Death of the TonB Shuttle Hypothesis

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Repository Citation
Larsen, Ray A.; Gresock, Michael G.; Savenkova, Marina I.; Ollis, Anne A.; and Postle, Kathleen, "Death of the TonB Shuttle Hypothesis" (2011). _Biological Sciences Faculty Publications_. 6.
https://scholarworks.bgsu.edu/bio_sci_pub/6

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INTRODUCTION

The TonB system energizes the transport of large, scarce, and important nutrients such as vitamin B12 and siderophores complexed with iron across the outer membrane (OM) of Escherichia coli. A complex of the cytoplasmic membrane (CM) proteins ExbB and ExbD harnesses energy derived from the proton motive force (pmf) and transmits this energy to TonB. TonB then physically contacts a ligand-loaded TonB-gated transporter (TGT) in the OM, thus enabling release of the ligand into the periplasmic space. The per-cell copy number ratio of the TonB:ExbB:ExbD proteins is 1:7:2, although the precise ratio in an energy-transducing complex is unknown (Higgs et al., 2002). In E. coli, one set of tonB–exbB–exbD genes supports transport for multiple TGTs. But in certain other species, logistics are more complex; for example, Xanthomonas campestris has eight tonB genes and genes for 48 different transporters (Schauer et al., 2008).

Escherichia coli TonB has a topology consistent with that of an inter-membrane energy transducer: a short cytoplasmic region (residues 1–11), an N-terminal transmembrane domain (TMD, residues 12–32), a periplasmic linker region from 33–149, and a C terminus (residues 150–239) capable of interacting with TGTs. The TMD is essential for localization and maintaining interactions with integral CM proteins ExbB/D (Karlsson et al., 1993; Larsen et al., 1999; Ollis et al., 2009). The periplasmic linker domain is predicted to be largely disordered and contains a non-essential proline-rich domain from 70–102 (Larsen et al., 1993, 2007; Peacock et al., 2005). The C terminus is essential for interaction with TGTs, but none of the 90 residues in the C terminus are individually essential for function (Postle et al., 2010). The molecular mechanism by which TonB transduces energy from the CM to TGTs and facilitates subsequent ligand transport is largely unknown.

The question of how TonB participates in energy transduction has been fertile ground for researchers’ imaginations since the idea of energy transduction from the CM pmf to the OM first emerged (Hancock and Braun, 1976). In 1980, the Bradbeer laboratory suggested the possibility that TonB might synthesize a mobile messenger that would diffuse from CM to OM (Reynolds et al., 1980). Wookey (1982) proposed that TonB might be a periplasmic permutase. Kadner (1990) proposed the idea that TonB might shuttle in a review article. Subsequent evidence for the shuttle model came from sucrose density gradient fractionation experiments where our lab showed TonB to localize in a CM:OM ratio of 60-40%, and exclusively at the OM in conditions in which ExbB/D and TolQ/R were absent (Letain and Postle, 1997). These initial findings were
supported by in vivo demonstrations that a cyst substitution in the extreme TonB N terminus, L3C, could be labeled with the probe Oregon Green® 488 maleimide (OGM; Larsen et al., 2003). As a control, truncated TonB L3C, consisting of the N-terminal 168 amino acids, was unable to interact with TGTs and could not be labeled although it formed a normal complex with ExbB. The degree of labeling for TonB L3C also increased substantially in the absence of the CM proteins ExbB/D and TolQ/R. Taken together, the results suggested that the extreme N terminus of TonB was labeled in the periplasm after it had departed from the CM. We suggested that energy transduction involved the physical cycling between the CM and OM compartments, a process called “shuttling.”

Alternative models have arisen since that time, including a propeller model, a pulling model, and a PBP-assisted model (reviewed in Krewulak and Vogel, 2011). What the later models have in common is that the TonB TMD never leaves the CM while accomplishing its functions. None of the models for TonB-dependent energy transduction has been widely embraced or ruled out, because of a lack of conclusive supporting evidence.

