[Frontiers in Bioscience 8, s472-483, May 1, 2003]

ON THE MECHANISM OF SOLUTE UPTAKE IN PSEUDOMONAS

Sandeep Tamber and Robert E.W. Hancock

Department of Microbiology and Immunology, University of British Columbia, #300 6174 University Boulevard, Vancouver, BC, Canada V6T 1Z3

TABLE OF CONTENTS

1. ABSTRACT

Pseudomonas species have over 300 known and putative nutrient uptake systems enabling them to metabolize a large number of organic compounds, and thus inhabit many diverse ecological niches. The outer membrane of these organisms acts as a semi-permeable barrier, excluding many classes of potentially toxic molecules from the cell. Nutrients use specialized water-filled channels called porins to traverse this barrier. Entry into the cell is mediated by one of four classes of cytoplasmic membrane transporters: glycerol facilitators, phosphotransferase systems, primary active transporters, and secondary active transporters. The class of transporter used is dependent on the environmental conditions, as well as the type and concentration of solute. The recent advances in elucidating the structures and functional mechanisms of these uptake systems will be discussed in this review.

2. INTRODUCTION

Pseudomonads are renowned for their ability to metabolize over 80 organic carbon compounds including sugars, amino acids, carboxylic acids, simple aromatics, parafinic hydrocarbons, and terpenes (1). Another hallmark of the genus is its intrinsic resistance to many antibiotics, detergents, dyes and other toxic compounds due to the low permeability of Pseudomonas outer membranes combined with secondary resistance mechanisms such as efflux and enzymatic hydrolysis that take advantage of the slow movement of compounds into the cell. Therefore, one of the more intriguing aspects of Pseudomonas physiology is how these organisms take up the myriad of structurally diverse compounds found in their environments despite the barrier properties of the outer membrane.

Genes encoding transporters account for 10% of the P. aeruginosa genome (2). Different substrates are taken up by different transporters. In addition, some substrates can have more than one transport system that differ from each other in mode of action, transport kinetics, and/or expression patterns, allowing the organism to optimize its uptake capabilities depending on the environmental conditions.

This review will begin by discussing the barrier and permeability properties of the Pseudomonas outer membrane. Then the four major classes of transporters housed in the cytoplasmic membrane: glycerol facilitators, phosphotransferase systems, primary active transporters, and secondary active transporters (3) will be reviewed. The mechanistic aspects of well-characterized transporters will be emphasized rather than listing the various Pseudomonas transporters. These findings will be generalized to the pseudomonads, particularly P. aeruginosa as its genome has been sequenced, and therefore provides the most complete catalogue of these organisms' transport capabilities.

3. ROLE OF THE OUTER MEMBRANE IN UPTAKE

3.1. Barrier Properties

As the outer membrane is at the interface of the environment and the cell interior, it plays a key role in determining which compounds enter the cell. The outer membrane is a semi-permeable barrier to hydrophobic substances and in collaboration with efflux systems limits the passage of small charged, hydrophobic, and amphipathic molecules, giving Gram-negative bacteria a selective advantage over their Gram-positive counterparts

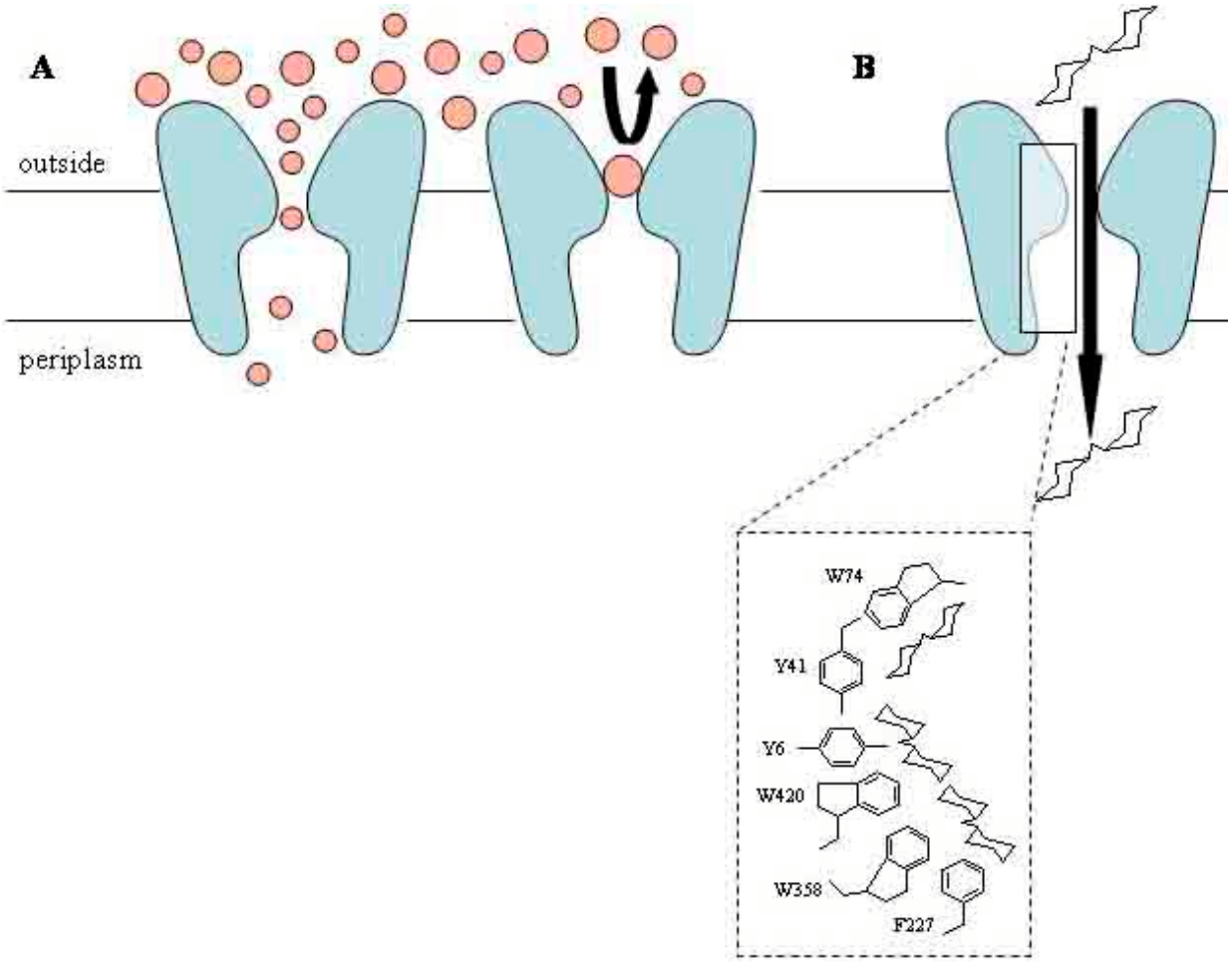

Figure 1. The passage of solutes through porins. **A**. Solutes of a particular size, charge, and solubility freely diffuse through general porins as shown on the left. Molecules not meeting these requirements may enter the external vestibule of the porin but cannot pass the eyelet region and will eventually diffuse out of the channel as is shown on the right. **B**. Specific porins have binding sites for their substrates as is shown with the LamB maltodextrin specific porin of E. coli. This channel has six contiguous aromatic amino acid residues (the greasy slide) that stabilize and facilitate the movement of the pyranose rings as they travel down the channel.

in environments rich in antibiotics, detergents, or other toxic compounds. Unlike other biological membranes, the outer membrane is an asymmetric bilayer. The inner leaflet is composed of phospholipids and is similar in composition to that of the cytoplasmic membrane. The outer leaflet may have a few phospholipid molecules but is mostly composed of lipopolysaccharide (LPS). It is the chemical properties of LPS that largely determine the barrier properties to the outer membrane. Pathogens that produce a truncated form of LPS called lipooligosaccharide (LOS) are more susceptible to hydrophobic antibiotics and detergents than LPS producing pathogens (4). Also studies of Escherichia coli and Salmonella enterica serovar Typhimurium mutants with defects in LPS synthesis, have shown that strains lacking the core region of LPS are much more (approximately 30 to 100 fold) sensitive to hydrophobic antibiotics, dyes, and detergents (5) than the wild-type strains. The phosphates in the core region of LPS bind magnesium ions and form non-covalent electrostatic cross-bridges with adjacent LPS molecules. These phosphate moieties also contribute to the large net negative surface charge of the bacteria.

