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FUNCTION OF *PSEUDOMONAS* PORINS IN UPTAKE AND EFFLUX

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■ **Abstract** Porins are proteins that form water-filled channels across the outer membranes of Gram-negative bacteria and thus make this membrane semipermeable. There are four types of porins: general/nonspecific porins, substrate-specific porins, gated porins, and efflux porins (also called channel-tunnels). The recent publication of the genomic sequence of *Pseudomonas aeruginosa* PAO1 has dramatically increased our understanding of the porins of this organism. In particular this organism has 3 large families of porins: the OprD family of specific porins (19 members), the OprM family of efflux porins (18 members), and the TonB-interacting family of gated porins (35 members). These familial relationships underlie functional similarities such that well-studied members of these families become prototypes for other members. We summarize here the latest information on these porins.

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INTRODUCTION

Pseudomonas aeruginosa is an exceptionally versatile organism that can adopt many ecological niches (33). It is known to exist in the environment, including in soil and attached to rocks in streams, and can opportunistically infect diverse organisms from grasshoppers to humans. It has become especially notorious as a human pathogen, being the third-most-common hospital pathogen, causing approximately 10% of the 2 million hospital infections in the United States annually. It is also the major cause of chronic lung infections in individuals with the genetic disease cystic fibrosis, and it is thought to be associated with progressive deterioration of lung function and eventual death in such individuals. A major reason for its prominence as a hospital pathogen is its high intrinsic resistance to antimicrobials, including antibiotics and disinfectants (16). Antibiotic resistance makes *P. aeruginosa* one of the most difficult organisms to treat. Similarly resistance to disinfection makes it difficult to remove from a hospital environment.

The average hospital strain can be susceptible to several antibiotics such as the recently introduced β-lactam antibiotics, aminoglycosides such as gentamicin, tobramycin, and amikacin, and fluoroquinolones such as ciprofloxacin (16). However, the organism is naturally less susceptible than most bacteria to such agents and naturally resistant to many others, a phenomenon termed "intrinsic resistance." Table 1 compares the minimal inhibitory concentrations for several antimicrobials of typical strains of *Pseudomonas* and *Escherichia coli*. The major impact of high intrinsic resistance is that, even for agents to which *P. aeruginosa* is initially susceptible, a mutation that causes a moderate increase in resistance can make this organism clinically untreatable.

The recent publication of the genomic sequence of *P. aeruginosa* (49) has provided a quantum increase in our knowledge of this organism. Overall, the genome of strain PA01 encodes 5570 genes (on 6.3 mega base pairs of DNA). The nature of the genes that have been annotated is consistent with the versatility of this organism, including four motility systems, a large number of systems for metabolism of carbon sources, the highest percentage of regulators (nearly 1 gene in 9) of any genome to date, and a plethora of transport systems (49). At the time of writing this review, the partial sequence of *P. putida* had been released as an unfinished genome (http://tigrblast.tigr.org/ufmg/index.cgi), and examination of this revealed similar trends for this bacterium. Indeed although none of the other type 1 fluorescent pseudomonads are significant hospital pathogens, they share similarities in having large genomes and substantial metabolic diversity. Being less well studied, especially with respect to outer membrane proteins (17), they will be covered only in passing here. Information supplemental to this review is posted

at www.cmdr.ubc.ca/bobh/omps/, and we largely reference articles subsequent to our last major review of this topic (17).

PSEUDOMONAS OUTER MEMBRANES

More than two decades ago we demonstrated (35) that *P. aeruginosa* had low outer membrane permeability (approximately 8% that of *E. coli*) but a large exclusion limit (permitting passage of compounds of around 3000 molecular weight compared to an exclusion limit of around 500 molecular weight for *E. coli*). This at first seemed contradictory, and indeed was challenged in the literature, but was subsequently confirmed (4, 36). In particular, investing *P. aeruginosa* with a raffinose metabolic operon permitted relatively rapid growth on tri- and tetra-saccharides (compared to *E. coli*), confirming the large exclusion limit (4). Furthermore, such studies indicated that the major porin contributing to this large exclusion limit was OprF (see below). Similarly cloning highly permeable porins, either deletion mutants of OprD (19) or *E. coli* OmpF (41), into *P. aeruginosa* led to substantial decreases in minimal inhibitory concentration (MIC) for multiple antibiotics, indicating that outer membrane permeability was limiting for antibiotic susceptibility.

However, low outer membrane permeability is insufficient by itself to explain high intrinsic antibiotic resistance (15). Even a poorly permeable outer membrane, like that of *P. aeruginosa*, will permit antibiotics to diffuse and equilibrate their concentrations across the membrane in 1–100 sec (compared to a doubling time of 2000 sec or more). Other secondary resistance mechanisms have to exist that will take advantage of the relatively low rate of permeation of antibiotics across the outer membrane; two have been described (15, 42). *P. aeruginosa* contains a periplasmic β-lactamase that is inducible (by some β-lactams, notably imipenem). Because this enzyme works catalytically, it will hydrolyze β-lactams at a steady rate that will benefit from the slow passage of β-lactam into the periplasm (15). Indeed it has been demonstrated for β-lactams such as imipenem and panipenem that β-lactamase is the major secondary determinant of intrinsic resistance (34), and for many but not all β-lactams, knockout of efflux has no apparent effect in β-lactamase derepressed mutants.

Conversely, some β-lactams and many other antibiotics are far more influenced by multidrug efflux systems (34, 41). The major system of note for *P. aeruginosa* intrinsic antibiotic resistance is the MexAB-OprM, RND efflux system, which benefits from relatively slow uptake of antibiotics and actively effluxes them from the cell (Table 1).

The consequence of a poorly permeable outer membrane is that many substrates have to utilize specialized pathways to cross the outer membrane at a rate sufficient to support growth. Indeed *P. aeruginosa* utilizes a diversity of outer membrane permeation pathways to support growth (17). As mentioned above, OprF is the major channel for larger substrates and can be considered a general or nonspecific porin (a porin is defined as a trans-outer-membrane protein that encloses a water-filled channel—general porins lack substrate specificity). Other proteins that also

TABLE 1 Susceptibility of *P. aeruginosa* and *E. coli* strains to antibiotics

	MIC (μg/ml)[a]					
Strains	**CTX**	**CB**	**CIP**	**NAL**	**TC**	**CAM**
P. aeruginosa WT	4	32	2	25	8	6.4
P. aeruginosa/oprDΔL5[b]	1	—	0.02	—	0.8	0.8
*P. aeruginosa oprM::*Ω[c]	1	0.4	0.1	2	0.5	0.2
E. coli WT	0.13	—	0.03	4	2	8

[a]Abbreviations: CTX, cefotaxime β-lactam; CB, carbenicillin β-lactam; CIP, ciprofloxacin fluoroquinolone; NAL, nalidixic acid quinolone; TC, tetracycline; CAM, chloramphenicol; PXB, polymyxin B; GM, gentamicin; WT, wild type. The symbol "—" signifies no data available.

[b]OprDΔL5 has a large channel such that when cloned into *P. aeruginosa* it increases outer-membrane permeability (19, 20).

[c]*oprM::*Ω mutations delete the major efflux system such that this *P. aeruginosa* strain lacks the major intrinsic efflux pathway of *P. aeruginosa* (22).

function as substrate-specific porins can serve as general porins for small substrates. For example, OprD acts as a rate-limiting porin for gluconate (18) and possibly some other low-molecular-weight substrates (S. Tamber & R.E.W. Hancock, unpublished observations), whereas OprB acts as a porin for monosaccharide passage (59). These proteins probably account for the nonspecific passage of most substrates across the outer membrane because *P. aeruginosa* utilizes few substrates larger than ~200 Da, the size of a monosaccharide.

