5 Protein-mediated Siderophore Uptake in Gram-negative Bacteria: A Structural Perspective

José D. Faraldo-Gómez

5.1 Introduction

Iron is amongst the most important nutrients of bacteria and also one of the most abundant chemical elements composing the Earth's crust. Under normal conditions, however, free iron is scarce due to its rapid oxidation and the subsequent formation of insoluble hydroxides. Thus, bacteria have had to evolve highly efficient mechanisms of iron uptake in order to ensure a sufficient supply.

These mechanisms generally rely on receptor proteins residing in the various compartments of the bacterial cell envelope that specifically bind and/or translocate a wide range of iron-containing molecules (Fig. 5.1). For many pathogens, these ligands can be the iron-binding proteins that the host itself utilizes to keep iron in solution, or other small proteins that capture heme. Alternatively, bacteria and other microorganisms are able to synthesize, excrete and finally reabsorb low-molecular-weight compounds that chelate environmental iron with outstanding affinity, known as siderophores (Wandersman and Delepelaire 2004).

This chapter will focus on the mechanisms of protein-mediated siderophore uptake in Gram-negative bacteria, from the perspective of structural biology. Consistent with iron's scarcity and importance, these mechanisms involve all the challenging and often poorly understood complexities characteristic of biology at the atomic level, such as allosteric regulation, signaling mechanisms, protein-protein recognition, energy transduction, etc.

The chapter's organization follows the uptake pathway, starting from the outermost surface and then moving towards the cytoplasm. It has been my intention to provide an overview of this process that is accessible, and hopefully not too tedious, to non-specialists in the field of structural protein biology. As usual, I refer those seeking a more detailed understanding to the research papers and reviews in the bibliography list, and to the references therein.

Center for Integrative Sciences, University of Chicago, Chicago, USA,
email: faraldo@uchicago.edu

Soil Biology, Volume 12
Microbial Siderophores
A. Varma, S.B. Chincholkar (Eds.)
© Springer-Verlag Berlin Heidelberg 2007

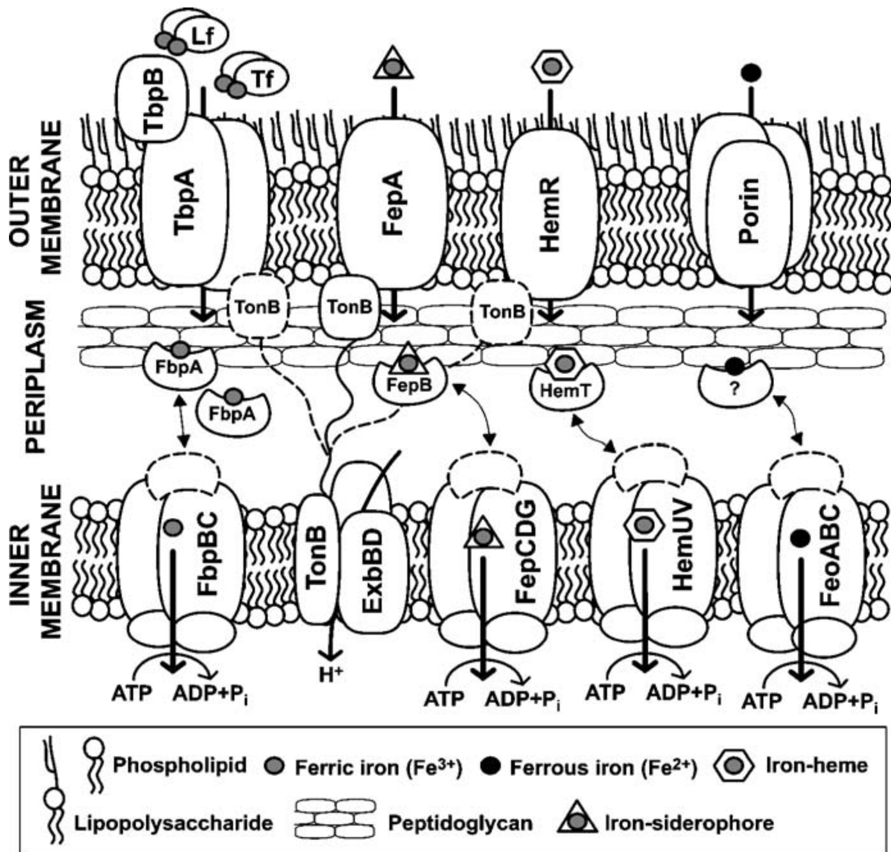

Fig. 5.1. Representative systems for uptake of iron in Gram-negative bacteria (Braun and Killmann 1999; Ratledge and Dover 2000; Crichton 2001; Wandersman and Delepelaire 2004). Under aerobic conditions, iron uptake is mediated by various high-affinity receptor proteins that bind Fe^{3+}-containing compounds on the cell's surface and that subsequently facilitate their translocation into the periplasm. This process is thought to require an input of mechanical energy that is provided by the TonB-ExbBD complex, or homologues thereof, anchored in the inner membrane. Periplasmic binding proteins and ATP-driven transporters in the cytoplasmic membrane ensure further transport into the cell. For example, *Escherichia coli* reabsorbs the endogenous siderophore enterobactin via the FepA receptor and the FepBCDG system. Pathogenic bacteria can also use iron sources from the host; for instance, in *Neisseria* species ferric iron is removed from transferrin (Tf) and lactoferrin (Lf), and transported into the cell by the TbpAB-FbpABC system; in *Yersinia enterocolitica* heme can be taken up directly through the HemRTUV system. Under anaerobic conditions, soluble Fe^{2+} can diffuse across outer membrane porins, and is subsequently imported by energy-dependent systems such as the FeoABC

5.2 Transport Across the Outer Membrane

5.2.1 The Outer-Membrane Receptor and Transporter Proteins

In contrast to Gram-positive bacteria, the outermost surface of Gram-negative bacteria is a double-layered lipid membrane, which encloses both the *murein sacculus* and the inner or cytoplasmic membrane. The external layer of this outer membrane is primarily composed of lipopolysaccharide (LPS) lipids. The strong interactions between LPS lipids, mediated by Mg^{2+} and Ca^{2+}, as well as their reduced intramolecular mobility, make the outer membrane highly impermeable. The outer membrane thus serves as a resistance barrier against environmental hazards such as toxic agents, host-defense proteins and digestive enzymes (Nikaido 1996).

