34 Hirano, T., Kobayashi, R. and Hirano, M. (1997) *Cell* 89, 511–521
35 Melby, T. E., Ciampaglio, C. N., Briscoe, G. and Erickson, H. P. (1998) *J. Cell Biol.* 142, 1595–1604
36 Uhlmann, F. and Nasmyth, K. (1998) *Curr. Biol.* 8, 1095–1101
37 Hirano, T. (1995) *Trends Biochem. Sci.* 20, 357–361
38 Guacci, V., Hogan, E. and Koshland, D. (1994) *J. Cell Biol.* 125, 517–530
39 Kumada, K. *et al.* (1998) *Curr. Biol.* 8, 633–641
40 Liang, H. *et al.* (1993) *Exp. Cell Res.* 204, 110–120
41 Sluder, G. *et al.* (1997) *J. Cell Sci.* 110, 421–429
42 Stratmann, R. and Lehner, C. F. (1996) *Cell* 84, 25–35
43 Weinstein, J. (1997) *J. Biol. Chem.* 272, 28501–28511
44 Prinz, S., Hwang, E. S., Visintin, R. and Amon, A. (1998) *Curr. Biol.* 8, 750–760
45 Yamamoto, A., Guacci, V. and Koshland, D. (1996) *J. Cell Biol.* 133, 85–97
46 Nicklas, R. B. (1997) *Science* 275, 632–637
47 Waters, J. C., Chen, R. H., Murray, A. W. and Salmon, E. D. (1998) *J. Cell Biol.* 141, 1181–1191
48 Wells, W. A. and Murray, A. W. (1996) *J. Cell Biol.* 133, 75–84
49 Li, R. and Murray, A. W. (1991) *Cell* 66, 519–531
50 Hoyt, M. A., Trotis, L. and Roberts, B. T. (1991) *Cell* 66, 507–517
51 Hardwick, K. G. *et al.* (1996) *Science* 273, 953–956
52 Tavormina, P. A. and Burke, D. J. (1998) *Genetics* 148, 1701–1713
53 Chen, R. H., Waters, J. C., Salmon, E. D. and Murray, A. W. (1996) *Science* 274, 242–246
54 Taylor, S. S. and McKeon, F. (1997) *Cell* 89, 727–735
55 Jin, D. Y., Spencer, F. and Jeang, K. T. (1998) *Cell* 93, 81–91
56 Taylor, S. S., Ha, E. and McKeon, F. (1998) *J. Cell Biol.* 142, 1–11
57 Straight, A. F., Belmont, A. S., Robinett, C. C. and Murray, A. W. (1996) *Curr. Biol.* 6, 1599–1608
58 Li, Y. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 12431–12436
59 Kim, S. H. *et al.* (1998) *Science* 279, 1045–1047
60 Hwang, L. H. *et al.* (1998) *Science* 279, 1041–1044
61 Gorbsky, G. J., Chen, R. H. and Murray, A. W. (1998) *J. Cell Biol.* 141, 1193–1205
62 Moore, D. P. and Orr-Weaver, T. L. (1998) *Curr. Top. Dev. Biol.* 37, 263–299
63 Molnar, M., Bahler, J., Sipiczki, M. and Kohli, J. (1995) *Genetics* 141, 61–73
64 Seitz, L. C., Tang, K., Cummings, W. J. and Zolan, M. E. (1996) *Genetics* 142, 1105–1117
65 Kerrebrock, A. W., Moore, D. P., Wu, J. S. and Orr-Weaver, T. L. (1995) *Cell* 83, 247–256
66 Lengauer, C., Kinzler, K. W. and Vogelstein, B. (1997) *Nature* 386, 623–627
67 Cahill, D. P. *et al.* (1998) *Nature* 392, 300–303
68 Angell, R. (1997) *Am. J. Hum. Genet.* 61, 23–32

Bacterial solutions to the iron-supply problem

Volkmar Braun and Helmut Killmann

The insolubility of Fe^{3+} necessitates special mechanisms for iron acquisition in most organisms. Bacteria use siderophores to chelate Fe^{3+} and iron in heme, hemoglobin, transferrin and lactoferrin, and employ novel mechanisms for receptor-dependent iron transport and iron-regulated gene expression. These mechanisms involve transfer of energy from the cytoplasmic membrane to the outer membrane to drive active transport and might induce transcription of transport genes by transmitting a signal from the cell surface.

IRON PLAYS A central role in many redox enzymes that function in electron-transport chains of intermediary metabolism. It is particularly suited to participating in a wide range of electron-transfer reactions because, depending on the ligand and protein environment, the redox potential of Fe^{3+}/Fe^{2+} spans +300 mV to −500 mV. Although iron is abundant in nature, the extremely low solubility (10^{-18} M) of Fe^{3+} at pH 7 means that most organisms face the problem of obtaining enough iron from their environment. After absorption of iron through the gut, humans use proteins to keep iron in solution: transferrin is the iron carrier in the blood; lactoferrin is the carrier in secretory fluids; and ferritin stores iron within cells. Disturbances of iron metabolism result in severe diseases, such as congenital hemochromatosis.