3.2. Selective Permeability

In addition to its role as a barrier, the outer membrane acts as a selectivity filter, permitting passage of small, hydrophilic solutes (6). This selective permeability is mediated by water filled protein channels called porins. Hydrophilic molecules up to 600 Da traverse these channels by simple diffusion, thus the kinetics of uptake are largely dependent on the concentration gradient of the solute. Also, porins are weakly ion selective due to the presence of charged amino acids at the mouth of the channel (7, 8), . There are four classes of porins, general, specific, gated, and efflux porins. General porins discriminate between solutes purely based on their physicochemical properties (i.e. size, charge, hydrophilicity, and concentration gradient). Specific porins have saturatable, stereospecific, substrate-binding sites and are often associated (perhaps transiently) with periplasmic

Table 1. Experimentally characterized uptake transporters in Pseudomonas aeruginosa

Name	PA number	Transporter Class	Substrates	References
OprB	PA3186	Specific porin	Glucose, carbohydrates	58
OprD	PA0958	Specific porin	Basic amino acids, gluconate	59, 60
OprF	PA1777	General porin	Various small molecules, weakly cation selective	61
OprP	PA3279	Specific porin	Phosphate	62
OprO	PA3280	Specific porin	Polyphosphate	63
GlpF	PA3581	Facilitator	Glycerol	22
AotJMQP	PA0888-0892	ABC transporter	Arginine, ornithine	64
BraCDEFG	PA1070-1074	ABC transporter	Branched chain amino acids	55
GltBFGK	PA3187-3190	ABC transporter	Glucose	53
PstABC	PA5366-5368	ABC transporter	Phosphate	65
SpuDEFGH	PA0300-0304	ABC transporter	Polyamines	66
ArcD	PA5170	Secondary	Arginine/ornithine antiporter	67
BraB	PA1590	Secondary	Branched chain amino acid/Na^+ symport	56
BraZ	PA1970	Secondary	Branched chain amino acid/H^+ symport	57

substrate binding proteins and cytoplasmic membrane transporters. These porins are required for growth at low substrate concentrations because the diffusion of substrates in these conditions is accelerated. Otherwise, the periplasmic binding proteins of high affinity cytoplasmic membrane transporters would be at most 5% saturated (5, 9), making outer membrane permeability rate limiting for growth. In addition, like general porins, specific porins can also act as selectivity filters by taking up low levels of structurally unrelated compounds (10). Gated porins take up large molecules such as iron-siderophore complexes and vitamin B_{12}. These channels specifically bind their substrates, and with energy input via the TonB protein, open and allow the substrate to pass through (11). Efflux porins, also called channel tunnels, are involved together with inner membrane components (pumps and linker proteins) in excretion of toxic molecules from the cell, helping to determine the barrier properties of the outer membrane (6, 12). These proteins will not be further discussed in this review, as it concerns uptake.

The crystal structures of several porins from different organisms have been solved and they reveal that despite very little primary sequence similarity, porins share a remarkably similar 3D structure (13-15). Porins are generally trimeric molecules that form beta-barrels with 8 to 22 beta-strands. The transmembrane beta-strands are amphipathic; the hydrophobic amino acid side chains point towards the membrane interior while the polar amino acid side chains face the interior of the channel, providing a hydrophilic environment. The beta-strands range from 12 to 25 amino acids in length and are usually capped by aromatic residues that are believed to anchor the porin in the membrane. Unlike general and specific porins, gated porins are monomers. Also, these channels have an N-terminal four stranded beta-sheet domain called the plug that folds into the channel interior to form part of the gating mechanism (16, 17).

The beta-strands of the barrels are connected at the periplasmic face by short turns ranging from 1 to 12 amino acids in length and by long, hydrophilic loops ranging from 2 to 46 amino acids in length (16). It is the loop regions that largely determine the properties of the porins and therefore the permeability characteristics of the membrane. Some loops (e.g. L2) fold away from the channel and interact with the LPS, or other monomers of the porin, thus stabilizing the structure. Other loops fold into the channel and constrict the opening and may form part of the substrate-binding site, such as L3 and L2 of OprD, the basic amino acid specific porin of P. aeruginosa (18).

A vertical cross-section of the general porin pore resembles an hourglass with three main regions, the external mouth and vestibule, the constriction zone or eyelet region, and the internal vestibule and exit (Figure 1A). The mouth of the pore acts as a coarse filter, preventing large, hydrophobic, potentially toxic molecules from entering the cell. It contains a large number of charged amino acids. In addition, some of the long, rigid, extracellular loops obstruct the opening both physically and electrostatically by contributing a number of charged residues to the region (7). The mouth region opens up to a conical shaped vestibule whose primary function is to present the constriction zone with a high concentration of pre-screened solutes, thereby increasing the overall rate of transport.

The constriction zone or eyelet ultimately determines which solutes enter the cell. Its properties are largely determined by one of the extracellular loops, L3, that folds into the eyelet constricting the diameter (to approximately 50 to 100 angstroms depending on the hydration state) and determines the maximal molecular weight and overall shape of permeable solutes. Also, a strong local electric field in this region produced by electrostatic interactions between the side chains of L3 and the barrel wall makes the entry of non-polar solutes energetically unfavourable. This local field is seen in all of the general porin structures solved to date, with the net charge of the pertinent residues in L3 being negative and the net charge of the residues in the pertinent region of the barrel wall being positive.

The internal vestibule and exit is the most conserved region among porin structures. Since its purpose is to allow the selected solutes to enter the cell with the greatest ease, it is wider and contains fewer charged residues than the external mouth. Also, the periplasmic