OUTER MEMBRANE PROTEINS

Knowledge of the *P. aeruginosa* genome (49) permits us to predict all of the outer membrane proteins in this organism, by homology to known *P. aeruginosa* outer membrane proteins or those from other organisms, and/or possession of certain motifs (particularly signal sequences plus a propensity for predicted β-strands and/or a conserved C-terminal β-strand). Table 2 describes all of the known outer membrane proteins of *P. aeruginosa* together with their *Pseudomonas* ID number, which will let the reader search for information on these proteins at www.pseudomonas.com. In Table 3 a list of possible additional porins is provided (see also www.cmdr.ubc.ca/bobh/omps/ for additional information about all known and predicted outer membrane proteins). Investigation of porins from other species has revealed that they contain transmembrane anti-parallel β-strands that wrap into a barrel (22) (Figure 1). Between 8 and 22 β-strands (from 1 to 3 subunits) make up this β-barrel embedded in the outer membrane bilayer. Generally speaking, these β-strands are interconnected by short turn sequences on the periplasmic side and larger loop sequences on the external face of the membrane, although a somewhat different picture is observed for the efflux channel-tunnels

TABLE 2 Known outer-membrane proteins

Gene	PAID[a]	Protein function and name	Position in genome nucleotides		No. of AAs	Medline Ref. ID	Known porin (class)[b]
			From	To			
algE	PA3544	Alginate production protein AlgE	3968448	3969920	490	92077417	P
aprF	PA1248	Alkaline protease secretion protein AprF	1353827	1355272	481	93051361	EP, PI
fliF	PA1101	Flagella M-ring protein	1192405	1194201	598	96239027	
fptA	PA4221	Fe(III)-pyochelin receptor	4726800	4724638	720	94117363	GP
fpvA	PA2398	Ferripyoverdine receptor	2655187	2657634	815	93328663	GP
hasR	PA3408	Heme uptake receptor HasR	3817335	3814660	891		GP
icmP	PA4370	Insulin-cleaving metalloproteinase; ICMP	4898192	4899532	446	10452958	
lppL	PA5276	Lipopeptide LppL	5941335	5941475	46	90279511	
omlA	PA4765	Lipoprotein OmlA	5352176	5352706	176	9973334	
oprB	PA3186	Glucose/carbohydrate porin OprB; protein D1	3577275	3575911	454	95286479	SP
oprC	PA3790	Putative copper transport porin OprC	4249873	4247702	723	96349120	GP?
oprD	PA0958	Basic amino acid, basic peptide and imipenem porin OprD; also named Porin D, Protein D2	1045314	1043983	443	90368779	SP, P
oprE	PA0291	Anaerobically induced porin OprE; Porin E1	327284	328666	460	93360827	SP
oprF	PA1777	Major porin and structural porin OprF; Porin F	1921174	1922226	350	88086862	P
oprG	PA4067	Outer-membrane protein OprG	4544606	4545304	232	99277900	
oprH	PA1178	PhoP/Q and low Mg^{2+} -inducible outer-membrane protein H1	1277006	1277608	200	89255086	GP?
oprI	PA2853	Outer-membrane lipoprotein OprI	3206914	3207165	83	89327122	
oprJ	PA4597	Multidrug efflux protein OprJ	5151071	5149632	479	97032139	EP
oprL	PA0973	Peptidoglycan-associated lipoprotein OprL	1057400	1057906	168	97312009	
oprM	PA0427	Major intrinsic multiple antibiotic resistance efflux protein OprM	476333 476333	477790 477790	485 485	97312458 97312458	EP EP
oprO	PA3280	Pyrophosphate-specific porin OprO	3674323	3673007	438	93023860	SP
oprP	PA3279	Phosphate-specific porin OprP; protein P	3672548	3671226	440	86296709	SP
pfeA	PA2688	Ferric enterobactin receptor PfeA	3040241	3042481	746	93123148	GP

(*Continued*)

TABLE 2 (*Continued*)

Gene	PAID[a]	Protein function and name	Position in genome nucleotides		No. of AAs	Medline Ref. ID	Known porin (class)[b]
			From	To			
phuR	PA4710	Heme/Hemoglobin uptake receptor PhuR	5289216	5291510	764		GP
pilQ	PA5040	Type 4 fimbrial biogenesis protein PilQ	5677857	5675713	714	94049125	
popD	PA1709	Translocator protein PopD; PepD	1854849	1855736	295	98449523	
popN	PA1698	Type III secretion protein PopN	1847227	1848093	288	98037517	
pscC	PA1716	Type III secretion protein PscC	1859493	1861295	600	97126825	PIII
xcpQ	PA3105	General secretion pathway protein D	3484353	3486329	658	95020542	PII
xcpU	PA3100	General secretion pathway protein H; PilD-dependent protein PddB	3480238	3479720	172	92269572	
xqhA	PA1868	Secretion protein XqhA	2028968	2031298	776	98343806	PII

[a]PAID; *Pseudomonas aeruginosa* gene identity number (see www.pseudomonas.com).

[b]P, general porin; SP, specific porin; GP, putative gated porin; EP, OprM family member of efflux and protein secretion porins; PI, putative type I secretion subfamily; PII, type II secretion channel; PIII, type III secretion channel.

like OprM (Figure 1*B*). The central area of the β-barrels of the general and specific porins contains stretches of amino acids from one of the interconnecting regions, often loop 3, that folds back into the channel region and gives this region many of its important characteristics.

A major finding from the genome sequence is that of the 163 known or predicted *P. aeruginosa* outer membrane proteins, 64 are found as part of 3 families of porins, the OprD-specific porin family, the TonB-dependent gated porin family, and the OprM efflux/secretion family.

GENERAL PORINS

OprF

OprF is a major outer membrane protein in *P. aeruginosa* that has been studied extensively due to its proposed utility as a vaccine component, role in antimicrobial drug resistance, and porin function (17). OprF has been described as a multifunctional protein, as gene disruption and gene deletion analysis has indicated that it is required for cell growth in low-osmolarity medium and for the maintenance of cell shape (44). In addition it appears to have a nonspecific porin function and binds to the underlying peptidoglycan (4, 17). Additional functions of OprF have been

identified in other *Pseudomonas* sp., for example, in *P. fluorescens* OprF is a root adhesion.

Many studies of OprF suggest that it resembles *E. coli* OmpA in both function and structure, and it is a structural member of the OmpA family of proteins (17). Through epitope-mapping experiments, and linker-insertion mutagenesis, a 16-β-stranded membrane topology model for *P. aeruginosa* OprF was originally proposed (17). However, subsequent deletion studies and secondary structure predictions indicated that there are 3 domains to this protein: (*a*) a N-terminal domain (first $\sim$160 aa) containing 8 anti-parallel sheets proposed to form a β-barrel structure (7, 46), (*b*) a loop or hinge region (161 to 209 aa) containing a poly-proline-alanine repeat region and two disulfide bonds, and (*c*) a C-terminal domain (210 to 326 aa) highly conserved with the corresponding domains of other OmpA family proteins (44, 50). This latter C-terminal region has also been shown to be the domain that forms the noncovalent linkage with peptidoglycan in the periplasm (44, 50). It is linked to the N-terminal domain by a proline-rich hinge and a loop region that contains two disulfide bonds (note that these disulfide bonds are not found in all *Pseudomonas* species OprF proteins) (44). This three-domain structural model for OprF has been further supported by circular dichroism spectroscopy analysis (7, 50) and three-dimensional modeling of the N terminus of OprF to the crystal structure of the proposed orthologous sequences in *E. coli* OmpA (Figure 1*D*).

Clinical isolates of *P. aeruginosa* that are multiply antibiotic resistant and deficient in the major outer membrane protein OprF have been obtained (17, 43). Sequencing of the *oprF* gene in such a clinical isolate has shown that the *oprF* gene and promoter are intact, indicating that a regulatory mutation may be involved (8). This regulatory mutation has not yet been revealed, although recent analysis of the promoter region of OprF has indicated that it is not just constitutively expressed from a sigma 70 promoter, as originally proposed (8). In addition, there is an extracellular factor (ECF) sigma factor promoter upstream of the gene that appears to be affected by disruption of an upstream ECF sigma factor gene named sigX. This sigma factor gene is not mutated in the clinical isolates that are multiply antibiotic resistant, so the mechanism for such resistance remains unknown.

