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Minireview

Mechanisms of TonB-Catalyzed Iron Transport through the Enteric Bacterial Cell Envelope

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The recent solution of enteric bacterial porin structure, and new insights into the mechanism by which outer membrane receptor proteins recognize and internalize specific ligands, advocates the re-evaluation of TonB-dependent transport physiology. In this minireview we discuss the potential structural features of siderophore receptors and TonB, and use this analysis to evaluate both existing and new models of energy and signal transduction from the inner membrane to the outer membrane of gram-negative bacteria.

KEY WORDS: TonB; iron transport; bacterial transport.

INTRODUCTION

Cell envelope transport systems play a crucial role in the survival of bacteria, because they concentrate nutrients, vitamins, and minerals intracellularly, without compromising the barrier properties of the outer cell wall. The gram-negative bacterial outer membrane (OM)[5] is one such uniquely evolved, asymmetric bilayer that contains sophisticated multi-component protein complexes for the uptake of metal chelates into the cell (Nikaido and Saier, 1992). Outer membrane receptor proteins exist for the acquisition of iron chelates of microbial origin, called siderophores (Greek "iron carrier"), as well as the cobalt-containing vitamin B_{12} (Neilands, 1981; Bradbeer, 1991). The biochemical mechanism by which these proteins accomplish their transport function is a paradigm of membrane physiology, because it ostensibly involves energy and signal transduction between two membranes, across an aqueous compartment. The key to the understanding of this process is an unusual and enigmatic cell envelope protein, TonB (Luria and Delbruck, 1943).

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[5] Abbreviations: OM, outer membrane; OMP, outer membrane protein; MAb, monoclonal antibody.

AN OVERVIEW OF SIDEROPHORE-MEDIATED IRON UPTAKE

The Siderophore Transport Cycle

During the 1970s and 1980s the components of cell envelope siderophore and vitamin B_{12} transport systems were identified, genetically and biochemically analyzed, cloned, and sequenced (for review, see Konisky, 1979; Kadner, 1990; Postle, 1990b). The results of this research established that all TonB-dependent receptor proteins function by a similar, if not identical, basic mechanism. Ferric enterobactin, the indigenous *Escherichia coli* siderophore, is transported and utilized by the vast majority of enteric bacteria (Rutz *et al.*, 1991), and the mechanism of its passage through the *E. coli* cell envelope is prototypic of the TonB-dependent uptake process. The ferric enterobactin transport cycle follows:

1. Enterobactin, synthesized and excreted by iron-deficient bacteria, complexes Fe^{+++} in the extracellular milieu (Neilands, 1981).

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2. Ferric enterobactin binds to its outer membrane receptor protein, FepA, at a site that is centrally located in its primary structure, and cell surface-exposed (Murphy *et al.*, 1990).
3. FepA releases ferric enterobactin to an underlying hydrophilic channel that is open to the periplasm, in a TonB-dependent, energy-dependent step (Rutz *et al.*, 1992).
4. FebB, a periplasmic protein, binds ferric enterobactin and delivers it to the Fep permease in the cytoplasmic membrane (Pierce and Earhart, 1986).
5. The Fep permease complex, consisting of FepC, FepD, FepG, and P43 (Shea and McIntosh, 1991), passes ferric enterobactin to the cytoplasm by an energy-dependent, but otherwise uncharacterized, mechanism.
6. Fe^{+++}-enterobactin is cleaved and reduced by Fes in the cytoplasm, releasing Fe^{++} to Fur, which represses transcription of enterobactin biosynthetic and transport genes (de Lorenzo *et al.*, 1988).

BIOCHEMISTRY OF TonB-DEPENDENT OUTER MEMBRANE TRANSPORT

Bioenergetics of Siderophore Uptake

Since ferric siderophore transport systems include an outer membrane transport protein, a periplasmic binding protein, and an inner membrane permease complex, from an energetics standpoint they are comparable to certain amino acid or sugar transport systems, which contain the same components (Nikaido and Saier, 1992). That is, the transport thermodynamics require the input of energy to concentrate the substrate across the inner membrane into the cytoplasm. However, an exception exists to this correspondence that defines a unique aspect of siderophore transport systems: ferric siderophores cross the OM by high-affinity uptake through specific receptor proteins (Neilands, 1981). Amino acids and sugars, on the other hand, passively diffuse across the OM through nonspecific porin channels (Nikaido and Saier, 1992). Since TonB-dependent receptor proteins bind ligands tightly at the cell surface, it is reasonable to assume that they require the input of additional energy to dislodge ligands into the periplasm. This inference agrees with existing data (Bradbeer, 1993; Woolridge *et al.*, 1992), and has led to the theory that TonB functions to transform the protonmotive force (Bradbeer, 1993) into a mechanical action or chemical reaction that energizes the operation of OM receptor proteins.