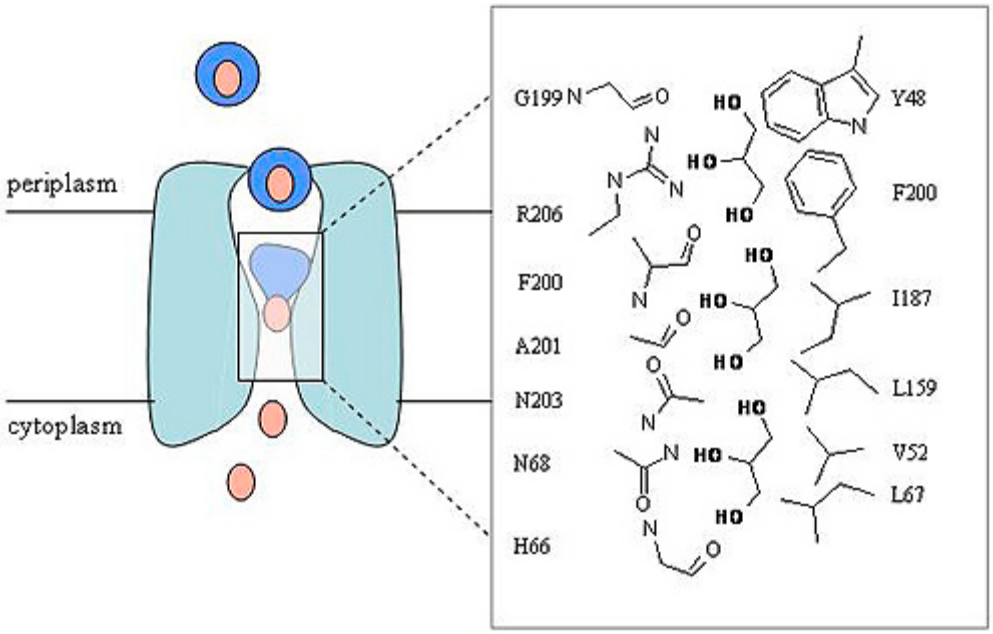

Figure 2. Glycerol uptake by the E. coli GlpF glycerol facilitator. The glycerol enters the channel via simple diffusion and loses its hydration shell as it passes through the constriction zone. However, the molecule is stabilized by forming non-covalent interactions with the amino acid side chains lining the channel wall, the hydroxyl moieties by the polar amino acids on one side to the channel, and the alkyl backbone by the non-polar amino acids lining the other side

turns are almost flush with the inner leaflet of the outer membrane, and therefore do not hinder the passage of solutes from entering the cell (19).

The functional mechanism of specific porins differs from that of the general porins. For example, the eyelet of the maltodextrin specific LamB porin of E. coli is constricted to a diameter of 50 angstroms by the folding of 2 additional loops into the channel interior (15). Substrate binding in this porin is aided by the presence of 6 contiguous aromatic amino acids, dubbed the greasy slide (Figure 1B), stretching from the external vestibule down to the internal (periplasmic) exit. As their name implies, these 6 amino acids form the path down which the pyranose rings of the maltodextrins travel. There are a high proportion of charged residues that line the channel wall but they are not segregated as they are in the general porins. The purpose of these residues termed the polar track is to form hydrogen bonds with the hydroxyl groups of the sugar and thus stabilize them (20).

3.3. Pseudomonas porins

The outer membrane of P. aeruginosa is approximately 13 fold less permeable than that of E. coli. This difference in permeability can be attributed in part to the porin composition of these organisms' outer membranes. The E. coli outer membranes contains a large number of general porins (OmpA, OmpC, OmpF), a few inducible or weakly active general porins (OmpE, OmpG, NmpE) and a few porins specific for large macromolecules (LamB- maltodextrins, Tsx- nucleotides). In contrast, the P. aeruginosa outer membrane only contains one weakly active general porin, OprF and a large number of specific porins (OprB, OprD, OprP, OprO, Table 1). In addition to these known specific porins, P. aeruginosa has a novel 19 member family of porins believed to be specific for a variety of Pseudomonas metabolites (6). Analysis of the partially sequenced genomes of P. putida, P. fluorescens, and P. syringae indicate that large paralogous families of porins are common to this genus.

4. ROLE OF THE CYTOPLASMIC MEMBRANE IN UPTAKE

4.1. Glycerol Facilitator

Glycerol facilitators are alpha-type channels (3) that facilitate the diffusion of small polyalcohols, such as glycerol, into the cell. These channels are interesting because of their exquisite substrate selectivity. They do not permit the passage of charged compounds through them, a feature essential for the maintenance of the electrochemical gradient across the membrane (21). P. aeruginosa is known to have a glycerol facilitator, GlpF (22) (PA3581). This facilitator is part of an operon encoding proteins involved in glycerol catabolism (www.pseudomonas.com) and is induced during growth on glycerol.

The crystal structure of the homologous E. coli GlpF channel with its substrate glycerol was recently solved at a 2.2 angstrom resolution (23). This channel is 80% similar to its homologe in P. aeruginosa and the elucidation of its structure has suggested the mechanism of glycerol uptake and ion exclusion.

In E. coli, GlpF is a tetramer composed of 4 channels. Each monomer displays an internal two-fold axis of symmetry and consists of six transmembrane alpha-helices and two half-membrane spanning helices in a right-handed helical bundle. One of the half helices enters the

membrane from the periplasmic face, the other from the cytoplasmic side. At their interface, they each contain a conserved asparagine-proline-alanine (NPA) motif that is important for substrate binding. These amino acids are arranged so that the proline rings of one half helix forms Van der Waals contacts with the proline and alanine of the other half helix, thus constraining the two asparagines so that their side chain amides are oriented towards the path of the glycerol. Also important for substrate binding is a cytoplasmic loop between transmembrane segments 2 and 3 that folds into the channel and provides the cytoplasmic vestibule with 3 successive carbonyl groups. A similar periplasmic loop between helices 6 and 7 provides the periplasmic vestibule with 3 carbonyl groups. Other polar residues along the one side of the channel wall have their hydrogen bond donor groups oriented towards the hydroxyl groups of the glycerol. On the opposite side of the channel wall, in the narrowest region, a phenylalanine is arranged perpendicularly to a tryptophan, thus forming a hydrophobic corner for the alkyl backbone of the glycerol molecule. Additional hydrophobic amino acids line this side of the channel, giving it an amphipathic quality.

The shape of the GlpF channel resembles a chalice (Figure 2). The periplasmic vestibule has a diameter of 15 angstroms. The channel reaches its narrowest point of 3.8 angstroms near the center of the transmembrane region. After this point, the channel has a slightly wider, variable diameter (less than 7 angstroms) before opening up at the cytoplasmic face of the membrane.

As glycerol passes through the GlpF channel, it gradually losses its hydration shell so it can pass through the constriction zone, which is only wide enough to accommodate a single CH-OH moiety. However, since one side of the channel is lined with polar hydrogen bond donors, the loss of the water molecules is not energetically unfavorable. Additionally, the hydrophobic contacts on the other side of the channel serve to stabilize the glycerol's alkyl backbone. Ions are excluded from GlpF, because like glycerol, they must lose their hydration shells. However, the hydrophobic channel lining imposes a thermodynamic barrier since half of the ion cannot be stabilized.

4.2. Phosphotransferase system

The phosphotransferase system (PTS), also referred to as group translocation, is unique to the bacterial kingdom and is the main sugar uptake system used by facultative aerobes (24). This mode of transport involves a series of phosphotransfers from phosphenolpyruvate (PEP) to the sugar; the phosphorylation of the sugar is coupled to its translocation into the cell. The PTS has been widely studied in E. coli, Salmonella enterica serovar Typhimurium, and many Gram-positive organisms. Three proteins are required to carry out the phosphotransfer reaction (25). First, enzyme I catalyzes the transfer of a phosphate molecule from PEP to a protein carrier called HPr (histidine-phosphorylatable protein). Then enzyme II (a sugar specific permease and kinase) catalyzes the concomitant phosphotransfer and translocation of the sugar. The exact mechanism of this last coupling step is not known and will remain elusive until the crystal structure of an enzyme II is solved. Some organisms may have an additional carrier protein called enzyme III that may or may not be peripherally associated with enzyme II (26), however this does not apply to the pseudomonads.

Pseudomonads metabolize sugars mainly via the Entner-Douderoff pathway (27). Because this pathway yields less PEP than glycolysis, the PTS is not a widely used mode of transport by these organisms. P. aeruginosa only has 2 complete phosphotransferase systems, one with N-acetylglucosamine as its putative substrate and one for fructose (28). Both of these systems lack the Hpr carrier protein. The putative N-acetylglucosamine transport system (PA3760 and PA3761) has not been characterized yet. It was identified after the release of the P. aeruginosa genome sequence based on the putative enzymes' homology with characterized phosphotransferase enzymes from Xanthomonas campestries and Klebsiella pneumoniae. Also, these two genes are found in a cluster of genes encoding N-acetylglucosamine metabolizing enzymes.

