ESCHERICHIA COLI IN DOMESTIC ANIMALS AND HUMANS

Edited by

C.L. GYLES

Department of Veterinary Microbiology and Immunology
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

CAB INTERNATIONAL

18

Outer Membrane Proteins

R.E.W. HANCOCK AND K. PIERS
Department of Microbiology and Immunology,
University of British Columbia, Vancouver, BC,
Canada V6T 1W5

Introduction

The outer membranes of Gram-negative bacteria are intimately involved in the lifestyles of these organisms, including their lifestyle within a human or animal host. Although outer membranes are not often named as virulence determinants, they have several roles that merit such a designation including those in exclusion of bile salts, resistance to proteases and other enzymes, resistance to serum bactericidal killing, excretion of other bacterial virulence factors, limitation of antibiotic uptake, uptake of important nutrients in short supply in the host, endotoxicity, and anchoring of adhesins and flagella (Nikaido and Vaara, 1985; Inouye, 1987; Hancock and Bell, 1989; Hancock, 1991). In this chapter we will summarize the properties of the outer membrane proteins of *E. coli* with special attention to those important in virulence and/or growth *in vivo*. Only a limited discussion of regulation or mechanisms of secretion will be included and the readers are referred to more detailed reviews on these subjects (Igo *et al.*, 1990; Mizuno and Mizushima, 1990; Pugsley, 1993). *E. coli* was the prototype for the first investigations on outer membranes and is by far the best studied of all organisms. For detailed discussion of outer membranes in other organisms three recent reviews may be consulted (Nikaido and Vaara, 1985; Hancock, 1991; Hancock *et al.*, 1993).

Outer Membrane Structure

The *E. coli* outer membrane constitutes a typical asymmetrical bilayer studded with proteins (Fig. 18.1; Hancock *et al.*, 1993). The outer

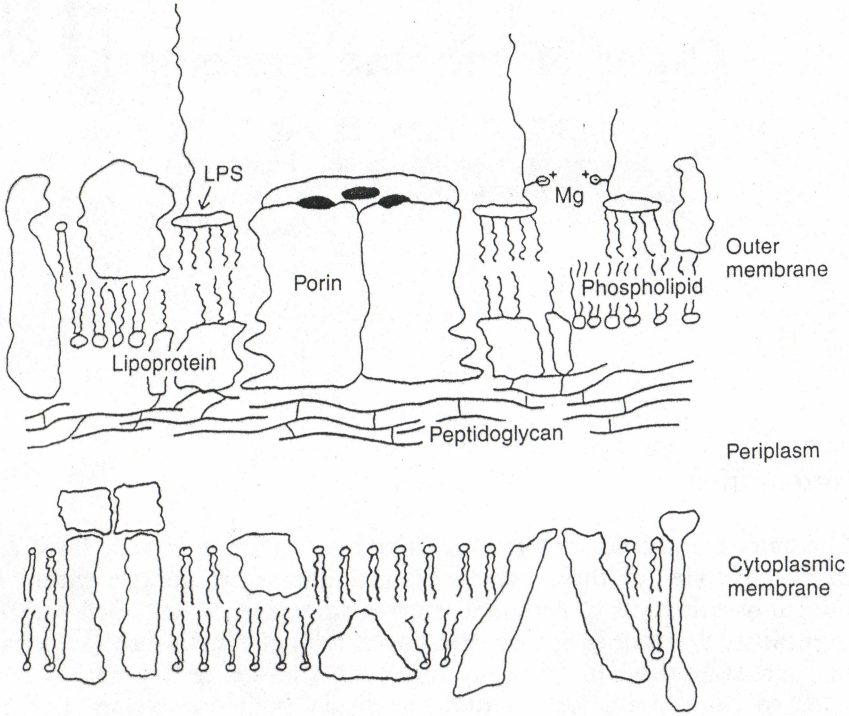

Fig. 18.1. Schematic diagram of the Gram-negative outer membrane.

monolayer of this asymmetrical bilayer contains the glycolipid molecule lipopolysaccharide (LPS) (discussed in Chapter 17), while the inner monolayer contains phospholipids, primarily phosphatidyl ethanolamine, phosphatidyl glycerol and cardiolipin (Cronan, 1979). This partitioning of lipidic species is almost complete as demonstrated by chemical and enzyme accessibility studies, immuno-electron microscopy, and freeze fracture studies (Avrameas, 1969; Funahara and Nikaido, 1980). The negatively charged LPS in the outer monolayer and its tight non-covalent association with itself via divalent cation crossbridging and with proteins via hydrophilic, charge-charge and hydrophobic forces (Nikaido and Vaara, 1985; Hancock, 1991; Hancock *et al.*, 1993), confer several important properties on the outer membrane. These include exclusion of many hydrophobic substances, such as dyes, detergents and bile salts, and resistance to attack by phospholipases and other enzymes. In addition, the uptake of cationic substances including antibiotics, such as polymyxins, aminoglycosides and azithromycin, and antibacterial peptides, such as defensins and bactenicins, involves a self-promoted uptake pathway that is initiated

by initial interaction of these cationic peptides at the divalent cation crossbridging sites on LPS (reviewed in Hancock and Bell, 1989; Hancock, 1991).

Proteins constitute nearly 60% by weight of the *E. coli* outer membrane. The predominant protein species are present at a copy number of 5×10^4 to 2×10^5 polypeptides per cell and are termed 'major' outer membrane proteins (Lugtenberg *et al.*, 1975). The major outer membrane proteins of *E. coli* cells grown on rich broth are OmpF, OmpC, OmpA and Lpp. However other proteins can become predominant under specific growth conditions or when *E. coli* harbours phages or plasmids that encode these proteins (see below). In addition to the major proteins, 50 or more other polypeptides can be observed in lower copy number on two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Many of these proteins are exposed on the surface of the outer membrane as judged by their function as phage or colicin receptors (Table 18.1), or their reactivity in intact cells with antibodies. However, this must be qualified since such assessments of surface localization are usually performed in *E. coli* K-12, which is a rough, LPS O-antigen-deficient variant, and such variants are rarely found *in vivo*. *E. coli* strains derived from infections, containing smooth LPS and/or capsules, fail to react with a variety of phages, colicins or protein-specific antibodies (see below).

Table 18.1. Functions of *E. coli* outer membrane proteins.

Function	Major components
Non-specific passage of small hydrophilic compounds	OmpF, OmpC
Specific uptake of hydrophilic substances	FepA, FecA, Fiu, FhuA, BtuB, LamB, Tsx
Uptake of fatty acids	FadL
Structural role	OmpA, Lpp, ExcC, OmpH
Protein excretion	TolC
Export and anchoring of surface appendages	FimD, FlgH
Enzymes	OmpT, PldA
Receptors for phages	FhuA, BtuB, LamB, FadL, OmpC, OmpF, OmpA, Tsx
Receptors for colicins	FepA, FhuA, Cir, BtuB, OmpF, Tsx
F pilus mating aggregate stabilization	OmpA

Outer Membrane Protein Functions

Outer membrane proteins have a variety of known functions, as summarized in Table 18.1. The first functions described were receptor functions for phages and bacteriocins (Konisky, 1979). However, since such functions would result in eventual cell death, it seemed unlikely that these were the major roles of the outer membrane proteins. Indeed, each of these receptors has now been ascribed an alternative function. For example, the receptors for phages T6 (Tsx) and λ (LamB) function in nucleoside and maltose/maltodextrin uptake, respectively, whilst the receptors for colicins B and E1 function in ferri-enterobactin and vitamin B_{12} uptake, respectively. In general, *E. coli* outer membrane proteins serve the following functions: the general porins mediate passage of hydrophilic solutes in a size-dependent fashion; the substrate-specific porins have a binding site within their channels that imparts selectivity for a given substrate; they may have roles in cell shape and structural stability; and specific proteins possess enzymatic activities, are involved in secretion and/or anchoring of surface appendages, or mediate excretion of haemolysin into the environment (Table 18.2). In addition, outer membrane proteins expressed from plasmid or lysogenic phage genes can have functions of importance in pathogenesis, including adherence and complement resistance (Table 18.3).

Outer Membrane Proteins

E. coli K-12 has by far the best characterized outer membrane of any organism, with 25 chromosomally encoded proteins for which the genes have been cloned and sequenced, and two other genetically defined proteins (Table 18.2). This section discusses the known properties of these proteins.

