The Biochemical and Physiological Characteristics of Surface Receptors of Gram Negative Bacteria

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Abstract: This review focuses on the properties of ferric iron surface receptors of Gram negative bacteria. We discuss the different strategies to acquire iron, and the fundamental role of these receptors in pathogenicity. The structure of some of these receptors, iron transport and regulation mechanisms are presented here.

INTRODUCTION

Gram negative bacteria are surrounded by two membranes which confine the periplasm and the peptidoglycan, a structure responsible for the cell shape and rigidity [1]. These two membranes differ both in lipid and in protein composition. The inner (cytoplasmic) membrane is constituted of a phospholipid bilayer in which proteins are embedded. Ions and solutes are actively transported through the inner membrane by means of channels, pumps and transporters which use ATP and the electrochemical gradient of protons generated by the electron transfer chain as the driven force [2]. The outer membrane (OM) constitutes a permeability barrier which protects the cell against noxious agents. Its lipid composition is atypical since the inner layer contains phospholipids and the outer layer lipopolysaccharides (LPS). The LPS which consists of a lipidic part (lipid A), an oligosaccharide core and a 0-specific polysaccharide chain, confers to the bacteria a polar and negatively charged surface. As a consequence, the outer membrane prevents the penetration of lipophilic compounds like bile salts and digestive enzymes found in the intestinal tract of animals. While protecting the bacteria against harmful components, the lipopolysaccharide also acts as a barrier against the influx of nutrients as well as of antibiotics which are in majority hydrophobic [3]. Proteins represent 50% of the outer membrane mass. Table 1 summarizes the main features of the most representative proteins of the *Escherichia coli* outer membrane. Most of them are integral proteins. Some are anchored to the peptidoglycan or are spanning the periplasm. Some of them play a structural role (OmpA) or have an enzymatic activity (OMPLA). Others, like porins and high affinity receptors, transport nutrients. OM proteins such as TolC are elements of complex envelope machineries which secrete proteases and lipases, and participate in efflux of drugs. Besides their physiological functions almost all of these proteins are potential receptors for pathogenic agents such as bacteriophages and bacteriocins.

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The importance of surface proteins in pathogenicity and in participating in antibiotic resistance has stimulated studies on their functioning and regulation. Furthermore, the high resolution structures of representatives of almost all families of OM proteins belonging either to *E.coli* or other Gram negative bacteria have now been solved [4,5]. Many criteria are now fulfilled to consider these proteins as the potential targets for drugs or to design new targets that would allow to bypass the OM barrier. Numerous reviews have focused on the properties of OM proteins. We will therefore only briefly survey their main features and more specifically focus on the high affinity receptors for which major advances have been made during the last few years. References will be cited throughout according only to the most recent publications or reviews.

GENERAL FEATURES OF SURFACE PROTEINS FROM GRAM NEGATIVE BACTERIA

Solving the high resolution structure of OM proteins belonging to six major families has revealed that all proteins share the same basic architecture [4,5]: a closed barrel formed by a variable number of antiparallel amphipathic transmembrane strands ranging from 4 to 22. The barrels are generally obstructed by hydrophilic surface-exposed loops which are involved in ligand binding (high affinity receptors) or selectivity towards ions or solutes (porins). With the exception of TolC and OmpA (see below) short turns point out on the periplasmic face. It is generally assumed that this sheet organization prevents the polypeptides, which have to be targeted to the OM during biogenesis, from remaining stuck in the inner membrane.

Non-selective porins belong to the most abundant class of proteins. They were among the first membrane proteins for which the 3D structure at high resolution was solved [6,7]. These pore-forming proteins, exemplified by the *E.coli* porin OmpF, allow the passive diffusion of small (< 600 Da) hydrophilic molecules. Selective porins, such as the *E.coli* maltoprotein LamB, also permit the diffusion of small hydrophilic solutes but they contain specific binding sites for the ligands inside the pore. Proteins of both families share the same homotrimeric organization, the

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Table 1. Structural and Functional Features of Outer Membrane Proteins from *E.coli* for which the 3D Structure has been Solved
(adapted from [4] and [64])

family	structural protein	enzyme	porins		high affinity receptors		channel-tunnel
			non selective	selective			
protein	OmpA	OMPLA	OmpF	LamB	FhuA	FepA	TolC
function	links the OM to the peptidoglycan	lipid hydrolysis	diffusion pore	oligosaccharide uptake	iron transport	iron transport	toxin export drug efflux
bacteriophages	K3, M1, Ox2, TuII		TuIa, T2	, K10, SS1	T1, T5, 80, UC-1		
bacteriocins	colicin K, L		colicin N		colicin M, microcin J25	colicin B, colicin D	colicin A
antibiotic					albomycin		
oligomeric state	monomer	monomer/dimer	homotrimer	homotrimer	monomer	monomer	homotrimer
TM [a]	8	12	16	18	22	22	4
3D structure	2000	1999	1992	1995	1998	1999	2000
reference	[10]	[12]	[7]	[8]	[26,27]	[28]	[14]

[a] number of transmembrane strands per monomer.

monomers of OmpF [7] and of LamB [8] being composed by 16 and 18 strands respectively. Besides their solute diffusion function, OmpF and LamB are specific receptors for diverse phages (Table 1). The importance of porins is highlighted in the case of *Pseudomonas aeruginosa* strains selected in the hospital environment, which become resistant to antibiotics due to mutations or deletions in one of the selective channel-forming protein OprD [9].

Amongst the most abundant proteins from the *E.coli* outer membrane is OmpA, a monomeric protein involved in the integrity of the OM. The 3D structure of the N-terminal domain of OmpA (residues 1-171) has been solved by X ray diffraction [10] and NMR [11]. It is composed of a small barrel of 8 antiparallel strands. The barrel is connected to a large periplasmic domain (residues 172-325) which ensures the physical linkage to the peptidoglycan. Four extracellular loops are involved in toxins (colicins) and phage binding and in F-mediated bacterial conjugation.

OMPLA (outer membrane phospholipase A) is the only outer membrane enzyme for which the 3D structure has been solved [12]. This protein, which is organized in a -barrel of 12 transmembrane strands, is implicated in the virulence of Campylobacter and Helicobacter strains. It has the particularity to exist both as a monomer and a dimer, the latter being the functional form.

TolC belongs to a highly conserved family of outer membrane proteins that ensure the transit of large substrates from the inner membrane to the external environment [5,13]. TolC is part of the so-called Type I secretion machinery, a protein complex that also comprises an inner membrane ABC transporter and a periplasmic protein. Its 3D structure, called "channel-tunnel" or "chunnel" [14] is remarkable. TolC forms a hollow cylinder 140 Å long that comprises a 100 Å long -helical barrel (the tunnel domain) anchored in the OM by a 40 Å long barrel (the channel domain). Three monomers, each contributing 4 -strands, form a single 12-stranded -barrel. Solving the 3D-structure has brought an unambiguous answer to how efflux of large molecules can take place from the inner membrane to the outer membrane while bypassing the periplasm.

HIGH AFFINITY RECEPTORS : FROM FUNCTION TO STRUCTURE

The high affinity receptors (also called ligand-gated porins or TonB-receptors) are involved in the uptake of molecules that are present at a very low concentration in the growth medium and that are too large to diffuse through the porins. Among the nutrients that are transported by these proteins are vitamin B_{12} and ferric iron [15].

Ferric iron receptors are central to this family. They are found across a broad range of Gram negative bacteria and play an essential role in growth and pathogenicity [16]. Strategies to acquire iron differ from one micro-organism to the other, a consequence of their necessary adaptations to variable environments. Despite this diversity iron receptors display common features. For a long time, most of our knowledge of their functioning has come from studies on *E.coli*. It is only recently that iron transport mechanisms in other pathogens have been investigated, opening the way to the design of new antimicrobial agents.

Strategies for iron Transport in Gram Negative Bacteria

Acquisition of ferric iron is one of the key steps in bacterial growth and in the development of a pathogen in its host. It is so crucial to the cellular mechanisms that the competition for iron between a host and a pathogen is one of the most important factors determining the course of a bacterial infection [17]. Iron differs from all other nutrients which are needed for bacterial growth since it is not available in a free form. The concentration of free Fe^{3+} in solution at physiological pH is too low ($< 10^{-18}$M) to permit growth of the micro-organisms which requires a minimum concentration of 10^{-8} M [18]. To cope with this shortage, bacteria have developed two strategies of iron acquisition. Some bacteria synthesize and secrete iron chelators (siderophores) to sequester and solubilize iron or use siderophores which are synthesized by other bacteria, yeasts or fungi. Other pathogenic micro-organisms make use of exogenous sources of iron provided by the host like haem, hemoglobin, transferrin or lactoferrin [19].

Siderophores-Mediated Iron Transport

Siderophores

To date, several hundred siderophores (from the Greek: 'iron carrier') have been isolated and characterized. These small molecules (MM < 1000 Da) belong to four major groups which are defined according to the chemical nature of the chelating ligand: catecholates, hydroxamates, hydroxypyridonates and aminocarboxylates. The dissociation constant of siderophores for ferric iron ranges from 10^{22} to 10^{50}. Since the affinity of iron for transferrin or lactoferrin or ferric hydroxide (the source of iron under laboratory growth conditions) is lower than for siderophores, iron provided by these sources can easily be removed [20]. Siderophore biosynthesis is depressed in response to iron deficiency. Following their synthesis, siderophores are secreted in the external medium where they chelate iron. The ferric siderophore is further transported across the bacterial envelope using a complex protein machinery (see below). The ferric iron is then released from the siderophore in the cytoplasm after being reduced into ferrous iron by reductases. It is used as a cofactor in metabolic functions [21]. Desferri-siderophores are then recycled for further iron utilisation.

Siderophores as Drug Agents

Siderophores and their analogs can behave as antibiotic agents. Their chelating power is used to deprive pathogenic micro-organisms of the iron essential for growth. Depletion of iron is either accomplished by competitive chelation of iron or by blocking the siderophore site on the receptor with a non functional siderophore analog. Alternatively, a non metabolizable metal ion such as ScIII or InIII may be used to chelate the naturally active siderophore and to prevent iron uptake by competitive inhibition on the OM receptor [19,20]. Siderophores may themselves be antibiotics. This is the case for albomycin, which is formed by the association of residues of the siderophore ferrichrome to a toxic moiety and is recognized by the ferrichrome receptor FhuA (see below) [22]. Such combination has served as a model to design new antimicrobial agents in which lethal compounds are attached to siderophores allowing the drug to be transported using the siderophore transport system. The use of siderophore-drug conjugates originates from work showing that -lactams antibiotics (penicillin and cephalosporin), associated to a catechol moiety had excellent antibacterial activity. It was further developed by Miller and his group [20]. The fact that these molecules are actively transported (see below) decreases the minimum inhibitory concentration by a factor of several hundred, in comparison to that of the unmodified antibiotic which enters by passive diffusion [23].

Mechanism of Transport of Ferric Siderophores Across the Envelope

A striking feature of ferric siderophore transport systems is their redundancy. In *E.coli* K12, the standard laboratory strain, there exists at least three different pathways for iron transport “Fig (**1**)”. Binding of ferric-siderophores on the surface of the bacteria is ensured by high-affinity receptors, each recognizing a specific siderophore. *E.coli* synthesizes three receptors for ferric hydroxamates (FhuA, FhuE, Iut), three receptors for ferric catecholates (FepA, Fiu, Cir) and one receptor for ferric citrate (FecA) [15]. *E.coli* synthesizes only one siderophore (enterochelin), the others (ferrichrome, coprogen, aerobactin) being brought by fungi or bacteria. The affinity of the receptors for siderophores is in the range of 0.3 to 50 nM. Once bound to its specific receptor, the iron siderophore is transported across the outer membrane. This transport requires a protein complex (TonB-ExbB-ExbD) anchored in the cytoplasmic membrane and is coupled to the electrochemical gradient of protons in the cytoplasmic membrane (see below). After being released in the periplasm, the iron-siderophore binds to a periplasmic-binding protein (FhuD, FecB or FepB). This protein donates the ferric siderophore to cytoplasmic membrane-anchored proteins (FhuBC, FepDGC, FecCDE) belonging to the superfamily of ABC transporters. Transport through the cytoplasmic membrane is coupled to ATP hydrolysis. Iron is then dissociated from the siderophore in the cytoplasm and the iron chelator recycled in the external medium.

E.coli Siderophore Receptors

High Resolution Structure of FhuA and FepA

Our view of how siderophore receptors function has considerably improved since the recent determination of the high resolution structure of FhuA and FepA by X ray diffraction. On the basis of secondary structure predictions and of genetic and biochemical experiments, topological models of the two proteins had been previously proposed [24,25]. FepA and FhuA were predicted to be organized as -barrels formed by 29 and 32 -strands respectively, that were gated by a large flexible external loop. Clearly these models which were much inspired by the structure of porins, were far from reality. Indeed, determination of the 3D structure at 2.5 Å resolution of FhuA [26,27] and FepA [28] has revealed an unexpected and unique structural organization of this family of proteins. FhuA “Fig (**2**)” is composed of a barrel domain with a cross section of 35 x 25

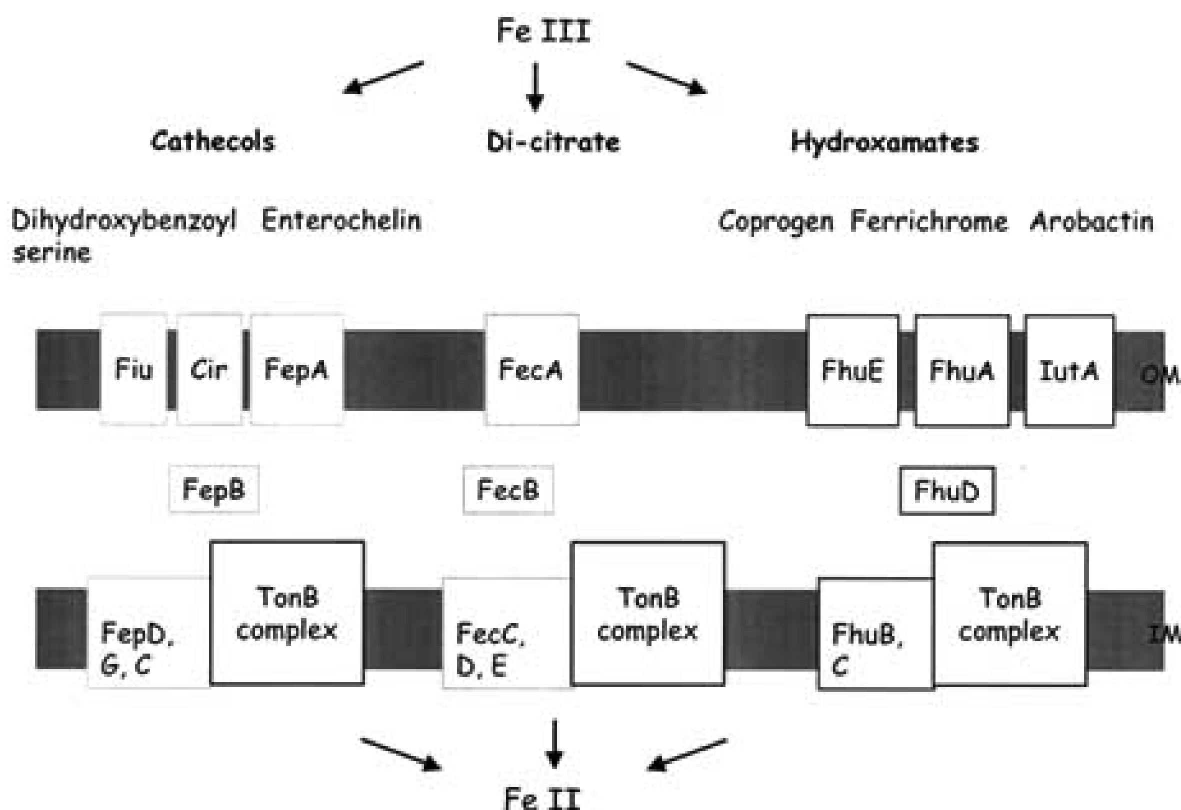

Fig (1). The different mechanisms of transport of ferric iron through the *Escherichia coli* envelope. OM : outer membrane; IM : inner membrane.

Å. It consists of 22 antiparallel -strands lodged in the membrane and of an amino-terminal globular domain that folds inside the barrel and occludes it. This "plug" or "cork" domain, which spans most of the interior of the -barrel, consists of a four-stranded -sheet and four short helices which are connected to the -barrel and to the hydrophilic loops. These large loops, which face the external medium, are involved in ligand binding. Ferrichrome binds on the top of the plug domain, but its binding induces only a few structural changes except for a short helix located in the periplasmic pocket that completely unwinds. FepA displays a crystal structure very similar to that of FhuA.

Role of the N-Terminal "Plug" Domain of FhuA and FepA in Function

In an attempt to decipher the role of the plug domain in the receptor function, the N-terminal domains of FhuA and FepA were either deleted or genetically exchanged [29-32]. The data obtained indicate that the N-termini of FepA and FhuA are not needed for the transport of the siderophores and that ligand selectivity is only dictated by the surface loops. Nevertheless the presence of the plug appears to enhance the binding capacity of the siderophores. In addition to this role, it was proposed that the plug domain may constitute a physical barrier preserving the cell from entry of the noxious compounds which are excluded from porins.

The question remains of whether the plug exits from the barrel upon ligand transport. Electrophysiological experiments using planar lipid bilayer in which FhuA was incorporated suggested that FhuA was converted into an open channel upon binding of phage T5, a phage that has parasited the FhuA receptor (see below). The conductance of the channel was compatible with a pore of ~ 2 nm in diameter. Furthermore, the channel conductance was strongly decreased when ferrichrome was added to the bilayer set, an event indicating that ferrichrome could diffuse through the pore [33]. These data suggest that the conformational changes induced to FhuA by binding of the phage [34] could trigger the removal of the plug from its initial position. The fact that FhuA and FepA deleted of their N-terminal domain transported the siderophores, led Scott *et al* as well [32] to propose that the transport activity of the wild type proteins requires the exit of the N-terminal domain as ligands traverse the outer membrane. 40 hydrogen bonds ensure the contact between the globular domain and the barrel. Removal of the plug requires energy which could be donated to the protein via the Ton complex.

The Ton Complex : An "Energy Transducer" between the Inner and Outer Membrane

The mechanism that supports iron and also vitamin B_{12} transport across the outer membrane, seems like a non-solved paradox. This transport is an energy-dependent process in which the substrates are taken up into the periplasm against their concentration gradients [35]. However, energy sources are neither available within this membrane nor in the periplasm. Moreover, the presence of

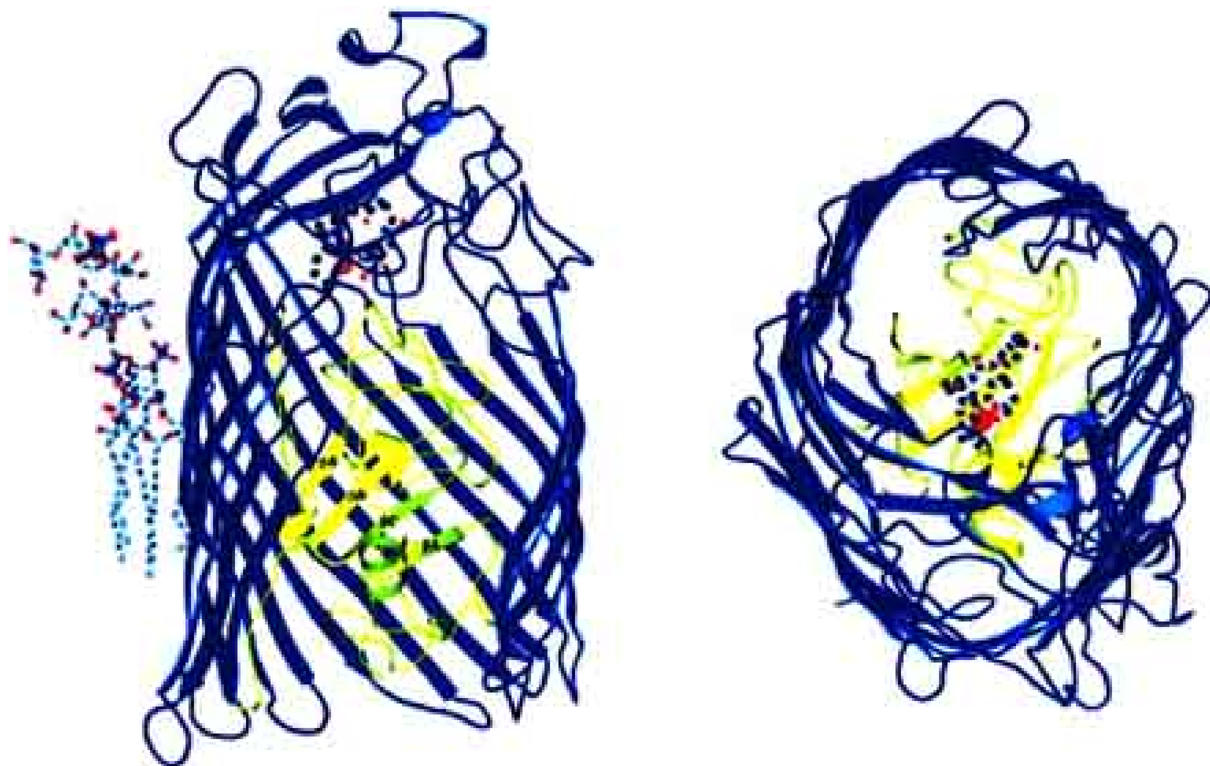

Fig (2). Crystal structure of the FhuA-ferrichrome iron complex (adapted from reference [27]).

Left figure : FhuA in ribbon representation. Residues 621 to 723 have ben removed to allow a view on the plug domain. The iron-ferrichrome (inside the barrel) and the lipopolysaccharide (outside the barrel) are represented as ball-and-stick models. The position of the membrane bilayer is delineated by dotted lines. The extracellular space is located at the top of the figure.

Right figure : FhuA as viewed from the external medium along the barrel axis. The ferrichrome iron is represented as ball-and-stick model.

porins in the OM makes any ion gradient untenable. Gram-negative bacteria have solved this dilemma by evolving a system whereby the energy derived from the electrochemical gradient of protons generated by the electron transfer chain in the cytoplasmic membrane is transduced across the periplasm to the OM receptors. Energy is transduced by means of a protein complex consisting of three cytoplasmic membrane-anchored proteins TonB, ExbB and ExbD (Ton complex).

Ton-dependent transport systems are widely spread. To date more than 20 outer membrane proteins whose function depends upon TonB have been identified in Gram-negative bacteria [36,37]. Besides its role in physiological substrates transport, TonB is implicated in the uptake of some bacteriocin proteins (colicin M, microcin J25 or cobalamin, for example) [38] and in infection by some phages (T1, 80) (Table 1).

TonB is a 26-kDa protein. Its N-terminus is in the cytoplasm and the protein is anchored in the inner membrane by its uncleaved N-terminal signal sequence. The middle part of the protein contains a large proline-rich region that is thought to confer to TonB a conformational rigidity and an extended shape allowing its C-terminal domain to contact the OM receptor [39].

ExbB is a 26 kDa protein predicted to have 3 transmembrane segments, a periplasmic N-terminal domain and a large C-terminal cytoplasmic domain.

ExbD is the smallest protein of the complex (MM: 15 kDa). It is predicted to have a unique transmembrane segment and a N-terminal domain facing the cytoplasm.

How does the Ton complex exert its function of energy transducer? In spite of many efforts carried out to unravel the mechanism using FhuA, FepA or BtuB as models, only a few facts are well established.

Extensive studies have allowed the determination of the regions of TonB and of the receptors that physically interact. *In vitro* studies, in non energized systems, have shown that the periplasmic domain of TonB is sufficient for interaction with the receptors but that TonB recognizes preferentially the ligand-loaded receptors [40,41]. The N-terminal-membrane anchor of TonB is however required for siderophore transport [42].

Many studies emphasized the importance of a short stretch of conserved amino acids in the N-terminal region of the OM receptors, known as TonB box, that could be responsible for the physical interaction with TonB [43]. Crystallographic studies have revealed dramatic ligand-induced conformational changes on the periplasmic face of FhuA which occur just C-terminally to its TonB box [26,27]. Similarly, the helical conformation of the TonB box of BtuB, the vitamin B_{12} receptor, is converted into a disordered structure that extends into the periplasm upon vitamin B_{12} addition [44]. Nonetheless, numerous mutagenesis analyses of TonB [45] and transport studies on receptors missing the N-terminal globular domain [32] have questioned the role of the TonB box.

The crystal structure of the C-terminal domain of TonB has recently been solved [46], showing a novel fold without homology to any known structure. It forms a tightly intertwined dimer [47]. This result is in agreement with those showing that a soluble form of TonB missing the transmembrane region may also form dimers that interact preferentially with FhuA *in vitro* [41]. This reopens the discussion about the mechanism allowing the interaction of TonB with the receptors.

Although it is well stated that ExbB and ExbD should be present for TonB activity, their function is poorly understood. It has been proposed that ExbB and TonB physically interact at the level of their transmembrane segments. A possible function for ExbB would be to modulate the conformation changes in TonB and to protect ExbD and TonB from proteolysis [23,48]. Experimental data point towards the involvement of these proteins both in the stabilization of TonB and in its recycling between a non-energized and an energized conformation. Different models of how TonB (monomer or dimer) would cycle between different energetic states have been proposed [35,45,46] but the way in which the energy is transduced remains hypothetical.

Siderophore-independent Iron Transport in Gram Negative Pathogens

To survive in the iron-limited environment of their host, pathogens, rather than using siderophores, use iron sources provided by the host. The main sources of iron are provided by lactoferrrin and transferrin or by haem. Although the mechanisms of iron acquisition differ they all share common features: (i) one or several outer membrane receptors are involved in binding the iron provided by the different sources; (ii) the transport through the outer membrane is TonB-dependent; (iii) in some, but not all cases, the iron is captured by a periplasmic binding protein; (iv) transport through the cytoplasmic membrane is driven by ATP hydrolysis and appears to depend on ABC transporters. These mechanisms are only beginning to be characterized at a molecular level but given the characteristics they share with siderophore-mediated transports one can expect rapid progress. Learning how to inhibit iron acquisition and therefore how to kill pathogens is certainly an essential challenge for the pharmaceutical industry.

Haem-dependent Iron Acquisition

Whereas intracellular pathogens use haem directly, extracellular pathogens must acquire the haem from haem-containing proteins (mainly haemoglobin and haemopexin) [49]. Haem capture involves its binding to a specific outer membrane receptor (OMR). There are at least 18 different pathogens for which a receptor has been characterized biochemically and/or genetically. Among these are Hemophilus, Neisseria, Vibrio and Yersinia species and *Pseudomonas aeruginosa*. Haem is transported through the outer membrane by three different mechanisms [50]:

(i) Haem and haemoglobin, which are liberated upon degradation of red blood cells by the toxin haemolysin, bind to separate sites on the same OMR.

(ii) Haemophores produced by the bacterium are secreted in the external medium where they capture haemoglobin (or haemopexin). The haemophore-haemoglobin complex then binds to the OMR. The best described system of haemophore mediated transport is that of *S. marcescens* for which the crystal structure of the haemophore (HasA) and its interactions with haemoglobin and the receptor HasR have been characterized [51,52].

(iii) Haemoglobin or haemopexin are degraded by bacterial proteases either located in the outer membrane or secreted. Free haem is then captured by the receptor.

Transferrin and Lactoferrin-dependent Iron Acquisition

Pathogens such as members of the Neisseriaceae or Pasteurellaceae species synthesize outer membrane receptors that bind specifically the two phylogenetically-related iron-binding glycoproteins transferrins (Tf) and lactoferrin (Lf) [53]. Tf and Lf , unlike siderophores, are large molecules (MM 80 kDa). These bilobed proteins bind two ferric ions per molecule. The Tf and Lf-mediated transports are associated with distinct receptor complexes consisting of two outer membrane proteins. Iron uptake takes place without internalisation of Tf (or Lf) [54]. The Tf (or Lf) receptor consists of a complex of two proteins, a relatively conserved protein TbpA (or LbpA) and an antigenically variable protein TbpB (or LbpB). TbpA and LbpA show sequence identity with FepA and other members of the TonB-dependent receptors. TbpB is a lipoprotein that lacks obvious membrane spanning stretches suggesting that it is anchored to the outer membrane *via* its lipid moiety. A model describing the mechanism of Tf-dependent iron transport has recently been proposed [19]. Tf first binds to TbpB at the surface of the bacteria. The bilobed Tf is then recognized by a dimer of TbpA. Iron is then removed from Tf and transported through TbpA in a manner similar to the siderophore-mediated transport i.e. depending on the Ton-energy transducer complex. Further transport through the periplasm and the cytoplasmic membrane depends on a periplasmic protein FpbA and on an ABC transporter FbpBC. After loss of its iron apo-Tf is released from the outer membrane receptor, this release requiring the Ton complex.

Regulation of Iron Transport: Transducing a Signal from the Bacterial Surface to the Cytoplasm

Iron, although being essential for bacteria, can also be deleterious. Indeed, accumulation of hydroxyl free radicals catalysed by iron results in the oxidative destruction of DNA, lipids and proteins and ultimately to cell death. To avoid iron toxicity, bacteria have developed regulation mechanisms that act in either a negative (under iron-rich conditions) or positive (under iron starvation) fashion. One important regulation comes from iron itself. Iron overload triggers the shut-off of the expression of many genes involved in iron uptake. This occurs *via* the cytoplasmic protein Fur which acts as a repressor together with iron. Fur-like proteins have been identified in a wide spectrum of bacterial species [55]. In most cases, once iron becomes limiting, iron-regulated genes become derepressed and the iron transport proteins and siderophores start to be synthesized. However, in some cases, synthesis of proteins requires positive gene regulation. The most documented study is that of the ferric dicitrate *E.coli* transport system [15] in which transcription of the transport genes *fecABCDE* is induced by ferric citrate in the culture medium. On the basis of genetic and biochemical experiments a model of regulation was proposed [15]. Binding of ferric dicitrate to the outer membrane receptor FecA, triggers a conformational change in the protein allowing its interaction with the regulatory protein FecR anchored in the cytoplasmic membrane. FecR then transmits an induction signal across the cytoplasmic membrane to the cytoplasm causing the activation of the *fec*-specific sigma factor FecI and its binding to the RNA polymerase core enzyme. The FecI-RNA polymerase complex binds to the promoter upstream of *fecA* and initiates transcription of the *fec* transport genes. Importantly, induction depends on the Ton complex and on the electrochemical gradient of protons across the cytoplasmic membrane.

Siderophore Receptors as Multifunctional Proteins

Most of the outer membrane receptors are multifunctional proteins: they transport compounds structurally related to the metal chelates such as antibiotics and are receptors for phage and toxins (Table 1). A striking example is the ferrichrome-receptor FhuA, which is the receptor for phage T1, T5, 80 and UC-1, for the toxins colicin M and microcin J25 and for the antibiotic albomycin [29]. Interestingly, phage T5 is the only FhuA ligand which does neither require TonB nor an energized cytoplasmic membrane to be active. Indeed, the mere interaction of phage T5 with isolated and purified FhuA triggers the release of the phage genome (a double stranded DNA molecule of 121 kbp) within a few seconds in the surrounding medium [56,57]. In an attempt to decipher the mechanism of phage DNA transport across membranes FhuA was reconstituted into liposomes. Phage T5 bound to reconstituted FhuA and its DNA was transferred into the liposomes [58]. Cryo-electron microscopy studies have already been used to visualize the binding of T5 to FhuA and to propose a model about the route used by DNA to cross the membrane during viral genome infection [59-61]. These experiments have also allowed, for the first time , the design of an *in vitro* assay of the functionality of an outer membrane receptor.

OUTLOOKS

All Outer membrane proteins display large hydrophilic surface accessible regions. These regions not only serve as binding sites for phage and toxins but they are ALSO considered as possible motifs to present foreign peptide epitopes on the bacterial cell surface. Foreign gene products have been fused to surface-accessible regions of several outer membrane proteins including OmpA and the porins OmpC, PhoE, LamB. Short epitopes and even large insertions of more than 100 amino acids have been inserted into these outer membrane proteins and shown to induce epitope-specific antibody responses [62,63]. Outer membrane receptors of pathogenic bacteria are also potentiel targets for vaccine development. Sera from patients with gonococcal infections contain antibodies to FbpA. Antibodies against TbpA and TbpB have also been found in patients with meningococcal disease [54]. These proteins also appear to be susceptible to attack by bactericidal antibodies. As stated by Gray-Owen and Schryvers, [53], the apparent conservation of receptor-ligand interactions raises the possibility that the vaccine antigen mimicking regions of the receptor involved in ligand binding can induce a broadly cross-reactive response against heterologous bacterial pathogens of a common host.

ACKNOWLEDGEMENTS

We thank Pascale Boulanger, Mélanie Bonhivers, Greg Moeck, Chantal Janmot and Laure Plançon who participated to the work done in our laboratory on the *E.coli* receptors. M.S. is a recipient of fellowships from the Ministère de l'Enseignement Supérieur and from the European Community (Marie Curie Reserach Training).

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