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Review

TonB-dependent receptors—structural perspectives

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Abstract

Plants, bacteria, fungi, and yeast utilize organic iron chelators (siderophores) to establish commensal and pathogenic relationships with hosts and to survive as free-living organisms. In Gram-negative bacteria, transport of siderophores into the periplasm is mediated by TonB-dependent receptors. A complex of three membrane-spanning proteins TonB, ExbB and ExbD couples the chemiosmotic potential of the cytoplasmic membrane with siderophore uptake across the outer membrane. The crystallographic structures of two TonB-dependent receptors (FhuA and FepA) have recently been determined. These outer membrane transporters show a novel fold consisting of two domains. A 22-stranded antiparallel β-barrel traverses the outer membrane and adjacent β-strands are connected by extracellular loops and periplasmic turns. Located inside the β-barrel is the plug domain, composed primarily of a mixed four-stranded β-sheet and a series of interspersed α-helices. Siderophore binding induces distinct local and allosteric transitions that establish the structural basis of signal transduction across the outer membrane and suggest a transport mechanism.

Keywords: TonB-dependent receptor; Outer membrane protein; Siderophore; Transporter; Signal transduction

1. Introduction

Iron has played an essential role in the evolution of life on earth, and is required by most microorganisms [1]. As a transition element, iron can reversibly modify its oxidation state (Fig. 1). The ability to lose or gain a single electron generates a wide range of favorable reduction–oxidation potentials. Accordingly, this essential trace element is often used as a cofactor in key metabolic processes, including deoxyribonucleotide synthesis, oxidative phosphorylation, and electron transport [2]. Despite its relative abundance in nature, atmospheric oxygen rapidly oxidizes iron to form stable, but sparingly soluble, ferric oxyhydroxides. As a result, the concentration of biologically available iron may be as low as 10^{-18} M under physiological conditions [3].

To colonize iron-restricted regions within hosts and to satisfy their nutritional requirement, bacteria have developed extensive siderophore-mediated iron acquisition strategies [4]. Under iron-limiting conditions, most bacteria secrete at least one siderophore. These low molecular weight compounds (500–1500 Da) can be divided into three groups based on chemical composition: (i) catechols (e.g. enterobactin); (ii) hydroxycarboxylates (e.g. citrate); and (iii) hydroxamates (e.g. ferrichrome). Of the approximately 200 known siderophores, most possess three bidentate iron chelating groups [3]. Although these agents display considerable structural diversity, all form six coordinate complexes with ferric iron of extraordinary affinity (formation constants range from $\approx 10^{23}$ to 10^{49}).

Siderophores avidly scavenge ferric ions and are bound by specific outer membrane receptors with high affinity ($K_d \sim 0.1$ μM). In contrast to porins which utilize passive diffusion for solute uptake, outer membrane receptors actively pump siderophores into the periplasm against a concentration gradient by utilizing an energy-dependent transport mechanism. Gram-negative bacteria have multiple siderophore-mediated iron acquisition pathways consisting of an outer membrane receptor, a periplasmic binding protein, and a complex of one or two cytoplasmic membrane proteins with an associated ATP-binding cassette, which together form an ABC transporter. Most

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$$Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \quad \text{Fenton}$$

$$Fe^{3+} + O_2^{-\cdot} \Rightarrow Fe^{2+} + O_2$$

$$O_2^{-\cdot} + H_2O_2 \Rightarrow O_2 + OH^{\cdot} + OH^{-} \quad \text{Haber-Weiss}$$

$$O_2^{-\cdot} + O_2^{-\cdot} + 2H^{+} \Rightarrow H_2O_2 + O_2 \quad \text{Superoxide dismutase}$$

$$H_2O_2 + H_2O_2 \Rightarrow 2H_2O + O_2 \quad \text{Catalase}$$

$$H_2O_2 + AH_2 \Rightarrow 2H_2O + A \quad \text{Peroxidase}$$

Fig. 1. The Fenton and Haber–Weiss reactions coordinate the reduction of Fe^{III} (ferric) to Fe^{II} (ferrous) iron in the presence of hydrogen peroxide with the production of molecular oxygen, hydroxyl anions, superoxide and hydroxyl radicals [4]. Unregulated intracellular iron metabolism causes lipid peroxidation, oxidation of sulfhydryl groups, DNA-damaging lesions, membrane disruptions, and ultimately, cell death. Superoxide dismutases normally degrade superoxide radicals [66]. Peroxide anions are subsequently converted into molecular oxygen and water by catalases and peroxidases. These systems protect bacteria against potent oxidizing agents that are generated either endogenously during respiration or exogenously by host organisms [67].

bacteria express parallel systems each specific for a single siderophore, underscoring the redundancy of these systems, their biological significance, and their importance in establishing infection and virulence. Although *Escherichia coli* encodes the genes for the biosynthesis of a single endogenous siderophore (enterobactin), this bacterium also

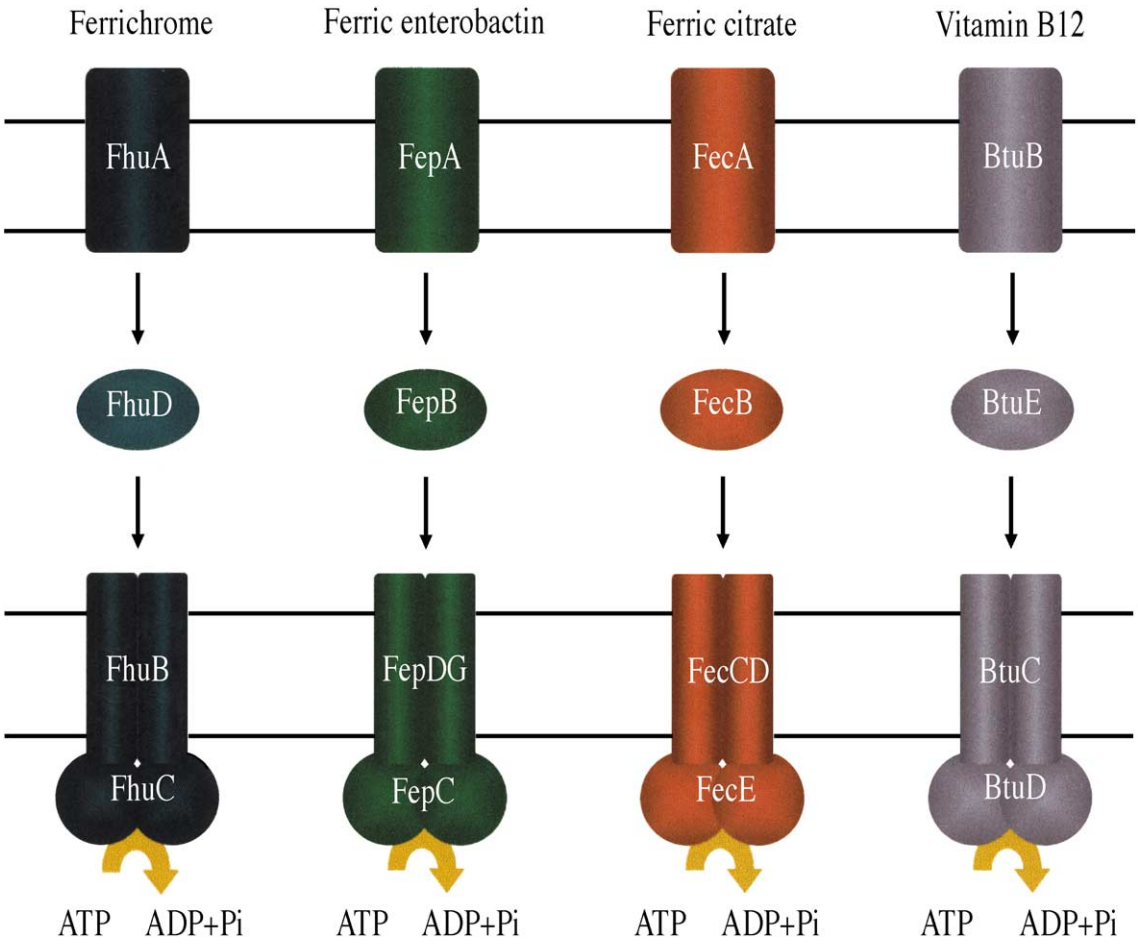


Fig. 2. Select siderophore-mediated iron acquisition systems of *E. coli*. Ligand transport across the outer membrane is TonB-dependent and requires the chemiosmotic potential of the cytoplasmic membrane. Hydroxamates—FhuA transports ferrichrome, ferricrocin, and the antibiotics albomycin, rifamycin CGP4832 and antibiotic microcin J25 [68]. Catechols—FepA transports ferric enterobactin [18]. Hydroxycarboxylates—FecA transports ferric citrate [61]. Cyanocobalamin (vitamin B_{12} contains cobalt) is grouped with those for siderophores, as they share a similar organization of proteins and utilize an analogous mechanism of ligand transport. The vitamin B_{12} receptor in the outer membrane of *E. coli* is BtuB [20].

utilizes siderophores secreted by co-existing microbes. For example, the ferric hydroxamate uptake system allows *E. coli* to utilize the fungal siderophore ferrichrome as an iron source.