The porin function of OprF has been extensively studied through liposome swelling experiments and planar lipid bilayer analysis of both the full-length protein and the N-terminal β-barrel domain (17). The size of the channels has been controversial (17). However, OprF channels have been shown to be nonspecific in nature, with weak cation selectivity. Both small (0.36 nS) channels and, rarely, large (2–5 nS) channels appear to form in planar lipid bilayer experiments, with only the small channels forming when the N-terminal domain of the protein is examined for porin function (7, 46). Because the full length of the protein is required for large channel formation and there is evidence that the C terminus of the protein contains both surface-exposed and peptidoglycan-binding regions, it seems possible that OprF forms more than one conformation varying in both structure and channel formation. Evidence supporting this concept of more than one structure and channel size has also been presented for *P. fluorescens* OprF (12).

OprF is also noted for its antigenicity, and vaccine candidates containing portions of OprF have been constructed (e.g., 14, 26). Due to its antigenicity, multifunctional nature, and apparently complex structure, OprF continues to be a fascinating protein that merits further study.

SPECIFIC PORINS

The best-characterized specific porin is *E. coli* LamB, which contains within its channel a substrate-binding site for maltose and maltodextrins (48). The crystal structure of LamB indicates that this porin is rather analogous to the nonspecific porins with quite modest differences (Figure 1). LamB is an 18 (compared to 16)-stranded β-barrel, which contains about 30% extra ($\sim$100) amino acids compared to the nonspecific porin OmpF (22). These extra residues are largely found in the surface loops that fold over to constrict the entrance of the channel (loops 4, 6, and 9) or reach over to the adjacent monomer in the LamB trimer (loop 2) (2). The substrate-binding site includes parts of loops 4, 5, and 6 (which are slightly longer than in OmpF) and the barrel wall, and it involves several hydrophobic residues that collectively are termed the "greasy slide." *Pseudomonas* has at least three well-characterized specific porins, OprB, OprP, and OprD, each of which contains one or more less-characterized homologs.

OprP/O

OprP (protein P) is a protein of 48,000 molecular weight that is induced under conditions of low phosphate (<0.15mM) (17). It is involved in the high-affinity, phosphate-starvation inducible transport system (PTS), as studied using an *oprP*::Tn*501* mutant. Purification of OprP and studying it using the planar lipid bilayer model membrane system indicated that the OprP channel contains a binding site for phosphate with a Kd of approximately 0.15 μM. While the channel is permeable to small anions, it is blocked by the binding of phosphate to its binding site (51). Molecular modeling and insertion mutagenesis have led to the proposal that OprP is a 16-stranded β-barrel (17). Systematic site-directed mutagenesis of all the lysine residues in the N-terminal half of OprP to glutamate and glycine revealed that lys-121 in the proposed loop 3 region was part of the phosphate-binding site (51). Two other basic lysine residues, lys-74 and lys-126, when changed to the acidic residue glutamate, but not when changed to the neutral residue glycine, affected the movement of anions through the OprP channel, which indicates that these other lysine residues probably represent secondary (non-rate-limiting) phosphate-binding sites. Thus the OprP channel probably acts as an electrical wire transmitting negatively charged phosphate residues from one positively charged binding site to the next of higher affinity. The lys-121-binding site is the highest-affinity binding site in OprP, but it still is of lower affinity than the periplasmic phosphate-binding protein, and thus, the phosphate will flow along the concentration gradient toward the periplasm. The *oprO* gene resides immediately

upstream of *oprP* and shares 76% identical amino acids (17). It is induced under conditions of phosphate starvation in the stationary phase of growth and like *oprP* has upstream pho-box sequences for binding of the regulator PhoB to its promoter. The OprO channel prefers pyrophosphate to phosphate (for OprP the situation is reversed).

OprD Family

OprD was first identified as a protein that was lost when *P. aeruginosa* clinical isolates became resistant to the broad-spectrum β-lactam imipenem (30). This β-lactam strongly resembles a dipeptide containing a positively charged residue. Consistent with this finding, Trias & Nikaido demonstrated that OprD is a specific porin that binds basic amino acids, dipeptides containing a basic residue and imipenem and related zwitterionic carbapenems (including meropenem) (54). This was confirmed in part by planar lipid bilayer analysis (19).

OprD is the closest *P. aeruginosa* homolog of the *E. coli* nonspecific porin OmpF, a fact that assisted in building a model for this porin as a 16-stranded β-barrel (20). This model was tested with reasonable success by PCR-directed site-specific (4–8 amino acid) deletion mutagenesis. Investigation of OprD mutants with deletions in specific loops demonstrated that both loop 2 and loop 3 deletions lose the ability to bind imipenem and mediate imipenem susceptibility (19, 37). Thus, OprD differs from other specific porins (22) in that loop 2 has a role in substrate binding to the channel. Also loops 5, 7, or 8 deletion variants of OprD have increased susceptibility of *P. aeruginosa* to multiple antibiotics, and they correspondingly produce larger channels (that still bind imipenem), which indicates that these loops constrict the channel entrance to limit nonspecific movement of molecules through OprD channels (20).

OprD is found as a moderately expressed outer membrane protein but is regulated by multiple systems. It is repressed by MexT (which also induces the MexEF-OprN efflux system), salicylate, and catabolite repression (23, 39), and it is activated by arginine/ArgR and a variety of other amino acids as carbon and nitrogen sources (38).

The genome sequence (49) revealed that OprD is part of a 19-member family of outer membrane proteins in *P. aeruginosa* which are 46%–57% similar to OprD at the amino acid level. Phylogenetic analysis has revealed two subfamilies, the OprD group and the OpdK group (F.S.L. Brinkman, S. Tamber & R.E.W. Hancock, unpublished data). Eight homologs are more closely related to OprD, and those studied have roles in amino acid or peptide transport. Eleven homologs are more similar to the PhaK porin of *P. putida* that is required for growth on phenyl acetic acid (including the previously studied anaerobically induced porin OprE) (17), and those studied have roles in transport of organic carbon sources (S. Tamber & R.E.W. Hancock, unpublished data). However, study of mutants in each gene indicate that only OprD is involved in antibiotic uptake, in contrast to earlier conclusions made regarding certain OprD homologs (17). Microarray analysis has indicated that only OprD, OprQ, OpdP, OpdQ, and OprE are even moderately

produced in minimal medium with succinate as a carbon source (M. Brazas & R.E.W. Hancock, unpublished data).

OprB

The closest homolog in *P. aeruginosa* of the crystallized specific porin LamB (Figure 1*C*) is OprB (17). It is induced by growth on minimal medium supplemented with glucose as the sole carbon source, and catabolite repressed by succinate. In contrast, *E. coli* LamB is induced by maltose, which is not a growth substrate for *P. aeruginosa*. Nevertheless, studies of both OprB and LamB indicate that they form rather similar channels, with small single-channel conductance for KCl that can be blocked by maltodextrins of four sugars more effectively than by glucose. Structural predictions based on regions of homology with LamB indicate that OprB has a cluster of five tryptophan and seven phenylalanine residues that resemble the so-called "greasy slide," which is proposed to guide the diffusion of sugars through the LamB channel.

Interposon mutants lacking OprB were deficient in passage across the outer membrane of a variety of sugars inducing mannitol, fructose, and glycerol (59). Similarly the liposome swelling experiments of Trias et al. indicated that OprB was selective for glucose and xylose (55). Thus, OprB is a general carbohydrate-selective porin. The equivalent porin of *P. putida* was highly similar in many properties (17) and is 80% identical (*P. putida* unfinished genome sequence). Interestingly, *P. aeruginosa* contains one other close OprB homolog, PA2291, which demonstrates 96% identity to OprB, and another, PA4099, which has 24% identical and 12% similar amino acids.