A large number of pore-forming proteins exist in the outer membrane in order to facilitate the uptake of nutrients across the LPS barrier. Ions and other small molecules such as sugars permeate the membrane across water-filled protein channels known as porins, driven by self-diffusion (Koebnik et al. 2000; Nikaido 2003). For larger solutes, or for molecules that are too scarce to rely on diffusion-driven transport, more sophisticated protein architectures are employed. These may include specific binding sites, as well as gating mechanisms whereby a permeation pore is opened or closed depending on whether the appropriate substrate is present, and, in some cases, also on whether an external source of mechanical energy is available.

Regardless of their function, all of the integral outer membrane proteins known to date are structurally distinct in that they fold in the so-called β-barrel architecture, instead of forming bundles of transmembrane α-helices like other membrane proteins do (Schulz 2000; Wimley 2003). The β-barrel architecture results from the lower-than-usual content of hydrophobic amino acids of these proteins, which in turn may be required for the successful translocation of newly synthesized proteins from the cell interior to the outer membrane (Koebnik et al. 2000; Postle 2002).

From the structural and functional viewpoints, the β-barrels involved in siderophore uptake are distinct from other outer membrane proteins in several aspects. First, they display a nanomolar-range affinity for their substrates, in contrast to, e.g., fatty-acid transporters (μM) (van den Berg et al. 2004) or the various porins (mM) (Nikaido 2003). Second, they form very large β-barrels (ca. 25 Å in diameter) that contain an additional protein domain in their interior, which provides the recognition site for the substrates to be transported; this so-called “plug” domain effectively blocks the pore formed by the barrel, and thus constitutes the permeation gate. And third, they require an external input of mechanical energy in order for the permeation gate to open. Incidentally,

uptake of vitamin B_{12} across the outer membrane is analogous to that of siderophores (Kadner 1990).

To date, four members of the family of outer-membrane siderophore transporters have been characterized structurally at the atomic level, by means of X-ray crystallography; these are FhuA (Ferguson et al. 1998; Locher et al. 1998), FepA (Buchanan et al. 1999) and FecA (Ferguson et al. 2002; Yue et al. 2003) from *Escherichia coli*, and FpvA from *Pseudomonas aeruginosa* (Cobessi et al. 2005). These membrane proteins mediate the uptake of the siderophores known as ferrichrome, enterobactin, ferric citrate and pyoverdin, respectively. In addition to these, the structure of the receptor for vitamin B_{12} is also known (Chimento et al. 2003a).

Schematic representations of some of these structures are shown in Fig. 5.2. In all cases, the β-barrel domain is made up of 22 strands; adjacent β-strands are generally connected by short stretches of amino acids on the periplasmic face of the proteins, and with longer stretches in the extracellular side. Interestingly, the conformation of some of these extracellular loops is strongly dependent on the presence of the iron-loaded substrate; specifically, these loops appear to change their structure upon ligand binding, precisely so as to obstruct any possible release of the ligand back into the extracellular solution.

In the interior of the β-barrel, the plug domain is held in place by numerous hydrogen bonds and polar contacts, and effectively prevents any significant permeation (Faraldo-Gómez et al. 2002; Chimento et al. 2005). Thus, translocation of siderophores across these proteins appears to involve a significant structural rearrangement of the plug domain, though the nature of these structural changes is unknown. It is possible that the plug domain comes out of the barrel in its entirety, dragging the bound siderophore with it; alternatively, partial unfolding of its structure while still residing within the barrel domain might be sufficient to open up a permeation pore (Faraldo-Gómez and Sansom 2003; Chimento et al. 2005).

Whatever the case may be, it has been long established that this step in the transport process necessitates the interaction of the outer membrane receptors with a protein anchored in the cytoplasmic membrane, known as TonB (Postle 1990), which is believed to provide the mechanical energy required to alter the conformation of the plug domain (see next section).

That the energy required for transport across the outer membrane is transduced from the cytoplasmic membrane is not striking, since no energy is available in the outer membrane in the form of electrochemical gradients, due to the numerous porins through which ionic species can diffuse freely. However, it is less obvious how the plug domain provides a functional advantage at the outer membrane level, given the energetic cost that it imposes on the uptake process.

One possible rationale is that, in terms of self-protection against environmental hazards, bacteria cannot afford to employ porins of size large enough to accommodate relatively large solutes such as siderophores, vitamin B_{12} or long-chain fatty acids, since these would also allow for the non-specific permeation of other toxic compounds (Nikaido 2003). Alternatively, or in addition, com-