All siderophore-mediated iron acquisition pathways are arranged such that iron chelates are transported across the outer membrane, periplasm, and the cytoplasmic membrane in discrete steps. The proteins required for each transport stage are localized to different cell envelope compartments and have specific energetic requirements (Fig. 2). Localized within the outer membrane is a diverse family of outer membrane receptors that transport siderophores into the periplasm. Siderophore uptake across the outer membrane is dependent upon the presence of a complex of three cytoplasmic membrane proteins TonB, ExbB and ExbD, and their ability to couple a proton gradient with siderophore transport [5]. Hence, this family of active outer membrane transport proteins has been termed TonB-dependent receptors.

The aim of this review is to provide an analysis of the relationship between the three-dimensional structure of the ferrichrome receptor FhuA and the ferric enterobactin receptor FepA, and their biological function as TonB-dependent transporters in the outer membrane of *E. coli* [6,7]. A discussion of the TonB–ExbB–ExbD complex and its role in energy transduction will also be presented.

2. Molecular architecture

The three-dimensional structures of unliganded FhuA [8,9] and unliganded FepA [10], and FhuA in complex with various siderophores [8,9,11] and antibiotics [11,12] have recently been determined by X-ray crystallography (Table 1). Both receptors show similar molecular architectures composed of two domains (Figs. 3 and 4). Embedded within the outer membrane is a monomeric C-terminal β-barrel with a shear number of 24 [13]. Formed by 22 antiparallel β-strands, the barrel is approximately 70 Å in height (Table 1). The right-handed twist of the β-strands produces an elliptical-shaped barrel with a diameter of 35×47 Å (measured between equivalent Cα-positions on opposite β-strands). Adjacent β-strands are connected by 11 long solvent-accessible loops at the external membrane surface and 10 short turns directed toward the periplasm. The tilt of the β-strands relative to the barrel axis is approximately 45°, and all transmembrane strands extend beyond the outer leaflet of the bilayer (Figs. 3 and 4).

Table 1
Statistical comparison of the three-dimensional structures of FepA and FhuA as determined by X-ray crystallography

Structural	FepA	FhuA
Barrel residues/Total residues	571/723	553/714
Transmembrane β-strands (β1–β22)		
Minimal length	6	7
Maximal length	20	25
Average length	12.5	14.4
Percentage of barrel (%)	48.0	57.1
Extracellular loops (L1–L11)		
Minimal length	3	3
Maximal length	38	35
Average length	22.1	17.0
Percentage of barrel (%)	42.6	33.8
Periplasmic turns (T1–T10)		
Minimal length	2	2
Maximal length	11	9
Average length	5.4	5.0
Percentage of barrel (%)	9.5	9.0

Crystallographic	Unliganded		Liganded				
	FepA	FhuA[a]	Ferrichrome	Ferricrocin[a]	Phenylferricrocin[a]	Albomycin[a]	CGP4832[a,b]
Resolution (Å)	2.40	2.50 and 2.75	2.60	2.70	2.95	3.10	2.90
R_{work}/R_{free} (%)	23.4/28.5	22.1/27.1 and 18.6/24.3	18.3/23.0	23.1/27.6	22.5/27.8	22.2/28.3	23.3/27.5
PDB code	1FEP [10]	1QFG [8,14] and 1BY3 [9]	1BY5 [9]	1QFF [8]	1QJQ [11]	1QKC [11]	1FI1 [14]

The crystallographic structure of FepA in complex with ferric enterobactin has not been described [10].

[a] These structures were determined in complex with a single lipopolysaccharide molecule non-covalently bound to the external membrane-embedded surface of the receptor [8,11,12,14].

[b] CGP4832 is a potent functional derivative of the antibiotic rifamycin, and does not contain ferric iron [14]. A statistical comparison of FepA and FhuA to other bacterial outer membrane proteins has been presented elsewhere [69].

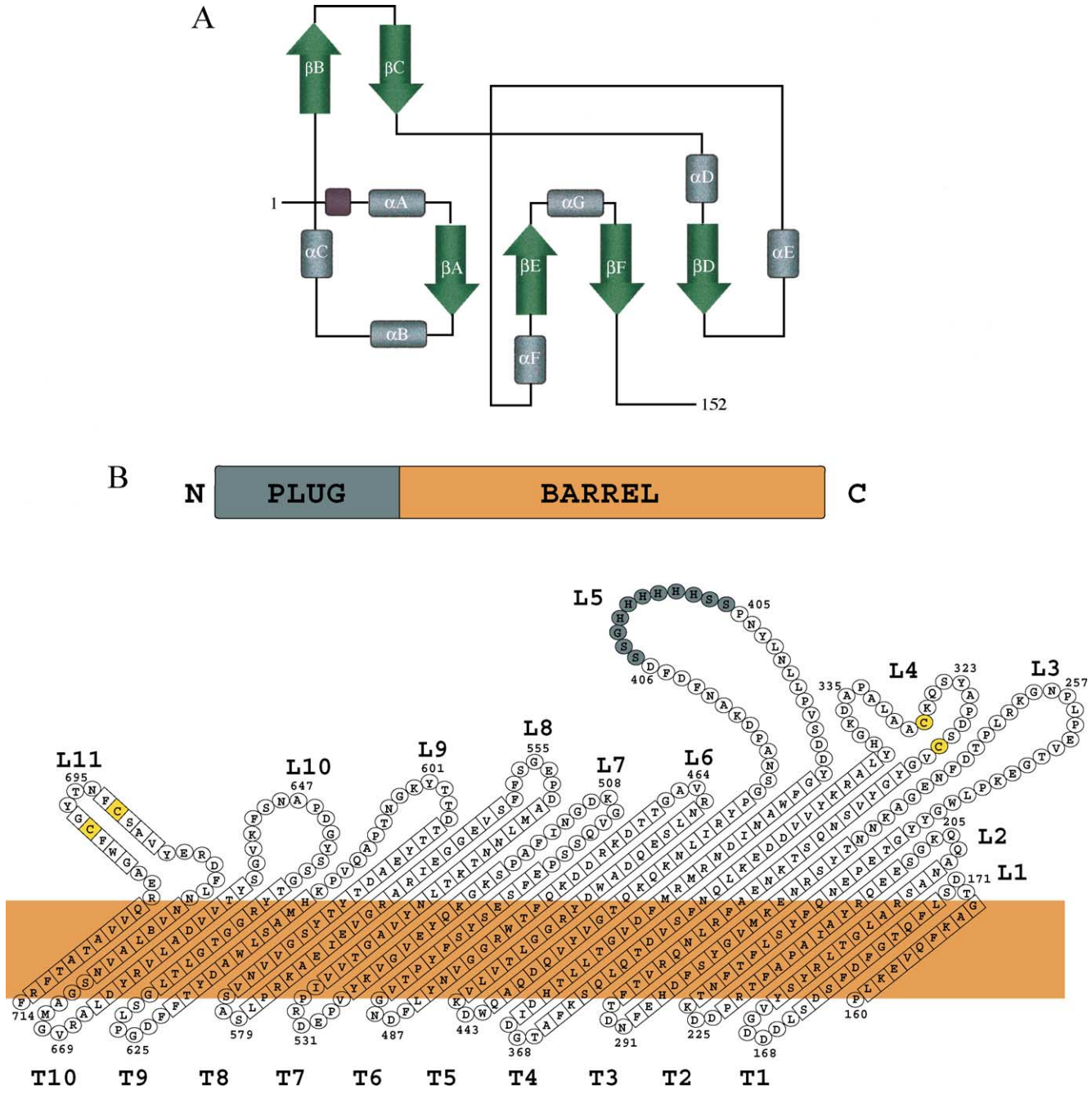


Fig. 3. Molecular architecture of FhuA and FepA. (A) Secondary structure representation of the plug domain of FepA. The TonB-box is colored purple. The equivalent domain of FhuA shows a similar fold. (B) Secondary structure of the β-barrel of FhuA. Long loops are exposed to the solvent (top), whereas short turns are directed toward the periplasm (bottom). FepA also presents a 22-stranded β-barrel of similar dimensions, although the extracellular loops of these receptors differ markedly. An affinity-tag was inserted into L5 [8,11,12,14] and is shown in red.

As observed with other integral outer membrane proteins, two girdles of aromatic residues extend into the outer membrane, inscribing a 25-Å hydrophobic band upon the membrane-exposed surface of FhuA and FepA (Fig. 5). The crystallographic structure of FhuA in complex with a single non-covalently bound lipopolysaccharide molecule shows electrostatic protein–lipid interactions between the phosphorylated glucosamine moieties and van der Waals contacts with the acyl chains of lipopolysaccharide [14]. These findings precisely define the membrane-embedded and solvent-accessible surfaces of the receptor, thereby orienting FhuA within the outer membrane.

