut shares homology, but is consistent with a role of the transporter in catabolism (see also Uptake of compatible solutes). In contrast, the Prb system, with a periplasmic-binding protein, PrbA, two membrane-integral subunits, PrbB and PrbC, and an ATP-binding subunit, PrbD, is a member of the oligopeptide transporter family (Alloing *et al.*, 2006). *prb* gene expression is induced by both proline betaine and sodium chloride, thereby differing from the *cho* genes. Moreover, uptake experiments with intact cells revealed that besides proline betaine, glycine betaine and choline are substrates.

EDTA. Bacterium BNC1, closely related to *Mesorhizobium* and *Agrobacterium* sp., was demonstrated to degrade the metal-chelating agent EDTA for use as a nitrogen source. Uptake of EDTA is probably mediated by the EppABCD transporter, whose encoding genes are adjacent to the gene for EDTA monooxygenase. Purified SBP, EppA, binds free EDTA with a K_d of 25 nM, but not stable metal–EDTA complexes (Zhang *et al.*, 2007).

Nitrogen sources

Nitrate, nitrite, cyanate, urea. Besides amino acids, prokaryotes can utilize a wide range of nitrogen sources that are transported by specific ABC uptake systems belonging to the NitT family.

Cyanobacteria require nitrate for growth, which is usually severely limited in aquatic environments. Organisms such as *Synechococcus* sp. and others are equipped with a high-affinity ABC transporter for the uptake of nitrate and nitrite. The Nrt system consists of a solute-binding lipoprotein, NrtA, localized to the outer leaflet of the inner membrane, which is an unusual feature of binding proteins from gram-negative bacteria, but typical for cyanobacteria, a membrane integral subunit, NrtB, a canonical ATPase subunit, NrtD, and a unique NBD-SBP fusion protein, NrtC (Omata, 1995;

Maeda & Omata, 1997). As already mentioned (Uptake of compatible solutes), the Nrt system shows significant similarities to the CmpA-BCD transporter, mediating the uptake of bicarbonate in cyanobacteria (Omata *et al.*, 1999). NrtA has a signal sequence containing a double arginine motif at its N-terminus, which marks it as a substrate for the Tat export system. The crystal structure of NrtA was resolved in complex with nitrate (Koropatkin *et al.*, 2006) and revealed an α/β protein composed of two domains that encompass the binding cleft that belongs to family 2 of SBPs (Wilkinson & Verschueren, 2003). The nitrate flux through the transporter is inhibited by ammonium ions that bind to the C-terminal solute-binding domain of NrtC (Kobayashi *et al.*, 1997).

The N-terminal domain of NtrC from *Phormidium laminosum*, when expressed separately, was found to bind ATP, but no ATPase activity could be detected (Nagore *et al.*, 2003).

A transporter displaying sequence similarity to the Nrt (and Cmp) system, NasFED, was reported for the enterobacterium *K. oxytoca*, which can utilize nitrate and nitrite as nitrogen sources (Wu & Stewart, 1998). Here, the periplasmic-binding protein, NasF, lacks an N-terminal cysteine residue required for modification with fatty acids and thus, as expected for gram-negative bacteria, is not a lipoprotein. NasE is homologous to NrtB and NasD is about 47% identical to NrtD. No NrtC-like component was found, although nitrate uptake by the NasFED transporter was inhibited by ammonium.

In the cyanobacterium *Synechococcus elongatus*, an ABC transporter, previously identified as a cyanate porter, CynABD (Espie *et al.*, 2007), was also shown to transport nitrite, in addition to the Nrt system (Maeda & Omata, 2009). CynA displays a high degree of sequence similarity to the periplasmic SBPs NrtA and CmpA. The transporter is thought to play a role in utilizing low concentrations of cyanate under nitrogen-limiting conditions and to allow those cyanobacteria that lack an Nrt system to assimilate nitrite.

Urea is a readily available nitrogen source due to its excretion by a variety of organisms in the environment. It can be metabolized by many microorganisms. An ABC transporter mediating the uptake of short-chain amides and urea (FmdDEF) was found in *Methylophylus methylotrophus* (Mills *et al.*, 1998) and characterized in more detail in cyanobacteria (Valladares *et al.*, 2002). The UrtABCDE porter of *Anabaena* sp. PCC 7120 consists of the periplasmic-binding protein, UrtA, two transmembrane subunits, UrtB and UrtC, and two ATP-binding subunits, UrtD and UrtE. The proteins share the highest sequence identities with the components of the branched chain amino acid transporter from *P. aeruginosa*. Uptake experiments with intact cells revealed a K_m of about 0.11 μM. Expression of the genes was induced under nitrogen-limiting conditions. A similar system was identified in *C. glutamicum*, whose expression is under the control of the global nitrogen regulator of the organism, AmtR (Beckers *et al.*, 2004).

Sources of phosphorus

Phosphate, phosphite, phosphonate, glycerophosphates. ABC transporters mediating the uptake of phosphorus-containing compounds are grouped within the PhoT and PhnT families. The high-affinity phosphate ABC transporter Pst – a member of the PhoT family – is present in many bacteria. Current knowledge on the prototype *E. coli* PstSCAB porter will be summarized here. The role of Pst in pathogenesis is discussed in Uptake of nutrients from the host.

The Pst system is part of the Pho regulon in *E. coli*, which is considered as a global regulatory network involved in phosphate metabolism (Lamarche *et al.*, 2008). It consists of the binding protein, PstS, whose structure is known (Wang *et al.*, 1994), the inner membrane proteins, PstA and PstC, and the ATPase subunit, PstB, for which enzymatic activity was demonstrated (Chan & Torriani, 1996) as was for the PstB homolog of *M. tuberculosis* (Sarin *et al.*, 2001). The transporter was studied by mutational analysis (Webb *et al.*, 1992), but biochemical characterization was never reported. The Pst system is involved in the regulation of the Pho regulon. This notion is based on the observation that mutations in *pst* genes result in the constitutive expression of the Pho regulon in many bacteria (Wanner, 1996). The current model implies that excess phosphate ($> 4\,\mu M$) causes the Pst transporter to form an inhibition complex with the sensor kinase PhoR, thereby preventing activation (phosphorylation) of the cognate transcriptional regulator, PhoB. The complex dissociates at limiting concentrations of phosphate, resulting in autophosphorylation of PhoR and subsequent activation of PhoB by a phosphotransfer reaction. Dephosphorylation of PhoB-P, which is return to repression under phosphate-rich conditions, is thought to occur through the phosphatase activity of PhoR in concert with PstB and PhoU. The latter is part of the *pst-phoU* operon (Lamarche *et al.*, 2008). The molecular basis of the proposed protein–protein interactions including the Pst complex remains elusive. Genetic evidence for the modulation of Pst transporter activity by PhoU was recently provided (Rice *et al.*, 2009).

ABC transporters are also involved in the utilization of alternative sources of phosphorus such as phosphite and phosphonates (reviewed in Kononova & Nesmeyanova, 2002). They are members of the PhnT family. A transport system with homology to the *E. coli* PhnCDE proteins was identified in *Mycobacterium smegmatis* as a second phosphate transporter besides several copies of the Pst system that does not recognize phosphite or phosphonates

(Gebhard *et al.*, 2006). Expression of the system is controlled by a specific repressor, PhnF, but also by the global two-component system SenX3-RegX3 (Gebhard & Cook, 2008).

The UgpBAEC transporter of *E. coli* allows the uptake of glycerol-3-phosphate (Schweizer & Boos, 1983) and glycerol-2-phosphate (Yang *et al.*, 2009a) as sources of phosphate. The transporter displays strong sequence similarity to the maltose transporter and thus belongs to the CUT1 family. In fact, UgpC, the NBD of the system, was demonstrated to substitute for MalK in maltose transport (Hekstra & Tommassen, 1993).

Sources of sulfur

Prokaryotes obtain sulfur either from inorganic sulfate or from organosulfur compounds including sulfonates, sulfate esters, sulfur-containing amino acids and glutathione (Kertesz, 2001).

Sulfate/thiosulfate/taurine. Sulfate/thiosulfate ABC transporters are members of the SulT family. In *E. coli*, the transporter consists of two binding proteins with preferred specificities for sulfate (SuBP) and thiosulfate (CysP), respectively, two membrane components, CysT and CysW, and the ATPase, CysA. The crystal structures of SuBP of *S. typhimurium* and CysA from *Alicyclobacillus acidocaldarius* have been resolved. In SuBP, sulfate is bound only by hydrogen bonds to neighboring amino acids and entirely dehydrated (Pflugrath & Quiocho, 1985, 1988). CysA revealed a C-terminal extension similar to those observed in the ABC proteins from CUT1 family members, but with an as yet unknown function (Scheffel *et al.*, 2005). The *cysPTWA* genes, together with other *cys* genes required for the synthesis of cysteine, are repressed in the presence of cysteine. Expression is activated by action of the positive regulator, CysB, and the coinducer, *N*-acetylserine (van der Ploeg *et al.*, 2001).

The utilization of taurine (2-aminoethanesulfonic acid) and alkanesulfates in *E. coli* and other bacteria requires the ABC transporters TauABC and SsuABC, belonging to the TauT family (Kertesz, 2001). Expression of the encoding genes is derepressed under conditions of sulfate or cysteine starvation (van der Ploeg *et al.*, 2001). Analysis of deletion mutants revealed that components of both transport systems are not functionally interchangeable (Eichhorn *et al.*, 2000).

Cysteine/cystine/methionine. In *E. coli* and *S. typhimurium*, experiments with intact cells revealed the participation of two binding protein-dependent transport systems in the uptake of cystine. The FliY/YecS/YecC system, one of the *E. coli* porters, has a rather broad substrate specificity also recognizing diaminopimelic acid. None of the systems has been characterized further, which also holds for cysteine transporters (Hosie & Poole, 2001).

A cysteine-binding protein (CjaA) was purified and crystallized from *Campylobacter jejuni*, a food-borne pathogen and a leading cause of acute human gastroenteritis. CjaA, which is a conserved immunodominant protein in *C. jejuni*, was shown to specifically bind L-cysteine with a K_d of ~0.1 μM, while the affinity for L-serine was > 200-fold lower. Binding of other amino acids could not be detected (Müller *et al.*, 2005).

In *B. subtilis*, two ABC transporters were identified by mutational analysis and uptake experiments with intact cells to mediate L-cystine transport (Burguière *et al.*, 2004): TcyJKLMN and TcyABC. The former includes two SBPs, TcyJ and TcyK, exhibiting 57% identity with unknown specificities. However, besides cystine, the system accepts cystathionine, djenkolic acid and *S*-methyl-cysteine, while the TcyABC porter seems to be more specific for cystine. Expression of the genes encoding the TcyJKLMN system was high in the presence of methionine, but reduced in the presence of sulfate or cystine (Even *et al.*, 2006). Expression of the TcyABC-encoding genes was low under all the conditions tested. TcyABC-like transporters are found in many gram-positive bacteria. The putative YxeMNO transporter that is most similar to the *E. coli* FliY/YecS/YecC is not involved in cystine uptake (Burguière *et al.*, 2004). The first crystal structure of a cystine-binding protein. NGO372 from *Neisseria gonorrhoeae*, which displays 50% and 41% sequence identity, respectively, to TcyA of *B. subtilis* (Burguière *et al.*, 2004) and BspA of *Lactobacillus fermentum* (Turner *et al.*, 1999), was recently resolved (H. Bulut, F. Scheffel, Moniot, W. Saenger & E. Schneider, unpublished data).

ABC transporters that transport L- and D-methionine, formyl-L-methionine and likely organic sulfur compounds in gram-positive bacteria are clustered in the MUT family (Zhang *et al.*, 2003). In *E. coli*, the long-known *metD* locus was shown to encode the MetNIQ transporter (Gál *et al.*, 2002; Merlin *et al.*, 2002) with the ATPase subunit, MetN, the membrane subunit, MetI, and the receptor, MetQ. These proteins were formerly known as ABC, YaeE and YaeC, respectively. Interestingly, the receptor, MetQ, is proposed to be a lipoprotein. The crystal structure of the $Met(NI)_2$ complex at a 3.7 Å resolution was resolved and revealed an explanation for an allosteric regulatory mechanism that operates at the level of transport activity (Kadaba *et al.*, 2008). Increased cellular concentrations of the transported ligand stabilize an inward-facing conformation of the transporter by binding to a site located in between the C-terminal extensions of the respective NBD dimers. As a consequence, the transporter is locked in an inactive state, thereby preventing further ligand uptake (Kadaba *et al.*, 2008). A similar mechanism was found for the molybdate/tungstate

transporter of *Methanosarcina acetivorans*, $Mod(AB)_2$ (see Trace elements).

The MetNPQ transporter of *B. subtilis*, which is also distributed among other gram-positive bacteria, was shown to also transport methionine sulfoxide (Hullo *et al.*, 2004). In *S. mutans*, the homologous AtmBDE system is involved in the uptake of L- and D-methionine, selenomethionine and homocysteine (Sperandio *et al.*, 2007).

A methionine-binding lipoprotein (TP32) was identified from its crystal structure in *T. pallidum*. The protein shares significant similarity to the putative methionine-binding protein MetQ (YaeC) of *E. coli* and is thus suggested to be a constituent of a methionine ABC transporter (Deka *et al.*, 2004). The crystal structure of a related protein, GNA1946, from *Neisseria meningitidis*, which is assumed to be a lipoprotein, also revealed L-methionine as the most likely substrate (Yang *et al.*, 2009b).

Glutathione. Mutational analysis and transport assays revealed a novel type of ABC transporter in *E. coli*, mediating the uptake of glutathione that is used as the sole source of sulfur (Suzuki *et al.*, 2005). The transporter is a member of the PepT family and consists of the solute receptor, YliB, two inner membrane components, YliC and YliD, and an ATP-binding subunit, YliA. Homologs are found in other enterobacteria.

Iron

Iron has a vital function for almost all prokaryotes due to its role in electron transfer proteins, for example cytochromes, and in some proteins, such as ribonucleotide reductase and soluble methane monooxygenase. Although iron is one of the most abundant elements on earth, its requisition is a major challenge for many prokaryotes because it is not readily available under most growth conditions. In the presence of oxygen and at neutral pH, iron forms insoluble hydroxides and most bacteria and certain fungi are forced to synthesize and secrete low-molecular-weight chelators (siderophores) that sequester Fe^{3+}-ions with a high affinity. Only under anaerobic conditions and at pH values below 3 can ferric ions be transported without chelators (see Crosa *et al.*, 2004 for review). Sufficient iron supply was also shown to contribute to the virulence of many pathogens. Consequently, iron transporters of pathogenic bacteria are virulence factors and are discussed in Uptake of nutrients from the host. Here, we will summarize the current knowledge on iron ABC transporters of *E. coli* K-12 and other nonpathogens.

Escherichia coli is equipped with three ABC transporters mediating the uptake of Fe^{3+}-siderophores, namely, Fe^{3+}-hydroxamate (FhuBCD), Fe^{3+}-enterobactin (FepBCDG) and Fe^{3+}-dicitrate (FecBCDE) (Köster, 2001). They are grouped within the FeCT family. While mutational and biochemical analyses have been performed for individual components of the transporters, an *in vitro* characterization of the assembled transport complexes has not been reported as yet. All three systems have in common the dependence of a specific outer membrane receptor, which, powered by the proton-motive force, translocates the substrate into the periplasm. Removal of a so-called plug, a domain of the polypeptide blocking the channel formed of β-strands (β-barrels), was suggested to be energy-consuming (Braun *et al.*, 2004; Ferguson & Deisenhofer, 2004). The molecular basis of this process, which requires the presence of a cytoplasmic membrane protein complex comprising TonB, ExbB, and ExbD remains elusive (Postle & Larsen, 2007).

The purified Fe^{3+}-hydroxamate-binding protein, FhuD, binds its substrates (ferric coprogen, ferric aerobactin, ferrichrome and albomycin) with dissociation constants in the low μM range (Braun *et al.*, 2004). The crystal structures of FhuD that have been resolved in complex with several substrates place the protein into family 3 of solute receptors (Krewulak *et al.*, 2004). The transmembrane subunit, FhuB, has about double the molecular mass of a typical TMD, each half spanning the membrane 10 times, and is proposed to represent a fusion of two TMDs with internal sequence homology. It was demonstrated that separate expression of the N- and C-terminal halves resulted in an active protein, thereby corroborating the above notion. The FhuC protein is a typical ABC ATPase (reviewed in Köster, 2001).

Fe^{3+}-dicitrate is passing the outer membrane by the action of the FecA receptor. It is assumed that in the periplasm only Fe^{3+} is delivered to the binding protein, FecB, based on the finding that 10 times more radiolabeled Fe^{3+} is transported into the cytoplasm than citrate (Braun *et al.*, 2004). The membrane-bound transport complex is composed of the transmembrane subunits FecB and FecC and the ATPase, FecE. The interaction of FecB with FecCD, as suggested for other ABC importers to initiate the transport cycle, was proposed based on the BtuF-CD structure to involve the formation of salt bridges. Evidence for this notion was presented recently by mutational analysis (Braun & Herrmann, 2007). The Fec system is induced by ferric citrate in the periplasm through a signaling pathway comprising the transmembrane protein FecR (Härle *et al.*, 1995).

The first crystal structure of a binding protein in complex with a native catecholate siderophore was reported recently. The FeuA protein of *B. subtilis* binds its substrate, ferribacillibactin, by electrostatic interactions through two lysine and one arginine residue (basic triad) from the N- and C-terminal lobes, respectively, and via hydrogen bonds from two glutamine residues (Peuckert *et al.*, 2009). A basic triad was also found in the structure of the enterobactin-binding protein CeuE from *C. jejuni*, which was crystallized in complex with an artificial substrate (Müller *et al.*, 2006).

FeuA belongs to family 3 solute receptors and is a component of the FeuABC-YusV transporter, which also imports ferrienterobactin (Ollinger *et al*., 2006).

Bacillus subtilis can also utilize the exogenous siderophore petrobactin, which, among others, is produced by members of the *Bacillus cereus* group, including the pathogen *B. anthracis*. Fe-petrobactin is internalized by the YclNOPQ transporter, comprising two transmembrane subunits, YclON, an ATPase, YclP and a solute-binding protein, YclQ. The crystal structure of YclQ revealed CeuE from *C. jejuni* as its closest relative, but also a similarity in shape to FeuA. The importance of three basic residues for interaction with the substrate was confirmed (Zawadzka *et al*., 2009).