Several iron-supply strategies are available to bacteria. Under anaerobic conditions, Fe^{2+} is present. It is sufficiently soluble that it can be taken up by anaerobic bacteria without the help of iron chelators. Similarly, acid-tolerant bacteria might find enough Fe^{3+}, which has a solubility of 10^{-8} M at pH 3, to cover their iron needs. Alternatively, bacteria (and fungi) synthesize a wide variety of low-molecular-weight iron ligands that are called siderophores. These form three major structural types: hydroxamates, catecholates and α-hydroxycarboxylates[1]. The siderophores, as in the cases of the transferrins and lactoferrins, bind Fe^{3+} very strongly; the free Fe^{3+} concentration is in the order of 10^{-24} M (the calculated pM value $\{-\log[M(H_2O)_n]^{m+}$, where M is the metal ion, m the metal valence and n the number of water molecules bound$\}$ for 1 μM Fe^{3+} and 10 μM ligand at pH 7.4)[2].

Certain pathogenic bacteria employ iron sources that occur in their hosts, and do not use siderophores. For example, *Serratia marcescens*, *Yersinia enterocolitica*, *Yersinia pestis*, *Shigella dysenteriae*, *Escherichia coli* O157 and *Vibrio cholerae* use heme; *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Haemophilus influenzae* use hemoglobin – both alone and bound to haptoglobin; *H. influenzae* also uses heme bound to hemopexin, and *Neisseria* and *H. influenzae* also use iron bound to transferrin and lactoferrin (Fig. 1)[3–5]. We do not know how iron is released from the iron proteins or whether it is transported in free or complexed form into the bacteria. A protein that is secreted into the culture medium of *S. marcescens* releases heme from hemoglobin[6], and heme bound to hemopexin is released by a secreted protein of *H. influenzae* type b (Ref. 3).

Initial binding of Fe^{3+} chelates to receptor proteins

Fe^{3+} siderophores bind to highly specific receptor proteins and are then transported into the cytoplasm (Fig. 1). In Gram-positive bacteria, which lack an outer membrane, the receptors are bind-

V. Braun and **H. Killmann** are at Lehrstuhl Mikrobiologie/Membranphysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany. Email: volkmar.braun@mikrobio.uni-tuebingen.de

0968–0004/99/$ – See front matter PII: S0968-0004(99)01359-6

ing proteins that are anchored to the cytoplasmic membrane by a covalently linked lipid of the type originally identified in the murein lipoprotein[7]. The binding proteins deliver the siderophores to ATP-binding cassette (ABC) importers in the cytoplasmic membrane that display striking structural similarities to eukaryotic ABC exporters, such as the human multiple-drug-resistance P glycoprotein[8].

In Gram-negative bacteria, access to the binding proteins is prevented by the outer membrane, which forms a permeability barrier for substrates of >600 Da. Fe^{3+} proteins, and most Fe^{3+} siderophores, are too large to be accommodated by the water-filled channels formed by porin proteins. In addition, under most environmental conditions, the concentrations of the Fe^{3+} chelates are too low for diffusion to meet the cell's iron requirements (10^5–10^6 ions per bacterial cell per generation). Receptors in the outer membrane therefore bind the Fe^{3+} chelates, and transport Fe^{3+} and chelated Fe^{3+} actively. Binding to these receptors, which have K_D values for Fe^{3+} siderophores of <0.1 μM, concentrates the chelates at the cell surface; Fe^{3+} siderophores, heme and iron released from transferrin and lactoferrin are then taken up into the cells. Most receptors involved in iron transport in *E. coli* also serve as binding sites for phages and allow entry of toxic proteins and peptides[3]. They are therefore attractive membrane proteins to study. The FhuA protein, for example, transports ferrichrome and the structurally related antibiotic albomycin, and binds the phages T1, T5, ϕ80 and UC-1, colicin M and microcin 25 (Ref. 3).