General porins

Porins represent a ubiquitous class of outer membrane proteins that form channels across the outer membrane (Hancock, 1986; Benz *et al.*, 1988). They are highly conserved in both general structure and function, although their primary sequences vary substantially from organism to organism (Jeanteur *et al.*, 1993). Porins are characterized as outer membrane proteins that are capable of forming channels when reconstituted into model membrane systems (Table 18.4). These proteins have a number of properties in common:

1. They all contain substantial β-sheet structure.
2. They have similar molecular weights (31 000–48 000).

Table 18.2. Chromosomally encoded outer membrane proteins of *E. coli*.

Name	Mol. wt	DNA seq acc^N no.	Map position	Function	Regulation	Other[a]
Eae	102,000	Z11541	—	Attachment and effacement of epithelial layers	By plasmid pMAR5	MW94
FimD	97,260	X51655	98.1′	Required for surface localization of type 1 fimbriae	Fim B/E; phase variation	—
Fiu	83,000	—	18′	Ferric iron uptake – scavenger pathway	Low iron derepressible (*fur*)	MW83
FecA	81,718	M20981	93′	Ferric citrate uptake (*fur*), citrate inducible	Low iron derepressible	MW80.5
FepA	79,908	J04216	13.6′	Ferric-enterobactin receptor/ permeation; colicins B,D receptor; gated porin	Low iron derepressible (*fur*)	MW81
FhuA	78,992	XO5810 M12486	3.7′	Ferri-ferrichrome receptor/ permeation; phages T1, φ80, T5 receptor; colicin M receptor	Low iron derepressible (*fur*)	MW78
FhuE	77,453	X17615	24.7′	Ferri-coprogen, ferri-rhodoturulic acid, ferrioxamine B receptor/ permeation	Low iron derepressible (*fur*)	MW76
Cir	67,179	J04229	46.4′	Ferric iron uptake-savenger pathway; colicin Ia, Ib receptor	Low iron derepressible (*fur*)	MW74

Table 18.2. *continued*

Name	Mol. wt	DNA seq acc^N no.	Map position	Function	Regulation	Other[a]
BtuB	66,412	M10112	89.7′	Vitamin B12 receptor/ permeation; phage BF23 receptor; colicins E1, E2, E3 receptor	—	MW60
TolC	66,000	X54049	66.4′	Required for haemolysin secretion	Constitutive minor protein	MW66, TRI
Hag	51,172	M14358	42.4′	Flagellin subunit	Constitutive	MW55; HM
LamB	47,932	V00298	91.5′	Maltodextrin-specific porin; phages λ, K10 receptor	Maltose induced (malT) PG	MW55, TRI, PG
FadL	45,969	M37714	50.6′	Fatty acid binding/ permeation; phage T2 receptor	Fatty acid induced, glucose repressed	MW43, HM
K	40,000	—	—	General porin	Found in encapsulated strains	MW40, TRI, PG
NmpC	39,500	M13457	12.6′	General porin (defective Tsr′ encoded)	Normally inactivated by IS5 insertion	MW39.5, TRI, PG
OmpC	38,307	K00541	47.7′	General porin, Phage TulB receptor, Phage K20, K21, K22 receptor	High salt induced (*envZ, OmpR*); acid pH	MW37, TRI, PG
OmpG (CE1248)	37,000	—	29′	General porin – weakly expressed	*Cog*	MW37, TRI, PG
PhoE	36,782	X00786	5.7′	General porin – anion selective (*phoB*, *phoR*, *phoM*)	Low phosphate induced	MW38, TRI, PG
OmpF	35,705	J01655	20.7′	General porin, colicin N receptor, phage TuI receptor temperature induced (*mic* F)	Low salt induced (*envZ, OmpR*); high growth	MW36, TRI, PG
OmpT	35,567	X06903	12.9′	Endoprotease	Constitutive	MW37
OmpA	35,159	J01654	21.7′	Porin; structural role – cell shape, stability; Phage K3, TulI receptor; stabilizes F pilus mating aggregates	Constitutive	MW33, HM, PG (TRI)
Tsx	31,418	M57685	9′	Nucleoside-specific porin; albicidin uptake; phage T6 receptor, colicin K receptor	Dual promoters: (1) cAMP + CAP induced; *cytR* repressed; (2) *desR* repressed	MW28
PldA	30,809	X00780 X02143	86.1′	Phospholipase A	Constitutive low level	MW27
FlgH	27,000	—	24′	Flagella L-ring	—	MW27
Pal	18,748	X65796	17′ (*excC*)	Outer membrane stability	Constitutive	MW17, PG, LP
OmpH (HlpA)	15,692	M21118	38′	LPS binding protein – possible structural role	Constitutive	MW17
Lpp	6,961	J01645	36.7′	Structure, osmotic stability	Constitutive	MW9, LP
OsmB	6,949	M22859	28.0′	Unknown	High osmolarity inducible	MW8, LP

[a] Code for other:
MW33 = apparent mol. wt = 33 K after boiling prior to SDS-PAGE;
TRI = trimers observed when run on SDS-PAGE;
(TRI) = evidence of native trimers but not observed on SDS-PAGE;
HM = heat modifiable (moves from low apparent molecular weight position to high apparent molecular weight position upon heating);
PG = peptidoglycan associated;

Table 18.3. Plasmid and phage encoded outer membrane proteins of *E. coli*.

Name	Mol. wt	DNA seq acc^N no.	Encoded by	Function	Regulation	Other[a]
FanD	84,500	X13560	pK99	Export/expression of pK99 functions	—	—
FaeD	82,100	X56003	pK88ab	Export of K88ab fimbrial subunits	—	—
PapC	81,000	X61239	pF13	Export/expression of P fimbriae	—	—
IutA	74,000	X05814	pColV	Ferric aerobactin receptor/ permeation (*fur*)	Low iron derepressible	MW74
ScrY	55,408	S44133	pUR400	Sucrose/maltodextrin specific porin; general porin	Sucrose induced (*scR*)	MW55, TRI
TraB	55,000	—	F	F pilus assembly/biosynthesis	—	—
Lc(HK253)	39,000	—	ΦHK253	General porin; lambdoid phage receptor	Growth temperature	MW39, TRI
Lc(PA102)	36,500	JO2580	ΦPA102	General porin	—	MW36.5 TRI, PG
TraT	25,000	JO1769	pR100	Complement resistance; surface exclusion	—	LP
	25,000	X52553	pR6–5	Surface exclusion	—	LP
	25,000	M13465	pED208	Surface exclusion	—	LP
	26,017	X14566	F	Complement resistance; surface exclusion	—	LP
TraF	25,000	M20787	F	F pilus assembly/biosynthesis	—	—
TraK	24,000	X54458	F	F pilus assembly/biosynthesis	—	—
		X54459[b]				
TraP	23,500	—	F	Function unknown	—	—
TraL	10,350	K01147	F	F pilus assembly/biosynthesis	—	—
BRP	2,900	X04466	pCloDF13 (geneH)	Bacteriocin release protein	—	LP
		J01566	pColE1 (*kil*)	Bacteriocin release protein	—	LP
		M29885	pColE2 (*ceiB*)	Bacteriocin release protein	—	LP
		J01574	pColE3 (*hic*)	Bacteriocin release protein	—	LP
		Xo2391	pColA (*cal*)	Bacteriocin release protein	—	LP

[a] See Table 18.2 for codes.
[b] Genes from related plasmids.

3. They form native trimers in the outer membrane as assessed by cross-linking studies or crystallography.
4. All contain channels that allow the passage of ions.

The two possible exceptions to these rules are an as yet uncharacterized voltage-sensitive channel (Buechner *et al.*, 1990) and the Tsx protein (Bremer *et al.*, 1990), both of which have rather unusual properties.

The general porins lack known substrate specificity (Hancock, 1986; Nikaido, 1992). Five of these have had their sequences defined genetically, namely OmpF, OmpC, PhoE, NmpC and Lc(PA-2) (Tables 18.2 and 18.3). This group of proteins shares the following structural properties: they have sequence similarity with about 56% identical amino acids, they are immunologically cross-reactive, they have very similar molecular weights of 35 000–40 000, and they form trimers that are resistant to denaturation by the detergent SDS. In addition, their functional properties are quite analogous. Model membrane studies have indicated that they have similar channels, with single channel conductances ranging from 1.5 to 2 nS in 1 M KCl solution, and weak ion selectivity (Table 18.4),

Table 18.4. Conductance characteristics of *E. coli* porins.