Located within the β-barrels of FhuA and FepA is an N-terminal domain, termed the plug (Fig. 4B). The plug domain consists of a mixed four-stranded β-sheet with short interspersed α-helices and connecting loops (Figs. 3A and 4B). The plane of the β-sheet is tilted by approximately 45° relative to the membrane normal, such

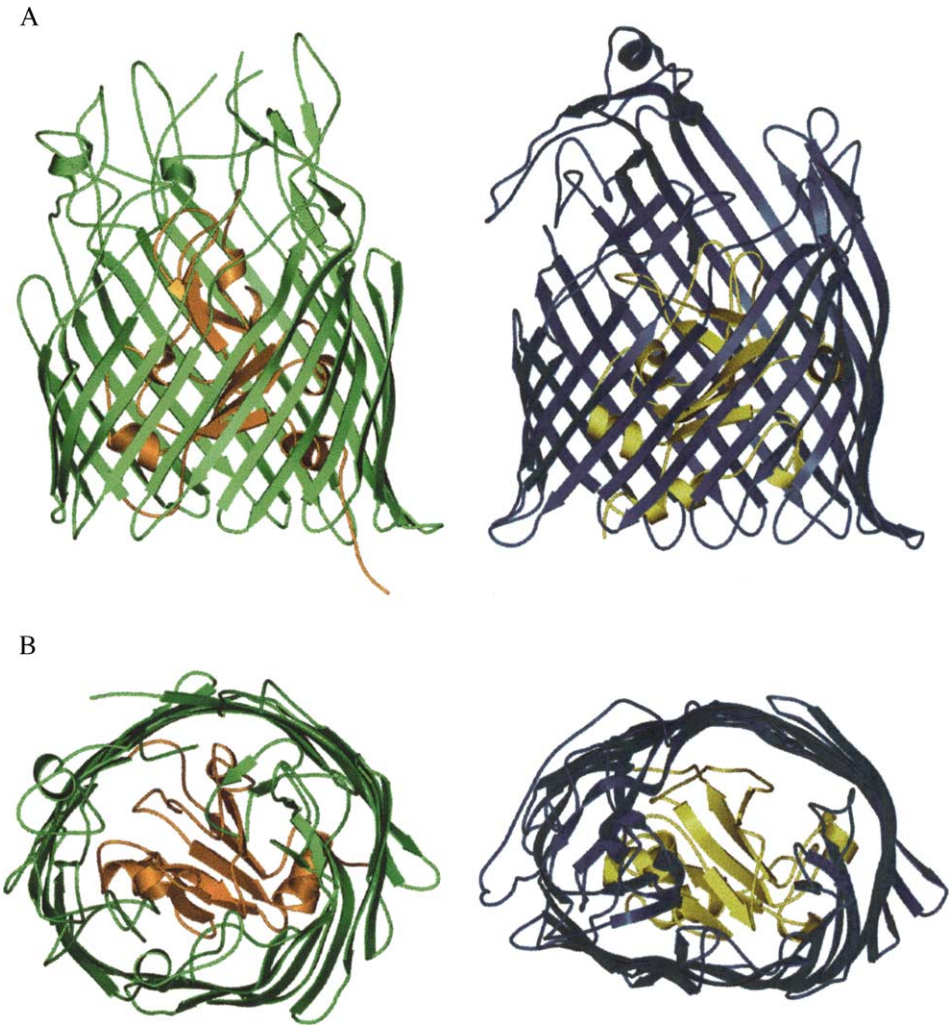


Fig. 4. Crystallographic structures of unliganded FepA and unliganded FhuA. (A) These receptors are presented as found in the outer membrane with the extracellular loops (top) and periplasmic turns (bottom) oriented toward the solvent and periplasm; and (B) View is given from the external solvent. The β-barrels are colored blue (FhuA) and green (FepA), and the plug domains shown in yellow (FhuA) and orange (FepA).

that its presence blocks the direct passage of siderophores through the outer membrane. Two loops of the plug domain of FepA extend approximately 20 Å beyond the outer membrane interface [10]. FhuA has three equivalent loops [apices A (R81), B (Q100) and C (Y116)] that are important for ligand binding [8,9,11,12].

The plug domain also delineates two pockets within the β-barrels of FhuA and FepA (Fig. 4A). Located above the plug domain and open to the solvent is the conical-shaped extracellular pocket. The electrostatic pocket linings for these receptors are different, and are tailored to their respective siderophores (ferrichrome is uncharged at physiological pH, whereas ferric enterobactin is negatively charged). A periplasmic pocket is found below the mixed β-sheet of the plug domain and is exposed to the periplasm. The N-termini of FepA (residues 1–10) and FhuA (residues 1–18) are localized to the periplasm, disordered, and could not be modeled [8–12,14].

3. Ligand-binding site

The crystallographic structure of FepA in complex with ferric enterobactin has not been described [10]. However, structures of FhuA in complex with the siderophores ferrichrome [9], ferricrocin [8], phenylferricrocin [11], and the antibiotics albomycin [11] and rifamycin CGP4832 [12] are available (Table 1). Non-covalently bound within the extracellular pocket of FhuA and approximately 20 Å beyond the outer membrane is a single ligand molecule. The iron chelating portions of ferrichrome, ferricrocin, phenylferricrocin, and albomycin (all ferric hydroxamates) are bound in identical orientations with the ferric ion buried, whereas the peptide components of these siderophores remain solvent-accessible within the extracellular pocket (Fig. 6). The structure of FhuA in complex with the semi-synthetic rifamycin derivative CGP4832 shows that this antibiotic occupies a similar position within the extracellular pocket

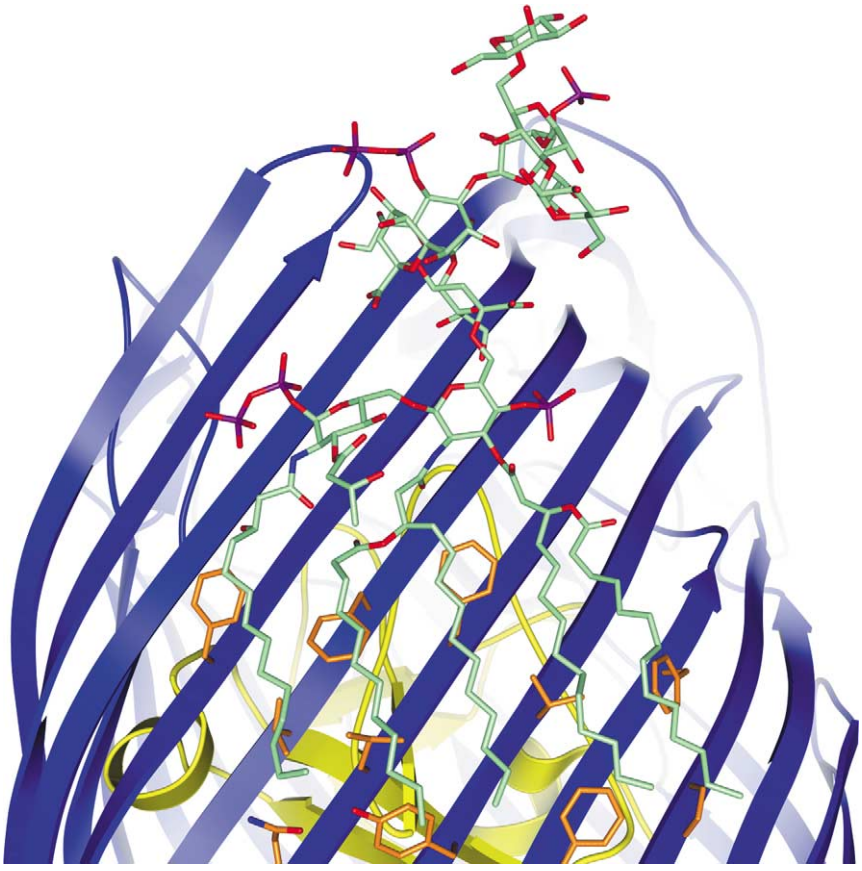


Fig. 5. Crystallographic structure of FhuA in complex with lipopolysaccharide. A single *E. coli* K-12 lipopolysaccharide molecule was observed non-covalently bound to the membrane-embedded surface of the receptor [14], and is shown as bond model with carbon atoms green, oxygen atoms red, nitrogen atoms blue, and phosphorous atoms purple. The β-barrel and plug domain of FhuA are colored blue and yellow, respectively. Side chains forming the upper and lower aromatic girdles are presented in orange.

[12,15]. Although chemically dissimilar, these compounds form electrostatic and van der Waals contacts with common side chains from FhuA (Fig. 7 and Table 2). Within the plug domain, apices A, B and C form most protein–ligand interactions. Additional contacts between FhuA and its ligand are provided by residues from the β-barrel located in L3, L4, L5, L7, L8 and L11, and β7, β8, β9 and β10. Given the structural diversity of these siderophores and antibiotics, these data illustrate the limited structural specificity of the binding site for this multifunctional transporter.