Physical Contact between Siderophore Receptors and TonB

It has been postulated that TonB promotes OM transport by physically contacting TonB-dependent proteins. These receptors show distinct regions of homology throughout their primary structures (Kadner, 1990; Bradbeer, 1991) that likely correspond to transmembrane polypeptides in their tertiary structures (Rutz *et al.*, 1991). The evolution of an outer membrane protein (OMP) occurs rapidly in its cell surface peptides, and relatively slowly in regions of the protein that interact with bilayer lipids (Jenteur *et al.*, 1991; Weiss *et al.*, 1990; Cowan *et al.*, 1992). It is therefore not surprising that the transmembrane strands of TonB-dependent receptors are similarly conserved (Rutz *et al.*, 1991). One such peptide, which comprises the first putative transmembrane strand of TonB-dependent OMP and is conserved among them in terms of overall hydrophobicity, has been suggested as a site of direct interaction between such receptors and TonB. The evidence for this physical interaction is primarily genetic: Mutations within this "TonB-box" domain (Sauer *et al.*, 1989) that inactivate the receptor's function may be weakly compensated by mutations in TonB. The mutations in question are proposed to counteract one another because the residues that they encode physically interact (Kadner, 1990; Braun *et al.*, 1991; Bradbeer, 1991). Other interpretations of these data are plausible, however. For example, in the histidine transport system of *Salmonella typhimurium*, mutations in HisJ, the periplasmic binding protein, are suppressed by mutations in HisP, the ATPase component of the cytoplasmic permease complex. These results were initially viewed as strong evidence for direct contact between the two proteins, at the periplasmic surface of the cytoplasmic membrane (Ames and Spudich, 1976). Recent studies, however, have established that the mutations of interest do not improve physical contact between HisJ and HisP. Rather, the compensating mutations in HisP increase the rate of ATP hydrolysis by this protein (Speiser and Ames, 1991; Petronilli and Ames, 1991). Hence the sites of mutation in the two proteins are not involved in physical interactions between them. This example demonstrates that genetic suppression is not conclusive evidence that two proteins physically contact each

other. Alternative models of TonB function may rationalize genetic suppression equally well.

THE STRUCTURE OF SIDEROPHORE RECEPTOR PROTEINS

Prediction of OMP Structure

OMP sequences manifest unusual, almost paradoxical properties for macromolecules that reside in membranes: They contain a preponderance of hydrophilic amino acids and few regions with sufficient hydrophobicity to span a bilayer. These properties were explained by structural models that predicted the existence of amphiphilic β-strands crossing the OM (Paul and Rosenbusch, 1985; Vogel and Jahnig, 1986; Klebba *et al.*, 1990). Certainly the most compelling recent advance in bacterial cell envelope physiology was the X-ray crystallographic solution of porin structure (Weiss *et al.*, 1990; Cowan *et al.*, 1991), which essentially confirms this theory. Archetypal porins, like *E. coli* OmpF or the *Rhodobacter capsulatus* porin, contain 16 membrane-crossing β-strands arranged as an amphiphilic β-barrel, with a hydrophilic interior surface and a hydrophobic exterior. These findings agree well with prior concepts of porin structure, channel size, and channel selectivity (Nikaido and Saier, 1992). Unfortunately, a variety of obstacles block the path to the X-ray solution of TonB-dependent OMP structure. Even in iron-deficient conditions siderophore receptors are not expressed at levels comparable to porins, and they are easily denatured upon purification. Porins tolerate high concentrations of ionic detergents at elevated temperatures without detectable alteration in conformation. Purified FepA, on the other hand, is rapidly denatured in 1% lauryl sulfate, even at room temperature ($t_{1/2} = 30$ min; C. K. Murphy and P. E. Klebba, unpublished data). In spite of these problems, FepA has been crystallized (Jalal and van der Helm, 1989), but these crystals are not yet of high enough quality to begin X-ray studies (Dick van der Helm, personal communication), making structural information unlikely in the near future. These difficulties led to an alternative methodology for the characterization of OMP structure: immunochemical mapping of epitopes with monoclonal antibodies (MAbs). The approach was to raise MAbs to the target OMP, determine the location of the respective epitopes in the intact, native protein *in vivo* (external surface, bilayer, or periplasmic surface), and then map the position of the epitopes within the protein's sequence (Klebba *et al.*, 1990). In this way peptides localized on the OM surfaces were identified in primary structure, and the folding of the polypeptide was inferred. A theoretical contribution was provided by an analysis of the distribution of amino acids in proteins at various environmental interfaces, and by the derivation of algorithms predicting the localization of peptides within proteins of unknown structure (Eisenberg, 1984; Rees *et al.*, 1989). The combination of these experimental and theoretical methods allowed the prediction of the folding of the FepA polypeptide in some detail (Murphy *et al.*, 1990). Slight adjustments to this approach, based on current knowledge of porin structure, lead to the following method for the prediction of OMP folding:

1. Identify regions of significant hydrophilicity (i.e., sequences of two or more residues with mean hydrophobicity < 0). The program MOMENT (Eisenberg, 1984) is well suited to this task. Strongly hydrophilic sequences are likely to reside on either the periplasmic or external surface of the OM. Among these, distinguish the long hydrophilic stretches (i.e., > 5 residues); analysis of porin structure suggests that these lie on the external surface.
2. Note the aromatic residues in the sequence of interest. In porins, the majority of aromatic amino acids, especially Trp and Phe, reside in the bilayer lipids (Weiss *et al.*, 1990; Cowan *et al.*, 1991).
3. Analyze the amphiphilicity of the sequence, again with MOMENT, searching for potential helices (17–23 residues) and sheets (7–15 residues) of sufficient length to cross a lipid bilayer. The MOMENT program is not perfectly suited to this task, because of the tendency of the hydrophilic residues Ser, Thr, and Tyr to reside on the hydrophobic surface of β-strands. Therefore, also inspect the sequence for otherwise amphiphilic regions that contain these amino acids.
4. Identify potential turns in the sequence (Wilmot and Thornton, 1988). Also mark prolines; the presence of a Pro does not guarantee a turn, but it ensures at least a bend in the secondary structure. Strongly predicted turns ($p > 1.0$) probably cannot reside in the OM bilayer, but the affinity of bilayer lipids for the hydrophobic surface of a putative transmembrane strand may overcome the tendency of a predicted turn to occur, and melt it into the bilayer.
5. If experimental data exist relative to the location of peptides or epitopes within the bilayer lipids or

on the membrane surfaces, incorporate these into the analysis.

6. When designating potential transmembrane β-strands, note that the length of known transmembrane sequences ranges from 7–15 residues, that these may be fully hydrophobic or amphiphilic, and that periplasmic peptide loops are usually 2–5 residues long (Weiss *et al.*, 1990; Cowan *et al.*, 1991). This fact tends to force transmembrane β-strands into antiparallel sheets.

Functional Domains within Siderophore Receptors

The application of the method described above to the ferric enterobactin receptor suggested that it contains two distinct functional domains: a cell surface region that recognizes and binds ligands, and a previously unrecognized underlying transmembrane channel. The ferric enterobactin-binding site has been localized on the basis of immunochemical and genetic studies to a central, surface-exposed region of the protein, bounded by residues 258–339 (Murphy *et al.*, 1990). The channel domain, which was originally demonstrated *in vivo* (Rutz *et al.*, 1992), has recently been reconstituted and characterized *in vitro* (Liu *et al.*, 1993). It is predicted to consist of a series of 29 hydrophobic or amphiphilic β-strands, 7–12 residues in length, that circumscribe a large pore, approximately 20 Å in diameter. The substantial sequence homologies among TonB-dependent OM proteins (Kadner, 1990) implies that all siderophore receptors contain this fundamental, bifunctional domain structure. That is, TonB-dependent OMPs are porins that are closed at the cell surface by their ligand-binding peptides. The perception of FepA as a gated porin does not imply that siderophore receptors function by facilitated diffusion; siderophore concentrations in the natural environment are too low to drive diffusion-mediated accumulation at a rate sufficient to support growth. In addition, available evidence suggests that opening and closing of TonB-dependent channels is an energy- and TonB-dependent phenomenon (Woolridge *et al.*, 1992; Bradbeer, 1993). The likely role of TonB, then, is to open the surface gate and facilitate the release of ligands into the channel, which is ultimately continuous with the periplasm (Rutz *et al.*, 1992).