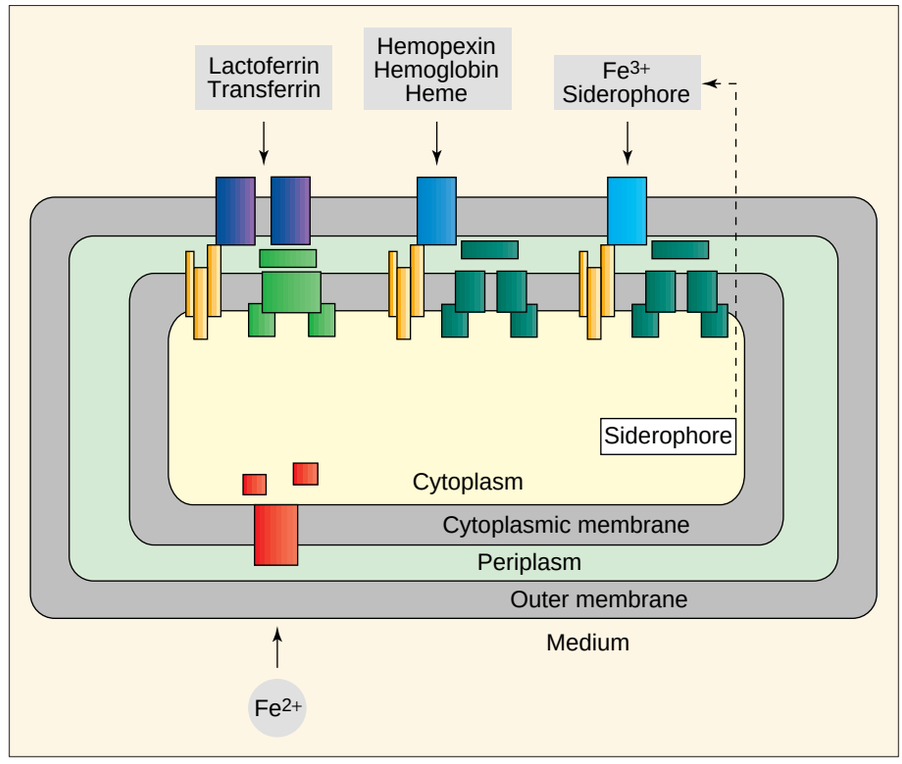

Figure 1

The Fe^{3+}-uptake systems of Gram-negative bacteria. Fe^{3+} is delivered by lactoferrin or transferrin, heme or heme bound to hemoglobin or hemopexin of the host organisms, or siderophores synthesized by the transporting strains or provided by other bacteria and fungi. The receptor proteins (shown in blue) transport iron/iron–ligand across the outer membrane: TbpA–TbpB (shown in darker blue) transports Fe^{3+}; HasR (shown in mid-blue) transports heme; and FhuA (shown in lighter blue) transports ferrichrome. Active transport across the outer membrane is energized by the proton gradient across the cytoplasmic membrane. Presumably, conformational energy is transferred from the cytoplasmic membrane to the outer membrane by a protein complex consisting of the TonB, ExbB and ExbD proteins (shown in yellow). In the periplasm, Fe^{3+}, heme or Fe^{3+} siderophores bind to binding proteins – for example, FhuD (see Fig. 2) – that are specific for Fe^{3+}, heme or Fe^{3+} siderophores of the hydroxamate, catecholate or hydroxycarboxylate type. The binding proteins deliver the iron compounds to transport proteins in the cytoplasmic membrane (shown in green); these transport proteins – for example, FhuB (Fig. 2) – translocate the iron compounds into the cytoplasm at the expense of ATP. The respective ATPases are associated with the inside of the cytoplasmic membrane. Fe^{2+} presumably diffuses across the outer membrane and is actively transported across the cytoplasmic membrane by proteins (shown in red).

Transport across the outer membrane by regulated channels

We obtained evidence for the existence of channels in outer-membrane receptor proteins by producing a FhuA mutant that lacked a 34-residue fragment. This altered receptor, FhuAΔ322–355, inserts into the outer membrane of *E. coli* and renders cells sensitive to SDS, bacitracin and novobiocin, to which wild-type cells, at the inhibitor concentrations used, are not sufficiently permeable for growth to be inhibited. Furthermore, in artificial lipid bilayers, FhuAΔ322–355 causes a stepwise increase in the conductance of the membranes, which is caused by the formation of single channels. The results of these studies suggest that FhuA contains a closed channel that has an inner diameter at least three times as large as the diameter of the porin channels[9]. Wild-type FhuA, by contrast, forms no channels[9]. Lucienne Letellier[10] and coworkers have obtained *in vivo* data that strongly support our findings. They showed that addition of phage T5 to wild-type FhuA that was integrated in lipid-bilayer membranes opens channels that display electrophysiological properties very similar to those of the channels formed by FhuAΔ322–355 (Ref. 10); the phage DNA enters liposomes[11], and internal ferrichrome diffuses out of the liposomes[12]. The peptide fragment deleted in FhuAΔ322–355 is exposed to the external surface: insertion of peptides into this region renders FhuA sensitive to proteases added at the cell surface; studies of biotin labeling, monoclonal-antibody binding, and adsorption of T1, T5 and ϕ80 phages support the same conclusion. Peptides identical in sequence to three regions of the loop inactivate the phages by triggering release of phage DNA[3].

Very recently, two groups[13,14] independently determined the crystal structure of FhuA at atomic resolution. The three-dimensional structure of the protein is novel. Residues 160–714 form a β-barrel composed of 22 antiparallel β-strands. The β-barrel is closed by the N-terminal region of FhuA (up to residue 159), which enters the β-barrel from the periplasmic side and has been termed the cork[13]. The crystal structure shows that the loop that forms the phage-binding site is indeed located at the cell surface. Binding of ferrichrome occurs at a site above the external outer-membrane interface, and triggers a small (1.7 Å) conformational

change close to the ferrichrome-binding site and a large conformational change in the periplasmic pocket of FhuA (which includes unwinding of a short helix and bending of the residues 180° to the former helix axis, and movement of Glu19 17.3 Å from its former α-carbon position). However, ferrichrome binding does not open the channel (see below). The crystal structure does not reveal a transport mechanism for ferrichrome, phage DNA or colicin M. One can envisage a small structural change that allows ferrichrome translocation through the channel. For transport of phage DNA and colicin M through the channel, most of the cork would have to move out of the β-barrel, which would consume much energy.

P. Klebba, M. McIntosh and co-workers have performed similar studies to ours, using the FepA protein – the receptor for Fe^{3+} enterobactin and colicins B and D. Deletion of residues 202–340 converts FepA to an open channel[15]. Despite this large deletion, FepAΔ202–340 inserts into the outer membrane and renders cells sensitive to SDS and antibiotics. It also increases the permeability of liposomes to stachyose (which has a mass of 666 Da) and ferrichrome (which has a mass of 749 Da). As in FhuA, this region of FepA is exposed at the cell surface. The crystal structure of FepA has been determined[16] and is very similar to that of FhuA. All these proteins, therefore, most probably form closed channels that have similar structures and open for the transport of Fe^{3+} siderophores, heme or iron.