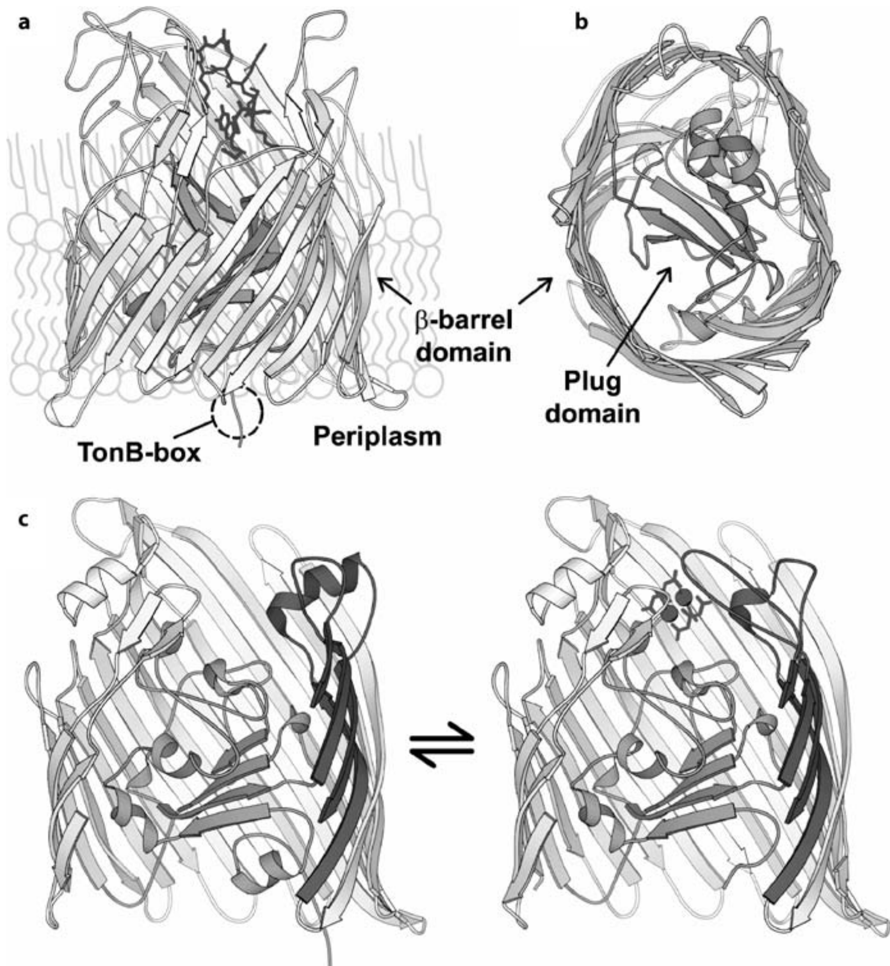

Fig. 5.2. **a** Schematic representation of the three-dimensional atomic structure of FpvA, the outer membrane receptor and transporter of pyoverdin from *Pseudomonas aeruginosa* (Cobessi et al. 2005). *Ribbons* follow the trace of the protein's backbone; *coil-shaped ribbons* represent α-helices and *arrow-shaped forms* represent β-strands; for clarity, amino-acid side chains are omitted. Pyoverdin (*dark grey sticks*) is shown in its binding site at the extracellular side of the plug domain. **b** Structure of FhuA, the outer membrane TonB-dependent transporter for ferrichrome from *E. coli*, viewed from the periplasm (Ferguson et al. 1998; Locher et al. 1998). **c** Ligand-free (*left*) and ligand-bound (*right*) states of FecA, the ferric citrate receptor in *E. coli* (Ferguson et al. 2002). Note the change in the conformation of some of the extracellular loops upon ligand binding

parison of the transport kinetics in porins and TonB-dependent transporters suggests that the presence of the plug domain and the reliance on an energy input might address the challenge of ensuring a sufficient uptake of nutrients such as iron under conditions of starvation.

Transport rates across porins are, within a wide range of solute concentrations, largely correlated with the electrochemical gradients across the membrane. Some specific porins, such as LamB, enhance their efficiency by possessing low-affinity binding sites, but at very low concentrations, as for iron-complexes under physiological conditions, porin-mediated transport systems are inefficient. By contrast, TonB-dependent receptors such as FepA are rather insensitive to the transmembrane concentration gradient, and reach saturation levels at relatively low concentrations and slow rates, which make them generically worse transporters than porins (Klebba and Newton 1998). However, it is their strong dependence on an external input of energy, rather than on concentration gradients, that, together with the high-affinity binding site on the extracellular face of the plug domain, seem to enable these receptors to operate more efficiently than porins at very low solute concentrations, and thus sustain cell growth.

5.2.2 Energy Transduction and TonB

Although it is widely accepted that TonB plays an essential role in the activation of siderophore uptake across the outer membrane, the actual mechanism of energy transduction remains sketchy. TonB is known to be anchored in the inner membrane by a single N-terminal transmembrane helix, and to project into the periplasm by virtue of a long polypeptide stretch rich in proline amino acids (Postle 1993). At the C-terminal end, the protein folds into a globular domain, which interacts with the outer membrane receptors, namely via a specific sequence of amino acids at the periplasmic side of their plug domain, termed the TonB-box (Fig. 5.2) (Pawelek et al. 2006; Shultis et al. 2006). To date, the only available structural information of TonB at the atomic level corresponds to this C-terminal domain, based on X-ray crystallography and NMR spectroscopy analyses (Ködding et al. 2005; Peacock et al. 2005).

The TonB-box has been shown to adopt alternative configurations dependent on whether the outer membrane receptors are loaded with siderophores, and it is believed that only in the ligand-bound form can TonB interact constructively with the plug domain (Ferguson et al. 1998; Locher et al. 1998; Merianos et al. 2000; Coggshall et al. 2001; Ferguson et al. 2002; Chimento et al. 2003b). Although a detailed structural understanding of this allosteric effect would require further investigations (Fanucci et al. 2003a, b), it would certainly be consistent with the notion that energy must not be transduced to ligand-free receptors in order to ensure efficient transport (Moeck et al. 1997; Braun 1998).

