Shuttling between Membranes, a New Energy Transduction Paradigm in Gram Negative Bacteria

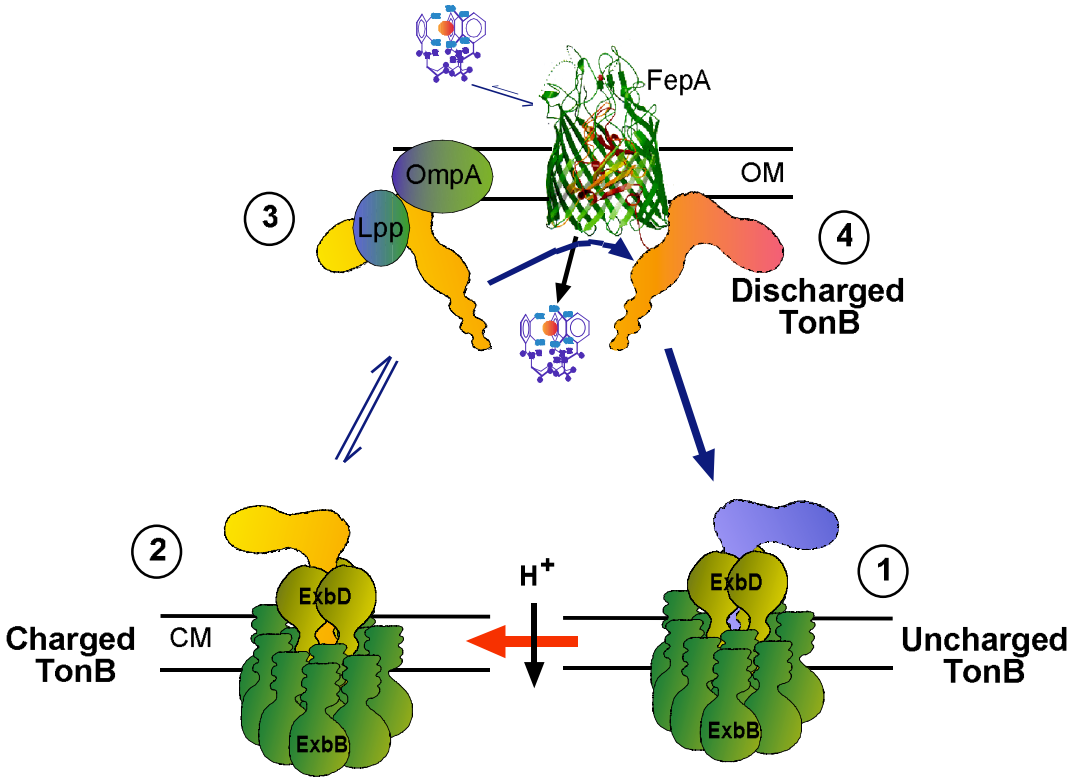
Kathleen Postle*
School of Molecular Biosciences
Washington State University
Pullman, WA 99164-4234
U.S.A.

Gram negative bacteria such as *E. coli* have both a cytoplasmic and an outer membrane. While processes in the cytoplasmic membrane can be energized by ion gradients or ATP hydrolysis, the outer membrane lacks access to these energy sources. Nonetheless, active transport of important nutrients occurs across the outer membrane. Surprisingly, the energy source for this transport turns out to be the protonmotive force (pmf) of the spatially separate cytoplasmic membrane. A large complex of made up of two cytoplasmic membrane proteins--ExbB and ExbD--appears to harvest the cytoplasmic membrane pmf and use it instill a third protein, TonB, with meta-stable conformationally stored potential energy. The energized TonB delivers stored energy to the outer membrane transporters, with subsequent movement of nutrients into the aqueous periplasmic space between the two membranes. From that point, the nutrients are retrieved by soluble binding proteins and actively transported into the cytoplasm by classical ABC transporter mechanisms.

Previous studies based on membrane fractionations suggested a model wherein TonB transduces energy by making sequential and cyclic contact with proteins in each membrane, a process called shuttling (1). A key feature of shuttling is that the amino terminal signal anchor (N-terminus in the cytoplasm) is released from the cytoplasmic membrane ExbB/ExbD complex as TonB becomes associated entirely with the outer membrane. To test this model *in vivo*, a sole cysteine was engineered in TonB at the extreme amino terminus. This cysteine could be labeled in whole cells by Oregon Green® 488 maleimide, but only under circumstances where TonB could shuttle to the outer membrane. Mutant TonB that could not shuttle also could not be labeled. In addition, more TonB was labeled under conditions where a greater proportion of TonB was found at the outer membrane (2). To our knowledge, this is the first description of a shuttle mechanism for energizing active transport.

1. Letain, T. E., and K. Postle. (1997) Mol. Microbiol. **24**:271-283.
2. Larsen, R.A., Letain, T.E., and K. Postle (2003) Mol. Microbiol. **49**: 211-218.

Model for Energy Transduction between Membranes of Gram-negative Bacteria



Uncharged TonB (1) is converted to charged TonB (2) by the passage of a proton through the ExbB/D complex. Charged TonB can shuttle to the outer membrane and dock with non-transporter proteins, Lpp and OmpA (3). Ligand binding to the TonB-gated transporter FepA results in a conformational change in FepA, that induces productive interaction with charged TonB, and release of the conformationally stored potential energy in TonB(4). Discharged TonB is recovered at the cytoplasmic membrane as uncharged TonB (1) by ExbB/D and begins a new energy transduction cycle.