Cyanobacteria have a high demand for iron to sustain photosynthesis. In *Synechocystis* PCC 6803, an iron transport system operates consisting of two SBPs, FutA1 and FutA2, which are 52% identical, a membrane-integral subunit, FutB, and an ATPase, FutC (Katoh *et al*., 2001). FutA1 and FutA2 bind iron directly without siderophores or anions, respectively, as revealed from their crystal structures. Iron is coordinated by four tyrosine and one histidine residue, as also observed in the crystal structure of the homologous FbpA protein of *C. jejuni* (Tom-Yew *et al*., 2005). Whether ferrous or ferric irons are the preferred ligands under physiological conditions is controversially discussed (Koropatkin *et al*., 2007a; Badarau *et al*., 2008). Moreover, the function of FutA1 as a periplasmic iron-binding protein has been questioned. Instead, it was suggested to act intracellularly under conditions of iron limitations. Accordingly, only FutA2, which has an export signal targeting it to the Tat secretion machinery, is proposed to deliver iron to the FutBC transporter (Badarau *et al*., 2008).

Vitamins

Vitamin B_{12}. Coenzyme B_{12} or adenosylcobalamin (vitamin B_{12} is the cyanocobalamin analogue), containing a corrin ring, serves as a cofactor for enzymatic radical reactions. The uptake of vitamin B_{12} in *E. coli* is mediated by the Btu system, which is closely related to the FeCT family comprising iron-chelate ABC transporters (Köster, 2001), and for which by far the most experimental data have been accumulated. Both families share the necessity of a specific substrate receptor in the outer membrane that requires the proton-motive force established at the cytoplasmic membrane for translocation of the substrate to the periplasm (see Iron). The $Btu(CD)_2$ transporter was the first ABC transporter whose crystal structure could be resolved (Borths *et al*., 2002). It consists of two copies of the transmembrane subunit, BtuC, each spanning the membrane 10 times, and of two ATPase subunits, BtuD. Characterization of the purified transporter in proteoliposomes revealed that, like in other cases, the $Btu(CD)_2$ complex exhibits a basal ATPase activity that is significantly stimulated by the cognate-binding protein, BtuF, and is sensitive to the inhibitor vanadate (Borths *et al*., 2005). BtuF was found to bind tightly to the transporter under all conditions tested. BtuF belongs to family 3 of SBPs (Borths *et al*., 2002; Karpowich *et al*., 2003) (see SBPs).

Thiamine pyrophosphate (TPP) (Vitamin B_1). TPP is indispensable in central metabolism as a cofactor of enzymes such as pyruvate dehydrogenase and oxo-glutarate dehydrogenase. An ABC transporter, consisting of the SBP, ThiB, a membrane-integral subunit, ThiP, and an ATPase subunit, ThiQ, mediating the uptake of thiamine and TPP was first identified in *S. typhimurium* mutants defective in *de novo* synthesis of TPP (Webb *et al*., 1998). The *E. coli* homolog was originally designated SfuABC to reflect its similarity to the ferric ion transporter of *Serratia marcescens*. Uptake experiments with intact cells demonstrated that both thiamine and TPP are accepted as substrates. Expression of the encoding operon is repressed by excess thiamin. The *E. coli* thiamine-binding protein designated TbpA (identical to ThiB of *S. typhimurium*) was shown to exhibit similarly high affinities for thiamin (K_d, 3.8 nM), thiamin monophosphate (K_d, 2.3 nM) and TPP (K_d, 7.4 nM) (Soriano *et al*., 2008). Its crystal structure was resolved in complex with thiamin monophosphate. Both lobes of TbpA are connected by two crossovers (Soriano *et al*., 2008). The protein is structurally most similar to the Fe^{3+}-binding protein from *Haemophilus influenzae*, but similarity was also found to thiaminase-I, a thiamine-degrading enzyme, suggesting a common evolutionary origin. Additional aspects of transport and salvage of thiamine and thiamine-related compounds are discussed in Transport of water-soluble vitamins and vitamin-derived compounds of this review.

Trace elements

Manganese, zinc. Manganese plays a crucial role in the water-splitting enzyme associated with photosystem II of oxygen-evolving phototrophic bacteria and is an important metal for an oxidative stress response (Horsburgh *et al*., 2002). Zinc is not involved in electron transfer reactions due to its single oxidation state in solution. Rather, it is a cofactor in a variety of enzymes with diverse functions such as alkaline phosphatase, RNA polymerase, zinc finger proteins and some ribosomal proteins. Both transition metals (like iron) are toxic at higher intracellular concentrations and thus metal homeostasis pathways must operate. Among others, high-affinity ABC transporters for the uptake of these metals are part of such pathways. They cluster in the MCT family of the TC system and, based on sequence alignments, their cognate extracellular SBPs constitute cluster 9 of solute receptors (Claverys, 2001). Some have been

recognized as important virulence factors of pathogens and are addressed in Uptake of nutrients from the host.

In the cyanobacterium *Synechocystis* sp. PCC 6803, a manganese ABC transporter, MntCAB, operates with a high affinity (K_m, 1–3 μM), which is induced under manganese-starvation conditions (Bartsevich & Pakrasi, 1996). The crystal structure of its SBP, MntC, was resolved with bound Mn^{2+} and revealed the presence of an unusual, but functionally crucial disulfide (Rukhman *et al*., 2005).

Manganese ABC transporters related to an oxidative stress response were found in numerous bacteria (Claverys, 2001; Horsburgh *et al*., 2002). The putative iron/manganese transporter of the root–nodule symbiont *S. meliloti* was shown to be important for the organism's response to oxidative stress. The SitABCD system for which a homolog exists in *S. enterica* serovar Typhimurium (Kehres *et al*., 2002) (Uptake of nutrients from the host) favors manganese over iron as a substrate. A mutant lacking the solute-binding subunit, SitA, is symbiotically defective and displays elevated sensitivity to superoxide due to decreased levels of superoxide dismutase B. The *S. meliloti* SodB (also called SodA in older references) can operate with iron or manganese, therefore termed 'cambialistic', but exhibits a higher activity with the latter. The mutant was rescued by exogenous addition of 10 μM $MnSO_4$, but not $FeSO_4$, although the latter was not applied under reducing conditions to maintain the Fe(II) redox state. The authors concluded that the SitABCD transporter plays a crucial role in manganese uptake (Davies & Walker, 2007), which is consistent with data obtained for the *Salmonella* Sit system.

Escherichia coli and others have a ZnuABC system, consisting of the extracellular binding protein, ZnuA, a membrane-integral subunit, ZnuB, and an ATPase, ZnuC. Expression of the *znuABC* gene cluster is regulated by zinc and a specific repressor, Zur (Hantke, 2005). Crystal structures of Ec-ZnuA are available and show that both β-/α-domains are connected by an α-helix as it is characteristic of family 3 receptors (Wilkinson & Verschueren, 2003). Three conserved histidine residues and a glutamate were found to coordinate the zinc ion (Chandra *et al*., 2007; Li & Jogl, 2007; Yatsunyk *et al*., 2008). The glutamate was replaced by water in a structure of the homologous ZnuA protein from *Synechocystis* (Banerjee *et al*., 2003). Similar to MntC, the Ec-ZnuA structures show an unusual disulfide bond in the C-terminal domain, which might be important for structural integrity or regulation of Zn^{2+} binding. In one structure, a second metal-binding site with an unclear function was observed (Yatsunyk *et al*., 2008). Ec-ZnuA binds Zn^{2+} with an estimated K_d of < 20 nM and other divalent cations, but not manganese. Only Zn^{2+}, Cd^{2+} and Cu^{2+} caused conformational changes in the protein thought to be required for metal delivery to the cognate transport complex (Yatsunyk *et al*., 2008). A highly charged and mobile loop observed in the vicinity of the binding cleft is proposed to act as a zinc chaperone to facilitate acquisition (Banerjee *et al*., 2003).

The crystal structures of two other proteins, PsaA from *S. pneumoniae* and TroA from *T. pallidum*, were resolved in complex with Zn^{2+}, but the nature of the physiological substrate (zinc or manganese) is still controversially discussed (Hantke, 2005). A binding study with TroA purified from *E. coli* revealed that Zn^{2+}, Mn^{2+} and possibly iron might be substrates, but that the transcriptional regulator of the *tro* operon, TroR, is likely to bind Zn^{2+} (Hazlett *et al*., 2003).

Nickel. In *E. coli*, Ni^{2+} is an essential cofactor for NiFe-hydrogenases that operate only under anaerobic conditions. To fulfill the demand for Ni^{2+} ions, the *nikABCDE* operon encoding an ABC transporter is expressed in close correlation with the hydrogenase expression levels. Positive regulation of *nikABCDE* is achieved by the redox sensor FNR while transcription is repressed in response to excess Ni^{2+} by NikR (reviewed in Eitinger & Mandrand-Berthelot, 2000). Furthermore, *nikABCDE* expression is also under the control of the nitrate-regulatory system (Rowe *et al*., 2005). The transporter consists of two heterodimers representing the transmembrane (NikBC) and nucleotide-binding subunits (NikDE), and of a SBP, NikA. Because of sequence similarities to oligopeptide transporters, it is grouped within the PepT family of the TC system. NikA was purified and a K_d for Ni^{2+} was estimated to be around 0.1 μM based on equilibrium dialysis and quenching of intrinsic tryptophan fluorescence, while that for Co^{2+} was 10-fold higher (de Pina *et al*., 1995). In a subsequent study, using isothermal titration calorimetry, unphysiologically high K_d values of ∼10 and 250 μM were reported for Ni^{2+} and Co^{2+}, respectively (Heddle *et al*., 2003). The lower affinity for Ni^{2+} found by this group corroborates with a study using fluorescently labeled NikA (Salins *et al*., 2002). Although several crystal structures of NikA have thus far been reported, a clear-cut answer on how Ni^{2+} is coordinated in the protein remains elusive. Heddle *et al*. (2003) presented structures obtained in the absence and presence of Ni^{2+}. In the latter, the ligand is not occluded in the binding cleft, but is rather accessible to the solvent, which is in contrast to other binding proteins. Also, the rotational motion between both lobes upon binding Ni^{2+} was less than that observed for related binding proteins. In 2005, Cherrier *et al*. (2005) reported on a refined structure (1.8 Å resolution) of NikA in complex with $FeEDTA(H_2O)^-$. These authors, on close inspection of the data deposited by Heddle *et al*. (2003), arrived at the conclusion that the nature of the ion in the 'Heddle' structure remains unclear and could very well be an iron. Among other considerations, this was based on the fact that both groups used EDTA-containing buffers for the

purification of NikA. Moreover, it was speculated that Ni^{2+} binding by NikA might require a chelating cofactor. This notion was further corroborated by the same group by resolving the crystal structure of NikA prepared in the absence of EDTA (Cherrier *et al*., 2008). Here, an unidentified chelator that might be butane-1,2,4-tricarboxylate was found to contribute to the binding of a transition metal ion of unknown identity. To make the picture even more complex, NikA was also shown to bind heme under anaerobic growth conditions, although the binding site is predicted to be remote from the nickel-binding cleft (Shepherd *et al*., 2007).

Homologs of the NikABCDE system with an experimentally confirmed function in nickel uptake were reported for *Brucella suis* (Jubier-Maurin *et al*., 2001) and *Yersinia pseudotuberculosis* (Sebbane *et al*., 2002). Because of the similarity among the SBPs within the PepT family, it is difficult to divide its members by amino-acid sequence comparisons into subclasses with substrate specificity for either metal ions or peptides. Based on genomic colocalization with genes for nickel-dependent enzymes or the presence of upstream NikR-binding sites, a subset of the PepT family was identified as metal transporters. A function in Ni^{2+} uptake has been ascribed to the vast majority of the members of this subset (Rodionov *et al*., 2006; Zhang *et al*., 2009). (see Uptake of Ni^{2+} and Co^{2+} ions, additional aspects of Ni^{2+} and Co^{2+} uptake).

Copper. Methanobactin, a siderophore-like molecule with a peptidic nature, is implicated in copper uptake in methanotrophic methylotrophs. Cu^{2+} is an essential cofactor of particulate methane monooxygenase. The acquisition of copper ions is achieved by methanobactin. Although nothing is currently known on the nature of the transporters involved, a TonB-dependent outer-membrane transporter and an ABC transporter in the cytoplasmic membrane were predicted for internalization of the Cu–methanobactin complex (Balasubramanian & Rosenzweig, 2008).

Molybdate/tungstate/vanadate. Molybdenum serves as a cofactor in a variety of enzymes, including (1) nitrogenase of nitrogen-fixing bacteria such as cyanobacteria or root–nodule symbionts and (2) a large group of pterin-based molybdenum enzymes in all kingdoms of life that are grouped into three families represented by sulfite oxidase, xanthine oxidase and dimethyl sulfoxide reductase (DMSOR). The DMSOR family includes many more enzymes involved in electron-transport chains under anoxic conditions, such as nitrate reductase and formate dehydrogenase (see Zhang & Gladyshev, 2008; Schwarz *et al*., 2009 for recent reviews). Molybdenum is never directly bound by an enzyme, but always via a cofactor, the 'FeMoco' (nitrogenase only) or a molybdenum cofactor (Moco), whose structure is derived from a metal-binding pterin (MPT). The same holds true for tungsten, which is found as a relative of Moco (called Wco) in tungsten-containing formate dehydrogenase (a member of the DMSOR family) and Wco-containing aldehyde : ferredoxin oxidoreductase (see Andreesen & Makdessi, 2008 for a review).

The *E. coli* ModABC transporter is the best-characterized uptake system for the naturally predominant form of Mo, the anoxyion molybdate (Self *et al*., 2001). It consists of the binding protein, ModA, the transmembrane subunit, ModB, and the ATPase, ModC. ModA binds molybdate with a very high affinity in the nanomolar range and, at equimolar quantities, also tungstate. Crystal structures of ModA in complex with molybdate and tungstate are available (Hu *et al*., 1997). Expression of the *modABC* genes is tightly regulated by the repressor, ModE, and requires molybdate starvation. Interestingly, the *E. coli* sulfate ABC transporter mediates molybdate uptake with a low affinity while, likewise with a low affinity, the ModABC system accepts sulfate as a substrate (Kertesz, 2001; Self *et al*., 2001).

The crystal structure of a molybdate/tungstate transporter, ModBC, from the sulfate-reducing archaeon *Archaeoglobus fulgidus* in complex with its cognate-binding protein, ModA/WtpA, has been resolved (Hollenstein *et al*., 2007). In contrast to ModA proteins from *E. coli* and *Azotobacter vinelandii*, which bind molybdate and tungstate as a tetrahedral complex, the crystal structures of ModA/WtpA of *A. fulgidus* and four other archaebacterial molybdate/tungstate-binding proteins revealed an octahedrally coordinated central metal ion (Hollenstein *et al*., 2009).

The crystal structure of a homolog from *M. acetivorans*, ModBC, revealed a C-terminal extension of the ATPase subunit, ModC, which provides the basis for the mechanism of trans-inhibition of transport similar to the methionine transporter of *E. coli* (Gerber *et al*., 2008) (Sources of sulfur). These regulatory domains provide two anoxy-binding pockets for molybdate/tungstate. When occupied by a substrate, the transporter is locked in the 'inward-facing' conformation, resulting in an inactive ATPase activity. Transport activity i.e. energy costs, can thus be controlled by cellular levels of the substrate.

A clear preference for tungstate over molybdate is exhibited by the receptor TupA of the TupABC transporter from the amino acid-degrading bacterium *Eubacterium acidaminophilum* (Makdessi *et al*., 2001). Unpublished data obtained by isothermal titration calorimetry revealed a K_d for tungstate of 0.2–1 nM, at least 1000-fold lower than that for molybdate. The crystal structure of TupA in the unliganded form was resolved (reported in Andreesen & Makdessi, 2008).

The genome of *C. jejuni*, which causes gastrointestinal infections, contains two distinct ABC transport systems (Cj0300–0303 and Cj1538–1540) with similarity to molybdate

transporters and the tungstate transporter of *E. acidaminophilum*, respectively (Smart *et al.*, 2009). Binding studies with the purified SBPs using isothermal titration calorimetry and intrinsic tryptophan fluorescence revealed equal affinities of Cj0303 (ModA) for molybdate and tungstate (K_d~4–8 nM), but a clear preference of Cj1540 (TupA) for tungstate over molybdate (5×10^4-fold). Tungstate is bound with an unusually low K_d of 1 pM. By mutational analysis, it was demonstrated that a *tupA* strain displays a significant reduction in formate dehydrogenase activity, suggesting a role of tungstate as a cofactor of this enzyme (Smart *et al.*, 2009). These data were corroborated by a study of Taveirne *et al.* (2009), who monitored the activities of different molydate- and tungstate-dependent enzymes in strains carrying mutations in the *mod* and/or *tup* genes.

Another tungstate and molybdate uptake system, WtpABC, was first identified in *P. furiosus* (Bevers *et al.*, 2006). In contrast to the ModABC transporters, which are present in the vast majority of bacteria, the WtpABC transporter appears to be the predominant ABC transporter for these substrates in archaea (Zhang & Gladyshev, 2008). WtpA, the solute receptor, binds tungstate in the low picomolar range, while the dissociation constant for molybdate is about 10^3-fold higher (Bevers *et al.*, 2006).

Bioinformatics identified a new subclass of putative molybdate transporters (ModA-like) in species of the hyperthermophilic archaeon *Pyrobaculum*. No experimental data on these systems are currently available (Zhang & Gladyshev, 2008).

A few nitrogen-fixing organisms produce an alternative nitrogenase containing FeVco (a vanadium-containing variant of the aforementioned FeMoco) when molybdenum is unavailable. The natural vanadium source vanadate is not transported by the known molybdate transport systems. A high-affinity vanadate ABC transporter, VupABC, was identified in the genome of the cyanobacterium *Anabaena variabilis* in the region near the genes encoding V-nitrogenase. It is not found in completely sequenced genomes from other prokaryotes in which a V-nitrogenase is known to operate. Mutational analysis revealed that a *vupB* mutant cannot produce V-nitrogenase activity at low concentrations of vanadate (Pratte & Thiel, 2006).