4. Ligand-induced structural changes

Binding of siderophores and antibiotics to the binding site of FhuA induces local and allosteric transitions. Comparing the Cα-positions of unliganded FhuA to those of FhuA liganded with ferricrocin [8], ferrichrome [9], phenylferricrocin [11], albomycin [11] or CGP4832 [12] reveals near perfect superposition of the β-barrels. However, three distinct plug domain conformations are observed: unliganded, siderophore-liganded, and CGP4832-liganded. Transition from the unliganded to siderophore-liganded conformation causes local changes in the positions of the Cα-atoms of apices A and B, illustrating an induced-fit binding mechanism. Specifically, apices A and B move by 0.7–2.0 Å toward the siderophore, whereas apex C remains stationary (Fig. 8). In the FhuA–CGP4832 complex, only apex B is shifted vertically 0.5–1.5 Å toward the antibiotic, apex A remains fixed, thereby establishing the CGP4832-liganded conformation.

The upward translation of apex A is propagated to all plug domain loops between this point and the periplasmic pocket of FhuA. In the unliganded conformation, helix αA termed the switch helix (residues 24–29) is positioned within a cavity formed by the eighth and ninth periplasmic turns, βA, and helix αB. Siderophore binding disrupts the integrity of this cavity, causing all residues N-terminal of R31 to undergo a helix-to-coil transition within the periplasmic pocket (Fig. 8) (e.g. bending away from the previous helix axis by 180°, W22 is displaced 17 Å from its unliganded Cα-position). Apex A in the FhuA–CGP4832 complex remains fixed 4.6 Å away from the nearest ligand atom, as in the unliganded conformation. No upward movement of plug domain loops is induced, leaving the switch helix wound. However, increases in the relative *B*-

factors of the Cα-atoms composing the switch helix demonstrate that this segment is destabilized upon CGP4832 binding.

5. Transmembrane signaling and TonB

Given that TonB-dependent receptors must compete for a limited number of TonB molecules [16], the unwinding of the switch helix functions as a tightly regulated, transmembrane signal that distinguishes unoccupied from occupied outer membrane receptors. This pronounced allosteric transition likely recruits TonB to its principal site of known interaction with TonB-dependent receptors, the TonB-box [17]. This N-terminal five-residue sequence [TXXV(S/T); where X= hydrophobic side chain] is the primary signature of TonB dependence [18,19].

Initial evidence suggesting that TonB associated with the TonB-boxes of outer membrane receptors was provided by genetic suppressor mutation studies. Bacterial cells expressing TonB proteins with single amino acid substitutions at position 160 were capable of restoring transport activity to TonB-dependent receptors with altered TonB-boxes [20–25]. Biochemical and biophysical techniques later demonstrated that the TonB-boxes of outer membrane transporters physically interact with TonB. When treated with formaldehyde, FepA can be chemically cross-linked to TonB [26]. The introduction of point mutations within the TonB-box of FepA prevents cross-linking and the TonB-dependent transport of ferric enterobactin in vivo

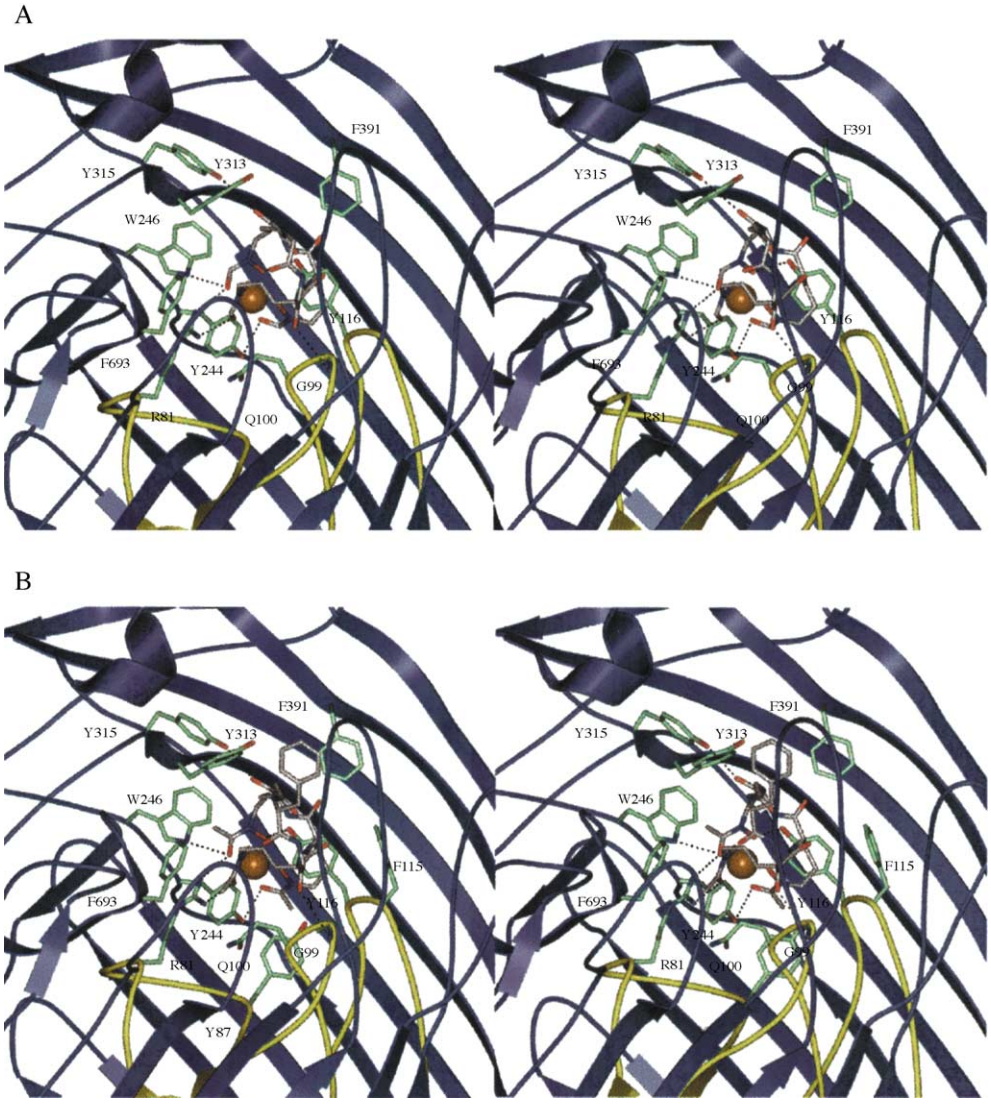


Fig. 6. Stereo representation of the binding sites for (A) ferricrocin; (B) phenylferricrocin; (C) albomycin; and (D) rifamycin CGP4832. The β-barrel is colored blue and the plug domain yellow. Ligand molecules are shown as bond models with carbon atoms gray, oxygen atoms red, nitrogen atoms blue, sulfur atoms green, and ferric ions as orange spheres. Protein–ligand hydrogen bonds are represented by dotted lines.