Porin	Growth conditions favouring production	Single channel conductance in 1 M KCl (n^S)	Selectivity (pK^+/pCl^-)	Binding
OmpF	Low salt	~ 1.9	Cation (3.7)	—[a]
OmpC	High salt	1.5	Cation (26)	—
PhoE	Low phosphate	1.8	Anion (0.33)	—
OmpG (CE1248)	Cog mutation	2.5	Cation (12.5)	—
NmpC	IS5B deletion	1.8	Anion (0.27)	—
OmpA	Constitutive	0.7	Anion (0.7)	—
LamB	Maltose	0.16	Cation	Maltose/ maltodextrins
Tsx	Various	0.01	Cation (4.2)	Bases/ nucleosides
Voltage sensitive	—[b]	0.6	Cation	—
Lc(PA-2)	Lysogeny	~ 2.0	Cation (6.5)	—
Lc(HK253)	Lysogeny	2.5	Cation (12)	—
K	Plasmid	1.8	ND	—
ScrY	Plasmid	1.4	Cation (8.6)	Sucrose maltodextrins

[a] No specific binding site.
[b] Actual protein has not been isolated.

indicating that all form large water-filled channels. Other porins, OmpG, protein K and Lc (HK253) share similar properties and may thus be related (Table 18.4).

The porins that have been investigated in the greatest detail are OmpF, OmpC and PhoE. OmpF and OmpC can be observed in the outer membranes of *E. coli* grown *in vitro* on many laboratory media. However, their production is influenced by medium constituents. The best studied of these is the medium osmolarity; high osmolarity favours OmpC production, whereas low osmolarity favours OmpF production. Osmolarity is sensed by the transmembrane sensor EnvZ which phosphorylates the OmpR activator, causing it to bind to the OmpC promotor and stimulate transcription (Igo *et al.*, 1990; Mizuno and Mizushima, 1990). Other regulatory elements include the micF antisense RNA which is transcribed from the upstream sequences of the *ompC* gene, and which down-regulates OmpF production when OmpC production is stimulated by high osmolarity or by changes in other environmental conditions (Andersen *et al.*, 1989). This inverse regulation is important since the high osmolarity found *in vivo* has been proposed to result in almost exclusive synthesis of OmpC (Nikaido and Vaara, 1985). Since OmpC has a smaller channel than OmpF (Nikaido and Rosenberg, 1983; Benz *et al.*, 1985) it has been proposed that this would restrict uptake of antibiotics, rendering *E. coli* more antibiotic resistant *in vivo* than *in vitro*.

PhoE which is highly similar to both OmpF and OmpC (they share >85% identical amino acids) is not found under most growth conditions and is absent *in vivo* (Robledo *et al.*, 1990). It is part of the phosphate-starvation-inducible Pho regulon (Tommassen *et al.*, 1987), and is activated upon growth in phosphate-deficient medium. However although anion selectivity of its channel favours uptake of anions, it does not contain a binding site for phosphates or polyphosphates (Darveau *et al.*, 1984; Bauer *et al.*, 1989).

Recently the crystal structures of OmpF and PhoE were published (Cowan *et al.*, 1992). They demonstrated remarkable similarity to the structure of the general porin from *Rhodobacter capsulatus* (Weiss *et al.*, 1991), despite the almost complete lack of sequence homology (n.b. it must be noted, however, that use of a refined amphipathicity prediction has permitted conceptual alignment of these sequences (Jeanteur *et al.*, 1993)). The general structure of the OmpF porin is shown in Fig. 18.2. Both OmpF and PhoE consist of 16 β-strands tilted at an angle of 35–50° and arranged in an ordered antiparallel fashion. The β-strands form a β-barrel structure that circles the central channel. Between these β-strands are eight short β-turns of two to three residues on the periplasmic side and eight longer loops on the outer surface side of the barrel. The loops are tightly packed and one long loop, L3, enters the channel to create the most constricted part of the channel and thus contribute to the channel's

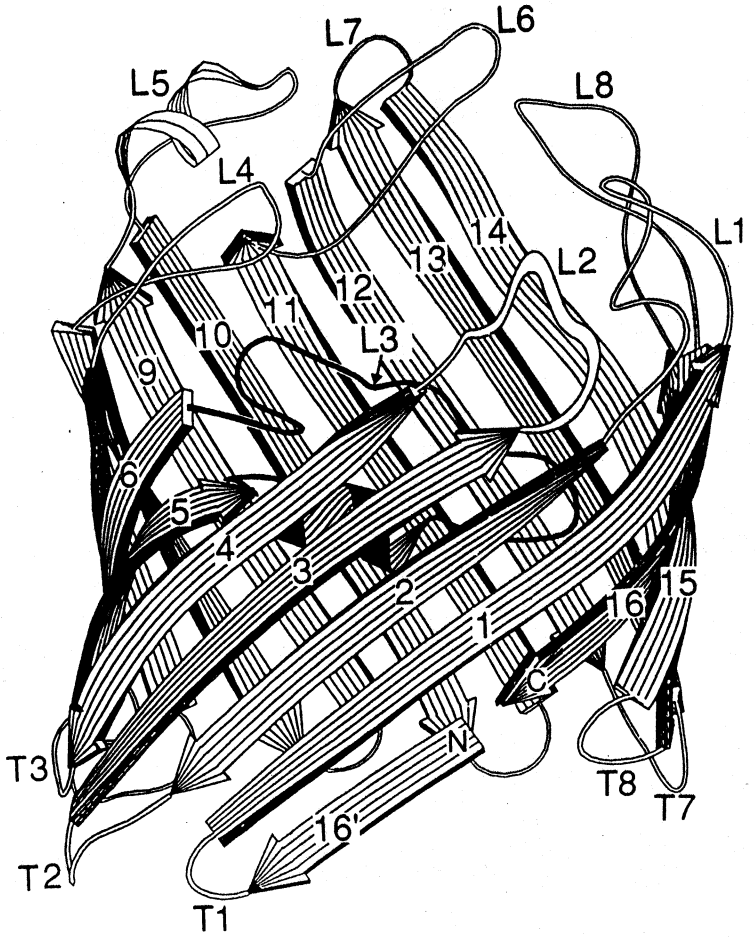

Fig. 18.2. General structure of the OmpF porin. Arrows represent β-strands and are labelled 1–16 starting from the strand after the first short turn. The long loops are denoted L1–L8 while the short turns at the other end are called T1–T8. Loop L2 protrudes to the viewer and is believed to be involved in monomer association, while L3 folds back into the barrel and is believed to contribute to the porin size. (Reproduced with permission from Cowan *et al.*, 1992.)

exclusion limit and ion selectivity (through charged amino acids in the loop). For example, the replacement in loop L3 of Gly-131 of OmpF with Lys-131 in PhoE, leading to an added positive charge at the channel constriction, explains the anion selectivity of PhoE, compared with OmpF (Table 18.3). Three monomeric subunits as described above are packed into a native trimer with strong hydrophobic interactions involving many residues of the subunits. This tight packing of monomers to form the native trimer probably explains the resistance of porins to denaturation, whereas the tight packing of the loop regions may explain the resistance of porins to proteolysis.

The other five general porins mentioned above are difficult to rationalize. Only one, OmpG, is found in wild-type *E. coli* K-12 strains. Misra and Benson (1989) observed a porin-like protein, OmpG, in *E. coli* cells that had acquired the ability to grow on large oligosaccharides in the absence of the specific porin LamB. Subsequently, Hancock *et al.* (1992) found a porin, which they called CE1248 porin, which was made in low levels in porin-deficient *E. coli* mutants. The large single channel conductance of this porin indicates that it should permit passage of larger sugars than OmpF or OmpC, and thus we hypothesize that this porin is OmpG, and is responsible for trans-outermembrane permeation of hydrophilic substances in the absence of OmpF or OmpC. Two other general porins, L_c (HK253) and L_c (PA-2), are encoded by lysogenic phages (Blasband *et al.*, 1986; Verhoef *et al.*, 1987), whereas the porin NmpC is encoded by the defective lambdoid prophage *Tsr*, but is inactivated in wild-type *E. coli* by insertion of IS5B (Blasband *et al.*, 1986). NmpC was found in pseudorevertants of porin-deficient *E. coli* strains which had spontaneously excised this insertion element. Protein K on the other hand is found almost exclusively in encapsulated *E. coli* strains (Sutcliffe *et al.*, 1983; Whitfield *et al.*, 1983). Although clearly related to OmpF, its regulation and genetics have not been well studied.

