dr(anoic acid) gene locus of *Pseudomonas aeruginosa* PAO1, *FEBS Eur. J. Biochem.* **209:** ..9–30.
Van Dyke, M. W., Couture, P., Brauer, M., Lee, H., and Trevors, J. T., 1993, *Pseudomonas aeruginosa* UG2 rhamnolipid biosurfactants: Structural characterization and their use in removing hydrophobic compounds from soil, *Can. J. Microbiol.* **39:** 1071–1078.
Wada, M., Fukunaga, N., and Sasaki, S., 1987, Effect of growth temperature on phospholipid and fatty acid composition in a phychrotrophic bacterium, *Pseudomonas* sp. strain E-3, *Plant Cell. Physiol.* **28:** 1209–1217.
Weber, F. J., Isken, S., and de Bont, J. A. M., 1994, Cis/trans isomerization of fatty acids as a defense mechanism of *Pseudomonas putida* strains to toxic concentrations of toluene, *Microbiology* **140:** 2013–2017.
White, D. C., Sutton, S. D., and Ringleberg, D. B., 1996, The genus *Sphingomonas*: Physiology and ecology, *Current Opinion in Biotechnology*, July.
Wilkinson, S. G., 1988, Gram-negative bacteria, in: *Microbial Lipids* (C. Ratledge and S. G. Wilkinson eds.), Academic Press, San Diego, Vol. 1, pp. 333–348.
Wilkinson, S. G., Galbraith, L., and Lightfoot, G. A., 1973, Cells walls, lipids, and lipopolysaccharides of *Pseudomonas* species, *Eur. J. Biochem.* **33:** 158–174.
Winson M. K., Camara, M., Latifi, A., Foglino, M., Chabra, S. R., Daykin, M., Bally, M., Chapon, V., Salmond, G. P., and Bycroft, B. W., 1995, Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*, *Proc. Natl. Acad. Sci. USA* **92:** 9427–9431.
Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T., and Yamamoto, Y., 1990, Proposals of *Sphingomonas paucimobilis* gen. nov. and comb., nov. *Sphingomonas parapaucimobilis* sp. Nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*, *Microbiol. Immunol.* **34:** 99–119.
Yabuuchi, E., Kosako, Y., Arakawa, M., Hotta, H., and Yano, I., 1992, Identification of Oklahoma isolate as a strain of *Pseudomonas pseudomallei*, *Microbiol. Immunol.* **36:** 1239–1249.
Yabuuchi, E., Kosaka, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, H., Ezaki, T., and Arakawa, M., 1994, Proposal of *Burkholderia* gen. nov. and transfer of seven species of the *Pseudomonas* hoimology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov., *Microbiol. Immunol.* **36:** 1251–1275.
Zhang, Y., and Miller, R. M., 1992, Enhanced octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid (biosurfactant), *Appl. Env. Microbiol.* **58:** 3276–3282.

Outer Membrane Proteins

5

ROBERT E. W. HANCOCK and
ELIZABETH A. WOROBEC

1. INTRODUCTION

Pseudomonas aeruginosa is a gram-negative bacterium and as such has an outer membrane. The general functions of the outer membrane include size-dependent exclusion of larger molecules, permitting selective permeability of smaller molecules, uptake of large and small polycations, specific facilitated uptake of certain substrates, export of excreted molecules including proteins, secondary metabolites and siderophores, maintenance of cell shape and growth in low osmolarity medium, binding of phages, bacteriocins, and pili during conjugation, serum resistance, and surface binding of antibodies and complement. Most of these functions have been described for *Pseudomonas aeruginosa* and result from the properties of outer membrane functions. It has been 5 years since the last review of the outer membrane proteins of *Pseudomonas aeruginosa* (*Hancock et al.*, 1990). Because of the vast amount of data available on this topic, we have concentrated on the information published since the previous review, but prior to the release of the *Pseudomonas aeruginosa* genomic sequence (http://www.pseudomonas.com).

2. ROLE IN ANTIBIOTIC SUSCEPTIBILITY

It is now well established that *Pseudomonas aeruginosa* is intrinsically antibiotic-resistant and that the low outer membrane permeability of this organism contributes to this high intrinsic (background) level of resis-

ROBERT E. W. HANCOCK • Department of Microbiology and Immunology, University of British Columbia, Vancouver V6T 1Z4, British Columbia. ELIZABETH A. WOROBEC • Department of Microbiology, University of Manitoba, Winnepeg R3T 2N2, Manitoba.

Pseudomonas, edited by Montie. Plenum Press, New York, 1998.

Table I. (*Continued*)

Protein name	Other names	Apparent molecular weight[a]	Sequence accession number	Map position	Production conditions	Function	Identified in follow fluorescent pseudomonads[d]
OprO		48,000	M86648	SpeI-C DpnI-N	Inducible by low phosphate, stationary phase	Pyrophosphate uptake	
OprB	D1	46,000	X77131	–	Inducible by glucose	Glucose/sugar uptake	PP, PF
OprD	OprD2, D2, D	45,500	Z14065 X63152	SpeI-F DpnI-A	Low level constitutive	Basic amino and imipenem uptake	
OprE	E1	43,500	D12711	–	Anaerobic induction	Unknown specific substrate	
E	E2	44,000	–	–	Constitutive	Structural?	
OprF	F	38,000	M94078 M18795	SpeI-L DpnI-C	Constitutive	Major nonspecific porin/structural	PP, PS, PF, PC, PST, AV, PT
OprG	G	25,000	–	–	Low level constitutive. Induced in high iron, high Mg^{2+}	Unknown	
OprH	H1	21,000	M26954	SpeI-M DpnI-A	Low Mg^{2+}, Sr^{2+}, Mn^{2+}, and Ca^{2+}	Gated porin?; polycation/EDTA resistance	PP, PS, PF, PC, PST
OprL	H2	20,500	Z50191	–	Constitutive	Structural	All
OprI	I	8,000	M25761	SpeI-V DpnI-D	Constitutive	Structural	All

[a]Apparent molecular weight on SDS-PAGE. Actual molecular weights can be obtained from the sequences. Apparent molecular weights vary according to the SDS-PAGE system used.
[b]Physical map position not determined.
[c]Gene cloned but accession number not available at press time.
[d]Abbreviations: PP - *Pseudomonas putida*; PF - *P. fluorescens*; PS - *P. syringae*; PC - *P. chloraphis*; PT - *P. tolasii*; PST - *P. stutzeri*; AV - *Azobacter vinelandii*; All - all of the above Pseudomonads.

aerobactin (Liu and Shokrani, 1978) produced by enteric bacteria, ferrioxamine B produced by *Streptomyces spp.* (Cornelis *et al.*, 1987), and pyoverdines produced by other fluorescent pseudomonads (Hohnadel and Meyer, 1988).

The induction of high molecular weight, iron-regulated, outer membrane proteins by *P. aeruginosa*, in response to the presence of non-pseudomonad siderophores, occurs with desferral, a derivative of ferrioxamine B, and ferri-enterobactin. The latter enterobactin-mediated protein has been characterized. Poole *et al.* (1990), upon studying enterobactin-mediated iron uptake by a pyoverdine-deficient strain of *P. aeruginosa*, identified a novel 80,000-Da outer membrane protein. Mutant strains incapable of enterobactin-dependent iron uptake were also lacking this protein, suggesting that this protein an enterobactin receptor. The structural gene for this putative enterobactin receptor was later cloned and characterized (Dean and Poole, 1993). The gene product was termed PfeA for *Pseudomonas* ferric enterobactin receptor. The structural gene for this putative enterobactin uptake was expressed in E. coli and was capable of complementing a mutation in the *E. coli fepA* gene which encodes the *E. coli* FepA, enterobactin receptor protein (an 81,000-Da outer membrane protein). The *pfeA* gene codes for an 81,000-Da protein which has more than 60% homology to its *E. coli* counterpart. The most significant homology is in regions involved in ligand binding. In addition, and not surprisingly, the two proteins cross-react. *P. aeruginosa* PfeA protein can replace FepA in *E. coli*, hence reinforcing the similarity between enterobactin-dependent uptake between the two organisms. This suggests the existence of analogous components in *P. aeruginosa*, such as periplasmic and cytoplasmic membrane proteins. The identification of a TonB box at the N-terminus of PfeA and Fur binding sites upstream of the *pfeA* gene also indicates the similarities between the two systems because FepA function depends on interaction with the TonB protein (Lundrigan and Kadner, 1986) and the expression of FepA is under control of the *fur* gene (Earhart, 1987). The extent of the analogy between the two systems is currently under investigation.

The concomitant loss of pyoverdine production and decreased production of a 90,000-Da IROMP in *P. aeruginosa*, led Poole *et al.* (1991) to believe that this protein is a ferri-pyoverdine receptor. In addition, a mutant strain deficient in the production of this protein shows a substantial decrease in pyoverdine-mediated iron transport. Previous to this Cornelis *et al.* (1987) assigned this function to an 80-KDa IROMP. An 85-KDa protein has also been identified as a pyocin S-receptor in a clinicalisolate of *P. aeruginosa* (Gensberg *et al.*, 1992). It is likely that these three proteins are the same. Using the above mutant strains, more re-

tance(iewed in Hancock *et al.*, 1990). It is clear that reduced outer membrane permeability, by itself, is not sufficient to explain resistance, because antibiotics equilibrate across even the weakly permeable outer membrane of *Pseudomonas aeruginosa* in a few seconds. Thus a secondary mechanism must exist that takes advantage of defective permeability. For β-lactams, inducible chromosomal β-lactamase has been proposed as such a mechanism (Bayer *et al.*, 1987), whereas for other antibiotics, efflux is involved (Li *et al.*, 1994a,b; Poole *et al.*, 1993). There is also evidence that efflux may be involved in intrinsic (basal) resistance of *Pseudomonas aeruginosa* to β-lactams, in that *mexA*, *mexB or oprM* interposon mutants are supersusceptible to many β-lactams (Li *et al.*, 1995). However, although efflux pumps have quite loose specificity, they favor hydrophobic (or amphipathic), weakly positively charged compounds, whereas β-lactams are generally anionic or zwitterionic and reasonably hydrophilic. Furthermore, β-lactams act in the periplasm and thus face only one uptake/efflux barrier whereas the usual substrates for efflux pumps must cross two membranous barriers. In addition, there are considerable disparities in the extent to which susceptibility of some β-lactams is affected by *mexA* or *oprM* deletion. Therefore in this case, it might be necessary to exclude other possible secondary effects, e.g., on β-lactamase levels or inducibility, penicillin binding protein levels, or peptidoglycan biosynthesis, before one can definitively conclude that β-lactams are subject to efflux. Nevertheless, it is clear that low outer membrane permeability is an absolute determinant in the intrinsic antibiotic resistance of *P. aeruginosa* because specifically increasing outer membrane permeability by cloning in a large channel porin (Loop 5 deletion mutant of OprD) increases antibiotic susceptibility to many antibiotics by eightfold or more (Huang and Hancock, 1996).