Here we show that in our hands a GFP–TonB fusion was too proteolytically unstable to allow meaningful interpretation of the data. In contrast, a fusion of the cytoplasmic domain of Vibrio cholerae ToxR (aa 1–181) to the N terminus of TonB was proteolytically stable, localized to the CM and had a relative specific activity that was essentially the same as wild-type TonB. These data indicated that TonB does not shuttle. We also discovered that our previous interpretation that TonB shuttled had been compromised by the fact, shown in these studies, that OGM could leak across the CM and label a cyst substitution in a cytoplasmically confined TonB derivative. In light of these findings, our previous data are reinterpreted here.

**MATERIALS AND METHODS**

**MEDIA, CULTURE CONDITIONS**

Saturated overnight cultures grown in Luria-Bertani (LB) broth were subcultured 1:100 into M9 minimal medium or MOPS medium with chloramphenicol added to 34 μg/ml as necessary to maintain plasmids (Shedlovsky and Brenner, 1963; Miller, 1972; Neidhardt et al., 1974). For M9 minimal medium, M9 salts were supplemented with 0.5% glycerol, 0.2% casamino acids (wt/vol), 40 μg/ml tryptophan, 0.4 μg/ml thiamine, 1 mM MgSO4, 0.5 mM CaCl2, and 1.85 μM FeCl3 (Postle, 2007). When necessary, l-arabinose or sodium propionate was added to induce expression of the T onB derivatives to normal chromosomal levels. For all assays, cells were grown to mid-exponential phase of 0.4–0.5 A550 units by the T onB derivatives to normal chromosomal levels. For all assays, a GFP–TonB fusion was too proteolytically unstable to allow meaningful interpretation of the data. In contrast, a fusion of the cytoplasmic domain of *Vibrio cholerae* ToxR (aa 1–181) to the N terminus of TonB was proteolytically stable, localized to the CM and had a relative specific activity that was essentially the same as wild-type TonB. These data indicated that TonB does not shuttle. We also discovered that our previous interpretation that TonB shuttled had been compromised by the fact, shown in these studies, that OGM could leak across the CM and label a cyst substitution in a cytoplasmically confined TonB derivative. In light of these findings, our previous data are reinterpreted here.

**STRAINS AND CONSTRUCTION OF PLASMIDS**

*Escherichia coli* K-12 strains W3110 [F− IN(rrnD−rrnE); Hill and Harnish, 1981]), KP1344 (W3110 ΔtonB::blaM; Larsen et al., 1999), KP1440 (W3110 ΔtonB::blaM exbB::Tn10, tolQ Δamr; Larsen et al., 2003), KP1035 (W3110 entA::mini-kan), KP1229 (W3110 Δana–tonB–trpC; Ahmer et al., 1995), and KP1231 (W3110 Δ(ana–tonB–trpC) entA::mini-kan) were the principal strains used in this study. KP1231 was constructed by sequential P1vir transduction of the entA::mini-kan cassette from MK3, a generous gift from Mark McIntosh, into W3110 to generate KP1035 and finally from KP1035 into KP1229 to yield KP1231 (Ahmer et al., 1995). Plasmids used in this study are shown in Table 1. Attempts were made to replicate construction of a GFP–TonB fusion protein previously described (Kaserer et al., 2008), where sgffp is connected to *tonB* by a *BamHI* site. The plasmid encoding the exact GFP variant (sgffp) used in that study was no longer available (discontinued by the manufacturer), so a gfpmut2 variant was used (encoded on pDR107B, a generous gift from P. de Boer). sgffp and gfpmut2 both encode derivatives of *Aequorea victoria* green fluorescent protein. Each has three substitutions to increase or enhance GFP fluorescence, but the substitutions are different – for sgffp, F64L, S65C, and I167T (Kaserer et al., 2008) and for gfpmut2, S65A, V68L, and S72A (Cormack et al., 1996) gfpmut2–tonB was constructed in three steps. First, *tonB* including the native RBS, from pKP325, was cloned into pPro33 using a 5′ *KpnI* site and 3′ *SphI* site, creating pKP1141 (primer sequences available upon request). Digestion of pPro33 with these restriction enzymes excised the unique *BamHI* site in the pPro33 MCS, since a unique *BamHI* site was needed in the final construct, as described above. In the second step, a *BamHI* site was added immediately before the *tonB* start codon in pKP1141, creating pKP1159. Forward and reverse primers were designed with the desired insertion flanked on both sides by 12–15 homologous bases. *DpnI* digestion was used to remove the template plasmid. Clones were screened for the presence of the *BamHI* site. In the third step, gfpmut2 from pDR107B was amplified, from the start codon through the final coding for Lys238 (no stop codon) and introducing flanking *BamHI*
pBAD33 (Guzman et al., 1995). As a result, ToxR–TonB expression was now regulated by the PBAD promoter. All other plasmids are derivatives of pKP325 (Larsen et al., 2003) into the unique XbaI site in pBAD33 (Guzman et al., 1995). As a result, ToxR–TonB expression was now regulated by the PBAD promoter. All other plasmids are derivatives of pKP325 (Larsen et al., 2007). Plasmid pKP544 was constructed by extra-long PCR, resulting in the removal of TonB expression. 