Another open question pertains to how TonB becomes energized, that is, competent to activate transport through the outer membrane receptors. At the present time, the prevailing model hypothesizes that TonB is able to alternate between several conformations (Holroyd and Bradbeer 1984; Larsen et al. 1999), by virtue of its association with the ExbBD protein complex, which also resides

in the inner membrane (Postle 1993; Braun 1995). The atomic structure of this macromolecular assembly is unknown, but biochemical analyses yielded a stoichiometry of one TonB per two ExbD per seven ExbB (Held and Postle 2002; Higgs et al. 2002). Sequence analyses also indicate that ExbD is a periplasmic protein anchored to the cytoplasmic membrane by an amino-terminal α-helix (Kampfenkel and Braun 1992), whereas the largest part of ExbB is thought to reside in the cytoplasm, with three membrane-spanning helices anchoring the protein to the cytoplasmic membrane (Kampfenkel and Braun 1993).

More importantly, however, the ExbBD complex is believed to couple the proton motive force sustained across the cytoplasmic membrane to conformational changes in TonB (Larsen et al. 1999; Held and Postle 2002). In other words, the translocation of protons from the periplasm into the cytoplasm, driven by their electrochemical gradient and mediated by ExbBD, would lead to an energized conformation of TonB in which the association of its carboxyl-terminal with the TonB-box of ligand-bound outer membrane receptors would be possible. The subsequent relaxation of TonB to its resting state would induce the rearrangements of the plug domain required for siderophore translocation.

5.3 Transport Across the Periplasm and Cytoplasmic Membrane

5.3.1 Periplasmic Binding Proteins

Once the outer membrane has been permeated, siderophores must traverse the periplasm, that is, the region that separates the outer and inner membrane. The periplasm is believed to be a gel-like environment, 15–25 nm wide, characterized by molecular diffusion rates that are 1000-fold smaller relative to the extracellular solution, and 100-fold smaller relative to the cytoplasm. This extreme viscosity appears to be due to its high protein content, as well as to the presence of the so-called *murein sacculus*, i.e. the complex multilayered network of covalently-linked peptides and sugars that define the shape and volume of the cell (Oliver 1996; Park 1996).

In order to translocate solutes across the periplasm efficiently, bacteria employ a wide range of high-affinity binding proteins that are specific for their respective ligands, which include sugars, amino acids, oligopeptides, ions and other compounds. These periplasmic binding proteins, or PBPs, subsequently deliver their cargo to specific ATP-dependent transporters or chemotaxis receptors residing in the inner membrane.

From the structural viewpoint, most PBPs are alike in that they contain two domains, arranged so as to form a suitable binding site in their interfacial region, although the precise fold and relative mobility of these domains vary across the PBP subfamilies (Dwyer and Hellinga 2004). Although the three-dimensional atomic structure of over 100 PBPs has been resolved by X-ray crystallography, only one member of the siderophore subfamily has been structurally characterized to date, namely the ferrichrome-binding protein FhuD (Clarke et al. 2000). However, the structure of the PBP for vitamin B_{12}, termed BtuF, is also known (Borths et al. 2002; Karpovich et al. 2003), and happens to be similar in its fold to that of FhuD. Since the uptake pathways for B_{12} and siderophores are analogous (see above and below), the similarity of these two PBPs indicates that all members of the siderophore subfamily might share a common architecture and binding mechanism (Köster 2001).

A schematic representation of the atomic structure of FhuD in complex with a gallium-loaded ferrichrome is shown in Fig. 5.3. As for other PBPs, the binding site is located in a cleft in the protein surface that is formed between the so-called N- and C-domains, each of which contains a central sheet of β-strands flanked by several α-helices. The amino acids lining the binding cleft determine the specificity and affinity of FhuD for its ligands, which also include other hydroxamate-type siderophores and derivatives such as coprogen, Desferal and

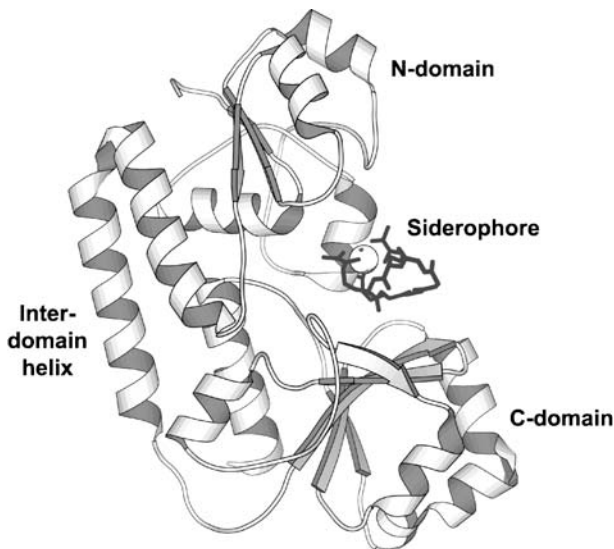

Fig. 5.3. Schematic representation of the three-dimensional atomic structure of the ferrichrome binding protein FhuD, in complex with a gallium-loaded siderophore (Clarke et al. 2000). As before, ribbons follow the trace of the protein's backbone and side chains are omitted for clarity. The gallium ion, which replaces ferric iron in the protein crystal, is shown as a sphere, and the interatomic bonds within ferrichrome are shown as *grey sticks*. The interdomain α-helix that traverses the protein is thought to confer PBPs of the siderophore subfamily with an increased mechanical rigidity

the naturally-occurring antibiotic albomycin (Clarke et al. 2002). Specifically, an arginine, a tyrosine and several tryptophan side chains provide both hydrogen-bonding interactions and a hydrophobic environment that match the chemical nature of the iron-chelating groups in these siderophores. Interestingly, the amino-acid composition of the binding pocket in FhuD is reminiscent of that of the outer membrane receptor FhuA, which illustrates how bacteria evolved very different protein architectures that can support a similar biological function in diverse environments.