One other general porin that is clearly unrelated to the OmpF family has recently come to light, namely OmpA. It was assumed for many years that OmpA lacked porin activity, but the strong homology of this protein to the well-characterized porin OprF of *P. aeruginosa* (Woodruff and Hancock, 1989) caused a re-examination of this assumption. OmpA was demonstrated to form channels in two different model systems (Sugawara and Nikaido, 1992; Saint *et al.*, 1993). These channels appear to be quite large but to have low permeability, an apparent contradiction that has been reconciled by proposing that the channel contains a bend which increases frictional interactions within the channel. OmpA seems to be a member of a family of proteins with homology in their C-terminal 150 amino acids (Woodruff and Hancock, 1989; Gentry-Weeks *et al.*, 1992) and due to its role in cell structure will be discussed in more detail below. It is worth noting that OmpA has many structural similarities to the OmpF family,

including high content of β-sheet structure, native trimer configuration, similar monomer size, association with the peptidoglycan and LPS, and lack of long stretches of hydrophobic amino acids (see Hancock, 1986; Woodruff and Hancock, 1989).

Substrate specific porins

Nikaido (1992) argued that outer membrane channel-forming proteins that contain, within their channels, a specific binding site, should be termed specific channels due to their fundamental differences in physiological behaviour. However these proteins retain many of the distinguishing features of porins including trimeric association, high content of β-sheet, association with the peptidoglycan and LPS and significant permeability to ions. Thus while the maltodextrin-specific channel-forming LamB protein has a rather distant evolutionary relationship to the OmpF-like porins or the OmpA family (Jeanteur *et al.*, 1993), we utilize the term substrate-specific porins here to reflect these structural similarities since it is often difficult to discriminate these families. For example the major porin of *Bordetella pertussis* is anion specific but related to the non-specific porins of *Neisseria* spp. (Li *et al.*, 1991). The plasmid encoded ScrY porin of *E. coli* on the other hand has the permeability characteristics of both specific and non-specific porins (Table 18.4).

Only three substrate-specific porins are known in *E. coli* (Table 18.4). An earlier conclusion that PhoE was phosphate and/or polyphosphate selective (Overbeeke and Lugtenberg, 1982; Dargent *et al.*, 1986) was in error (Darveau *et al.*, 1984; Bauer *et al.*, 1989), although PhoE, by virtue of its anion selectivity, demonstrates relatively high intrinsic permeability to large anions.

The best characterized substrate-specific porin is LamB, which is named for its role as the cellular receptor for bacteriophage λ. It is present in low amounts in cells growing on rich medium but is maltose inducible by a process that involves the *malT* apoactivator and maltose as a coactivator (Smelcman and Hofnung, 1975). Genetic studies including linker insertion mutagenesis, insertion of foreign epitopes, and sequencing of mutants that affect phage or antibody binding, have resulted in a sophisticated two-dimensional folding model for LamB (Saurin *et al.*, 1989). Cells deficient in LamB can still grow on maltose, since at high concentrations of external maltose, uptake through the general porins is sufficient to permit transit of maltose across the outer membrane. However LamB is obligately required (Smelcman and Hofnung, 1975) for growth on larger malto-oligosaccharides (maltodextrins).

Model membrane studies have confirmed that LamB channels contain a binding site for maltose and maltodextrins. For example, in planar lipid bilayer experiments, these sugars block the conductance of ions through the channel, by binding to sites within the channel. As the size of the maltodextrin increases, the binding affinity (concentration of sugar leading to a 50% decrease in ion conductance) increases, up to maltopentose (K_s = 59 μM) and stays high for larger sugars (Benz *et al.*, 1987). The existence of such binding sites has been confirmed by starch binding (Francis *et al.*, 1991a, b) and equilibrium flow dialysis (Gehring *et al.*, 1991) experiments. The latter have confirmed the existence of three independent binding sites, presumably one per monomer in the LamB trimer, and two-dimensional crystal reconstruction studies (Lepault *et al.*, 1988) have indicated an analogous structure (i.e. three independent channels) to that observed for OmpF (Fig. 18.2). Molecular genetic studies have identified specific amino acids that are involved in maltodextrin binding including residues 8–18, 74–82, 118–121, 152–154 and 360, suggesting that several regions of the polypeptide must collaborate in forming the binding site(s) (Dargent *et al.*, 1988; Heine *et al.*, 1988; Francis *et al.*, 1991a, b; Benz *et al.*, 1992; Chan and Ferenci, 1993). As discussed by Benz and colleagues (Benz *et al.*, 1987, 1992), the higher affinity of the channel for maltodextrins with four or more glucose residues implies that the channel may have a series of binding sites spaced in such a way that they can bind to more than one residue of the maltodextrin simultaneously.

The plasmid-encoded ScrY porin is involved in sucrose utilization by cells containing the sucrose regulon of plasmid pUR400 (Schmid *et al.*, 1988; Hardesty *et al.*, 1991). ScrY shows 23% amino acid identity with LamB. As mentioned above, the general permeation properties of Scr Y are similar to OmpF in that it forms large water-filled channels (Schülein *et al.*, 1991). However it is also similar to Lam B in having a maltodextrin binding site(s) for which binding affinity is a function of size of the maltodextrin.

The Tsx protein was originally characterized as the receptor for colicin K and bacteriophage T6. Hantke (1976) demonstrated that cells lacking Tsx had decreased rates of uptake of nucleosides and deoxynucleosides when these substrates were present at low (nM) extracellular concentrations. Model membrane channel studies (Benz *et al.*, 1988; Maier and Bremer, 1988) indicated that Tsx had limited permeability for ions, with a single channel conductance nearly 200-fold lower than that of OmpF. However this low conductance could be blocked by a variety of nucleosides and deoxynucleosides with a binding constant (K_s) as low as 50 μM. Despite these similarities to LamB, Tsx is unusual in that is quite susceptible to detergent denaturation (Bremer *et al.*, 1990) in contrast to all other porins. As befits a nucleoside channel, it is regulated by two repressors, DeoR and CytR (acting at separate, tandem promoters), which also control nucleoside transport/catabolism. Tsx is also involved in uptake of a plant-derived antibiotic albicidin which has no close resemblance to a nucleoside, but is a DNA synthesis inhibitor (Birch *et al.*, 1990).

The FadL protein is known to be involved in uptake of long-chain fatty acids from the medium (Black *et al.*, 1987). It is as yet unknown whether it functions as a substrate-specific porin or through another mechanism, although its role in outer membrane translocation has been definitively demonstrated and it binds fatty acids with very high affinity (K_d = 6.3 nM) (Black *et al.*, 1987; Mangroo and Gerber, 1992)). Unlike the porins, its sequence demonstrates an abundance of hydrophobic residues (Black, 1991). Nevertheless, it is heat modifiable on SDS-PAGE gels (Black *et al.*, 1987), a result consistent with its high content of β-sheet, while its molecular weight is similar to that of the substrate-specific porin LamB (Table 18.2). FadL is induced by the presence of fatty acids in the medium. Interestingly it demonstrates 42% identity with protein P1 from *Haemophilus influenzae* (Black, 1991).

Iron regulated outer membrane proteins

Iron is an essential nutrient of most bacteria and is available in limited amounts under most environmental conditions and *in vivo* (Braun *et al.*, 1976a). When *E. coli* cells are grown under conditions of low iron, they synthesize an enzyme system that produces, from shikimic acid, the siderophore enterobactin, a cyclic trimer of dihydroxybenzoylserine. In addition, five iron-regulated outer membrane proteins of apparent molecular weights 83 K (Fiu), 81 K (FepA), 78 K (FhuA and FhuE) and 74 K (Cir) are synthesized (Braun *et al.*, 1976a; Hancock *et al.*, 1976). The biosynthetic and outer membrane protein genes constitute a regulon which is repressed by the Fur repressor which binds to a consensus Fur-box nucleotide sequence present in the upstream region of each of the operons involved (Hantke, 1982). It has been proposed that iron is a corepressor such that iron deficiency causes the Fur repressor to dissociate from the Fur-box permitting transcription of all of the coregulated operons (Bagg and Neilands, 1987a, b). In addition to the above iron-regulated outer membrane proteins, the FecA protein is Fur-regulated and depressed by growth on low iron, providing citrate is present (Pressler *et al.*, 1988), whereas the col V-plasmid-encoded outer membrane protein Iut and the biosynthetic genes for the siderophore aerobactin are also iron regulated in *E. coli* cells harbouring this plasmid (de Lorenzo *et al.*, 1986).