\[ \text{TonB} \]

Plasmid pKP1529 was generated by cloning PCR-amplified ToxR–TonB (including the ToxR Shine–Dalgarno sequences) from pASAToxRTonB1 (Sauter et al., 2003) into the unique XbaI site in pBAD35 (Guzman et al., 1995). As a result, ToxR–TonB expression was now regulated by the Pnad promoter. All other plasmids are derivatives of pKP325 (Larsen et al., 2007). Plasmid pKP544 was constructed by extra-long PCR, resulting in the removal of TonB residues 13–35. Plasmid DNA sequences were verified at the Penn State Genomics Core Facility – University Park, PA, USA.

\[ \text{TonB} \]

In Vivo Formaldehyde Crosslinking

Formaldehyde crosslinking assays were performed essentially as described previously (Postle, 2007; Ollis et al., 2009). Mid-exponential phase cells were resuspended in sodium phosphate buffer pH 6.8. A portion of each culture served as a control for TonB expression levels. To examine crosslinking of ToxR–TonB in the absence of pmf, carbonyl cyanide-3-chlorophenylhydrazone (CCCP) was added to a final concentration of 50 μM. A control for solvent addition, dimethyl sulfoxide (DMSO) was added to each sample that was not treated with CCCP. Samples were incubated at 37°C for 5 min. and treated with 16% paraformaldehyde (final concentration 1%) for 15 min at room temperature. After centrifugation for 5 min at room temperature, crosslinked samples were solubilized in sample buffer (Laemmli, 1970) at 60°C for 5 min., and uncrosslinked samples were boiled at 95°C for 7 min. Complexes were visualized on immunoblots with the anti-TonB monoclonal (4F1) and anti-ExbD polyclonal antibodies (Larsen et al., 1996; Higgs et al., 2002).

\[ \text{TonB} \]

\[^{59}\text{Fe}]\text{Ferrichrome Transport}

Mid-exponential phase cells were harvested, suspended in buffer and initial rates of \[^{59}\text{Fe}]\text{ferrichrome transport} were determined in triplicate as described (Postle, 2007). A sample of each culture was TCA precipitated immediately prior to assay to determine TonB expression levels. Proteins were visualized on immunoblots of SDS-polyacrylamide gels with the anti-TonB monoclonal antibody 4F1. This experiment was performed independently three times and the same results obtained.

\[ \text{TonB} \]

\[ \text{Proteinase K Accessibility} \]

Strains expressing TonB derivatives were converted to either intact or lysed spheroplasts and were treated with or without proteinase K and with or without carbonyl cyanide-3-chlorophenylhydrazone (CCCP) as described previously (Larsen et al., 1999). Proteinase K-resistant forms were visualized on immunoblots with the monoclonal antibody 4F1.