3. IROMPs: FpvA, FptA, PfeA

Pseudomonas aeruginosa, like most bacteria, requires iron for growth. To sequester iron from its environment, this organism produces two iron chelators or siderophores, pyochelin and pyoveridine (Cox and Adams, 1985), which are produced under conditions of iron limitation. Up to seven iron-regulated outer membrane proteins have also been identified (Cornelis *et al.*, 1987), some of which have been classified as receptors for the iron-bound siderophores and hence are involved in the first step in the entry of iron into the cell. In addition to the two aforementioned siderophores, *P. aeruginosa* can utilize siderophores synthesized by other organisms, such as enterobactin (Polle *et al.*, 1990) and

Table I. Outer Membrane Proteins of *Pseudomonas aeruginosa*

Protein name	Other names	Apparent molecular weight[a]	Sequence accession number	Map position	Production conditions	Function	Identified in following fluorescent pseudomonads[d]
FpvA	IROMP	80,000	L10210 U07379	SpeI-J DpnI-B	Iron limitation	Ferripyoverdine uptake	PP, PF, PS, PC, PT
FptA	IROMP	75,000	U03161	SpeI-A DpnI-C	Iron limitation	Ferripyochelin uptake	
PfeA	IROMP	80,000	M98033	–[b]	Iron limitation, enterochelin present	Ferrienterobactin uptake	
OprC	C	70,000	Cloned[c]	–	Anaerobic induction, copper repressible	Copper transport	PST
OprJ	OprK	54,000	Cloned	–	Derepressed by mutation	Efflux	
AlgE	alg 76	54,000	M37181	SpeI-T DpnI-H	Coexpressed with alginate exopolysaccharide	Putative export of alginate	AV, PS, PP
OprN		50,000	–[b]	–	Derepressed by mutation	Efflux	
OprM	OprK	50,000	L23839	SpeI-H DpnI-E	Constitutive low level, can be derepressed	Efflux	
OprP	P	48,000	X53313	SpeI-C DpnI-N	Inducible by low phosphate	Phosphate uptake	PP, PS, PF, PC

(continued)

cently Poole *et al.* (1993) reported cloning the gene for the putative pyoverdine receptor by complementation. The product of the *fpvA* gene is consistent with an 86-kDa mature protein. Sequence analyses revealed homology to highly conserved domains found in TonB-dependent receptors, even though nothing resembling a TonB box was found. FpvA also has regions of homology with the PupA and PupB proteins of *P. putida*. PupA and PupB are IROMPs which function as receptors for ferric pseudobactin, a siderophore which is similar to pyoverdine (Bitter *et al.*, 1991). Some homology was also found with FhuE, the *E. coli* receptor for fungal siderophores coprogen, rhodoturilic acid, and ferrioxamine A.

Two different IROMPs have been described as involved in ferripyochelin uptake by *P. aeruginosa*. Originally, a 14-KDa protein, designated the ferripyochelin-binding protein (FBP) was assigned this role (Sokol and Woods, 1985). However, Heinrichs *et al.* (1991) demonstrated that a high MW protein (75,000 Da) interacts with pyochelin and supports pyochelin-mediated iron transport at nM levels of $FeCl_3$. This group also reported that a strain deficient in this protein transports ferripyochelin at higher ferric chloride concentrations, hence suggesting a putative role of the 14,000-Da protein in a second, lower affinity transport system. They also attributed the failure of earlier studies to observe the 75-Kda protein to the induction of the protein in the late log to stationary phase. The structural gene for the high-affinity ferripyochelin receptor (*fptA*) was cloned and the expression of a 75-Kda protein (Ankenbauer, 1992) substantiated the findings of Heinrichs *et al.* (1991). The mature FptA protein has a molecular mass of 76 KDa, and sequence homology studies revealed considerable homology with FpvA of *P. aeruginosa*, PupA and PupB of *P. putida*, and FhuE of *E. coli* (ankenbauer and Quan, 1994). Like FpvA and PupB, no TonB box was identified. Pyochelin and its receptor are both regulated by the transcriptional activator PchR which in turn is iron-regulated. The *fptA* gene is preceded by a sequence matching the *E. coli* fur-binding site, indicating the importance of iron concentrations in the expression of this receptor. Heinrich and Poole (1993) described a regulator PchR for both pyochelin and FptA biosynthesis.

In their study of the uptake and function of an antipseudomonal cephalosporin antibiotic, Yamano *et al.* (1994) found that the compound was best transported via the ferric iron transport pathway. They also found that the compound was best transported via the ferric iron transport pathway. They also found that mutant strains resistant to this antibiotic lacked a 66-kDa IROMP which, as they predicted, acts as a receptor for the antibiotic. *E. coli* IROMPS Fiu and Cir have been implicated

in the uptake of similar antibiotics (Curtis *et al.*, 1988; Nikaido and Rosenberg, 1990), hence suggesting a similarity between the uptake systems and the proteins in question. No further reports have emerged regarding the role of this 66-KDa IROMP.

4. OprC

OprC is a low copy number outer membrane protein which has been shown by Yoshihara and Nakai (1989) to form slightly anion-selective, small diffusion pores. The purified protein runs on SDS-PAGE with an apparent M_r of 70,000. The results, however, from this study are not without controversy because the investigators demonstrated in the same study that the major nonselective porin OprF, of *P. aeruginosa* did not exhibit porin function. In a later study, Nikaido *et al.* (1990) repeated the work of Yoshihara and Nakai and found that in their hands the levels of OprC were so low that they were not able to assess its function accurately. OprC has also been linked to the uptake of anionic antipseudomonal β-lactam antibiotics, such as cefsulodin, piperacillin, and azetreonam (Satake *et al.*, 1990). However, the role of OprC in antibiotic susceptibility was recently reexamined by Yoneyama *et al.* (1995).The *oprC*, *oprD*, and *oprE* genes were disrupted by gene replacement, and the resulting mutant strains were examined for susceptibility to a variety of antiotics. The results demonstrated that OprC is not involved in imipenem permeability nor the permeability of any of a number of antipseudomonal antibiotics, including quinilones, cephalosporins, chloramphenicol, and various penicillin derivatives.

Yamano *et al.* (1993) demonstrated that OprC levels increase fourfold during anaerobic growth and this increase is not caused by a concomitant decrease in iron concentration in the growth medium, nor reduced growth rate in a minimal medium. It was proposed that the increased production of this protein along with OprE aids in the growth of *P. aeruginosa* by enhancing the rate of entry of essential nutrients into the cell during oxygen stress. More recently, Nakae and collaborators sequenced OprC and showed that it is 65% homologous with NosA of *P. stutzeri* (Lee *et al.*, 1989), an outer membrane porin required for producing copper-containing nitrite reductase. Like NosA, it was shown that OprC is made only anaerobically and is repressed by high Cu^{2+} concentrations in the medium. Nitrite reductase is a key element in the anaerobic respiratory process termed denitrification. The predominant single-channel conductance increments of the OprC and NosA porins are virtually identical.

5. OprJ

The overproduction of a 54-KDa outer membrane protein has been reported in several mutant strains of *P. aeruginosa* belonging to the Type2 (*nfxB*) group (Hirai *et al.*, 1987; Legakis *et al.*, 1989; Yoshida *et al.*, 1994). These strains are generally cross-resistant to quinilones, such as norfloxacin, to newly developed cephems like cefpirome, and are hypersensitive to β lactams and aminoglycosides. This protein has only been recently been termed OprJ and its relationship to the other resistance-correlated outer membrane proteins OprK, OprM, and OprN examined (Masuda *et al.*, 1995). In some strains, OprJ is repressed by salicylate in conjunction with an increase in OprN production, suggesting some sort of linked control mechanism, not unlike that seen with OmpC/F in *E. coli*. It seems plausible to imply that OprJ is an efflux protein although no conrete information has been presented.

Purified OprJ retains its SDS-PAGE electrophoretic mobility regardless of reduction or heating. A monoclonal antibody specific for OprJ was used in immunoblot studies (Hosaka *et al.*, 1995) to demonstrate that OprJ is produced by fluoroquinolone-resistant NfxB mutant strains of *P. aeruginosa* but not by other types of fluoroquinolone-resistant strains (NalB, NfxC). This implies that OprJ is a protein novel to NfxB mutant strains.

6. AlgE

Mucoid strains of *Pseudomonas aeruginosa* produce a 54-kD protein termed AlgE (Rehm *et al.*, 1994). The *algE* gene is found as part of the 34 min alginate gene cluster and has been cloned and sequenced (Chu *et al.*, 1991). Purified AlgE forms a large, anion-selective channel. Therefore it has been proposed that it functions in enhancing the excretion of exopolysaccharide precursors out of the cell to a location where they are assembled into the mucoid material of *P. aeruginosa*. The protein has been modeled as an 18-β-strand porin, reminiscent of the Lam B porin (Schirmer *et al.*, 1995).

7. OprN

P. aeruginosa mutant strains possessing cross-resistance to quinilones, imipenem, and chloramphenicol plus a hypersensitivity to cephems, aminoglycosides, and carbenicillin overproduce a 50-kDa outer membrane protein, OprN (Fukuda *et al.*, 1990, 1995). In these strains a decrease in norfloxacin accumulation and in the rate of imipenem uptake was noted, suggesting the presence of an efflux process. Also in these strains, a decrease in OprD was noted, hence the reduced imipenem uptake. Although it has the same apparent molecular weight as OprM, this protein does not change SDS-PAGE mobility after heating and is positively regulated by salicylate (Masuda *et al.*, 1995). In conjunction with salicylate induction, increased resistance to quinolones and carbapenems is linked to the decrease in OprD production and the increased production of OprN. This mirrors the phenotype observed for the OprN-overproducing Type 3 (*nfxC*) mutant strains. OprN levels are also elevated when OprJ overproducing mutant strains are treated with salicylate, suggesting that the two proteins are regulated to obtain a constant copy number in the outer membrane. Masuda *et al.* (1995) have proposed that OprJ, OprM, and OprN act as independent efflux transporters and that OprJ and OprN are cooperatively regulated, much like OmpF and OmpC from the enteric bacteria.