Siderophores bind iron with high affinity, and when released into the medium, can capture iron from other weaker iron binding systems such as the human serum protein transferrin. Subsequently, the iron–siderophore complex binds to a specific outer membrane protein and this triggers translocation across the outer membrane. Of the *E. coli* proteins mentioned above, FepA binds the complex of iron with enterobactin (Braun *et al.*, 1976a; Armstrong *et al.*, 1990), whereas FecA binds ferric–citrate complexes (Pressler *et al.*, 1988) and Iut binds ferric–aerobactin

complexes (Bagg and Neilands, 1987b). The proteins Fiu and Cir apparently have quite loose specificity and bind complexes of iron with enterobactin breakdown products (Nikaido and Rosenberg, 1990) as well as complexes of iron with a variety of catechol-containing β-lactams (Curtis *et al.*, 1988; Nikaido and Rosenberg, 1990; Critchley *et al.*, 1991), and have thus been proposed to serve as scavenger systems to enhance iron influx. However the two other iron-regulated outer membrane proteins, FhuE and FhuA, function strictly as agents of parasitism since they demonstrate specificity for the ferrated siderophores of fungi (Sauer *et al.*, 1990; Killmann and Braun, 1992).

The translocation of iron across the outer membrane depends in all cases on a protein called TonB (Postle, 1990). It was proposed, based on the influence of *tonB* mutations on the energized, irreversible binding of bacteriophages T1 and ϕ80 to FhuA (formerly called TonA) that TonB serves to couple the proton motive force to this outer membrane protein (Hancock and Braun, 1976). Subsequently this proposal was extended to the translocation of siderophore–iron complexes and vitamin B_{12} (via BtuB) across the outer membrane (Schöffler and Braun, 1989; Hannavy *et al.*, 1990; Kadner, 1990). It was demonstrated that TonB is a cytoplasmic membrane protein anchored by a single N-terminal hydrophobic anchor (Postle, 1990). However, it was proposed that an unusual segment of X-Pro dipeptide repeats served to span the periplasm and contact the iron-regulated outer membrane proteins and BtuB, permitting the transmission of 'protein conformational changes from the cytoplasmic membrane across the periplasm' as a means of coupling the proton motive force to outer membrane transport (Hannavy *et al.*, 1990). Consistent with this, it was previously demonstrated that point mutations in the TonB boxes of specific iron-regulated outer membrane proteins and BtuB could suppress point mutations in the *tonB* gene which otherwise inactivated TonB (Gudmundsdottir *et al.*, 1989) and vice versa (Bell *et al.*, 1990).

Recently evidence was presented that FepA might function as a gated porin, in which TonB would serve in a gatekeeper role (Rutz *et al.*, 1992). Thus deletion of those cell surface loops that were involved in binding of ferric enterobactin resulted in mutant proteins that were incapable of high affinity uptake. Instead these mutant FepA derivatives formed non-specific diffusion channels indicating that the parent channel might be a gated channel.

FepA, FhuA and the related TonB-dependent outer membrane vitamin B_{12} translocator BtuB, have been subjected to genetic manipulations designed at mapping the transmembrane and surface topology of these proteins (Maier and Bremer, 1988; Murphy and Klebba, 1989; Armstrong *et al.*, 1990; Carmel *et al.*, 1990; Murphy *et al.*, 1990; Koebnik and Braun, 1993). Each protein has been confirmed as having similarities to the two-dimensional maps of the porins with antiparallel β-sheet regions

separated by loop regions. However, they are proposed to contain 29–32 membrane-spanning β-chains (Murphy *et al.*, 1990; Koebnik and Braun, 1993). Ligand, monoclonal antibody and bacteriocin binding domains have been localized on the two-dimensional maps.

Detailed studies have been performed on the events that occur subsequent to translocation across the outer membrane (see Postle, 1990), but are not within the scope of this chapter. However it is worth noting that two proteins, ExbB and TolQ, have been proposed to potentially be involved in translocation of specific siderophore–iron complexes across the outer membrane (Postle, 1990).

Structural proteins

Deletion of both outer membrane proteins, OmpA and Lpp, resulted in cells that adopted a rounded morphology and were unable to grow in medium with low divalent cation concentrations (Sonntag *et al.*, 1978). This suggested a structural role for both sets of proteins.

Subsequently it was demonstrated that OmpA is a multifunctional outer membrane protein that serves as the parent of a family of proteins in other bacteria with related carboxy-terminal sequences (Gentry-Weeks *et al.*, 1992), one of which, *P. aeruginosa* OprF, has a similar structural role to that proposed for OmpA (Woodruff and Hancock, 1989). Interestingly, studies with deletion variants of OprF have indicated that the sequences required to complement the structural consequences of the lack of OprF in *P. aeruginosa* OprF:Ω mutants, reside within the OmpA homologous carboxy-terminal region (E. Rawling and R.E.W. Hancock, unpublished). The way in which OmpA serves such a structural role in shape determination is unknown, but apparently does not involve a loss of the other known shape-determining protein of *E. coli*, namely penicillin-binding protein 2 (Sonntag *et al.*, 1978). Nevertheless, since the peptidoglycan is involved in shape maintenance, it is possible that the tight association of OmpA with the underlying peptidoglycan (Endermann *et al.*, 1978) may be of some importance. In addition to this structural role, OmpA serves as a porin (Sugawara and Nikaido, 1992; Saint *et al.*, 1993), as a phage receptor (Skurray *et al.*, 1974), is involved in stabilization of mating aggregates between F-plasmid containing donors and plasmidless recipients (Skurray *et al.*, 1974; Ried and Henning, 1987), and plays a role in pathogenesis (see below).

The N-terminal portion of OmpA is arranged as a porin-like sequence of eight antiparallel β-strands separated by small periplasmic or larger external loop regions. However the proposed periplasmic localization of the carboxy-terminal region of OmpA (Morona *et al.*, 1985) should be re-examined based on its high content of β-sheet and the demonstration that the homologous region of OprF contains at least four surface-localized

epitopes (Finnen *et al.*, 1992), whereas the homologous region of *Neisseria* protein PIII is partly involved in the serum blocking activity of this protein (Virji and Heckels, 1989), a function shared by OmpA (see below).

The other protein involved in cell structure is Lpp, otherwise known as the Braun lipoprotein. Lpp is a 58 amino acid protein containing three covalently bound fatty acids at the N-terminal cysteine. It is present in *E. coli* in high copy number (7×10^5 copies per cell) with one-third of Lpp molecules being covalently linked by the ε-amino group of its C-terminal lysine to the carboxyl group of diaminopimelate of the peptidoglycan; the other two thirds are not covalently linked to the peptidoglycan (Inouye *et al.*, 1972; Braun, 1975). Both forms of Lpp are largely organized in alpha-helices (Braun *et al.*, 1976b) and are probably associated with the outer membrane primarily via insertion of the fatty acids into the inner monolayer of the outer membrane. Loss of lipoprotein causes cells to become leaky (Suzuki *et al.*, 1978), consistent with the structural role mentioned above, in *lpp ompA* double mutants (Sonntag *et al.*, 1978). Again the association of lipoprotein with the peptidoglycan seems to be of importance in this structural role, and it seems likely that the covalently bound form is most important in this regard (Fung *et al.*, 1978; Woodruff and Hancock, 1989). Another small lipoprotein found in the *E. coli* outer membrane is OsmB (Jung *et al.*, 1989). Although osmotically inducible, its precise function is at present unknown.

In addition to these two structural proteins, *E. coli* also contains an outer membrane protein, the peptidoglycan associated lipoprotein (PAL), which is part of a family of related higher-molecular-weight lipoproteins that are non-covalently peptidoglycan associated (Mizuno, 1979; Chen and Henning, 1987). Mutants (*exc*C) lacking this protein demonstrate leakage of several periplasmic enzymes and increased sensitivity to deoxycholate and other compounds (Lazzaroni and Portalier, 1992). Use of Tn*phoA* fusion techniques demonstrated that the N-terminal portion is associated with the outer membrane, whereas the carboxy-terminal portion is necessary for interaction with the peptidoglycan. Of great interest is the recent observation that the carboxy terminus of PAL can be partially aligned with that of OmpA (Gentry-Weeks *et al.*, 1992) (Fig. 18.3).