Polyamines

Polyamines, especially spermidine (NH_2-$(CH_2)_4$–NH-$(CH_2)_3$-NH_2) and putrescine (NH_2-$(CH_2)_4$–NH_2), both derived from arginine, are the major polycations in the cell and have long been associated with nucleic acid and protein biosynthesis and structure, cell growth and differentiation (Tabor & Tabor, 1985). Together with Mg^{2+}, polyamines can bind to intracellular polyanions such as nucleic acids and ATP. Other processes involving polyamines include incorporation into the cell wall, biosynthesis of siderophores or acid resistance (Wortham *et al.*, 2007). New results with an *E. coli* mutant carrying deletions of all genes involved in polyamine biosynthesis demonstrated a requirement of polyamines under strictly anaerobic conditions while no effect was observed under aerobic conditions (Chattopadhyay *et al.*, 2009).

Two ABC transport systems mediating the uptake of spermidine and putrescine in *E. coli* have been studied extensively (reviewed in Igarashi *et al.*, 2001). The PotABCD porter preferentially transports spermidine with a K_m value of 0.1 μM, but also accepts putrescine with a 15 times lower affinity. In contrast, the PotFGHI system is specific for putrescine ($K_m = 0.5$ μM). The crystal structures of the respective SBPs, PotD and PotF, have been resolved in complex with spermidine and putrescine, respectively. There is evidence that spermidine uptake is inhibited by excess of intracellular spermidine through binding to the C-terminal domain of the NBD, PotA (trans-inhibition). Expression of the *potABCD* genes is unusually repressed by the PotD precursor through binding to two regions close to the transcription initiation site of the operon.

A polyamine-binding lipoprotein with a preference for putrescine over spermidine was identified by structural analysis and binding studies in *T. pallidum*. Based on these data, together with the X-ray structures of the *E. coli* PotD and PotF proteins, the authors proposed several key residues that determine specificity for spermidine or putrescine (Machius *et al.*, 2007).

Maintenance of outer membrane integrity

Asymmetrical distribution of lipids in the outer membrane of gram-negative bacteria, with lipopolysaccharides in the outer leaflet and phospholipids in the inner leaflet, is thought to be crucial for the barrier function of the outer membrane (Nikaido, 2005). An ABC importer that is conserved in most gram-negative bacteria was proposed to play an important role in a newly discovered pathway for maintaining the lipid asymmetry of the *E. coli* outer membrane (Malinverni & Silhavy, 2009). Based on mutational analysis of the *mlaFEDCB* gene cluster, it was shown that deletion of *mlaC* or *mlaB* caused sensitivity of the cells against SDS/EDTA. Moreover, deletion of *mlaC* resulted in increased levels of palmitoylated lipid A, which reflects increased phospholipids in the outer leaflet of the outer membrane. The putative transporter consists of an SBP located at the periplasmic side of the cytoplasmic membrane via its uncleaved signal sequence, MlaD, a transmembrane subunit, MlaE, and an NBD, MlaF. A cytoplasmic protein of still unknown function, MlaB, is hypothesized to be a part of the transport complex. MlaC is a second SBP that is likely to bind phosphatidylethanolamine, as demonstrated by the crystal structure of an MlaC ortholog

(PDB ID: 2QGU). However, the structure bears no resemblance to canonical SBPs. A genetically unrelated gene, *mlaA*, encodes an outer-membrane lipoprotein of unknown function. The authors hypothesize that the transporter mediates retrograde phospholipid transport from the outer leaflet of the outer membrane to the cytoplasmic membrane (Malinverni & Silhavy, 2009). The evidence for the transporter as a virulence factor is summarized in Maintenance of cell integrity.

Chemotaxis

Escherichia coli, *S. typhimurium* and other motile bacteria respond to chemical gradients by moving toward higher concentrations of attractants and lower concentrations of repellents, a phenomenon known as chemotaxis. The signaling cascade mediating this behavior comprises chemoreceptor proteins localized to the cytoplasmic membrane that sense and transmit the presence of chemotactically active compounds. Sensing is achieved either by direct binding of the chemicals or indirectly via SBP components of ABC import systems. As a result, the chemoreceptors that are stable homodimers undergo a conformational change that is transmitted to a series of kinases, eventually leading to a change of the rotational state of the flagellar rotary motor (reviewed in Falke & Hazelbauer, 2001).

SBPs including those for maltose, ribose, glucose/galactose and dipeptides, respectively, are known to interact with the chemoreceptors Tar, Trg and Tap in case of attractant chemotaxis (Falke & Hazelbauer, 2001). The binding protein of the nickel ABC transporter of *E. coli*, NikA, was shown to be involved in the repellent reaction mediated by Tar (de Pina *et al.*, 1995). Maltose-binding protein (MalE) interacts with Tar, which also senses free aspartate, by two well-defined regions located on each domain of the protein. These regions partially overlap with those involved in contacting the MalFG transmembrane subunits. Mutational analysis and modeling of the interaction between MalE and the periplasmic domain of Tar based on available crystal structures suggested a mechanism by which this interaction could trigger a conformational change similar to aspartate (Zhang *et al.*, 1999). In case of ribose-binding protein, four distinct regions in the N- and C-terminal domains are involved in interaction with the transporter, the chemoreceptor, Trg, or both (Binnie *et al.*, 1992; Eym *et al.*, 1996).

Cell-to-cell communication and cell differentiation

Quorum sensing

Bacteria communicate with one another using secreted chemical signal molecules termed autoinducers in a process called 'quorum sensing' (Waters & Bassler, 2005; Williams *et al.*, 2007). While a large number of gram-negative bacteria produce acyl-homoserine lactones, gram-positive bacteria synthesize oligopeptide autoinducers. Furthermore, parallel quorum-sensing circuits are known that rely on derivatives of 4,5-dihydroxy-2,3-pentadione, some of which contain boron (AI-2), as signals. AI-2 plays a universal role as a signal used for interspecies communication in many gram-negative and -positive bacteria. In *S. typhimurium* and other enteric bacteria, an AI-2-specific ABC transporter, LsrABCD, operates, which displays homology to the ribose ABC transporter, RbsABC, of *E. coli* (Taga *et al.*, 2001). Expression of the encoding genes that are part of the *lsr* operon is repressed by a specific transcriptional regulator, LsrK, which becomes inactivated by binding of phosphorylated AI-2 due to the action of a cytoplasmic kinase, LsrK (Taga *et al.*, 2003; Xavier & Bassler, 2005). It is proposed that internalization and subsequent enzymatic inactivation of AI-2 causes a decline in the external concentration, which is indicative of a monospecies environment (Waters & Bassler, 2005).

The SBP LsrB of the gram-negative bacterium *Aggregatibacter actinomycetemcomitans*, a causative agent of periodontitis, was shown to differ from ribose-binding protein, RbsB, in the interaction with AI-2 from different organisms (James *et al.*, 2006; Shao *et al.*, 2007).

Competence and sporulation

Competence, which is the capability of cells to import DNA fragments from the environment, is a phenomenon largely distributed among gram-positive bacteria. Competence is not a constitutive trait, but is regulated on a community-wide scale by quorum sensing. In gram-positive bacteria, this process includes the secretion of short peptides (pheromones) acting either from the outside or after internalization by oligopeptide ABC transporters (Sturme *et al.*, 2002).

In *B. subtilis*, the Opp transporter encoded by *spo0K* takes up the competence-stimulating factor (CSF), one of two peptides known to be involved in competence. CSF is a pentameric peptide (ERGMT) and has different functions depending on its intracellular concentration. At low nanomolar concentrations, it stimulates the expression of genes involved in competence whereas at higher concentrations (up to 1 μM), it inhibits genes required for competence and stimulates the expression of genes required for sporulation (reviewed in Detmers *et al.*, 2001; Waters & Bassler, 2005). Another pentameric peptide, PhrA (ARNQT), is internalized by the Opp system in *B. subtilis* and acts as an effector of a transcriptional regulator in the phosphorelay signal transduction pathway that controls competence/sporulation.

In a recent study, the oligopeptide transporter called Ami in *Streptococcus thermophilus* was shown to be involved in the development of natural competence in a peptide-free medium (Gardan *et al.*, 2009). The authors demonstrated

that the Ami transporter controlled the expression of *comX*, encoding a peptide that regulated the transcription of competence genes. One out of two oligopeptide-binding proteins, AmiA3, is predominantly involved in this process.

Bacillus subtilis produces acetoin as an external carbon storage compound that is reused during the stationary phase and sporulation. Mutational analysis suggested that the products of the *ytrABCDEF* operon constitute an ABC transporter consisting of a solute-binding lipoprotein, YtrF, two transmembrane subunits, YtrCD, and two NBDs, YtrBE, which is required for the reuptake of acetoin (Yoshida *et al.*, 2000).

Conjugation

Conjugation is a mechanism of cell-to-cell transfer of certain plasmids, which, in *Enterococcus faecalis* and related bacteria, is initiated by the secretion of hydrophobic peptides by the recipient cells and internalization of these peptides by the donor cells. The peptides, also called sex pheromones, contain six to eight amino acids and are transported into the donor cells by components of the Opp system. However, a particular binding protein, PrgZ in case of plasmid pCF10, is involved, which, in contrast to OppA, displays a high specificity for the respective peptide (Leonard *et al.*, 1996). Another pheromone-binding protein, TraC, which shares a high sequence identity with PrgZ, delivers its peptide to an unknown ABC transporter because a strain lacking the *oppD* gene that encodes a nucleotide-binding subunit exhibited normal conjugative activity (Nakayama *et al.*, 1998).

Swarming

The process of bacterial swarming is distinct from swimming as it is a multicellular event that takes place on solid supports and requires the differentiation of vegetative cells into specialized swarmer cells (Rather, 2005). For *E. coli*, it was shown that swarming depends on polyamines such as spermidine (Kurihara *et al.*, 2009). A polyamine-deficient mutant carrying a deletion of the *potABCD* genes encoding the polyamine ABC transporter (see Polyamines) was shown to display a swarming-negative phenotype in the presence of exogenous spermidine. The result suggests a role of the transporter in the induction of swarming.

Multicellular differentiation in *Streptomyces*

Strains of the gram-positive, filamentous soil bacteria from the genus *Streptomyces* undergo complex morphological differentiation, resulting in the formation of sporulating aerial hyphae. Two basic classes of developmental mutants of *Streptomyces coelicolor* A3(2) are known: bald mutants (*bld*), which fail to produce fuzzy aerial hyphae, and white mutants (*whi*), which form aerial hyphae, but no gray-pigmented spores (Ohnishi *et al.*, 2002).

The *bldK* locus encodes an ABC transporter (BdlK-ABCDE) with sequence similarity to oligopeptide porters Opp from *S. typhimurium* and SpoOK of *B. subtilis*. Mutations in the *bldK* locus caused a bald phenotype and resistance of cells to the toxic peptide bialaphos, known to be internalized by oligopeptide transporters. It was suggested to mediate the uptake of an extracellular signal required for the formation of aerial hyphae (Nodwell *et al.*, 1996). BdlK and other putative oligopeptide ABC transporters are upregulated by exogenous *S*-adenosylmethionine (SAM), and documented to activate secondary metabolism in *Streptomyces* (Shin *et al.*, 2007).

An ABC transporter was demonstrated in *Streptomyces griseus* to be involved in the regulation of morphological differentiation in response to glucose (Seo *et al.*, 2002). It consists of a solute-binding lipoprotein, DasA, and two membrane-spanning subunits, DasBC, which are encoded by the *dasRABC* cluster. A *dasA* mutant failed to produce aerial hyphae, thus displaying a bald phenotype. Overexpression of *dasA* in a wild-type strain caused a severe defect in normal development in the presence of glucose, resulting in an early ectopic septation of young substrate hyphae and subsequent sporulation. The observation that overproduction of DasA was sufficient to cause the phenotype led to the proposal that DasA, besides a proposed role in substrate transport, might interact with a two-component sensor–regulator system under certain growth conditions (Seo *et al.*, 2002).

DasA was later identified as a component of the DasBC-MsiK ABC transporter mediating the uptake of chitobiose in *S. coelicolor* (Colson *et al.*, 2008; Saito *et al.*, 2008). MsiK is a nucleotide-binding protein that is shared with other sugar ABC transporters in *Streptomyces* (Schlösser *et al.*, 1997; Saito *et al.*, 2008) (see Carbon and energy sources). DasR, a transcriptional regulator also included in the *das* gene cluster, represses the expression of *dasA*. Colson *et al.* (2008) found induction of *dasA* expression by chitin, a readily available biopolymer, for soil-dwelling bacteria. A *dasA* mutant showed increased chitinolytic activity. It was proposed that DasA, in the absence of chitin, binds to the chitin sensor, ChiS, thereby preventing activation of the cognate regulator, ChiR, resulting in the chitinolytic system of the organism to be shut off. When the chitin-derived disaccharide chitobiose is available, DasA is engaged in its transport, and thus ChiS is relieved and the chitinolytic system will subsequently be turned on as a consequence of ChiR-P action. Furthermore, a role of DasABC in the control of development was suggested based on the observation that extracellular NAG arrests the cells in the vegetative growth phase due to a complex regulatory control involving the sugar-transporting PTS (Rigali *et al.*, 2006). In contrast,

development was normal in the presence of chitin, a polymer of NAG. The authors propose that DasABC-mediated uptake of chitobiose, internal hydrolysis to NAG and subsequent phosphorylation by a kinase bypass the PTS, and thus enable the latter to target developmental proteins for phosphotransfer (Colson *et al.*, 2008).

Response to environmental stress

Uptake of compatible solutes

Prokaryotes have developed mechanisms to maintain their cell volume and turgor pressure when facing sudden osmotic changes in the water content of their environment, termed 'osmoadaptation', 'osmoregulation' or 'osmoprotection'. Because of the lack of active transport systems for water, turgor can only be controlled by altering the concentration of osmotically active compounds in the cytoplasm, such as potassium ions and so-called 'compatible solutes' (Wood, 1999). The latter include certain sugars, polyols, amino acids and their derivatives, quartenary amines and others (Welsh, 2000). The uptake of K^+ ions is often an immediate first response to an increase in environmental osmolarity, which is followed by synthesis or, energetically favored, internalization of compatible solutes mediated in many prokaryotes at least in part by specific ABC transporters belonging to the QAT family of the TC system (Wood, 1999).

The prototype of an ATP-driven glycine betaine uptake system is the ProVWX transporter, encoded by the *proU* operon of *E. coli*. While proline betaine is likewise a major substrate, proline and ectoine are much poorer substrates as is also reflected by the binding properties of the periplasmic receptor, ProX. The transporter is indirectly induced by high osmolality (reviewed in Csonka & Epstein, 1996). Crystal structures of ProX in complex with glycine betaine and proline betaine, respectively, have been reported (reviewed in Horn *et al.*, 2005).

The best-characterized ABC transporter mediating the uptake of the compatible solutes glycine betaine and proline, the latter with a low affinity, as a response to high-osmolarity growth conditions is the OpuA system of *L. lactis* (reviewed in Poolman *et al.*, 2004). The transporter, which was intensively studied at the level of purified proteins in proteoliposomes, is composed of an unusual transmembrane subunit to which a solute-binding domain is fused, OpuAB, and an ATP-binding subunit, OpuAA (van der Heide & Poolman, 2002). Moreover, the transporter is known to sense intracytoplasmic ionic strength through tandem cystathionine-β-synthase (CBS) domains fused to the C-terminus of OpuAA (Biemans-Oldehinkel *et al.*, 2006b). An anionic surface of the membrane is also required. Mutational analysis identified residues from both a surface-exposed cationic region of the CBS domain as well as of its C-terminal anionic tail to be crucial for an electrostatic switching mechanism that was proposed to underlie the activation/inactivation cycle of the transporter (Mahmood *et al.*, 2006, 2009). According to this proposal, the transporter is locked in an inactive state by interaction of the CBS domains with the membrane under 'normal' physiological conditions. Upon an increase in ionic strength as a consequence of an osmotic upshift, the CBS domain is repulsed from the membrane, thereby turning on the transporter's activity (Biemans-Oldehinkel *et al.*, 2006b). It was also speculated that the CBS domains might contribute to OpuAA dimer formation (Biemans-Oldehinkel *et al.*, 2006b) as it was shown for the C-terminal domain of MalK of the maltose transporter (Chen *et al.*, 2003).

The gram-positive soil bacterium *B. subtilis* is equipped with five active transport systems involved in the uptake of compatible solutes. Among these are two ABC transporters with specificities for choline (OpuB), and proline betaine/glycine betaine (OpuA) and a third ABC transporter exhibiting a rather broad specificity range (OpuC). The OpuA system displays homology to the ProU ABC transporter of *E. coli* and the OpuA transporter of *L. lactis*, and transcription of the encoding genes that are organized in an operon is induced under high-osmolarity growth conditions. OpuA is composed of an SBP, OpuAC, a transmembrane subunit, OpuAB, and a nucleotide-binding subunit, OpuAA (reviewed in Horn *et al.*, 2005). OpuAC binds glycine betaine with a K_d of 19 μM while the dissociation constant for proline betaine is about 10 times higher. Crystal structures of OpuAC in complex with both substrates are available, revealing a missing hydrogen bond in the structure with proline betaine as a possible explanation for the differences in affinity. Coordination of the quaternary trimethylammonium group of glycine betaine is achieved by cation-stacking involving conserved Trp residues, slightly different from the arrangement found in the *E. coli* homolog, ProX (Horn *et al.*, 2006). The transporter was intensively characterized biochemically. Results on monomer/dimer formation of the purified OpuAA protein (reviewed in Horn *et al.*, 2005) contributed to the alternating access model of ABC importers (see The 'alternating access'-model of transport). Because OpuAA of *B. subtilis* also contains the C-terminal CBS domains as does OpuAA from *L. lactis*, their contribution to the dimerization of OppAA cannot be excluded (Horn *et al.*, 2005; Mahmood *et al.*, 2006).

In the gram-negative bacterium *P. syringiae*, a plant pathogen, an OpuC transporter was identified by uptake experiments with salt-stressed intact cells as the sole or the predominant porter involved in osmoregulation (Chen & Beattie, 2007). OpuC is more closely related to the *Bacillus* system than to osmoprotectant transporters from other gram-negative bacteria. It displays a high affinity for

glycine betaine and a broad substrate specificity, accepting choline, acetylcholine, carnitine and proline betaine. Like nucleotide-binding subunits from other Opu systems, the OpuCA subunit contains tandem CBS domains and a C-terminal tail. However, unlike in OpuAA of *L. lactis*, the C-terminal tail of the *P. syringae* protein and those from other *Pseudomonas* sp. is positively charged. Thus, the mechanism by which ionic strength is sensed by the CBS domains might differ between organisms (Chen & Beattie, 2007).