When isolated and analyzed at low temperatures, FepA exists as a compact protein with an apparent molecular weight of 63 kDa (Murphy *et al.*, 1990); we perceive this structure as the native, monomeric receptor. In this respect it is comparable to OmpA, which shows analogous heat modifiability. Recent analysis of the ferric enterobactin receptor in nondenaturing conditions reveals FepA as a high-molecular-weight oligomer *in vivo* (Liu *et al.*, 1993). Its relative mobility in LDS-PAGE suggests that the native ferric enterobactin receptor is a homotrimer.

Conformational Change to Internalize Ferric Siderophores

Conformational change seems to be an inescapable aspect of siderophore receptor function. Since TonB-dependent OMPs bind their ligands with high affinity, conformational change is envisioned as a mechanism to release ligands and concomitantly internalize them through the outer membrane bilayer (Bradbeer, 1991). The gated-porin model of siderophore receptor transport provides a conceptual framework for the evaluation of possible mechanisms of conformational change.

The ligand-binding peptides of FepA apparently overlay other surface loops that originate upstream in its sequence. In deletion mutants (ΔMC and ΔRV) that eliminate the FepA ligand-binding site (residues 258–339), epitopes in region 100–142 that are not surface accessible in wild-type FepA are recognized on the cell surface by antibodies (Rutz *et al.*, 1992). Conversely, deletion of residues 58–142 abrogates ferric enterobactin binding by the receptor, even though the downstream ligand-binding domain is intact and properly localized on the cell surface. These results suggest that the ligand-binding domain comprises a complex assembly of folded and juxtaposed polypeptides. Conformational changes that open the channel may therefore involve movement of multiple surface peptides and/or transmembrane strands. Nevertheless, uptake studies with the same mutants demonstrate that removal of the outermost, ligand-binding surface loop opens the channel to the diffusion of small molecules. Elimination of the underlying surface peptides does not.

A novel feature of outer membrane proteins is that even though they reside in a bilayer, the amphiphilic nature of their transmembrane β-strands makes their mean hydrophobicity comparable to that of a soluble protein (Vogel and Jahnig, 1986; Rees *et al.*, 1989). This property of OMP structure imparts a degree of plasticity to the potential localization of amphiphilic β-strands. That is, amphiphilic peptides may exist stably in either a membrane or an aqueous environment, a fact that is reiterated within the pro-