\[ \text{TonB} \]

\[ \text{Electron Microscopy Preparations} \]

Plasmid-bearing mid-exponential phase KP1344 cells were pelleted and fixed with glutaraldehyde and paraformaldehyde at concentrations of 2% w/v, each according to standard protocols from the Franceschi Microscopy and Imaging Center, Washington State University, Pullman, WA, USA. Samples were dehydrated in an ethanol series and then embedded in LR-White resin. Cured samples were thin sectioned and adhered to nickel grids. Immunolabeling was performed with 1:100 4F1, followed by 1:100 goat anti-mouse antibody conjugated with 20 nm gold particles, Grids were stained with uranyl acetate and lead citrate and visualized with a JEOL transmission electron microscope (JEOL USA Inc.) with images captured with a MegaViewIII Digital camera at the Franceschi Microscopy and Imaging Center.

\[ \text{TonB} \]

\[ \text{Oregon Green® Maleimide Labeling} \]

In vivo labeling was performed as described previously (Larsen et al., 2003). Briefly, 75 ml cells were grown in LB broth with 34 μg/ml chloramphenicol to an A₅₅₀ of 0.50. Buffer A (50 mM KPO₄, 100 mM K₂SO₄, pH 7.0) was added to 25 ml of cells and OGM in DMSO was added to a final concentration of 172 μM. Samples were incubated for 1 h on ice with shaking and were quenched with 2-mercaptoethanol. After washing three times with Buffer A, samples were suspended in Tris–LSB, boiled for 10 min and frozen at −30°C.

In vitro labeling was performed in a manner similar to the in vivo labeling protocol but differed in that RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 1% SDS in 150 mM NaCl 50 mM Tris-acetate pH 7.6) was added to harvested cells, the cells were passed twice through a French Pressure cell, and lysate was collected. Samples were incubated with OGM and frozen as above except that no LSB–Tris was added prior to freezing. The results did not change for either in vivo or in vitro labeling regardless of whether or not 2-mercaptoethanol was added to quench the reaction.

\[ \text{TonB} \]

\[ \text{Immunoprecipitation} \]

In vivo and in vitro samples were thawed, and TonB was immunoprecipitated with a cocktail of 4F1 and 4H4 monoclonal antibody as described previously (Larsen et al., 2003). Samples for GroEL analysis were prepared the same way as the TonB samples but immunoprecipitated with anti-GroEL antibody (Stressgen Bioreagents, Victoria, BC, Canada). Samples were divided, resolved on SDS-polyacrylamide gels and immunoblots were subsequently developed with either anti-TonB or anti-GroEL antibodies and anti-OGM (Pierce) antibodies.

\[ \text{TonB} \]

\[ \text{Results} \]

GFP–TonB CAN GIVE RISE TO “SHUTTLEABLE” DEGRADATION PRODUCTS

A recent study fused GFP from A. victoria to the N terminus of TonB and asked whether or not this GFP–TonB construct was active (Kaserer et al., 2008). The fusion of the TonB cytoplasmic N terminus to a large cytoplasmically localized protein should prevent potential shuttling by keeping TonB retained in the CM. The GFP–TonB construct supported TonB activity and the authors concluded that TonB did not shuttle. To extend this work, we reconstructed a GFP–TonB fusion with the same linker sequence between GFP and TonB as was used in that study and used TCA to precipitate bacteria immediately upon harvest to prevent the degradation of GFP–TonB (and presumably GFP–TonB fragments) that could occur in gel sample buffer at 95°C (Skare and Postle, 1991). This allowed for accurate determination of the
steady-state levels of all TonB-specific peptides. In our hands, expression of GFP–TonB at chromosomal levels resulted in the detection of several prominent degradation products (Figure 1). The results did not change regardless of whether M9 or MOPS medium was used, nor if GFP–TonB was expressed in the tonB enter strain KP1231 (data not shown). The degradation products that were the size of full-length TonB or slightly larger might support TonB activity by shuttling. Because of the challenges and problems with proteolysis and because the mechanism of TonB energy transduction is still considered an open question (Krewulak and Vogel, 2011), we wanted to identify a system that could answer the question of shuttling more definitely.