Two rather than one transporter, Ota and Otb, with homology to the OpuA system of bacteria were identified in the methanogenic archaeon *Methanosarcina mazei* Gö1. The Ota system is composed of a substrate-binding protein, OtaC, a transmembrane subunit, OtaB, and the ATPase, OtaA (Spanheimer *et al.*, 2008). In addition to genes encoding the A and C components, the *otb* operon contains two genes coding for a transmembrane subunit (Saum *et al.*, 2009). When overproduced in an *E. coli* strain lacking a glycine betaine transport system, the Ota transporter exhibited high-affinity glycine betaine uptake with a K_m around 10 μM and was activated only by salinity gradients (Schmidt *et al.*, 2007). This finding is in line with the observation that *M. mazei* accumulates glycine betaine in response to hypersalinity. The Ota system was found by genetic analysis to be the only glycine betaine transporter of *M. mazei*. The function of Otb remains elusive (Saum *et al.*, 2009).

In *S. meliloti*, an ABC transporter, EhuABCD, was identified that is induced by ectoine (a tetrahydropyrimidine) and hydroxyectoine, but not by glycine betaine or high osmolality (Jebbar *et al.*, 2005). The transporter displays similarity to amino acid porters of the PAAT family. The SBP EhuB binds ectoine and hydroxyectoine with similarly high affinity (K_d values 1.6 and 0.5 μM, respectively; Hanekop *et al.*, 2007). The crystal structures of EhuB in complex with both substrates were determined and revealed a ligand coordination by cation-stacking involving aromatic amino acid residues as has been found for ProX from *E. coli* and OpuAC from *B. subtilis* with bound glycine betaine (Hanekop *et al.*, 2007). Mutational analysis of key residues from the binding site confirmed their importance in ligand interaction, although most residues were more tolerant to changes than observed in ProX. This result was attributed to an additional stabilization of the delocalized positive charge of the ligands by salt bridges and hydrogen bonds, which is missing in ProX.

ABC transporter involved in response to pH stress

A regulatory role of a putative sugar ABC transporter (CUT2 family), encoded by the *supABCD* operon, in potassium transporters of *S. meliloti* was suggested in the context of alkaline stress (Lin *et al.*, 2009). A *supA* mutant lacking the predicted SBP is impaired in survival at pH 10, which is enhanced in the presence of high levels of potassium ions. Expression of potassium transport systems and putative cation/proton antiporters, thought to be involved in alkaline resistance, was reduced in the mutant. How the SupABCD system might regulate the potassium transporters remains elusive.

A glutamate transporter was found by BLAST search in the oral lactic acid bacterium *S. mutans* using glutamine transporter genes as a query. Accordingly, the transporter genes were termed *glnQHMP*, encoding two transmembrane subunits, GlnMP, an ATPase, GlnQ, and a solute-binding protein, GlnH. Competition experiments with intact cells revealed that, besides glutamate, glutamine, aspartate and cysteine might also be transported (Krastel *et al.*, 2010). In the presence of glutamate, wild-type strain UA159 showed increased production of lactate and displayed a decreased acid-tolerance response (ATR). The latter finding was interpreted to mean that the cells might have difficulties in coping with higher 'acid load' due to glutamate degradation (via phosphoenol pyruvate) by the glycolytic pathway. The observation that a *glnQHMP* deletion mutant produced less lactate in the presence of glutamate and exhibited increased ATR was taken as evidence for this notion. Furthermore, transcriptional downregulation of the transporter genes at acidic pH is also consistent with a link between glutamate transport and ATR (Krastel *et al.*, 2010).

ABC importers as virulence factors in pathogens

ABC importers play various roles in the survival of pathogens within mammalian hosts. Moreover, components of ABC importers are candidates for the development of vaccines and might be targets for novel antimicrobials (Garmony & Titball, 2004).

Uptake of nutrients from the host

Amino acids. The uptake of certain amino acids from the host has been implicated in bacterial virulence. *Neisseria meningitidis* is a colonizer of nasopharyngeal mucosa in about 10% of healthy humans, which, in a few cases, may cause meningitis and sepsis. Genome analysis identified a gene cluster encoding a putative four-component ABC transporter for the uptake of glutamate, termed GltT. A mutant strain (serogroup B) deficient in the integral membrane subunit (NMB 1965) displayed reduced glutamate transport at a low sodium concentration and was essential for growth in infected epithelial cells (Monaco *et al.*, 2006). In a mouse model, a mutation inactivating the homologous protein (NMC1937) of a serogroup C strain caused an attenuated phenotype in mixed infections, indicating that

the wild-type bacteria outcompeted the GltT-deficient cells (Colicchio *et al.*, 2009). Similarly, a mutant with a defective ATP-binding protein (NMB1966) was also demonstrated to be crucial for virulence *in vivo* (Li *et al.*, 2009). Glutamate may be required as a precursor for the synthesis of the antioxidant glutathione.

Besides glutamate, glutamine is a primary nitrogen source of *S. enterica* serovar Typhimurium. Together with glutamine synthetase, the glutamine ABC transporter GlnHPQ (> 95 sequence identity with the *E. coli* system; see Carbon and nitrogen sources) was shown by mutational analysis to be essential for virulence of the organism. While a single mutation in *glnH*, encoding the periplasmic-binding protein, allowed full virulence in mice, a combination with a mutation in *glnA*, encoding glutamine synthetase, caused an attenuated phenotype. Moreover, the double mutant protected mice against a subsequent challenge with wild-type bacteria (Klose & Mekalanos, 1997).

In *N. gonorrhoeae*, two putative glutamine ABC transporters displaying homology to the Gln system were shown to be transcriptionally upregulated twofold in a *pilT* mutant deficient in a protein required for retraction of type IV pili that triggers responses of epithelial cells during infection. Glutamine reversed downregulation of the transporter genes by PilT. The physiological meaning of this observation is not yet understood (Friedrich *et al.*, 2007).

In group B streptococci, which cause neonatal sepsis and meningitis, transposon mutagenesis inactivating the *glnQ* gene of a glutamine transporter, GlnPQ, resulted in decreased adherence to and invasion of respiratory epithelial cells *in vitro* and in decreased virulence *in vivo* (Tamura *et al.*, 2002). GlnP is an unusual fusion protein composed of two solute-binding domains, followed by a TMD (van der Heide & Poolman, 2002), while GlnQ is the ATPase subunit. Glutamine transport was demonstrated to be strongly impaired in the *glnQ* mutant. The role of GlnQ in adhesion remains elusive (Tamura *et al.*, 2002).

Disruption of genes encoding nine ABC transporters in *S. pneumoniae* resulted in identification of the LivJHMGF transporter as a virulence factor. The SBP was demonstrated by binding assays to accept leucine, isoleucine and valine as substrates, which is in agreement with its sequence similarity to the *E. coli* branched-chain amino acid transporter. A deletion strain showed reduced virulence in pneumonia and septicemia models when tested by competitive infection (Basavanna *et al.*, 2009).

In the gram-positive bacterium *Listeria monocytogenes*, a food-borne pathogen, the product of the *imo0135* gene was shown to contribute to virulence in a murine model of intravenous infection. The protein, termed CtaP, displays homology to oligopeptide-binding proteins, but was identified by mutational analysis to be required for high-affinity cysteine uptake. CtaP was also shown to contribute to host cell adhesion, colonization of the gastrointestinal tract of mice and acid resistance (Xayarath *et al.*, 2009).

Phosphate. The high-affinity phosphate transporter, PstSCAB, has been implicated in the virulence of both extracellular and invasive pathogenic bacteria, such as avian pathogenic *E. coli* (APEC) (Lamarche *et al.*, 2005), *Proteus mirabilis* (Jacobsen *et al.*, 2008; O'May *et al.*, 2009), *Shigella flexneri* (Runyen-Janecky *et al.*, 2005) and *M. tuberculosis* (Peirs *et al.*, 2005). Strains carrying mutations in the periplasmic-binding protein, PstS, but also in other transporter components are generally attenuated in virulence. The suggested roles of Pst include regulation of invasion, resistance to cationic antimicrobial peptides, colonization and biofilm formation (recently summarized in Lamarche *et al.*, 2005).

Iron. Pathogenic bacteria have developed three mechanisms for acquiring iron from the environment involving ABC import systems: (1) secretion of siderophores to chelate iron and their subsequent uptake as it is widely found in nonpathogens, (2) capturing iron directly from storage molecules of the host and (3) uptake of iron from free heme or heme-containing proteins (Tong & Guo, 2009). Usually, the genetic equipment of pathogenic bacteria allows more than one mechanism to operate.

In gram-negative pathogens, the synthesis and secretion of several siderophores are generally observed, which require uptake into the periplasm by a specific outer-membrane receptor by a TonB-dependent mechanism. The subsequently operating ABC transporters share sequence similarities to Fhu, Fec and Fep transport systems described for *E. coli* (see Iron). For example, the ViuPDGC system, identified in *Vibrio cholerae*, the causative agent of cholera, is required for the internalization of catechol siderophores such as vibriobactin and enterobactin. The transporter consists of the periplasmic-binding protein, ViuP, two membrane-integral subunits, ViuDG, and ATPase, ViuC, which display the highest sequence identity to the Fep system. Interestingly, ViuP was demonstrated to be a lipoprotein (Wyckoff *et al.*, 1999). In pathogenic *E. coli* strains derived from human isolates, a putative iron uptake system, FitABCDE, was identified by an *in vivo* expression technology protocol. Although expressions of the encoding genes were found to be induced by iron limitation, its precise role including the transported substrate remained unclear (Ouyang & Isaacson, 2006).

Siderophores are also produced and secreted by gram-positive pathogens. *Staphylococcus aureus*, an opportunistic human pathogen of surgical wounds and implanted medical devices, produces two polycarboxylate-type siderophores termed staphyloferrin A and B [the latter might be identical to staphylobactin (see Beasley *et al.*, 2009)]. Staphyloferrin A is internalized by the HtsABC transporter as demonstrated by

uptake experiments. Although the HtsABC transporter had previously been implicated in heme acquisition, a deletion strain showed no growth defect in the serum (Beasley *et al.*, 2009). The crystal structure of the solute-binding component HtsA identified it as a member of class III receptors with similarities to other siderophore- and heme-binding proteins. The *htsABC* operon lacks a gene encoding an ATPase component, but results from mutational analysis suggested that FhuC of the iron-hydroxamate transporter FhuCBG also assists in staphyloferrin A uptake (Beasley *et al.*, 2009). Staphyloferrin B (staphylobactin) was shown to require the SirABC transporter, consisting of a binding protein, SirA, and two membrane-integral subunits, SirBC, for internalization (Dale *et al.*, 2004). Again, experimental evidence indicated that FhuC is also shared by the Sir system. Moreover, an *fhu* deletion strain had decreased virulence in a mouse model (Speziali *et al.*, 2006). A third putative siderophore transporter, SstABCD, has been found in *S. aureus*, but the chemical nature of its substrate(s) remained elusive (Morrissey *et al.*, 2000).

In *M. tuberculosis*, the causative agent of tuberculosis, the *irtAB* system, when mutated, decreased the ability of *M. tuberculosis* to survive under iron-deficient conditions *in vitro* as well as in human macrophages or mouse lungs (Rodriguez & Smith, 2006). The authors postulated a role of IrtAB as an importer for siderophores. This view was subsequently challenged by (Farhana *et al.*, 2008), who identified IrtA as an exporter for carboxymycobactin, while IrtB was shown to mediate the transport of iron-loaded carboxymycobactin both *in vivo* and in proteoliposomes. Interestingly, IrtB is composed of a TMD that is C-terminally fused to an NBD. The protein Rv2895c was demonstrated by a pulldown assay to interact with IrtB as a ferrated siderophore-binding protein (Farhana *et al.*, 2008). Most recent *in vivo* experiments by the Rodriguez lab, which also indicated an FAD-binding domain in the N-terminus of IrtA, were taken as further evidence in favor of their original proposal of IrtAB as a heterodimeric importer (Ryndak *et al.*, 2010). Thus, the precise role of IrtA is still a matter of debate.

Some gram-negative pathogens, including *H. influenzae*, *Neisseria* sp., *Mannheimia haemolytica* and others, deprive iron ions from host-storage proteins such as transferrin and lactoferrin by a siderophore-independent mechanism. The system involves a specific outer-membrane receptor that binds and releases iron from proteins directly. Uptake of free iron into the cytoplasm requires transport to the periplasm by a TonB-dependent process and subsequent translocation across the cytoplasmic membrane via an ABC transporter. The latter is usually composed of a ferric iron-binding protein, FbpA, a transmembrane subunit, FbpB, and an ATPase, FbpC. When assayed in an *E. coli* strain deficient in siderophore synthesis, the *H. influenzae* FbpABC transporter displayed an apparent K_m for Fe^{3+} uptake of 0.9 μM. The transporter prefers trivalent (Fe^{3+}, Ga^{3+}, Al^{3+}) over divalent cations (Anderson *et al.*, 2004). The crystal structure of hFbp(A), the first of its kind, revealed coordination of Fe^{3+} by two conserved tyrosines: one histidine and one glutamate residue. In addition, a water molecule and an anion (phosphate) are part of the binding site. An anion (carbonate) involved in ligand binding is present in human transferrin as well, suggesting a common evolutionary origin of both protein families, which also include ferric ion-binding proteins from nonpathogens, such as cyanobacteria (see Iron) (Bruns *et al.*, 2001). The synergistic anion, together with the arrangement of the other ligand-binding residues, is thought to be responsible for the extremely high affinity for ferric ions (Bruns *et al.*, 2001). For recombinant Fbp(A) from *Neisseria*, the effective binding constant for ferric ion was determined to be in the order of 10^{18} per mol, similar to that of transferrin (Taboy *et al.*, 2001). A carbonate ion was also found in *Mycobacterium haemophila* FbpA, but otherwise, the ligand is bound only by three tyrosine residues. Comparison with the unliganded open form suggested a role of carbonate in modulating the overall conformation of the protein (Shouldice *et al.*, 2004). Q10

Less knowledge exists on transport systems for free iron in gram-positive pathogens. Most recently, the crystal structure of a binding lipoprotein, MtsA, a component of an ABC transporter, MtsABC, of *Streptococcus pyogenes*, the causative agent of scarlet fever, was reported (Sun *et al.*, 2009). The structure was refined at a 1.8 Å resolution and the bound metal was assumed to be Fe^{2+}. The ligand-binding site includes two histidines, a glutamate and an aspartate, as it is characteristic for Fe–Mn-specific receptors (Claverys, 2001). Furthermore, a bicarbonate ion participates in the coordination of the metal. Binding studies confirmed a 10-fold preference for Fe^{2+} over Mn^{2+}. Mutational analysis revealed that the glutamate residue within the binding site is dispensable (Sun *et al.*, 2009) (see Trace elements).

Utilization of heme, a tetrapyrrole complexing a ferrous ion, and its oxidized form, hemin, as iron sources has mostly been studied in gram-negative bacteria. Hemolysins, secreted by the bacteria, lyse erythrocytes thereby releasing hemoglobin, which, then in a complex with serum haptoglobin, is delivered to the liver for degradation. Heme, after being released from hemoglobin, is sequestered by hemopexin or serum albumin. At least two mechanisms of heme acquisition that differ in their initial step are known: (1) Heme-containing proteins or free heme are bound directly to a specific outer membrane receptor or (2) extracellular hemophores bind free heme or extract heme from carrier proteins and shuttle it to hemophore-specific outer-membrane receptors (reviewed in Cescau *et al.*, 2007). Subsequently, transfer to the periplasm is achieved by a TonB-dependent process, followed by internalization via an ABC transporter. Once in the cytoplasm, heme is bound by a specific binding protein and

delivered to an oxygenase, which opens the porphyrin ring, yielding free iron (Tong & Guo, 2009).

In *P. aeruginosa*, the Phu system operates, which represents a well-studied model for direct heme uptake. It involves the outer-membrane heme receptor, PhuR, a periplasmic-binding protein, PhuT, and an ABC transporter, composed of an integral membrane protein, PhuU and an ATPase, PhuV. In addition, a cytoplasmic heme-binding protein, PhuS, is required for heme storage (Ochsner *et al.*, 2000; Tong & Guo, 2009). Crystal structures of PhuT and of its homolog, ShuT, from *Shigella dysenteriae*, have been resolved and identified the proteins as members of class III of periplasmic-binding proteins (Ho *et al.*, 2007). In both proteins, heme is coordinated by a tyrosine residue, although the architecture of the binding site is somewhat different between PhuT and ShuT. Structurally, PhuT/ShuT resemble BtuF more than other class III members, which might reflect ligand similarity. Apo-PhuT binds heme with a K_d of ~1.2 nM (Tong & Guo, 2009). The ShuUV transport system was purified and reconstituted into proteoliposomes (Burkhard & Wilks, 2008). Basal ATPase activity was observed with ShuT enclosed in the lumen of the vesicles, which was about fourfold increased in the presence of heme. Moreover, transport of heme as monitored by binding to the (cytoplasmic) heme-binding protein ShuS occurred concomitantly with ATP hydrolysis, providing clear evidence for a functional heme transport system.

A (direct) heme transport system (HugBCD) from *Pleisiomonas shigelloides*, a gram-negative bacterium that causes diarrheal disease in humans, was recently shown to enhance recombinant hemoglobin production when overexpressed in *E. coli*. Such studies are aimed to establish an alternative to human blood for patients requiring blood transfusions (Villarreal *et al.*, 2008).

Heme utilization was studied only in a few gram-positive pathogens, with the Isd system of *S. aureus* and the Hts (Sia) system of *S. pyogenes* being the best understood. In *S. aureus*, hemoglobin and haptoglobin–hemoglobin are first bound to specific cell surface receptors, from which heme is extracted and passed to a cell wall protein, subsequently delivering it to an ABC transporter. The latter is composed of a binding lipoprotein, IsdE, an integral membrane protein, IsdF, and an ATPase, IsdD (Tong & Guo, 2009). The crystal structure of soluble IsdE in complex with heme revealed a class III receptor, with the heme iron being uniquely coordinated by a methionine and a histidine residue. The histidine residue was shown by mutational analysis to be essential for the IsdE-mediated growth of *S. aureus* on heme as the sole iron source. Unlike PhuT/ShuT of gram-negative bacteria, IsdE is structurally rather distantly related to BtuF (Grigg *et al.*, 2007).

In *S. pyogenes*, only two surface proteins with little sequence similarity to the Isd proteins, Shr and Shp, deliver heme to the solute-binding lipoprotein component HtsA (SiaA) of the ABC transporter HtsBC (SiaBC) (Lei *et al.*, 2003; Nygaard *et al.*, 2006). Characterization of HtsA by biophysical means suggested that heme is six-coordinate with methionine and histidine as axial ligands, which is similar to IsdE, but different from PhuT (Sook *et al.*, 2008).