Energy input into outer-membrane transport

Release of the strongly bound Fe^{3+} siderophores, heme and iron from receptors, and their subsequent transport, requires energy. In addition, cells must control the permeability of the outer membrane because large, permanently open channels would make the cells vulnerable to toxic agents. Controlled opening of the channels and/or closing of the channels also requires energy. However, no energy source in the outer membrane has been identified; in fact, the proton motive force across the cytoplasmic membrane drives transport through the outer membrane. Three proteins, TonB, ExbB and ExbD (the Ton system), are involved in transfer of energy from the cytoplasmic membrane to the outer membrane (Fig. 1). TonB and ExbD are located in the periplasm, and their N-termini are anchored to the cytoplasmic membrane. ExbB spans the cytoplasmic membrane three times; its N-terminus is in the periplasm, and most of the protein lies in the cytoplasm[17]. It is not known how the three proteins respond to the proton gradient across the cytoplasmic membrane or how the intermembrane energy transfer is achieved. The Ton complex somehow responds to the proton gradient, and TonB might assume an energized conformation that opens the receptor channels[17–20]. Interactions between TonB and outer-membrane receptors have been demonstrated. All TonB-dependent receptors contain a motif known as the TonB box proximal to the N-terminus. Various point mutations in the TonB boxes of a variety of receptors can be suppressed by Glu160→Leu/Lys TonB mutants[17,20]. This suggests that the TonB box interacts with the region around residue 160 of the TonB protein. The crystal structures of FhuA and FepA indicate that the TonB boxes are located in the periplasm, where they can interact with TonB.

Electron-spin-resonance spectroscopy has revealed that structural changes in FepA occur upon binding of Fe^{3+}–enterobactin to FepA and subsequently during transport. Jiang[21] resolved the two steps by showing that binding of Fe^{3+}–enterobactin to unenergized cells and *tonB* mutants, and transport in energized *tonB*$^+$ cells, resulted in different spin-label motions.

Iron bound to transferrin is delivered to *Neisseria* and *Haemophilus* species by the TbpA and TbpB outer-membrane proteins (Fig. 1). TbpA contains a TonB box in the N-terminus and therefore probably responds to the proton gradient across the cytoplasmic membrane. TbpA prevents degradation of TbpB by trypsin, which indicates that TbpA interacts with TbpB. The interaction between TbpA and TbpB is affected by the proton gradient: the trypsin sensitivity of TbpB increases significantly when energy transfer from the cytoplasmic membrane to the outer-membrane receptors is impaired (e.g. in TbpA TonB-box mutants and in strains that lack the TonB and ExbB activities). In addition, the affinity of TbpA for transferrin is higher (in the subnanomolar range) in *exbB* and *tonB* mutants than in the wild type[22]. These data indicate that energy-dependent conformational changes occur in both transferrin-mediated-iron-transport proteins; these conformational changes reduce the binding affinity of TbpA–TbpB for transferrin and might facilitate transfer of iron from transferrin to TbpA–TbpB, which then transport iron into the periplasm.

Transport across the cytoplasmic membrane

In contrast to transport across the outer membrane, transport of Fe^{3+} siderophores, heme and iron across the cytoplasmic membrane is driven by ATP hydrolysis. The most common substrate-transport mechanism in bacteria – through ABC transporters – is used for all iron sources[3,23–25]. Iron, Fe^{3+} siderophores and heme bind to proteins that deliver the iron compounds to integral cytoplasmic-membrane proteins. Deletion of the genes that encode these binding proteins inactivates transport. Ferrichrome binds to the *E. coli* periplasmic protein FhuD, renders FhuD resistant to proteolysis and changes its tryptophan fluorescence[3]. An ABC transporter that is encoded by the *sfuABC* genes and transports apparently unchelated Fe^{3+} across the cytoplasmic membrane was first characterized in *Serratia marcescens*[3]. Proteins encoded by homologues of *sfuABC* were then shown to transport iron into *N. gonorrhoeae* (the *fbpABC* genes), *H. influenzae* (the *hitABC* genes), *Y. enterocolitica* (the *yfuABC* genes), and *Actinobacillus pleuropneumoniae* (the *afuABC* genes)[3]. Unchelated iron appears to be able to bind reversibly to the periplasmic FbpA protein of *N. gonorrhoeae* and to HitA of *H. influenzae*[24]. Bacterial periplasmic iron-binding proteins and human iron-binding transferrin display remarkably similar structures. The crystal structure of the HitA protein (also known as hFBP)[28] is similar to one lobe of transferrin, and the iron ligands – two tyrosines, a histidine, a carboxylate group, and a carbonate or phosphate group – are the same[24,27].

In vitro studies of reconstituted maltose- and histidine-transport systems have demonstrated that substrate-loaded binding proteins trigger ATP hydrolysis[25]. Such a mechanism prevents wasteful ATP hydrolysis when no transport substrates are present. Triggering of ATP hydrolysis implies that substrate-loaded binding proteins contact the ATPases directly or that a signal is transmitted through the integral transport protein from the periplasm to the cytoplasm. Given that ATP is provided in the cytoplasm, the ATP-binding sites of the ATPases must be exposed to the cytoplasm. Are the ATPases integrated in the cytoplasmic membrane, and do they contact the substrate-loaded binding proteins when the latter are bound to the integral transport proteins?