GATED PORINS

Iron is a requirement for virtually all microorganisms and it is of particular importance in aerobic metabolism (56). Thus bacteria have evolved a series of elegant strategies for acquiring iron, including the production and secretion of powerful iron-binding compounds called siderophores, and the direct acquisition of iron from heme or hemoglobin, *Pseudomonas* sp., being aerobes, employ a wide variety of uptake systems for acquisition of iron in conjunction with siderophores (both known and unknown) and heme/hemoglobin. The initial step in uptake involves association with an outer membrane receptor protein. Prototypes of these receptors have been crystallized (22) and form 22-stranded β-barrels, into the center of which folds a 4-stranded β-sheet domain (visualized as a gate) (Figure 1*A*). Engagement of the receptor by the ferric-iron-loaded compound and energy input through the auspices of a periplasm-spanning inner-membrane protein called TonB (in conjunction with ExbB and ExbD) leads to a conformational change that opens the gate and lets the iron-loaded compound through the outer membrane (21). Such receptor proteins are termed TonB-dependent receptors and/or iron-regulated outer membrane proteins (IROMP) and function

as gated porins. A major surprise arising from knowledge of the genome sequence was the large number [35] of such gated porin homologs (Tables 2 and 3). Here we describe only those members of this family that have been functionally characterized.

FpvA

P. aeruginosa pyoverdine is a 6,7-dihydroxyyquinolone-containing fluorescent compound joined to a partly cyclic octapeptide. It has high affinity for Fe^{3+}. It is probably the predominant siderophore for iron acquisition from transferrin or serum in vivo (56). Although many *Pseudomonas* sp. produce pyoverdine siderophores, there is chemical heterogeneity and considerable specificity in that each bacterium tends to utilize its own siderophore and few others (13). This specificity is mediated at the level of the outer membrane receptors/gated porins. The receptor for the pyoverdine of *P. aeruginosa* PA01 is FpvA (13).

It was demonstrated that FpvA copurified with iron-free pyoverdine, but this did not lead to productive transport (47). Ferric-pyoverdine displaces this iron-free pyoverdine with rapid kinetics to form FpvA-pyoverdine-Fe^{3+} complexes in a reaction that is dependent on TonB. (In fact, *P. aeruginosa* has two TonB homologs, and one, TonB1, is preferred over TonB2 for this displacement). This then presumably leads to ferric-pyoverdine translocation across the membrane. Insertion mutagenesis of *fpvA* has identified two sites, Y359 and Y402 (13), where incorporation of an 18–amino acid–encoding sequence compromised ferric pyoverdine binding and uptake. These residues are presumed to be extramembranous, and it was hypothesized that they are in a region that is involved in ligand binding.

PupA, PupB

The characterized ferric-pyoverdine receptors of *P. putida* are called PupA and PupB, although pyoverdines are also called pseudobactins in this species (6, 25). These proteins are homologous to FpvA and other putative TonB receptors of *P. aeruginosa* (Table 3). These receptors have different specificities in that PupA is a specific receptor for ferric pseudobactin 358 while PupB facilitates transport via two siderophores, pseudobactin BN7 and BN8, as well as being inducible by a variety of heterologous siderophores. Interestingly, Bitter et al. (6) constructed hybrid siderophores with the *E. coli* ferric-coprogen receptor FhuA, and these hybrids were active and helped define domains of these proteins. For example, it was concluded that the ligand-binding domains were located in different regions of these proteins. Both the genome sequence and specific PCR experiments indicate that *P. putida* contains multiple ferric-pseudobactin (pyoverdine) receptors. This is certainly also true for *P. aeruginosa* PA01 (Table 3).

FptA/PfeA/Heme

Another *P. aeruginosa* siderophore named pyochelin is structurally distinct in possessing neither hydroxamate nor catecholate-chelating groups. When loaded

TABLE 3 Probable outer-membrane porins

PAID	Gene name	Range from	Range to	Similarity	No. of AAs	Probable porin class[a]
PA2760	oprQ	3120072	3121349	59% similar to OprD, named OprE3 in Genbank	425	SP
PA2291	opbA	2522616	2521258	62% similar to OprB of *P. aerguinosa*	452	SP
PA2700	opdB	3053843	3055150	57% similar to OprD	435	SP
PA0162	opdC	184594	185928	58% similar to OprD	444	SP
PA1025	opdD	1110947	1112197	62% similar to PhaK of *P. putida*; OprD family	416	SP
PA0240	opdF	271838	270573	53% similar to OprE; OprD family	421	SP
PA2213	opdG	2432312	2433562	60% similar to PhaK of *P. putida*; OprD family	416	SP
PA0755	opdH	824198	822915	58% similar to OprE; OprD family	427	SP
PA0189	opdI	216908	215550	55% similar to OprD	452	SP
PA2420	opdJ	2702925	2704343	51% similar to OprD	472	SP
PA4898	opdK	5495712	5494459	56% similar to PhaK of *P. putida*; OprD family	417	SP
PA4137	opdL	4626661	4627917	69% similar to PhaK of *P. putida*; OprD family	418	SP
PA4179	opdN	4674943	4676238	58% similar to PhaK of *P. putida*; OprD family	431	SP
PA2113	opdO	2324783	2323554	62% similar to PhaK of *P. putida*; OprD family	409	SP
PA4501	opdP	5038900	5040354	52% similar to OprD	484	SP
PA3038	opdQ	3400683	3401948	65% similar to PhaK of *P. putida*; OprD family	421	SP
PA3588	opdR	4021918	4020668	56% similar to OprE; OprD family	416	SP
PA2505	opdT	2823919	2822573	57% similar to OprD, named OprD3 in Genbank	448	SP
PA1288	fadL	1400505	1399231	47% similar to fatty acid transport protein FadL of *E. coli*	424	SP
PA4589		5140440	5139049	41% similar to fatty acid transport protein FadL of *E. coli*	463	SP
PA1764		1906842	1908440	40% similar to fatty acid transport protein FadL of *E. coli*	532	SP
PA4099		4581392	4582696	36% similar to glucose porin OprB	434	SP
PA0165		189120	189956	46% similar to region of OMP Tsx of *S. typhimurium*	278	SP?
PA2522	czcC	2843304	2842018	59% similar to cation efflux protein CzcC of *R. eutropha*; OprM family	428	EP

TABLE 3 (*Continued*)

PAID	Gene name	Range from	Range to	Similarity	No. of AAs	Probable porin class[a]
PA2837	opmA	3190210	3191649	53% similar to OprN	479	EP
PA2525	opmB	2847778	2846282	50% similar to OprM	498	EP
PA4208	opmD	4710620	4712083	56% similar to OprN	487	EP
PA3521	opmE	3939494	3938019	52% similar to OprN	491	EP
PA4592	opmF	5144533	5143052	40% similar to type I secretion protein CyaE of *B. pertussis*; OprM family	493	EP, PI?
PA5158	opmG	5805679	5807157	53% similar to putative aromatic efflux pump OMP of *S. aromaticivorans*; OprM family	492	EP
PA4974	opmH	5584100	5585548	54% similar to efflux porin TolC of *E. coli*	482	EP, PI?
PA3894	opmI	4362983	4361493	51% similar to putative aromatic efflux pump OMP of *S. aromaticivorans*; OprM family	496	EP
PA1238	opmJ	1340527	1339079	51% similar to OprN	482	EP
PA4144	opmK	4636297	4637712	49% similar to Type I secretion protein CyaE of *B. pertussis*; OprM family.	471	EP, PI?
PA1875	opmL	2043847	2045124	41% similar to AprF; OprM family	425	EP, PI?
PA3404	opmM	3810612	3809257	68% similar to AprF; OprM family	451	EP, PI
PA2391	opmQ	2645303	2646727	48% similar to OprM	474	EP
PA0931	pirA	1018230	1020458	72% similar to ferric enterobactin receptor PfeA	742	GP
PA4514	piuA	5055876	5053615	49% similar to putative iron transport receptor of *E. coli*	753	GP
PA1910	ufrA	2084267	2081853	99% similar to undefined iron transport receptor UfrA of *P. aeruginosa*	804	GP
PA1322	pfuA	1433166	1435364	44% similar to ferrichrome-iron receptor of *S. paratyphi*	732	GP
PA0674	pigC	734159	734875	53% similar to FpvA	238	GP
PA1922	cirA	2097491	2099452	56% similar to iron-regulated colicin I receptor of *E. coli*.	653	GP
PA3901	fecA	4368836	4371190	75% similar to ferric citrate receptor FecA of *E. coli*	784	GP
PA0470	fiuA	532437	530029	98% similar to ferrioxamine receptor of *P. aeruginosa*	802	GP
PA1302	hxuC	1411585	1414140	57% similar to Ton-dependent heme receptor TdhA of *H. ducreyi*	851	GP
PA4675	optH	5243177	5245405	62% similar to ferric aerobactin receptor IutA *E. coli*	742	GP
PA4897	optI	5491345	5494314	52% similar to OM hemin receptor of *P. aeruginosa*	989	GP