Manganese, zinc. It is well established that ABC transporters mediating the uptake of manganese and zinc are of importance for pathogenic bacteria during infection of host tissues in which the concentrations of these metals are generally low (Claverys, 2001).

The first systems that have been described in this respect were the AdcCBA and PsaABC of *S. pneumoniae* that transport Zn^{2+} and Mn^{2+}, respectively (Dintilhac *et al.*, 1997; Novak *et al.*, 1998) (but see discussion in Trace elements). The crystal structure of the solute-binding lipoprotein PsaA revealed a novel structural type of SBPs, termed class III receptors (reviewed in Claverys, 2001) (see Solute-binding proteins). Orthologs of the Psa system were subsequently identified in other *Streptococci*. In *S. mutans*, which is associated with dental caries and human endocarditis, a putative Mn/Fe transporter, composed of a solute-binding lipoprotein, SloC, a membrane-integral subunit, SloB, and an ATPase, SloA, was shown to be required for endocarditis virulence in a rat model. Growth experiments and transport assays using intact cells revealed a preference for Mn^{2+} over Fe^{2+} and repression of the system by Mn^{2+} only, suggesting that the latter is the physiological substrate (Paik *et al.*, 2003).

A similar system, MtsABC, was found in *S. pyogenes* (Janulczyk *et al.*, 1999) and characterized at the level of intact cells and mutants. An *mtsABC* mutant displayed increased susceptibility to reactive oxygen species due to the reduced activity of manganese-dependent superoxide dismutase (Janulczyk *et al.*, 1999).

In *S. enterica* serovar Typhimurium, the SitABCD transporter, composed of the periplasmic-binding protein, SitA, two membrane-integral subunits, SitCD, and an ATPase, SitB, is encoded on a pathogenicity island and its mutation attenuates the virulence of the bacterium. Transport assays revealed an apparent affinity constant of ~0.1 μM and an optimal activity at a slightly alkaline pH (Kehres *et al.*, 2002). The Sit system also transports Fe^{2+}, but the physiological relevance of this activity is controversially discussed (Boyer *et al.*, 2002; Kehres *et al.*, 2002). Together with the pmf-driven manganese transporter MntH, SitABCD also contributes to resistance to oxidative stress (Boyer *et al.*, 2002). Similarly, the Sit transporter, which is encoded on a conjugative plasmid in the APEC O78 strain χ7122, was shown to contribute to virulence in a chicken infection model using isogenic mutants (Sabri *et al.*, 2008). In *N. gonorrhoeae*, the etiological agent of the sexually transmitted disease gonorrhea, the MntABC transporter was shown by mutational analysis to be required for the intracellular

survival of the bacteria in a human cervical epithelial cell model and also showed reduced ability to form a biofilm. The solute-binding lipoprotein, MntC, binds Mn^{2+} and Zn^{2+} with equal dissociation constants (~0.1 μM), similar to those described for the TroA protein of *T. pallidum*. The specificity for both metal ions is in line with the apparent lack of other class III receptors in the genome of *N. gonorrhoeae* (Lim *et al*., 2008).

In *S. pneumoniae*, a second Zn-binding protein, besides the AdcA of the AdcCBA transporter, was identified by whole-genome analysis (Loisel *et al*., 2008). AdcAII is not genetically included in a classical ABC operon structure and displays only ~30% sequence identity to AdcA and PsaA. The crystal structure in complex with one Zn^{2+} was resolved and revealed a tetrahedral coordination geometry composed of three histidine and one glutamate residue. Together with results from binding experiments using biophysical means, a role of AdcAII as a Zn^{2+}-binding protein was suggested. *adcAII* is colocalized with two *pht* genes encoding proteins of an as yet unknown function, which, however, induce immune protection in animal models. Pht proteins (also termed Htp) only exist in *Streptococci* and contain several His-triad motifs that might form potential binding sites for Zn^{2+} delivered by AdcAII. Thus, a role of AdcAII in Zn^{2+} homeostasis was postulated (Loisel *et al*., 2008; Linke *et al*., 2009).

In *S. pyogenes*, a homolog of AdcAII, Lbp (or Lsp) was identified as a zinc receptor that also binds to laminin, a highly glycosylated multidomain protein found in all human tissues (Linke *et al*., 2009). Adhesion to laminin by *S. pyogenes* is an initial step in infection. The crystal structure of Lbp shows one Zn^{2+} coordinated as in AdcAII. These results are corroborated by a study demonstrating that mutations in Lsp affecting the Zn-binding pocket cause attenuated virulence in a murine soft tissue infection model (Weston *et al*., 2009). Although genetically not included in an operon encoding an ABC transporter, a role of Lbp/Lsp as the binding protein component of a Zn transporter is suggested.

The zinc transporter, ZnuABC, of *S. enterica* serovar Typhimurium is required for virulence as has been demonstrated by an increase in the 50% lethal dose of a *znuC* mutant, defective in the ATPase subunit, when inoculated orally or intraperitoneally in mice (Campoy *et al*., 2002). Furthermore, it was shown that a *znuA* mutant, lacking the SBP, is impaired in its ability to grow in epithelial cells (Ammendola *et al*., 2007). Similar results were reported in a mutational study on the role of the ZnuABC transporter in a uropathogenic *E. coli* strain (Sabri *et al*., 2009). Most recently, ZinT (formerly known as YodA), which is induced under zinc starvation conditions, was shown to assist ZnuA in zinc recruitment in *S. typhimurium* (Petrarca *et al*., 2010). ZinT is homologous to the N-terminus of ZnuA and other Zn-binding proteins, including AdcA from streptococci, and forms a binary complex with ZnuA *in vitro*. Together, the data suggest a role of the ZnuABC transporter in environments poor in zinc or with limited zinc availability such as the intracellular compartment of host cells.

Determinants of resistance against antimicrobial peptides and bile

Small cationic peptides with antimicrobial properties are produced by a variety of animal species, including humans. These peptides are responsible for a potent, nonspecific humoral immune response against invading microorganisms. Although differing in length and amino acid sequence, many of these peptides attack pathogens by the same postulated mode of action, that is, by destabilizing the cytoplasmic membrane and, in case of gram-negative species, also the outer membrane. Successful pathogens have evolved mechanisms to withstand the antibiotic activity of these molecules (Otto, 2009).

In *S. enterica* serovar Typhimurium, the *sap* operon (sensitivity to antimicrobial peptides) is required for virulence and resistance to antimicrobial peptides such as protamine and melittin (Groisman *et al*., 1992). The gene products, including an SBP, SapA, two membrane-integral subunits, SapBC, and two nucleotide-binding subunits, SapDF, display homology to oligopeptide ABC transporters (Parra-Lopez *et al*., 1993). It was proposed that host defense peptides are internalized by the transporter, followed by degradation in the cytoplasm (Groisman, 1994). In a subsequent study, Parra-Lopez *et al*. (1993) identified the *sapG* locus of *S. typhimurium*, also found to be required for protamine resistance, as being identical to the *E. coli* TrkA protein, implicated in potassium transport.

The role of the *sap* operon in *E. coli* remains unknown. However, it was shown that the ATP-binding subunit SapD is recruited by the potassium transport systems Trk^H and Trk^G, thereby explaining the dependence of both systems on the proton-motive force and ATP. Mutational analysis suggested that ATP binding rather than ATP hydrolysis is sufficient to promote potassium transport activity (Harms *et al*., 2001). Moreover, Stumpe & Bakker (1997) demonstrated that in *E. coli* K-12, this Trk-related function is responsible for protamine resistance, thereby challenging the above proposal by Groisman and colleagues. According to their hypothesis, protamine causes the leakage of potassium from the cytoplasm by forming a channel, but the cells can be rescued by high potassium influx until the peptide is detoxified.

The *sap* operon was also studied in nontypeable *H. influenzae* and shown to be upregulated upon exposure of the cells to antimicrobial peptides (Mason *et al*., 2006). The organism is a nasopharyngeal commensal, but also an opportunistic invader of the middle ear space, where it is likely to

become exposed to antimicrobial peptides (AMPs). It was further demonstrated that purified recombinant SapA binds to AMPs and that SapD is required for both resistance and potassium transport. The authors conclude that SapABCDE is likely an importer for AMPs to be degraded internally, but also emphasize a role of SapD in potassium uptake as hypothesized by Stumpe & Bakker (1997) for *E. coli*.

A transporter of unknown cellular function, YejABEF, was found in *E. coli* that is responsible for uptake and thus sensitivity against the translation inhibitor microcin C, a peptide–nucleotide antibiotic (Novikova *et al*., 2007). Mutations in any of the four components resulted in a microcin-resistant phenotype. The transporter with YejA being an SBP, YejBE, the transmembrane subunits and YejF, an ATPase is related to oligopeptide porters. It is also the target of regulation by the small RNA RydC at the mRNA level (Antal *et al*., 2005). RydC degrades the message that was found to be associated with growth defects on certain sugars.

In *S. typhimurium*, the *yejABEF* operon was shown to be upregulated in host cells and to confer resistance to AMPs (Eswarappa *et al*., 2008). A *yejF* deletion strain exhibited higher sensitivity to protamine, melittin and polymyxin B, a phenotype that was not observed with a Ä*yejA* strain. The capability of the Ä*yejF* strain to proliferate in activated macrophages and epithelial cells was compromised and it was attenuated for survival in a murine model of typhoid fever. The authors suggest that the transporter might play a role in resistance by mediating the uptake of AMPs for subsequent internal degradation, similar to the model proposed for the Sap complex. However, the missing phenotype of the Ä*yejA* mutant points to a specific function of the ATPase subunit YejF, reminiscent of that of SapD for the potassium transporter Trk^{H} in *E. coli* (Stumpe & Bakker, 1997).

A transporter exhibiting sequence similarity to betaine ABC transporters was found in *L. monocytogenes* (Sleator *et al*., 2005). The BilE proteins also share a modular organization with the Opu system of *L. lactis*, that is, besides a putative ATPase, BilA, it consists of a fusion of the transmembrane and solute-binding domains, BilEB. Although transcriptional upregulation was observed in response to salt and low-temperature stress, the authors found no evidence for osmolyte transport. Rather, the transporter seems to confer resistance to bovine bile (oxgall) as suggested from a bile-sensitive phenotype, a *bilE* mutant. Furthermore, wild-type *L. monocytogenes* cells accumulated lower levels of radiolabeled bile than the mutant, leading the authors to propose a role in bile exclusion by an unknown mechanism.

Oligopeptide transporters

An oligopeptide-binding protein (Hly-OppA) was identified in *V. furnissii*, thought to cause acute gastroenteritis in humans, which exhibits hemolytic activity (Wu *et al*., 2007). The protein was purified and found to share high sequence identity with orthologs from other *Vibrio* sp. and to contain the signature sequence of cluster 5 SBPs (Tam & Saier, 1993). Binding of a nonapeptide by the purified protein and Hly-OppA-dependent accumulation of fluorescent substrates in intact cells were taken as evidence for a functional oligopeptide-binding protein. Deletion of the encoding gene caused less mortality of BALB/c mice and also effected biofilm formation.

In *Vibrio fluvialis*, a halophilic human pathogen, an *opp* gene cluster was identified that appears to be involved in biofilm formation. A mutation in the *oppA* gene encoding the oligopeptide-binding component, resulted in the formation of much more biofilm compared with the wild type for currently unknown reasons (Lee *et al*., 2004).

Osmoregulation

In *M. tuberculosis*, mRNA levels of *proXVWZ*, encoding a glycine betaine transporter, are elevated in response to host interactions, such as phagocytosis. A deletion strain is impaired in the accumulation of betaine under conditions of osmotic stress, resulting in a reduced growth within human macrophages. It was further shown that the ProXVWZ transporter is the means by which *M. tuberculosis* acquires betaine from the host cells (Price *et al*., 2008).

Maintenance of cell integrity

The recently discovered Mla system, encompassing the ABC importer MlaDEF, was suggested to be involved in maintaining the lipid asymmetry in *E. coli* (Malinverni & Silhavy, 2009) (see Maintenance of outer membrane integrity). Orthologs are present in pathogenic bacteria, including enteroinvasive *E. coli*, *S. flexneri* and *Burkholderia pseudomallei*. In *S. flexneri*, VacJ, the ortholog of the outer-membrane protein MlaA, was found to be essential for intercellular spreading of the bacteria within the host (Suzuki *et al*., 1994), leading to the speculation that an intact outer membrane is a prerequisite for escape from the host double membrane (Malinverni & Silhavy, 2009).

In *Haemophilus ducreyi*, the causative agent of a sexually transmitted disease termed chancroid, which is most prevalent in developing countries, an ABC transporter mediating the uptake of sialic acid (*N*-acetyl-neuraminic acid) was identified by transposon mutagenesis (Post *et al*., 2005). In bacteria of the genera *Haemophilus* and *Neisseria*, sialic acid is added to the terminal galactose residue of lipooligosaccharides in their outer membrane, thereby promoting resistance to the antimicrobial activity of human serum (Severi *et al*., 2007). The transporter consists of an SBP, SatA, two membrane-integral subunits, SatBC, and an ATPase, SatD. Interestingly, the SatC protein is a fusion of an N-terminal TMD and a C-terminal ATPase domain,

suggesting that the latter forms a heterodimer with SatD. Transport of radiolabeled sialic acid was verified for the wild type, while mutants carrying insertions in any of the four genes displayed a transport-negative phenotype (Post *et al.*, 2005).

SBPs

Agrobacterium tumefaciens is the causative agent of crown gall tumors in a wide range of dicotyledonous plants. The bacteria transfer and integrate T-DNA, originating from their tumor-inducing (Ti) plasmid into the plant genome in response to environmental (chemical) signals produced at the wound site of a plant (Brencic & Winans, 2005). The Ti plasmid carries virulence (*vir*) operons, whose induction by plant-released signals requires the combined action of the VirA/VirG sensor-regulator system and the periplasmic sugar-binding protein, ChvE. It is hypothesized that sugar-loaded ChvE interacts with periplasmic regions of VirA, thereby increasing its sensitivity for plant-derived phenolic compounds. ChvE displays homology to type I binding proteins, such as the glucose/galactose- and ribose-binding protein (Peng *et al.*, 1998) and is suggested to be associated with the putative sugar ABC transporter, GguAB (Kemner *et al.*, 1997). GguAB displays homology to CUT2 family members, which is consistent with ChvE being a monosaccharide-binding protein, but its role in sugar-dependent growth is still a matter of debate (Kemner *et al.*, 1997; He *et al.*, 2009). However, the homologous system of *B. suis* was demonstrated by mutational analysis to be required for growth on monosaccharides (Alvarez-Martinez *et al.*, 2001). Mutational analysis and fluorescence-based sugar-binding studies with purified wild type and mutant ChvE variants identified two overlapping sites on ChvE for interaction with VirA and the cognate transporter (He *et al.*, 2009). ChvE is also involved in chemotaxis to the sugar it binds.

Mycoplasma hyorhinis is a swine pathogen, but has also been proposed to be a source of oncogenic transformation. Mycoplasmas lack a cell wall and survive attached to or within eukaryotic host cells. Protein p37 was found to induce invasiveness in cancer cells and identified by X-ray crystallography as a thiamine-binding protein related to TbpA of *S. typhimurium* p37 (renamed CypI) is encoded in an operon together with genes for components of an ABC transporter (Dudler *et al.*, 1988). It has been hypothesized that the CypI might sequester thiamine from the surrounding of host cells, thereby increasing the pathogen's chances for invasion (Sippel *et al.*, 2009).

Substrate-binding lipoproteins from gram-positive pathogenic bacteria were shown to function as native ligands of Toll-like receptors (TLR) of mammals, which play a crucial role in sensing microorganisms. These include SitC, a triacylated substrate-binding protein from an iron transporter in *S. aureus* (Kurokawa *et al.*, 2009). The purified protein was capable of stimulating specifically TLR2, responsible for recognizing lipopolysaccharides and lipoproteins. Two other solute-binding lipoproteins from *B. subtilis* and *Microccocus luteus* displayed the same properties, suggesting to the authors a general role as TLR ligands for the binding proteins of ABC transporters from gram-positive bacteria.

SBPs as targets for immunotherapy and for the development of vaccines

Immunogenic ABC transporter proteins, mostly SBPs and a few NBDs, have been identified as immunodominant proteins through screening with immune sera raised against whole bacterial cells (summarized in Garmony & Titball, 2004). SBPs were also studied as potential antigens for the development of vaccines. Two binding lipoprotein components (PiuA, PiaA) of iron transport systems from *S. pneumoniae* that are essential for virulence were shown after immunization to protect mice against a systemic challenge similar to an established control vaccine (Brown *et al.*, 2001). In a subsequent report, the same group provided evidence for the notion that the antibodies raised against PiuA and PiaA enhanced bacterial opsonophagocytosis as analyzed with a human neutrophil cell line rather than inhibiting iron transport (Jomaa *et al.*, 2005). Similarly, mice were protected against systemic infection with *S. pneumoniae* by immunization with the polyamine-binding protein PotD, which contributes to virulence as shown in two model systems (Shah & Swiatlo, 2006).

An immunochemical analysis of sera from rabbits immunized with inactivated cells of *Yersinia pestis*, the causative agent of plague, identified four SBPs as immunoreactive proteins: OppA, PstS, YrbD and PiuA. Only OppA was found to increase the mean time to death of mice when challenged with *Y. pestis* in a correlation with the level of IgGs (Tanabe *et al.*, 2006).

ECF-type transporters

Uptake of Ni^{2+} and Co^{2+} ions

Nickel ions are essential components of various enzymes involved in nitrogen and energy metabolism and in detoxification processes. This list includes urease, [NiFe] hydrogenase, anaerobic carbon monoxide dehydrogenase, acetyl-CoA synthase/decarbonylase, [Ni] superoxide dismutase, methyl-coenzyme M reductase and glyoxalase I (see Hausinger & Zamble, 2007) for a review). Uptake of Ni^{2+} ions into microbial cells is mediated by primary (ABC-type) and secondary active transporters (reviewed by Eitinger & Mandrand-Berthelot, 2000; Eitinger, 2001; Eitinger *et al.*, 2005).