The FhuC ATPase of the ferrichrome transport system is found in the membrane fraction and can be localized to

the cytoplasmic membrane by electron microscopy[3] (Fig. 2). To identify sites at which the periplasmic FhuD protein contacts the integral FhuB membrane protein of the ferrichrome-transport system, we[26] examined the binding of peptides identical in sequence to segments of FhuB to FhuD. The results indicated that a few distinct regions of FhuB interact with FhuD (shown in red and as red asterisks in Fig. 2). These regions correspond to segments exposed to the periplasm and, unexpectedly, to transmembrane segments and the cytoplasmic adjoining regions[26]. Region 7 is probably a binding site for the FhuC ATPase (Ref. 23). Figure 2 emphasizes the transmembrane topology of FhuB, as derived from mapping by using FhuB fragments fused to β-lactamase[27]. In reality, the loops probably fold back into the FhuB structure; they might be part of an FhuB channel into which FhuD inserts from the outside, and FhuC inserts from the inside. In such a model, FhuD comes into close contact with FhuC and might activate FhuC through a direct interaction. Recent three-dimensional structural data for the multiple-drug-resistance P glycoprotein support this model. The structure of the P glycoprotein is analogous to that of a single polypeptide that contains FhuB and two copies of FhuC. Electron microscopy and single-particle image analysis of the P glycoprotein have revealed the existence of an open cylinder that has an inner diameter of ~10 nm and is closed at the cytoplasmic face of the membrane[8]. If FhuB adopts a similar structure, then the cylinder presumably accommodates a portion of FhuD – given that FhuD very probably displays a structure similar to that of HitA, which has external dimensions of 3.2 nm × 4 nm × 5.8 nm (Ref. 28, and D. McRee, pers. commun.).

Transport of Fe^{2+} across the cytoplasmic membrane

Transport of Fe^{2+} differs from transport of Fe^{3+}. The first molecular evidence for an Fe^{2+}-transport system was obtained in *E. coli*. The *feoB* gene encodes an 84-kDa cytoplasmic-membrane protein that contains an ATP-binding motif; transport of Fe^{2+} might be coupled to ATP hydrolysis. Two other genes in the operon, *feoA* and *feoC*, encode small (<10 kDa) proteins, whose functions are unknown[3]. Fe^{2+} can also be transported by an Mg^{2+}-transport system, which also accommodates other divalent cations[29].

Regulation of iron transport

The iron content of cells must be regulated to conserve energy and substrates, and to avoid iron toxicity. The easy one-electron conversion of Fe^{2+} to Fe^{3+} results in the formation of oxygen radicals, of which the hydroxyl radical is the most active radical in the oxidative destruction of DNA, lipids and proteins. Synthesis of iron-transport proteins and siderophore-biosynthesis enzymes is repressed by iron. Under iron-depleted growth conditions, synthesis can increase 30-fold. The Fur protein present in Gram-negative and certain Gram-positive bacteria and the DtxR protein in Gram-positive bacteria repress gene transcription when loaded with Fe^{2+} (Ref. 3). Fur and DtxR share very little sequence similarity; however, the N-terminal DNA-binding sites and the metal-binding domains might assume similar structures. Both proteins are active as dimers. Fur and DtxR regulate transcription of bacterial toxin genes, including those that encode *Serratia marcescens* hemolysin, *Shigella dysenteriae* shiga toxin, *Pseudomonas aeruginosa* exotoxin A and diphtheria toxin.

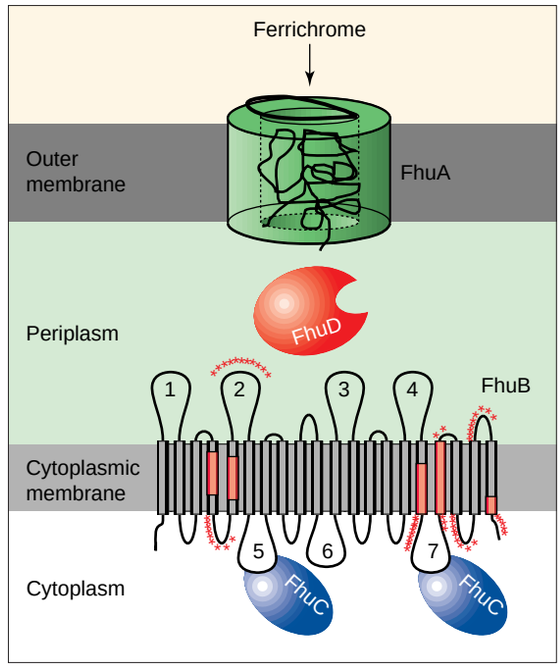

Figure 2
Arrangement of the FhuABCD transport proteins that transport ferrichrome into the cytoplasm of *Escherichia coli* K-12. FhuA forms a β-barrel that has a closed channel that is opened by interaction with the Ton system. Ferrichrome binds to the FhuD protein, which delivers ferrichrome to the FhuB protein in the cytoplasmic membrane. The sites of interaction between FhuD and FhuB, which are revealed by peptide mapping[26] (A. Mademidis, H. Killmann and V. Braun, unpublished) are shown in red and by red asterisks. Note the asymmetry of the interaction sites, and the access of FhuD to the transmembrane regions of FhuB. Loops 1–7 are not exposed (as shown in the figure) but indicate the regions localized experimentally in the periplasm and cytoplasm[27]. The FhuC ATPase (Ref. 3, and C. Dangelmayr *et al.*, unpublished) drives transport through FhuB.