**ToxR (1–181) – TonB IS STABLE AND FULLY ACTIVE**

During the course of studies on TonB dimerization, we identified a fusion of full-length TonB to the cytoplasmic domain (aa 1–181) of *V. cholerae* ToxR (Miller et al., 1987), that was extremely stable as expressed from pASAToxRTonB1 and analyzed by immunoblot (data not shown). This plasmid was a kind gift of V. Braun from earlier studies on TonB dimerization where the expression levels and activities of the ToxR–TonB constructs were not reported (Sauter et al., 2003). Because ToxR–TonB was overexpressed from pASAToxRTonB1 (data not shown), we cloned toxR–*tonB*, including its putative Shine–Dalgarno sequences into pBAD33 so that its expression could be regulated by the PBAD promoter (Guzman et al., 1995). Like wild-type TonB, virtually all of the ToxR–TonB could be converted into a proteinase K-resistant conformation by collapse of the pmf due to CCCP addition (Figure 2). Because ExbB and ExbD are required for this proteinase K-resistant conformation (Larsen et al., 1999), it suggested that ToxR–TonB was located in the CM. The pmf is also required for TonB and ExbD to form stable, full length ToxR–TonB. The amount of TonB in the uninduced sample (pTonB no ara) far exceeded the amount of the only detectable degradation product that derived from ToxR–TonB and supported only 12% activity (Figure 4). Thus, stable, full length ToxR–TonB had the same relative specific activity as chromosomally encoded wild-type TonB.

**MEMBRANE PERMEABILITY OF OGM EXPLAINS PREVIOUS RESULTS**

The strongest prior evidence for in vivo shuttling was the demonstration that a cys substitution predicted to be cytoplasmically localized could be labeled by OGM in full-length TonB but not in a truncated TonB derivative missing much of its C terminus and incapable of interaction with the OM (Larsen et al., 2003). OGM has been considered a “generally membrane impermeant” probe, and other groups have used this reagent to discriminate between free cysteines in the periplasm or cytoplasm of Gram-negative bacteria (Ye et al., 2001; Ye and Maloney, 2002; Yang et al., 2005; Liu et al., 2007; Nanatani et al., 2007, 2009). Given the contrast in findings between the ToxR fusions and the OGM labeling, we tested the possibility that OGM leakage across the CM could account for our previous findings (Larsen et al., 2003).

To assess the potential for OGM to leak across the CM, we constructed a TonB variant lacking its transmembrane domain but
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FIGURE 3 | ToxR–ToxB makes the pmf-dependent crosslink with ExbD.

Crosslinking was performed as described in Section “Materials and Methods” with the following strains: cToxB (W3110 expressing chromosomally encoded ToxB and ExbD); ΔToxB (KP1344); pToxB (KP1344/pKP325); pToxR–ToxB (KP1344/pKP1529); and pToxR–ToxB CCCP (KP1344/pKP1529 treated with 50 μM CCCP). L-Arabinose was added to induce expression of plasmid-encoded ToxB as follows: 0.00075% for pToxB and 0.00046% for pToxR–ToxB. Complexes were detected on immunoblots of 13% SDS-polyacrylamide gels using ExbD polyclonal antibodies (upper two panels). Uncrosslinked monomeric ToxB and ToxR–ToxB levels were visualized with the monoclonal anti-ToxB antibody 4F1 (bottom panel). The molecular mass standards (kDa) are shown on the left.

FIGURE 4 | ToxR–ToxB supports as much activity as plasmid-encoded wild-type ToxB.