In many cases, synthesis of the transporters is controlled by the Ni^{2+}-responsive transcriptional repressor NikR (Rodionov *et al.*, 2006; Zhang *et al.*, 2009). The NikABCDE system of *E. coli*, a member of the peptide/opine/nickel (PepT, TC 3.A.1.5) transporter family, is the prototype of canonical ABC-type Ni^{2+} transporters (Navarro *et al.*, 1993) and has been discussed in Trace elements. It is composed of the periplasmic SBP NikA, the transmembrane subunits NikB and NikC and the NBDs NikD and NikE (Fig. 8a).

In 1993, sequence analysis of the cobalamin synthesis gene cluster of *S. enterica* serovar Typhimurium identified the *cbiMNQO* genes encoding an ABC ATPase (CbiO) and three membrane proteins, but no periplasmic substrate-binding protein (Roth *et al.*, 1993). Because of the similarity of CbiO to the NBDs of canonical ABC importers and the fact that mutations in the *cbiMNQO* region could be corrected by increasing the concentration of Co^{2+} ions in the medium, the authors suggested that the *cbiMNQO* genes encode a transporter for Co^{2+}. This hypothesis was confirmed >10 years later by experimental analysis, and the CbiMNQO system may be considered the founding member of the emerging class of ECF transporters (Rodionov *et al.*, 2006). Because *cbiQ* encodes the T component of the transporter, homologs are widespread in prokaryotic genomes and flanked by one or two ABC ATPase genes in most cases. This situation led to substantial confusion during annotation of genome sequences. Independent of the presence or the nature of an adjacent S-unit gene, a multitude of T components are misannotated as cobalt transporters or as components of cobalt transporters.

Homologs of CbiMNQO with a function in nickel uptake were described for *Streptococcus salivarius* (Chen & Burne, 2003) and *Actinobacillus pleuropneumoniae* (Bosse *et al.*, 2001). In both organisms, the metal transporter is encoded adjacent to genes for the synthesis of the nickel-dependent urease and was shown to be essential for urease activity under nickel limitation. Systematic analysis of colocalization and coregulation of 'MNQO' genes in prokaryotic genomes and of the phylogeny of the 'M' proteins revealed that these systems fall into two major branches. The members of one branch are colocalized with genes for cobalamin synthesis and/or are regulated by coenzyme B_{12}-responsive riboswitch elements, and thus are clearly implicated in Co^{2+} uptake. The second branch contains operons that are colocalized with genes for nickel-dependent enzymes or under control of a NikR repressor. As a consequence, those systems were predicted to function as Ni^{2+} transporters and were renamed 'Nik' (Rodionov *et al.*, 2006). Experimental analyses of the metal specificity of the CbiMNQO systems of *R. capsulatus* and *S. enterica* serovar Typhimurium and the NikMNQO systems of *R. capsulatus* and *M. thermoautotrophicus* are in full agreement with the bioinformatic predictions (Rodionov *et al.*, 2006; Hebbeln, 2009).

CbiM and NikM are homologous membrane proteins with seven predicted membrane-spanning domains in their mature form and a strongly conserved extracytoplasmic N-terminus containing a His residue at position 2 (Fig. 9). This His residue is essential for the activity of both CbiMNQO and NikMNQO systems (P. Hebbeln, D.A. Rodionov & T. Eitinger, unpublished data). CbiN and NikN proteins have a similar topology, with two TMDs separated by an extracytoplasmic loop, but they are unrelated at the sequence level. NikN is fused to NikM in many cases and is replaced by NikL or NikK and NikL, two other small transmembrane proteins, in a number of prokaryotes (Rodionov *et al.*, 2006). A heterooligomeric structure of the S unit is restricted to the metal transporters among the ECF systems (Fig. 8), but the distinct functions of the 'M', 'N', 'K' and 'L' proteins are currently unknown.

A number of NikO ATPases from sulfate reducers, methanogens and some other organisms contain a histidine-rich C-terminal tail that may function in metal binding. Assuming a metal-sensing and regulatory role of these tails is an attractive hypothesis. Indeed, initial characterization of the NikO from *M. thermoautotrophicus* pointed to such a function (M.M.P. Murphy, University of British Columbia, Vancouver, Canada, pers. commun.).

Transport of water-soluble vitamins and vitamin-derived compounds

Substrate specificities of most ECF transporters were originally predicted by bioinformatic techniques including studies of genomic colocalization, coregulation by proteinaceous transcription factors or substrate-sensing riboswitch elements, and co-occurrence. These analyses suggested prevalence among the ECF systems of transporters for water-soluble vitamins, vitamin-derived cofactors and cosubstrates, and intermediates of vitamin-salvage pathways. In many gram-positive bacteria including pathogenic *Streptococcus* and clostridial species, ECF systems provide a means of transport for vitamins and vitamin precursors that are essential for growth because these bacteria do not contain all of the pathways for *de novo* biosynthesis (Rodionov *et al.*, 2009). For a number of transporters (biotin, riboflavin, thiamine, folate and pantothenate transporters), the predicted substrate specificity was subsequently verified by physiological and biochemical analyses. These systems as well as the predicted transporters for thiamine precursors, niacin, vitamin B_6, vitamin B_{12}-related compounds and lipoate will be discussed in the ensuing paragraphs.

Biotin

Biotin (also known as vitamin B_7, vitamin B_8 or vitamin H) is an essential cofactor in carboxylation and decarboxylation

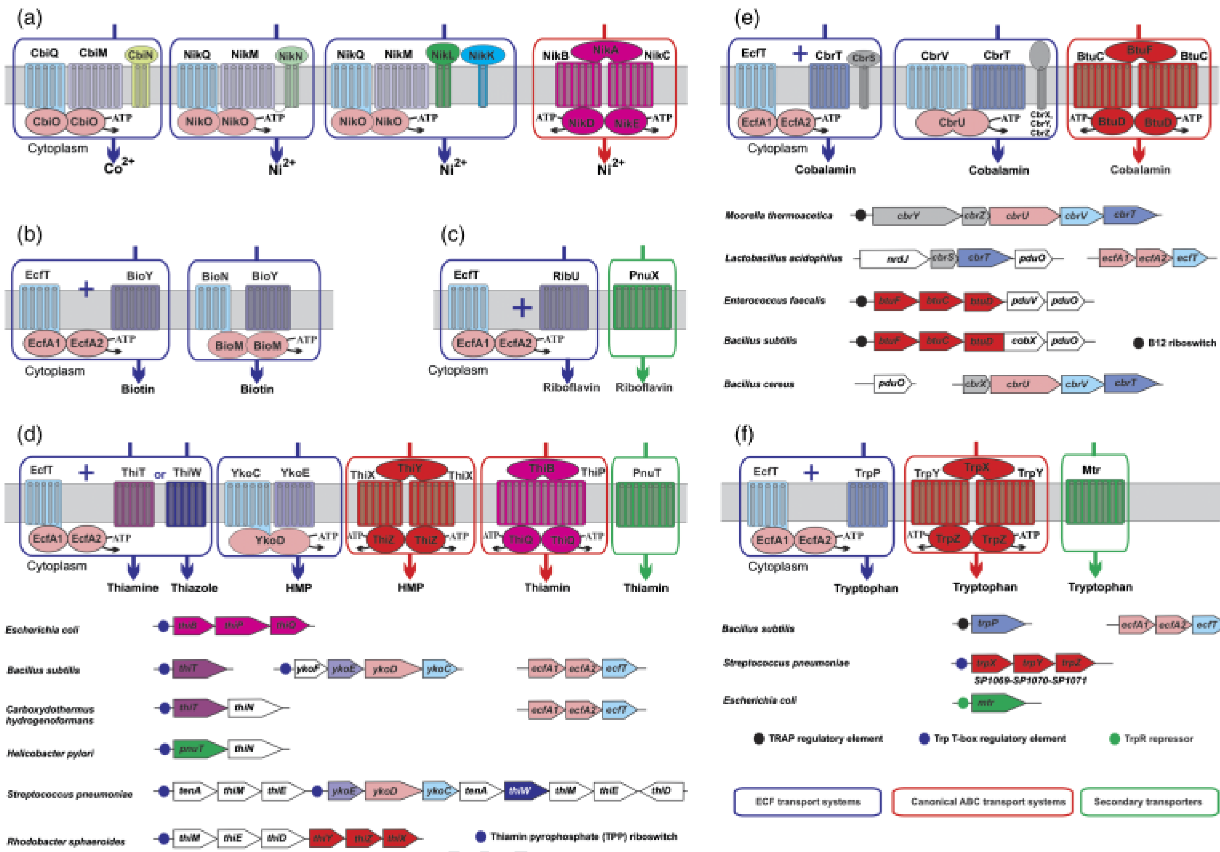

Fig. 8. ECF, canonical ABC (and some secondary) transport systems in prokaryotes mediating the uptake of Co^{2+} and Ni^{2+} (a), biotin (b), riboflavin (c), thiamine and its metabolic precursors (d), cobalamin (e) and tryptophan (f). Components from different protein families are shown by different colors. Examples of genomic clusters of genes encoding the thiamine, cobalamin and tryptophan transport systems are shown by stretches of arrows with colors corresponding to the protein families. Candidate regulatory elements for these genes – either transcription factor-binding sites or riboswitches – are shown by colored dots.

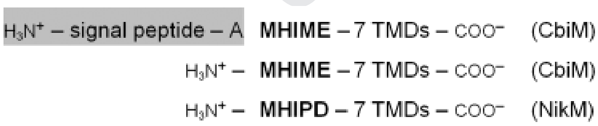

Fig. 9. Schematic view of CbiM and NikM sequences. Roughly half of the bacterial and archaeal CbiM proteins contain a predicted N-terminal signal peptide cleavage of which between Ala and Met releases the strongly conserved N-terminus with MHIME as the consensus sequence. NikM proteins have a similar N-terminus, but do not contain signal peptides. TMDs, transmembrane domains.

reactions and is thus necessary, for example for fatty acid biosynthesis. It is produced by many bacteria, certain archaea, fungi and plants but can also be taken up from environmental sources. Contrasting the situation in eukaryotes, the mechanisms behind biotin transport into prokaryotic cells have, until recently, not been understood. Active transport of biotin into *E. coli* K-12 cells was reported about 35 years ago (Prakash & Eisenberg, 1974), but the carrier responsible for uptake has not been identified until now. Evidence pointing to a role of BioM (A unit) and BioN (T unit), which are homologs of CbiO and CbiQ, respectively, in biotin uptake, was reported for the root-nodulating *Alphaproteobacteria S. meliloti* (Entcheva *et al.*, 2002) and *Rhizobium etli* (Guillén-Navarro *et al.*, 2005). Comparative genomic analyses of biotin-responsive transcriptional repression by the widespread regulator BirA and the regulators BioR in *Alphaproteobacteria* and BioQ in actinobacteria predicted a role for the transmembrane protein BioY in biotin uptake (Rodionov *et al.*, 2002a; Rodionov & Gelfand, 2006; Rodionov, 2007). A six-transmembrane-domain architecture with the N-terminus inside is predicted for the majority of BioY proteins (Fig. 8b). *bioMNY* operons encode subclass I ECF transporters in many proteobacteria, actinobacteria and Euryarchaeota (Table 3). In many *Firmicutes*, *Thermotogales* and in archaea from the *Thermococcus* genus, BioY is predicted to interact with the endogenous EcfA1A2T module to form subclass II ECF biotin-transport systems

(Rodionov *et al.*, 2009). Many *Alphaproteobacteria*, some actinobacteria and chlamydia contain BioY homologs in the absence of any recognizable A and T unit of ECF transporters, suggesting that those BioY proteins function as transporters in a solitary state and independent of ATP hydrolysis.

As described above, the best-investigated prokaryotic biotin transporter is the BioMNY system of *R. capsulatus*. Analysis of the contributions of the individual subunits showed that BioY functions as the biotin-capture protein and acts as a transporter in its solitary state at high (i.e. above 1 nM) substrate concentrations. BioMN represent the ECF. A functional BioM ATPase is required for high-affinity transport at biotin concentrations in the pM range (Hebbeln *et al.*, 2007).

bioY is clustered with biotin synthesis genes in many prokaryotes. Positional clusters of *bioY* with *bioB* are found in *Alphaproteobacteria* and *Clostridia*. BioB encodes biotin synthase, which inserts a sulfur atom into dethiobiotin in the last step of the biosynthetic path. This genomic organization points to a role of BioY in the uptake of dethiobiotin in addition to biotin.

Riboflavin

Riboflavin (vitamin B_2) is the precursor of the redox cofactors FMN and FAD, which are indispensable for all organisms and involved in many metabolic reactions (Fraaije & Mattevi, 2000). The vitamin is synthesized by many microorganisms and plants, but is also taken up from the environment. The S unit RibU (termed YpaA in some cases and found mostly in *Firmicutes*) and the secondary active transporter PnuX (found in *Actinobacteria*) were predicted to mediate riboflavin uptake because the respective genes encode integral membrane proteins and are regulated by the FMN-responsive riboswitch (*RFN* element) in many bacteria (Gelfand *et al.*, 1999; Kreneva *et al.*, 2000; Vitreschak *et al.*, 2002). With only a few exceptions, for example in *Bifidobacterium* sp. and in the archaeon *Caldivirga maquilingensis* in which *ribU* is located adjacent to an *ecfA1A2T* cluster, RibU is the S unit of subclass II ECF transporters. *In silico* topological analyses of individual RibU proteins predict between four and six TMDs. Experimental investigation using fusions of GFP, PhoA and LacZ to the *B. subtilis* RibU suggests that the protein has five TMDs and that the C-terminus faces the cytoplasm (Vogl *et al.*, 2007). Comparing riboflavin uptake of wild-type and RibU$^-$ *B. subtilis* strains confirmed the function of RibU as the central component of the riboflavin transporter (Vogl *et al.*, 2007; Rodionov *et al.*, 2009). In contrast to RibU (K_m for riboflavin in the lower nM range), PnuX has a much lower affinity for its substrate (K_m in the lower μM range) and does not seem to depend on an energy source (Fig. 8c) (Vogl *et al.*, 2007).

Two lines of evidence support the conclusion that RibU proteins interact with EcfA1A2T to form subclass II systems. Disruption of *ecfA1* in *B. subtilis* abolishes riboflavin uptake as does disruption of *ribU* (Rodionov *et al.*, 2009). Coproduction of RibU and EcfA1A2T from *L. mesenteroides* in *E. coli* allows the isolation of stable complexes containing all four subunits from membranes of the recombinants (Neubauer *et al.*, 2009; Rodionov *et al.*, 2009).

Transport and salvage of thiamine and derived compounds

TPP, the active form of vitamin B_1, is required for central metabolic reactions including the decarboxylation of 2-oxo-acids in sugar catabolism and in the citric acid cycle. TPP is produced by prokaryotes, fungi and plants in two separate branches, yielding the intermediates hydroxyethylthiazole phosphate (HET-P) and hydroxymethylpyrimidine pyrophosphate (HMP-PP), which are combined to yield thiamine monophosphate (TMP) that, in the final step, is phosphorylated to the active cofactor (Jurgenson *et al.*, 2009). Bacteria can salvage thiamine and a number of components of TPP. In the absence of central parts of the TPP biosynthetic pathway, for example in several pathogens among the *Firmicutes*, salvage is essential. A canonical ABC transporter for thiamine, TMP and TPP, consisting of the NBD ThiQ, the transmembrane protein ThiP and the SBP ThiB, was identified in *S. enterica* serovar Typhimurium (Webb *et al.*, 1998). Crystal structure analysis characterized the *E. coli* ThiB (also known as TbpA) as a group II periplasmic-binding protein that exhibits similarity to thiaminase I, an enzyme involved in thiamine degradation (Soriano *et al.*, 2008).

Reconstruction of the regulons controlled by the TPP-responsive riboswitch (*THI* element) identified various candidate transporters involved in thiamine salvage pathways in bacteria, in addition to the ThiBPQ system, which is widespread among gram-negative bacteria (Fig. 8d) (Rodionov *et al.*, 2002b). A predicted secondary transporter for thiamine (PnuT) was found in several lineages of proteobacteria that lack the ThiBPQ system. The subclass II ECF transporter for thiamine, ThiT (also known as YuaJ), was identified in *Firmicutes* that lack both ThiBPQ and PnuT, at that in some thiamine-auxotrophic species (e.g. *Streptococcus pyogenes*), it complements the absence of thiamine biosynthesis genes. The prediction that ThiT proteins bind thiamine and represent the S units of subclass II ECF transporters was confirmed in a series of biochemical assays with the *L. casei* and *L. monocytogenes* homologs (Eudes *et al.*, 2008; Rodionov *et al.*, 2009; Schauer *et al.*, 2009).

At least two types of transporters, the canonical ABC system ThiXYZ and the subclass I ECF system YkoEDC, are implicated in recycling pyrimidine moieties of TPP that

arise through base degradation of the cofactor (Fig. 8d) (Rodionov *et al.*, 2002b). In a recently discovered pathway, base-degraded hydrolysis products of thiamine in soil such as formylaminopyrimidine are transported into the cells, deformylated by an amidohydrolase and subsequently hydrolyzed by thiaminase II (TenA) to HMP, which, as the pyrophosphate, is a building block of TPP (Jenkins *et al.*, 2007). A canonical ABC system encoded adjacent to *tenA* in *B. cereus* and *Bacillus halodurans* consisting of the SBP ThiY, the integral membrane protein ThiX and the NBD ThiZ serves as the transporter for formylaminopyrimidine (Jenkins *et al.*, 2007). The subclass I ECF system YkoEDC may play this role in other bacteria. It is composed of the S unit YkoE, the A unit YkoD containing duplicated NBDs and the T component YkoC. In *E. faecalis*, *Streptococcus* sp. and *Geobacillus kaustophilus*, the *ykoEDC* genes are colocalized with *tenA*. In *B. subtilis* and *Oceanobacillus iheyensis*, the *ykoFEDC* operon contains an additional gene. YkoF of *B. subtilis* is a thiamine-binding protein that is unrelated to substrate-binding proteins of canonical ABC transporters. It binds thiamine at two sites with dissociation constants of 10 and 250 μM (Devedjiev *et al.*, 2004). Because these values are by far above physiological requirements, it is unlikely that YkoF contributes to the transport of thiamine precursors.

ThiW (S unit)-containing ECF transporters are predicted to function in the salvage of the thiazole moiety of TPP. The assumption is based on positional clustering of *thiW* with *thiM* encoding HET kinase (Rodionov *et al.*, 2009). ThiW proteins from numerous *Firmicutes* and a single archaeon (*Korarchaeum cryptofilum*) belong to subclass II ECF systems, whereas their orthologs from *Crenarchaeota* and *Chloroflexi* are encoded by operons including genes for A and T components and are thus classified as subclass I ECF transporters.