A protein previously termed histone-like protein I (HLP-I) was found to have 91% identical amino acids to the OmpH outer membrane protein of *S. typhimurium*, suggesting it may be an outer membrane protein (Hirvas *et al.*, 1990). Its high basicity (pI > 10) indicates a potential structural role in binding to negatively charged LPS. The protein, termed Protein III by Hindennach and Henning (1975), may possibly be OmpH, but confirmation of this and its possible function awaits the isolation of mutants lacking OmpH.

```
OmpA    apkdntwytgaklgwsqyhdtgfinnngpthenqlgagafggyqvnpyvgfemgydwlgr   60
OprF    qqqnsveieafgkryftdsvrnmknadlyggsigyfltddvelalsygeyhdvrgtyetg   60
PAL     cssnknasndgsegmlgagtgmdanggngnmsseeqar--------------------     38
PIII    geasvqgytvsgqsneivrnnygecwknayfdkasqgrvecgdavavpepepapvavveq   60

OmpA    mpykgsvengaykaqgvqltaklgypitddldiytrlggmvwradtksnvygknhdtgvs   120
OprF    nkkvhgnltsldaiyhfgtpgvglrpyvsaglahqnitninsdsqgrqqmtmanigaglk   120
PAL     ------------------------------------------------------------    38
PIII    apq-------------------------------------------------------     63

OmpA    pvfaggveyaitpeiatrleyqwtnnigdahtigtrpdngmlslgvsyrfgqgeaapvva   180
OprF    yyftenffakasldgqyglekrdnghqgewmaglgvgfnfggskaapapepvadvcsdsd   180
PAL     ------------------------------------------------------------    38
PIII    ------------------------------------------------------------    63

OmpA    papapape----------------VQTKHFTLKSDVLFNFNKATLKPEGQAALDQLYS   222
OprF    ndgvcdnvdkcpdtpanvtvdangcpAVAEVVRVQLDVKFDFDKSKVKENSYADIKNLAD   240
PAL     -------------------------LQMQQLQQNNIVYFDLDKYDIRSDFAQMLDAHAN    72
PIII    -------------------------YVDETISLSAKTLFGFDKDSLRAEAQDNLKVLAQ    97

OmpA    QLSnldpkdgSVVVLGYTDRIGSDAYNQGLSERRAQSVVDYLISK-GIPADKISARGMGE   281
OprF    FMKqypst--STTVEGHTDSVGTDAYNQKLSERRANAVRDVLVNEyGVEGGRVNAVGYGE   298
PAL     FLRsnpsy--KVTVEGHADERGTPEYNISLGERRANAVKMYLQGK-GVSADQISIVSYGK   129
PIII    RLSrtnvq--SVRVEGHTDFMGSEKYNQALSERRAYVVANNLVSN-GVPASRISAVGLGE   154

OmpA    SNPVTGNTcdnvkqraalidclapdrrveievkgikdvvtqpqa----------------   325
OprF    SRPVADNAtaegrainrrveaeveaeak------------------------------   326
PAL     EKPAVLGHdeaaysknrravlvy-------------------------------------   152
PIII    SQAQMTQVcqaevaklgakaskakkrealiaciepdrrvdvkirsivtrqvvparnhhqh   214
```

Fig. 18.3. Sequence alignment of OmpA, PIII, OprF and PAL amino acid sequences. The signal sequence has been removed to simplify the alignment. Upper case letters represent blocks of homology as defined by the program MACAW, version 1.06 (Schuler *et al.*, 1991).

Enzymes

Only two well-characterized enzymes (PldA and OmpT) are known to be associated with the outer membrane of *E. coli*. PldA is the so-called detergent-resistant phospholipase A of *E. coli* (Homma *et al.*, 1984). OmpT is identical to protease VII from *E. coli* (Grodberg *et al.*, 1988; Sugimura, 1988), and is well known for its role in degradation of secreted recombinant proteins (Baneyx and Georgiou, 1990). However, neither enzyme has been definitively identified as having a physiological function.

Appendages

Most *E. coli* cells contain flagella which are involved in directed movement (chemotaxis) towards attractant chemicals (McNab, 1987a, b). The structural protein of flagella, flagellin (FliC), may often be observed in outer membranes as a doublet of approximately 52,000 molecular weight, although strictly speaking it is not an outer membrane protein. The flagellin traverses the outer membrane through the outer membrane ring protein, FlaY, which has been proposed to form a tight greaseless bearing that permits flagella rotation without disrupting the outer membrane (McNab, 1987a, b).

About 70% of wild-type *E. coli* strains contain type 1 or common fimbriae that mediate mannose-inhibitable binding to eukaryotic cells (Pallesen *et al.*, 1989). The structural subunit of type 1 fimbriae, FimA, is apparently assembled by the outer membrane FimD protein (Klemm, 1985). FimD has general homology to the PapC protein which is part of the operon for expression of P (pyelonephritis associated) pili and to FaeD and FanD which are involved in expression of K88 and K99 pili, respectively (Krogfelt, 1991). The best studied of these proteins, PapC, has been proposed to function as an outer membrane usher that releases the pilin structural subunit from the PapD protein (the PapD protein serves to 'chaperone' the pilin subunit across the periplasm), and then orchestrates an ordered progression of pilin subunits into the growing pilus (Jones *et al.*, 1992). Another outer membrane protein, PapH, which is associated with the synthesis of P fimbriae, has been proposed to serve as an outer membrane anchor for the pilus. Given the general homology of the pilus expression systems in *E. coli*, we assume that analogous proteins are present for other pili. While the type 1 fimbriae are by far the most common, other chromosomally encoded pili operons have been found in *E. coli* (including the P-pili mentioned above) and as many as three separate operons have been demonstrated in a single uropathogenic *E. coli* strain (Rhen *et al.*, 1983).

Many *E. coli* contain the F plasmid. This plasmid encodes as many as eight different Tra proteins which reside in the outer membrane and have general functions in pilus assembly or surface exclusion (Willetts and Skurray, 1987).

Protein export

There are few proteins that are exported across the outer membrane of *E. coli*, other than the appendage proteins discussed above. In the case of fimbriae, there are specific proteins involved in the translocation across the outer membrane and assembly of these fimbriae as discussed above. However the haemolysin of *E. coli* is secreted from cells by an independent pathway. In this case, it is thought that the minor outer membrane protein TolC is involved in secretion in some as-yet undetermined fashion (Wandersman and Delepelaire, 1990). Interestingly, recent data has suggested that TolC may be a peptide-specific porin (R. Benz, unpublished results).

Role of Outer Membrane Proteins in Pathogenesis

The functions served by outer membrane proteins during growth of cells *in vitro* may also be of some importance during growth in a host. For example the regulation and pore functions of porins play a role in antibiotic susceptibility *in vivo*, the export of pilin and flagellin is important in the adhesive and motility functions of these appendages, and the excretion of haemolysin provides *E. coli* with its only excreted virulence factor. In addition to these general functions, there is a variety of functions more specifically associated with pathogenesis.

Attachment and effacement

In most cases, the adherence of *E. coli* to eukaryotic cells is mediated by a minor protein subunit that is attached as part of a complex to the tip of pili (Chapter 16). For example, the adhesin for type 1 pili is actually the FimH protein (Krogfelt *et al.*, 1990), whereas the adhesin for P fimbriae is the PapG protein (Jones *et al.*, 1992). In the latter case, it has been demonstrated that transposon mutants lacking the actual fimbrial subunit PapA, can still adhere (Jones *et al.*, 1992). Under such circumstances, the PapG adhesin subunit would be in close juxtaposition to the outer membrane, and it would be difficult to prove definitively whether such an adhesin were an outer membrane protein or not.

There is strong evidence that a specific outer membrane protein is involved in a process known as attachment and effacement (Chapter 20). This process involves a 94-kDa outer membrane protein (Eae or intimin), which is the product of the chromosomal *eaeA* gene, and a plasmid-encoded regulator called Per (Jerse and Kaper, 1991). The Eae protein may be involved in the more intimate adherence of *E. coli* with epithelial cells that causes cytoskeletal rearrangements but it is uncertain whether this protein is an actual adhesin, or whether it acts indirectly in this process. The *eaeA* gene has been cloned and sequenced from both enteropathogenic and enterohaemorrhagic *E. coli* and has substantial homology to *Yersinia* proteins called invasins that are involved in invasion (Isberg *et al.*, 1987). Interestingly, an outer membrane protein of similar molecular weight has been proposed to function in epithelial cell invasion by enterotoxigenic *E. coli* (Elsinghorst, 1992), suggesting quite broad distribution of this outer membrane function in pathogens.