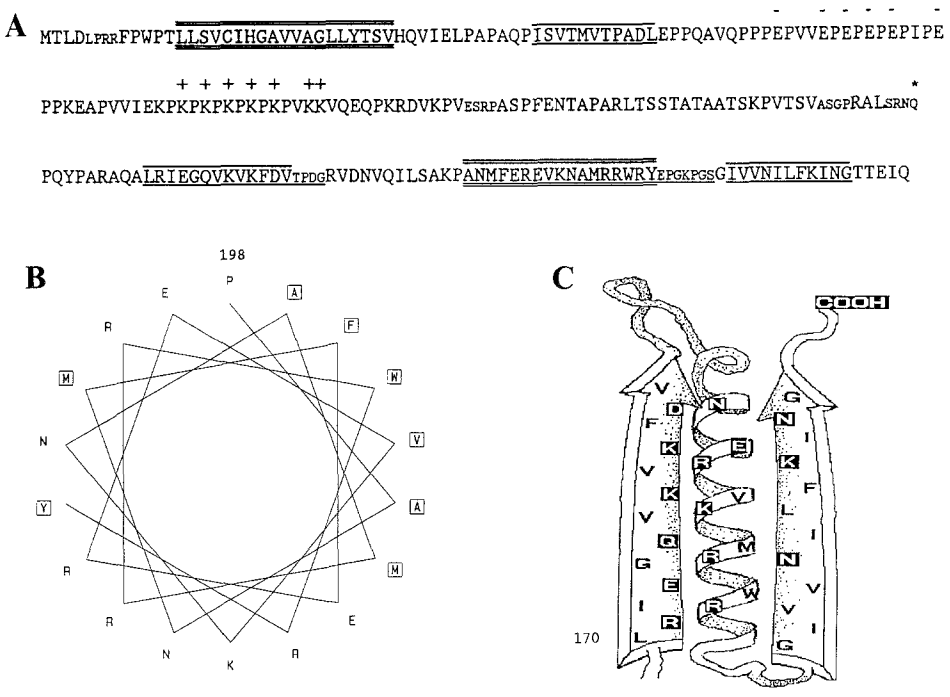

Fig. 1. A. Sequence and structural features of E. coli TonB. Potential transmembrane β-strands (underlined), α-helices (double underlined), weakly predicted β-turns (small caps), strongly predicted β-turns (small caps, underlined), and clustered charges are indicated. The site within TonB that gives rise to mutations that compensate deficiencies in the OM receptor protein TonB-box (Gln 160) is starred. B. Helical wheel display of the potential amphiphilic α-helix within TonB. The structure is near the C-terminus (residues 198–215); hydrophobic residues are shown in boxes. C. Proposed β-strand–α-helix–β-strand motif penultimate to the TonB C-terminus. Significantly hydrophilic residues are shown in black boxes.

posed surface peptides of FepA, which contain numerous regions of amphiphilicity. Thus, in addition to the obvious opening of the surface gate region, conformational changes in TonB-dependent OMPs may involve the relocalization of residues from the β-barrel domain to either the cell surface, or the hydrophilic barrel interior. Alternatively, residues may relocalize from the cell surface to the transmembrane β-barrel domain; the pattern of OMP folding that has been demonstrated for porins and hypothesized for TonB-dependent proteins (substantial external surface loops and small periplasmic surface peptides) suggests that this latter change may push strands from the membrane bilayer into the periplasmic space.

THE STRUCTURE OF TonB

TonB contains unusual structural features that have created an air of mystery around its biochemical function. For example, for a protein comprised of 17% Pro, it contains few recognizable β-turns (Wilmot and Thornton, 1988; Fig. 1). Secondly, the sequence of TonB includes two rigid, highly charged internal domains, a negatively charged Glu-Pro repeat closely followed by a positively charged Lys-Pro repeat, whose functions are unknown. Few distinct biochemical functions can be ascribed to TonB, but when the algorithm described above for the study of OM proteins is applied to it, various distinct structural features are apparent:

1. An N-terminal hydrophobic domain that may span a membrane bilayer as a hydrophobic helix, or as a pair of β-strands, followed by another potential transmembrane β-strand at residues 45–55. Evidence points to localization of this region in the cytoplasmic membrane (Roof *et al.*, 1991; Plastow and Holland, 1979).

2. A central, elongated core region containing a structurally rigid linear sequence of negative charges (Glu-Pro), an intervening region with slight conformational flexibility (Brewer *et al.*, 1990), a rigid linear sequence of positive charges (Lys-Pro), and a

fourth hydrophilic domain containing several predicted turns. As recognized by Brewer *et al.*, (1990), who suspected interaction between the sequential negative and positive domains but could not demonstrate it, understanding of the Glu-Pro and Lys-Pro repeats may be pivotal to comprehension of the TonB mode of action. The principal structural feature of this central domain, which likely resides in the periplasm, is its rigid elongated nature.

3. A C-terminal domain consisting of three amphiphilic, potentially transmembrane structures (β-strand–α-helix–β-strand), separated from each other by strongly predicted β-turns. The location of this $\beta\alpha\beta$-core within the cell envelope is unknown. Although the C-terminus of TonB is dispensable (Anton and Heller, 1991), the terminal amphiphilic β-strand is essential to TonB function.

MECHANISMS OF TonB FUNCTION

For over five decades physiologists have recognized the importance of TonB in the transport of molecules into the bacterial cell (Luria and Delbruck, 1943; Wang and Newton, 1971; Guterman and Dann, 1973). Several diverse theories of TonB participation in metal assimilation have arisen during this period (Konisky, 1979; Wookey, 1982; Kadner, 1990; Postle, 1990), but without knowledge of the structure of the outer membrane siderophore receptors, or their transport mechanism, the molecular understanding of TonB participation in uptake has remained baffling. During the last few years, however, new perceptions of both OMP structure and the mechanism of siderophore passage through TonB-dependent receptors prompt a new evaluation of TonB function.