The PTS is the sole route of fructose entry into P. aeruginosa (29). Mutants in the PTS pathway failed to take up fructose but were able to grow on mannitol (which is converted to fructose before being metabolized). The fructose phosphotransferase system consists of a 72kD soluble enzyme I (ptsI or fruI, PA3562) and a membrane bound enzyme II (fruA, PA3560) that are induced by fructose (30). A number of other Pseudomonas species also utilize the PTS for fructose uptake (31).

4.3. Primary Active Transporters

Primary active transporters use a primary source of energy (chemical, electrical, or solar) to transport solutes against a concentration gradient. The largest family by far are the ATP binding cassette (ABC) transporters that use the energy released from ATP hydrolysis to drive transport (3). The P. aeruginosa genome encodes 91 potential ABC transporters. Of these, five uptake systems have been functionally characterized: PstABC, GltBFGK, SpuDEFGH, BraCDEFG, and AotJMPQ (Table 1). There is strong bioinformatic evidence implicating 26 other systems in nutrient uptake. Many of these systems are proposed to take up amino acids, peptides, and other nitrogenous compounds such as taurine and polyamines (http://www-biology.ucsd.edu/~ipaulsen/transport/).

A typical ABC transporter invovled in nutrient uptake consists of 5 polypeptides. First, there is the periplasmic substrate binding protein that determines the specificity of the transporter complex. These proteins tightly bind their substrates with dissociation constants in the micromolar range (32) and then deliver them to the appropriate porter in the cytoplasmic membrane. By limiting the diffusion of the substrate to two dimensions rather than three, and facilitating the diffusion of substrates through the gel-like matrix of the periplasm, binding proteins increase the overall efficiency of the transport process (9). In addition to these roles, studies on the E. coli maltose ABC transporter indicate that the binding protein acts as a catalyst by stabilizing the transition state of the transporter during ATP hydrolysis (33, 34).

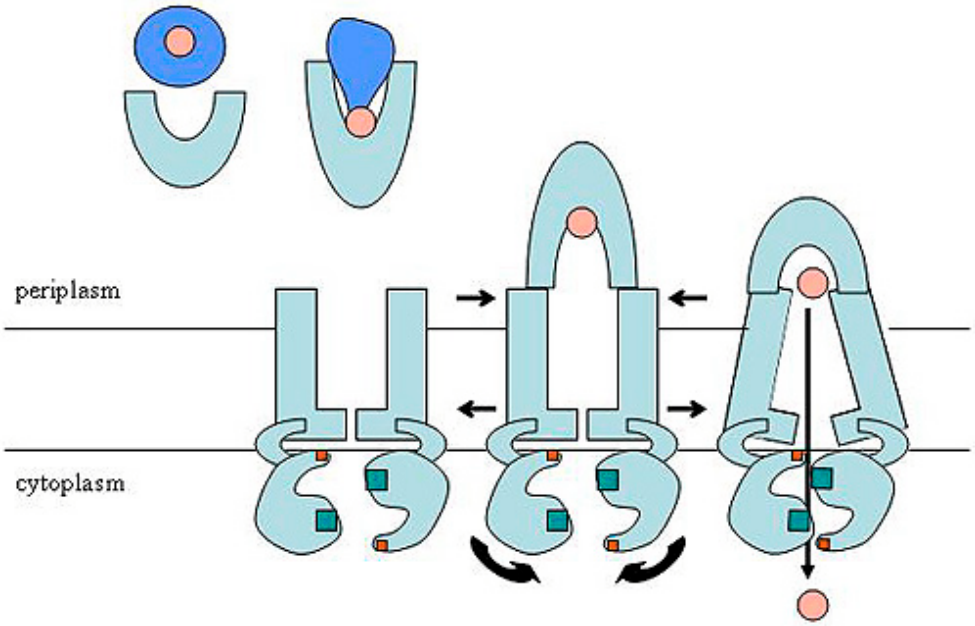

Figure 3. The proposed mechanism of ABC transporters. Upon substrate binding, the periplasmic binding protein changes its conformation to one recognized by the cytoplasmic porter. Once docked, the porter relays this information to the ATP binding subunits, which then bind ATP. Since the nucleotide-binding site is composed of the Walker A box (red squares) of one subunit and the LSGGQ motif (green squares) of the other, the two ATP binding subunits are brought closer together. This movement causes the cytoplasmic gate of the porter to open up, narrowing the periplasmic entrance and releasing the substrate from the binding protein

The crystal structures of several periplasmic binding proteins from other bacteria have been solved and reveal a striking similarity in their structures (35). Each binding protein has an ellipsoid shape, composed of two globular domains that are connected by three separate short stretches of amino acids (the cleft) similar to the Rossmann fold of nucleotide binding proteins. When the protein binds the substrate in the cleft, it undergoes a conformational change and closes around the substrate removing its hydration shell (the Venus-flytrap model) (36). The substrate is stabilized by forming hydrogen bonds with the main chain atoms in the cleft (35).

Two highly hydrophobic integral membrane proteins constitute the porter. The ABC transport system porters for a particular substrate in different bacteria exhibit high sequence similarity, and are thought to function as a pseudodimer. This assumption is supported by ABC transporter permeases from other organisms that are fused into one polypeptide chain (e.g. Mycoplasma, Homo sapiens) (36). Despite their conservation within a given substrate system, ABC porters for other substrates share virtually no sequence similarity (37). The name ABC transporter is derived from the next set of polypeptides, the 2 ATP binding proteins. Unlike the porters, the ATP binding domains exhibit a high degree of sequence similarity (30-50%) (37). The most highly conserved region of these proteins is called the ATP binding cassette, a sequence of 200 amino acids flanked by the two nucleotide-binding, Walker A and B motifs. The sequence between the two Walker motifs is considerably larger than in other ATP binding proteins and contains the signature sequence of ABC transporters (LSGGQ) (38). In addition, there is a conserved aspartate (the D-loop) followed by a stretch of 9 amino acids followed by a histidine (the switch region) located carboxy terminal to Walker B motif that are important for function. The ATP binding components do not contain any membrane-spanning segments but are associated with the cytoplasmic face of the membrane where they cooperatively hydrolyze ATP and provide the porter with the energy needed for translocation.

How the hydrolysis of ATP is coupled to the transport process has been the subject of numerous biochemical investigations over the past 30 years. The recent solution at a resolution of 3.2 angstroms, of the crystal structure of the E. coli BtuCD complex, the vitamin B_{12} ABC porter and ATP binding component has allowed us to re-evaluate these biochemical data and begin to elucidate the transport mechanisms of these systems (39). The complex resembles an inverted portal with the dimensions 90 Å tall, 60Å wide, and 39 Å thick (Figure 3). The two integral membrane protein porter molecules, BtuC, are closely associated and form a homodimer composed of 20 alpha-helices, many more than the 12 predicted from topological analyses of ABC transporter porters (37). This discrepancy may be a consequence of the difficulty involved in predicting the number of transmembrane segments. Alternatively, the extra helices may be required to increase the size of the channel to accommodate the relatively large vitamin B_{12} molecule. The channel is defined by two helices from each porter subunit (TM5 and TM10) and is open to the periplasm. At the cytoplasmic face, however, the channel is closed by a serine and

threonine from the loops connecting TM4 and TM5 from both BtuC monomers (the gate). Two sets of loops are involved in the interaction of the porter with the other components of the ABC transporter. The long, relatively flexible periplasmic loops connecting TM5 to TM6 are good candidates for binding the periplasmic binding protein. The conserved EAA motif (40) of bacterial ABC importers is found in the loops connecting TM6 to TM7. These cytoplasmic loops fold into two short alpha-helices (termed the L-loop) that form the contact regions for the ATP binding subunits. Sequence alignments show that the L-loop is conserved among ABC porters, however, its location in the different proteins varies. Therefore, if all ABC transporters adopt a similar fold, the order of the connecting loops may not be conserved.