8. OprK

Upon examining a pyoverdine-deficient strain of *P. aeruginosa*, Poole *et al.* discovered the iron-inducible expression of OprK, a 50-kDa outer membrane protein. The same strain is resistant to a number of structurally diverse antimicrobial agents (e.g., ciprofloxacin, nalidixic acid, tetracycline and chloramphenicol). It was originally though that the *oprK* gene belongs to an operon (*mexA-mexB-oprK*) responsible for the efflux of a variety of antibiotics and secondary metabolites (e.g., pyoverdine). However, it was recently found that the *oprK* gene expresses OprM and thus was renamed *oprM* (Li *et al.*, 1995). The OprK protein derives in fact from an *nfxB* mutation derepressing the *oprJ* gene, and thus the protein has now been renamed OprJ (Poole, K., personal communication).

9. OprM

Analysis of nalidixic acid (*nalB* or Type 1) multiple antibiotic-resistant strains of *P. aeruginosa* revealed the overexprssion of a 49-KDa protein termed OprM (Masuda and Ohya, 1995). OprM has been partially characterized (Gotoh *et al.*, 1994b) and found to be heat modifiable. OprM changes molecular mass on SDS-PAGE from 50 kDa to more

than 100 kDa upon heating before loading on gels. Strains deficient in the production of OprM are highly susceptible to a number of antibiotics, including quinilones, β-lactams, tetracycline, chloramphenicol, and several penems (Gotoh *et al.*, 1994a). OprM production is not influenced by salicylate, the known synthesis suppressor of some outer membrane proteins (Masuda *et al.*, 1995). Initially evidence indicated that OprM acts much like OprK in a separate, but related, efflux system that Li *et al.* (1994a) call MexC-MexD-OprJ. However it was recently shown that the product of the cloned *oprK* gene is identical to OprM (Li *et al.*, 1995). Li *et al.* (1995) examined the accumulation of a variety of drugs in several mutant strains in the presence and absence of a proton ionophore and found that MexA-MexB-OprM forms an energy-dependent complex capable of pumping various antibacterial agents out of *Pseudomonas aeruginosa*. MexA and MexB resemble cytoplasmic membrane export proteins. A proposed model for efflux has the antibiotics pumped from the cytoplasm to the external face of the cell through a direct channel (Li *et al.*, 1994b). MexB would form a cytoplasmic membrane transporter with the lipoprotein MexA acting as a link between the outer membrane channel, OprM (formerly OprK), and the MexB transporter. Indeed a disruption of any of the three gene makes the strain extremely susceptible to the effluxable antibiotics. Components similar to OprM, MexA, and MexB have also been identified by Morshed *et al.* (1995) using norfloxacin-resistant and *nalB* multiply resistant strains of *P. aeruginosa*. Active efflux has been very well documented in *E. coli* (Thanassi *et al.*, 1995), and the MexA/MexB proteins have homologues in many bacteria (Poole *et al.*, 1993) Although the finding of a similar process in *P. aeruginosa* is not completely unexpected, the concept of efflux as a major player in intrinsic resistance provided a substantial breakthrough in our thinking about how *P. aeruginosa* escapes killing by multiple antibiotics.

10. OprP

OprP was first identified as an outer membrane protein induced upon growth of *P. aeruginosa* in a low phosphate medium (see Hancock *et al.*, 1990; Siehnel *et al.*, 1990 for most citations). A Tn501 mutant lacking OprP was shown to be deficient in the high-affinity, phosphate-starvation-inducible (PST) transport system. Detailed model membrane studies have made this one of the best characterized anion channels in nature. The OprP channel was shown to contain a phosphate-binding site with a K_i of approximately 0.15μM (approximately the medium concentration of phosphate at which OprP is optimally induced). Chemical modification studies of OprP indicated that the phosphate-binding site contains a lysine residue. Therefore we have started to change the lysines of OprP systematically to glutamates and glycines (Sukhan and Hancock, 1996). A single lysine, residue-121, changed to either a glutamate or a glycine residue, causes loss of the phosphate-binding site, as judged by model membrane studies.

To determine where lysine-121 exists, a membrane topology model (Fig. 1a) was created by predicting transmembranous amphipathic β-strands (typical of porins; Huang *et al.*, 1995) and constructing linker and epitope insertion mutants at various sites through the protein (utilizing the observation that loop regions interconnecting β-strands can tolerate insertion of extra amino acids, but that the β-strand regions cannot) (Sukhan and Hancock, 1995). Interestingly, in this model, lysine-121 is right in the middle of loop 3 which, in the porins were with defined-three-dimensional structurs, form the narrowest (constriction) region of the porin channel. Indeed lysine-131 of PhoE, which is substituted for glycine in the related *E. coli* porin OmpF (Cowan *et al.*, 1992), is the major contributor to the anion selectivity of PhoE and the cation selectivity of OmpF.

OprP is coregulated with the periplasmic phosphate-binding protein. Biochemical data suggests that these two proteins can interact whereas genetic data suggest they collaborate in phosphate uptake. A mathematical model of phosphate passage through the OprP channel has been developed.

11. OprO

OprO was first identied as an open reading frame upstream of the OprP gene. It shares 76% identical amino acids with OprP (Siehnel *et al.*, 1992). Model membrane studies on the purified OprO porin, after overexpression in *E. coli*, indicate that it is quite similar to OprP in that it forms anion-specific channels with a phosphate-binding site (Hancock *et al.*, 1992). However, the OprO channel is somewhat larger and prefers pyrophosphate over phosphate (cf. OprP for which the situation is reversed).

Utilizing an OprP::Tn501 mutant of *P. aeruginosa*, OprO expression was demonstrated after growth of this mutant under phosphate-starvation conditions into the stationary phase (Siehnel *et al.*, 1992). Like the *oprP* gene, *oprO* contains an inverted repeat element overlapping this

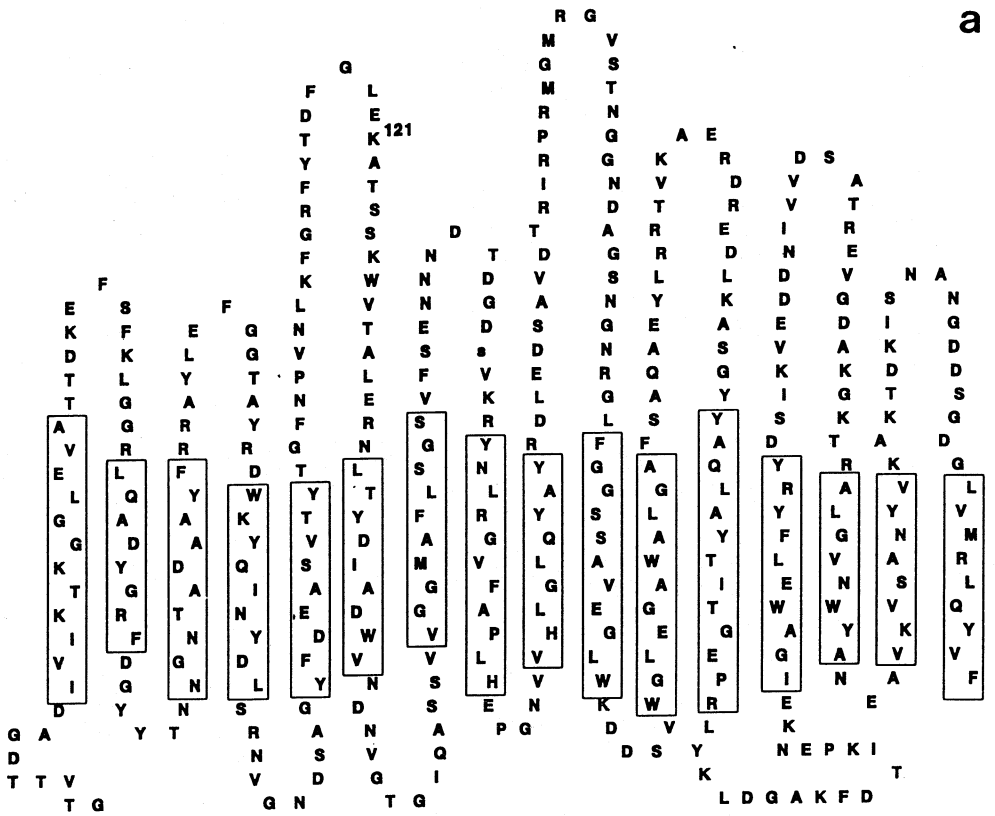

Figure 1. Membrane topology models of selected outer membrane porin proteins of *Pseudomonas aeruginosa*. The boxed regions represent proposed membrane spanning β-strands. The top interconnecting stretches of amino acids represent proposed surface exposed loop regions and the bottom stretches are proposed periplasmic turns. (a) OprP; (b) OprD; (c) OprF.

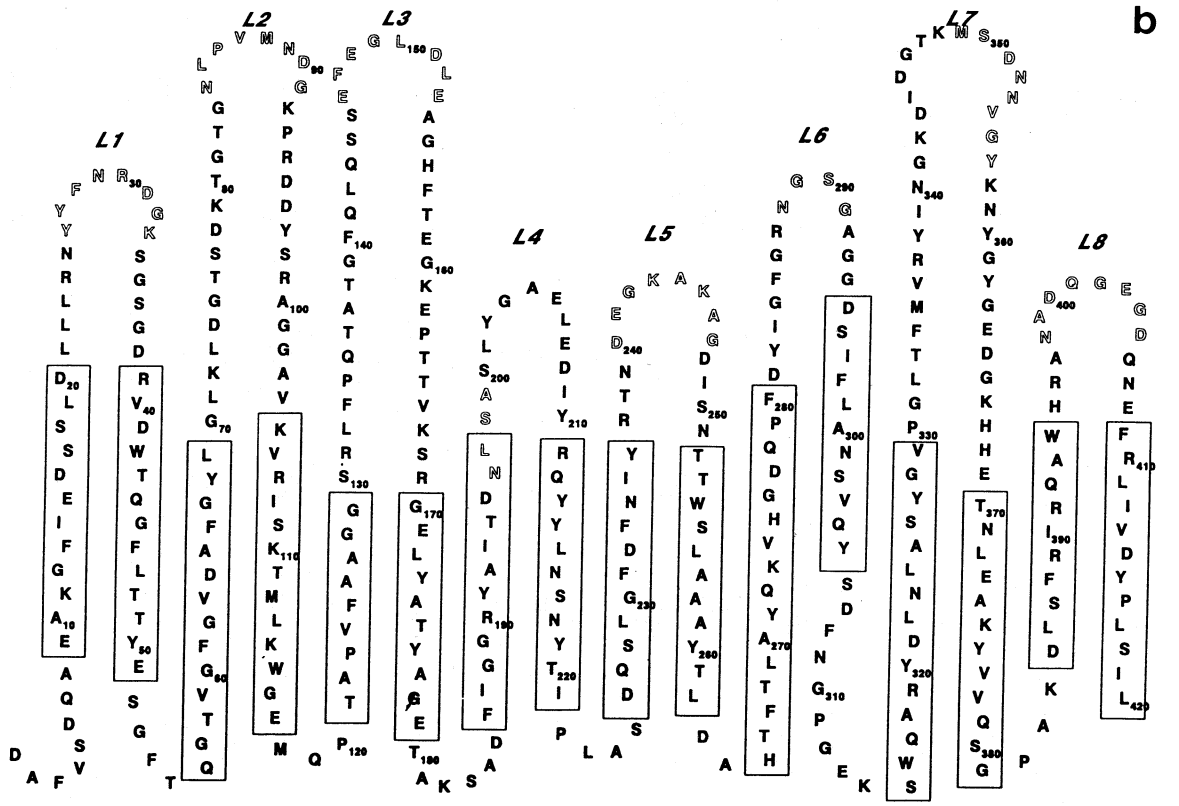

Figure 1. (*Continued*).

