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Review

# Bacterial cleanup: lateral diffusion of hydrophobic molecules through protein channel walls

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## Abstract

The outer membrane (OM) of Gram-negative bacteria forms a very efficient barrier against the permeation of both hydrophilic and hydrophobic compounds, owing to the presence of lipopolysaccharides on the outside of the cell. Although much is known about the OM passage of hydrophilic molecules, it is much less clear how hydrophobic molecules cross this barrier. Members of the FadL channel family, which are widespread in Gram-negative bacteria, are so far the only proteins with an established role in the uptake of hydrophobic molecules across the OM. Recent structural and biochemical research has shown that these channels operate according to a unique lateral diffusion mechanism, in which the substrate moves from the lumen of the barrel into the OM via an unusual opening in the wall of the barrel. Understanding how hydrophobic molecules cross the OM is not only of fundamental importance but could also have applications in the design of novel, hydrophobic drugs, biofuel production and the generation of more efficient bacterial biodegrader strains.

**Keywords:** biodegradation; lateral diffusion; lipopolysaccharide; outer membrane; transport.

## Introduction

The production of toxic xenobiotics ('compounds foreign to life') in modern, industrialized society is causing a large burden on the environment and is posing substantial risks for human health. The metabolization of such compounds by certain bacteria (biodegradation) and the utilization of these bacteria in the removal of xenobiotics from the environment (bioremediation) have therefore been the focus of an enormous amount of research interest. The vast majority of xenobiotics are hydrophobic, which contributes to the persistence of these compounds within the environment.

Although the intracellular fates of many xenobiotics (e.g., mono- and poly-aromatic hydrocarbons) are well established, it was until recently completely unclear how such compounds enter bacterial cells, an obvious prerequisite for their biodegradation. In the case of Gram-negative bacteria, to which many biodegrading bacteria belong (e.g., *Pseudomonas* species), the outer membrane (OM) forms an efficient permeability barrier for hydrophobic molecules, largely preventing the spontaneous diffusion of hydrophobic compounds (1). This is due to the composition of the outer leaflet of the OM, which consists almost exclusively of lipopolysaccharides (LPS), which are glycolipids composed of lipid A with attached sugars. LPS have two properties that make the OM a particularly efficient barrier. First, the lipid A moiety has a large number of acyl chains (typically 5–7 depending on the organism), resulting in tight packing and giving the OM a wax-like rather than a fluid character that is typical for regular phospholipid bilayers. Second, the many sugar molecules that are attached to lipid A contain phosphate and carboxyl groups that are crosslinked by divalent metal ions, creating a substantial (>25 Å thick) polar barrier on the outside of the cell (1, 2).

For the cell to acquire the nutrients necessary for growth and function there are a large number of channels within the OM of Gram-negative bacteria (1). Most of these channels, however (e.g., porins), are water-filled conduits that do not allow efficient passage of hydrophobic compounds such as long chain fatty acids (LCFAs) and xenobiotics destined for biodegradation. Therefore, specialized channels are required for uptake of such molecules. So far, channels of the FadL family (3) are the only proteins with an established involvement in the uptake of hydrophobic molecules across the OM (4). How the uptake of hydrophobic molecules across the OM occurs is not only a fundamental problem in biology but it also has implications for biodegradation and bioremediation, as well as for the design of potential novel hydrophobic antibacterial drugs. In this review, I will summarize the current knowledge about the structure and transport mechanism of FadL channels, with an emphasis on channels dedicated to the uptake of mono-aromatic hydrocarbons (MAHs) in biodegrading bacteria.

## Occurrence and function of FadL channels

FadL channels are widespread in Gram-negative bacteria and are present in α- through ε-proteobacteria. Interestingly, many bacteria have multiple (up to three) FadL orthologs in their genomes, suggesting that FadL channels can be substrate specific. The archetype of the FadL family, *Escheri-*

*chia coli* FadL (EcFadL) was discovered more than 30 years ago (5) and confers the ability to *E. coli* to grow on LCFAs as the sole source of carbon (6). In *E. coli* and related bacteria, FadL works in concert with the inner membrane-bound acyl CoA synthetase FadD to activate LCFAs in an ATP-dependent manner, a process termed vectorial acylation (7). Within the cytosol, the energy-rich long chain acyl CoAs are primarily degraded by β-oxidation, although they can also be incorporated into phospholipids. So far, the only proteins that are known to be absolutely required for LCFA transport are FadL and FadD (7). Furthermore, the transport across the OM as mediated by FadL does not seem to be dependent on external energy input and most likely occurs by diffusion.

FadL orthologs have also been described in several biodegrading bacteria, such as *Pseudomonas putida* F1 and *Ralstonia pickettii* PKO1. Such orthologs are located in chromosomal or plasmid-based operons dedicated to the degradation of xenobiotics, suggesting a role for these channels in OM transport. Direct experimental evidence implicating FadL channels in the uptake of xenobiotics has so far been very limited, however. Inactivation of the *todX* gene in *P. putida* F1 resulted in a strain that grew more slowly than wild type on toluene (8), suggesting that TodX mediates toluene entry into the cells. Likewise, the *R. pickettii* PKO1 TbuX channel has been implicated in the uptake of toluene (9) and the XylN channel was proposed to be required for the uptake of xylene in *P. putida* (10).

## Structure and transport mechanism of *E. coli* FadL

X-ray crystal structures of *E. coli* FadL, the first of any FadL channel, were reported in 2004 in two different space groups (11). As is the case for virtually all OM proteins, FadL was found to form a β-barrel, in this case consisting of 14 β-strands (Figure 1). Although FadL is a typical OM protein in some respects (e.g., regarding the presence of long extracellular loops and short periplasmic turns), it has three features that are highly unusual (11). First, the N-terminal ~40 residues form a compact domain, termed 'hatch', that plugs the lumen of the FadL barrel on the periplasmic side (Figure 1). The specific combination of a hatch and a relatively narrow, 14-stranded barrel is unique to FadL. Second, the N-terminus is located inside the barrel and occupies a position on the extracellular side of the OM. This is in marked contrast to TonB-dependent receptors, which have a large (~150 residues) N-terminal hatch domain of which the N-terminus is located in the periplasmic space and which is accessible for interaction with TonB. The third unusual structural feature of FadL is the presence of a sizeable hole in the side of the barrel wall (~6×10 Å; atom-center to atom-center distance; Figure 1E). This hole is formed due to an inward-pointing kink in one of the β-strands (S3), breaking the hydrogen bonding interactions of strand S3 with the neighboring strands S2 and S4. The kink in strand S3 interacts with the N-terminus of the hatch via a small β-sheet (Figure 1).

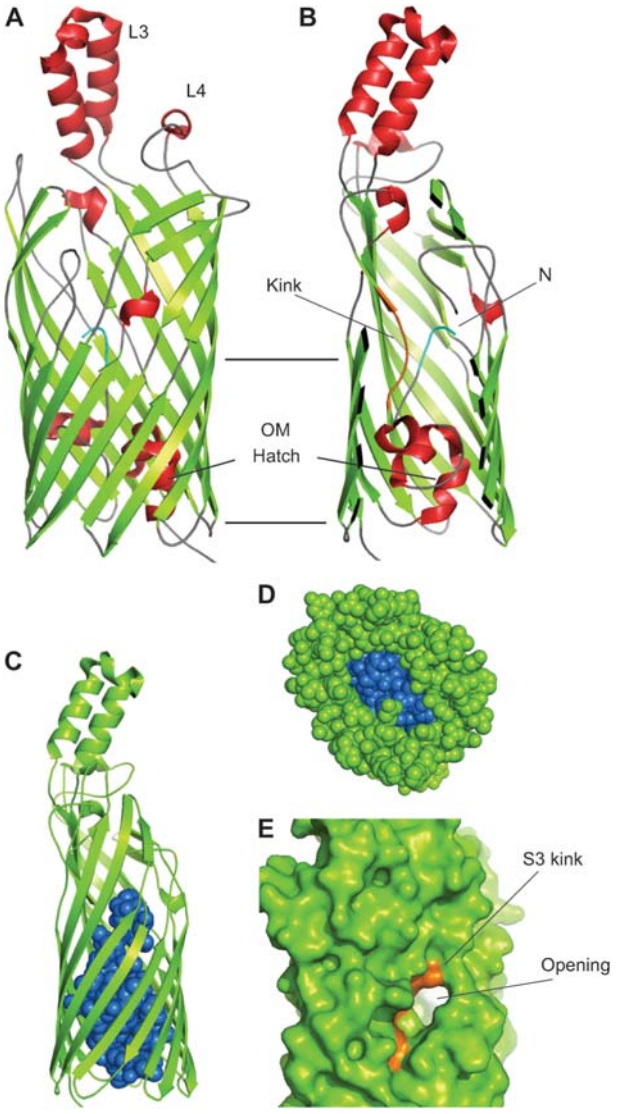


**Figure 1** Structure of *E. coli* FadL.
(A) Cartoon representation viewed from the side, with β-strands colored green, helices red and loops gray. Extracellular loops L3 and L4 are indicated. The N-terminus (residues 1–5) is colored cyan. The approximate positions of the OM interface regions are indicated by horizontal lines. (B) Cut-away view from the side, 90° rotated relative to (A). The kink in β-strand S3 (residues 99–107) is colored orange. (C) View as in (B), showing the hatch domain occupying the lumen of the barrel as a space-filling model in blue. (D) Space-filling view from the periplasmic side, demonstrating that the hatch domain fully occludes the FadL barrel. (E) Side surface view of the region surrounding the lateral opening between strands S2 and S3. All images were made with PyMOL (12).

Because LCFAs are detergents (although with very low critical micelle concentrations), it was not surprising that the FadL structures contained bound detergent molecules (used for purification and crystallization) at three different locations in the protein [(11); Figure 2A]. The first detergent binding site in FadL is a solvent-exposed hydrophobic groove located between two long extracellular loops (L3 and L4). This groove probably forms the initial interaction site

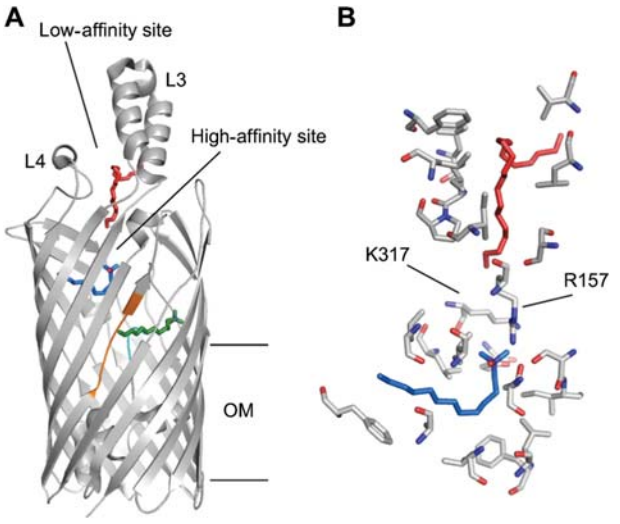


**Figure 2** Detergent binding sites in *E. coli* FadL.
(A) Overview showing a $C_8E_4$ molecule (red) in the low-affinity binding groove between loops L3 and L4, the LDAO molecule in the high-affinity binding pocket (blue), and an LDAO molecule bound in the region of the lateral opening (green). (B) Close-up of the low- and high-affinity binding sites with the residues shown (as stick models, with carbon gray, oxygen red and nitrogen blue) that are 4 Å or closer to the detergent molecules. Residues Arg157 and Lys317, probably involved in binding the LCFA carboxyl group, are indicated. The orientation is identical to that in panel (A).

of FadL with the LCFA substrates in the external milieu. The second detergent binding site is located within the lumen of the FadL barrel. This site forms a well-defined hydrophobic pocket with more than 15 hydrophobic residues lining its walls (Figure 2A and B). The detergent bound at this position (LDAO) is structurally closely related to a $C_{12}$ saturated fatty acid. LDAO was used only during the initial purification stages of the protein (11), suggesting that this site has a high-affinity for substrates. Recent intrinsic fluorescence titrations of oleate using single-Trp FadL mutants support the high-affinity of EcFadL for LCFA substrates ($K_d$ ~0.2 μM; unpublished data). Interestingly, there are several charged residues within hydrogen bonding distance to the zwitterionic head group of the LDAO molecule; of these, Arg157 and Lys317 are likely to interact with the negatively charged head group of an LCFA substrate (Figure 2B). The high-affinity binding pocket is directly connected to the surface-exposed hydrophobic groove, suggesting that the LCFA substrates diffuse from this groove into the high-affinity binding site (11). The detergents in the low- and high-affinity binding sites are observed in both EcFadL crystal structures. In addition, well-defined density for an additional detergent (LDAO) molecule is observed (only in monoclinic FadL) at the site of the lateral opening, with the detergent tail protruding from the hole in the barrel wall (Figure 2A). This observation has important mechanistic implications, because it shows that the lateral opening is large enough to function as a potential transport channel.

Considering that FadL-mediated OM transport does not require exogenous energy input (6), how would the substrate be released from the high-affinity binding site? The two original crystal structures of wild-type FadL provide a possible answer to this question. Although the structures of monoclinic and hexagonal FadL are virtually identical for most of the protein ($C_\alpha$ r.m.s.d. 0.55 Å), large differences exist for the conformation of the first seven residues [(11); Figure 3A]. In the hexagonal FadL structure, the N-terminus has undergone a translation and rotation relative to the N-terminus in monoclinic FadL (Figure 3A). Importantly, the LDAO molecule bound in the high-affinity binding site has also moved relative to its position in monoclinic FadL; specifically, the LDAO head group has shifted over a distance of more than 10 Å and is not within hydrogen bonding distance of Arg157 and Lys317 anymore. These spontaneous structural changes of the N-terminus can be interpreted as causing the release of the substrate from the high-affinity binding site. It is important to realize that crystal packing is unlikely to affect the conformation of the N-terminus,

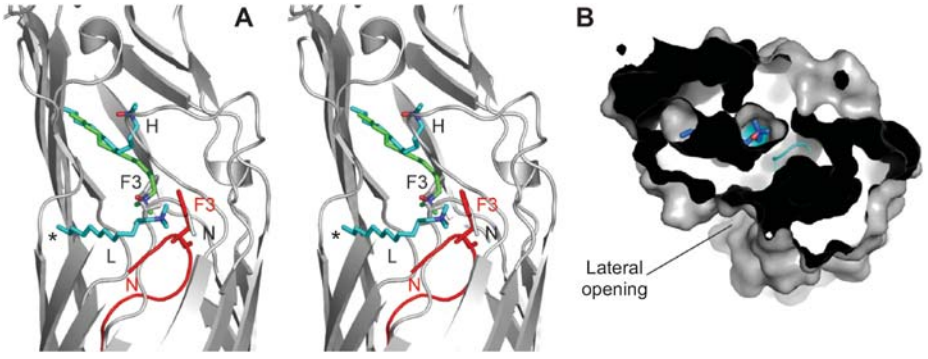


**Figure 3** Conformational changes within the N-terminus of *E. coli* FadL.
(A) Stereoview from the side, showing the different conformation of the N-terminus in monoclinic FadL and hexagonal FadL (red). Residue Phe3 is shown as a stick model and labeled. The detergent molecules present in monoclinic FadL are colored cyan (H, high-affinity binding site; L, lateral opening), and that in hexagonal FadL is colored green. The lateral opening is indicated with an asterisk. (B) Surface cutaway view of monoclinic FadL from the extracellular side, showing the interruption of the hydrophobic tunnel by the N-terminus of FadL (cyan). The detergent molecule in the high-affinity binding site is shown.

because it is buried inside the lumen of the barrel; therefore, the observed structural differences are likely to occur spontaneously.

Is the lateral opening part of the substrate transport pathway? How does the substrate get into the periplasm? Two possible models can be envisioned for FadL-mediated transport (13). The first model, termed 'lateral diffusion', is inferred from the presence of a detergent molecule in the lateral opening of the barrel and predicts a crucial role for this opening during transport. After release from the high-affinity binding site by conformational changes of the N-terminus, the substrate would diffuse laterally into the OM via the opening in the barrel wall (13). The second possible model can be considered 'classical'. Here, spontaneous conformational changes in the hatch (other than those occurring within the N-terminus) would open up a transient substrate diffusion pathway into the periplasmic space (Figure 4). The direction of transport would be perpendicular to the plane of the membrane, analogous to virtually all other membrane transport proteins (hence the term 'classical' model). To experimentally distinguish between the two possible models, a number of site-directed mutants were made, focusing on the hatch domain and the lateral opening (13). The oleate uptake characteristics of the mutants were assayed *in vivo*, using a *fadL* knockout strain with the mutant proteins expressed from an inducible plasmid. The two mutants that were designed to close the lateral opening (ΔS3 kink; E77E/S100R) were inactive for oleate transport. Importantly, the crystal structure of the E77E/S100R mutant showed it to be identical to wild-type FadL, with the sole exception of a much smaller lateral opening (~3×4 Å) in the mutant. Thus, the biochemical and structural data demonstrate that constricting the lateral opening is sufficient to block LCFA transport, providing strong support for the lateral diffusion transport model (13). Additional support for the lateral diffusion model was obtained from the structure of a *Pseudomonas aeruginosa* FadL channel (PaFadL). In this structure, three bound $C_8E_4$ detergent molecules clearly delineate a hydrophobic tunnel that runs all the way from the extracellular surface to the lateral opening in the barrel wall (13). As expected, the walls of the tunnel consist mostly (~80%) of hydrophobic amino acids. A closer inspection of PaFadL reveals that the hydrophobic tunnel, like the one in (monoclinic) EcFadL (Figure 3B), is interrupted by the N-terminal three residues (in particular Phe3), blocking access of the substrate to the lateral gate and necessitating conformational changes in the N-terminus to generate an uninterrupted passageway. Interestingly, the hydrophobic tunnel is continuous in hexagonal FadL (11), suggesting that the release of the substrate from the high-affinity binding site and formation of a continuous diffusion channel are coupled. The lateral opening in FadL channels is located in the region of the outer leaflet interface (Figure 2A). This location makes sense, because it would provide favorable environments for the hydrophilic and hydrophobic parts of an amphipathic substrate (e.g., an LCFA) upon emergence from the lateral opening.

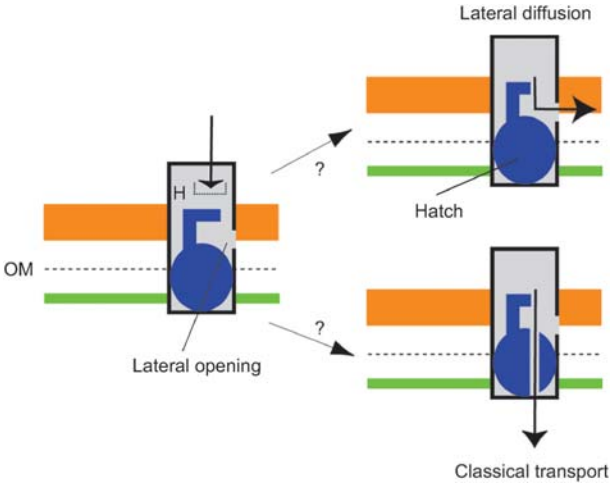


**Figure 4** Two possible transport mechanisms for FadL channels. After initial substrate recognition by the low-affinity binding groove and substrate diffusion into the adjacent high-affinity binding site (H), substrate transport can occur either via lateral diffusion or via a classical mechanism. The polar layer of the LPS, representing the principal barrier for transport, is shown in orange. See text for details.

The lateral diffusion model implies that the hatch domain does not undergo conformational changes. Indeed, the crystal structures of several hatch mutants were determined and suggest that the hatch is rigid and does not form a channel for LCFA transport (13). Owing to the composition of the hatch, any channel through this domain would be relatively polar, which would make the diffusion of LCFAs energetically unfavorable. What then could be the function of the hatch? A hatchless EcFadL mutant expresses only to very low levels in the OM, suggesting that the hatch could be important for protein folding and/or stability. The absolutely conserved NPA signature sequence of the hatch [residues 33–35 in EcFadL; (11)] could be particularly important in this respect, as NPA mutants are active in LCFA transport but show very low expression levels (13). A second probable function of the hatch is to prevent substrate diffusion directly into the periplasmic space. Instead, this domain serves as a plug to direct the substrates, via the lateral opening, into the OM. The unique architecture of FadL channels makes perfect sense when one considers that the polar part of the LPS on the outside of the cell is the sole barrier for entry of hydrophobic molecules into a Gram-negative bacterial cell. The FadL system is fundamentally different from the TonB-dependent receptors, which also have a globular domain inside the (22-stranded) β-barrel. In the latter class of proteins, energy derived from the proton-motive-force across the inner membrane is used by TonB to somehow generate conformational changes in the hatch that result in a passageway for the substrate into the periplasmic space (14).

Like all lipid bilayers, the OM is an efficient sink for hydrophobic compounds, providing the driving force for transport. Considering the position of the lateral opening, the LCFAs are likely to enter the outer leaflet of the OM. Subsequently the LCFAs will probably move to the inner leaflet of the OM by spontaneous flip-flop, a process which has

been shown to be very efficient in regular phospholipid bilayers (15). From the OM, desorption of the LCFAs into the periplasmic space will probably occur by simple mass action. Although desorption from the membrane in liposomes has been shown to become slower with longer acyl chains, it is still fast due to the high concentrations of LCFAs within the membrane (15, 16). LCFAs can cross the periplasmic space by diffusion, although it is possible that periplasmic binding proteins are involved: a previous transposon mutagenesis study has suggested that the periplasmic protein Tsp increases the efficiency of LCFA transport (17). Interestingly, although Tsp functions as a protease it has homology to retinoid-binding proteins, which are known to bind hydrophobic ligands (including LCFAs). After passage of the periplasmic space, the LCFAs partition into the inner membrane, flip-flop to the inner leaflet and are subsequently activated by fatty acyl CoA synthetase (FACS) (7). Flip-flop is much more efficient for uncharged LCFAs (15), which is probably the reason why LCFA uptake was found to be more efficient in the presence of the proton motive force (PMF) (18).

## Structures of the mono-aromatic hydrocarbon channels TodX and TbuX

The structures of TodX from *P. putida* F1 and TbuX from *R. pickettii* have been determined by X-ray crystallography (19). These channels are present in operons dedicated to the degradation of toluene and other MAHs, suggesting that they form uptake channels for these compounds (8, 9). TodX and TbuX are similar both in sequence (42% identity) and structure ($C_\alpha$ r.m.s.d. 1.4 Å). Both MAH channels have low (15–20% identity) sequence similarity to EcFadL, prompting the question as to the similarity of their structures with that of EcFadL. A structural comparison clearly shows that although the membrane-embedded parts of the channels are very similar (including the presence of a hatch/plug domain), there are large differences in the extracellular loops between FadL and both MAH channels (Figure 5A and B). The most obvious explanation for the differences in these loops is that they are the result of differences in substrate specificities. This notion is supported by the fact that the MAH channels do not transport LCFAs, the first evidence that FadL channels are indeed substrate specific (19).

Like *E. coli* FadL, the MAH channels also contain bound detergents. Interestingly, despite considerable structural differences in the proteins, the detergents are bound at similar positions in the structures of *E. coli* FadL and TodX/TbuX (Figure 5C), suggesting that the substrate diffusion pathways are similar (19). In TodX/TbuX, the residues that are close (<4.5 Å) to the detergent molecules are exclusively hydrophobic. The two basic amino acids coordinating the LDAO head group in EcFadL (R157/K317) are leucine residues in TodX/TbuX (L166/L326 in TodX), in accordance with the neutral character of their substrates. It should also be noted that no (zwitterionic) LDAO molecules are bound to TodX/TbuX; instead, only neutral $C_8E_4$ molecules are present in the MAH channels. Together, the bound detergent molecules delineate a long hydrophobic tunnel from the extracellular surface of the channels down into the lumen of the barrels, as present in the structures of all FadL channels.

## Do MAH channels transport their substrates by lateral diffusion?

Although the inward-pointing S3 kink is not as pronounced in TodX/TbuX as it is in FadL, the neighboring strand S2 in TodX/TbuX has a prominent outward bulge that is partially disordered, with the result that both TodX and TbuX have a lateral opening in the barrel at the same position as EcFadL (Figure 5D). Thus, the structures of TodX/TbuX are entirely consistent with a lateral diffusion mechanism for transport of MAHs. It should be noted that, like in most structures of FadL channels, access to the lateral opening in TodX/TbuX is blocked by the N-terminus, necessitating a conformational change to generate a continuous pathway for lateral diffusion.

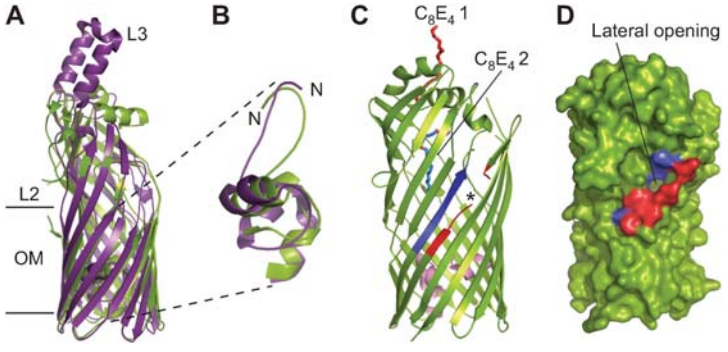


**Figure 5** Structural comparison of the toluene channels TodX and TbuX with *E. coli* FadL.
(A) Cartoon representations of *P. putida* F1 TodX (green) superimposed on *E. coli* FadL (purple). The locations of loops L2 and L3 are indicated. (B) Close-up of the hatch domains, with coloring and orientation as in (A). (C) $C_8E_4$ detergent binding in TodX (colored as in Figure 2). Strand S2 (residues 69–82) is colored red, strand S3 dark blue. The location of the lateral opening in TodX is indicated by an asterisk and the hatch domain is shown in pink. (D) Surface view of TodX showing the lateral opening in the wall of the channel.

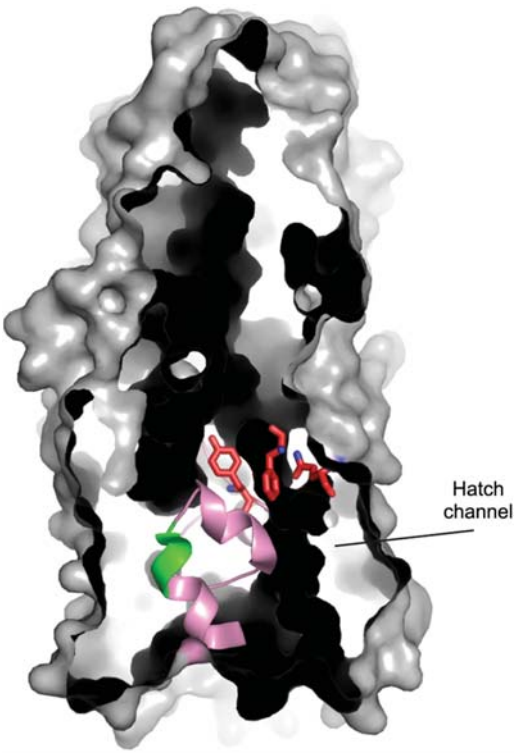


**Figure 6** Surface cut-away view of TodX showing a possible transport channel through the hatch domain.
Residues Tyr9, Gln83 and Phe100, forming the constriction of this channel, are shown as stick models (red). The location of the NPA sequence of the hatch domain (pink) is shown in green.

Strikingly, both TodX and TbuX also have a channel through the hatch domain that could be indicative of a classical transport mechanism (Figure 6). Even at the constriction (formed by residues Tyr9, Q83 and F100), this channel is wide enough to allow passage of a MAH molecule. Distinguishing which mechanism operates in the MAH channels will require development of a toluene uptake assay combined with site-directed mutagenesis as done for *E. coli* FadL. Because the *E. coli* OM is too permeable for MAHs due to the presence of porins, such an assay will need to be carried out in, e.g., *P. putida* F1, which has (i) a low-permeability OM (like other pseudomonads, *P. putida* F1 lacks porins) and (ii) the MAH degradative genes required to establish a sink to drive transport. In Gram-negative bacteria that have a low-permeability OM, channels with a classical transport mechanism could have evolved in FadL proteins dedicated to the transport of compounds that are, unlike LCFAs, relatively water-soluble [the aqueous solubility of toluene is ~5 mM, whereas that of palmitate is ~10 nM; (20)]. The diffusion of such MAH compounds through a relatively polar hatch channel would be much more favorable than for LCFAs. If a classical transport mechanism operates in a subset of FadL channels, it would be the first example of different proteins from the same family exhibiting fundamentally different transport mechanisms, underscoring the unique nature of this family of OM channels. Moreover, it is possible that in certain FadL channels both pathways are used for transport, depending on the hydrophobicity of the substrate (with more hydrophobic substrates preferentially using the lateral diffusion pathway). This would make FadL channels remarkably similar to Sec61/SecYEG protein translocation channels, where, depending on the hydrophobicity of a polypeptide segment, lateral diffusion into the inner membrane or secretion through a polar channel occurs (21).

## Outlook

The most fundamental mechanistic question to be addressed by future studies is whether lateral diffusion is employed by all FadL channels or only for those channels that transport very hydrophobic substrates such as LCFAs. The MAH channels (TodX/TbuX) provide an excellent model system to address this question, because their substrates are relatively polar and they have a channel through the hatch domain. Once a toluene uptake assay has been established in a suitable *P. putida* F1 knockout strain, answering the question which transport pathway operates should be relatively straightforward via the use of site-directed mutants with constricted/blocked hatch channels or lateral openings.

The substrate specificity of FadL channels is another area that warrants future study. Although it is clear that FadL channels are substrate specific (i.e., MAH channels do not transport LCFAs), it is not clear what the extent is of the specificity and which structural features are responsible for the specificity. Addressing these questions will require establishing *in vivo* uptake systems for the substrates to be tested, with the important constraint that a substrate-degrading machinery should be present inside the cell to provide a sink. Interesting classes of substrates are LCFAs (and analogs), alkanes, MAHs and polyaromatic hydrocarbons, most of which are also important from the point of view of biodegradation. Sequence alignments should allow identification of structurally similar subclasses of FadL channels (with likely similar specificities). In addition, focusing on FadL channels from the same organism (e.g., *P. putida* F1 having three FadL orthologs) can also be a good strategy for identification of channels with different specificities. Ultimately, the generation of site-directed mutants (including loop-swapped chimeras) should lead to the modification/alteration of the substrate specificities of FadL channels (e.g., convert TodX into a channel that transports LCFAs).

Recently, the production of biofuels from sugars in *E. coli* was reported (22). These biofuels, which are predominantly long chain ($>C_{12}$) fatty esters, fatty alcohols and waxes and are therefore very hydrophobic, are secreted into the extracellular environment by an unknown mechanism. Although it is possible that these compounds are secreted via OM vesicles (23), it is attractive to speculate that FadL channels mediate secretion of such compounds across the OM, because there is no reason *a priori* that FadL-mediated dif-

fusion would be unidirectional. Demonstrating the involvement of FadL channels in secretion of hydrophobic molecules should be relatively straightforward by generating FadL knockouts in biofuel-producing *E. coli* strains.

One potentially interesting avenue of clinical research concerns the possible importance of FadL channels during infection by pathogenic Gram-negative bacteria. FadL channel-mediated uptake of, e.g., arachidonic acid, liberated by the action of cellular phospholipases at an infection site, could provide a means for the bacteria to suppress the local immune response of the host, thereby providing an advantage for the bacteria early during infection. The involvement of FadL channels in the establishment of infection has never been tested thus far. However, upregulation of several *fad* genes has been demonstrated during *Salmonella* infection of mice (24), suggesting that FadL can be important during bacterial infections. Intriguingly, several pathogenic bacteria (e.g., *Vibrio cholerae*, *P. aeruginosa*) have three FadL orthologs, reinforcing the notion that these channels could be important for infectivity.

On a practical level, several future applications of FadL channels could be envisioned. First, it might be possible to utilize FadL channels for the delivery of novel, hydrophobic drugs directed against Gram-negative bacteria. As an example, LCFA analogs could be designed that could specifically inhibit bacterial enzymes involved in fatty acid synthesis and degradation, which are essential cellular processes. Second, it should be possible to engineer FadL channels in biodegrading bacteria to alter and/or widen substrate specificity, or to make substrate uptake more efficient (it should be noted that it is unclear whether OM passage is rate-limiting during biodegradation). Of course, the usefulness of the engineered bacteria for biodegradation would still depend on the presence of the appropriate catabolic genes inside the cytosol.

Finally, recent evidence suggests that FadL channels might not be the only OM proteins involved in the uptake of (small) hydrophobic molecules (25–27). Likely candidates for such channels are members of the OmpW family, which form small (8-stranded) β-barrels that are, like FadL channels, widespread in Gram-negative bacteria. Importantly, two X-ray crystal structures have been solved for OmpW members and they both show a lateral opening in the barrel wall at a similar position as those in FadL channels, suggesting that OmpW channels can also employ lateral diffusion for substrate uptake (28). Another example of channels involved in uptake of hydrophobic molecules could be proteins from COG4313, a group of uncharacterized OM proteins that may be involved in uptake of small aromatic compounds. One family member was very recently implicated in the uptake of polychlorophenols (29). No structural information is available for any COG4313 protein, but sequence alignments suggest that they are distinct from both OmpW and FadL channels. With more data from genome sequencing efforts becoming available, more hydrophobic transporter families could be discovered in the future. Channels that employ lateral diffusion for the uptake of hydrophobic compounds could therefore turn out to be widespread in Gram-negative bacteria.

## Highlights

- The OM of Gram-negative bacteria is an efficient barrier for hydrophobic compounds.
- Channels of the FadL family are widespread in Gram-negative bacteria and are involved in the uptake of hydrophobic molecules such as LCFAs and xenobiotics destined for biodegradation.
- Structural features unique to FadL are a plug domain occluding a 14-stranded β-barrel and a hole in the barrel wall caused by a kink in one of the β-strands. These features are crucial for the transport mechanism.
- Hydrophobic compounds are transported across the OM via lateral diffusion through the hole in the wall of the channel.
- The hatch domain in EcFadL channels prevents diffusion of hydrophobic substrates directly into the periplasmic space.
- FadL channels are substrate specific.
- Channels involved in uptake of MAHs are structurally similar to FadL yet show differences that could be related to substrate specificity.
- Compounds that are less hydrophobic than LCFAs could be transported according to a classical mechanism by FadL orthologs in bacteria with a low-permeability OM.
- In addition to FadL channels, other families of channels could be involved in the uptake of hydrophobic molecules across the OM.

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## References

1. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003; 67: 593–656.
2. Snyder S, Kim D, McIntosh TJ. Lipopolysaccharide bilayer structure: effect of chemotype, core mutations, divalent cations, and temperature. Biochemistry 1999; 38: 10758–67.
3. Busch W, Saier MH. The IUBMB-endorsed transporter classification system. Mol Biotechnol 2004; 27: 253–62.
4. van den Berg B. The FadL family: unusual transporters for unusual substrates. Curr Opin Struct Biol 2005; 15: 401–7.
5. Nunn WD, Simons RW. Transport of long-chain fatty acids by Escherichia coli: mapping and characterization of mutants in the fadL gene. Proc Natl Acad Sci USA 1978; 75: 3377–81.
6. Black PN, Said B, Ghosn CR, Beach JV, Nunn WD. Purification and characterization of an outer membrane-bound protein involved in long-chain fatty acid transport in *Escherichia coli*. J Biol Chem 1987; 262: 1412–9.

7. Black PN, DiRusso CC. Transmembrane movement of exogenous long-chain fatty acids: proteins, enzymes, and vectorial esterification. Microbiol Mol Biol Rev 2003; 67: 454–72.
8. Kasai Y, Inoue J, Harayama S. The TOL plasmid pWWO xylN gene product from *Pseudomonas putida* is involved in *m*-xylene uptake. J Bacteriol 2001; 183: 6662–6.
9. Kahng HY, Byrne AM, Olsen RH, Kukor JJ. Characterization and role of TbuX in utilization of toluene by *Ralstonia pickettii* PKO1. J Bacteriol 2000; 182: 1232–42.
10. Eaton, RW. p-Cymene catabolic pathway in Pseudomonas putida Fl: cloning and characterization of DNA encoding conversion of p-cymene to p-cumate. J Bacteriol 1997; 179: 3171–80.
11. Van den Berg B, Black PN, Clemons WM Jr, Rapoport TA. Crystal structure of the long-chain fatty acid transporter FadL. Science 2004; 304: 1506–9.
12. DeLano WL. The PyMOL molecular graphics system. San Carlos, CA, USA: DeLano Scientific, 2002. Available at http://www.pymol.org.
13. Hearn EM, Patel DR, Lepore BW, Indic M, van den Berg B. Transmembrane passage of hydrophobic compounds through a protein channel wall. Nature 2009; 458: 367–70.
14. Noinaj N, Guillier M, Barnard TJ, Buchanan SK. TonB-dependent transporters: regulation, structure, and function. Annu Rev Microbiol 2010; in press.
15. Hamilton JA. New insights into the roles of proteins and lipids in membrane transport of fatty acids. Prostaglandins Leukot Essent Fatty Acids 2005; 77: 355–61.
16. Hamilton JA, Cistola DP. Transfer of oleic acid between albumin and phospholipid vesicles. Proc Natl Acad Sci USA 1986; 83: 82–6.
17. Azizan A, Black PN. Use of transposon TnphoA to identify genes for cell envelope proteins of *Escherichia coli* required for long-chain fatty acid transport: the periplasmic protein Tsp potentiates long-chain fatty acid transport. J Bacteriol 1994; 176: 6653–62.
18. Azizan A, Sherin D, DiRusso CC, Black PN. Energetics underlying the process of long-chain fatty acid transport. Arch Biochem Biophys 1999; 365: 299–306.
19. Hearn EM, Patel DR, van den Berg B. Outer-membrane transport of aromatic hydrocarbons as a first step in biodegradation. Proc Natl Acad Sci USA 2008; 105: 8601–6.
20. Vorum H, Brodersen R, Kragh-Hansen U, Pedersen AO. Solubility of long-chain fatty acids in phosphate buffer at pH 7.4. Biochim Biophys Acta 1992; 1126: 135–42.
21. Rapoport TA. Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. Nature 2007; 450: 663–9.
22. Steen EJ, Kang Y, Bokinsky G, Hu Z, Schirmer A, McClure A, Del Cardayre SB, Keasling JD. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 2010; 463: 559–62.
23. Ellis TN, Kuehn MJ. Virulence and immunomodulatory roles of bacterial outer membrane vesicles. Microbiol Mol Biol Rev 2010; 74: 81–94.
24. Mahan MJ, Tobias JW, Slauch JM, Hanna PC, Collier RJ, Mekalanos JJ. Antibiotic-based selection for bacterial genes that are specifically induced during infection of a host. Proc Natl Acad Sci USA 1995; 92: 669–73.
25. Van Beilen JB, Eggink G, Enquist H, Bos R, Witholt B. DNA sequence determination and functional characterization of the OCT-plasmid-encoded alkJKL genes of *Pseudomonas oleovorans*. Mol Microbiol 1992; 6: 3121–36.
26. Eaton RW. Organization and evolution of naphthalene catabolic pathways: sequence of the DNA encoding 2-hydroxychromene-2-carboxylate isomerase and trans-*o*-hydroxybenzylidenepyruvate hydratase-aldolase from the NAH7 plasmid. J Bacteriol 1994; 176: 7757–62.
27. Neher TM, Lueking DR. *Pseudomonas fluorescens* OmpW: plasmid localization and requirement for naphthalene uptake. Can J Microbiol 2009; 55: 553–63.
28. Hong H, Patel DR, Tamm LK, van den Berg B. The outer membrane protein OmpW forms an eight-stranded β-barrel with a hydrophobic channel. J Biol Chem 2006; 281: 7568–77.
29. Belchik SM, Schaeffer SM, Hasenoehrl S, Xun L. A β-barrel outer membrane protein facilitates cellular uptake of polychlorophenols in *Cupriavidus necator*. Biodegradation 2010; 21: 431–9.