The two ATP binding subunits closely associate with each other to form the homodimer of BtuD just below the surface of the membrane, in the cytoplasm. Each monomer consists of a six-stranded beta-sheet. This beta-sheet is surrounded by nine alpha-helices and a peripheral three-stranded beta-sheet. The BtuD monomers are aligned in a head to tail manner such that the nucleotide binding site is composed of a Walker A motif of one monomer and the LSGGQ motif of the other, explaining the cooperative nature of ATP binding and hydrolysis by ATP binding cassettes (41). Other residues found at the BtuD interface include the conserved residues found in the D-loop and switch region, implying that this structure may be conserved among all ABC transporters. The BtuD residues that interact with the porter are mainly located between the Walker A and B motifs, providing a mechanism for coupling ATP binding and hydrolysis with the conformation of the L-loop in the porter.

The proposed uptake mechanism of ABC transporters involves a series of conformational changes (Figure 3). The uptake reaction begins when a periplasmic binding protein binds its substrate and closes around it. The ligand-bound, closed form of the binding protein is recognized by the cytoplasmic membrane porter. Once docked, the porter transmits this information to the ATP binding subunits via a conformational change of the L-loop. The ATP binding subunits then bind ATP, which causes them to come closer to each other. Because the ATPases are tightly associated with the porter, their inward movement moves the porter subunits, opening the gate to the cytoplasm. Locher *et al*. have used the analogy of a clothespin to describe the action of the cytoplasmic porter. Once the cytoplasmic gate has been opened, the periplasmic entrance narrows, causing the substrate to be released from the binding protein.

4.4. Secondary Active Transporters

Secondary active transporters couple the energy derived the movement of an ion down its concentration gradient (a secondary energy source) to the uphill movement of a solute against its concentration gradient. In the absence of an electrochemical gradient, these transporters use the energy derived from the downhill movement of the solute to drive the uphill movement of ions outside of the cell in order to generate a membrane potential. The transport of the solute may simply be facilitated (uniport), co-transported with an ion (symport), or counter-transported with a structurally related compound (antiport). There are a large number (107) of secondary transporter families that are defined by strict evolutionary criteria (3). Members of one family tend to take up the same type of substrate. Interestingly, there are some families in which the members take up different coupling ions (i.e. either H^+ or Na^+) (42). Despite the differences in coupling ion, and direction of transport, many secondary carriers are homologous and are thought to share a common tertiary structure and uptake mechanism (43).

The genome of P. aeruginosa putatively encodes a potential 143 secondary transporters (http://www.biology.ucsd.edu/~ipaulsen/transport/). Three of these transporters, ArcD, BraB, and BraZ have been characterized functionally (Table 1). Of the remaining transporters, there is strong bioinformatic evidence implicating 83 in solute uptake. The putative substrates for the majority of these permeases include amino acids, small aromatic molecules, Kreb's cycle intermediates, di- and tricarboxylic acids, nucleosides, and inorganic ions. In comparison to E. coli, very few P. aeruginosa transporters are predicted to take up sugars, thus reflecting this organism's metabolic capabilities.

Secondary transporters consist of a single polypeptide with 11-15 predicted transmembrane segments (42). Recently, the three dimensional structure of the Oxalobacter formigenes oxalate-formate antiporter, OxlT was solved by electron crystallography at a resolution of 6.5 angstroms (44). This transporter consists of twelve transmembrane alpha-helices. The helices are tightly packed at the membrane boundaries giving the monomer an oval shape. The molecule has a central cavity that is also widest at the center of the membrane (Figure 4A). The periplasmic and cytoplasmic openings of the proposed channel are each obscured by a pair of helices. There are three mutually perpendicular pseudo two-fold axes of symmetry. Two pass through the center of the molecule, parallel to the plane of the membrane, suggesting that the periplasmic and cytoplasmic ends of OxlT are similar and may provide a structural explanation for the bi-directional transport carried out by this protein. The other axis of symmetry also passes through the center of the molecule but is perpendicular to the plane of the membrane, lending support to the idea that the N- and C-termini of secondary transporters are evolutionarily related (45).

Despite the lack of structural data, there have been tremendous advances in elucidating the uptake mechanism of secondary transporters, mainly focusing on LacY, the lactose transport protein of E. coli. In an effort to identify side chains important substrate binding, Kaback and coworkers created a library of LacY mutants with a cysteine residue at each position of the protein. Interestingly, only six residues are required for transport. Glu126 (helix 4) and Arg144 (helix 5) are required for substrate binding. Glu269 (helix 8), Arg302 (helix 9), His322 (helix 10), and Glu325 (helix 10) are essential for proton translocation and coupling. There are other residues