(*Continued*)

TABLE 3 (*Continued*)

PAID	Gene name	Range from	Range to	Similarity	No. of AAs	Probable porin class[a]
PA2335	optO	2577150	2579519	37% similar to pesticin receptor of *Y. pestis*	789	GP
PA2466	optS	2785225	2782763	63% similar to ferrioxamine receptor FoxA of *Y. enterocolitica*	820	GP
PA4837		5429841	5427715	45% similar to ferrichrome iron receptor FhuA of *E. agglomerans*	708	GP
PA0151		171047	173434	43% similar to ferric-pseudobactin receptor PupB of *P. putida*	795	GP
PA0192		219172	221544	39% similar to pesticin receptor FyuA of *Y. enterocolitica*	790	GP
PA0434		484964	487156	43% similar to ferric-pseudobactin receptor PupB of *P. putida*	730	GP
PA0781		851319	849256	37% similar to PhuR	687	GP
PA0982		1065103	1064555	46% similar to 27-kDa OMP of *Coxiella burnetii*; probable TonB-dependent receptor	182	GP
PA1271		1381804	1383654	46% similar to BtuB, OM receptor for transport of vitamin B12 of *E. coli*	616	GP
PA1365		1476384	1478825	68% similar to the ferric alcaligin receptor AleB of *R. eutropha*	813	GP
PA1613		1758597	1756489	37% similar to OM receptor for colicin I CirA of *E. coli*	702	GP
PA2057		2251275	2253815	43% similar to ferric-pseudobactin receptor PupB of *P. putida*	846	GP
PA2089		2298012	2300663	40% similar to ferric enterobactin receptor of *B. pertussis*	883	GP
PA2289		2518561	2516429	56% similar to putative OM receptor for iron transport in *E. coli*	710	GP
PA2911		3265847	3268003	42% similar to putative hydroxamate-type ferrisiderophore receptor of *P. aeruginosa*	718	GP
PA3268		3658150	3655985	61% similar to *E. coli* ferric citrate receptor FecA	721	GP
PA4156		4652457	4650373	48% similar to ferric vibriobactin receptor ViuA of *V. cholerae*	694	GP
PA4168		4663853	4666261	54% similar to ferripyoverdine receptor FpvA	802	GP
PA2590		2933461	2930807	50% similar to ferric enterobactin receptor of *Xylella fastidiosa*	884	GP
PA0685	hxcQ	741925	744336	49% similarity to type II secretion protein XcpQ	803	PII
PA1382		1498813	1501092	49% similar to S-protein secretion D of *Aeromonas hydrophila*	759	PII
PA4304		4829628	4828378	47% similar to type II secretion protein of *Mesorhizobium loti*	416	PII

[a]P, general porin; SP, specific porin; GP, putative gated porin; EP, OprM family member of efflux and protein secretion porins; PI, putative type I secretion subfamily; PII, type II secretion channel; PIII, type III secretion channel.

with iron it is taken up by the FptA receptor (3). Interestingly, FptA is a known virulence determinant (56).

The *E. coli* siderophore enterobactin can utilize the PfeA receptor in iron uptake (11). Indeed PfeA shows more than 60% homology to its *E. coli* counterpart, the crystallized gated porin FepA, with especially high homology in the ligand-binding regions. Consistent with this, the cloned *pfeA* gene complemented an *E. coli fepA* mutant to permit enterobactin-dependent iron uptake. It has been postulated that a second, lower-affinity ferric-enterobactin uptake system exists in *P. aeruginosa* (56), and a protein named PirA, which has 72% similarity to PfeA, is a candidate for being responsible for this uptake system.

Another characterized uptake system in *P. aeruginosa* is the heme iron uptake system. This involves two outer membrane receptors, HasR and PhuR (40). Both systems mediate growth on hemin or hemoglobin as its sole iron source, but it requires a double knockout to eliminate growth on hemin and hemoglobin. Another ORF in *P. aeruginosa* termed OptI is 52% similar to HasR (Table 3) but has not been characterized. A variety of other TonB dependent iron uptake receptors exist but only one, Fiu, has been defined. This apparently acts as the receptor for the uptake of ferrioxamine B (56).

OprC

OprC was first described as a nonselective porin that formed slightly anion-selective, small diffusion pores (17). However, later work disproved a role in antibiotic uptake (61, 62). Nakae and collaborators demonstrated that OprC is 65% homologous with *P. stuzeri* NosA (27, 61), an outer membrane porin required for production of the Cu^{2+}-containing nitrate reductase. OprC is only made anaerobically and is repressed by high medium Cu^{2+} concentrations (61). It is interesting that it shows substantial homology to PfeA (25% identity, 40% similarity over 504 amino acids from the N and C termini) and thus appears to be a member of the large TonB-dependent family of proteins, most of which are involved in uptake of complexed iron. It seems likely that the substrate for OprC is actually Cu^{2+}. Another unusual member of the TonB family of outer membrane receptors is BtuB, the *E. coli* receptor for vitamin B12 (10). *P. aeruginosa* contains a gene PA1271 that is 46% similar to BtuB.

OprH

OprH is an outer membrane protein that is upregulated upon Mg^{2+} starvation by the PhoPQ two-component regulatory system, with which it forms the *oprH phoP phoQ* operon (31). Insertion and deletion mutagenesis have demonstrated that it forms an eight-stranded β-barrel (45). While devoid of porin activity in its native form, it forms channels when surface loop 4 is deleted (B. Rehm & R.E.W. Hancock, unpublished observations). Thus, it is possible that OprH is a gated porin for divalent cations.

EFFLUX PORINS

As described above, active efflux is a major contributor to intrinsic multiple antibiotic resistance in *P. aeruginosa*. In addition overexpression of any of at least three efflux operons leads to even higher resistance to a wide range of clinically useful antibiotics (42). The most important efflux systems in *P. aeruginosa* are members of the resistance-nodulation-division (RND) family. This series of efflux systems involves a three-component efflux pathway, which includes a cytoplasmic-membrane pump protein, a peripheral cytoplasmic-membrane linker (sometimes called a membrane-fusion protein), and an elaborate outer membrane/periplasmic channel protein. Each of these proteins is so highly conserved (at around the 20% or greater identity level) that sequence homology searching can easily identify them. We largely concern ourselves here with the outer membrane channel proteins (termed here efflux porins). The best studied of these is the *E. coli* TolC channel-tunnel, which was recently crystallized (24) and is discussed below. This protein has a dual function in multiple antibiotic efflux and as a component of the type I secretion system for hemolysin.