Fur and DtxR are the principal iron-regulatory proteins and mainly act negatively, repressing gene transcription when cells iron requirements are met. Additional regulatory devices act positively: transcription of the transport genes is induced only when the Fe^{3+}-siderophores are available[30–32]. These more sophisticated regulatory systems still respond to iron limitation.

The best-studied example of regulation is that of citrate-mediated Fe^{3+} transport in *E. coli* K-12 (Ref. 30). Under iron-limiting growth conditions and in the presence of ferric citrate, synthesis of a ferric-citrate-transport system is induced. The ferric-citrate-transport system is a typical Gram-negative Fe^{3+}-siderophore-transport system. It consists of the outer-membrane protein FecA, the Ton energy-transfer device, the periplasmic binding protein FecB, the cytoplasmic-membrane proteins FecC and FecD, and the FecE ATPase. FecA plays a dual role: it transports ferric citrate across the outer membrane into the periplasm, and it is required for initiation of *fecABCDE* gene transcription. Initiation of transcription does not require transport of ferric citrate into the cytoplasm: mutants that cannot transport iron across the cytoplasmic membrane are fully inducible; however, mutants of any of the *fecA*, *tonB*, *exbB* and *exbD* genes involved in transport across the outer membrane are no longer inducible. This does not reflect the need to transport ferric citrate into the periplasm: missense mutations in *fecA* that lead to constitutive induction of *fec*-transport-gene transcription but do not transport ferric citrate have been isolated. FecA contains an extended N-terminus of 40 residues that is not present in other Fe^{3+}-siderophore receptors of *E. coli* K-12. Deletion of this portion of FecA blocks induction but does not affect transport. These results indicate that the transport activity of FecA is not required for induction and imply that ferric citrate

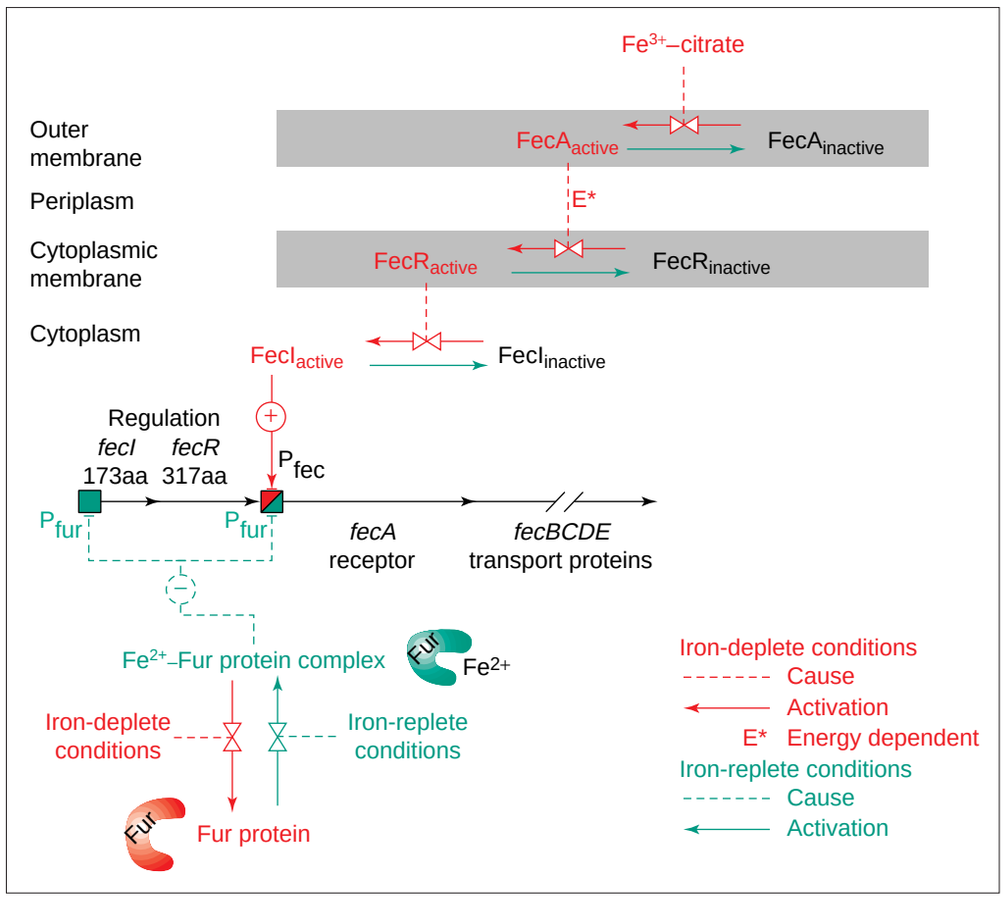

Figure 3

Model of transmembrane signaling for transcriptional control of the ferric-citrate-transport system. The FecA protein is the first component in the signaling cascade, which starts at the cell surface. Binding of ferric citrate to FecA induces a conformation such that FecA interacts with FecR, which in turn converts FecI into an active sigma factor that directs the RNA polymerase to the P_{fec} promoter and initiates transcription of the *fecABCDE* transport genes. When the iron supply is sufficient, the Fe^{2+}-loaded Fur repressor inhibits transcription of the *fecIR* regulatory genes and of the *fecABCDE* transport genes. P_{fur} denotes the promoters to which the Fe^{2+}-loaded Fur repressor binds. aa, amino acids encoded.

need only bind to FecA to induce transcription from the cell surface.