Ferrichrome transport assays were performed as described in Section “Materials and Methods” with the following strains: cToxB (W3110 expressing chromosomally encoded ToxB); ΔToxB (KP1344); pToxB (KP1344/pKP325); pToxB no ara (KP1344/pKP325 uninduced); and pToxR–ToxB (KP1344/pKP1529). L-Arabinose was added to induce expression of plasmid-encoded ToxB as follows: 0.00075% for pToxB and 0.00045% for pToxR–ToxB. Initial ferrichrome transport rates are as follows, expressed as counts per minute (cpm)/0.05 A550 ml cells/minute: cToxB 453 ± 31; pToxB 326 ± 13 (standard to 100%); pToxB no ara 39 ± 7 (12%); pToxR–ToxB 333 ± 15 (102%); ΔToxB −4 ± 4 (0%). ToxB expression levels were visualized on immunoblots of 11% SDS-polyacrylamide gels with anti-ToxB monoclonal antibody 4F1. A longer exposure is shown to provide a comparison between uninduced pKP325 and the lack of full-length degradation product from pKP1529. Mass standards are shown on the right. Degradation products are marked with an asterisk (*).

ToxB L3C ΔTMD expressed from pKP544 should be cytoplasmically localized (Karlsson et al., 1993). The cytoplasmic localization of the ToxB L3C ΔTMD construct was tested by two methods: proteinase K accessibility assays in intact spheroplasts and transmission electron microscopy immunolocalization. Whereas wild-type ToxB (ToxB L3C C18G) is sensitive to proteinase K in intact spheroplasts, the ToxB L3C ΔTMD was sequestered from proteinase K (Figure 5A). When spheroplasts were lysed, both ToxB L3C C18G and ToxB L3C ΔTMD were sensitive to proteinase K, indicating that ToxB L3C ΔTMD was cytoplasmically localized (Figure 5A). The same results were also obtained in KP1344 (data not shown). Consistent with that result, electron microscopy revealed that the ToxB L3C ΔTMD was confined to the cytoplasm whereas gold particles detecting wild-type ToxB localized to the envelope (Figure 5B).

Oregon Green® 488 maleimide was able to label the cytoplasmically localized ToxB L3C ΔTMD both in vitro and in vivo. This showed that in our previous experiments, OGM was indeed capable of crossing the CM and ToxB L3C did not need to shuttle to become labeled by it (Figure 6). In addition, OGM labeled the cytoplasmic chaperone GroEL in vitro and in vivo, confirming its ability to leak across the CM (Figure 6).
The fate of the TonB N terminus during energy transduction has been speculated about and investigated for many years. Today, the primary models that explain its role include several mechanical models where TonB remains continuously associated with the CM and the shuttle model, where TonB cyclically dissociates from the CM and shuttles to the OM. The mechanical models suggest direct use of the pmf in energizing TGTs with TonB as a bridge between the two. The shuttle model suggests that TonB somehow stores potential energy from the pmf prior to shuttling to the OM. The TonB TMD was proposed to store the potential energy through response of residues S16 and H20 to the pmf (Larsen and Postle, 2001). Because these two models have different consequences and make different predictions, it is important to determine which is correct.

Since the emergence of the shuttle model, we have learned that the TonB TMD is highly tolerant to mutation. In particular, the only sidechain that cannot be replaced by ala and retain activity is H20, yet H20 can be replaced by asn and retain full activity, suggesting that the TonB TMD is not on a proton translocation pathway (Larsen et al., 2007; Swayne and Postle, 2011). These results cast doubt on the shuttle hypothesis by eliminating the TMD as the region of TonB that needed to be delivered to the OM. Here we demonstrate that, in contrast to GFP–TonB, a fusion of the cytoplasmic domain of ToxR (1–181) to TonB produced virtually no degradation products when expressed at levels characteristic of native TonB. ToxR–TonB appeared to be anchored in the CM at all times because it could be fully converted to a proteinase K-resistant form in de-energized cells. ToxR–TonB could also form a pmf-dependent crosslink with ExbD and was fully active in ferrichrome transport assays. This eliminated the possibility that somehow the entire ~180 residue folded cytoplasmic domain of ToxR could be pulled out of the CM to shuttle. Even if the ToxR domain could be pulled out of the CM, it would not be possible for it to recross back into the cytoplasm and allow the TonB portion to assume its rightful relationship with ExbB and ExbD such that it had 100% activity. Because ToxR–TonB had an equivalent relative specific activity to TonB, we conclude that TonB does not shuttle. These results confirm and expand the conclusions of Kaserer et al. (2008).