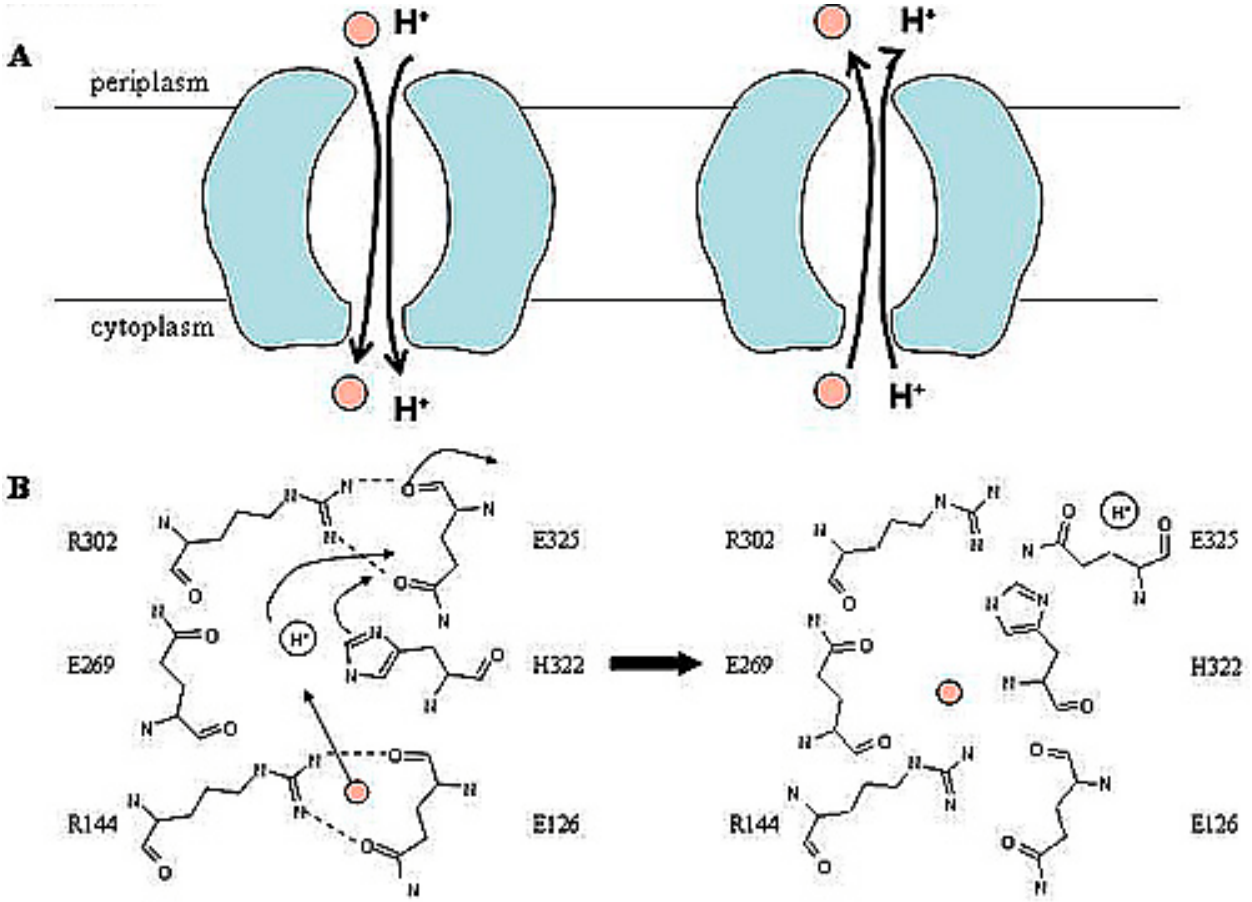

Figure 4. The proposed structure and function of secondary transporters. **A**. Proposed structure based on the OxlT oxalate-formate antiporter of O. formigenes. Both the transporter and channel are oval in shape. The symmetrical nature of the channel provides a structural explanation for the bi-directional transport it mediates. **B**. Proposed mechanism based on biochemical studies on the LacY lactose-proton symporter of E. coli. Only the six residues essential for lactose transport are shown. The ground state of the channel has two charge paired amino acid groups (indicated by dashed lines) and is protonated. Upon substrate binding, the protein undergoes a series of conformational changes that culminates in the release of both proton and substrate. Briefly, E325 and H322 rotate towards the plane of the membrane, disrupting the charged pairs, displacing the proton, which then releases the lactose from its binding site.

Table 2. Properties of the transporter classes found in the cytoplasmic membrane of Pseudomonas

Class	Substrates	Advantages to cell	Disadvantages to cell	Optimum environment
Facilitator	Small polyalcohols (e.g. glycerol)	High capacity Low energy cost	Low affinity Low concentrative potential	High substrate concentrations
Phosphotransferase	Fructose, N-acetylglucosamine	High capacity Couples transport with first metabolic step	Multiple polypeptides High energy cost	Low substrate concentrations
Primary (ABC)	Various	High affinity High concentrative potential	Multiple polypeptides High energy cost	Low substrate concentrations
Secondary	Various	High capacity Low energy cost	Low affinity Low concentrative potential	Intermediate substrate concentrations H^+: neutral or acid Na^+: alkaline

involved in substrate binding but these are not essential for function (46). Using a number of techniques aimed at determining which residues neighbour each other in space, the same group was able to generate a model of how the LacY helices pack, which is very similar to the published structure of OxlT. In the LacY model, the side chains of the six essential residues are positioned to stabilize the proton and lactose molecule through a hydrogen-bonding network. These six residues also stabilize each other in the channel by forming two sets of charged pairs (47).

The current uptake model (Figure 4B) proposes that the ground state of LacY is protonated and this primes the transporter to bind a lactose molecule. Upon lactose binding, the transporter undergoes a conformational change that disrupts the Glu269-His322 and Arg322-Glu325 charged pairs. This disruption transfers the proton to another residue, causing the substrate-binding site to be exposed to the membrane interior, thus displacing the lactose. The proton is then released and the permease relaxes back to its ground state (47). Functional studies of the E. coli sodium ion-proline symporter, PupP, are in agreement with this model (42). Therefore it is proposed that all secondary transporters share this common uptake mechanism.

5. WHY HAVE MULTIPLE TRANSPORT SYSTEMS

Generally, bacteria tend to have multiple transport systems for metabolites that are commonly found in their environmental niches. Which transport system the organism uses is ultimately dependent on the current nutrient conditions and often involves a compromise between the energetic costs of a particular transport system and the efficiency of its uptake capabilities (see Table 2, (9, 48, 49)). ABC transporters, while having the highest affinities for their substrates, are the most costly to the cell. In addition to the ATP required to drive the transport reaction, the synthesis of these multiple polypeptide systems requires a significant amount of the cell's resources. Therefore, to conserve cellular energy, primary active transport is mainly used in nutrient limited environments. In environments where the nutrient concentrations are higher and thus high substrate affinities are not required, secondary active transporters are mainly used. These transport systems require less energy for synthesis and function. Also, these permeases have higher uptake capacities than ABC transporters since they do not require periplasmic binding proteins to deliver solute to them.

Pseudomonas aeruginosa has multiple transport systems for a number of substrates including arginine/ornithine, di- and tricarboxylates, phosphate, and various other amino acids and sugars (www.pseudomonas.com). To date, the uptake systems for glucose and branched chain amino acids are the best characterized.

5.1. Glucose

Glucose enters the periplasm of P. aeruginosa through the specific porin OprB. This porin is induced in the presence of glucose and is down-regulated by Kreb's cycle intermediates (e.g. succinate, citrate). Depending on the glucose concentration in the periplasm, one of two transport systems may be used. At high concentrations, a membrane bound glucose dehydrogenase oxidizes the glucose to gluconate (50), which enters the cell via a gluconate specific secondary transporter (putatively PA2290, GnuT, GntT, or GntU) (www.pseudomonas.com). This transporter is induced by both glucose and gluconate (51). Alternatively, the gluconate may be oxidized to 2-ketogluconate by a second membrane bound dehydrogenase and enter the cell via KguT (putatively PA2262) a 2-ketogluconate specific secondary transporter. Interestingly, the P.aeruginosa glucose dehydrogenase gene, gcd (PA2290), is upstream of opbA (PA2291), an uncharacterized homologue of oprB. Whether this putative porin plays a role in the low affinity pathway remains to be determined.

At low glucose concentrations, the sugar enters the cell via a high affinity ABC transporter. The characterized components of the system include, a periplasmic binding protein GltB (PA3190) (52), and GltK (PA3187) (53), an ATP binding protein. The other putative components of this system are GltG, (PA3188), which is 58% similar to an ABC permease from Pyrococcus horikoshii and GltF (PA3189), which is 91% similar to an ABC permease from Pseudomonas putida. This system is induced by glucose via a two-component regulatory system. The response regulator of this system (GltR, PA3192) is known to be required for glucose uptake (54), the sensor kinase (PA3191) has not been studied to date. Gluconate represses the high affinity glucose uptake pathway (50), thus preventing the gratuitous use of ATP for transport in glucose rich environments.

5.2. Branched chain amino acids

There are three uptake systems for branched amino acids in P. aeruginosa. There is a high affinity ABC transporter (55) (BraCDEFG, PA1070 to PA1074) and two secondary transporters with intermediate affinities (BraB (56) and BraZ (57)). As BraB (PA1590) uses Na^+ as the coupling ion and BraZ (PA1971) uses H^+ as the coupling ion, it is likely that environmental conditions dictate which system is used. Therefore, the ABC transporter is probably expressed during nutrient limited conditions and the secondary transporters when the branched chain amino acid concentration is higher, with BraB expressed in alkaline environments and BraZ expressed in neutral or acid ones. The regulatory network controlling these transport systems remains to be determined.

6. PERSPECTIVE

Pseudomonas aeruginosa is one of the most nutritionally versatile organisms known and this is reflected in its large number (greater than 300) of known and putative solute uptake systems. An outstanding feature of this organism is its large number of regulatory genes (one in nine of the 5570 total open reading frames). This permits Pseudomonas to orchestrate its metabolism to suit particular environments and one major strategy for accomplishing this would be regulation of solute uptake systems. Therefore, to fully understand the versatility of this organism, it will be necessary to determine the substrate specificities of transporters and how these respond to environmental conditions.