If FecA transmits information across the outer membrane, how does this information reach the cytoplasm, where *fecABCDE* transcription takes place? Two regulatory genes lie upstream of the *fec* transport genes (Fig. 3). The *fecR* gene encodes a protein that spans the cytoplasmic membrane once and, thus, could transmit information across it. Cells that synthesize only the N-terminal portion of FecR, which is located in the cytoplasm, constitutively transcribe the *fec* transport genes. This N-terminal fragment might assume the conformation that it adopts in the complete FecR protein upon ferric-citrate induction.

FecR does not interact with DNA; rather, it activates FecI, the second Fec regulatory protein. FecI is a sigma factor and, when activated, binds to a promoter upstream of the *fecA* gene (Fig. 3). FecI belongs to a subfamily of sigma factors [the extracytoplasmic function (ECF) sigma factors] that sense a signal generated at or outside the cytoplasmic membrane. FecI seems to regulate only *fec* transport-gene transcription.

The level of intracellular iron regulates, through Fur, transcription of the *fecR* and *fecI* genes and, in addition, regulates transcription of the *fec* transport genes directly[30]. This is an economic method of gene regulation because the regulatory proteins are synthesized only when iron is needed. When sufficient levels of iron are present in the cells, transcription of transport genes is repressed immediately by Fur; cells do not wait until the inducing proteins are diluted out during growth.

Studies of *Pseudomonas putida* have provided evidence for the presence of a regulatory system similar to the ferric-citrate regulatory mechanism, but the system has not been characterized in detail[32]. *Pseudomonas aeruginosa* also encodes at least two genes that are homologous to *fecI* and *fecR*. Regulation of Fe^{3+}-siderophore biosynthesis and transport can also proceed by other, as yet incompletely characterized, mechanisms[32]. The most-complex regulation yet observed is present in *Vibrio anguillarum* and involves a leucine-zipper protein and an antisense RNA[31].

Outlook

The transfer of energy from the cytoplasmic membrane to the outer membrane in order to drive active transport across the outer membrane is a challenging scientific problem and has practical applications. The antibiotic albomycin, a structural analogue of ferrichrome, is actively transported through the outer membrane and the cytoplasmic membrane. β-Lactam antibiotics, such as penicillin, which need enter only the periplasm to inhibit murein biosynthesis can be actively transported across the outer membrane when they are covalently linked to Fe^{3+} siderophores[33]. Active transport decreases the minimum inhibitory concentration by a factor of several hundred (in comparison to that of unmodified antibiotics, which enter by diffusion). The crystal structures of outer-membrane iron receptors provide the structural basis for performing experiments aimed at understanding the way that the receptors function in iron, heme and Fe^{3+}-siderophore transport, release of iron from transferrin and lactoferrin, transduction of regulatory signals from the surface to the cytoplasm, infection by phages, and uptake of phage DNA and bacterial protein toxins. Iron transport across the cytoplasmic membrane provides important insights to our understanding of the function of ABC transporters. An understanding of the transport mechanisms might also provide the basis for chemotherapeutic interference with the growth of pathogenic bacteria for which iron represents a scarce nutrient. Iron-transport mutants might provide attenuated vaccine strains for the presentation of homologous and heterologous antigens.

Acknowledgements

We thank the Deutsche Forschungs-

gemeinschaft (SFB 323 project B1, Schwerpunktsprogramm 'Molekulare Analyse von Regulationsnetzwerken in Bakterien' and Graduiertenkolleg 'Mikrobiologie') and the Fonds der Chemischen Industrie for support, Wolfgang Köster, Athanasios Mademidis and Stefan Plantör for help in designing figures, and Uwe Stroeher and Karen A. Brune for critical reading of the manuscript.

