protein similar to that of a number of bacterial membrane transporters (*5*). The PIN1 predicted structure is a hydrophilic central domain flanked by five to six predicted membrane-spanning domains (*5*). Consistent with the model, PIN1 is most abundant on the basal membrane of cells in the central vascular region of the shoot. Although direct biochemical evidence that PIN1 transports auxins outwardly is still lacking, the indirect evidence presented is compelling.

From the complexity of the auxin pathway, multiple auxin efflux carriers are to be expected that are specific to tissues, individual cells, and even plasma membrane locations. On the basis of the large number of similar *PIN* genes already in the sequence database, these expectations will certainly be met soon, and the new results point to the first realization. *AGR1* (*6*)—now independently cloned by four labs—can be added to the list of putative auxin efflux carriers, and the evidence indicates that its function is specific to roots. *AGR1,* also called *EIR1* (*7*) and *PIN2* (*8*), encodes a membrane protein that is 64% identical to PIN1, but unlike PIN1, it is found on the apical, rather than basal, membrane of cells. But this distribution actually fits the model: "Out" in cortical root cells is "up" because in this part of the root, the auxin pathway is moving upward toward the shoot (see the figure). Further support for AGR1's identity as an efflux carrier is that yeast cells expressing AGR1 transport out auxin (*6*) and the toxin 5-fluoro-indole (*6*, *7*) more rapidly than do control yeast cells. In addition, *agr1* roots retain preloaded auxin longer than wild-type roots (*6*), and Utsuno *et al.* (*9*) report that *agr1* roots are sensitive to 2,4-D but not IAA or NAA (a characteristic of the efflux carrier). Müller *et al.* (*8*), however, did not observe convincing differences in the auxin sensitivity profile among these inhibitors, so all the expectations for an efflux carrier are not yet unequivocally met.

The work by these labs marks a breakthrough in the field and has transformed the Darwins' recalcitrant topic of gravitropisms and phototropisms to one where answers to the next set of questions are within reach: In which cell type is each carrier located, and how do they operate to establish the longitudinal and radial auxin gradients? Which are the lateral carriers predicted to set up asymmetrical auxin gradients, and how do extracellular signals act upon them? What are the endogenous regulation mechanisms? New mutant genes, soon to be cloned (*13*), will certainly shed light on these questions with gravity.

References

1. C. Darwin, *Power of Movement in Plants* (Murray, London, 1880).
2. F. W. Went, *Rec. Trav. Bot. Ned.* **25**, 1 (1928).
3. C. Uggla, T. Moritz, G. Sandberg, B Sandberg, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9282 (1996); L. Wolpert, in *The Molecular Basis of Positional Signalling*, R. Key and J. Smith, Eds. (Company Biology, Cambridge, UK, 1989), pp. 3–12; H. Meinhardt, in *Positional Controls in Plant Development*, P. W. Barlow and D. J. Carr, Eds. (Cambridge Univ. Press, Cambridge, UK, 1984), pp. 1–32.
4. J. A. Raven *New Phytol.* **74**, 163 (1975); P. H. Rubery and A. R. Sheldrake, *Planta* **118**, 101 (1974).
5. L. Gälweiler *et al.*, *Science* **282**, 2226 (1998).
6. R. Chen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15112 (1998).
7. C. Luschnig, R. A. Gaxiola, P. Grisafi, G. R. Fink, *Genes Dev.* **12**, 2175 (1998).
8. A. Müller *et al.*, *EMBO J.* **17**, 6903 (1998).
9. K. Utsuno, T. Shikanai, Y. Yamada, T. Hashimoto, *Plant Cell Physiol.* **39**, 1111 (1998).
10. M. H. M. Goldsmith, *Annu. Rev. Plant Physiol.* **28**, 439 (1977); T. L. Lomax, G. K. Muday, P. H. Rubery, in *Plant Hormones*, P. J. Davies, Ed. (Kluwer Academic, Boston, 1995), p. 509; A. Delbarre, P. Muller, V. Imhoff, J. Guern, *Planta* **19**, 532 (1996).
11. A. M. Jones, *Plant Physiol.* **93**, 1154 (1990); H. Kaldewey, *Encyl. Plant Physiol.* **10**, 80 (1984).
12. K. Okada and J. Ueda, M. K. Komaki, C. J. Bell, Y. Shimura, *Plant Cell* **3**, 677 (1991); C. J. Bell and P. Maher, *Mol. Gen. Genet.* **220**, 289 (1990); K. Okada and Y. Shiimura, *Science* **250**, 274 (1990).
13. E. L. Stowe-Evans, R. M. Harper, A. V. Motchoulski, E. Liscum, *Plant Physiol.* **118**, 1265 (1998); M. Ruegger *et al.*, *Plant Cell* **9**, 745 (1997); H. Fujita and K. Syono, *Plant J.* **12**, 583 (1997).