7. ACKNOWLEDGMENTS

S.T. is the recipient of a studentship from the Canadian Cystic Fibrosis Foundation. R.E.W.H. holds a Canada Research Chair in Microbiology.

8. REFERENCES

1. Stanier R.Y., N.J. Palleroni, and M. Doudoroff, The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol*, 43(2), 159-271 (1966)

2. Stover C.K., X.Q. Pham, A.L. Erwin, S.D. Mizoguchi, P. Warrener, M.J. Hickey, F.S. Brinkman, W.O. Hufnagle, D.J. Kowalik, M. Lagrou, R.L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L.L. Brody, S.N. Coulter, K.R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G.K. Wong, Z. Wu, I.T. Paulsen, J. Reizer, M.H. Saier, R.E.W. Hancock, S. Lory, and M.V. Olson, Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature*, 406(6799), 959-64 (2000)

3. Saier M.H., Jr., A functional-phylogenetic classification system for transmembrane solute transporters. *Microbiol Mol Biol Rev*, 64(2), 354-411 (2000)

4. Hancock R.E.W., Bacterial outer membranes: evolving concepts. *ASM News*, 57(4), 175-182 (1991)

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

6. Hancock R.E.W. and F.S.L. Brinkman, Function of *Pseudomonas* porins in uptake and efflux. *Annu Rev Microbiol*, 56, 17-38 (2002)

7. Schirmer T., General and specific porins from bacterial outer membranes. *J Struct Biol*, 121(2), 101-9 (1998)

8. Bauer K., M. Struyve, D. Bosch, R. Benz, and J. Tommassen, One single lysine residue is responsible for the special interaction between polyphosphate and the outer membrane porin PhoE of *Escherichia coli*. *J Biol Chem*, 264(28), 16393-8 (1989)

9. Hengge R. and W. Boos, Maltose and lactose transport in *Escherichia coli*. Examples of two different types of concentrative transport systems. *Biochim Biophys Acta*, 737(3-4), 443-78 (1983)

10. Nikaido H., Porins and specific diffusion channels in bacterial outer membranes. *J Biol Chem*, 269(6), 3905-8 (1994)

11. Klebba P.E. and S.M. Newton, Mechanisms of solute transport through outer membrane porins: burning down the house. *Curr Opin Microbiol*, 1(2), 238-47 (1998)

12. Poole K., Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. *J Mol Microbiol Biotechnol*, 3(2), 255-64 (2001)

13. Cowan S.W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R.A. Pauptit, J.N. Jansonius, and J.P. Rosenbusch, Crystal structures explain functional properties of two *E. coli* porins. *Nature*, 358(6389), 727-33 (1992)

14. Kreusch A., A. Neubuser, E. Schiltz, J. Weckesser, and G.E. Schulz, Structure of the membrane channel porin from *Rhodopseudomonas blastica* at 2.0 A resolution. *Protein Sci*, 3(1), 58-63 (1994)

15. Schirmer T., T.A. Keller, Y.F. Wang, and J.P. Rosenbusch, Structural basis for sugar translocation through maltoporin channels at 3.1 A resolution. *Science*, 267(5197), 512-4 (1995)

16. Koebnik R., K.P. Locher, and P. Van Gelder, Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Mol Microbiol*, 37(2), 239-53 (2000)

17. Ferguson A.D., E. Hofmann, J.W. Coulton, K. Diederichs, and W. Welte, Siderophore-mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide. *Science*, 282(5397), 2215-20 (1998)

18. Ochs M.M., M. Bains, and R.E.W. Hancock, Role of putative loops 2 and 3 in imipenem passage through the specific porin OprD of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*, 44(7), 1983-5 (2000)

19. Jap B.K. and P.J. Walian, Structure and functional mechanism of porins. *Physiol Rev*, 76(4), 1073-88 (1996)

20. Van Gelder P., F. Dumas, I. Bartoldus, N. Saint, A. Prilipov, M. Winterhalter, Y. Wang, A. Philippsen, J.P. Rosenbusch, and T. Schirmer, Sugar transport through maltoporin of *Escherichia coli*: role of the greasy slide. *J Bacteriol*, 184(11), 2994-9 (2002)

21. Hohmann I., R.M. Bill, I. Kayingo, and B.A. Prior, Microbial MIP channels. *Trends Microbiol*, 8(1), 33-8 (2000)

22. Schweizer H.P., R. Jump, and C. Po, Structure and gene-polypeptide relationships of the region encoding glycerol diffusion facilitator (glpF) and glycerol kinase (*glpK*) of *Pseudomonas aeruginosa*. *Microbiology*, 143 (Pt 4), 1287-97 (1997)

23. Fu D., A. Libson, L.J. Miercke, C. Weitzman, P. Nollert, J. Krucinski, and R.M. Stroud, Structure of a glycerol-conducting channel and the basis for its selectivity. *Science*, 290(5491), 481-6 (2000)

24. Meadow N.D., D.K. Fox, and S. Roseman, The bacterial phosphoenolpyruvate: glycose phosphotransferase system. *Annu Rev Biochem*, 59, 497-542 (1990)

25. Saier M.H., Jr. and J. Reizer, The bacterial phosphotransferase system: new frontiers 30 years later. *Mol Microbiol*, 13(5), 755-64 (1994)

26. Hays J.B.: Group translocation transport systems, In: Bacterial Transport. Eds: Rosen B. P., Marcel Dekker, Inc., New York (1978)

27. Clarke P.H. and L.N. Ornston: Metabolic pathways and regulation- part I, In: Genetics and Biochemistry of *Pseudomonas*. Eds: Clarke P. H. and Richmond M. H., John Wiley & Sons, Toronto (1975)

28. Reizer J., A. Reizer, M.J. Lagrou, K.R. Folger, C.K. Stover, and M.H. Saier, Jr., Novel phosphotransferase systems revealed by bacterial genome analysis: the complete repertoire of *pts* genes in *Pseudomonas aeruginosa*. *J Mol Microbiol Biotechnol*, 1(2), 289-93 (1999)

29. Hoshino T.: Transport systems in *Pseudomonas*, In: *Pseudomonas*. Eds: Montie T. C., Plenum Press, New York (1998)

30. Roehl R.A. and P.V. Phibbs, Jr., Characterization and genetic mapping of fructose phosphotransferase mutations in *Pseudomonas aeruginosa*. *J Bacteriol*, 149(3), 897-905 (1982)

31. Sawyer M.H., P. Baumann, L. Baumann, S.M. Berman, J.L. Canovas, and R.H. Berman, Pathways of D-fructose catabolism in species of Pseudomonas. *Arch Microbiol*, 112(1), 49-55 (1977)

32. Ames G.F., Bacterial periplasmic transport systems: structure, mechanism, and evolution. *Annu Rev Biochem*, 55, 397-425 (1986)

33. Chen J., S. Sharma, F.A. Quiocho, and A.L. Davidson, Trapping the transition state of an ATP-binding cassette transporter: evidence for a concerted mechanism of maltose transport. *Proc Natl Acad Sci U S A*, 98(4), 1525-30 (2001)

34. Davidson A.L., Mechanism of coupling of transport to hydrolysis in bacterial ATP-binding cassette transporters. *J Bacteriol*, 184(5), 1225-33 (2002)

35. Quiocho F.A., Atomic structures of periplasmic binding proteins and the high-affinity active transport systems in bacteria. *Philos Trans R Soc Lond B Biol Sci*, 326(1236), 341-51; discussion 351-2 (1990)