References

1 Drechsel, H. and Winkelmann, G. (1997) in *Transition Metals in Microbial Metabolism* (Winkelmann, G. and Carrano, C. J., eds), pp. 1–49, Harwood Academic
2 Raymond, K. N., Müller, G. and Matzanke, B. (1984) *Top. Curr. Chem.* 123, 249–302
3 Braun, V., Hantke, K. and Köster, W. (1998) in *Metal Ions in Biological Systems. Iron Transport and Storage in Microorganisms, Plants and Animals* (Vol. 35) (Sigel, A. and Sigel, D., eds), pp. 67–145, Marcel Dekker
4 Gray-Owen, S. D. and Schryvers, A. B. (1996) *Trends Microbiol.* 4, 185–191
5 Genco, C. and Desai, P. J. (1996) *Trends Microbiol.* 4, 179–184
6 Letoffe, S., Redeker, V. and Wandersman, C. (1998) *Mol. Microbiol.* 179, 3572–3579
7 Schneider, R. and Hantke, K. (1993) *Mol. Microbiol.* 8, 111–121
8 Rosenberg, M. F., Callaghan, R., Ford, R. C. and Higgins, C. F. (1997) *J. Biol. Chem.* 272, 10685–10694
9 Killmann, H., Benz, R. and Braun, V. (1993) *EMBO J.* 12, 3007–3016
10 Bonhivers, M., Ghazi, A., Boulanger, P. and Letellier, L. (1996) *EMBO J.* 15, 1850–1856
11 Plancon, L., Chami, M. and Letellier, L. (1997) *J. Biol. Chem.* 272, 16868–16872
12 Letellier, L., Locher, K., Plancon, L. and Rosenbusch, J. P. (1997) *J. Biol. Chem.* 272, 1448–1445
13 Ferguson, A. *et al.* (1998) *Science* 282, 2215–2220
14 Locher, K. P., Rees, B., Koebnik, R., Mitschler, A., Moulinier, L., Rosenbusch, P. and Moras, D. (1998) *Cell* 95, 771–778
15 Rutz, J. M. *et al.* (1992) *Science* 258, 471–475
16 Buchanan, S. *et al.* (1999) *Nat. Struct. Biol.* 6, 56–63
17 Braun, V. (1995) *FEMS Microbiol. Rev.* 16, 295–307
18 Postle, K. (1993) *J. Bioenerg. Biomembr.* 25, 591–601
19 Moeck, G. S. and Coulton, J. W. (1998) *Mol. Microbiol* 28, 675–681
20 Kadner, R. J. (1990) *Mol. Microbiol.* 4, 2027–2033
21 Jiang, X. *et al.* (1997) *Science* 276, 1261–1264
22 Cornelissen, C. A., Anderson, J. E. and Sparling, P. F. (1997) *Mol. Microbiol.* 26, 25–35
23 Köster, W. (1991) *Biol. Metals* 4, 23–32
24 Mietzner, T. A. *et al.* (1998) *Curr. Top. Microbiol. Immun.* 225, 113–135
25 Boos, W. and Lucht, J. M. (1996) in *Escherichia coli and Salmonella typhimurium* (Neidhardt, F.C., ed.), pp. 1175–1209, ASM Press
26 Mademidis, A. *et al.* (1997) *Mol. Microbiol.* 26, 1109–1123
27 Groeger, W. and Köster, W. (1998) *Microbiology* 144, 2759–2769
28 Bruns, C. M. *et al.* (1998) *Nat. Struct. Biol.* 4, 919–924
29 Hantke, K. (1997) *J. Bacteriol.* 179,

Mechanisms of lipid-body formation

Denis J. Murphy and Jean Vance

Most organisms transport or store neutral lipids as lipid bodies – lipid droplets that usually are bounded by specific proteins and (phospho)lipid. Neutral-lipid bodies vary considerably in their morphology and are associated with an extremely diverse range of proteins. However, the mechanisms by which they are generated in plants, animals and microorganisms appear to share many common features: lipid bodies probably arise from microdomains of the endoplasmic reticulum (or the plasma membrane in prokaryotes) that contain lipid-biosynthesis enzymes, and their synthesis and size appear to be controlled by specific protein components.

LIPID-BODY FORMATION occurs at some point in the life cycle of nearly all organisms and is an integral part of energy storage and/or transport in most eukaryotes. Malfunctions in neutral-lipid storage are implicated in several serious human diseases, such as fatty liver, obesity, atherosclerosis and type 2 diabetes. There is also considerable biotechnological interest in manipulation of lipid storage for both medical and agricultural purposes. Storage and transport lipids are contained in spheroidal droplets, which can range in diameter from 0.1 μm to 50 μm. The neutral lipids contained in these droplets are primarily triacylglycerol (TAG), diacylglycerol (in some insect tissues) and cholesteryl esters. Despite its evident importance, however, there have, until recently, been surprisingly few studies of the fundamental mechanism(s) of lipid-body biogenesis. During the past few years, this situation has begun to change, and several common themes are now emerging. Here, we compare recent findings from a range of cell types from plants, animals and microorganisms (see Table 1).

D. J. Murphy is at the Dept of Brassica and Oilseeds Research, John Innes Centre, Norwich Research Park, Norwich, UK NR4 7UH; and **J. Vance** is at the Lipid and Lipoprotein Research Group and Dept of Medicine, 315 HMRC, University of Alberta, Edmonton, Alberta, Canada T6G 2S2. Email: denis.murphy@bbsrc.ac.uk

Plants

Seeds and fruits. Intracellular storage-lipid bodies in plants are particularly abundant in oil-rich fruit and seed tissues, which can contain as much as 50–75% (w/w) lipid. As in animals, such cytosolic lipid bodies are believed to arise from specific microdomains of the endoplasmic reticulum (ER) membrane that contain the full complement of TAG-biosynthesis enzymes[1] (Fig. 1). Data from several labeling studies suggest that these enzymes channel intermediates towards lipid-body formation and, hence, segregate them from the bulk lipid-bilayer components[2].

Lipid bodies from all desiccation-tolerant seeds analysed to date are bounded by a continuous surface layer of unique amphipathic proteins, which are termed oleosins[2]. Recent evidence suggests that oleosins are cotranslationally inserted into the ER membrane and have an unusual topology: both termini are directed towards the cytoplasm. Site-directed mutagenesis studies have shown that targeting of oleosin to lipid bodies is regulated by the protein's characteristic hydrophobic central domain and, in particular, by a triple-proline knot motif[3]. Recent studies on the ectopic expression of oleosin in

0968–0004/98/$ – See front matter PII: S0968-0004(98)01349-8