P. aeruginosa has 18 outer membrane proteins with putative functions in efflux (49). Eleven of these, including OprM, OprN, and OprJ, fall into one phylogenetic subclass (www.cmdr.ubc.ca/bobh/omps/phylogenetic.htm) and are presumed to be parts of specialized multiple antibiotic efflux systems. Of the other seven, one is a homolog of CzcC that is involved in cation efflux (as a detoxification mechanism), one is AprF, which is involved in the type I secretion of alkaline protease, and a third OpmH is the closest *P. aeruginosa* homolog of *E. coli* TolC (54% similar). The four others, OpmF, OmpK, OpmL, and OpmM, are similar to CyaE of *Burkholderia pertussis* or to AprF (Table 3), and thus are likely to also be components of type I protein secretion pathways for as-yet-unknown substrates. As is clear for TolC, a single efflux outer membrane protein can serve more than one secretion/efflux system (24) in part because specificity is determined by the pump component in combination with the linker (45).

OprM

OprM is the major outer membrane efflux porin involved in intrinsic multiple antibiotic resistance in *P. aeruginosa*. Deletion of OprM leads to 10–1000-fold increases in susceptibility to many antibiotics from different classes (29), and the cloned OprM gene can complement such deletions. Conversely, mutations in the *nalB* (*mexR*) gene can lead to overexpression of OprM and its neighboring linker and pump proteins, MexA and MexB, and cause resistance to a broad range of antibiotics (42).

It has also been reported that OprM collaborates with the MexX-MexY system to mediate aminoglycoside resistance (1), although certain results (57) and our own unpublished studies are not entirely consistent with this conclusion. OprM shares only 21% identity with TolC, but it can be structurally modeled based on the TolC

crystal structure (58) (Figure 1*B*) using a procedure called threading. Studies involving insertions and deletions in two laboratories (28, 58) indicate that the resultant model is reasonably accurate. Thus we can define OprM function by reference to the TolC-like model (24). OprM is assumed to be a trimer of three subunits that comprises a single channel-tunnel spanning the outer membrane and periplasm. The trimer forms a 12-stranded β-barrel (4 β-strands per monomer) that lodges in the outer membrane and sits atop a coiled 12-helix α-helical barrel that spans the periplasm and is presumed to contact the MexB-pump/MexA-linker complex in the cytoplasmic membrane. The α-helical barrel twists into a constricted point at the base proximal to the cytoplasmic membrane and is proposed to open like an iris diaphragm upon contact with the pump/linker complex, energy input, and possibly substrate engagement. Indeed freshly purified OprM formed nice large channels in planar lipid bilayer experiments, but over time of storage the channel conductance became much smaller (58), in fact similar to the conductance of *E. coli* TolC (5), a result that is consistent with open and closed states of the OprM channel.

Mutagenesis of OprM has contributed substantially to the overall picture of how this protein operates (28, 58). Insertions in the surface loop regions of the outer membrane barrel do not influence function, whereas insertions or deletions in most locations within the α-helical barrel are nonpermissive. As shown for TolC, there is a putative girdle around the periplasmic α-helical barrel segment, but deletions or insertions in this region seem to be well tolerated. Similarly, deletions and insertions at both the N and C termini, including removal of the putative N-terminal acylation site (such that OprM cannot become a lipoprotein), are tolerated and largely without functional consequences.

Other Multidrug Efflux Porins

P. aeruginosa has at least two other efflux porins, OprJ and OprN, that are normally silent but can be highly expressed due to mutation, as part of the MexCD-OprJ and MexEF-OprN operons, leading to multidrug resistance (17, 42). Most overexpressing mutants are in the *nfxB* repressor and *mexT* (*nfxC*) activator genes. The latter system is very interesting since *mexT* mutations lead to coordinate upregulation of the MexEF-OprN efflux system and downregulation of OprD (23, 39).

P. putida has several homologous systems that have been largely studied because of their ability to efflux aromatic hydrocarbons (42). However, systems involving the efflux porins ArpC, MepC, TtgC, and TtgI all influence antibiotic susceptibility when overexpressed. We have also gathered preliminary evidence linking OpmG, OpmH, and OpmI to aminoglycoside efflux in *P. aeruginosa* (J. Jo & R.E.W. Hancock, unpublished data).

AprF and Protein Secretion

P. aeruginosa secretes many proteins involved in virulence, utilizing largely type II secretion systems (52, 53). However, alkaline protease, the product of the *aprA* gene, is secreted by a three-component type 1 secretion system, AprDEF, where

AprF is the outer membrane component (53). Based on homology modeling and the known dual function of TolC (24), it seems possible to conclude that the outer membrane efflux component AprF functions similarly to TolC, although clearly folding of AprA must be avoided during secretion, suggesting a potential chaperone-like function. As mentioned above, there are five other homologs that could be engaged in type I secretion.

In addition to type I secretion, there are both type II and type III protein secretion pathways in *P. aeruginosa* (49). Both utilize outer membrane proteins that form ring-like structures with multiple subunits (9, 53). These presumably form the channels for secretion of proteins; however, because a large channel would compromise the low outer membrane permeability of *P. aeruginosa*, it is presumed that these channels are gated, probably by engagement of the specific secreted protein with other components of the secretion apparatus. In *P. aeruginosa*, the major outer membrane channel for the type I general secretion pathway is XcpQ (9). There is one other XcpQ homolog named XqhA (32). For type III secretion, the XcpQ homolog PscC acts as a channel (60).

CONCLUDING REMARKS

Publication of the genome sequence of *P. aeruginosa* (49) has dramatically expanded the extent of our understanding of the *P. aeruginosa* outer membrane and its porins. With the genomic sequencing of *P. putida* finished, and that of *P. syringae* and *P. fluorescens* underway, we are rapidly moving to where we can better understand how the outer membrane contributes to the extraordinary versatility of this group of organisms. The biggest surprise in the genomic sequence of *P. aeruginosa* was the finding of three large families of outer membrane proteins with 18–35 individual members. These families arose from distant gene duplication events followed by evolutionary divergence, almost as if *Pseudomonas* was operating on a rather simple blueprint. Thus these families likely reflect the versatility of this organism as each protein diverged to have slightly differing functions and was selected to be maintained in the organism. A major topic of research will be to attempt to understand how the functions of these proteins relate to their diverged sequences and how the regulatory network permits them to be expressed when they are needed.