The permeability of OGM to the CM explained the apparent contradiction between the ToxR–TonB results, and the previous ones suggesting that TonB shuttles in vivo (Larsen et al., 2003). The data from our 2003 study can be reinterpreted in the light of this new information. The labeling seen for full-length TonB L3C can be explained by the observation that OGM diffuses into the cytoplasm. A more interesting explanation may pertain to the observation that TonBam168 L3C did not become labeled with OGM, even though it still retained the ability to respond conformationally to pmf in spheroplasts and formaldehyde crosslink to ExbB, indicating that the truncation did not radically interfere with assembly with ExbB/D. The reinterpretation of the data raises the question of whether there is signal transduction occurring from the OM to the CM ExbB/D complex through TonB. It could be that when full-length TonB is present, the cytoplasmic domains of ExbB allow access of OGM to the extreme N terminus of TonB. In this model, when the C terminus of TonB is deleted and TonB does not associate with the OM, then the cytoplasmic domains of ExbB are conformationally rearranged such that the TonB L3C residue is sequestered. Consistent with that idea, TonB
TonB must be disordered in order to span the periplasm, then it is likely that TonB C terminus participates in an energy transduction cycle of association and dissociation from the TGTs rather than being constantly associated with the OM. In support of this idea, the number of OM transporters in a given cell exceeds the number of available TonB molecules (Kadner and Heller, 1995; Higgs et al., 2002; Postle et al., 2010).

In a detailed analysis of four TGT structures, Chimento et al. (2005) noted that the β-strands of the cork domain that fills the TGT barrel are mostly parallel to the periplasmic space. They propose that only a modest perpendicular force supplied by TonB would be necessary to unfold the cork (Chimento et al., 2005). Now that we know that TonB does not shuttle, and that it may contact the TGT in a disordered conformation, it suggests that the mere folding of TonB upon contact might be sufficient to allow transport.

These new data also raised the question of what the 30–40% of TonB that fractionates with the OM represents. It could possibly represent the fraction of TonB that is strongly associated with the OM transporters at any one point in the energy transduction cycle. Alternatively it could represent TonB that has temporarily dissociated from the ExbB/D complex while its C terminus is associated with the OM. In either case, it must mean that TonB is being artifactually pulled out of the CM because ExbB and ExbD remain associated with the CM (Letain and Postle, 1997).

A remaining mystery is why, in the absence of ExbB/D and TolQ/R, nearly all of the TonB is found associated with the OM. It could be that the conformation of the TonB C terminus is such that its default interaction is at the OM. Alternatively it may be that the action of ExbB/D is primarily required to assist the TonB C terminus in dissociating from an OM transporter after an energy transduction event. The demonstration that TonB does not shuttle eliminates debate concerning how energy would be used to physically remove TonB from a CM complex of ExbB/D, and also how the N terminus would somehow target TonB back to an ExbB/D complex at the CM (Larsen et al., 2003; Postle and Kadner, 2003).

ACKNOWLEDGMENTS

We thank Volkmar Braun and Silke Patzer for pASAToxRTonB1. We thank Jay Keasling for pPro33, Piet de Boer for pDR107B, and Mark McIntosh for strain MK3. Penelope Higgs created strain KP1231. This work was supported by grant GM42146 to K.P. from the National Institute of General Medical Sciences.

REFERENCES


Frontiers in Microbiology | Microbial Physiology and Metabolism

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Conflict of Interest Statement: The authors are aware of no personal or monetary issues which arose during preparation of this manuscript that could represent a conflict of interest.

Received: 02 July 2011; paper pending published: 09 August 2011; accepted: 15 September 2011; published online: 12 October 2011.


This article was submitted to Frontiers in Microbial Physiology and Metabolism, a specialty of Frontiers in Microbiology.

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