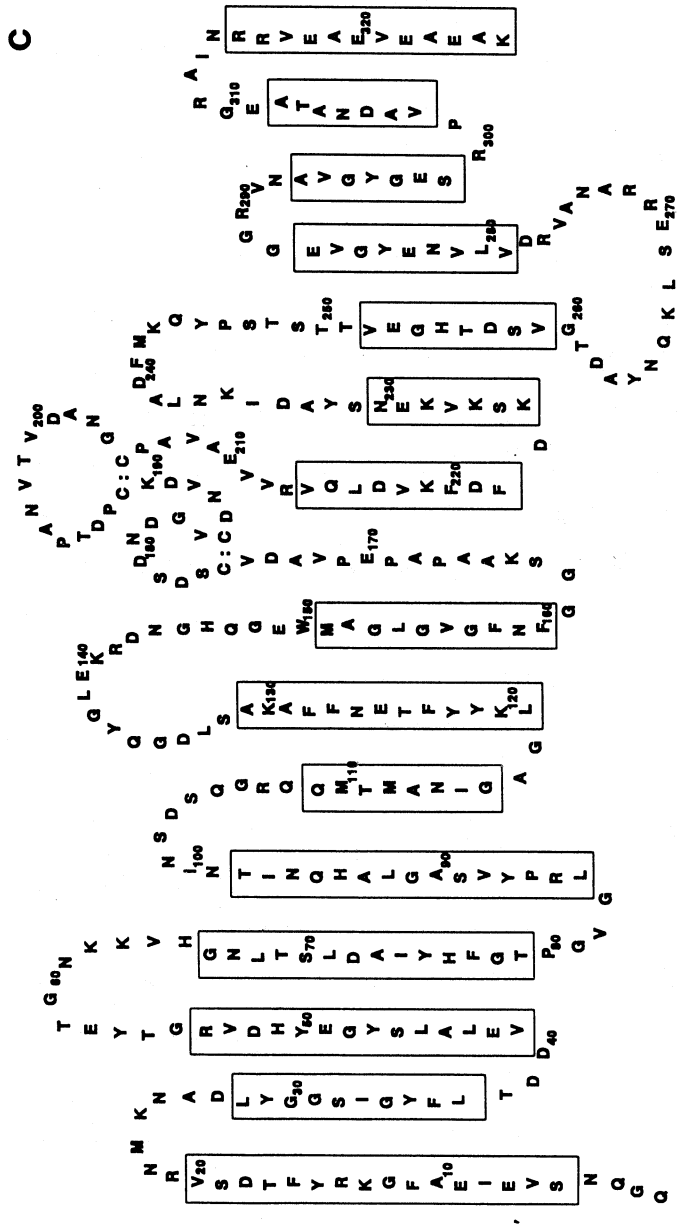

Figure 1. (*Continued*).

Pho box with homology to the cyclic AMP receptor-binding site of the *E. coli rpoH* gene.

12. OprB

OprB was first identified by Hancock and Carey (1980) following the growth of *P. aeruginosa* in a minimal medium supplemented with glucose as the sole carbon source. The protein was identified as a heat-modifiable protein, whose M_r changes from 30,000 to 47,000 upon heating to 100 °C before SDS-PAGE. OprB is coregulated with a periplasmic glucose-binding protein as part of a high-affinity glucose uptake system. Hancock and Carey (1980) originally demonstrated the porin function of OprB by following the efflux of several carbohydrates from phospholipid vesicles reconstituted with purified OprB. Using the liposome swelling assay, Trias *et al.*, (1988) characterized OprB as selective for glucose and xylose. Further analyses of purified OprB from *P. aeruginosa* (Wylie *et al.*, 1993) and *P. putida* (Saravolac *et al.*, 1991) revealed many similarities between the two proteins, including high β-sheet content (40%), as determined by circular dichroism, immunochemical cross-reactivity, amino terminal sequence homology, and a glucose-binding site (K_s = 380 mM for *P. aeruginosa* and 110 mM for *P. putida*). The two proteins differ in molecular weight (*P. aeruginosa* OprB is 4500 Da larger) and in ion selectivity. The *P. aeruginosa* OprB is anion-selective, and *P. putida* OprB is cation-selective. The reason for this difference in selectivity is still unknown.

The sequence of the cloned *P. aeruginosa oprB* gene revealed some homology to the maltodextrin-selective LamB porin of *E. coli* (Wylie and Worobec, 1994). Topological modeling by the prediction rules of Paul and Rosenbusch predicted 18 potential membrane-spanning β-strands when placing both N-and C-termini on the periplasmic face of the membrane, similar to that deduced from the crystal structue of LamB (Schirmer *et al.*, 1995). As with LamB, extra membranous loops joining the β-strands are short on the periplasmic face compared with those facing extracellularly. Amino acid comparison between OprB and LamB revealed several conserved and identical residues implicated in sugar binding to LamB. Likewise, OprB has a cluster of five tryptophan and seven phenylalanine residues which resembles what is termed the "greasy slide" of LamB, a region which is though to guide the diffusion of sugars. The aromatic ring of phenylalanine and tryptophan residues contribute to protein–sugar interactions in carbohydrate-binding proteins. The residues stack on one or both sides of the pyranoside rings of sugars, con-

tributing to the Van der Waals contacts (Quiocho, 1989). OprB also has two cysteine residues (C148 and C156) which are fairly rare in porin proteins but are also found in LamB (Gerbl-Rieger *et al.*, 1991). No function has been attributed to these residues in LamB (Ferenci and Stretton, 1989), and the analysis of their function in OprB is presently underway.

Using interposon mutagenesis, *P. aeruginosa* strains were created which either lacked or overexpressed OprB (Wylie and Worobec, 1995). These strains were used to study the in vivo role of OprB in the diffusion of glucose and other carbohydrates across the outer membrane. These experiments demonstrated that OprB acts as a substrate-selective porin for a variety of different sugars, including mannitol, glycerol, and fructose. Indeed, Williams *et al.* (1994), found the production of OprB in conjunction with prolonged growth on glycerol. Thus, rather than acting exclusively as a glucose-selective proin, OprB facilitates the diffusion of a wide range of carbohydrates and thus has been labeled a carbohydrate-selective porin.

Other members of the *Pseudomonadaceae* rRNA group I carry a gene which hybridizes with the *P. aeruginosa oprB* structural gene and produce proteins with strong homology to OprB (based on SDS-PAGE mobility, immunochemical cross-reaction, and N-terminal sequence), when grown on glucose or other carbohydrates, such as xylose or maltose (Wylie and Worobec, 1994). This again reinforces the role of OprB as a general component of carbohydrate uptake in pseudomonads.

13. OprD

OprD was first identified as a modestly expressed outer membrane protein called D2 (Hancock and Carey, 1979). It was subsequently demonstrated that mutants of *P. aeruginosa*, which had become specifically resistant to a broad-spectrum β-lactam called imipenem, had lost OprD (Quinn *et al.*, 1986). Interestingly imipenem strongly resembles a dipeptide of a basic amino acid plus one other amino acid, so much so that a renal dipeptidase inhibitor, cilastatin, must be coadministered with imipenem to improve its stability in vivo (N.B: to some extent all β-lactams mimic the D-ala-D-Ala peptide in peptidoglycan precursors). Thus it seemed quite reasonable when Trias and Nikaido (1990a,b) demonstrated that OprD is a specific porin that recognizes basic amino acids, peptides containing basic amino acids, and imipenem and related carbapenem β-lactams (e.g., meropenem) as substrates. It was confirmed that these two classes of substrates bind to the same site on OprD (Trias and Nikaido, 1990b) and that high concentrations of basic amino acids substantatively increase the minimal inhibitory concentration (MIC) of imipenem and micropenem (Fukuoka *et al.*, 1991). Competition for binding to a common site within the OprD channel was definitively demonstrated by model membrane studies (Huang and Hancock, 1996; Nakae *et al.*, 1996; Trias and Nikaido, 1990b). Overexpression of OprD from the cloned gene was used to prove that the channel is quite specific for carbapenem β-lactams and does not permit passage of other β-lactams or quinolones (Huang and Hancock, 1993). This corrects some misleading conclusions in the literature (Michea-Hamzehpour *et al.*, 1991). Indeed the observation of cross-resistance between imipenem and fluoroquiinolones is more likely caused by regulatory or efflux mutants (Masuda and Ohya, 1995). Nevertheless, OprD overexpression studies indicate that gluconate can pass through this channel, possibly in a nonspecific manner (Huang and Hancock, 1993).

OprD is the first specific porin that has sequence alignment with the (generally) non-specific porin superfamily (Huang *et al.*, 1995). This has permitted constructing a membrane topology model (Fig. 1b). The model was tested with reasonable success by PCR-mediated site-specific deletion. By studying the resultant 4–8 amino acid deletion mutants, the following general conclusions were drawn: (1) loop 2 has a role in imipenem binding and (2) loops 5, 7, and 8 fold in to constrict the size of the channel and prevent high nonspecific movement of molecules through the OprD channel (Huang *et al.*, 1995; Huang and Hancock, 1996).