Although the atomic structure of a ligand-free FhuD has not been reported as yet, comparison with the structures of ligand-free and ligand-bound BtuF (Fig. 5.4a), as well as with other PBPs in the same structural subfamily, indicates that substrate binding is not likely to result in dramatic structural changes

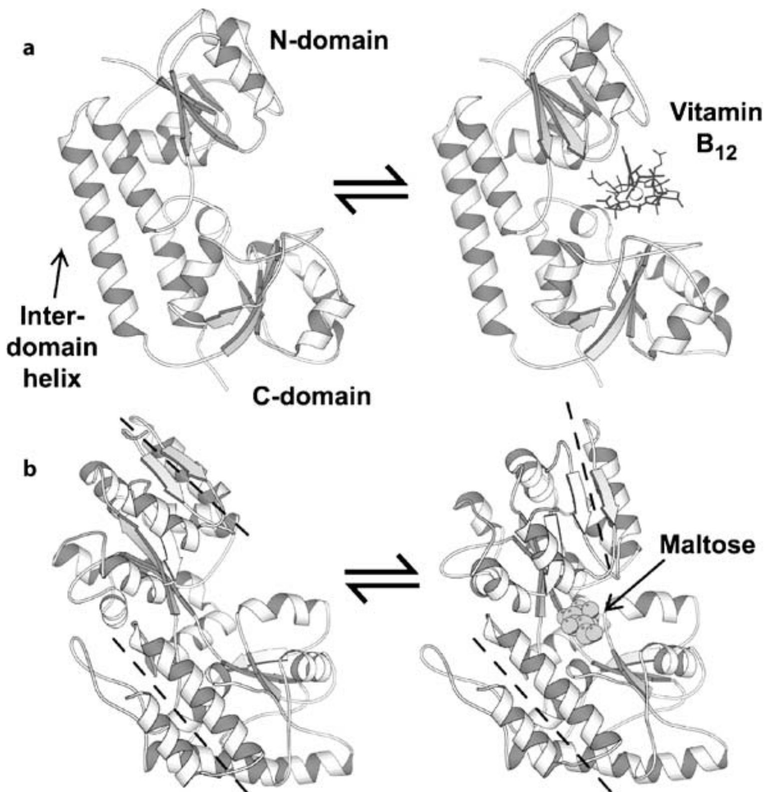

Fig.5.4. **a** Schematic representation of the three-dimensional atomic structure of BtuF, the PBP for vitamin B_{12}, in the ligand-free (*left*) and ligand-loaded (*right*) states (Karpovich et al. 2003). Interatomic bonds within vitamin B_{12} are shown as *sticks*, and the cobalt ion is shown as a *sphere*. **b** Structures of the periplasmic maltose-binding protein MBP, in the ligand-free (*left*) and ligand-bound (*right*) states (Spurlino et al. 1991; Sharff et al. 1992). Maltose atoms are shown as *grey spheres*. Note the dramatic change in the relative orientation and position of the amino- and carboxyl-terminal domains upon ligand binding

beyond the locality of the binding cleft, though a reduction in the overall flexibility in the protein structure is expected. This is in contrast with most of the two-domain PBPs of known structure, which do undergo substantial conformational changes upon ligand binding (Quiocho and Ledvina 1996; Felder et al. 1999; Dwyer and Hellinga 2004); in particular, their binding site, initially exposed to the solvent environment, becomes buried within the protein as a result of the spatial rearrangement of the two flanking domains (Fig. 5.4b). Since PBPs typically deliver their cargo to transport proteins residing in the inner membrane, the different mechanical properties of siderophore PBPs may well correlate with a distinct mechanism of ligand release and transport into the cytoplasm, compared with other members of the family. However, to date no structural information is available that elucidates the nature of the interaction between PBPs and their inner membrane counterparts, so these mechanistic aspects remain a matter of debate.

5.3.2 The Cytoplasmic-membrane ABC Transporters

In contrast to the outer membrane, which primarily serves as a permeability barrier that protects the cell, the inner or cytoplasmic membrane of Gram-negative bacteria is involved in a very wide range of important cellular functions, such as the regulated transport of nutrients and metabolic products, the propagation of signals upon external stimuli, and the generation and conservation of energy. Consistent with this function, a much greater diversity of proteins can be found to reside in this membrane, where they also are in large numbers. In fact, detailed analyses of the inner membrane composition indicate that only three or four layers of phospholipid molecules might separate proteins from each other (Kadner 1996).

In order to deal with this logistical complexity in such a crowded environment, inner-membrane transporters associate specifically with loaded PBPs and pump their ligands into the cytoplasm (Higgins 2001; Davidson and Chen 2004). In their resting state, these membrane transporters, which are in fact aggregates of two identical membrane proteins (i.e. homodimers), are not competent for permeation, but they do provide a suitable docking site for their corresponding PBPs on the membrane surface. Subsequent to this association, large structural changes are thought to take place in the complex that result in (a) the exchange of the substrate between the PBP and transporter, (b) the opening of a translocation pathway across the membrane between the homodimers, (c) the release of the substrate into the cytoplasm, and (d) the disassociation of the PBP from the transporter, which then goes back to the resting state.

Although much remains to be elucidated concerning the actual mechanism of transport, it is well established that this process requires, at one or more stages, the energy derived from the hydrolysis of ATP into ADP. (ATP and ADP stand for adenosine tri- and di-phosphate respectively; the conversion of ATP into

ADP by hydrolysis, i.e. the water-mediated release of the terminal phosphate group from ATP, is a spontaneous reaction provided an appropriate chemical environment. Typically, this reaction results in structural changes in the locality of the ATP/ADP binding sites, which then propagate and become amplified elsewhere in the protein's structure.) Thus, in addition to the two-protein transmembrane domain that provides the permeation pathway, ABC transporters include two extra-membranous nucleotide-binding domains (NBD), which provide suitable sites for ATP binding and hydrolysis. These domains, each of which is non-covalently bound to one of the transmembrane proteins, are also known as ATP binding cassettes – hence the term ABC transporter.