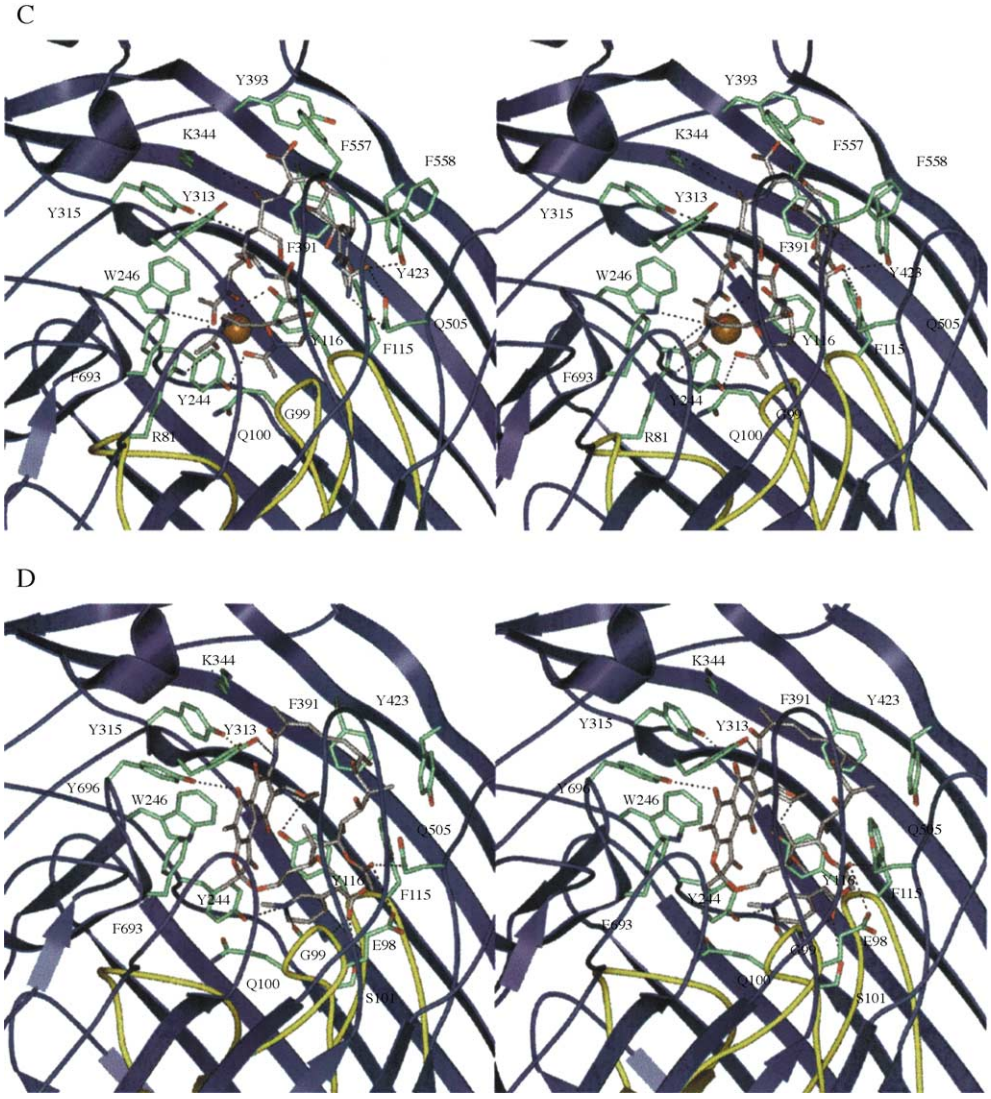


Fig. 6 (*continued*).

[27]. Similar studies have been carried out with FhuA [28]. The three-dimensional structures of FhuA and FepA revealed that the TonB-box is localized to the periplasm [8–12,14]. Following this advance, site-directed disulfide cross-linking was used to unequivocally demonstrate direct physical interactions between the TonB-box of BtuB and TonB [29]. Cysteine residues introduced at successive positions within the TonB-box form disulfide bridges with a single cysteine residue when inserted at or near to position 160 of TonB [29,30].

Recent studies, in which the plug domains of FhuA and FepA were genetically removed, showed residual TonB-dependent activity for these mutant receptors [31,32]. In the absence of the plug domain and the TonB-box, TonB physically interacts with the β-barrel of FhuA and FepA. Although secondary sites of interactions remain to be determined, it is likely that an energized TonB molecule interacts with at least one periplasmic turn, which drives TonB-dependent siderophore transport [33].

6. TonB–ExbB–ExbD complex and energy transduction

Different from solute transport across the cytoplasmic membrane where primary or secondary energy-dependent transporters utilize ion translocation or the hydrolysis of ATP for uptake, there is no known energy source within the outer membrane to drive the translocation of siderophores into the periplasm. Thus, Gram-negative bacteria couple the chemiosmotic gradient of the cytoplasmic membrane with siderophore transport across the outer membrane [5]. Siderophore uptake into the cytoplasm is mediated by periplasmic binding protein-dependent ABC transporters. The division of ener-

getic requirements reflects the compartmentalization of the transport processes (Fig. 2). While the precise mechanism by which chemiosmotic potential and ATP catalysis energize transport remains to be determined, it is known that the TonB–ExbB–ExbD complex is essential for the transfer of energy to the outer membrane.

Alignment of Enterobacteriaceae tonB sequences identifies three domains that contribute in distinct ways to TonB's function as an energy transducer [34,35]. The N-terminus of TonB functions as an uncleaved export signal that anchors the protein in the cytoplasmic membrane [36]. Although deletion of this anchor does not inhibit the formation of cross-linked FepA–TonB complexes [37], this segment is required for energy transduction [38]. The second domain contains proline-rich repeats. These motifs were predicted to have functional significance as synthetic peptides corresponding to this segment were shown by nuclear magnetic resonance to be capable of spanning the periplasm [39,40]. However, deletion of this domain does not affect TonB activity [41]. The structure of the C-terminal domain of TonB was recently described; it forms a cylindrical-shaped intertwined dimer that physically interacts with the TonB-boxes of outer membrane receptors [42,43]. Genetic deletion of this domain prevents formation of cross-linked complexes of TonB with outer membrane receptors [44].

TonB's association with the cytoplasmic membrane, stability, and biological function is dependent upon two additional membrane proteins with unusual transmembrane topologies, ExbB and ExbD [45]. Different from TonB, the N-terminus of ExbB is localized to the periplasm [46,47]. Two additional segments of this protein span the cytoplasmic membrane; the remainder resides in the cytoplasm [38]. Similar to TonB, ExbD is anchored in the cytoplasmic membrane by its N-terminus; the remainder of the protein

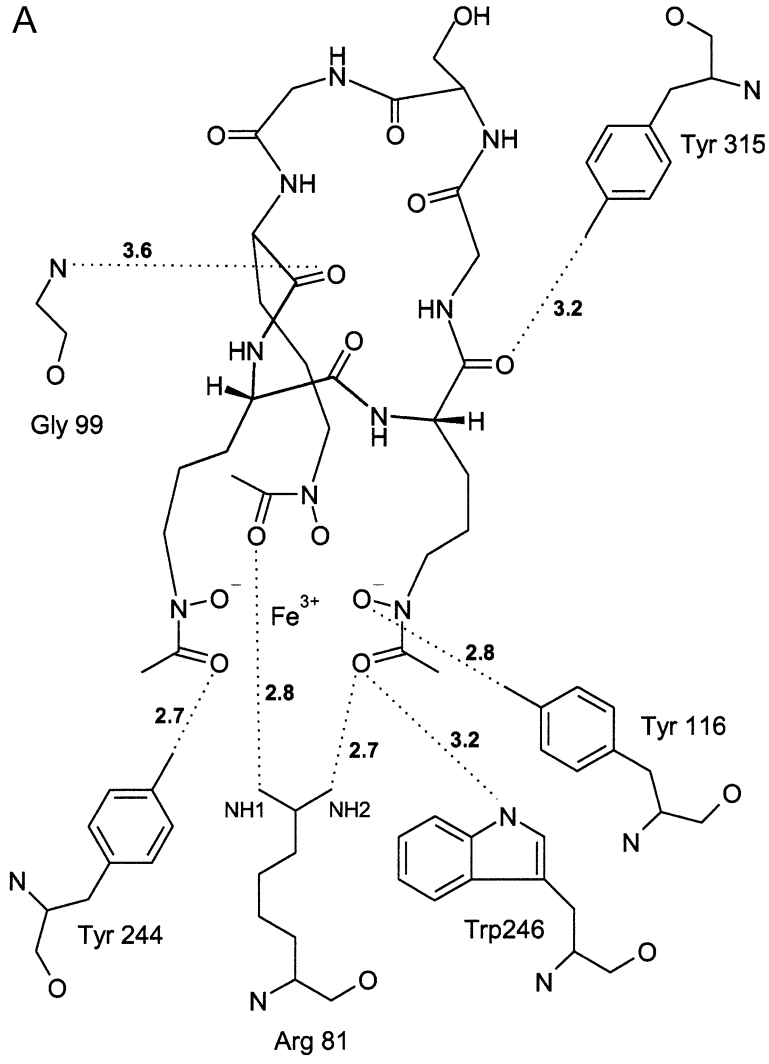
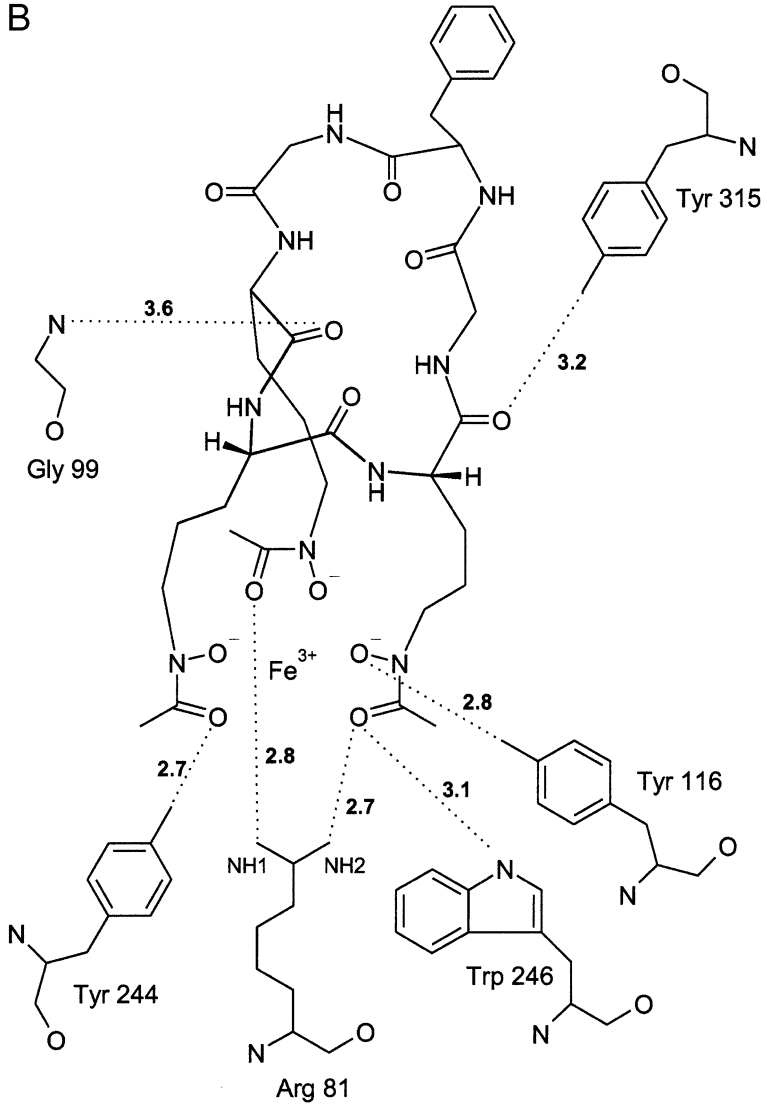