Recently Nakae and callaborators (1996) observed very weak protease activity (turnover number 10^{-5}/sec) for OprD that is inhibitable by imipenem. We propose that this "protease" site actually functions as a surface-binding site for basic amino acids and imipenem and is the loop 2 binding site. We further hypothesize that the binding site within the OprD channel is actually located in loop 3 (which for the known crystallized porins, both specific and nonspecific, usually forms the narrowest constriction zone of the porin channel).

14. OprE

OprE was first identified as a porin (Yoshihara and Nakae, 1989) with an exclusion limit of approximately a di- to trisaccharide (see Hancock *et al.*, 1990 for an overview), but it is now clear that it differs from outer membrane protein E described by Hancock and Carey (1979). Yamano *et al* (1993) demonstrated that it is a non-heat-modifiable pro-

tein with an overall 39% identity and 51% similarity with OprD. The protein was induced under anaerobic conditions and the upstream region of the *oprE* gene contains a weak FNR box sequence and a sigma 54-RNA polymerase-binding region. It should be noted that *Pseudomonas aeruginosa*, although termed an "obligate" aerobe, grows anaerobically in the presence of nitrate or arginine as terminal electron acceptors, and on occasion may grow in this fashion in vivo.

There is no prescribed function for this porin, although its homology to OprD suggests that it may be a specific porin with an unknown substrate. Yamano *et al.* (1993) suggested that it may be involved in the uptake of ferrous iron because anaerobic conditions favor the reduced form of iron and depress the synthesis of IROMPs. Interposon mutants lacking OprE did not change in antibiotic susceptibility, although these studies were somewhat ambiguous because they were not done under anaerobic conditions (Yoneyama *et al.*, 1995).

15. E2

Protein E2, formerly E, has not been well studied. It is present in reasonable concentrations in outer membrane samples grown under normal condition (Hancock and Carey, 1979), but it is the most prominent band in the whole cell protein gels (R. Hancock, unpublished), and thus may not be a true outer membrane protein. In experiments involving progressive solubilization of outer membrane proteins, protein E2 remained as the major detergent-insoluble protein in the residue, even after peptidoglycan digestion (Hancock *et al.*, 1981). In some antibiotic-resistant mutants, protein E levels were reduced (Yamano *et al*, 1993).

16. OprF

OprF, the most predominant outer membrane protein in *P. aeruginosa* under most media conditions, is present at around 200,000 copies/cell. It has a major role in maintaining correct cell structure and stability because mutants lacking OprF do not grow in a low osmolarity medium and are significantly shorter, giving them an almost rounded morphology. These properties and sequence similarity place OprF in the OmpA superfamily (Woodruff and Hancock, 1988). The sequence similarity to OmpA occurs entirely within the carboxy-terminal half and

extends to more distantly related proteins, including OprL (the peptidolycan associated lipoprotein), pIII of *Neisseria gonorrhoeae* (the serum blocking protein), and *motB* of *Bacillus subtilis* (a non-outer membrane protein involved in motility). Interestingly, like OmpA, OprF can be progressively deleted down to an N-terminal core structure of 168 amino acids which it is predicted, forms an eight-stranded β-barrel where the eighth β-strand has high homology to the 16th (C-terminal β-strand) of members of the porin superfamily (Rawling, 1995). Deletions into this homologous β-strand, e.g., in PhoE (Bosch *et al.*, 1989) are nonpermissive and prevent a protein product from being formed. Therefore, this represents both a similarity and a difference between members of the OmpA and porin superfamilies.

Study of the above series of C-terminal deletion mutant of OprF has demonstrated that this region of OprF has a substantial role in determining both cell shape and the ability to grow in a low osmolarity medium. One explanation for these properties would be if OprF somehow determines the pattern and rate of peptidoglycan growth. With regard to this, OprF is noncovalently associated with the peptidoglycan, as demonstrated by cofractionation after solubilization of outer membrane/peptidoglycan complexes in SDS at moderate temperature. It shares this property with OprL and OprI. Any deletion of the C-terminus, even one with as few as 36 amino acids, resulted in loss of peptidoglycan association defined in this way.

The folding of OprF in the membrane has evoked some controversy. First, the model for OmpA places the C-terminus in the periplasm, albeit using indirect evidence (Freudl *et al.*, 1986). Second, at least one of the members of the OmpA superfamily is not at all an outer membrane protein. Third, one might expect that the strong noncovalent association of OprF with the peptidoglycan would require that a significant portion of OprF is present in the periplasm. Fourth, only two regions in the C-terminus are strongly predicted to be transmembranous amphipathic β-strands as found in other outer membrane proteins. Despite this there is considerable evidence that several parts of the C-terminus of OprF are exposed on the surface of the outer membrane and that a significant portion of the OprF C-terminus (AAs 164–326) must be folded into β-sheets. This data involves (1) the mapping of linear, surface-localized, monoclonal antibody binding sites to regions involving amino acids 55 to 62, 237 to 244, and 297 to 314 (Rawling *et al.*, 1995; seven other antibodies with conformational epitopes were also mapped to larger amino acid stretches of the C-terminus); (2) the mapping of a surface-located protease cleavage site in a conserved region of *P. fluorescens* OprF to

amino acids 188 to 197 (DeMot *et al.*, 1994); 93) the demonstration that 10 amino acid malarial epitopes inserted at residues 188, 196, 213, 215, 231, 290, and 310 can be localized to the surface by immunofluorescence (Wong *et al.*, 1995); and (4) the creation of surface-reactive antibodies using, amino acids 261 to 274, 305 to 318 (Hughes *et al.*, 1995), 188 to 216, and 308 to 326 as immunogens (von Specht *et al.*, 1995) (N.B: the first of these is disputed; see Hughes *et al.*, 1992 and von Specht *et al.*, 1995). In addition, circular dichroism spectra clearly indicate a β-strand content of approximatley 60% (Siehnel *et al.*, 1990), that can be accommodated only if one ensures that approximately 50% of the C-terminal residues are located in β-strands. Taking all of the above data together, a membrane topology model is presented in Figure 1c. One interesting part of this model is the cysteine disulfide region in its center. This insertion, missing in most *P. fluorescens* OprF sequences and in OmpA, has homology to a calcium-binding region in several eukaryotic proteins (De Mot and Vandeleyden, 1994).

In addition to its structural role, OprF functions as a porin. Although this was disputed for some time, there is now a consensus that OprF is the major porin of *P. aeruginosa* permitting nonspecific passage of substances larger than disaccharides (Bellido *et al.*, 1992; Nikaido *et al.*, 1991) and that it contributes substantially to, but is not the sole determinant of, the large exclusion limit and low outer membrane permeability of the *P. aeruginosa* outer membrane (Bellido *et al.*, 1992). These two properties of OprF and the outer membrane have led to considerable confusion because they indicate that even minor outer membrane proteins, or those with small channels, may contribute to the net permeation of substrates in *P. aeruginosa*. This is further complicated by efflux systems which are expected to influence net antibiotic uptake. Thus it is not surprising that defined OprF-deficient mutants have at most a two-to threefold increase in MIC to β-lactam antibiotics (Woodruff and Hancock, 1988; Yoneyama *et al.*, 1995). Nevertheless there is some evidence that OprF is the major uptake route for antibiotics, in that trained mutants selected for quinoline resistance acquire a multiple antibiotic resistance (MAR) phenotype (i.e., cross-resistance to other antibiotic classes) and OprF deficiency simultaneously (Piddock *et al.*, 1991; Zhanel *et al.*, 1994). Such MAR resistance can be reversed by complementing with the cloned *oprF* gene (G. Zhanel, personal communication). MAR mutants with OprF deficiencies can be isolated in the clinic (Piddock *et al.*, 1991).

In addition to these porin functions, evidence has been presented by comparing isogenic OprF-deficient mutants and wild-type that OprF is the route of uptake for ironsiderophore complexes, for which no specific IROMP exists (Meyer, 1992), and is possibly the route of toluene uptake (L. Li, personal communication).

17. OprG

OprG is a 25,000-Da protein which is expressed in the outer membrane of *P. aeruginosa* under a variety of growth conditions. OprG levels are affected by temperature, carbon sources, LPS content, the presence of iron, and growth in the stationary phase. OprG production has been linked to a low-affinity, iron-uptake system and to quinilone uptake. It was also reported that OprG levels are lowered in an OprN-overproducing, norfloxacin resistant *nfxC* mutant strain of *P. aeruginosa*, along with the decrease of OprD and possibly OprJ (see below). No definite function has yet been attributed to OprG.

18. OprH

OprH is a minor outer membrane protein that becomes dramatically overexpressed when cells are grown on media with low (50μM) Mg^{2+} concentrations (providing Ca^{2+}, Sr^{2+} and Mn^{2+} are also low). Under such conditions (and in mutant H181 which overexpresses OprH in a normal medium), cells become cross-resistant to polymyxins, aminoglycosides, and EDTA. Studies with an isogenic OprH-deficient interposon mutant have shown that OprH is necessary for these resistances (Young *et al.*, 1992). Overexpression of OprH from its cloned gene, however, does not fully replicate the resistance phenotype suggesting that an alteration in another outer membrane component (possibly LPS) is required. Presumably the constitutive OprH-overexpressing mutant H181 is actually a regulatory mutant, a suggestion consistent with cloning results (Bell and Hancock, 1989). We have hypothesized that OprH actually replaces divalent cations in the outer membrane, under conditions of medium insufficiency, to permit outer membrane stabilization (Nicas and Hancock, 1983).

OprH has no apparent porin activity (Young and Hancock, 1993). Our recent studies (Rehm and Hancock, manuscript in preparation), however, have caused us to reevaluate this conclusion. Circular dichroism studies indicate that OprH contains 50% β-sheet. A model of OprH as an eight-stranded β-barrel was created and tested using a combination of the methods utilized above for OprD and OprF. Deletion of external loops 1 and 3 had no effect on OprH's ability to form channels.

However, an OprH mutant with a 10 amino acid deletion of loop 4 formed large channels with a single-channel conductance of 1.3 nS. An analogous observation for the *E. coli* iron-regulated outer membrane protein FepA has led to the conclusion that FepA is a gated porin (Rutz *et al.*, 1992), and thus we suggest the same for OprH.

19. OprL

OprL is a constitutively produced, peptidoglycan-associated lipoprotein which contains covalently bound fatty acyl chains (Mizuno, 1979, 1981). The protein runs on SDS-PAGE with an apparent MW of 20,500 Da (Hancock and Carey, 1979). Like OprI, this protein is highly conserved among the pseudomonads found in the rRNA group I, as detected by immunochemical cross-reactivity studies (Mutharia and Hancock, 1985). The gene has been cloned and sequenced (Lim *et al.*, 1995) and demonstrates good homology to members of the PAL family of peptidoglycan-associated lipoproteins (and consequent C-terminal homology to OprF). Although no direct function has been assigned to OprL, it appears tobe involved in the structural integrity of the *P. aeruginosa* outer membrane (L. Ramos, personal communications).

20. OprI

Lipoprotein I (OprI) is a highly abundant, 8-KDa outer membrane protein which has become an important vaccine candidate for *P. aeruginosa* (Finke *et al.*, 1990, 1991; Rahner *et al.*, 1990; von Specht *et al.*, 1995). The *oprI* structural gene has been cloned and sequenced by three groups (Cornelis *et al.*, 1989; Duchene *et al.*, 1989; Saint-Onge *et al.*, 1992) and has many similarities to its *E. coli* counterpart, Braun's lipoprotein. The mature OprI protein has a molecular weight of 5,950 Da and a single, covalently linked fatty acid. The two proteins differ in amino acid sequence with only 30% identical residues. The majority of identical residues are located at the signal sequence cleavage site and at the C-terminus, which is the peptidoglycan linkage point of the *E. coli* lipoprotein. Using PCR and probes derived from the *P. aeruginosa oprI* gene, DeVos *et al.* (1993) extensively surveyed members of the *Pseudomondaceae* and close relatives to find that the gene could be amplified only in members of the rRNA group I, those species considered to be authentic pseudomonads. Much interest has been generated in using recombinant OprI, OprI–OprF fusion proteins, or OprI specific monoclonal antibodies as vaccine candidates for *P. aeruginosa*. OprI is antigenically cross-reactive among all 17 known serotypes of *P. aeruginosa*, according to the International Antigenic Typing Scheme (Finke *et al.*, 1990), and large quantities of recombinant OprI can be rapidly purified to homogeneity from *E. coli* (Toth *et al.*, 1994). Most recently, von Specht *et al.*, (1995) found that a glutathione S-transferase (GST)-linked fusion of OprF and OprI protects immunized animals 975-fold against *P. aeruginosa*.

ACKNOWLEDGMENTS. The financial assistance of the Medical Research Council of Canada and the Canadian Cystic Fibrosis Foundation in our research is gratefully acknowledged.

REFERENCES

Ankenbauer, R. G., 1992, Cloning of the outer membrane high-affinity Fe (III)-pyochelin receptor of *Pseudomonas aeruginosa.*, *J. Bacteriol.* **174:**4401–4409.

Ankenbauer, R. G., and Quan, H. N., 1994, FptA, the Fe(III)-pyochelin receptor of *Pseudomonas aeruginosa*: A phenolate siderophore receptor homologous to hydroxamate siderophore receptors, *J. Bacteriol.* **176:** 307–319.

Bayer, A. S., Peter, J., Parr, T. R., Chan, L., and Hancock, R. E. W., 1987, In vivo development of ceftazidime resistance in an experimental *Pseudomonas aeruginosa* endocarditis model, *Antimicrob. Agents Chemother.* **31:** 253–258.

Bell, A., and Hancock, R. E. W., 1989, Outer membrane protein H1 of *Pseudomonas aeruginosa*: Expression, gene cloning, and nucleotide sequence, *J. Bacteriol.* **171:** 3211–3217.

Bellido, F., Martin, N. C., Siehnel, R. J. and Hancock, R. E. W., 1992, Reevaluation, using intact cells, of the exclusion limit and role of porin Opr in *Pseudomonas aeruginosa* outer membrane permeability, *J. Bacteriol.* **174:** 5196–5203.

Bitter, W., Marugg, J. D., de Weger, L. A., Tommassen, J. and Weisbeek, P. J., 1991, The ferric-pseudobactin receptor PupA of *Pseudomonas putida*: Homology to TonB-dependent *Escherichia coli* receptors and specificity of the protein, *Mol. Microbiol.* **5:** 647–655.

Bosch, D., Scholten, M., Verhagen, C. and Tommassen, J., 1989, The role of carboxy-terminal membrane-spanning fragment in the biogenesis of *Escherichia coli* K-12 outer membrane protein PhoE, *Mol. Gen. Genet.* **216:** 144–148.

Chamberland, S., Bayer, A. S., Schollaardt, T., Wong, S. A. and Bryan, L. E., 1989, Characterization of mechanisms of quinolone resistance in *Pseudomonas aeruginosa* strains isolated in vitro and in vivo during experimental endocarditis, *Antimicrob. Agents Chemother.* **33:** 624–634.

Chu, L., May, T. B., Chakrabarty, A. M. and T. K., 1991, Nucleotide sequence and expression of the algE gene involved in alginate biosynthesis by *Pseudomonas aeruginosa*, *Gene* **107:** 1–10.

Cornelis, P., Bouia, A., Belarbi, A., Guyonvarch, A., Kammerer, B., Hannaert, V. and Hubert, J. C., 1989, Cloning and analysis of the gene for the major outer membrane lipoprotein from *Pseudomonas aeruginosa*, *Mol. Microbiol.* **3:** 421–428.

Cornelis, P., Moguilevsky, N., Jacques, J. F., and Masson, P. L., 1987, Study of the side-

rophores and receptors in different clinical isolates of*Pseudomonas aeruginosa*, in: *Basic Research and Clinical Aspects of Pseudomonas aeruginosa* (G. Döring, I. A. Holder, and K. Botzenhart, eds.) S. Karger, Basel, pp. 290–306.

Cowan, S. W., Schirmer, T., Rummel, G., Steiert, M., Ghosh, R., Paupitt, P. A., Janosonius, J. N., and Rosenbusch, J. P., 1992, Crystal structures explain functional properties of two *Escherichia coli* porins, *Nature* (London) **358:** 727–733.

Cox, C. D., 1980, Iron uptake with ferripyochelin and ferric citrate by *Pseudomonas aeruginosa*, *J. Bacteriol.* **142:** 581–587.

Cox, C. D., and Adams, P., 1985, Siderophore activity of pyoverdine for *Pseudomonas aeruginosa*, Infect. Immun. **48:** 130–138.

Curtis, N. A. C., Eisenstadt, R. L., East, S. J., Cornford, R. J., Walker, L. A. and White, A. J., 1988, Iron-regulated outer membrane proteins of K-12 and mechanism of action of catechol-substituted cephalosporins, *Antimicrob. Agents Chemother.* **32:** 1879–1886.

Dean, C. R., and Poole, K., 1993, Cloning and characterization of the ferric enterobactin receptor gene (*pfeA*) of *Pseudomonas aeruginosa*, *J. Bacteriol.* **175:** 317–324.

De Mot, R., Schoofs, G., Roelandt, A., Declerk, P., Proost, P., van Damme, J., and Vanderleyden, J., 1994, Molecular characterization of the major outer membrane protein OprF from plant-root colonizing *Pseudomonas fluorescens*, *Microbiology* **140:** 1377–1387.

De Mot, R. and Vanderleyden, J., 1994, A conserved surface-exposed domain in outer membrane proteins of pathogenic *Pseudomonas* and *Branhamella* species shares sequence homology with the calcium-binding repeats of the eukaryotic extracellular matrix protein thrombospondin, *Mol. Microbiol.* **13:** 379–380.

De Vox, D., Lim, A., Jr., De Vox, P., Sarniguet, A., Kersters, K., and Cornelis, P., 1993, Detection of the outer membrane lipoprotein I and its gene in fluorescent and non-fluorescent pseudomonads: Implications for taxonomy and diagnosis, *J. Gen. Microbiol.* **139:** 2215–2223.

Duchene, M., Barron, C., Schweizer, A., von Specht, B.–U., and Domdey, H., 1989, *Pseudomonas aeruginosa* outer membrane lipoprotein I gene: Molecular cloning, sequence, and expression in *Escherichia coli*, *J. Bacteriol.* **171:** 4130–4137.

Earhart, C. F., 1987, Ferrienterobactin transport in *Escherichia coli*, in: *Iron Transport in Microbes, Plants, and animals (G. Winkelmann, D. van der Helm, and J. B. Neilands, eds.), VCH Verlagsgellschaft mbH, Weinheim, Germany, pp. 67–84.*

Ferenci, T. and Stretton, S., 1989, Cysteine-22 and cysteine-38 are not essential for the function of maltoporin (LamB protein), FEMS Microbiol. Lett. **61:** 335–340.

Finke, M., Duchene, M., Eckhardt, A., Domdey, H. and vonSpecht, B.–U., 1990, Protection against experimental *Pseudomonas aeruginosa* infection by recombinant *P. aeruginosa* lipoprotein I exxpressed in *Escherichia coli*, *Infect. Immun.* **58:** 2241–2244.

Finke, M., Muth G., Reichhelm, T., Thomas, M., Duchene, M., Hungerer, K.–D., Domdy, H., and von Specht, B. U., 1991, Protection of immunosuppressed mice against infection with *Pseudomonas aeruginosa* by recombinant *P. aeruginosa* by recombinant *P. aeruginosa* lipoprotein I and lipoprotein I-specific monoclonal antibodies, *Infect. Immun.* **59:** 1251–1254.

Freudl, R., MacIntyre, S., Degen, M., and Henning, U., 1986, Cell surface exposure of the outer membrane protein OmpA of *Eschericha coli* K12, *J. Mol. Biol.* **188:** 491–494.

Fukuda, H., Hosaka, M., Hirai, K., and Iyobe, S., 1990, New norfloxacin resistance gene in *Pseudomonas aeruginosa* PAO, *Antimicrob. Agents Chemother.* **34:** 1757–1761.

Fukuda, H., Hosaka, M., Iyobe, S., Gotoh, N., Nishino, T., and Hirai, K., 1994, *nfxC*-type quinolone resistance in a clinical isolate of *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **39:** 790–792.

Fukuoka, T., Masuda, N., Takenouchi, T., Sekine, N., Iijima, M., and Ohya, S., 1991, Increase in susceptibility of *Pseudomonas aeruginosa* to carbapenem antibiotics in low amino acid media, *Antimicrob. Agents Chemother.* **35:** 529–532.

Gensberg, K., Hughes, K., and Smith, A. W., 1992, Siderophore-specific induction of iron uptake in *Pseudomonas aeruginosa*, *J. Gen. Microbiol.* **138:** 2381–2387.

Gerbl-Rieger, S., Peters, J., Kellerman, J., Lottspeich, F., and Baumeister, W., 1991, Nucleotide and derived amino acid sequences of the major porin of *Comamonas acidovorans* and comparison of porin primary structures, *J. Bacteriol.* **173:** 2196–2205.

Glaser, P., Sakamoto, H., Bellalou, J., Ullmann, A., and Danchin, A., 1988, Secretion of cyclolysin, the calmodulin-sensitive adenylate cyclase-haemolysin bifunctional protein of *Bordatella pertussis*, *EMBO J.* **7:** 3997–4004.

Gotoh, N., Itoh, N., Tsujimoto, H., Yamagishi, J.-I., Oyamada, Y., and Nishino, R., 1994a, Isolation of OprM-deficient mutants of *Pseudomonas aeruginosa* by transposon insertion mutagenesis: Evidence of involvement in multiple antibiotic resistance, *FEMS Microbiol. Lett.* **122:** 267–274.

Gotoh, N., Itoh, N., Yamada, H., and Nishino, T., 1994b, Evidence for the location of OprM in the *Pseudomonas aeruginosa* outer membrane, *FEMS Microbiol. Lett.* **122:** 309–312.

Grabert, E., Wingender, J., and Winkler, U. K., 1990, An ouiter membrane protein chracterisitic of mucoid strains of *Pseudomonas aeruginosa*, *FEMS Microbiol. Lett.* **68:** 83–88.

Hancock, R. E. W., and Carey, A. M., 1979, Outer membrane of *Pseudomonas aeruginosa*: Heat- and 2-mercaptoethanol-modifiable proteins, *J. Bacteriol.* **140:** 901–910.

Hancock, R. E. W., and Carey, A. M., 1980, Protein D1 - a glucose inducible, pore-forming protein from the outer membrane of *Pseudomonas aeruginosa*, *FEMS Microbiol. Lett.* **8:** 699–707.

Hancock, R. E. W., Elgi, C., Benzy, R., and Siehnel, R. J., 1992, Overexpression in *Escherichia coli* and functional analysis of a novel PPi-selective porin, OprO, from *Pseudomonas aeruginosa*, *J. Bacteriol.* **174:** 471–476.

Hancock, R. E. W., Irvin, R. T., Costerton, J. W., and Carey, A. M., 1981, The outer membrane of *Pseudomonas aeruginosa*: Peptidoglycan associated proteins, *J. Bacteriol.* **145:** 628–631.

Hancock, R. E. W., Siehnel, R., and Martin, N., 1990, Outer membrane proteins of *Pseudomonas aeruginosa*, *Mol. Microbiol.* **4:** 1069–1075.

Heinrichs, D. E., and Poole, K., 1993, Cloning and sequence analysis of a gene (*pchR*) encoding an AraC family activator of pyochelin and ferripyochelin receptor synthesis in *Pseudomonas aeruginosa*, *J. Bacteriol.* **175:** 5882–5889.

Heinrichs, D. E., Young, L., and Poole, K., 1991, Pyochelin-mediated iron transport in *Pseudomonas aeruginosa*: Involvement of a high-molecular-mass outer membrane protein, *Infect. Immun.* **59:** 3680–3684.

Hirai, K., Suzue, S., Irikura, T., Iyobe, S., and Mitsuhashi, S., 1987, Mutations producing resistance to norflaxacin in *Pseudomonas aeruginosa*, *Antimicrob.* Agents Chemother, **31:** 582–586.

Hohnadel, D., and Meyer, J.–M., 1988, Specificity of pyoverdine-mediated iron uptake among fluorescent *Pseudomonas* strains, *J. Bacteriol.* **170—** 4865–4873.

Hosaka, M., Gotoh, N. and Nishino, R., 1995, Purification of a 54-kilodalton protein (OprJ) produced in NfxB mutants of *Pseudomonas aeruginosa* and production of a monoclonal antibody specific to OprJ, *Antimicrob. Agents Chemother.* **39:** 1731–1735.

Huang, H., and Hancock, R. E. W., 1996, Role of specific loop regions in determining the function of the imipenem specific pore-protein OprD of *Pseudomonas aeruginosa*, *J. Bacteriol.*, in press.

Huang, H., and Hancock, R. E. W., 1993, Genetic definition of the substrate selectivity of

outer membrane protein OprD of *Pseudomonas aeruginosa*, *J. Bacteriol.* **175:** 7793–7800.

Huang, H., Jeanteur, D., Pattus, F., and Hancock, R. E. W., 1995, Membrane topology and site-specific mutagenesis of *Pseudomonas aeruginosa* porin OprD, *Mol. Microbiol.* **16:** 931–941.

Hughes, E. E., Gilleland, L. B., and Gilleland, H. E., 1992, Synthetic peptides representing epitopes of outer membrane protein F of *Pseudomonas aeruginosa* that elicit antibodies reactive with whole cells of heterologous immunotype strains of *P. aeruginosa*, *Infect. Immun.* **60:** 3497–3503.

Kropinski, A. M. B., Lewis, V., and Berry, D., 1987 Effect of growth temperature on the lipids, outer membrane proteins, and lipopolysaccharides of *Pseudomonas aertuginosa*, *J. Bacteriol.* **169:** 1960–1966.

Lee, H. S., Hancock, R. E. W., and Ingraham, J. L., 1989, Properties of a *Pseudomonas stutzeri* outer membrane channel-forming protein (NosA) required for production of copper containing N_2O reductase, . *Bacteriol.* **171:** 2096–2100.

Legakis, N. J., Tzouvelekis, L. S., Makris, A., and Kotsifaki, H., 1989, Outer membrane alterations in mutiresistant mutants of *Pseudomonas aeruginosa* selected by ciprofloxacin, *Antimicrob. Agents Chemother*, **33:** 124–127.

Létoffé, S., Delepelaire, P., and Wandersman, C., 1990, Protease secretion by *Erwinia chrysanthemi*: The specific secretion functions are analogous to those of *Escherichia coli* α-hemolysin, *EMBO J.* **9:** 1375–1382.

Li, X.-Z., Livermore, D. M., and Nikaido, H., 1994a, Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: Resistance to tetracycline, chloramphenicol, and norfloxacin, *Antimicrob. Agents Chemother.* **38:** 1732–1741.

Li, X.-Z., Nikaido, H., and Poole, K., 1995, Role of MexA-MexB-OprM in antibiotic efflux in *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **39:** 1948–1953.

Lim, A., de Vox, D., Brauns, M., Gabella, A., Hamers, R., and Comelis, P., 1995, unpublished cited in Genbank release, Accession No. 250191.

Liu, P. V., and Shokrani, F., 1978, Biological activities of pyochelins: Iron-chelating agents of *Pseudomonas aeruginosa*, *Infect. Immun.* **22:** 878–890.

Lundrigan, M. D., and Kadner, R. J., 1986, Nucleotide sequence of the gene for the ferrienterochelin receptor FepA in *Escherichia coli*: Homology among outer membrane receptors that interact with TonB, *J. Biol., Chem.* **137:** 653–657.

Masuda, N., and Ohya, S., 1995, Outer membrane proteins responsible for multiple drug resistance in *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **39:** 645–649.

Meyer, J. M., 1992, Exogenous siderophore-mediated iron uptake in *Pseudomonas aeruginosa*: Possible involvement of porin OprF in iron translocation, *J. Gen. Microbiol.* **138:** 951–958.

Meyer, J.-M., Hohnadel, D., Khan, A., and Cornelis, P., 1990, Pyoverdin-facilitated iron uptake in *Pseudomonas aeruginosa*: Immunological characterization of th ferripyoverdin receptor, *Mol. Microbiol.* **4:** 1401–1405.

Michea-Hamzehpour, M., Furet, Y. X., and Pechere, J. C., 1991, Role of protein D2 and lipopolysaccharide in diffusion of quinolones through the outer membrane of *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **35:** 2091–2097.

Mizuno, T. 1979, A novel peptidoglycan-associated lipoprotein found in the cell envelope of *Pseudomonas aeruginosa* and *Escherichia coli*, *J. Biochem.* **86:** 991–1000.

Mizuno, T., 1981, A novel peptidoglycan-associated lipoprotein (PAL) found in the outer membrane of *Proteus mirabilis* and other gram-negative bacteria, *J. Biochem.* **89:** 1039–1049.

Mizuno, T., and Kageyama, M., 1979, Isolation and characterization of a major outer membrane protein of *Pseudomonas aeruginosa*. vidence for the occurence of a lipoprotein, *J. Biochem.* **85:** 115–122.

Morshed, S. R. M., Lei, Y., Yoneyama, H., and Nakae, T., 1995, Expression of genes associated with antibiotic extrusion in *Pseudomonas aeruginosa*, *Biochem. Biophys. Res. Commun.* **210:** 356–362.

Mutharia, L. M., and Hancock, R. E. W., 1985, Monoclonal antibody specific for an outer membrane lipoprotein of the *Pseudomonas fluorescens* group of the family *Pseudomonodaceae*, *Int. J. Syst. Bacteriol.* **35:** 530–532.

Nakae, T., Yoshihara, E., Yoneyama, H., and Ishii, J., 1996, Protein D2 of the outer membrane of *Pseudomonas aeruginosa*. In: *Abstracts 5th Int. Pseudomonas Meeting*, Tsukuba City, Japan, 1995.

Nicas, T. L., and Hancock, R. E. W., 1983, Alteration of susceptibility to EDTA polymyxin B and gentamicin in *Pseudomonas aeruginosa* by divalent cation regulation of outer membrane protein H1, *J. Gen. Microbiol.* **129:** 509–517.

Nicas, T. L., and Hancock, R. E. W., 1989, Outer membrane protein H1 of *Pseudomonas aeruginosa*: Involvement in adaptive and mutational resistance to ethylenediaminetetraacetate, polymisin B, and gentamycin, *J. Bacteriol.* **143:** 872–878.

Nikaido, H., Nikaido, T., and Harayama, S., 1991, Identification and characterization of porins in *Pseudomonas aeruginosa*, *J. Biol. Chem.* **266:** 770–779.

Nikaido, H., and Rosenberg, Y., 1990, cir and Fiu proteins in the outer membrane of *Escherichia coli* catalyse transport of monomeric catechols: Study with β-lactam antibiotics containing catechol and analogous groups, *J. Bacteriol.* **172:** 1361–1367.

Ohkawa, I., Shiga, S., and Kageyama, M., 1980, Effect of iron concentration in the growth medium on the sensitivity of *Pseudomonas aeruginosa* to pyocin S2, *J. Biochem.* **87:** 323–331.

Piddock, L. V. J., Hall, M. C. Bellido, F., Bains, M., and Hancock, R. E. W., 1992, A pleiotropic, posttherapy, enoxacin-resistant mutant of *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **36:** 1057–1061.