Serum resistance

E. coli cells harbouring F plasmid or F-like plasmids are more resistant to the bactericidal action of serum. One of these F-like plasmids, Col V, is strongly associated with virulence (Binns *et al.*, 1979). This serum

(complement) resistance is mediated by a plasmid-encoded protein TraT (Moll *et al.*, 1980), an oligomeric lipoprotein that is exposed on the surface of the outer membrane (Sukupolvi and O'Connor, 1990). Colony hybridization studies using gene probes have shown that as many as 70% of strains from patients with bacteraemia, septicaemia, or enteric infections contain a homologue of TraT, whereas only 20–40% of *E. coli* strains from normal faeces gave positive hybridization signals (Montenegro *et al.*, 1985). The natural function of TraT in F-plasmid biology is to mediate surface exclusion, which inhibits DNA transfer from cells harbouring a closely related plasmid (Harrison *et al.*, 1992). This property is independent of serum resistance per se, since F-like plasmids fall into surface exclusion specificity groups that appear to depend on differences in a 5-amino acid region of TraT (Harrison *et al.*, 1992).

The molecular mechanism whereby TraT mediates resistance to serum is unclear, although it has been suggested that TraT influences the correct functioning or assembly of the membrane attack complex (components C5–9) (Moll *et al.*, 1980; Ogata *et al.*, 1982). One possible mechanism could be the blocking of specific LPS divalent cation binding sites which have been proposed to be involved in complement insertion into outer membranes (Hancock, 1984). Alternatively, TraT could support the role of OmpA in serum resistance (see below), since Riede and Eschbach (1986) have demonstrated that TraT is capable of interacting with OmpA.

Recently, Weiser and Gotschlich (1991) demonstrated that OmpA-deficient mutants of an *E. coli* K-1 strain demonstrated a substantially increased susceptibility to killing via the classical pathway of complement killing. Coincident with this, the OmpA-deficient strain demonstrated a significantly reduced ability to cause chick embryo lethality and bacteraemia in neonatal rats. Thus OmpA has a clear role in serum resistance. Interestingly, OmpA shows substantial carboxy-terminal sequence homology to protein pIII from *Neisseria gonorrhoeae* which mediates resistance to complement-mediated killing (Virji and Heckels, 1989). Indeed protein pIII has been termed the serum-blocking protein since specific IgG antibodies to pIII block the bactericidal action of even immune serum (Rice *et al.*, 1986). Preliminary data suggest this is also true for OmpA (Weiser *et al.*, 1992).

In vivo *expression/antigenicity*

Many studies have examined heterogeneity in the SDS-PAGE profiles of *E. coli* isolates from normal faeces (Hofstra and Dankert, 1980; Jann and Jann, 1980), neonatal meningitis (van Alphen *et al.*, 1983), urinary tract (Jann and Jann, 1980; Achtman *et al.*, 1983, 1986), intestinal disease (Jann and Jann, 1980; Chart *et al.*, 1988) and septicaemia (Kapur *et al.*, 1992). In every case substantial variability was observed in the apparent molecular

weights and amounts of the major outer membrane proteins. This was evident for outer membrane proteins, identified by immunological methods or from the influence of solubilization temperature on the electrophoretic mobilities, including OmpA, the porins (Hofstra and Dankert, 1980; van Alphen *et al.*, 1983) and the iron-regulated outer membrane proteins (Chart *et al.*, 1988). Although this has been suggested to indicate a clonal relationship for similar isolates (Achtman *et al.*, 1983), no unique relationship between such clonal groups and virulence or disease specificity has been identified. In addition there is no obvious utility of clonal subgrouping by outer membrane protein patterns, in direct contrast to results for other bacteria.

E. coli have been isolated directly from animal models (Griffiths *et al.*, 1983; Sciortino and Finkelstein, 1983) and from infected human urine (Lam *et al.*, 1984; Robledo *et al.*, 1990) and characterized without subculturing. With one major exception, their outer membrane profiles demonstrate a similar complement of proteins to those observed in cells grown *in vitro* in rich broth. Iron-regulated outer membrane proteins are highly induced, indicating that bacteria grow *in vivo* under iron-deficient conditions, at least when growing at the densities required to permit subsequent analysis. Another consequence of *in vivo* growth is apparently down-regulation of OmpF, as indicated by the phenotype of a β-lactam-resistant isolate obtained from an infected patient (Medeiros *et al.*, 1987) and by the outer membrane protein profiles of bacteria obtained directly from the urine of bacteriuric patients (Robledo *et al.*, 1990).

The expression of outer membrane proteins *in vivo* is also revealed by the immune response to surface antigens. Taplits and Michael (1979) suggested that the immune response to the surface proteins of *E. coli* B, a rough non-encapsulated bacterium, was dominant. Proteins recognized include OmpA (Puohiniemi *et al.*, 1990), the porins, Pal and Lpp (Nicolle *et al.*, 1988; Henriksen and Maeland, 1990). Extensive investigations have demonstrated strong conservation of most outer membrane protein epitopes, including those of OmpF, OmpC (Hofstra and Dankert, 1980; Bentley and Klebba, 1988), PhoE (van der Ley *et al.*, 1986a), Pal, Lpp (Henriksen *et al.*, 1989), FepA (Rutz *et al.*, 1991), OmpA (Hofstra and Dankert, 1980; Overbeeke and Lugtenberg, 1980), TraT (Bitter-Suermann *et al.*, 1984), Fiu and Cir (Chart *et al.*, 1988). Nevertheless, with few exceptions, outer membrane proteins usually make poor vaccines (Bolin and Jensen, 1987; Vuopio-Varkila *et al.*, 1988). Moreover, it appears that antigenic epitopes on the surface of outer membrane proteins are weakly or not-at-all accessible to antibodies (Hofstra *et al.*, 1979; van der Ley *et al.*, 1986b; Gómez-Miguel *et al.*, 1987; Bentley and Klebba, 1988), high-molecular-weight substrates (Ferenci and Lee, 1986) and phages (van der Ley *et al.*, 1986a) due to shielding by LPS O-side-chains, and furthermore,

the most surface-exposed epitopes have undergone the greatest antigenic variation (Pagès *et al.*, 1988; Rutz *et al.*, 1991).

Endotoxin-associated proteins

Almost all outer membrane proteins, when extracted, remain non-covalently associated with LPS (e.g. Yamada and Mizushima, 1980) and this interaction appears to be tight and specific (Datta *et al.*, 1977; Schweizer *et al.*, 1978; Parr *et al.*, 1986). Lipopolysaccharide isolated by various techniques (Strittmatter and Galanos, 1987) often contains outer membrane proteins. Likewise LPS (and its Lipid A portion, endotoxin) may be released together with proteins *in vivo* (Leive *et al.*, 1968). Although this association can modulate the effects of endotoxin, it is still uncertain as to whether it is physiologically important.

Other roles

A variety of other roles in pathogenesis have been ascribed to outer membrane proteins, based largely on *in vitro* assays. However it is not known whether these functions have any *in vivo* significance. For example, reactive arthritis is caused, in patients with the HLA-B27 type, by a variety of Gram-negative bacteria, but not *E. coli*. This is thought to be due to molecular mimicry in which a bacterial surface antigen induces self antibodies directed against HLA-B27. It is of interest that antisera specific for HLA-B27 reacts with two *E. coli* outer membrane proteins of apparent molecular weights 35,000 and 23,000. The former is OmpA (Zhang *et al.*, 1989) and the specific epitopes have been shown to comprise two peptide regions, both of which contain two consecutive arginine residues (Yu *et al.*, 1991).

Outer membrane proteins are capable of interacting directly with B lymphocytes as judged by their ability to stimulate B cell replication (mitogenicity). *E. coli* proteins that have been ascribed this function are OmpF, OmpA (Bessler and Henning, 1979), Lpp (Melchers *et al.*, 1975) and endotoxin protein (Sultzer and Goodman, 1976). Evidence has also been presented that porins can specifically bind Clq and activate antibody-independent killing via the classical complement pathway (Loos and Clas, 1987).

Use as vaccines and carriers of epitopes

As discussed above, outer membrane proteins have demonstrated limited promise as vaccines against heterologous bacteria. However a variety of *E. coli* outer membrane proteins including LamB, TraT, PhoE and OmpA,

have been shown to be able to accept extra amino acids without compromising expression or correct assembly into the outer membrane (see Hofnung, 1991 for review). Comparisons of the PhoE sites accepting extra amino acids (which are introduced as oligonucleotides into the corresponding location in the gene) with the three-dimensional structure have demonstrated that it is the surface loops connecting adjacent antiparallel β-strands which exclusively accept insertion of epitopes (Cowan *et al.*, 1992). A wide variety of foreign epitopes have been expressed in outer membrane proteins and, when the resultant recombinant antigens are used as vaccines, specific protective antibodies can be elicited in animals (Hofnung, 1991).