The Theory of Periplasmic Interactions

One popular concept of TonB function is that it spans the periplasm and tickles the underbelly of outer membrane receptor proteins (at the periplasmic face of the OM), inducing them to release their substrates to periplasmic binding proteins. The evidence for this idea is primarily threefold:

(i) The existence of a highly conserved TonB-box domain within siderophore receptors argues that they have been evolutionarily preserved to interact with another specific macromolecule, in a lock and key scenario (Heller *et al.*, 1988; Sauer *et al.*, 1989).

(ii) Genetic suppressors exist between TonB and outer membrane receptor proteins that compensate for mutational deficiencies (Di Girolamo *et al.*, 1971; Heller and Kadner, 1985; Heller *et al.*, 1988; Bell *et al.*, 1990; Braun *et al.*, 1991).

(iii) Evidence exists for direct binding between the Lys-Pro domain of TonB and purified FhuA (Hannavy *et al.*, 1990).

Analysis

1. Careful examination of the primary structures of TonB-dependent outer membrane proteins reveals two important characteristics of the TonB-box. First, the region is not truly conserved among all TonB-dependent OMPs, but rather, is homologous. Many subtle variations exist among all the residues that compose it. Second, when their potential transmembrane domains are identified, the sequences of TonB-OMPs are homologous throughout their length. The finding that evolution in these proteins occurs more slowly in their transmembrane strands is logical, because transmembrane residues must interact with the lipophilic environment of the outer membrane bilayer, populated by the fatty acids of Lipid A and phospholipids. Dramatic changes in transmembrane β-strand composition will locally disrupt OM structure by destabilizing these thermodynamic forces. Such detrimental mutations will be eliminated by natural selection. Therefore, an alternative view of the TonB-box, and the many other similarly conserved regions within TonB-dependent outer membrane proteins, is that they represent the transmembrane domains of these receptor proteins. The first transmembrane strand of outer membrane receptors, the site of the TonB-box, may furthermore assume special biochemical significance, because it may be crucial to the targeting of these proteins to the correct (outer membrane) secretory pathway. These points do not disprove physical contact between TonB and siderophore receptors, but they illustrate the uncertainties of the TonB-box concept.

2. The existence of genetic suppressors between TonB and OM receptor proteins is circumstantial evidence for physical interaction between them, as discussed above. Another notable result of these studies was the isolation of suppressors to TonB-box deficiencies *at other positions within the TonB-box itself* (Bell *et al.*, 1990). In every case, these internal suppressors increase the overall hydrophobicity of the region. These data support the idea that TonB-box mutations disrupt the stability of the first transmembrane strand of TonB-dependent receptors, causing allosteric effects in other regions that impair the inter-

nalization of bound ligands. Mutations in TonB may suppress these defects by increasing the efficiency of its (currently unknown) biochemical action, while compensating mutations within the TonB-box itself simply restore the stability of the first strand in the bilayer.

3. Knowledge of cell envelope structure and properties raises conceptual objections to the ability of an inner membrane-anchored protein to reach across the periplasmic space and physically bind multiple outer membrane proteins. First, how does TonB find and service as many as eight (Fiu, FepA, FecA, FhuE, TonA, Cir, IutA, BtuB) different outer membrane receptor proteins, which are each regulated to maximum levels of 100–10,000 copies/cell? Since the maximum level of TonB expression maybe as high as 3000 copies/cell, the ratio of receptors/TonB in an iron-deficient environment is at least 10/1. The enrichment of siderophore receptors under iron stress is clearly not compensated by equivalent increases in the concentration of TonB (Postle, 1990a). This fact alone tends to preclude direct interaction between TonB and individual siderophore receptors. At least, TonB must rapidly identify siderophore receptors (presumably with adsorbed ligands), bind them with high affinity and energize ligand entry to the periplasm, and then dissociate for energetic recharging, at which time the cycle can repeat. The complexity of this process raises doubts about the kinetic feasibility of such a mechanism.