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LITERATURE CITED

1. Aires JR, Köhler T, Nikaido H, Plesiat P. 1999. Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. *Antimicrob. Agents Chemother.* 43:2624–28
2. Andersen C, Bachmeyer C, Tauber H, Benz R, Wang J, et al. 1999. In vivo and in vitro studies of major surface loop deletion mutants of the *Escherichia coli* K-12 maltoporin: contribution to maltose and maltooligosaccharide transport and binding. *Mol. Microbiol.* 32:851–67
3. Ankenbauer RG, Quan HN. 1994. FptA, the Fe(III)-pyochelin receptor of *Pseudomonas aeruginosa*: a phenolate siderophore receptor homologous to hydroxamate siderophore receptors. *J. Bacteriol.* 176:307–19
4. Bellido F, Martin NL, Siehnel RJ, Hancock REW. 1992. Reevaluation, using intact cells, of the exclusion limit and role of porin OprF in *Pseudomonas aeruginosa* outer membrane permeability . *J. Bacteriol.* 174:5196–203
5. Benz R, Maier E, Gentschev I. 1993. TolC of *Escherichia coli* functions as an outer membrane channel. *Zentralbl. Bakteriol.* 278:187–96
6. Bitter W, van Leeuwen IS, de Boer J, Zomer HW, Koster MC, et al. 1994. Localization of functional domains in the *Escherichia coli* coprogen receptor FhuE and the *Pseudomonas putida* ferric-pseudobactin 358 receptor PupA. *Mol. Gen. Genet.* 245:694–703
7. Brinkman FS, Bains M, Hancock REW. 2000. The amino terminus of *Pseudomonas aeruginosa* outer membrane protein OprF forms channels in lipid bilayer membranes: correlation with a three-dimensional model. *J. Bacteriol.* 182:5251–55
8. Brinkman FS, Schoofs G, Hancock RE, De Mot R. 1999. Influence of a putative ECF sigma factor on expression of the major outer membrane protein, OprF, in *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. *J. Bacteriol.* 181:4746–54
9. Brok R, Van Gelder P, Winterhalter M, Ziese U, Koster AJ, et al. 1999. The C-terminal domain of the *Pseudomonas* secretin *XcpQ* forms oligomeric rings with pore activity. *J. Mol. Biol.* 294:1169–79
10. Cadieux N, Bradbeer C, Kadner RJ. 2000. Sequence changes in the ton box region of BtuB affect its transport activities and interaction with TonB protein. *J. Bacteriol.* 182:5954–61
11. Dean CR, Poole K. 1993. Cloning and characterization of the ferric enterobactin receptor gene (pfeA) of *Pseudomonas aeruginosa*. *J. Bacteriol.* 175:317–24
12. El Hamel C, Freulet MA, Jaquinod M, De E, Molle G, Orange N. 2000. Involvement of the C-terminal part of *Pseudomonas fluorescens* OprF in the modulation of its pore-forming properties. *Biochim. Biophys. Acta* 1509:237–44
13. Folschweiller N, Schalk IJ, Celia H, Keiffer B, Abdallah MA, et al. 2000. The pyoverdin receptor FpvA, a TonB receptor involved in iron uptake by *Pseudomonas aeruginosa*. *Mol. Membr. Biol.* 17:123–33
14. Gilleland HE, Gilleland LB, Staczek J, Harty RN, Garcia-Sastre A, et al. 2000. Chimeric animal and plant viruses expressing epitopes of outer membrane protein F as a combined vaccine against *Pseudomonas aeruginosa* lung infection. *FEMS Immunol. Med. Microbiol.* 27:291–97
15. Hancock REW. 1997. The bacterial outer membrane as a drug barrier. *Trends Microbiol.* 5:37–42
16. Hancock REW, Speert DP. 2000. Antibiotic resistance in *Pseudomonas aeruginosa*. Mechanisms and impact on treatment. *Drug Resist. Updat.* 3:247–55
17. Hancock REW, Worobec EA. 1998. Outer

membrane proteins. See Ref. 33, pp. 139–67

18. Huang H, Hancock REW. 1993. Genetic definition of the substrate selectivity of outer membrane porin protein OprD of *Pseudomonas aeruginosa*. *J. Bacteriol.* 175:7793–800
19. Huang H, Hancock REW. 1996. The role of specific surface loop regions in determining the function of the imipenem-specific pore protein OprD of *Pseudomonas aeruginosa*. *J. Bacteriol.* 178:3085–90
20. Huang H, Jeanteur D, Pattus F, Hancock REW. 1995. Membrane topology and site-specific mutagenesis of *Pseudomonas aeruginosa* porin OprD. *Mol. Microbiol.* 16:931–41
21. Klebba PE, Newton SM. 1998. Mechanisms of solute transport through outer membrane porins: burning down the house. *Curr. Opin. Microbiol.* 1:238–47
22. Koebnik R, Locher KP, Van Gelder P. 2000. Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Mol. Microbiol.* 37:239–53
23. Kohler T, Michea-Hamzehpour M, Henze U, Gotoh N, Curty LK, Pechere J-C. 1997. Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. *Mol. Microbiol.* 23:345–54
24. Koronakis V, Li J, Koronakis E, Stauffer K. 1997. Structure of TolC, the outer membrane component of the bacterial type I efflux system, derived from two-dimensional crystals. *Mol. Microbiol.* 23:617–26
25. Koster M, van de Vossenberg J, Leong J, Weisbeek PJ. 1993. Identification and characterization of the pupB gene encoding an inducible ferric-pseudobactin receptor of *Pseudomonas putida* WCS358. *Mol. Microbiol.* 8:591–601
26. Larbig M, Mansouri E, Freihorst J, Tummler B, Kohler G, et al. 2001. Safety and immunogenicity of an intranasal *Pseudomonas aeruginosa* hybrid outer membrane protein F-I vaccine in human volunteers. *Vaccine* 19:2291–97
27. Lee HS, Hancock REW, Ingraham JL. 1989. Properties of a *Pseudomonas stutzeri* outer membrane channel-forming protein (NosA) required for production of copper-containing N_2O reductase. *J. Bacteriol.* 171:2096–100
28. Li XZ, Poole K. 2001. Mutational analysis of the OprM outer membrane efflux component of the MexA-MexB-OprM multidrug efflux system of *Pseudomonas aeruginosa*. *J. Bacteriol.* 183:12–27
29. Li XZ, Poole K, Nikaido H. 1995. Role of MexA-MexB-OprM in antibiotic efflux in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 39:1948–53
30. Lynch MJ, Drusano GL, Mobley HLT. 1987. Emergence of resistance to imipenem in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 31:1892–96
31. Macfarlane ELA, Kwasnicka A, Ochs MM, Hancock REW. 1999. PhoP-PhoQ homologues in *Pseudomonas aeruginosa* regulate expression of the outer-membrane protein OprH and Polymyxin B resistance. *Mol. Microbiol.* 34:305–16
32. Martinez A, Ostrovsky P, Nunn DN. 1998. Identification of an additional member of the secretin superfamily of proteins in *Pseudomonas aeruginosa* that is able to function in type II protein secretion. *Mol. Microbiol.* 28:1235–46
33. Montie T, ed. 1998. *Pseudomonas*. *Biotechnology Handbooks*, Vol. 10. London: Plenum. 334 pp.
34. Nakae T, Nakajima A, Ono T, Saito K, Yoneyama H. 1999. Resistance to β-lactam antibiotics in *Pseudomonas aeruginosa* due to interplay between the MexAB-OprM efflux pump and β-lactamase. *Antimicrob. Agents Chemother.* 43:1301–3
35. Nikaido H, Hancock REW. 1986. Outer membrane permeability of *Pseudomonas aeruginosa*. In *The Bacteria: A Treatise on Structure and Function*, ed. JR Sokatch, pp. 145–93. London: Academic
36. Nikaido H, Nikaido T, Harayama S. 1991. Identification and characterization of