Fig. 7. Schematic comparison of the hydrogen bonding pattern and charge interactions of (A) ferricrocin; (B) phenylferricrocin; (C) albomycin; and (D) rifamycin CGP4832 with side chains of the FhuA ligand-binding site. The chemical structures of ferricrocin, phenylferricrocin, albomycin, and rifamycin CGP4832 are shown with hydrogen bonds and charge interactions with side chains as dotted lines (distances are given in Angstrom units). Rifamycin CGP4832 does not chelate iron.



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is largely placed in the periplasm [47]. In addition to forming TonB–ExbB and TonB–ExbD complexes [26,48–50], both ExbB and ExbD form oligomers in the absence or presence of TonB and TonB-dependent receptors [51]. In the absence of ExbB and ExbD, bacterial cells are resistant to bacteriophage and group B colicin infection, and manifest a 90% reduction in all TonB-dependent activities [26,49]. The residual level of TonB activity results from the presence of TolQ and TolR, which are functional analogues of the ExbB and ExbD proteins of *E. coli* [52–55].

7. Transport across the outer membrane

By integrating currently available data, a model of TonB-dependent siderophore transport can be proposed. TonB, embedded within the cytoplasmic membrane, is closely associated with the ExbB–ExbD complex. The binding of siderophores to the extracellular pockets of outer membrane receptors causes a TonB-independent allosteric transition that is propagated through the outer membrane. The unwinding of the switch helix and the translocation of the TonB-box signal the occupancy of the receptor in the periplasm.

The transfer of chemiosmotic potential from the cytoplasmic membrane to the outer membrane by the TonB–ExbB–ExbD complex likely occurs by a cooperative transition. The ExbB–ExbD complex presumably constitutes the entire proton translocation apparatus, which couples chemiosmotic potential with a series of conformational changes in TonB. Although ExbB has been shown to be involved in this TonB-charging cycle, the role of ExbD remains poorly defined [45,49,51,56]. Recently, it was suggested that this cycle involves the transition of TonB through three distinct conformations: uncharged, charged, and discharged [45]. Following the unwinding of the switch

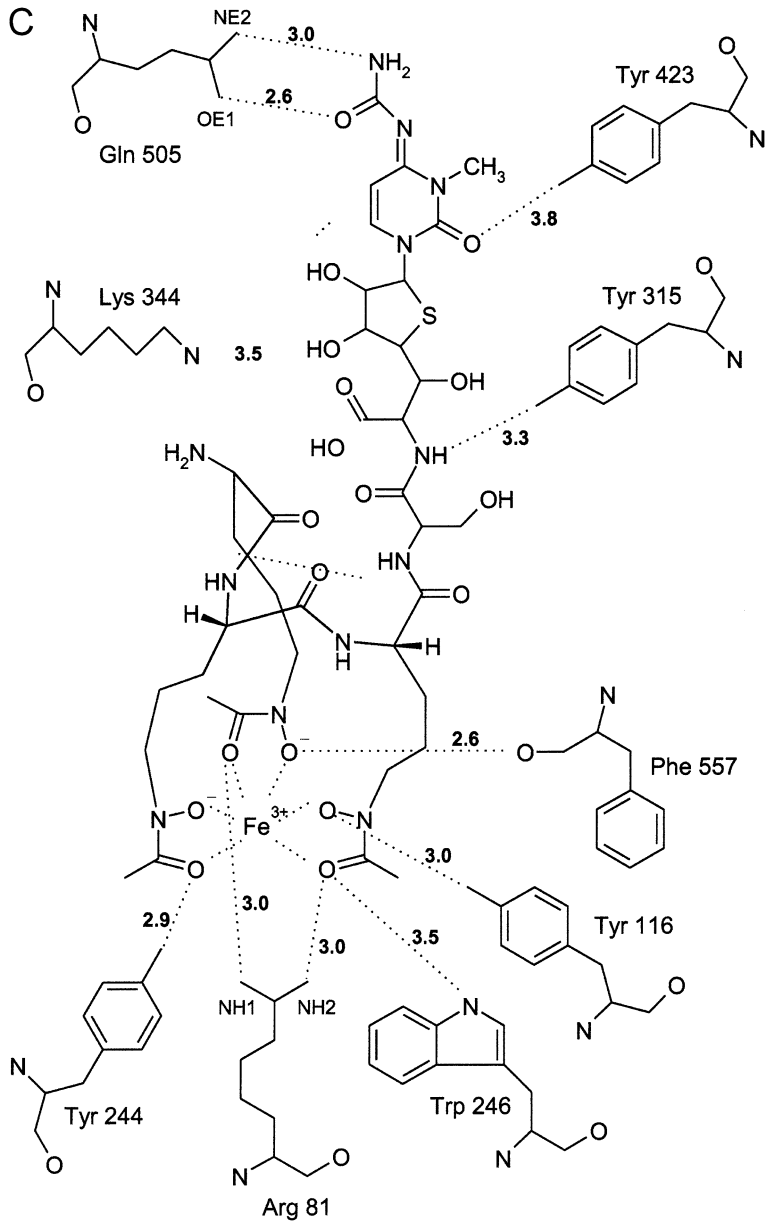
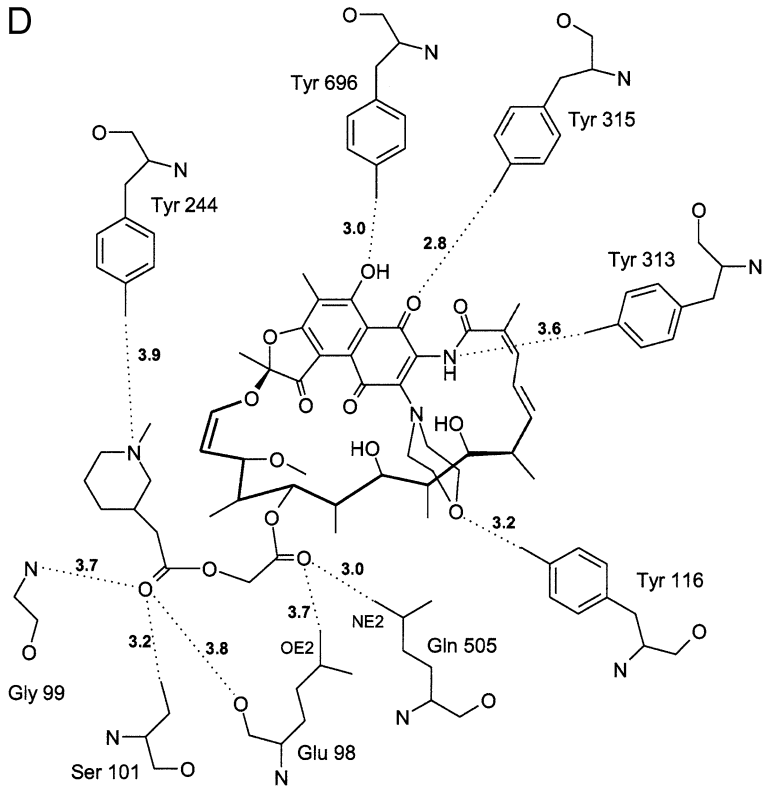


Fig. 7 (*continued*).

helix and the transition to the liganded receptor conformation, one charged TonB molecule preferentially interacts with outer membrane transporters [33,42,44]. However, a population of uncharged TonB molecules may also form binary complexes with liganded receptors with wound switch helices as observed with the FhuA–CGP4832 complex [14] and membrane fractionation studies [44,45]. In either case, the C-terminal domain of TonB physically contacts the TonB-box and probably other side chains found within the periplasmic turns of the β-barrel domain of TonB-dependent receptors [30,31]. This association occurs independently of the energized state of TonB and the ExbB–ExbD complex [33,42,44], but is enhanced by the presence of ligand [26,28,29,42,56].