36. Higgins C.F., S.C. Hyde, M.M. Mimmack, U. Gileadi, D.R. Gill, and M.P. Gallagher, Binding protein-dependent transport systems. *J Bioenerg Biomembr*, 22(4), 571-92 (1990)

37. Higgins C.F., ABC transporters: from microorganisms to man. *Annu Rev Cell Biol*, 8, 67-113 (1992)

38. Schneider E. and S. Hunke, ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiol Rev*, 22(1), 1-20 (1998)

39. Locher K.P., A.T. Lee, and D.C. Rees, The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science*, 296(5570), 1091-8 (2002)

40. Mourez M., M. Hofnung, and E. Dassa, Subunit interactions in ABC transporters: a conserved sequence in hydrophobic membrane proteins of periplasmic permeases defines an important site of interaction with the ATPase subunits. *Embo J*, 16(11), 3066-77 (1997)

41. Fetsch E.E. and A.L. Davidson, Vanadate-catalyzed photocleavage of the signature motif of an ATP-binding cassette (ABC) transporter. *Proc Natl Acad Sci U S A*, 99(15), 9685-90 (2002)

42. Jung H., Towards the molecular mechanism of Na(+)/solute symport in prokaryotes. *Biochim Biophys Acta*, 1505(1), 131-43 (2001)

43. Saier M.H., Jr., Computer-aided analyses of transport protein sequences: gleaning evidence concerning function, structure, biogenesis, and evolution. *Microbiol Rev*, 58(1), 71-93 (1994)

44. Hirai T., J.A. Heymann, D. Shi, R. Sarker, P.C. Maloney, and S. Subramaniam, Three-dimensional structure of a bacterial oxalate transporter. *Nat Struct Biol*, 9(8), 597-600 (2002)

45. Pao S.S., I.T. Paulsen, and M.H. Saier, Jr., Major facilitator superfamily. *Microbiol Mol Biol Rev*, 62(1), 1-34 (1998)

46. Frillingos S., M. Sahin-Toth, J. Wu, and H.R. Kaback, Cys-scanning mutagenesis: a novel approach to structure function relationships in polytopic membrane proteins. *Faseb J*, 12(13), 1281-99 (1998)

47. Kaback H.R., M. Sahin-Toth, and A.B. Weinglass, The kamikaze approach to membrane transport. *Nat Rev Mol Cell Biol*, 2(8), 610-20 (2001)

48. Ferenci T., Adaptation to life at micromolar nutrient levels: the regulation of Escherichia coli glucose transport by endoinduction and cAMP. *FEMS Microbiol Rev*, 18(4), 301-17 (1996)

49. Lengeler J.W., Carbohydrate transport in bacteria under environmental conditions, a black box? *Antonie Van Leeuwenhoek*, 63(3-4), 275-88 (1993)

50. Midgley M. and E.A. Dawes, The regulation of transport of glucose and methyl alpha-glucoside in *Pseudomonas aeruginosa*. *Biochem J*, 132(2), 141-54 (1973)

51. Guymon L.F. and R.G. Eagon, Transport of glucose, gluconate, and methyl alpha-D-glucoside by *Pseudomonas aeruginosa*. *J Bacteriol*, 117(3), 1261-9 (1974)

52. Stinson M.W., M.A. Cohen, and J.M. Merrick, Purification and properties of the periplasmic glucose-binding protein of *Pseudomonas aeruginosa*. *J Bacteriol*, 131(2), 672-81 (1977)

53. Adewoye L.O. and E.A. Worobec, Identification and characterization of the *gltK* gene encoding a membrane-associated glucose transport protein of *Pseudomonas aeruginosa*. *Gene*, 253(2), 323-30 (2000)

54. Sage A.E., W.D. Proctor, and P.V. Phibbs, Jr., A two-component response regulator, *gltR*, is required for glucose transport activity in *Pseudomonas aeruginosa* PAO1. *J Bacteriol*, 178(20), 6064-6 (1996)

55. Hoshino T. and K. Kose, Cloning, nucleotide sequences, and identification of products of the *Pseudomonas aeruginosa* PAO *bra* genes, which encode the high-affinity branched-chain amino acid transport system. *J Bacteriol*, 172(10), 5531-9 (1990)

56. Hoshino T., K. Kose, and Y. Uratani, Cloning and nucleotide sequence of the gene *braB* coding for the sodium-coupled branched-chain amino acid carrier in *Pseudomonas aeruginosa* PAO. *Mol Gen Genet*, 220(3), 461-7 (1990)

57. Hoshino T., K. Kose-Terai, and Y. Uratani, Isolation of the *braZ* gene encoding the carrier for a novel branched-chain amino acid transport system in *Pseudomonas aeruginosa* PAO. *J Bacteriol*, 173(6), 1855-61 (1991)

58. Wylie J.L. and E.A. Worobec, The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*. *J Bacteriol*, 177(11), 3021-6 (1995)

59. Trias J. and H. Nikaido, Protein D2 channel of the *Pseudomonas aeruginosa* outer membrane has a binding site for basic amino acids and peptides. *J Biol Chem*, 265(26), 15680-4 (1990)

60. Huang H. and R.E.W. Hancock, Genetic definition of the substrate selectivity of outer membrane porin protein OprD of *Pseudomonas aeruginosa*. *J Bacteriol*, 175(24), 7793-800 (1993)

61. Bellido F., N.L. Martin, R.J. Siehnel, and R.E.W.Hancock, Reevaluation, using intact cells, of the exclusion limit and role of porin OprF in *Pseudomonas aeruginosa* outer membrane permeability. *J Bacteriol*, 174(16), 5196-203 (1992)

62. Hancock R.E.W., K. Poole, and 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(2), 730-8 (1982)

63. Siehnel R.J., C. Egli, and R.E.W. Hancock, Polyphosphate-selective porin OprO of *Pseudomonas aeruginosa*: expression, purification and sequence. *Mol Microbiol*, 6(16), 2319-26 (1992)

64. Nishijyo T., S.M. Park, C.D. Lu, Y. Itoh, and A.T. Abdelal, Molecular characterization and regulation of an operon encoding a system for transport of arginine and ornithine and the ArgR regulatory protein in *Pseudomonas aeruginosa*. *J Bacteriol*, 180(21), 5559-66 (1998)

65. Nikata T., Y. Sakai, K. Shibat, J. Kato, A. Kuroda, and H. Ohtake, Molecular analysis of the phosphate-specific transport (pst) operon of *Pseudomonas aeruginosa*. *Mol Gen Genet*, 250(6), 692-8 (1996)

66. Lu C.D., Y. Itoh, Y. Nakada, and Y. Jiang, Functional analysis and regulation of the divergent *spuABCDEFGH-spuI* operons for polyamine uptake and utilization in *Pseudomonas aeruginosa* PAO1. *J Bacteriol*, 184(14), 3765-73 (2002)

67. Verhoogt H.J., H. Smit, T. Abee, M. Gamper, A.J. Driessen, D. Haas, and W.N. Konings, *arcD*, the first gene of the arc operon for anaerobic arginine catabolism in *Pseudomonas aeruginosa*, encodes an arginine-ornithine exchanger. *J Bacteriol*, 174(5), 1568-73 (1992)

Key Words: Pseudomonas, Uptake, Outer membrane, Porins, Glycerol facilitator, Phosphotransferase system, ABC transporters, Secondary transporters, Substrate specificity, Membrane protein structure, Transport mechanism, Review

Send correspondence to: Robert E.W. Hancock, Department of Microbiology and Immunology, University of British Columbia, #300 6174 University Boulevard, Vancouver, BC, Canada V6T 1Z3, Tel: 604-822 3489, Fax: 604-822 6041, E-mail: bob@cmdr.ubc.ca