porins in *Pseudomonas aeruginosa*. *J. Biol. Chem.* 266:770–79

37. Ochs MM, Bains M, Hancock REW. 2000. Role of putative loops 2 and 3 in imipenem passage through the specific porin OprD of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 44:1983–85
38. Ochs MM, Lu CD, Hancock REW, Abdelal AT. 1999. Amino acid–mediated induction of the basic amino acid–specific outer membrane porin OprD from *Pseudomonas aeruginosa*. *J. Bacteriol.* 181:5426–32
39. Ochs MM, McCusker MP, Bains M, Hancock REW. 1999. Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. *Antimicrob. Agents Chemother.* 43:1085–90
40. Ochsner UA, Johnson Z, Vasil ML. 2000. Genetics and regulation of two distinct haem-uptake systems, *phu* and *has*, in *Pseudomonas aeruginosa*. *Microbiology* 146:185–98
41. Okamoto K, Gotoh N, Nishino T. 2001. *Pseudomonas aeruginosa* reveals high intrinsic resistance to penem antibiotics. Penem resistance mechanisms and their interplay. *Antimicrob. Agents Chemother.* 45:1964–71
42. Poole K. 2001. Multidrug resistance in Gram-negative bacteria. *Curr. Opin. Microbiol.* 4:500–8
43. Pumbwe L, Everett MJ, Hancock REW, Piddock LJ. 1996. Role of gyrA mutation and loss of OprF in the multiple antibiotic resistance phenotype of *Pseudomonas aeruginosa* G49. *FEMS Microbiol. Lett.* 143:25–28
44. Rawling EG, Brinkman FS, Hancock REW. 1998. Roles of the carboxy-terminal half of *Pseudomonas aeruginosa* major outer membrane protein OprF in cell shape, growth in low-osmolarity medium, and peptidoglycan association. *J. Bacteriol.* 180:3556–62
45. Rehm BHA, Hancock REW. 1996. Membrane topology of the outer membrane protein OprH from *Pseudomonas aeruginosa*: PCR-mediated site-directed insertion and deletion mutagenesis. *J. Bacteriol.* 178:3346–49
46. Saint N, El Hamel C, De E, Molle G. 2000. Ion channel formation by N-terminal domain: a common feature of OprFs of *Pseudomonas* and OmpA of *Escherichia coli*. *FEMS Microbiol. Lett.* 190:261–65
47. Schalk IJ, Hennard C, Dugave C, Poole K, Abdallah MA, et al. 2001. Iron-free pyoverdin binds to its outer membrane receptor FpvA in *Pseudomonas aeruginosa*: a new mechanism for membrane iron transport. *Mol. Microbiol.* 39:351–60
48. Schirmer T, Keller TA, Wang YF, Rosenbusch JP. 1995. Structural basis for sugar translocation through maltoporin channels at 3.1 Å resolution. *Science* 267:512–14
49. Stover KC, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, et al. 2000. Complete genome sequence of *Pseudomonas aeruginosa*: an opportunistic pathogen. *Nature* 406:959–64
50. Sugawara E, Steiert M, Rouhani S, Nikaido H. 1996. Secondary structure of the outer membrane proteins OmpA of *Escherichia coli* and OprF of *Pseudomonas aeruginosa*. *J. Bacteriol.* 178:6067–69
51. Sukhan A, Hancock REW. 1996. The role of specific lysine residues in the passage of anions through the *Pseudomonas aeruginosa* porin OprP. *J. Biol. Chem.* 271: 21239–42
52. Thanassi DG, Hultgren SJ. 2000. Multiple pathways allow protein secretion across the bacterial outer membrane. *Curr. Opin. Cell Biol.* 12:420–30
53. Tommassen J, Filloux A, Bally M, Murgier M, Lazdunski A. 1992. Protein secretion in *Pseudomonas aeruginosa*. *FEMS Microbiol. Rev.* 9:73–90
54. Trias J, Nikaido H. 1990. Protein D2 channel of the *Pseudomonas aeruginosa* outer membrane has a binding site for basic amino acids and peptides. *J. Biol. Chem.* 265:15680–84
55. Trias J, Rosenberg EY, Nikaido H. 1988. Specificity of the glucose channel formed

by protein D1 of *Pseudomonas aeruginosa*. *Biochim. Biophys. Acta.* 938:493–96

56. Vasil M, Ochsner UA. 1999. The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry, and virulence. *Mol. Microbiol.* 34:399–413
57. Westbrock-Wadman S, Sherman DR, Hickey MJ, Coulter SN, Zhu YQ, Warrener P. 1999. Characterization of a *Pseudomonas aeruginosa* efflux pump contributing to aminoglycoside impermeability. *Antimicrob. Agents Chemother.* 43:2975–83
58. Wong KKY, Brinkman FSL, Benz RS, Hancock REW. 2001. Evaluation of a structural model of *Pseudomonas aeruginosa* outer membrane protein OprM, an efflux component involved in intrinsic antibiotic resistance. *J. Bacteriol.* 183:367–74
59. Wylie JL, Worobec EA. 1995. The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*. *J. Bacteriol.* 177:3021–26
60. Yahr TL, Goranson J, Frank DW. 1996. Exoenzyme S of *Pseudomonas aeruginosa* is secreted by a type III pathway. *Mol. Microbiol.* 22:991–1003
61. Yoneyama H, Nakae T. 1996. Protein C (OprC) of the outer membrane of *Pseudomonas aeruginosa* is a copper-regulated channel protein. *Microbiology* 142:2137–44
62. Yoneyama H, Yamano Y, Nakae T. 1995. Role of porins in the antibiotic susceptibility of *Pseudomonas aeruginosa*: construction of mutants with deletions in the multiple porin genes. *Biochem. Biophys. Res. Commun.* 213:88–95
63. Zgurskaya HI, Nikaido H. 2000. Multidrug resistance mechanisms: drug efflux across two membranes. *Mol. Microbiol.* 37:219–25

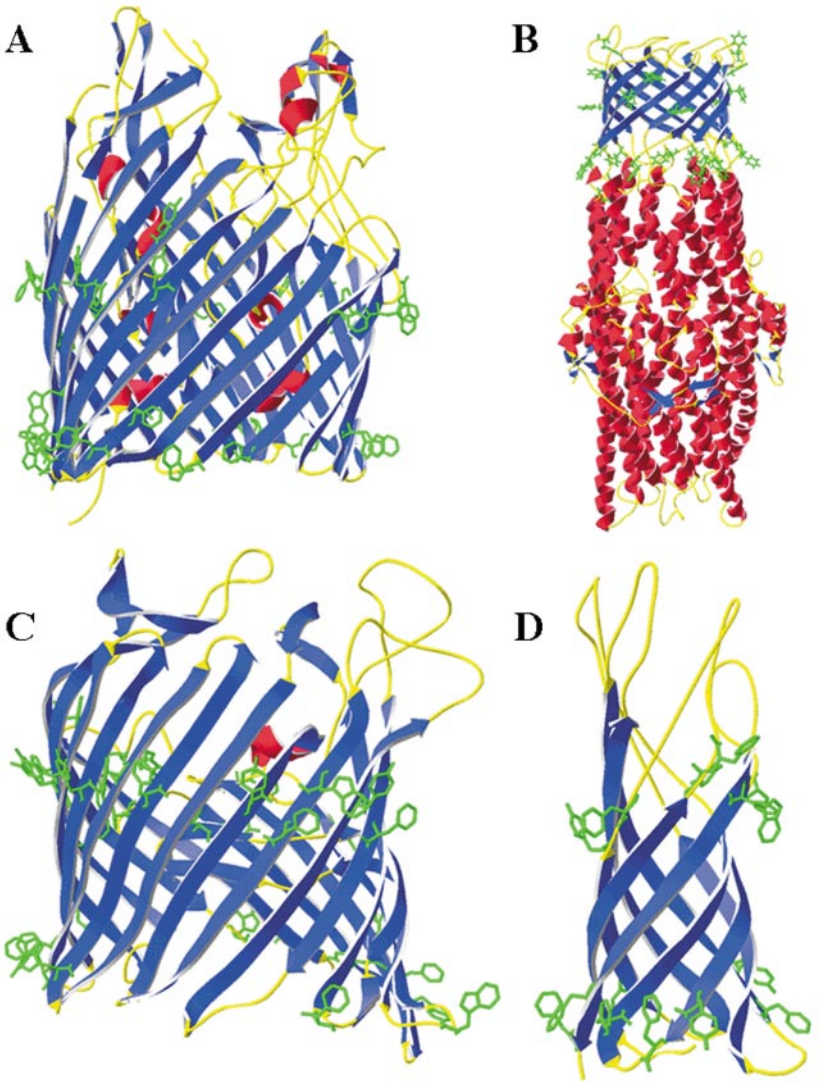

Figure 1 Representative models of the four classes of porins, based on crystal structures of *E. coli* gated porin FepA (*A*) and specific porin LamB (*C*) (22) and homology models of *P. aeruginosa* efflux porin OprM (*B*) and the N-terminal domain of the nonspecific porin OprF (*D*). The homology models were developed by threading to orthologous *E. coli* proteins as previously described (7, 58). Structures are colored to aid visualization of β-strands (*blue*), α-helices (*red*), and loop regions (*yellow*) with aromatic residues that form "rings" around the β-barrels illustrated in *green*. Such rings are proposed to stabilize the barrel in the membrane, being situated at the lipid-solvent interface.

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