Folate

Tetrahydrofolate is a carrier of one-carbon units and involved in many central metabolic reactions including the formylation of methionyl-tRNA, the last step of methionine synthesis, thymidylate synthesis and synthesis of purines. Tetrahydrofolate is produced in plants and microorganisms in two branches: the pterin branch starting from GTP and the 4-aminobenzoate branch starting from chorismate. The resulting pterin and 4-aminobenzoate moieties are condensed to a pteroate, which is glutamylated and reduced twice to yield tetrahydrofolate. Tetrahydrofolate may become polyglutamylated in a subsequent step. Salvage pathways include the uptake and utilization of folates and of the degradation product 4-aminobenzoyl glutamate (Carter *et al.*, 2007; de Crecy-Lagard *et al.*, 2007). Folate transporters similar to related systems in plants were found in cyanobacteria and may also occur in proteobacteria (Klaus *et al.*, 2005). Uptake of folates by naturally auxotrophic organisms such as *Lactobacillus* sp. is known for >30 years ago (Henderson *et al.*, 1977; Kumar *et al.*, 1987), but only recently subclass II ECF transporters were shown to be the systems involved (Eudes *et al.*, 2008). FolT, which is widespread among the *Firmicutes*, was predicted to be the S unit based on three lines of evidence: the sequence similarity of *folT* to sequences predicted by reverse genetics of peptides derived from the *L. casei* 'folate-binding protein' (= FolT), the colocalization of *folT* with *folC*, encoding folylpolyglutamate synthase, in some genomes, and the strict co-occurrence of *folT* only in those genomes that encode an EcfA1A2T module. *Lactobacillus casei* FolT was shown to bind folate and folinate with a high affinity and to transport folate into *L. lactis* cells in the presence of a cognate EcfA1A2T module. Using a folate-auxotrophic (and naturally folate-transport deficient) *E. coli* strain as the host, it was unequivocally shown that heterologously produced FolT and EcfA1A2T from *L. mesenteroides* form a stable and functional folate transporter (Eudes *et al.*, 2008; Neubauer *et al.*, 2009; Rodionov *et al.*, 2009).

Pantothenate

Pantothenate (vitamin B_5) is a building block of coenzyme A and is thus essential for central metabolic reactions such as fatty acid synthesis. During *de novo* synthesis in a number of prokaryotes, in fungi and in plants, pantothenate arises by condensation of β-alanine and pantoate (see Spry *et al.*, 2008 for a recent review). In prokaryotes, β-alanine is either produced by decarboxylation of aspartate or taken up from the environment. In *E. coli*, β-alanine uptake is mainly mediated by the CycA amino-acid transporter (Schneider *et al.*, 2004). The pantoate moiety is derived from 2-oxo-isovalerate. Pantothenate is converted into coenzyme A in a five-step path via 4-phosphopantothenate, 4-phosphopantothenoylcysteine, 4-phosphopantetheine and dephospho-coenzyme A. Pantothenate and probably pantetheine can also be used in salvage pathways. Pantothenate is taken up either by a widespread Na^+/pantothenate symporter (PanF, TC 2.A.21.1.1), or as recently predicted, by ECF transporters (mostly subclass II) using PanT as the S component (Rodionov *et al.*, 2009). PanT is found in many pathogenic (e.g. *Streptococcus* sp.) and nonpathogenic lactobacteria and in clostridia including *Clostridium difficile*, *Clostridium perfringens* and *Clostridium tetani*. These organisms are known to depend on exogenous pantothenate for growth. Heterologous production of PanT and EcfA1A2T from *L. mesenteroides* in *E. coli* resulted in stable quadripartite complexes in membranes of the recombinant host (Rodionov *et al.*, 2009) and conferred pantothenate-transport activity on a PanF-deficient strain (Neubauer *et al.*, 2009). These results clearly

demonstrate the role of PanT-EcfA1A2T as a high-affinity pantothenate transporter.

Niacin

NAD is an important redox coenzyme, but also a substrate for certain enzymes such as bacterial DNA ligase and ADP-ribosyl transferases that decompose the molecule. NAD is synthesized *de novo* from aspartate via quinolinate and nicotinate mononucleotide. A variety of pathways for recycling of endogenous or exogenous NAD degradation products including nicotinate and nicotinamide (named niacin or vitamin B_3 independent of the acid or amide function) are known, but many of the transporters for the exogenous compounds are not characterized. A recent functional genomic analysis of the NAD synthesis and salvage regulons governed by the niacin-responsive transcriptional repressor NiaR in *Firmicutes* identified the ribosyl nicotinamide transporter gene *pnuC* and the genes for three putative transporters (*niaP*, *niaY* and *niaX*) as regulated targets (Rodionov *et al*., 2008). NiaP of *B. subtilis*, a member of the major facilitator transporter family, complemented an NAD-auxotrophic *E. coli* mutant in the presence of nicotinamide and is thus implicated in niacin transport. Experimental data for such a role of NiaY and the more widespread NiaX are not available. NiaX was found only in *Firmicutes* containing an EcfA1A2T module, and it was tentatively predicted to function as the S unit of a subclass II ECF transporter for niacin (Rodionov *et al*., 2009).

Vitamin B_6

Vitamin B_6 exists in the three forms: pyridoxine, pyridoxal and pyridoxamine. The active form, pyridoxal-5-phosphate (PLP), is a cofactor of a large number of enzymes mainly involved in amino-acid metabolism. PLP is synthesized by microorganisms and plants by one of two ways: most bacteria including *E. coli* combine 4-phosphohydroxy-L-threonine (derived from erythrose-4-phosphate) and deoxyxylulose-5-phosphate, a metabolite also used for isoprenoid and thiamine synthesis, to produce pyridoxine-5-phosphate, which is oxidized to PLP. Some other bacteria including *B. subtilis*, archaea and eukarya synthesize PLP directly from ribulose-5-phosphate, dihydroxyacetone phosphate and glutamine (see Mooney *et al*., 2009) for a recent review on the metabolic roles and biosynthesis of vitamin B_6). The various forms of vitamin B_6 and their 5-phosphates are interconvertible, which allows recycling of the endogenous compounds released for instance during protein degradation. Salvage of exogenous vitamin B_6 variants depends on the uptake of the metabolites. Surprisingly little is known on vitamin B_6 transport into prokaryotic cells including lactobacterial species that are natural auxotrophs. Although vitamin B_6 uptake was demonstrated for various lactobacteria (Mulligan & Snell, 1977) and *S. enterica* serovar Typhimurum (Mulligan & Snell, 1976), the transporters behind this process were not known. Homologous S units of ECF transporters, PdxT and HmpT, are predicted to function as vitamin B_6 transporters in *Firmicutes*, *Thermotogales* and some archaea (Rodionov *et al*., 2009). In many *Lactobacillales*, *pdxT*/*hmpT* genes are colocalized with genes encoding pyridoxine-related kinases of two different types (similar to PdxK from *E. coli* and to ThiD/PdxK from *B. subtilis*), both of which have specificity for pyridoxine, pyridoxal and pyridoxamine (Park *et al*., 2004; Safo *et al*., 2006). In our previous study, based on the colocalization with *thiD* kinase genes, we proposed that HmpT transporters are involved in the thiamine salvage pathways. Similarity searches using PSI-BLAST identified HmpT and PdxT as homologous proteins from the same family (COG4720). Thus, we revise our initial annotations and combine the HmpT and PdxT families into a single family of the pyridoxine-related transporters PdxU that may be specific to PLP precursors, pyridoxine, pyridoxal and pyridoxamine.

Vitamin B_{12}-related compounds

Corrinoid-containing cofactors are synthesized in prokaryotes, and utilized for essential reactions by prokaryotes, animals and algae, but not by higher plants. Reactions that rely on B_{12} cofactors include 5′-deoxyadenosyl cobalamin-dependent rearrangements, methyl-cobalamin- or methyl-cobamide-dependent methyl-group transfer and reductive dehalogenation (see Banerjee & Ragsdale, 2003; Matthews *et al*., 2008 for a review). B_{12} cofactors are produced *de novo* from uroporphyrinogen III, an intermediate of tetrapyrrole synthesis, by two complex metabolic routes, resulting in the formation of an adenosylated cobyric acid derivative as another intermediate. The two routes, known as an aerobic and an anaerobic pathway, differ in the cobalt-insertion reaction that occurs early in the anaerobic path and late in the aerobic path, and in the elimination of the C_{20} carbon atom that is present in tetrapyrroles, but absent from the corrin skeleton. An aminopropanol moiety derived from threonine is attached to the adenosylated cobyrate to yield adenosyl cobinamide, which is converted to 5′-deoxyadenosyl cobalamin by the addition of α-ribazole. The latter is derived from dimethylbenzimidazole and nicotinic acid mononucleotide (reviewed by Scott & Roessner, 2002). Prokaryotes, for example those that do not contain the complete pathway for *de novo* B_{12} synthesis (e.g. *E. coli* K-12), can take up corrinoids from the environment and many prokaryotes use a canonical ABC transporter for this purpose. In *E. coli*, corrinoids enter the periplasm through the TonB-dependent outer-membrane transporter BtuB and are transported into the cytoplasm via the BtuCD-BtuF ABC

transporter (Fig. 8e). These proteins have been analyzed in great detail and the crystal structures of BtuB (Chimento *et al.*, 2003), the SBP BtuF (Borths *et al.*, 2002), the complex of BtuC (TMD) and BtuD (NBD) (Locher *et al.*, 2002) and the BtuCD-BtuF (Hvorup *et al.*, 2007) holotransporter have been determined. Related ABC transporters are widespread among bacteria and archaea, and the respective genes are regulated by adenosylcobalamin-dependent riboswitch (*B12* element) in most cases (Rodionov *et al.*, 2003). The *E. coli* BtuCD-BtuF system was shown to transport adenosylcobalamin, the non-natural cyanocobalamin (vitamin B_{12}) and, although at a lower rate, cyanocobinamide (Bradbeer *et al.*, 1978).

CbrT is the predicted S component of subclass II ECF transporters for cobalamin in *Lactobacillales* (Rodionov *et al.*, 2009). Several *Lactobacillus* sp. contain an *nrdJ–cbrS–cbrT–pduO* gene cluster encoding adenosylcobalamin-dependent ribonucleotide reductase (NrdJ), a hypothetical lipoprotein (named CbrS), the putative S unit CbrT and a cobalamin adenosyltransferase (PduO) (Fig. 8e). The subclass I transport system CbrTUV was identified in other *Firmicutes*, in some *Actinobacteria* and in the archaeon *Methanosphaera stadtmanae*. It is accompanied by one or two distinct hypothetical lipoprotein components (e.g. CbrY-Z in *Moorella thermoacetica* and CbrX in *B. cereus*). The absence of *btuFCD* in the genomes that possess the *cbrT* or *cbrTUV* genes suggests the involvement of both transport systems in the uptake of corrinoids. The absence of CobU and CobS (enzymes converting adenosylcobinamide to adenosylcobalamin) and the presence of PduO [an enzyme that adenosylates cobalamin and cobinamide, for example in *Lactobacillus reuteri* (Park *et al.*, 2008)] in *Lactobacillus* sp. suggest that CbrT has specificity to cobalamin.

In a number of *Firmicutes*, CblT is predicted to be the S unit of subclass II ECF systems and in a *Desulfitobacterium* sp. it belongs to a subclass I system. CblT may be involved in the salvage of dimethylbenzimidazole (Rodionov *et al.*, 2009). A couple of these bacteria have adjacent *cblT* and *cblS* genes, but lack CobT that is required for the synthesis of α-ribazole-5′-phosphate from dimethylbenzimidazole. Thus, it was predicted that the *cblT* and *cblS* genes encode a salvage path for dimethylbenzimidazole consisting of a transporter and a novel type of α-ribazole-5′-phosphate synthase (Rodionov *et al.*, 2003).

Lipoate

Lipoic acid is a sulfur-containing cofactor that resembles biotin in structure and is essential for the function of a number of enzymes including pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. Lipoic acid is covalently bound via its carboxyl group to a lysine residue in the lipoyl domain of lipoate-dependent enzymes. This reaction is catalyzed by lipoate-protein ligase (LplA) if free lipoate is available. *De novo* synthesis of lipoate has been extensively investigated in *E. coli*. It is produced from *n*-octanoic acid by two routes. In the minor path, octanoate is bound by LplA to the lipoyl domain of the target protein. Subsequently, lipoate synthase (LipA) inserts two sulfur atoms into the octanoyl moiety in a complex reaction resembling the biotin-synthase reaction. The major route depends on LipB that transfers the octanoyl moiety from octanoyl-acyl carrier protein, derived from fatty acid synthesis, to the target with the subsequent transfer of sulfur by LipA (see Cronan, 2008, for an overview). Transporters that may enhance the salvage of exogenous lipoate are unknown, but ECF systems may be involved in certain organisms. Several *Phytoplasma* sp. that contain neither *lipA* nor *lipB* contain an *lplA–lipT* cluster encoding lipoate-protein ligase and the predicted S unit of a subclass II ECF transporter (LipT) that has a weak similarity to ThiT from *Phytoplasma* (Rodionov *et al.*, 2009).

Other salvage pathways

Methionine

Besides its role as a proteinogenic amino acid, methionine fulfills many other functions in the cell. The adenosylated form, SAM, is the major donor of methyl groups, but is also used to produce adenosyl radicals for reactions catalyzed by enzymes of the radical/SAM family, as a donor of the ribosyl group for the queuosine modification in tRNA, and as a donor of its aminobutyryl moiety used for the synthesis of spermidine, biotin and *N*-acyl-homoserine-lactones, and for tRNA modification (see Hondorp & Matthews, 2006, for a review). Many organisms contain salvage pathways for recycling the degradation products of SAM. Splitting off the aminobutyryl group of SAM produces methylthioadenosine (MTA), which is a starting point of the salvage cycle. In the first step, adenine is cleaved off to yield methylthioribose-1-phosphate (MTR-1-P), either in a one-step reaction catalyzed by MtnP or in a two-step path via methylthioribose (MTR) by the serial action of MtnN and MtnK. *Escherichia coli* does not further recycle MTR, but excretes this compound. In many other organisms, MtnA isomerizes MTR-1-P to methylribulose-1-phosphate, which is used to produce methionine in a series of four or five additional reactions (reviewed by Sekowska *et al.*, 2004).

Methionine is taken up by members of the MUT family (TC 3.A.1.24) of canonical ABC transporters encoded in *E. coli* by the *metNIQ* operon and in *B. subtilis* by the *metQPN* operon (see Sources of sulfur). In addition, secondary active transporters for methionine [MetPS (Trötschel *et al.*, 2008)], *S*-methylmethionine [in *E. coli* (Thanbichler *et al.*, 1999)] and SAM [in *Rickettsia prowazekii* (Tucker *et al.*, 2003)] have been described. Comparative genomic analyses

predicted the existence of subclass I ECF transporters for methionine precursors that arise by the aforementioned reactions (Rodionov *et al.*, 2004, 2009). In a few prokaryotes, the genes *mtaU* (encoding a duplicated ABC ATPase), *mtaV* (encoding a T component) and *mtaT* (encoding a putative S component) are clustered with *mtnA* and *mtnP*, suggesting that MtaTUV transporters are involved in the uptake of MTA, MTR and/or MTR-1-P. MtsTUV is another transporter under the control of the methionine regulons (SAM riboswitch or methionine T-box regulatory elements in *Firmicutes*; MetJ repressor in *Vibrio*). Comparative genomic analysis (gene co-occurrence, colocalization and coregulation) of the 'methionine metabolism' subsystem in the SEED database allowed us to tentatively predict that MtsTUV systems are transporters for SAM. The reduced genomes of *Phytoplasma* lack any methionine biosynthesis enzyme including the SAM synthetase MetK. The absence of these essential enzymes in *Phytoplasma* is complemented by two transport systems: the methionine transporter MetNPQ and the predicted SAM transporter MtsTUV.

Queuosine and archaeosine

Queuosine (Q) and archaeosine (G^+) are hypermodified 7-deaza-purine nucleosides present in the anticodon wobble position of bacterial and eukaryotic $tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{His}$ and $tRNA^{Tyr}$, and in the D-loop of archaeal tRNAs, respectively. Whereas mammals take up the free base queuine with the diet or from the intestinal flora and use it to replace a guanine base by means of a tRNA guanine transglycosylase (TGT) enzyme, the modified nucleosides are produced *de novo* from GTP in prokaryotes. The $PreQ_0$ precursor (a 7-cyano-7-deaza purine) is synthesized in a four-step pathway that is common to bacteria and archaea. In bacteria, $PreQ_0$ is converted to $PreQ_1$ (7-aminomethyl-7-deazaguanine), which is then transferred to tRNAs by TGT and subsequently converted to Q by the QueA enzyme. In archaea, $PreQ_0$ is incorporated into tRNA and converted to G^+ (Roth *et al.*, 2007; Phillips *et al.*, 2008; McCarty *et al.*, 2009). Nucleolytic cleavage of Q-containing tRNAs could result in $PreQ_1$, but not in $PreQ_0$ or other precursors. Degradation of G^+-containing tRNAs would hardly result in the free $PreQ_0$ base. Because bacterial, archaeal and eukaryotic TGT enzymes are specific for $PreQ_1$, $PreQ_0$ and queuine, respectively (Stengl *et al.*, 2005), variations of the predicted salvage pathway containing individual enzymes that produce the appropriate precursors are conceivable.

Two different families of ECF transporters operating with the S units QrtT and QueT were predicted to be involved in a hypothetical Q salvage pathway in prokaryotes (Rodionov *et al.*, 2009). QrtT occurs in many prokaryotic groups and is the predicted S unit of subclass II ECF transporters and of subclass I ECF systems in combination with QrtU (T unit), QrtV and QrtW (A units). Likewise, QueT is found in diverse bacteria and archaea and considered as an S component of subclass II ECF systems (Rodionov *et al.*, 2009). Predictions on the substrates of QrtT- and QueT-containing ECF systems are based on the genome-context evidences captured in the SEED database (Overbeek *et al.*, 2005). In the genomes of *Firmicutes*, the *qrtT* and *queT* genes are coregulated with the Q-biosynthetic operon by representatives of two classes of $PreQ_1$-responsive riboswitches (Roth *et al.*, 2007; Meyer *et al.*, 2008), and co-occur with the *tgt* and *queA* genes, but not with the *de novo* $PreQ_1$ biosynthesis genes, suggesting the involvement of the respective transporters in the $PreQ_1$ salvage. Colocalization of *qrtT* with a hypothetical homolog of the inosine-uridine nucleoside *N*-ribohydrolase gene *iunH* in many *Firmicutes* suggests that these organisms utilize queuosine salvage. Projection of the predicted specificities of QueT and QrtT in bacteria to archaeal homologs is challenging because the G^+ biosynthesis in *Archaea* does not utilize $PreQ_1$.