Poole, K., Krebes, K., McNally, C., and Neshat, S., 1993, Multiple antibiotic resistance in *Pseudomonas aeruginosa*: Evidence for involvement of an efflux operon, *J. Bacteriol.* **175:** 7363–7372.

Poole, K., Neshat, S., and Heinrichs, D., 1991, Pyoverdine-mediated iron transport in *Pseudomonas aeruginosa*: Involvement of high-molecular-mass outer membrane protein, *FEMS Microbiol. Lett.* **78:** 1–6.

Poole, K., Neshat, S., Krebes, K., and Heinrichs, D., 1993, Cloning and nucleotide sequence analysis of the ferripyoverdine receptor gene *fpvA* of *Pseudomonas aeruginosa*, *J. Bacteriol.* **175:** 4597–4604.

Poole, K., Young, L., and Neshat, S., 1990, Enterobactin-mediated ironn transport in *Pseudomonas aeruginosa*, *J. Bacteriol.* **172:** 6991–6996.

Quinn, J. P., Dudeck, E. J., Divincenzo, C. A., Lucks, D. A., and Lerner, S. A., 1986, Emergence of resistance to imipenem during therapy for *Pseudomonas aeruginosa* infections, *J. Infect. Dis.* **154:** 289–294.

Quiocho, F. A., 1989, Protein-carbohydrate interactions: Basic molecular features, *Pure Appl. Chem.* **61:** 1293–1306.

Rahner, R., Eckhardt, A., Duchêne, MN., Domdey, H., and von Specht, B. U., 1990, Protection of immunosuppressed mice against infection with *Pseudomonas aeruginosa* by monoclonal antibodies to outer membrane protein OprI, *Infection* **18:** 242–254.

Rawling, E. G., 1995, Outer membrane protein OprF of *Pseudomonas aeruginosa*, Ph.D. Thesis, University of British Columbia, Vancouver.

Rawling, E. G., Martin, N. L., and Hancock, R. E. W., 1995, Epitope mapping of the

Pseudomonas aeruginosa major outer membrane protein OprF. *Infect. Immun.* **63:** 38–42.

Rehm, B., Boheim, G., Tommassen, J., and Winkler, U. K., 1994, Overexpression of *algE* in *Escherichia coli*: Subcellular localization, purification, and ion channel properties, *J. Bacteriol.* **176:** 5639–5647.

Rutz, J. M., Liu, J., Lyons, J. A., Goranson, J., Armstrong, S. K., McIntosh, M. A., Feix, J. B., and Klebba, P. E., 1992, Formation of a gated channel by a ligand-specific transport channel in the bacterial outer membrane, *Science* **258:** 471–=475.

Saint-Onge, A., Romeyer, F., Lebel, P. Masson, L., and Brousseau, R., 1992, Specificity of the *Pseudomonas aeruginosa* PAO1 lipoprotein I gene as a DNA probe and PCR target region with the *Pseudomonodaceae*, *J. Gen. Microbiol.* **138:** 733–741.

Saravolac, E. G., Taylor, N. F., Benz, R., and Hancock, R. E. W., 1991, Purification of glucose-inducible outer membrane protein OprB of *Pseudomonas putida* and reconstitution of glucose-specific pores, *J. Bacteriol.* **173;** 4970–4976.

Satake, S., Yoshihara, E., and Nakae, T., 1990, Diffusion of β-lactam antibiotics through liposome membranes reconstituted from purified porins of the outer membrane of *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **34:** 685–690.

Schirmer, T., Keller, T. A., Wang, Y.–F., and Rosenbusch, J. P., 1995, Structural basis for sugar translocation through maltoporin channels at 3.1 Å resolution, *Science* **267:** 512–514.

Siehnel, R. J., Egli, C., and Hancock, R. E. W., 1992, Polyphospate-selective porin OprO of *Pseudomonas aeruginosa*: Expression purification and sequence, *Mol. Microbiol.* **6:** 2319–2326.

Siehnel, R. J., Martin, N. L., and Hancock, R. E. W., 1990, Function and structure of the porin proteins OprF and OprP of *Pseudomonas aeruginosa*, In: *Pseudomonas: Biotransformation, Pathogenesis and Evolving Biotechnology*, S. Silver, A. M. Chakrabarty, B. Iglewski, and S. Kaplan eds.) American Society for Microbiology, Washington, D.C., pp. 328–342.

Sokol, P. A., and Woods, D. E., 1983, Demonstration of an iron-siderophore-binding protein in the outer membrane of *Pseudomonas aeruginosa*, *Infect. Immun.* **40:** 665–669.

Sukhan, A., and Hancock, R. E. W., 1995, Insertion mutagenesis of the *Pseudomonas aeruginosa* phosphate-specific porin OprP, *J. Bacteriol.* **177:** 4914–4920.

Sukhan, A., and Hancock, R. E. W., 1996, The role of specific lysine residues in the passage of phosphate and chloride ions through the *Pseudomonas aeruginosa* phosphate starvation inducible porin OprP. *J. Biol. Chem.* **271:**21239–21242.

Thanassi, D. G., Suh, G. S., and Nikaido, H., 1995, Role of outer membrane barrier in efflux-mediated tetracycline resistance of *Escherichia coli*, *J. Bacteriol.* **177:** 998–1007.

Toth, A., Schödel, F., Duchêne, M., Massarrat, K., Blum, B., Schmitt, A., Domdey, H., and von Specht, B.–U., 1994, Protection of immunosuppressed mice against tanslocation of *Pseudomonas aerugginosa* from the gut by oral immunization with recombinant *Pseudomonas aeruginosa* outer membrane protein I expressing *Salmonella dublin*, *Vaccine* **12:** 1215–1221.

Trias, J., and Nikaido, H., 1990a, Outer membrane protein D2 catalyzes facilitated diffusion of carbapenems and penems through the outer membrane of *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **34:** 52–57.

Trias, J., and Nikaido, 1990b, Protein D2 channel of the *Pseudomonas aeruginosa* outer membrane has a binding site for basic amino acids and peptides, *J. Biol. Chem.* **265:** 15680–15684.

Trias, J., Rosenberg, E. Y., and Nikaido, H., 1988, Specificity of the glucose channel formed by protein D1 of *Pseudomonas aeruginosa*, *Biochem. Biophys. Acta* **938:** 493–496.

VonSpecht, B.-U., Knapp, B., Muth, G., Bröker, M., Hungerer, K.–D., Diehl, K.–D., Massarrat, K., Seemann, A., and Domdey, H., 1995, Protection of immunocompromised mice against lethal infection with *Pseudomonas aeruginosa* by active or passive immunization with recombinant *P. aeruginosa* outer membrane protein F and outer membrane protein I fusion proteins, *Infect. Immun.* **63:** 1855–1862.

Williams, S. G., Greenwood, J. A., and Jones, C. W., 1994, The effect of nutrient limitation on glycerol uptake and metabolism in continuous cultures of *Pseudomonas aeruginosa*, *Microbiology* **140:** 2961–2969.

Wong, R. S. Y., Wirtz, R. A., and Hancock, R. E. W., 1995, *Pseudomona aeruginosa* outer membrane protein OprF as an expression vector for foreign epitopes: The effects of positioning and length on the antigenicity of the epitope, *Gene* **158:** 55–60.

Woodruff, W. A., and Hancock, R. E. W., 1982, Construction and characterization of *Pseudomonas aeruginosa* protein F-deficient mutant after in vivo and in vitro insertion mutagenesis of the cloned gene, *J. Bacteriol.* **170:** 2592–2598.

Wylie, J. L., 1994, Characterization of the OprB porin of *Pseudomonas aeruginosa*, Ph.D. Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Wylie, J. L., Bernegger-Egli, C., O'Neil, J. D.J., and Worobec, E. A., 1993, Biophysical characterization of OprB, a glucose-inducible porin of *Pseudomonas aeruginosa*, *J. Bioenerg. Biomembr.* **25:** 547–556.

Wylie, J. L., and Worobec, E. A., 1994, Cloning and nucleotide sequence of the *Pseudomonas aeruginosa* glucose-selective OprB porin gene and distribution of OprB within the family *Pseudomonadaceae*, *Eur. J. Biochem.* **220:** 505–512.

Wylie, J. L., and Worobec, E. A., 1995, The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*, *J. Bacteriol.* **177:** 3021–3026.

Yamano, Y., Nishikawa, T. and Komastusu, Y., 1993, Cloning and nucleotide sequence of anaerobically induced porin protein E1 (OprE) of *Pseudomonas aeruginosa* PAO1, *Mol. Microbiol.* **8:**993–1004.

Yamano, Y., Nishkawa, T., and Komatsu, Y., 1994, Ferric iron transport system of *Pseudomonas aeruginosa* PAO1 that functions as the uptake pathway of a novel catechol-substituted chephalosporin, S-9096, *Appl. Microbiol. Biotechnol.* **40:** 892–897.

Yates, J. M., Morris, G., and Brown, M. R. W., 1989, Effect of iron concentration and growth rate on the expression of protein G in *Pseudomonas aeruginosa*, *FEMS Microbiol. Lett.* **58:** 259–262.

Yoneyama, H., Yamano, Y., and 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.

Yoshida, T., Muratani, T., Iyobe, S., and Mitsuhashi, S., 1994, Mechanisms of high-level resistance to quinolones in urinary tract isolates of *Pseudomonas aeruginosa*, *Antimicrob. Agents Chemother.* **38:** 1466–1469.

Yoshihara, E., and Nakae, T., 1989, Identification of porins in the outer membrane of *Pseudomonas aeruginosa* that form small diffusion pores, *J. Biol. Chem.* **264:** 6297–6301.

Young, M. L., Bains, M., Bell, A., and Hancock, R. E. W., 1992, Role of *Pseudomonas aeruginosa* outer membrane protein OprH in polymyxin and gentamicin resistance: Isolation of an OprH-deficient mutant by gene replacement techniques, *Antimicrob. Agents Chemother.* **36:** 2566–2568.

Young, M. L., and Hancock, R. E. W., 1992, Fluoroquinolone supersusceptibility mediated by Opr overexpression in *Pseudomonas aeruginosa*: Evidence for involvement of a non-porin pathway, *Antimicrob. Agents Chemother.* **36:** 2365–2369.

Zhanel, G. G., Karlowsky, J. A., Saunders, M. H., Davidson, R. J., Hoban, D. J., Hancock, R. E. W., McLean, I., and Nicolle, L. E., 1994, Development of multiple antibiotic-resistant (MAR) mutants of *Pseudomonas aeruginosa* after serial exposure to fluoroquinolones, *Antimicrob. Agents Chemother.* **39:** 489–495.