The existing structural information regarding ABC transporters is very limited, and sometimes contradictory (Davidson and Chen 2004). In particular, no three-dimensional atomic structures are available of any of the transporters involved in siderophore uptake across the inner membrane. Fortunately, one of few proteins in this family whose structure has been resolved at atomic resolution is that of the vitamin B_{12} transporter, known as BtuCD (Locher et al. 2002). It is expected that, as for the outer membrane receptors and the PBPs, the structure of siderophore inner-membrane transporters will be similar to that of BtuCD (Köster 2001).

As shown in Fig. 5.5, the transmembrane domain of BtuCD, that is, the BtuC dimer, differs from the outer membrane receptor BtuB in that the protein is folded in the conventional α-helical architecture. In particular, each of monomers contains 10 α-helices, which aggregate to form a so-called helical bundle. As for the outer membrane β-barrel proteins, loops of variable length connect pairs of α-helices, although these are not always adjacent. Each of the NBDs, in this case termed BtuD, binds at the cytoplasmic end of each BtuC monomer, forming an interface that appears to be energetically stabilized primarily by the close contact of hydrophobic amino acids.

From the functional viewpoint, each BtuC bundle and its corresponding BtuD domain is believed to operate as mechanical unit that changes its orientation with respect to the membrane plane during each transport event, opening and closing a permeation pathway that runs along the dimer interface. In particular, hydrolysis of ATP has been proposed to result in a rotational motion of the BtuD domains, which in turn would lead to an increased separation between the BtuC bundles at the cytoplasmic side of the membrane, and a closure at the periplasmic side. Provided that vitamin B_{12} is present in the periplasmic side of BtuC, the proposed structural rearrangements would effectively induce the release of BtuF back into the periplasm and the translocation of B_{12} into the cytoplasm, after which BtuCD would return to its initial state (Locher et al. 2002; Locher and Borths 2004).

Although this mechanism is plausible, it is at odds with previous models proposed for other ABC transporters (Chen et al. 2003), underlying the difficulties in assessing which state in the transport cycle is captured by the crystallographic analysis. In addition, crucial aspects of the transport process are unclear, such as the signalling mechanism that triggers ATP hydrolysis in the cytoplasm upon association of a ligand-loaded PBP at the periplasmic side of the transporter.

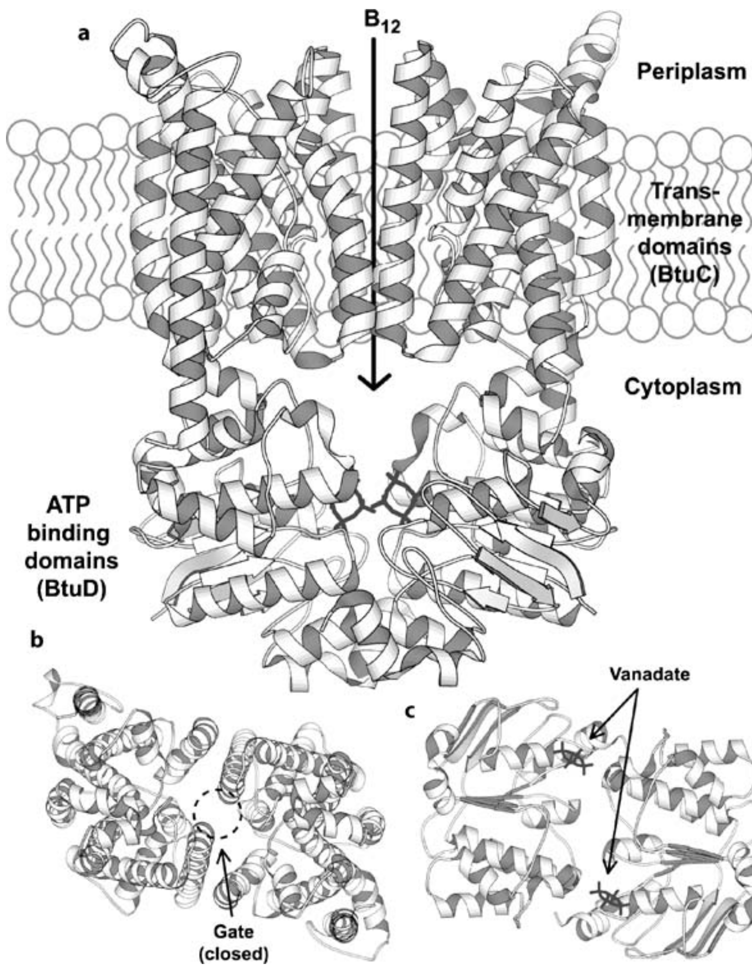

Fig.5.5. a Schematic representation of the structure of BtuCD, the inner membrane transporter of vitamin B_{12} (Locher et al. 2002), which is thought to be similar to those of the siderophore ABC transporters. A cyclotetravanadate molecule (shown in *sticks*) occupies the ATP binding site in each of the NBDs. **b** View of BtuC from the periplasm. The cytoplasmic end of the putative permeation pathway is effectively closed due to the close contact of several side chains in the gate region (not shown for clarity). **c** View of the BtuD nucleotide-binding domains from the cytoplasmic side of BtuC. Note that the ATP binding sites are located in the interface between the two BtuD domains, consistent with the hypothesis that rearrangement of this interface upon ATP hydrolysis may lead to structural changes in BtuC that open the gate and allow permeation of B_{12} into the cytoplasm

5.4 Conclusions

As appropriately put by Klebba (2003), siderophore uptake is "a high-affinity, multi-specific, multi-component, energy-dependent reaction that is a paradigm of ligand-gated transport" across biological membranes. In recent years, the determination of the atomic-resolution structure of several proteins involved in this fascinating process, alongside ongoing biochemical, genetic and spectroscopic investigations, has advanced very significantly our understanding thereof. Nonetheless, fundamental questions remain open with regard to signalling and energy transduction mechanisms, large and small-scale conformational changes, and protein-protein and protein-ligand recognition. As it happens, these questions are also the paradigm of twenty-first-century structural biology and biophysics, which suggest that the elucidation of siderophore uptake systems will continue to be at the forefront of research in molecular biology.

References

Borths EL, Locher KP, Lee AT, Rees DC (2002) The structure of *Escherichia coli* BtuF and binding to its cognate ATP binding cassette transporter. Proc Natl Acad Sci USA 99:16642–16647

Braun V (1995) Energy-coupled transport and signal transduction through the Gram-negative outer membrane via TonB-ExbB-ExbD-dependent receptor proteins. FEMS Microbiol Rev 16:295–307

Braun V (1998) Pumping iron through cell membranes. Science 282:2202–2203

Braun V, Killmann H (1999) Bacterial solutions to the iron-supply problem. Trends Biochem Sci 24:104–109

Buchanan SK, Smith BS, Venkatramani L, Xia D, Essar L, Palnitkar M, Chakraborty R, van der Helm D, Deisenhofer J (1999) Crystal structure of the outer membrane active transporter FepA from *Escherichia coli*. Nat Struct Biol 6:56–63

Chen J, Lu G, Lin J, Davidson AL, Quiocho FA (2003) A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle. Mol Cell 12:651–661

Chimento DP, Kadner RJ, Wiener MC (2003a) The *Escherichia coli* outer membrane cobalamin transporter BtuB: structural analysis of calcium and substrate binding, and identification of orthologous transporters by sequence/structure conservation. J Mol Biol 332:999-1014

Chimento DP, Mohanty AK, Kadner RJ, Wiener MC (2003b) Substrate-induced transmembrane signaling in the cobalamin transporter BtuB. Nat Struct Biol 10:394–401

Chimento DP, Kadner RJ, Wiener MC (2005) Comparative structural analysis of TonB-dependent outer membrane transporters: implications for the transport cycle. Proteins 59:240–251

Clarke TE, Ku SY, Dougan DR, Vogel HJ, Tari LW (2000) The structure of the ferric siderophore binding protein FhuD complexed with gallichrome. Nat Struct Biol 7:287–291

Clarke TE, Braun V, Winkelmann G, Tari LW, Vogel HJ (2002) X-ray crystallographic structures of the *Escherichia coli* periplasmic binding protein FhuD bound to hydroxamate-type siderophores and the antibiotic albomycin. J Biol Chem 277:13966–13972

Cobessi D, Celia H, Folschweiller N, Schalk IJ, Abdallah MA, Pattus F (2005) The crystal structure of the pyoverdine outer membrane receptor FpvA from *Pseudomonas aeruginosa* at 3.6 Å resolution. J Mol Biol 347:121–134

Coggshall KA, Cadieux N, Kadner RJ, Cafiso DS (2001) Site directed spin labeling reveals the first step in the transport pathway of a TonB-dependent transporter. Biophys J 80:213

Crichton RR (2001) Inorganic biochemistry of iron metabolism: from molecular mechanisms to clinical consequences. Wiley, New York

Davidson AL, Chen J (2004) ATP-binding cassette transporters in bacteria. Annu Rev Biochem 73:241–268

Dwyer MA, Hellinga HW (2004) Periplasmic binding proteins: a versatile superfamily for protein engineering. Curr Opin Struct Biol 14:495–504

Fanucci GE, Lee JY, Cafiso DS (2003a) Membrane mimetic environments alter the conformation of the outer membrane protein BtuB. J Am Chem Soc 125:13932–13933

Fanucci GE, Lee JY, Cafiso DS (2003b) Spectroscopic evidence that osmolytes used in crystallization buffers inhibit a conformation change in a membrane protein. Biochemistry 42:13106–13112

Faraldo-Gómez JD, Sansom MSP (2003) Acquisition of iron-siderophores in Gram-negative bacteria. Nat Rev Mol Cell Biol 4:105–116

Faraldo-Gómez JD, Smith GR, Sansom MSP (2002) Molecular dynamics simulations of the bacterial outer membrane protein FhuA: a comparative study of the ferrichrome-free and bound states. Biophys J 85:1406–1420

Felder CB, Graul RC, Lee AY, Merkle HP, Sadee W (1999) The Venus flytrap of periplasmic binding proteins: an ancient protein module present in multidrug receptors. AAPS PharmSci 1:A2

Ferguson AD, Hofmann E, Coulton JW, Diederichs K, Welte W (1998) Siderophore-mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide. Science 282:2215–2220

Ferguson AD, Chakraborty R, Smith BS, Esser L, van der Helm D, Deisenhofer J (2002) Structural basis of gating by the outer membrane transporter FecA. Science 295:1715–1719

Held KG, Postle K (2002) ExbB and ExbD do not function independently in TonB-dependent energy transduction. J Bacteriol 184:5170–5173

Higgins CF (2001) ABC transporters: physiology, structure and mechanism – an overview. Res Microbiol 152:205–210

Higgs PI, Larsen RA, Postle K (2002) Quantification of known components of the *Escherichia coli* TonB energy transduction system: TonB, ExbB, ExbD and FepA. Mol Microbiol 44:271–281