Tryptophan

Tryptophan is one of the rarest amino acids in most proteins and can be synthesized by most microorganisms, except some pathogenic and symbiotic species (e.g. *Lactobacillus*, *Streptococcus*). Both auxotrophic and prototrophic species often possess Trp transporters, for instance *E. coli* and related organisms possess the Mtr permease, whose production is under the control of the Trp repressor (Heatwole & Somerville, 1991; Sarsero *et al.*, 1991). The tryptophan-specific S unit TrpP (also known as YhaG) was identified as a member of the tryptophan regulon operated by the TRAP attenuation protein in *B. subtilis* (Sarsero *et al.*, 2000; Yakhnin *et al.*, 2004). Involvement of TrpP in tryptophan transport was confirmed by sensitivity tests of the *trpP* mutant to growth-inhibiting levels of tryptophan analogs (Sarsero *et al.*, 2000). *trpP* orthologs in clostridia are regulated by the Trp-specific T-box attenuator (Vitreschak *et al.*, 2008). TrpP proteins in *Firmicutes* are a part of subclass II ECF transporters for tryptophan (Fig. 8f), whereas its homologs in methanogenic archaea belong to the subclass I systems. A canonical ABC transporter for tryptophan (termed TrpXYZ) regulated by the Trp-specific T-box attenuator was predicted in some gram-positive bacteria that lack TrpP (Vitreschak *et al.*, 2008).

ABC and ECF importers in plants?

As noted at the outset, ABC transporters in eukaryotes were until recently considered to mediate substrate movement only in an inside-to-outside direction from the

cytoplasm across the plasma membrane or across the membranes of vacuoles and peroxisomes. Based on the genome sequences of *A. thaliana* and *O. sativa*, plants are considered to contain a total of >120 ABC transporters, the majority of which are involved in the defense against biotic and abiotic stresses (see Rea, 2007; Yazaki *et al.*, 2009, for a review). In recent times, however, experimental evidence accumulated, suggesting that distinct ABC systems in plants transport substrates across the plasma membrane into cells. The list of ABC importers in the plasma membrane includes the AtABCB4 and AtABCB14 systems in *A. thaliana* and the CjMDR1 transporter of the medicinal plant *Coptis japonica*. CjMDR1 is implicated in the transport of berberine, a benzylisoquinoline alkaloid with antibacterial and antimalarial activity, from xylem vessels into rhizome cells. The function of CjMDR1 as an uptake system was confirmed upon production in *Xenopus* oozytes (Shitan *et al.*, 2003). Heterologous production of the CjMDR1 homolog AtABCB4 resulted in indole-3-acetic acid (IAA)-uptake activity of HeLa cells (Terasaka *et al.*, 2005) and in the hypersensitivity of yeast cells to IAA and the toxic auxin analog 5-fluoroindole (Santelia *et al.*, 2005). These results suggest a function of AtABCB4 in auxin uptake. AtABCB14 mediates the uptake of malate from the apoplast into guard cells, a process involved in the CO_2-responsive regulation of closure of stomata. Recombinant production of AtABCB14 conferred malate-uptake activity on *E. coli* cells lacking the endogenous dicarboxylate transporter and enhanced malate uptake of HeLa cells (Lee *et al.*, 2008). These findings suggest that at least distinct full-size ABC transporters such as AtABCB4, AtABCB14 and CjMDR1 have the potential to couple intracellular ATP hydrolysis to the uptake of substrates across the plasma membrane.

The genomes of various plant lineages ranging from deeply branching algae (e.g. *Ostreococcus*) via mosses (e.g. *Physcomitrella*) to crops encode homologs of prokaryotic T components and thus it is tempting to speculate about the existence of ECF transporters in plants. With very few exceptions (e.g. a BioY homolog encoded in the chloroplast genome of *Mesostigma viride*, a freshwater flagellate and very deeply branching member of the green plants), homologs of prokaryotic S components with known or predicted substrate specificity have not been identified in plants. Thus, questions on potential substrates remain elusive. *In silico* analyses of the available plant T protein sequences predict plastid-specific transit peptides in several cases, suggesting that those T units may be located in the chloroplast envelope (Fig. 6). Plant organelles play important roles in vitamin synthesis. The entire *de novo* synthesis of folate, a central step in pantothenate synthesis and the final step of biotin synthesis, occur in the mitochondria (reviewed by Smith *et al.*, 2007). Because biotin is required for fatty acid synthesis in chloroplasts, the vitamin must be exported from the mitochondria and imported into chloroplasts. Central steps of thiamine synthesis are catalyzed in chloroplasts and in the mitochondria (Kong *et al.*, 2008; Huang *et al.*, 2009). These facts indicate that vitamins and vitamin precursors must be transported across the organellar envelopes. Given the substrate preference of prokaryotic ECF transporters and the existence of T components in plants, the assumption that ECF-related systems may be involved in vitamin transport across organellar membranes is an attractive speculation.

A group of T components of unknown function in cyanobacteria is most closely related to the aforementioned plant homologs (Fig. S2a). In a number of cyanobacteria, these T proteins are encoded in a conserved cluster containing *engA*, the T-unit gene and *pipX*. PipX is a small protein that interacts with PII and NtcA and is thus involved in nitrogen regulation. EngA is a widespread GTPase that plays an important role in ribosome assembly. At present, the genome context of this special type of cyanobacterial T components does not allow to draw conclusions on potential physiological roles.

Common features of canonical ABC and ECF importers, and perspectives

The structure and mechanism of many canonical ABC importers have been analyzed in great detail. On the other hand, and due to the fact that ECF systems as an abundant new class of transporters were described only recently, experimental analysis of the latter is still in its infancy. Nevertheless, the available data allow a comparative view of the two types of ABC importers.

Utilization of ABC ATPases is the immediately obvious common feature of the two types of ATP-dependent transporters. Thus, questions of evolutionary origins are of interest. Phylogenetic analyses of sets of ATPases of canonical ABC importers and of ECF transporters clearly show that the NBDs of the two transporter groups form distinct branches (Fig. S3), indicating that they have diverged early in evolution. Nevertheless, sequence alignments show that the NBDs of ECF-type transporters contain all the signatures that are known as being essential for ABC proteins. Structural data are available for a multitude of NBDs of canonical ABC importers, but for only a few NBDs of ECF systems. Structural alignments of CbiO, the NDB of the ECF-type Co^{2+} transporter of *C. perfringens* (Ramagopal *et al.*, unpublished data; PDB code 3GFO), with its canonical-type relatives, do not reveal significant differences in the catalytically important domains. Q12

At first glance, the property of at least certain S components to function as transporters in the solitary state seems to be completely different from canonical ABC

importers that strictly depend on an extracytoplasmic SBP and the NBDs in addition to the transmembrane components. However, distinct mutations in the genes for TMDs (Covitz *et al*., 1994) or NBDs (Speiser & Ames, 1991) of two canonical ABC importers are known, which render substrate uptake independent of the SBP. Substrate transport by the TMDs in the absence of both the SBP and the NBDs has not been reported, but preliminary results indicate that such functional variants of the maltose transporter might exist (E. Schneider, unpublished data). In this respect, it is noteworthy that a pmf-driven activity was demonstrated for LmrA, a multidrug ABC exporter from *L. lactis*, when lacking its NBDs (Venter *et al*., 2003).

Intramolecular signaling between the Q loops of the NBDs and the TMDs may follow similar principles. In canonical ABC systems, the Q loop interacts with the EAA loop, a conserved helical region in the TMDs. The conserved Ala-Arg-Gly signatures (with variations as regards the Ala and Gly residue, indicated in the legend to Fig. 6) in a helical segment of T units may play a similar role in ECF systems.

Questions of whether or not the transport cycles of canonical ABC and ECF importers follow similar principles need future experimentation. According to the standard model for the former systems, the substrate-charged SBP plays an important role in initiating the cycle. Obviously, the mechanism must be different in the case of ECF systems because soluble SBPs are not involved. The scarcely available data on a biotin importer suggest that ATP hydrolysis of the BioMNY holotransporter is independent of the addition of a substrate. Because of the extremely high affinity of ECF systems for their substrates, it cannot be excluded that biotin remains bound to the BioMNY complex during purification. Thus, questions of whether the substrate induces hydrolysis of ATP remain enigmatic.

Other questions concern the supramolecular assembly of ECF systems in comparison with canonical ABC importers. The latter consist of two TMDs, two NBDs and (except for the hitherto only few importers in plants) an SBP and thus have a five-domain structure. The subunit assembly of ECF systems is completely unclear. Core transporters (S units) are small proteins with six or less transmembrane helices. Certain S units are functional transporters in their solitary state, i.e. in the absence of a T unit and the NBDs (A units), and it is unlikely that they have a monomeric structure. Assuming an oligomeric structure for S units, questions on the number of subunits in the holotransporter complexes arise, and it is not unlikely that S components are dimeric. Because the T component is an additional integral membrane protein, ECF systems would contain at least three TMDs, which is another difference compared with canonical ABC importers.

Finally, it is worth comparing the substrate spectra of canonical ABC and ECF importers. Canonical systems in prokaryotes transport a wide variety of substrates ranging from carbon, energy and nitrogen sources, which are required in large amounts by the cells, to transition metal ions that are required and tolerated only in trace amounts. The majority of ECF systems are transporters of micronutrients, frequently vitamins, and nickel and cobalt ions. This specificity correlates with an extremely high affinity for the substrate in the low nanomolar or picomolar range of the S components. The specific contributions of the A and T components in flux control remain to be elucidated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Maximum likelihood phylogenetic trees of different families of S component proteins.
Fig. S2. (a) Maximum likelihood phylogenetic tree of T component proteins. (b) Maximum likelihood phylogenetic tree of A component proteins. Q31
Fig. S3. Maximum likelihood phylogenetic tree of A components of ECF transporters and ATPases of classical ABC importers from prokaryotes.

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Query No.	Description	Author Response
Q17	Author: Please provide the page range for the reference Dassa (2007).	
Q18	Author: Please provide the page range for the reference Eitinger (2001).	
Q19	Author: If this is not a one-page article please supply the last page for the reference Forward et al. (1993).	
Q20	Author: Please provide the volume and page range for the reference Gould & Shilton (2010).	
Q21	Author: Please provide the page range for the reference Hausinger & Zamble (2007).	
Q22	Author: Please provide the page range for the reference Hondorp & Matthews (2006).	
Q23	Author: Please provide the volume and page range for the reference Lewinson et al. (2010).	
Q24	Author: Please provide the location of the publisher for the reference Ponte-Sucre (2009).	
Q25	Author: Please confirm the change of author name ‘J W’ to ‘Wood JM’ in the reference Poolman et al. (2004).	
Q26	Author: Please provide the page range for the reference Postma et al. (1996).	
Q27	Author: Please provide the page range for the reference Schneider (2003).	
Q28	Author: Please provide the page range for the reference Sekowska et al. (2001).	
Q29	Author: Please provide the volume and page range for the reference Vahedi-Faridi et al. (2010).	
Q30	Author: Please provide the page range for the reference Wanner (1996).	
Q31	Author: As per style, only a one-line caption is allowed for supplementary information captions. Hence, please provide a one-line caption instead of the present one.	

Author Query Form

Journal FEMSRE

Article 230

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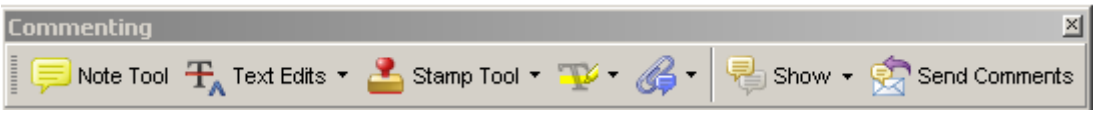
Query No.	Description	Author Response
Q32	Author: Please check whether ‘chapter 5’ deleted in the caption of Fig. 6 is OK.	
Q33	Author: Please check whether the placement of the closing quote is correct for ‘Cyano_T’.	
Q34	Author: Please provide the significance of ‘?’ in column 2 of Table 2.	

USING E-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required Software

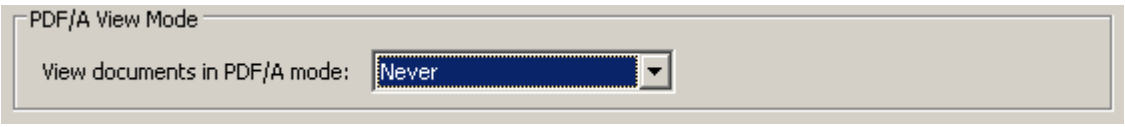
Adobe Acrobat Professional or Acrobat Reader (version 7.0 or above) is required to e-annotate PDFs. Acrobat 8 Reader is a free download: http://www.adobe.com/products/acrobat/readstep2.html

Once you have Acrobat Reader 8 on your PC and open the proof, you will see the Commenting Toolbar (if it does not appear automatically go to Tools>Commenting>Commenting Toolbar). The Commenting Toolbar looks like this:



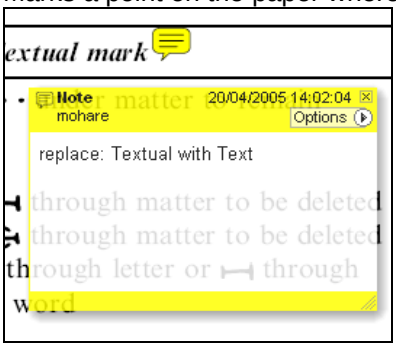
If you experience problems annotating files in Adobe Acrobat Reader 9 then you may need to change a preference setting in order to edit.

In the “Documents” category under “Edit – Preferences”, please select the category ‘Documents’ and change the setting “PDF/A mode:” to “Never”.



Note Tool — For making notes at specific points in the text

Marks a point on the paper where a note or question needs to be addressed.

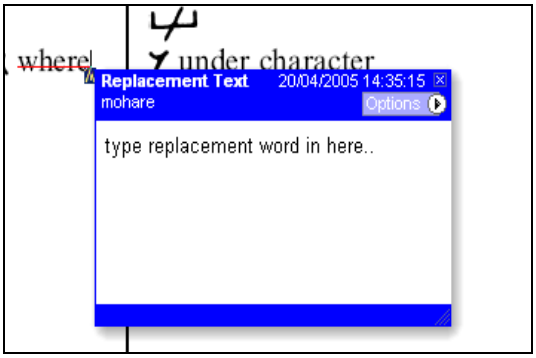


How to use it:

1. Right click into area of either inserted text or relevance to note
2. Select Add Note and a yellow speech bubble symbol and text box will appear
3. Type comment into the text box
4. Click the X in the top right hand corner of the note box to close.

Replacement text tool — For deleting one word/section of text and replacing it

Strikes red line through text and opens up a replacement text box.

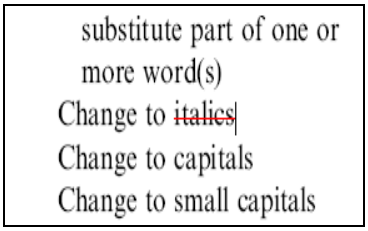


How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
5. Type replacement text in blue box
6. Click outside of the blue box to close

Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

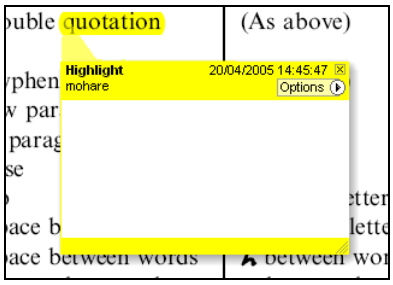
Approved tool — For approving a proof and that no corrections at all are required.

How to use it:

1. Click on the Stamp Tool in the toolbar
2. Select the Approved rubber stamp from the 'standard business' selection
3. Click on the text where you want to rubber stamp to appear (usually first page)

Highlight tool — For highlighting selection that should be changed to bold or italic.

Highlights text in yellow and opens up a text box.

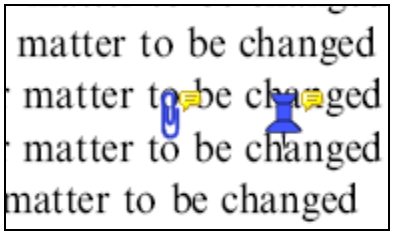


How to use it:

1. Select Highlighter Tool from the commenting toolbar
2. Highlight the desired text
3. Add a note detailing the required change

Attach File Tool — For inserting large amounts of text or replacement figures as a files.

Inserts symbol and speech bubble where a file has been inserted.

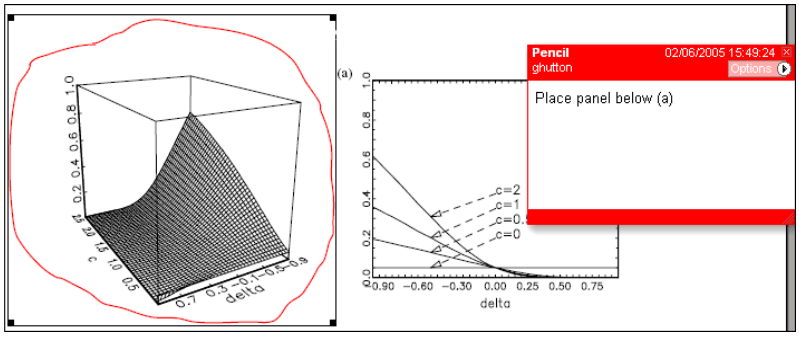


How to use it:

1. Click on paperclip icon in the commenting toolbar
2. Click where you want to insert the attachment
3. Select the saved file from your PC/network
4. Select appearance of icon (paperclip, graph, attachment or tag) and close

Pencil tool — For circling parts of figures or making freeform marks

Creates freeform shapes with a pencil tool. Particularly with graphics within the proof it may be useful to use the Drawing Markups toolbar. These tools allow you to draw circles, lines and comment on these marks.



How to use it:

1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
5. Select Open Pop-Up Note and type in a details of required change
6. Click the X in the top right hand corner of the note box to close.

Help

For further information on how to annotate proofs click on the Help button to activate a list of instructions: