[Frontiers in Bioscience 8, s265-274, May 1, 2003]

MALTODEXTRIN TRANSPORT THROUGH LAMB

Alain Charbit

Laboratoire de Microbiologie; INSERM U-570, Faculté de Médecine Necker, 156, rue de Vaugirard, 75730 Paris Cedex 15, France

TABLE OF CONTENTS

1. Abstract
2. Introduction
2.1. A few words of history: some important dates
2.2. The lambda receptor site
2.3. The sugar binding site
2.4. Topological studies
2.5. The functional domains of LamB involved in maltodextrin binding and transport.
3. Role of the greasy slide and polar track
4.Participation of the surface loops to sugar transport
5. In vivo interactions between loops L4, L5 and L6
6. In vitro studies of loop deletion mutants
7.Proposed mechanism of sugar translocation
7.1. Role of the L9 loop
8. Concluding remarks
9. Acknowledgments
10. References

1. ABSTRACT

The trimeric protein LamB of *E. coli* K12 (maltoporin) specifically facilitates the diffusion of maltose and maltooligosaccharides through the outer membrane and acts as a non-specific porin for small hydrophilic molecules. LamB serves also as a specific cell surface receptor for phages, including phage lambda. Each monomer consists of an eighteen-stranded antiparallel ß-barrel with nine surface loops (L1 to L9). Three loops fold into the ß-barrel, with loop L3 constricting the channel about half way. Monomers bind sugars independently of each other. Structural studies of maltoporin in complex with maltodextrin showed that the binding site, located at the channel constriction, was composed of : i) a “greasy slide”, a left-handed helical arrangement of aromatic residues extending along the channel providing a hydrophobic path to the glycosyl moieties; and ii) an “ionic track”, found on both sides of the channel constriction zone, providing residues available for forming hydrogen bonds with the sugars. The participation of the surface loops that cover the entry of the pore to phage binding and to sugar binding and transport has also been thoroughly investigated. Genetic and biochemical analyses suggest that some of the surface loops participate directly in the orientation and entry of maltooligosaccharides into the channel and, thus, control access to the binding site.

This non-exhaustive review will summarize briefly the major methodological and conceptual advances on the analysis of LamB, during the past two decades. The role of different domains of the protein that participate to sugar transport will be described; particular emphasis will be given to the role of the surface loops. Then, we will discuss how these domains may interact functionally and propose a comprehensive model of maltodextrin transport through the LamB pore.

2. INTRODUCTION

The outer membrane of Gram-negative bacteria acts as a molecular filter, which has a defined exclusion limit for the permeation of hydrophilic solutes. These molecular sieving properties are due to a major class of proteins called porins (1). Many porin pores form non-specific water-filled channels which allow the diffusion of ions and low molecular weight hydrophilic molecules (generally smaller than 600 daltons, 2). Other porin pores such as LamB (maltoporin) and Tsx of *Escherichia coli* (3, 4, 5) and the protein P of *Pseudomonas aeruginosa* (6, 7) form specific channels that contain binding sites for classes of solutes. These specific pores are either constitutively expressed in the outer membrane or they are induced when the cells are grown on particular growth media.

Like the general porins, LamB of *Escherichia coli* K12 is a trimeric protein that forms non-specific channels through the outer membrane. LamB is also a sugar-specific porin that facilitates the diffusion of maltose and maltodextrin into the cell (8, 9, 10), and references therein). In addition to these transport functions, LamB serves as a specific cell surface receptor for phage λ as well as for a number of other bacteriophages (10, 11, 12, 13, and references therein).

The structures of several general porins of Gram-negative bacteria have been determined by X-ray crystallography (14, 15, 16, 17, 18, 19) in the past ten years. These structures are variations of a 16-stranded antiparallel ß-barrel with nearest-neighbour connections between strands. In contrast, the crystallographic analyses of the maltose-specific LamB proteins from E. coli (20) and S. typhimurium (21) and of the sucrose-specific ScrY of enteric bacteria (22) revealed that each monomer within a trimer formed an 18-stranded anti-parallel ß-barrel; the ß-strands being linked by nine loops from the extracellular side, with variable lengths (called L1 to L9, from the N to the C terminus), and eight internal turns or loops (called I1 to I8). The resemblance of the maltoporin and sucrose porin tertiary and quaternary structures to those of non-specific porins is obvious, although there is no detectable

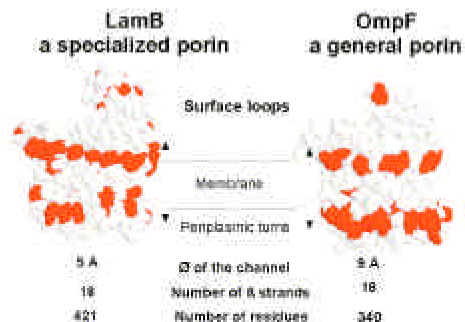

Figure 1. LamB and OmpF of *E. coli* K12. Space-filled 3D models (generated by the RasMac program) of the *E. coli* LamB (left part) and OmpF (right part) monomers. The aromatic residues are in red. The dotted lines represent the approximative limits of the lipid bilayer.

amino acid sequence similarity (Figure 1). Although, the determination of the crystallographic structure of maltoporin constituted undoubtly a major step toward the understanding of the molecular mechanisms of sugar translocation through LamB, genetic approaches and biochemical analyses provided essential information about the *in vivo* process of sugar translocation.

Here, we will place first the major genetic and structural studies on LamB in a historical perspective. Then, our current view of sugar binding and transport through LamB will be discussed.

2.1. A few words of history: some important dates

Various genetic, biochemical and structural studies, initiated more than 30 years ago, contributed - either independently or complementarily- to our present understanding of the mechanism of maltodextrin transport through LamB. Some key dates that correspond to major advances rergarding the phage receptor activity of LamB, its sugar-specific pore function as well as important topological and structural data, are listed in Table 1.

The LamB protein is part of the maltose uptake system (the *mal* system) in *E. coli* and other *Enterobacteriaceae* (see 23, for a recent review). The first function identified for the *lamB* gene product was to constitute a receptor for phage λ at the surface of *E. coli* K12. The protein was therefore initially called the lambda receptor (12, 24, 25). In parallel, Szmelcman and Hofnung showed that LamB of *E. coli* was also involved in maltose utilization. Mutants lacking this protein were impaired in maltose uptake when the concentration of maltose was in the micromolar range (9). The first quantitative analyses of the effect of LamB on maltose transport were then performed (26). This suggested a high specificity of LamB for carbohydrates (27) hence its other name, maltoporin. In fact, swelling experiments using reconstituted liposomes showed later that the LamB channel exhibited a considerable specificity for the permeation of maltose and maltooligosaccharides over that of other carbohydrates such as sucrose and lactose (28). Moreover, maltoporin was shown to possess a binding site for carbohydrates *in vivo* and *in vitro* (8). Experiments suggesting physical interactions between LamB and the periplasmic maltose-binding protein were also reported (29, 30). The determination of the limiting amount of LamB in maltose uptake *in vivo* (at micromolar concentrations) was then shown (31). Later, Boos and co-workers determined the *in vivo* kinetic constants for the flux of maltodextrins through LamB, using a maltohexaose analog (32).

A series of new residues in LamB, involved in phage adsorption (33) and/or sugar binding (34), were then identified.

2.2. The lambda receptor site

The extensive analysis of mutations conferring resistance to bacteriophages, performed in the laboratory of Maurice Hofnung, allowed the definition of the phage lambda receptor site on LamB. These studies revealed that the receptor site was discontinuous and composed of a series of residues mainly clustered in four surface loops of LamB (33). Remarkably, the X-ray structure confirmed that all the mutations conferring phage resistance are located on the extracellular side of the barrel. In some cases though, they affect residues at the base of the loop. These sites are not exposed and should cause an indirect effect on the surface of the loop structures (20, 35).

Furthermore, some of the residues constituting the phage receptor appeared to be also involved in maltose uptake *in vivo* (36) as well as *in vitro* (4). Three classes of mutants were identified: i) mutants with no effect on sugar binding and transport; ii) mutants with a significant but variable reduction in sugar binding and transport; and iii) mutants for which sugar binding and transport was almost completely abolished. The correlation between the level of impairment of dextrin binding (measured on starch columns) and impairment of maltose transport (measured with ^{14}C-labelled maltose, at various limiting concentrations) was consistent with the notion that the residues influencing starch binding were inside, or in close proximity to, the pore.

2.3. The sugar binding site

On the other hand, a series of mutations altering starch binding, analyzed in the laboratory of Tom Ferenci, provided crucial information on the nature of the sugar binding site of maltoporin (see for examples, 34, 37, 38). Most of these mutants were not affected in phage binding, demonstrating that the binding sites for starch and phage lambda overlapped but were distinct. Strikingly, among the residues identified, residues R8 and R82 were later shown by Xray analysis to belong to the ionisable track within the LamB constriction zone; and residue W74, the first aromatic of the greasy slide; demonstrating the efficiency of genetic selection procedures. In addition, it was also shown very recently that replacement of residue Y118, located at the tip of L3 in the central constriction of the channel (39), increased ion fluxes through lamB.

2.4. Topological studies

Other approaches, like the mapping of monoclonal antibody binding sites and proteolytic cleavage sites in LamB, were developed in the laboratory of Maxime Schwartz (40, 41, 42, 43, 44) to provide topological information on the tertiary and quaternary structure of LamB.

In 1986, I developed a novel general genetic procedure to probe the topology of LamB, in the laboratory of

Table 1. Several important dates on LamB studies

Event	Major contributors	Year
1. LamB, the phage λ receptor		
Discovery of phage λ in *E. coli K12*	Lederberg	1951
Genetic association and common regulation	Schwartz	1967
A single gene -*lamB*- determines the λ receptor	Thirion & Hofnung	1972
Purification of active λ receptor	Randall-Hazelbauer & Schwartz	1973
pel required for the injection of λ DNA	Scandella & Arber	1974
Host range mutants of λ and LamB mutants	Hofnung & Breton	1976
Sequence of the *lamB* gene of *E.coli*	Clément & Hofnung	1981
Three intact subunits constitute an active receptor	Marchal & Hofnung	1983
The λ receptor site is discontinuous	Charbit & Hofnung	1984
The permease IIC^{Man} / IID^{Man} is necessary for the injection of λ DNA	Erni	1987
Host range mutations in λ ?are located in the distal portion of the tail fiber protein J	Charbit & Hofnung	1994
2. LamB, maltoporin		
LamB required for maltose entry at ? M concentrations	Szmelcman & Hofnung	1975
LamB required for growth on dextrins (=3 G units)	Wandersman, Schwartz & Ferenci	1979
LamB, a porine specific for dextrins	Luckey & Nikaido	1980
Maltoporin binds maltodextrins	Ferenci	1980
The active form of Maltoporin is a homotrimer	Ishii & Nakae	1981
LamB possesses three dextrins binding sites	Ferenci & Lee	1989
Kinetic analyses of sugar binding to LamB	Benz	1994
Participation of the surface loops to sugar binding and transport	Charbit, Klebba & Hofnung Andersen & Benz	1994-1998
Sugar transport through LamB: roles of the greasy slide and of the polar track	Van Gelder, Schrimer & Rosenbuch	2000-2002
3. LamB, structure		
First 2D model of LamB folding	Charbit & Hofnung	1984
X-ray structure of the porin from *R. capsulatu*s	Weiss & Schulz	1990
X-ray structure of the porins OmpF and PhoE	Cowan, Schirmer & Rosenbush	1992
X-ray structure of LamB from *E.coli*	Schirmer & Rosenbush	1995
X-ray structure of LamB complexed with dextrins	Dutzler, Schirmer & Rosenbush	1996
X-ray structure of LamB from *S. typhimurium*	Meyer & Schultz	1997
X-ray structure of the sucrose porin from *S. typhimurium*	Forst &Welte	1998
sugar transport through maltoporin : role of the polar track	Dumas & Van Gelger,	2000
sugar transport through maltoporin : role of the greasy slide	Van Gelger, Dumas, Rosenbush & Schirmer	2002

Maurice Hofnung (45). This approach consisted in inserting a foreign epitope into permissive sites of the protein, allowing probing its topology with a single antibody (specific of the inserted epitope). In the absence of crystallographic data, this approach led us to identify the major loops of LamB (45, 46 47). Later on, a loop deletion mutagenesis approach was used (48, 49 50) to understand the role of these predicted loops in maltodextrin binding and uptake. The analysis of the *in vivo* properties of the various insertion or deletion mutants of LamB, combined with biochemical studies, carried out in collaboration with the laboratory of Roland Benz, led us to define the contribution of the surface loops to sugar transport through LamB (51, 52, 53). After the elucidation of the three dimensional structure of LamB of *E. coli* by X-ray crystallography (20) in 1995, systematic site-directed mutagenesis studies were carried out to determine the participation of each of the residues suspected to constitute the sugar binding site (the aromatics of the greasy slide and the charges of the polar track) to sugar binding and transport through the LamB pore (54, 55, 56).

2.5. The functional domains of LamB involved in maltodextrin binding and transport

The maltoporin oligomer of *E. coli* is formed by three polypeptide subunits composed of 421 amino acids (57). Each subunit forms a cylinder. The dimension of the channel in the subunits is limited by loop L3, which is folded inside the cylinder and restricts it to 3 x 5 Å. The specificity of maltoporin is mainly provided by three sets of factors in the interactions with maltooligosaccharide: i) apolar van der Waals contacts with a hydrophobic path composed of aromatic residues and located at the channel lining (also called the greasy slide); ii) hydrogen bonds in particular at the constriction of the channel and at the external orifice (55) and iii) a stereospecificity filter provided by the surface loops that control the access to the channel. We will come back to this notion of accessibility filter in the last paragraph. The entry of the LamB channel has a diameter of about 10 Å and is reduced to 5 Å in the transmembrane part, mainly due to the presence of two loops from the extracellular side, L1 and L3 as well as part of L6, which fold into the barrel and create a constriction zone. In the general diffusion porins, only one loop, L3, folds into the channel

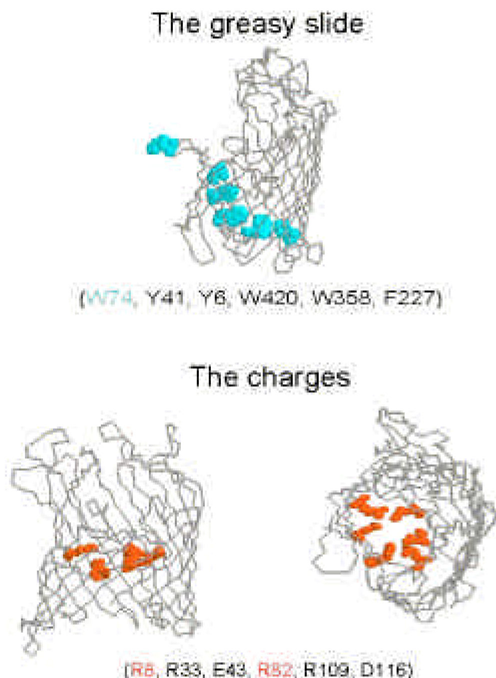

Figure 2. The sugar binding site. Upper part: The greasy slide. Side view of a 3D model of the LamB monomer (backbone). The aromatic residues are in space-filled representation (in cyan). W74 corresponds to the aromatic that is donated to an adjacent subunit. Lower part. The polar track. Side view (left) and top view (right) of a 3D model of the LamB monomer (backbone). The charged residues are in space-filled representation (in red). The labels that are colored correspond to residues identified by genetic screening (35).

and contributes to the reduced size of the constriction zone (to approximately 9 Å). Thus, in spite of their larger molecular weights, maltoporins form significantly narrower channels *ca*. 50% in the constriction zone (Figure 1). Interestingly, the LamB protein also contains two of four sequences that are conserved in amylases and form the maltodextrin binding site there (58). One of them, at positions 106 to 111 of LamB, is actually part of the sugar binding site as determined by the X-ray crystallography.

We will discuss below the respective contribution of the sugar binding site (greasy slide and polar track) and of the surface loops, to sugar translocation through LamB.

3. ROLE OF THE GREASY SLIDE AND POLAR TRACK

The greasy slide is composed of six contiguous aromatic residues which form an elongated apolar stripe extending from the vestibule through the channel constriction to the periplasmic outlet (Figure 2). The first residue W74, is provided by L2 of the neighboring subunit. The next three residues Y41, Y6 and W420, are situated in the middle of the channel. The last two residues W358 and F227, reside in the periplasmic vestibule. Alanine scanning mutagenesis on the six residues and mutants were characterized *in vivo* and *in vitro* (56). All the mutants which could still form trimers showed considerably reduced maltose uptake rates *in vivo* (<10% of the wild-type). Thus, both ends of the slide, although not part of the high-affinity binding site, facilitate diffusion of maltose and are of comparable importance.

The polar track, located at the constriction zone, is composed of two sets of ionisable residues, arranged pair-wise, but in most cases too far apart to form salt bridges and thus, likely to constitute hydrogen bond donors/acceptors with sugar substrates (Figure 2).

These residues (R8, R33, E43, R82, and R109, D116 (54) were systematically mutagenized and the effects of single or multiple mutations were investigated by current fluctuations, liposome swelling assays and *in vivo* transport assays. Analysis of the various mutations revealed a strong effect on the k*on* values (corresponding to the association rate of the sugar), indicating that the polar track residues were clearly involved in the fixation of the sugar to the binding site of the protein. As expected, all the mutations induced also a strong decrease of *in vivo* uptake of sugars.

The affinity of LamB for maltodextrins increases with sugar length but, on the disaccharide level, there is little difference between maltose, sucrose, trehalose and melibiose binding affinity (59). Compared with maltose, the rate of trehalose is similar while it is smaller by a factor of three for melibiose and by a factor of 40 for sucrose. It has been shown *in vivo* that trehalose utilizes LamB for passage across the outer membrane (60, 61), while sucrose uptake is facilitated by thesucrose porin ScrY (62, 63). Interestingly, in the sucrose porin of *S. typhimuirum*, ScrY, which transports sucrose (a non-reducing disaccharide composed of Glucosyl and fructosyl moieties), the four external of the six aromatic residues are strictly conserved, up to the level of a similar side chain conformation and the degree of conservation of ionisable residues -the ionic track- is also high (22). In spite of the very similar architecture of both glycoporins in the constriction zone, the cross-section of ScrY is larger than LamB, due to three major exchanges in the sucrose porin (residues 192, 201 and 204, in ScrY), explaining the much higher single channel conductance of ScrY in 1 M KCl as compared to LamB (1.4 versus 0.15 nS) (64). In consequence, while in the maltose-transporting porin LamB, the flow of the bulky sucrose molecules is severly impeded (mainly by sterical hindrance), in the larger sucrose porin, the sucrose molecules have the ability to slide easily through the channel, allowing thus to transport sucrose rapidly.

The structure of LamB and of ScrY show thus a large degree of structural conservation; the gross differences between both "glycoporins" lying in the size and sequence of their external loops which tend to tilt over the pore vestibule in both porins.

4. PARTICIPATION OF THE SURFACE LOOPS TO SUGAR TRANSPORT

The entry of the pore is covered by loops from the extracellular side that form a sort of “umbrella” (Figure 3). The presence of this umbrella, mainly composed of loops L4, L6 and L9, raises two major questions: i) does it constitute a selective filter or a

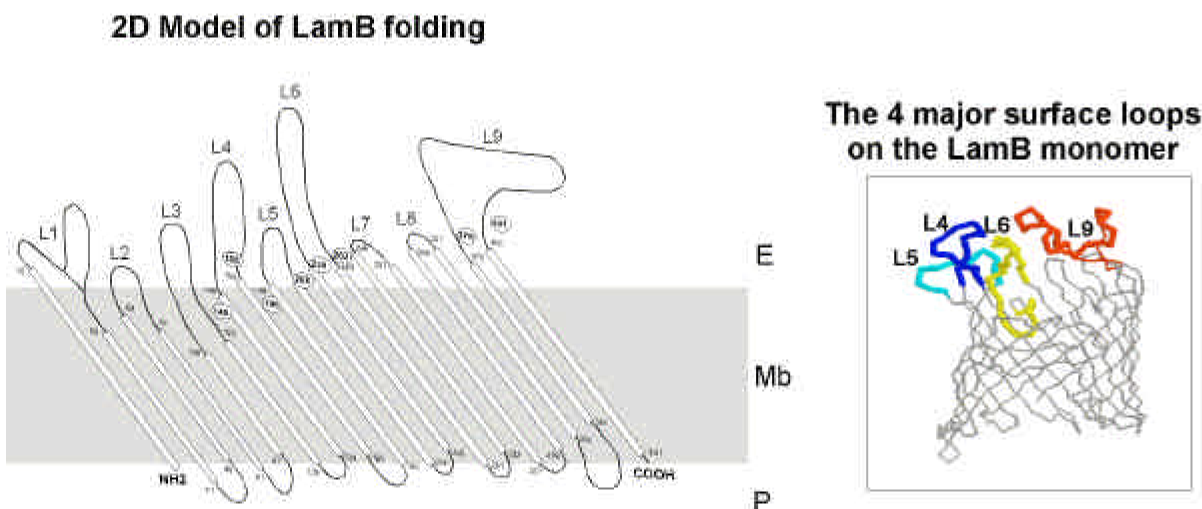

Figure 3. The surface loops. Left part: 2D model of LamB folding. L1 to L9 refer to the 9 cell surface loops. E means exterior; P, periplasm; Mb outer membrane (the region of the membrane is shaded in gray). The ß strands are represented by white rectangles (numbers indicate amino acid position). Right part: Side view of a 3D model of the LamB monomer (backbone). Surface loops L4 (blue), L5 (cyan), L6 (yellow) and L9 (red) are in thick.

barrier at the entry of the pore? ii) does it contain a primary weak binding site for sugars at the entry of the pore?

The role of these loops on sugar transport and binding was addressed by constructing and analysing the *in vivo* and *in vitro* properties of loop deletion mutants (49, 50, 51). The data obtained fit with a model where the maltooligosaccharides bind in the orientation where the non-reducing end points towards the periplasm and where absence of some of the loops lead to obstruction of the channel from the outside.

5. IN VIVO INTERACTIONS BETWEEN LOOPS L4, L5 AND L6

In vivo, single and double loop deletions including ?L4, ΔL5, ΔL6 abolished maltoporin functions, whereas the triple deletion ?L4+ΔL5+ΔL6 did not. Moreover, while deletion of the central variable portion of loop L9 affected only moderately maltoporin function, its combination with the double deletion of loops L4 and L6 strongly impaired maltoporin function (51). The *in vivo* transport and binding data suggested that the three surface loops L4, L5 and L6 of LamB interact together *in vivo*, but do not interact with loop L9, the most distal surface loop of the protein. One simple explanation for the loss of maltoporin functions in the single deletions of L4, L5 or L6, is that the conformation of each of the loops depends on the presence of the two other loops. Without one of the loops, at least one of the remaining ones would collapse and prevent access of the sugar to its binding site located within the channel. The *in vitro* results are in perfect agreement with this hypothesis. This idea also fits well with the observation that these three loops are packed together on the X-ray model. The absence of the three loops L4, L5 and L6 is not deleterious to maltoporin function. However, the simultaneous loss of L4, L6 and most of L9 strongly impaired maltoporin function *in vivo,* which suggests that these major surface loops of LamB contribute to some extent to the capture of the sugar molecules at the entry of the channel.

At this stage, it is interesting to compare the properties of the loop deletion mutants with earlier results on chromosomally-encoded single amino-acid substitutions in LamB. Some of the mutations conferring tight resistance to λ were also shown to significantly affect maltoporin function (36). In particular, changes at amino-acid positions 148 (located one residue upstream of the first residue of loop L4), 245, 247, 249 and 250 (all four located in the proximal portion of loop L6) were shown to provoke a strong decrease of maltoporin function. Initial rates of maltose uptake were strongly reduced and starch binding was completely abolished. The effects of this single amino-acid substitution on maltoporin function can be explained either by direct or indirect modifications of loops L4 and/or L5 and L6. The mutation could for example provoke the collapse of loop L4, thereby blocking the entry of the LamB channel. The mutation could also modify the orientation of the adjacent loop L4, so as to provoke a destabilization of the neighboring loop(s) (L5, L6), leading finally to the occlusion of the pore. Remarkably, the crystal structure shows that all these residues are located at the “mouth” of the pore.

Thus, even if the multi-loop structure does not play an active role in maltodextrin capture, its integrity seems essential for (optimal) maltoporin functions.

6. IN VITRO STUDIES ON LOOP DELETION MUTANTS

In vitro, reconstitution experiments, performed with lipid bilayer membranes in the presence of purified mutant proteins (51, 65), showed that the deletion of one of the loops L4 or L6 resulted in a considerable reduction of carbohydrate binding from the external side, whereas it had only little influence on the carbohydrate binding from the periplasmic side.

In addition, the results of amylose column purification and asymetric titration experiments suggest that carbohydrates enter the LamB channel from the cell surface side with the non-reducing end in advance (51). Reconstitution experiments with lipid bilayer membranes proved that the different loop deletion mutants formed ion-permeable channels in lipid bilayer membranes. The single-channel conductance of these channels was very similar to that of wild type maltoporin (6).

The loop deletion mutants LamBΔL4 and LamBΔL6 led to channels which exhibit asymmetry concerning carbohydrate binding from both sides of the channel. The binding of the reducing carbohydrate end from the periplasmic side of the channel appeared to be essentially unchanged, whereas the affinity of the binding site towards the non-reducing end from the external surface was drastically reduced. Two, non mutually exclusive, hypotheses can explain the mutant channel asymmetry. The first one consists in saying that the surface exposed loops are needed to direct the carbohydrates to the binding site localized inside the channel (53, 55). However, this explanation is not totally satisfactory because a LamB mutant which lacks all major surface loops (L4, L6, L9) has approximately the same *in vitro* carbohydrate binding affinity as wild type maltoporin and does not exhibit any binding asymmetry (51). The second hypothesis proposes that the deletion of certain surface exposed loops such as L4 or L6 leads to a collapse of the complex architecture of the surface-exposed structure which covers the external surface of the channel. This would then cause a sterical hindrance for the entrance of carbohydrates to the binding site inside the channel.

7. PROPOSED MECHANISM OF SUGAR TRANSLOCATION

In vitro measurement of malto-oligosaccharides binding to LamB suggest that the sugar molecules hit the channel many times before they are bound to the internal binding site. This is probably to due to the fact that the sugar must be: i) properly oriented -i.e. the non-reducing end in advance; and ii) in alignment with the channel axis, before it can bind efficiently. In bacteria, the sugar bound from the cell surface has two possibilities: to move back or to move further into the channel. The surface loops probably participate to the correct orientation of the sugar molecules via one or several discrete binding sites. The periplasmic space represents the bottleneck that converts the transport through the LamB channel into a vectorial one when sugars have reached the periplasm. Indeed, once in the periplasm, sugars bind with high affinity (K_D around 1 ?M) to maltose-binding protein (MBP). Subsequently, the MBP-sugar complexes reach the inner membrane transporter MalF-G-K_2, an ABC transporter made of two integral membrane proteins (MalF and MalG) and two copies of the ATP-hydrolyzing peripheral subunit MalK (see the reviews by Amy Davidson and Elie Dassa, in this isssue). Since neither the sugar nor any of the proteins involved in the passage across the inner membrane are phosphorylated during transport, it is commonly proposed that the energy gained through ATP hydrolysis is transferred to protein conformational changes in MalF and MalG, followed by the MBP-triggered release of the sugar, resulting in its unidirectional translocation through the membrane.

7.1. Role of the L9 loop

The thorough analysis of the L9 loop of LamB (49) led us to propose the existence of a primary weak binding site for sugars in the proximal portion of L9 (Figure 4) which would participate in sugar concentration and orientation at the rim of the channel.

Our results are consistent with the hypothesis that the functions of loop L9, both in the specific facilitation of maltodextrin uptake and the control of non-specific permeability, arise from the upstream conserved flanking sequence that contain charged and aromatic residues. The charged residues clustered in the proximal portion of L9 may create a sugar binding domain for maltose and maltodextrin, possibly together with another part of the protein. A biochemical rationale exists for the participation of this region of the protein in maltose binding: the charged and aromatic residues are capable of sugar binding by hydrogen bonding or ring stacking. However, in the structure, residues E374 and W376 are not exposed to the pore lumen: E374 forms a slat-bridge with an arginine and is covered by L3; and W376 is very central to the loop L9 protrusion (T. Shirmer, personal communication). Therefore, indirect structural effects caused by some of the mutations cannot be excluded.

The fact that this portion of LamB was not implicated in binding when maltoporin crystals were soaked with maltotriose (20), might be due : i) to a low affinity of this binding site ; ii) to a sterical hindrance provoked by trimer-trimer contacts within the crystals that prevent normal maltose binding; or iii) to the fact that the loop structure is modified by crystal contacts. The establishment of a binding site at the rim of the LamB vestibule umbrella, may provide a driving force for passage of linear maltodextrin into the channel.

We previously proposed that point or small insertions or deletions affecting the specific diffusion of maltodextrin through LamB could influence one or several of three main factors: accessibility from the outside of the pore, pore size, or affinity of the binding site. Our data are compatible with the idea that passage of maltose and maltodextrins through the LamB pore occurs in three major steps (Figure 5): i) the proximal portions of the three major surface loops of LamB L4, L6, and L9 participate to the first step of dextrins binding at the rim of the channel (the upstream conserved region of the D-loop containing the major binding site); ii) the sugar is transferred to the binding site, in the channel constriction zone below (this second site being composed of the greasy slide and polar track, at the channel lining); iii) the sugars are finally released to the other side of the constriction zone in the periplasm, where they can be taken up by the maltose-binding protein and addressed to the inner membrane transporter.

8. CONCLUDING REMARKS

One of the major aims of this review was to illustrate how genetic, biochemical and crystallographic approaches converged to elaborate a comprehensive view of the mechanism of sugar translocation across LamB. Indeed, while crystallographic studies helped define at the atomic level the nature of the interactions between the sugar molecules and the protein; the genetic and biochemical analyses of defined mutants provided

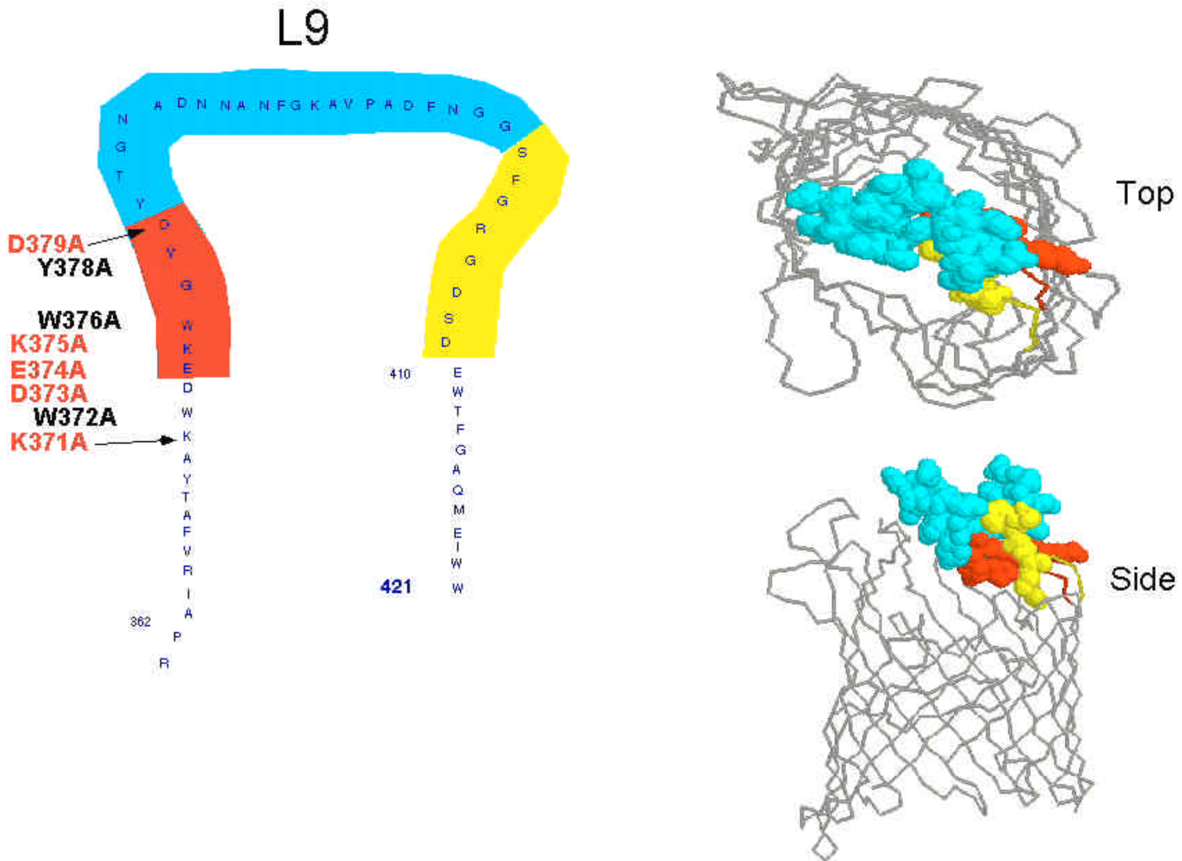

Figure 4. The L9 loop. Left part: 2D model and amino acid sequence of the L9 loop region. The mutagenized charged and aromatic residues of the proximal portion of L9 are indicated. Numbers indicate amino acid positions. Right part: Top and Side views of LamB monomer (backbone). Residues of L9 are in space-filled representation. The proximal and distal portions of L9 are in red and yellow, respectively. The large central variable portion of L9 is in blue (or cyan).

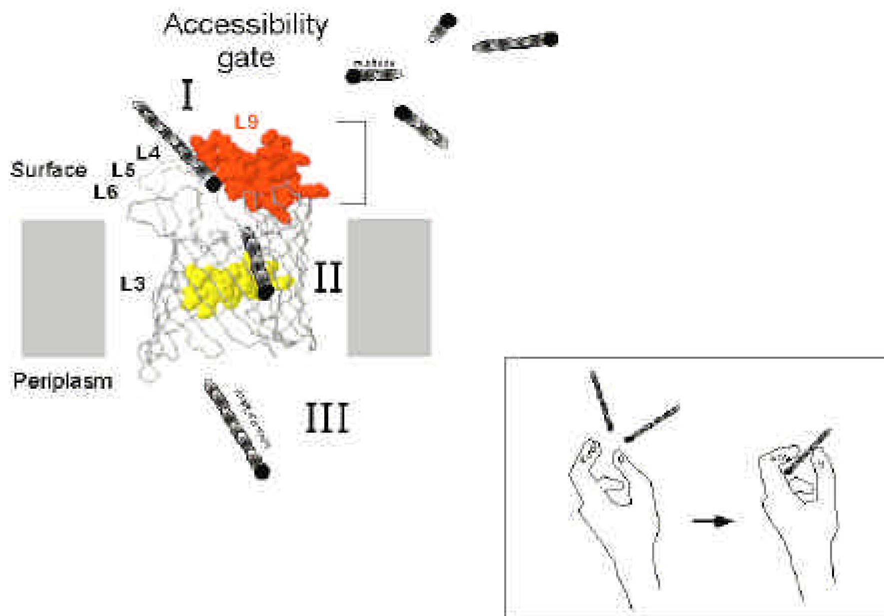

Figure 5. Mechanism of maltodextrin translocation through LamB. Side views of LamB monomer (backbone). Maltodextrin are translocated in three steps consisting of : I - the orientation of the sugar molecules with the non-reducing end in advance (black circles) mediated on the on hand, by the surface loops L4, L5, L6 and on the other hand by L9 (existence of a weak binding site at the rim of the channel?). II - the gliding through the channel mediated by interaction with the greasy slide (hydrophobic stacking to the aromatic residues) and the polar track (ionic interactions with the charged residues). III – the release in the periplasm. The central loop L3 (yellow) and loop L9 (red) are in space-filled representation. L1 to L9 refer to the nine cell surface-exposed loops. Boxed to the bottom right: a cartoon representing the LamB monomer as a hand where the major surface loops form fingers helping capture and proper access of the linear sugars to the channel interior.

information on the kinetic parameters influencing sugar translocation. However, in spite of these extensive studies on LamB during the past two decades, the actual mechanism of maltodextrin transport is not yet fully understood. At this stage, it is tempting to propose that the major surface loops of LamB (L4, L6 and also L9) may constitute an accessibility gate that controls the access of the sugars to the LamB channel. Since the length of a maltoheptaose molecule is of about 25 Å and the diameter of the constriction zone within the LamB channel of about only 5 Å, correct orientation of the sugar moleclules is obviously an absolute prerequisite for their efficient translocation inside the LamB channel (66). Interactions of the sugars with these LamB cell surface domains may participate in capture and correct orientation of sugars. The exact nature of this accessibility gate is still an open question. Its functioning could involve its conformation, a weak binding capacity or even perhaps movements of the corresponding loops.

9. ACKNOWLEDGMENTS

I have a respectful thought for the memory of Maurice Hofnung who was a pioneer in the studies on LamB and who inspired an important part of the work and ideas summarized in this review. I thank Colin Tinsley for careful reading of the manuscript. Work done in the laboratory of Maurice Hofnung was supported by the CNRS.

10. REFERENCES

1. Nikaido, H., & M. Vaara. Molecular basis of bacterial outer membrane permeability. *FEMS Microbiol Rev* 49, 1-32 (1985)

2. Nikaido, H., & M. H. J. Saier. Transport proteins in bacteria: common themes in their design. *Science* 258, 936-942 (1992)

3. Benz, R., A. Schmid, T. Nakae, & G. H. Vos-Scheperkeuter. Pore formation by LamB of *E. coli* in lipid bilayer membranes. *J Bacteriol* 165, 978-986 (1986)

4. Dargent, B., A. Charbit, M. Hofnung, & F. Pattus. Effect of point mutations on the *in vivo* pore properties of maltoporin, a protein of *E. coli* outer membrane. *J Mol Biol* 201, 497-506 (1988)

5. Maier, C., E. Bremer, A. Schmid, & R. Benz. Pore-forming activity of the Tsx protein from the outer membrane of *Escherichia coli.* Demonstration of a nucleoside-specific binding site. *J Biol Chem* 263, 2493-2499 (1988)

6. Benz, R., & R. E. Hancock. Mechanism of ion transport through the anion-selective channel of the *Pseudomonas aeruginosa* outer membrane. *J Gen Physiol* 89, 275-295 (1987)

7. Hancock, R. E., K. Poole, & R. Benz. Outer membrane protein P of *Pseudomonas aeruginosa*: regulation by phosphate deficiency and formation of small anion-specific channels in lipid bilayer membranes. *J Bacteriol* 150, 730-738 (1982)

8. Ferenci, T., & W. Boos. The role of the *Escherichia coli* lambda receptor in the transport of maltose and maltodextrins. *J Supramol Struct* 13, 101-116 (1980)

9. Szmelcman, S., & M. Hofnung. Maltose transport in *Escherichia coli* K12. Involvement of the bacteriophage lambda receptor. *J Bacteriol* 124, 112-118 (1975)

10. Werts, C., V. Michel, M. Hofnung, & A. Charbit. Adsorption of bacteriophage lambda on the LamB protein of *E. coli* K12 : point mutations in gene *J* of lambda responsible for extended host range. *J Bacteriol* 176, 941-947 (1994)

11. Charbit, A., & M. Hofnung. Isolation of different bacteriophages using the LamB protein for adsorption on *Escherichia coli* K12. *J Virol* 53, 667-671 (1985)

12. Hazelbauer, G. L. Role of the receptor for bacteriophage lambda in the functioning of the maltose chemoreceptor of *Escherichia coli. J Bacteriol* 124, 119-126 (1975)

13. Hofnung, M., A. Jezierska, & C. Braun-Breton. *lamB* mutations in *E. coli* K12: growth of lambda host range mutants and effect of nonsense suppressors. *Mol Gen Genet* 145, 207-213 (1976)

14. Cowan, S. W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. A. Pauptit, J. N. Jansonius, & J. P. Rosenbusch. Crystal structures explain functional properties of two *E. coli* porins. *Nature* 358, 727-733 (1992)

15. Dutzler, R., G. Rummel, S. Alberti, S. Hernandez-Alles, P. Phale, J. Rosenbusch, V. Benedi, & T. Schirmer. Crystal structure and functional characterization of OmpK36, the osmoporin of *Klebsiella pneumoniae. Structure Fold Des* 7, 425-434 (1999)

16. Hirsch, A., K. Diederichs, J. Breed, K. Saxena, O. M. Richter, B. Ludwig, & W. Welte. The structure of porin from *Paracoccus denitrificans* at 3.1 A resolution. *FEBS Lett* 404, 208-210 (1997)

17. Kreusch, A., A. Neubüser, E. Schiltz, J. Weckesser, & G. E. Schulz. Structure of the membrane channel porin from *Rhodopseudomas blastica* at 2.0 Å resolution. *Protein Sci* 3, 58-63 (1994)

18. Weiss, M. S., A. Kreusch, E. Schiltz, U. Nestel, W. Welte, J. Weckesser, & G. E. Schulz. 1991. The structure of porin from *Rhodobacter capsulatus* at 1.8 Å resolution. *FEBS Lett* 280:379-382.

19. Zeth, K., K. Diederichs, W. Welte, & H. Engelhardt. Crystal structure of Omp32, the anion-selective porin from *Comamonas acidovorans*, in complex with a periplasmic peptide at 2.1 A resolution. *Structure Fold Des* 8, 981-992 (2000)

20. Schirmer, T., T. Keller, Y. F. Wang, & J. P. Rosenbush. Structural basis for sugar translocation through maltoporin channels at 3.5 Å resolution. *Science* 267, 512-514 (1995)

21. Meyer, J. E. W., M. Hofnung, & G. E. Schulz. Structure of maltoporin from *Salmonella typhimurium* ligated with a nitrophenyl-maltotrioside. *J Mol Biol* 266, 761-775 (1997)

22. Forst, D., W. Welte, T. Wacker, & K. Diederichs. Structure of the sucrose-specific porin ScrY from *Salmonella typhimurium* and its complex with sucrose. *Nature Struc Biol* 5, 37-46 (1998)

23. Boos, W., & H. Shuman. Maltose/maltodextrin system of *Escherichia coli*: transport, metabolism, and regulation. *Microbiol Mol Biol Rev* 62, 204-229 (1998)

24. Randall-Hazelbauer, L., & M. Schwartz. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. *J Bacteriol* 116, 1436-1446 (1973)

25. Thirion, J. P., & M. Hofnung. On some genetic aspects of phage lambda resistance in *E. coli* K12. *Genetics* 71, 207-216 (1972)

26. Szmelcman, S., & M. Schwartz. Maltose transport in *Escherichia coli* K12. *Eur J Biochem* 65, 13-19 (1976)

27. von Meyenburg, K., & H. Nikaido. Outer membrane of gram-negative bacteria. XVII. Secificity of transport process catalyzed by the lambda-receptor protein in *Escherichia coli. Biochem Biophys Res Commun* 78, 1100-1107 (1977)

28. Luckey, M., & H. Nikaido. Specificity of diffusion channels produced by lambda receptor protein of *E. coli*. *Proc Natl Acad Sci* (USA) 77, 167-171 (1980)

29. Bavoil, P., C. Wandersman, M. Schwartz, & H. Nikaido. A mutant form of maltose-binding protein of *Escherichia coli* deficient in its interaction with the bacteriophage lambda receptor protein. *J Bacteriol* 155, 919-921 (1983)

30. Neuhaus, J. M., H. Schindler, & J. P. Rosenbusch. The periplasmic maltose-binding protein modifies the channel-forming characteristics of maltoporin. *Embo J* 2, 1987-1991 (1983)

31. Brass, J. M., M. D. Manson, & T. J. Larson. Transposon *Tn*10-dependent expression of the *lamB* gene in *Escherichia coli. J Bacteriol* 159, 93-99 (1984)

32. Freundlieb, S., U. Ehmann, & W. Boos. Facilitated diffusion of p-Nitrophenyl-? -D-maltohexaoside through the outer membrane of *Escherichia coli*. *J Biol Chem* 263, 314-320 (1988)

33. Gehring, K., A. Charbit, E. Brissaud, & M. Hofnung. Bacteriophage lambda receptor site on the *Escherichia coli* K-12 LamB protein. *J Bacteriol* 169, 2103-2106 (1987)

34. Francis, G., Brennan, S. Stretton, & T. Ferenci. Genetic mapping of starch -and lambda- receptor sites in maltoporin: identification of substitutions causing direct and indirect effects on binding sites by cysteine mutagenesis. *Mol Microbiol* 5, 2293-2301 (1991)

35. Charbit, A., C. Werts, V. Michel, P. E. Klebba, P. Quillardet, & M. Hofnung. A role of residue 151 of LamB in bacteriophage lambda adorption: possible steric effect of amino acid substitutions. *J Bacteriol* 176, 3204-3209 (1994)

36. Charbit, A., K. Gehring, H. Nikaido, T. Ferenci, & M. Hofnung. Maltose transport and starch binding in phage resistant point mutants of maltoporin fuctionnal and topological implications. *J Mol Biol* 201, 487-496 (1988)

37. Ferenci, T., & K. S. Lee. Channel architecture in maltoporin: dominance studies with *lamB* mutations influencing maltodextrin binding provide evidence for independent selectivity filters in each subunit. *J Bacteriol* 171, 855-861 (1989)

38. Heine, H. G., G. Francis, K. S. Lee, & T. Ferenci. Genetic analysis of sequences in maltoporin that contribute to binding domains and pore stucture. *J Bacteriol* 170, 1730-1738 (1988)

39. Orlik, F., C. Andersen, & R. Benz. 2002. Site-Directed Mutagenesis of Tyrosine 118 within the Central Constriction Site of the LamB (Maltoporin) Channel of *Escherichia coli*. I. Effect on Ion Transport. *Biophys J* 82, 2466-2475 (2002)

40. Desaymard, C., M. Debarbouillé, M. Jolit, & M. Schwartz. Mutations affecting antigenic determinants of an outer membrane protein of *Escherichia coli*. *Embo J* 5, 1383-1388 (1986)

41. Gabay, J., S. Benson, & M. Schwartz. Genetic mapping of antigenic determinants on a membrane protein. *J Biol Chem* 258, 2410-2414 (1983)

42. Gabay, J., & M. Schwartz. Monoclonal antibodies as a probe for structure and function of *Escherichia coli* outer membrane protein. *J Biol Chem* 257, 6627-6630 (1982)

43. Schenkman, S., E. Couture, & M. Schwartz. Monoclonal antibodies reveal LamB antigenic determinants on both faces of the *Escherichia coli* outer membrane. *J Bacteriol* 155, 1382-1392 (1983)

44. Schenkman, S., A. Tsugita, M. Schwartz, & J. P. Rosenbusch. Topology of phage lambda receptor protein. Mapping targets of proteolytic cleavage in relation to binding sites for phage or monoclonal antibodies. *J Biol Chem* 259, 7570-7576 (1984)

45. Charbit, A., J. C. Boulain, A. Ryter, & M. Hofnung. Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope; Expression at the cell surface. *Embo J* 5, 3029-3037 (1986)

46. Charbit, A., J. Ronco, V. Michel, C. Werts, & M. Hofnung. Permissive sites and the topology of an outer-membrane protein with a reporter epitope. J *Bacteriol* 173, 262-275 (1991)

47. Newton, S. M. C., P. E. Klebba, V. Michel, M. Hofnung, & A. Charbit. Topology of the membrane protein LamB by epitope tagging and a comparison with the X-ray model. *J Bacteriol* 178, 3447-3456 (1996)

48. Charbit, A., J. Wang, V. Michel, & M. Hofnung. A cluster of charged and aromatic residues in the C-terminal

portion of maltoporin participates in sugar binding and uptake. *Mol Gen Genet* 260, 185-92 (1998)

49. Klebba, P., E., S. Newton, M.C., A. Charbit, V. Michel, D. Pérrin, & D. Hofnung. Further genetic analysis of the C-terminal loop region in *Escherichia coli* maltoporin. *Res Microbiol* 148, 375-387 (1997)

50. Klebba, P. E., M. Hofnung, & A. Charbit. Mechanism of maltodextrin transport through the sugar-specific porin, LamB. *Embo J* 13, 4670-4675 (1994)

51. Andersen, C., C. Bachmeyer, H. Tauber, R. Benz, J. Wang, V. Michel, S. M. Newton, M. Hofnung, & A. Charbit. *In vivo* and *in vitro* studies of major surface loop deletion mutants of the *Escherichia coli* K12 maltoporin: contribution to maltose and maltooligosaccharide transport and binding. *Mol Microbiol* 32, 851-867 (1999)

52. Benz, R., G. Francis, T. Nakae, & T. Ferenci. Investigation of the selectivity of maltoporin channels using mutant LamB proteins: mutations changing the maltodextrin binding site. *Biochimica and Biophysica Acta* 1104, 299-307 (1992)

53. Jordy, M., C. Andersen, K. Schulein, T. Ferenci, & R. Benz. Rate constants of sugar transport through two LamB mutants of *Escherichia coli*: comparison with wild-type maltoporin and LamB of *Salmonella typhimurium*. *J Mol Biol* 259, 666-678 (1996)

54. Dumas, F., R. Koebnik, M. Winterhalter, & P. Van Gelder. Sugar transport through maltoporin of *Escherichia coli*. Role of polar tracks. *J Biol Chem* 275, 19747-19751 (2000)

55. Dutzler, R., Y. F. Wang, P. Rizkallah, J. P. Rosenbusch, & T. Schirmer. Crystal structures of various malto-oligosaccharides bound to maltoporin reveal a specific sugar translocation pathway. *Structure* 4, 127-34 (1996)

56. Van Gelder, P., F. Dumas, I. Bartoldus, N. Saint, A. Prilipov, M. Winterhalter, Y. Wang, A. Philippsen, J. P. Rosenbusch, & T. Schirmer. Sugar transport through maltoporin of *Escherichia coli*: role of the greasy slide. *J Bacteriol* 184, 2994-2999 (2002)

57. Clément, J-M., &d M. Hofnung. Gene sequence of the lambda receptor, an outer membrane protein of *E. coli* K12. *Cell* 27, 507-514 (1981)

58. Schneider, E., S. Freundlieb, S. Tapio, & W. Boos. Molecular characterization of the MalT-dependent periplasmic alpha-amylase of *Escherichia coli* encoded by *malS*. *J Biol Chem* 267, 5148-5154 (1992)

59. Benz, R., A. Schmid, & G. H. Vos-Scheperkeuter. Mechanism of sugar transport through the sugar-specific LamB channel of *Escherichia coli* outer membrane. *J Membr Biol* 100, 21-29 (1987)

60. Boos, W., U. Ehmann, H. Forkl, W. Klein, M. Rimmele, & P. Postma. Trehalose transport and metabolism in *Escherichia coli. J Bacteriol* 172, 3450-3461 (1990)

61. Klein, W., & W. Boos. Induction of the lambda receptor is essential for effective uptake of trehalose in *Escherichia coli*. *J Bacteriol* 175, 1682-1686 (1993)

62. Schmid, K., R. Ebner, K. Jahreis, J. W. Lengeler, & F. Titgemeyer. A Sugar-Specific Porin, ScrY, Is Involved in Sucrose Uptake in Enteric Bacteria. *Mol Microbiol* 5, 941-950 (1991)

63. Schülein, K., K. Schmid, & R. Benz. The sugar-specific outer membrane channel ScrY contains functional characteristics of general diffusion pores and substrate-specific porins. *Mol Microbiol* 5, 2233-2241 (1991)

64. Schülein, K., C. Andersen, & R. Benz. The deletion of 70 amino acids near the N-terminal end of the sucrose porin Scry causes its functional similarity to LamB *in vivo* and *in vitro*. *Mol Microbiol* 17, 757-767 (1995)

65. Andersen, C., B. Schiffler, A. Charbit, & R. Benz. PH-induced collapse of the extracellular loops closes Escherichia coli maltoporin and allows the study of asymmetric sugar binding. *J Biol Chem* 277, 41318-25 (2002)

66. Dutzler, R., T. Schirmer, M. Karplus, & S. Fischer. Translocation mechanism of long sugar chains across the maltoporin membrane channel. *Structure* (Camb) 10, 1273-84 (2002)

Key Words: LamB, porins, maltoporin, lambda receptor, sugar transport, Gram-negative bacteria, Review

Send correspondence to: Dr Alain Charbit, Laboratoire de Microbiologie; INSERM U-570, Faculté de Médecine Necker, 156, rue de Vaugirard, 75730 Paris Cedex 15, France, Tel.: 33 1 - 40 61 53 76 Fax: 33 1 - 40 61 55 92, E-mail: charbit@necker.fr