Holroyd CD, Bradbeer C (1984) Cobalamin transport in *Escherichia coli*. In: Schlessinger D (ed) Microbiology. American Society for Microbiology, Washington DC, pp 21–23

Kadner RJ (1990) Vitamin B_{12} transport in *Escherichia coli*: energy coupling between membranes. Mol Microbiol 4:2027–2033

Kadner RJ (1996) Cytoplasmic membrane. In: Neidhardt F (ed) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington DC, pp 58–87

Kampfenkel K, Braun V (1992) Membrane topology of the *Escherichia coli* ExbD protein. J Bacteriol 174:5485–5487

Kampfenkel K, Braun V (1993) Topology of the ExbB protein in the cytoplasmic membrane of *Escherichia coli*. J Biol Chem 268:6050–6057

Karpovich NK, Huang HH, Smith PC, Hunt JF (2003) Crystal structures of the BtuF periplasmic binding protein for vitamin B12 suggest a functional important reduction in protein mobility upon ligand binding. J Biol Chem 278:8429–8434

Klebba PE (2003) Three paradoxes of ferric enterobactin uptake. Front Biosci 8:1422–1436

Klebba PE, Newton SMC (1998) Mechanisms of solute transport through the outer membrane proteins: burning down the house. Curr Opin Microbiol 1:238–248

Koebnik R, Locher KP, van Gelder P (2000) Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Mol Microbiol 37:239-253

Ködding J, Killig F, Polzer P, Howard SP, Diederichs K, Welte W (2005) Crystal structure of a 92-residue C-terminal fragment of TonB from *Escherichia coli* reveals significant conformational changes compared to structures of smaller TonB fragments. J Biol Chem 280:3022–3028

Köster W (2001) ABC transporter-mediated uptake of iron, siderophores, heme and vitamin B_{12}. Res Microbiol 152:291–301

Larsen RA, Thomas MG, Postle K (1999) Proton motive force, ExbB and ligand-FepA drive conformational changes in TonB. Mol Microbiol 31:1809–1824

Locher KP, Borths EL (2004) ABC transporter architecture and mechanism: implications from the crystal structures of BtuCD and BtuF. FEBS Lett 564:264–268

Locher KP, Rees B, Koebnik R, Mitschler A, Moulinier L, Rosenbusch JP, Moras D (1998) Transmembrane signalling across the ligand-gated FhuA receptor: crystal structures of free and ferrichrome-bound states reveal allosteric changes. Cell 95:771–778

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

Merianos HJ, Cadieux N, Lin CH, Kadner RJ, Cafiso DS (2000) Substrate-induced exposure of an energy-coupling motif of a membrane transporter. Nat Struct Biol 7:205–209

Moeck G, Coulton JW, Postle K (1997) Cell envelope signalling in *Escherichia coli*: ligand binding to the ferrichrome-iron receptor FhuA promotes interaction with the energy-transducing protein TonB. J Biol Chem 272:28391–28397

Nikaido H (1996) Outer membrane. In: Neidhardt F (ed) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington DC. pp 29–47

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593–656

Oliver DB (1996) Periplasm. In: Neidhardt F (ed) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington DC, pp 88–103

Park JT (1996) The *murein sacculus*. In: Neidhardt F (ed) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington DC, pp 48–57

Pawelek PD, Croteau N, Ng-Thow-Hing C, Khursigara CM, Moiseeva N, Allaire M, Coulton JW (2006) Structure of TonB in complex with FhuA, *E. coli* outer membrane receptor. Science 312:1399–1402

Peacock RS, Weljie AM, Howard SP, Price FD, Vogel HJ (2005) The solution structure of the C-terminal domain of TonB and interaction studies with TonB-box peptides. J Mol Biol 345:1185–1197

Postle K (1990) TonB and the Gram-negative dilemma. Mol Microbiol 4:2019–2025

Postle K (1993) TonB protein and energy transduction between membranes. J Bioenerg Biomembr 25:591–601

Postle K (2002) Close before opening. Science 295:1658–1659

Quiocho FA, Ledvina PS (1996) Atomic structure and specificity of bacterial periplasmic receptors for active transport and chemotaxis: variation on common themes. Mol Microbiol 20:17–25

Ratledge C, Dover LG (2000) Iron metabolism in pathogenic bacteria. Annu Rev Microbiol 54:881–941

Schulz GE (2000) β-Barrel membrane proteins. Curr Opin Struct Biol 10:443–447

Sharff AJ, Rodseth LE, Spurlino JC, Quiocho FA (1992) Crystallographic evidence of a large ligand-induced hinge-twist motion between two domains of the maltodextrin binding protein involved in active transport and chemotaxis. Biochemistry 31:10657–10663

Shultis DD, Purdy MD, Banchs CN, Wiener MC (2006) Outer membrane active transport: structure of the BtuB:TonB complex. Science 312:1396–1399

Spurlino JC, Lu GY, Quiocho FA (1991) The 2.3-Å resolution structure of the maltose or maltodextrin binding protein, a primary receptor of bacterial active transport and chemotaxis. J Biol Chem 266:5202–5219

van den Berg B, Black PN, Clemons WM Jr, Rapoport TA (2004) Crystal structure of the long-chain fatty acid transporter FadL. Science 304:1506–1509

Wandersman C, Delepelaire P (2004) Bacterial iron sources: from siderophores to hemophores. Annu Rev Microbiol 58:611–647

Wimley WC (2003) The versatile β-barrel membrane protein. Curr Opin Struct Biol 13:404–411

Yue WW, Grizot S, Buchanan SK (2003) Structural evidence for iron-free citrate and ferric citrate binding to the TonB-dependent outer membrane transporter FecA. J Mol Biol 332:353–368