Upon forming a complex with an outer membrane receptor, TonB releases stored energy, possibly in the form of mechanical force, and assumes the discharged conformation. The structural basis of this event remains to be established. Energy transduction triggers an allosteric transition within the receptor such that binding site integrity is disrupted, reducing the binding affinity. In vivo, formation of a complex with TonB causes a high-conductance channel to open within TonB-dependent receptors [57]. Cooperative structural changes within the plug domain and/or β-barrel may eject the plug domain with bound siderophore into the periplasm [58]. Alternatively, the plug domain may remain inside the β-barrel, and both domains undergo allosteric transitions leading to the opening of an



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underlying transmembrane channel within the receptor through which the ligand permeates into the periplasm by a surface-diffusion mechanism similar to those characterized for glycoporins [8,59,60]. Upon arrival in the periplasm, siderophores are rapidly bound by specific periplasmic binding proteins, and shuttled to distinct

Table 2
Electrostatic and van der Waals contacts between FhuA and its cognate ligands bound in the extracellular pocket

Side chain	Ferrichrome/ Ferricrocin	Phenylferricrocin	Albomycin[a]	Albomycin[b]	Rifamycin CGP4832
R81—Apex A	+	+	+	+	–
Y87—Plug domain	–	+	–	–	–
E98—Apex B	–	–	–	–	+
G99—Apex B	+	+	+	+	+
Q100—Apex B	+	+	+	+	+
S101—Apex B	–	–	–	–	+
F115—Apex C	–	+	–	+	+
Y116—Apex C	+	+	+	+	+
Y244—L3	+	+	+	+	+
W246—L3	+	+	+	+	+
Y313—β7	+	+	+	+	+
Y315—L4	+	+	+	–	+
K344—β8	–	–	+	–	+
F391—β9	+	+	+	+	+
G392—β9	–	–	–	–	+
Y393—L5	–	–	+	+	–
Y423—β10	–	–	–	+	+
Q505—L7	–	–	–	+	+
F557—L8	–	–	+	–	–
F558—L8	–	–	+	–	–
F693—L11	+	+	+	+	+
Y696—L11	–	–	–	–	+

Listed are all side chains within 4 Å of ligand atoms: ferricrocin [8] or ferrichrome [9]; phenylferricrocin [11]; albomycin extended ([a]) and compact ([b]) conformational isomers [11]; or rifamycin CGP4832 [14].

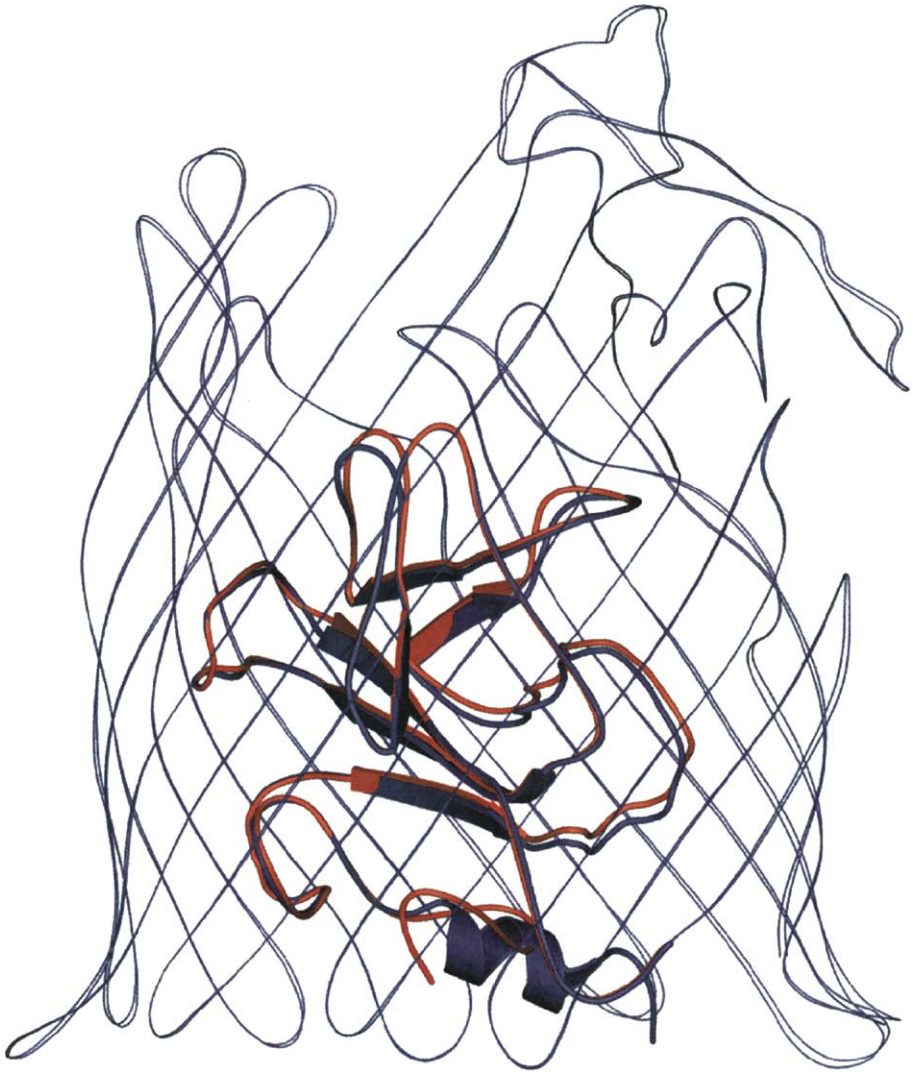

Fig. 8. Ligand-induced conformational changes in the plug domain of FhuA. The β-barrels of unliganded and liganded FhuA are shown as wire frame models. The plug domain of unliganded FhuA is colored blue and liganded FhuA is shown in red [8,9].

ABC transporters embedded within the cytoplasmic membrane for transport into the cytoplasm.

8. Unresolved questions

Although these multifunctional outer membrane receptors provide structural platforms to study the complex mechanism of TonB-coupled siderophore transport and transmembrane signaling, the currently available data do not clarify the precise mechanism of energy-dependent transport through the outer membrane.

Before the solution of the three-dimensional structures of FhuA and FepA, the β-barrels of these receptors were predicted to actively participate in siderophore transport. By analogy to the constriction loop of porins and their putative role in voltage-gating, an equivalent extracellular loop has been proposed to mediate channel opening and closing by TonB-dependent receptors. Allosteric transitions regulate channel formation, where siderophore binding and the transfer of potential energy from TonB, modulate these events. Gating refers to the process by which siderophore binding to its receptor induces allosteric transitions that enable transport by establishing or removing a physical channel obstruction referred to as the gate, thereby regulating transmembrane siderophore flow. However, comparison of the unliganded and liganded FhuA structures does not reveal any significant conformational changes within the β-barrel upon the binding of siderophores or antibiotics. No movement or rearrangement of the extracellular loops were observed, leaving the extracellular pocket of FhuA open to the solvent. How can directed transport be ensured in the absence of a gate that blocks the rerelease of siderophore into the solvent? Can siderophore binding cause allosteric transitions within the β-barrel in the absence of TonB?

One siderophore-mediated iron acquisition system of *E. coli* is responsive to both internal and external iron concentrations [61]. The outer membrane transporter of this system (FecA) can be distinguished from FhuA and FepA as it performs two independent functions: mediating ferric citrate uptake and transcription of the ferric citrate transport genes [62,63]. Excision of a linear segment of FecA termed the N-terminal extension abrogates transcription; however, TonB-dependent uptake of ferric citrate remains unaffected [64,65]. Does FecA assume a similar molecular architecture as FhuA and FepA? How can a two-domain arrangement (barrel and plug) influence transcription in the cytosol? Is the N-terminal extension an individual domain or an extension of the plug domain? Does ferric citrate binding to FecA also cause local and allosteric transitions as observed with FhuA? How is receptor occupancy signaled by the plug domain and the N-terminal extension? What is the structural basis of this signal transduction mechanism?

In conclusion, the determination of the three-dimensional structures of FhuA and FepA provided a working model for the TonB-dependent transport of siderophores across the outer membrane; however, energy-dependent transitions cannot be derived from crystallographic data. Thus, to fully understand this energy-dependent transport mechanism will require additional genetic, biochemical, and structural experiments.

Note added in proof

Recently the crystal structures of the unliganded and liganded forms of the outer membrane transporter FecA were reported (A.D. Ferguson et al., Science 295 (2002) 1715–1719). Binding of ferric dicitrate to FecA causes conformational changes near the TonB interaction site similar to those seen in liganded FhuA; in addition, large conformational changes in two extracellular loops of the barrel domain lead to a complete closure of the ligand blinding pocket.