PERSPECTIVES: PROTEIN STRUCTURE

Pumping Iron Through Cell Membranes

Volkmar Braun

Gated protein channels allow ions and small molecules to flow across a cell membrane in a regulated way. Despite the importance of these proteins, the three-dimensional structure at atomic resolution has been determined for only a few gated channel proteins, such as channel-forming bacterial protein toxins and a partial bacterial potassium channel. On page 2215 of this issue, Ferguson *et al.* describe the complete structure of a gated channel from the outer membrane of *Escherichia coli* (*1*).

The protein studied by Ferguson *et al.*, designated FhuA, has a number of extraordinary properties. It serves as a transporter for the uptake of an iron complex (ferrichrome) that belongs to a class of microbial compounds (siderophores) that solubilize Fe^{3+}, a structurally related antibiotic (albomycin), a protein toxin (colicin M), and a peptide toxin (microcin 25), and it functions as a receptor for a number of viruses (phages T1, T5, ϕ80, and UC-1) that multiply in *E. coli* (see the figure). Moreover, the activity of FhuA is dependent on energy input that is provided by the proton motive force of the cytoplasmic membrane because there is no energy source in the outer membrane.

Energy is transmitted from the cytoplasmic membrane to the outer membrane by a complex that consists of the three proteins TonB, ExbB, and ExbD, of which TonB and ExbD are located in the periplasm between the cytoplasmic membrane and the outer membrane with their NH_2-termini inserted in the cytoplasmic membrane (see the figure) (*2–4*). It has been genetically and biochemically shown that TonB interacts with FhuA, suggesting that TonB assumes an energized conformation in response to the proton motive force and allosterically opens the FhuA channel. Energy coupling is required for all FhuA ligands except phage T5. Only T5 infects TonB mutants, binds to isolated FhuA, releases its DNA, and opens a channel in FhuA (*5*) similar in size to a FhuA deletion mutant in which residues 322 to 355 were genetically excised (*6*).

The crystal structure consists of 22 antiparallel transmembrane β strands extending from residues 161 to 723 that form a β barrel. The β-barrel strands are interconnected by large loops at the cell surface and small turns in the periplasm. Such a β-barrel structure is also the principal arrangement of the outer membrane porins. However, in contrast to the porins, no loop folds inside the barrel to restrict the permeability of the channel. Rather, the FhuA barrel is entirely closed by the NH_2-terminal cork domain that enters the β barrel from the periplasmic side. More than half of the molecule is located above the bilayer membrane. It contains a cavity that is open to the external medium and separated from a periplasmic cavity by a thin layer of amino acid residues. It is tempting to propose that the channel is opened in this region by a conformational transition that is caused by the interaction of FhuA with TonB in the energized conformation. The region of FhuA (TonB box, residues 7 to 11) that according to a genetic suppression analysis in-