Finally, the enteric bacterial outer membrane is not a fluid mosaic, but a bilayer that is dominated on the surface by the hydrophilic properties of LPS core and O-antigen sugars, and internally by the β-hydroxymyristoyl fatty acids of Lipid A. Lateral diffusion of proteins does not occur in the outer membrane on a time scale that would permit rapid interactions of TonB with various proteins. On the other hand, the lipid composition of the inner membrane does create a fluid mosaic bilayer, capable of rapid diffusion of lipids and proteins. However, lateral mobility of TonB in the cytoplasmic membrane is also restricted by its proposed extension across periplasmic space, through the crosslinked network of peptidoglycan. It is likely that peptidoglycan restricts the mobility of molecules that penetrate through it, although the extent is not known.

The Theory of Adhesion Zones

Bayer and colleagues have demonstrated the existence of zones of adhesion between the inner and outer membranes. They have observed such adhesions by microscopy, reported their purification, and considered their possible relation to cell envelope physiology (Bayer, 1991). Certain bacteriophages, including TonB-dependent phages, have been observed to infect bacteria through ostensible zones of adhesion. Several observations suggest that the C-terminus of TonB may span the outer membrane bilayer, juxtaposing the inner and outer membrane:

(i) A well defined region of amphiphilicity capable of spanning a bilayer occurs penultimate to the TonB C-terminus (Fig. 1), and deletions into this region destroy TonB function (Anton and Heller, 1991).

(ii) Experimental evidence suggests that the TonB-box domain of siderophore receptor proteins is buried in the OM bilayer (Murphy *et al.*, 1990; Koebnik and Braun, 1993), implying that if physical contact occurs between TonB and receptor proteins at the TonB-box, it takes place within the outer membrane.

(iii) PhoA fusions to the C-terminus of TonB localize the enzyme to the periplasm. Although these data were interpreted to localize the C-terminus in the periplasm (Roof *et al.*, 1991), studies of PhoA fusion to OMPs (Murphy and Klebba, 1989) demonstrate that these results are also consistent with residence of the $\beta\alpha\beta$-core in the OM.

Analysis

The notion that TonB is an integral fixture of cell envelope structure that links the inner and outer membranes does not fit the formal definition of an adhesion zone, in which the two bilayers are proposed to physically fuse to create special sites of transport or secretion (Bayer, 1991; Konisky, 1979). This classical adhesion zone theory is inconsistent with the finding that cell siderophore transport systems include periplasmic binding proteins, while protein-mediated coupling of the two membranes fits these data, and solves other problems as well. Localization of the $\beta\alpha\beta$-core in the OM may resolve the stoichiometry dilemma discussed above, if TonB organizes outer membrane receptors into a higher-order transport complex, as the hub of a wheel organizes its spokes, or the barrel of a gun organizes its chambers. In this context TonB may act as a nucleus that unites trimers of outer membrane proteins by interacting with the exterior of their β-barrels in a

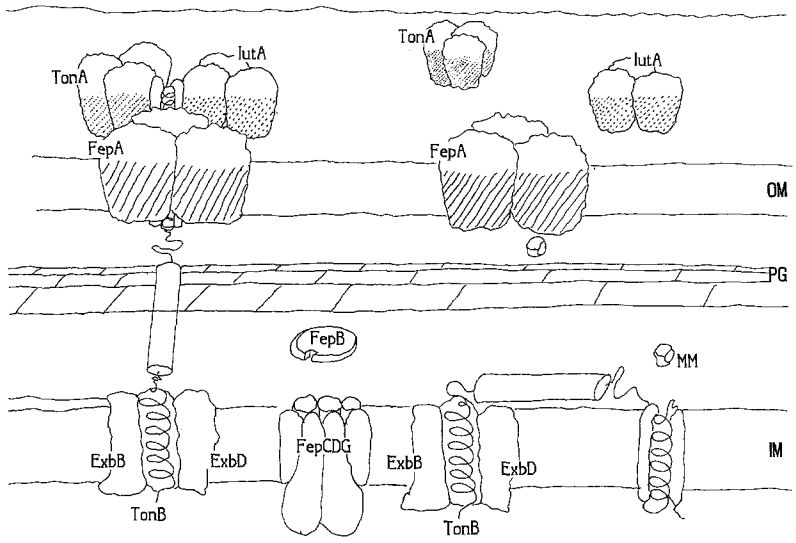

Fig. 2. Mechanisms of TonB function. On the left TonB extends through the peptidoglycan (PG); its C-terminal $\beta\alpha\beta$-core spans the outer membrane (OM), creating an adhesion within the cell envelope, and interacts with the exterior of OMP β-barrels to stimulate ligand transport. On the right the $\beta\alpha\beta$-core spans the inner membrane (IM), and catalyzes the formation of a mobile messenger (MM) that triggers ligand transport.