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References

[1] J.E. Posey, F.C. Gherardini, Science 288 (2000) 1651–1653.
[2] J.F. Briat, J. Gen. Microbiol. 138 (1992) 2475–2483.
[3] J.B. Neilands, J. Biol. Chem. 270 (1995) 26723–26726.
[4] V. Braun, Biol. Chem. 378 (1997) 779–786.
[5] C. Bradbeer, J. Bacteriol. 175 (1993) 3146–3150.
[6] V. Braun, Science 282 (1998) 2202–2203.
[7] K. Postle, Nat. Struct. Biol. 6 (1999) 3–6.
[8] A.D. Ferguson, E. Hofmann, J.W. Coulton, K. Diederichs, W. Welte, Science 282 (1998) 2215–2220.
[9] K.P. Locher, B. Rees, R. Koebnik, A. Mitschler, L. Moulinier, J. Rosenbusch, D. Moras, Cell 95 (1998) 771–778.
[10] S.K. Buchanan, B.S. Smith, L. Venkatramani, D. Xia, L. Esser, M. Palnitkar, R. Chakraborty, D. van der Helm, J. Deisenhofer, Nat. Struct. Biol. 6 (1999) 56–63.
[11] A.D. Ferguson, V. Braun, H.-P. Fiedler, J.W. Coulton, K. Diederichs, W. Welte, Protein Sci. 9 (2000) 956–963.
[12] A.D. Ferguson, J. Ködding, J.W. Coulton, K. Diederichs, V. Braun, W. Welte, Structure 9 (2001) 707–716.
[13] A.G. Murzin, A.M. Lesk, C. Chothia, J. Mol. Biol. 236 (1994) 1369–1400.
[14] A.D. Ferguson, W. Welte, E. Hofmann, B. Lindner, O. Holst, J.W. Coulton, K. Diederichs, Structure 8 (2000) 585–592.
[15] A.P. Pugsley, W. Zimmerman, W. Wehrli, J. Gen. Microbiol. 133 (1987) 3505–3511.
[16] R.J. Kadner, K.J. Heller, J. Bacteriol. 177 (1995) 4829–4835.
[17] E. Schramm, J. Mende, V. Braun, R.M. Kamp, J. Bacteriol. 169 (1987) 3350–3357.
[18] M.L. Lundrigan, R.J. Kadner, J. Biol. Chem. 261 (1986) 10797–10801.
[19] R.J. Kadner, Mol. Microbiol. 4 (1990) 2027–2033.
[20] K.J. Heller, R.J. Kadner, J. Bacteriol. 161 (1985) 904–908.
[21] K.J. Heller, R.J. Kadner, K. Gunther, Gene 64 (1988) 147–153.
[22] A. Gudmondsdottir, P.E. Bell, M.D. Lundrigan, C. Bradbeer, R.J. Kadner, J. Bacteriol. 171 (1989) 6526–6533.
[23] H. Schöffler, V. Braun, Mol. Gen. Genet. 217 (1989) 378–383.
[24] P.E. Bell, C.D. Nau, J.T. Brown, J. Konisky, R.J. Kadner, J. Bacteriol. 172 (1990) 3826–3829.
[25] M. Sauer, K. Hantke, V. Braun, Mol. Microbiol. 4 (1990) 427–437.
[26] J.T. Skare, B.M.M. Ahmer, C.L. Seachord, R.P. Darveau, K. Postle, J. Biol. Chem. 272 (1993) 16302–16308.
[27] R.A. Larsen, D. Foster-Hartnett, M.A. McIntosh, K. Postle, J. Bacteriol. 179 (1997) 3213–3221.
[28] G.S. Moeck, J.W. Coulton, K. Postle, J. Biol. Chem. 272 (1997) 28391–28397.
[29] N. Cadieux, R.J. Kadner, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10673–10678.
[30] H.J. Merianos, N. Cadieux, C.H. Lin, R.J. Kadner, D.S. Cafiso, Nat. Struct. Biol. 7 (2000) 205–209.
[31] M. Braun, H. Killmann, V. Braun, Mol. Microbiol. 33 (1999) 1037–1049.
[32] D.C. Scott, Z.H. Cao, Z.B. Qi, M. Bauler, J.D. Igo, S.M.C. Newton, P.E. Klebba, J. Biol. Chem. 276 (2001) 13025–13033.
[33] S.P. Howard, C. Herrmann, C.W. Stratilo, V. Braun, J. Bacteriol. 183 (2001) 5885–5895.
[34] I. Traub, S. Gaisser, V. Braun, Mol. Microbiol. 8 (1993) 409–423.
[35] R.A. Larsen, P.S. Myers, J.T. Skare, C.L. Seachord, R.P. Darveau, K. Postle, J. Bacteriol. 178 (1996) 1363–1373.
[36] K. Postle, J.T. Skare, J. Biol. Chem. 263 (1988) 11000–11007.
[37] R.A. Larsen, M.G. Thomas, G.E. Wood, K. Postle, Mol. Microbiol. 13 (1994) 627–640.
[38] M. Karlsson, K. Hannavy, C.F. Higgins, Mol. Microbiol. 8 (1993) 389–396.
[39] J.S. Evans, B.A. Levine, I.P. Trayer, C.J. Dorman, C.F. Higgins, FEBS Lett. 208 (1986) 211–216.
[40] S. Brewer, M. Tolley, I.P. Trayer, G.C. Barr, C.J. Dorman, K. Hannavy, C.F. Higgins, J.S. Evans, B.A. Levine, M.R. Wormald, J. Mol. Biol. 216 (1990) 883–895.
[41] R.A. Larsen, G.E. Wood, K. Postle, Mol. Microbiol. 10 (1993) 943–953.
[42] G.S. Moeck, L. Letellier, J. Bacteriol. 183 (2001) 2755–2764.
[43] C.S. Chang, A. Mooser, A. Pluckthun, A. Wlodawer, J. Biol. Chem. 276 (2001) 27535–27540.
[44] T.E. Letain, K. Postle, Mol. Microbiol. 24 (1997) 271–283.

[45] R.A. Larsen, M.G. Thomas, K. Postle, Mol. Microbiol. 31 (1999) 1809–1824.
[46] K. Eick-Helmerich, K. Hantke, V. Braun, Mol. Gen. Genet. 206 (1987) 246–251.
[47] K. Kampfenkel, V. Braun, J. Bacteriol. 174 (1992) 5485–5487.
[48] E. Fischer, K. Günther, V. Braun, J. Bacteriol. 171 (1989) 5127–5134.
[49] B.M.M. Ahmer, M.G. Thomas, R.A. Larsen, K. Postle, J. Bacteriol. 177 (1995) 4742–4747.
[50] V. Braun, S. Gaisser, C. Herrmann, K. Kampfenkel, H. Killmann, I. Traub, J. Bacteriol. 178 (1996) 2836–2845.
[51] P.I. Higgs, P.S. Myers, K. Postle, J. Bacteriol. 180 (1998) 6031–6038.
[52] V. Braun, J. Bacteriol. 171 (1989) 6387–6390.
[53] K. Eick-Helmerich, V. Braun, J. Bacteriol. 171 (1989) 5117–5126.
[54] V. Braun, C. Herrmann, Mol. Microbiol. 8 (1993) 261–268.
[55] K. Kampfenkel, V. Braun, J. Bacteriol. 175 (1993) 4485–4491.
[56] R.A. Larsen, K. Postle, J. Biol. Chem. 276 (2001) 8111–8117.
[57] M. Bonhivers, A. Ghazi, P. Boulanger, L. Letellier, EMBO J. 15 (1996) 1850–1856.
[58] K.C. Usher, E. Özkan, K.H. Gardner, J. Deisenhofer, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10676–10681.
[59] T. Schirmer, T.A. Keller, Y.F. Wang, J. Rosenbusch, Science 267 (1995) 512–514.
[60] D. Forst, W. Welte, T. Wacker, K. Diederichs, Nat. Struct. Biol. 5 (1998) 37–46.
[61] U. Pressler, H. Staudenmaier, L. Zimmermann, V. Braun, J. Bacteriol. 170 (1988) 2716–2724.
[62] M. Ochs, S. Veitinger, I. Kim, D. Welz, A. Angerer, V. Braun, Mol. Microbiol. 15 (1995) 119–132.
[63] M. Ochs, A. Angerer, S. Enz, V. Braun, Mol. Gen. Genet. 250 (1996) 455–465.
[64] C. Härle, I. Kim, A. Angerer, V. Braun, EMBO J. 14 (1995) 1430–1438.
[65] I. Kim, A. Stiefel, S. Plantör, A. Angerer, V. Braun, Mol. Microbiol. 23 (1997) 333–344.
[66] A.P. Sehn, B. Meier, Biochem. J. 304 (1994) 803–808.
[67] S.B. Farr, T. Kogoma, Microbiol. Rev. 55 (1991) 561–585.
[68] J.W. Coulton, P. Mason, D.R. Cameron, G. Carmel, R. Jean, H.N. Rode, J. Bacteriol. 165 (1986) 181–192.
[69] R. Koebnik, K.P. Locher, P. van Gelder, Mol. Microbiol. 37 (2000) 239–253.