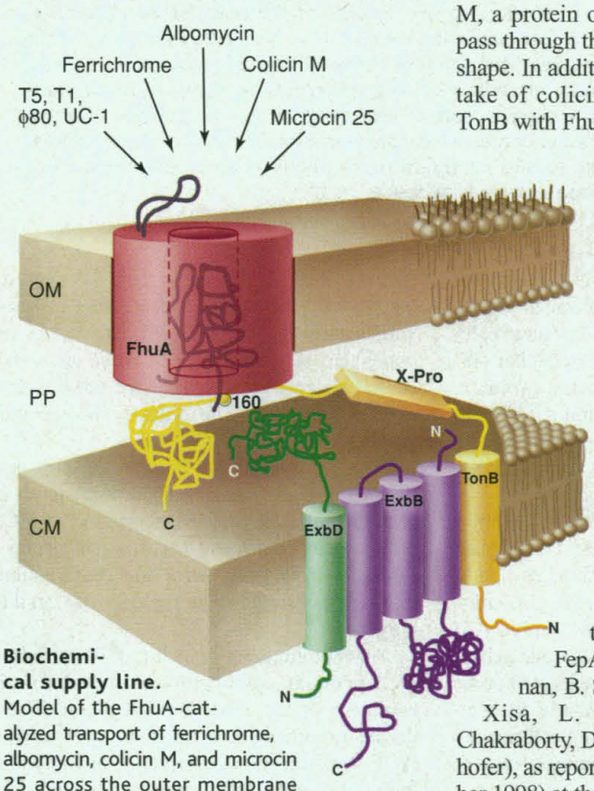
The author is in the department of Mikrobiologie/Membranphysiologie, Universität Tüebingen, D-72076 Tübingen, Germany. E-mail: volkmar.braun@mikrobio.uni-tuebingen.de

teracts with TonB (*7*) is located in the periplasm. It is not seen in the crystal structure, which starts at residue 19 and apparently does not assume a fixed structure.

A highlight of the crystal structure analysis is the comparison of the ferrichrome-loaded FhuA with the unloaded FhuA. The uncharged ferrichrome is fixed above the cork well outside the membrane by hydrogen bonds and van der Waals contacts in a binding site formed by eight aromatic residues and one residue each of arginine, glutamine, and glycine. Ferrichrome binding induces structural transitions that go through the entire FhuA molecule up to the NH_2-terminus. In particular, apex B formed by residues Glu^{98} to Gln^{100} of the cork domain makes an α-carbon translation of 1.7 Å toward ferrichrome. This and additional movements of residues in the cork domain and the barrel domain largely maintain the FhuA secondary structure up to the periplasmic cavity, where in contrast a short helix (residues 24 to 29) is completely unwound and the resulting coil bends away by 180° from the previous helix axis. A small structural change at the ferrichrome-binding site at the cell surface is amplified to a large structural change in the periplasmic pocket where, for example, Glu^{19} is 17.3 Å away from its former α-carbon position. These results are in line with the conversion of isolated FhuA by ferrichrome to trypsin resistance at Lys^{67} and the reduction of monoclonal antibody binding to residues 21 to 59 (*8*). Binding of ferrichrome enhances interaction of FhuA with TonB so that preferentially substrate-loaded FhuA is coupled to the energy-providing Ton system (*9*). The amounts of FhuA and the other TonB-dependent receptors are under natural iron-depleted conditions much higher than the amount of TonB, which makes it necessary and economic that the channels of only substrate-loaded receptors are opened by interaction with TonB. Ferrichrome binding to viable cells quenches fluorescence of fluorescein-labeled engineered Cys^{336} in loop L4 (*10*), which is the major FhuA loop at the cell surface, and intrinsic tryptophan fluorescence is quenched upon binding of ferrichrome to isolated FhuA (*11*). Loop 4 also serves as the principal binding site of the phages (*12*) whereby T1 and ϕ80 recognize the energized conformation of FhuA, which would indicate that not only the cork but also the structure of loop 4 is altered in response to the proton motive force and TonB.

The crystal structure of FhuA contains as a remarkable feature a lipopolysaccharide molecule (LPS) that is noncovalently bound. This is the first x-ray structure of an LPS, and it localizes FhuA in the outer membrane. The six fatty acid residues of LPS form mainly the lipid layer of the outer leaflet. The crystal structure precisely localizes the fatty acids at FhuA and thus the position of FhuA in the outer membrane.