functional manner (Fig. 2). In this scheme TonB transmutes protonmotive force into mechanical energy that triggers siderophore receptors to release bound ligands. For example, if the C-terminal amphiphilic domain of TonB exists at the hub of a receptor complex, then forces engendered by its physical movement (either rotation in the plane of the bilayer, transmembrane motion across the bilayer, or internal conformational change) will be transmitted to proteins that it contacts. This type of mechanical model rationalizes the hallmark structural feature of TonB, its rigid, elongated, central domain. What is the biochemical function of such a structure? It can transmit mechanical force, while a flexible polypeptide cannot.

A variation on this theme addresses the dramatic amphiphilicity of the C-terminal domain. If the peripheral, hydrophobic surface of this structure associates with the exterior of outer membrane proteins within the bilayer, then it is conceivable that it transiently integrates into the β-barrels of receptor proteins bearing ligands. The resulting physical stress placed on the target receptor may be sufficient to alter the topology of its cell surface ligand binding peptides, releasing substrate into its channel. Or, the numerous charges localized within the proposed strand-helix-strand motif may drive this transformation. We suggest that they form ionic bonds that stabilize the structure, and that neutralization of the negative charges within it (by protons?) induces internal (positive) charge repulsion that drives the strands apart. In this way protonmotive force is converted into mechanical energy. Of course, the current lack of a mechanism to channel protonmotive force to the neutralization of negative charges within the proposed membrane spanning amphiphilic domain of TonB represents a serious deficiency in this hypothesis.

The Theory of a Mobile Messenger

Reynolds *et al.* (1980) postulated the existence of a diffusible intermediate, formed by TonB, that energizes OM transport phenomena.

Analysis

The concept of a mobile messenger within the periplasm is an attractive one, because it solves the TonB/siderophore receptor stoichiometry quandary, and the kinetic problems associated with direct interaction between membrane proteins. Furthermore, a TonB-generated mobile messenger readily explains existing data on ferric siderophore and vitamin B_{12} transport. A diffusible intermediate may bind directly to sites on TonB-dependent receptor proteins, located at the periplasmic surface of the OM, or within OMP β-barrels, or at the interior surface of the ligand-binding gate. Just as direct inter-

actions with TonB at the TonB-box may trigger receptor functions, indirect interactions with TonB mediated by a mobile messenger may stimulate conformational changes that release bound substrates into the channel. According to this view, TonB-box deficiencies decrease the transport activity of siderophore receptors by altering their structure in a way that impedes access of the mobile messenger to its target. Such mutations may be phenotypically alleviated by mutations in TonB that increase periplasmic concentrations of the intermediate itself.

The mobile messenger hypothesis raises the possibility of a different topological model of TonB structure, in which both the N- and C-terminal domains are anchored in the cytoplasmic membrane (Fig. 2), where the N-terminus interacts with ExbB and ExbD, and the C-terminal $\beta\alpha\beta$-core spans the bilayer to perform a critical biochemical function. In this light, the intervening proline-rich domain is seen as a rigid spacer, physically separating the ongoing reactions between ExbB, ExbD, and the TonB upstream helix from those occurring within the $\beta\alpha\beta$-core, perhaps because they are incompatible to the production of the mobile messenger. TonB would then be a protein that changes the net environment of the periplasm in a manner that catalyzes OM receptor protein conformational change. Certain TonB-dependent OMPs manifest an affinity for cations (Bradbeer, 1991), and this property may be relevant to the notion of a mobile messenger. Although TonB-PhoA fusion results argue against localization of the $\beta\alpha\beta$-core in the cytoplasmic membrane (Roof *et al.*, 1991), these data must be interpreted taking into consideration that alkaline phosphatase possesses an overwhelming inherent tendency to localize in the periplasm, which in some cases is sufficient to completely distort the structure of membrane proteins to which it is fused (Murphy and Klebba, 1989).

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