There is no doubt that the crystal structure is a milestone in the understanding of the intriguing mechanisms underlying the multifunctional activities of FhuA, but, as good science usually does, it poses more questions than it answers. The most pertinent one is how the proton motive force of the cytoplasmic membrane opens the channel of FhuA in the outer membrane. This concerns the conversion of TonB to a form that interacts with FhuA such that the channel is opened. There may be additional, unknown proteins involved. It is not clear whether FhuA serves as a pump of ferrichrome or whether vectorial transfer across the outer membrane and accumulation in the periplasm results from binding of ferrichrome to the periplasmic FhuD protein (*13*). It is also difficult to visualize how the DNA of the phages enters the periplasm through the FhuA channel. Removal of the entire cork would be required, and this would consume much energy (>100 kcal). Provided that the osmotic pressure in the phage head is high enough, it is difficult to visualize how it can be converted into a force that removes the entire cork. Colicin M, a protein of 27 kD, is also too large to pass through the FhuA channel in a globular shape. In addition, there is evidence that uptake of colicin M requires interaction of TonB with FhuA and with colicin M as well (*2*). Structural and functional analyses of FhuA mutants designed according to the crystal structure and cocrystallization of FhuA with the complete structures or fragments of TonB, colicin M, and phage DNA will be required to answer these questions.



Biochemical supply line. Model of the FhuA-catalyzed transport of ferrichrome, albomycin, colicin M, and microcin 25 across the outer membrane (OM) of *E. coli* and of the FhuA-mediated infection by the phages T5, T1, ϕ80, and UC-1. The model illustrates the major surface loop 4, which serves as a binding site of the phages and of the cork domain that closes the FhuA channel. The NH_2-terminus of FhuA contains the TonB box that is thought to interact with the Gln^{160} of TonB in the periplasm (PP). The model proposes energy transfer from the cytoplasmic membrane (CM) to the outer membrane by the Ton complex (TonB, ExbB, and ExbD). The repetitive X-Pro motif of TonB is thought to bridge the periplasmic space.

The results obtained with FhuA by the Konstanz-Montreal group (*1*) are supported by a parallel FhuA x-ray analysis from a Basel-Strasbourg group (K. Locher, B. Rees, R. Koebnik, A. Mitschler, L. Moulinier, J. P. Rosenbusch, and D. Moras), and they agree largely with the FepA crystal structure (S. Buchanan, B. S. Smith, L. Venkatramani, D. Xisa, L. Esser, M. Palnikar, R. Chakraborty, D. van der Helm, and J. Deisenhofer), as reported at a conference (9 November 1998) at the University of Strasbourg and in (*14*). I strongly suspect that FhuA and FepA will prove to be the type structures for a large group of bacterial outer membrane transporters that take up bacterial ferric siderophores, ferric iron released from host transferrin and lactoferrin, heme and heme released from hemoglobin and hemopexin, and vitamin B_{12} in *E. coli* (*15*).

References

1. A. D. Ferguson, E. Hofmann, J. W. Coulton, K. Diederichs, W. Welte, *Science* **282**, 2215 (1998).
2. V. Braun, *FEMS Microbiol. Rev.* **16**, 295 (1995).
3. C. Bradbeer, *J. Bacteriol.* **175**, 3146 (1993).
4. K. Postle, *J. Bioenerg. Biomembr.* **25**, 591 (1993).
5. M. Bonhivers *et al.*, *EMBO J.* **15**, 1850 (1996).
6. H. Killmann, R. Benz, V. Braun, *ibid.* **12**, 3007 (1993).
7. H. Schöffler and V. Braun, *Mol. Gen. Genet.* **217**, 378 (1989).
8. G. S. Moeck and J. C. Coulton, *Mol. Microbiol.* **28**, 675 (1998).
9. ———, K. Postler, *J. Biol. Chem.* **272**, 28391 (1997).
10. C. Bös, D. Lorenzen, V. Braun, *J. Bacteriol.* **180**, 605 (1998).
11. K. L. Locher and J. P. Rosenbusch, *Eur. J. Biochem.* **247**, 770 (1997).
12. H. Killmann, G. Videnov, G. Jung, H. Schwarz, V. Braun, *J. Bacteriol.* **177**, 694 (1995).
13. W. Köster and V. Braun, *J. Biol. Chem.* **265**, 21407 (1990).
14. K. L. Locher *et al.*, *Cell* **95**, 771 (1998).
15. R. J. Kadner, *Mol. Microbiol.* **4**, 2027 (1990).