Future Perspectives

We are approaching a time when every single outer membrane protein in *E. coli* will have been characterized. For example, more than 25% of the *E. coli* K-12 genome has been sequenced (Médigue *et al.*, 1991). However while we now understand how many individual outer membrane proteins function, we still have an inadequate understanding of the interactions between components of the outer membrane, and the way outer membranes are functionally integrated with other cell compartments. It is in this area that we expect the majority of work will concentrate in the next decade.

Acknowledgements

The financial assistance of the Medical Research Council of Canada and Canadian Bacterial Diseases Network in the author's own research is gratefully acknowledged.

References

Achtman, M., Mercer, A., Kusecek, B., Pohl, B., Heuzenroeder, M., Aaronson, W., Sutton, A. and Silver, R.P. (1983) Six widespread bacterial clones among *Escherichia coli* K1 isolates. *Infection and Immunity* 39, 315–335.

Achtman, M., Heuzenroeder, M., Kusecek, B., Ochman, H., Cougant, D., Väisanen-Rhen, V., Korhonen, T., Stuart, S., Ørskov, F. and Ørskov, I. (1986) Clonal analysis of *Escherichia coli* O2:K1 isolated from diseased humans and animals. *Infection and Immunity* 51, 268–276.

Andersen, J., Forst, S.A., Zhao, K., Inouye, M. and Delihas, N. (1989) The function of *micF* RNA. *Journal of Biological Chemistry* 264, 17961–17970.

Armstrong, S.K., Francis, C.L. and McIntosh, M.A. (1990) Molecular analysis of the *Escherichia coli* ferric enterobactin receptor FepA. *Journal of Biological Chemistry* 265, 14536–14543.

Avrameas, S. (1969) Indirect immunoenzyme techniques for the intracellular detection of antigens. *Immunochemistry* 6, 825–831.

Bagg, A. and Neilands, J.B. (1987a) Ferric uptake regulation protein acts as a repressor, employing iron(II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. *Biochemistry* 26, 5471–5477.

Bagg, A. and Neilands, J.B. (1987b) Molecular mechanism of regulation of siderophore-mediated iron assimilation. *Microbiological Reviews* 51, 509–518.

Baneyx, F. and Georgiou, G. (1990) *In vivo* degradation of secreted fusion proteins by the *Escherichia coli* outer membrane protease OmpT. *Journal of Bacteriology* 172, 491–494.

Bauer, K., Struyvé, M., Bosch, D., Benz, R. and Tommassen, J. (1989) One single lysine residue is responsible for the special interaction between polyphosphate and the outer membrane porin PhoE of *Escherichia coli*. *Journal of Biological Chemistry* 264, 16393–16398.

Bell, P.E., Nau, C.D., Brown, J.T., Konisky, J. and Kadner, R.J. (1990) Genetic suppression demonstrates interaction of TonB protein with outer membrane transport proteins in *Escherichia coli*. *Journal of Bacteriology* 172, 3826–3829.

Bentley, A.T. and Klebba, P.E. (1988) Effect of lipopolysaccharide structure on reactivity of antiporin monoclonal antibodies with the bacterial cell surface. *Journal of Bacteriology* 170, 1063–1068.

Benz, R., Schmid, A. and Hancock, R.E.W. (1985) Ion selectivity of Gram-negative bacterial porins. *Journal of Bacteriology* 162, 722–727.

Benz, R., Schmid, A. and Vos-Scheperkeuter, G.H. (1987) Mechanism of sugar transport through the sugar-specific LamB channel of *Escherichia coli* outer membrane. *Journal of Membrane Biology* 100, 21–29.

Benz, R., Schmid, A., Maier, C. and Bremer, E. (1988) Characterization of the nucleoside-binding site inside the Tsx channel of *Escherichia coli* outer membrane. *European Journal of Biochemistry* 176, 699–705.

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

Bessler, W. and Henning, U. (1979) Protein I and protein II from the outer membrane of *Escherichia coli* are mouse B-lymphocyte mitogens. *Zeitschrift für Immunitätsforschung* 155, 398.

Binns, M.M., Davies, D.L. and Hardy, K.G. (1979) Cloned fragments of the plasmid ColV, I-K94 specifying virulence and serum resistance. *Nature (London)* 279, 778–781.

Birch, R.G., Pemberton, J.M. and Basnayake, W.V.S. (1990) Stable albicidin resistance in *Escherichia coli* involves an altered outer-membrane nucleoside uptake system. *Journal of General Microbiology* 136, 51–58.

Bitter-Suermann, D., Peters, H., Jürs, M., Nehrbass, R., Montenegro, M. and Timmis, K. (1984) Monoclonal antibody detection of IncF group plasmid-encoded TraT protein in clinical isolates of *Escherichia coli*. *Infection and Immunity* 46, 308–313.

Black, P.N. (1991) Primary sequence of the *Escherichia coli fadL* gene encoding

an outer membrane protein required for long-chain fatty acid transport. *Journal of Bacteriology* 173, 435–442.

Black, P.N., Said, B., Ghosn, C.R., Beach, J.V. and Nunn, W.D. (1987) Purification and characterization of an outer membrane-bound protein involved in long-chain fatty acid transport in *Escherichia coli*. *Journal of Biological Chemistry* 262, 1412–1419.

Blasband, A.J., Marcotte Jr, W.R. and Schnaitman, C.A. (1986) Structure of the *lc* and *nmpC* outer membrane porin protein genes of lambdoid bacteriophage. *Journal of Biological Chemistry* 261, 12723–12732.

Bolin, C.A. and Jensen, A.E. (1987) Passive immunization with antibodies against iron-regulated outer membrane proteins protects turkeys from *Escherichia coli* septicemia. *Infection and Immunity* 55, 1239–1242.

Braun, V. (1975) Covalent lipoprotein from the outer membrane of *Escherichia coli*. *Biochimica et Biophysica Acta* 415, 335–377.

Braun, V., Hancock, R.E.W., Hantke, K. and Hartmann, A. (1976a) Functional organization of the outer membrane of *Escherichia coli*: phage and colicin receptors as components of iron uptake systems. *Journal of Supramolecular Structure* 5, 37–58.

Braun, V., Rotering, H., Ohms, J.-P. and Hagenmeier, H. (1976b) Conformational studies on murien-lipoprotein from the outer membrane of *Escherichia coli*. *European Journal of Biochemistry* 70, 601–610.

Bremer, E., Middendorf, A., Martinussen, J. and Valentin-Hansen, P. (1990) Analysis of the *tsx* gene, which encodes a nucleoside-specific channel-forming protein (Tsx) in the outer membrane of *Escherichia coli*. *Gene* 96, 59–65.

Buechner, M., Delcour, A.H., Martinac, B., Adler, J. and Kung, C. (1990) Ion channel activities in the *Escherichia coli* outer membrane. *Biochimica et Biophysica Acta* 1024, 111–121.

Carmel, G., Hellstern, D., Henning, D. and Coulton, J.W. (1990) Insertion mutagenesis of the gene encoding the ferrichrome-iron receptor of *Escherichia coli* K-12. *Journal of Bacteriology* 172, 1861–1869.

Chan, W.C. and Ferenci, T. (1993) Combinatorial mutagenesis of the *lamB* gene: residues 41 through 43, which are conserved in *Escherichia coli* outer membrane proteins, are informationally important in maltoporin structure and function. *Journal of Bacteriology* 175, 858–865.

Chart, H., Stevenson, P. and Griffiths, E. (1988) Iron-regulated outer membrane proteins of *Escherichia coli* strains associated with enteric or extraintestinal diseases of man and animals. *Journal of General Microbiology* 134, 1549–1559.

Chen, R. and Henning, U. (1987) Nucleotide sequence of the gene for the peptidoglycan-associated lipoprotein of *Escherichia coli* K12. *European Journal of Biochemistry* 163, 73–77.

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

Critchley, I.A., Basker, M.J., Edmondson, R.A. and Knott, S.J. (1991) Uptake of a catecholic cephalosporin by the iron transport system of *Escherichia coli*. *Journal of Antimicrobial Chemotherapy (London)* 28, 377–388.

Cronan, J.E. (1979) Phospholipid synthesis and assembly. In: Inouye, M. (ed.) *Bacterial Outer Membranes*. John Wiley & Sons, New York, pp. 35–65.

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 *Escherichia coli* K-12 and mechanism of action of catechol-substituted cephalosporins. *Antimicrobial Agents and Chemotherapy* 32, 1879–1886.

Dargent, B., Hofmann, W., Pattus, F. and Rosenbusch, J.P. (1986) The selectivity filter of voltage-dependent channels formed by phosphoporin (PhoE protein) from *Escherichia coli*. *European Molecular Biology Organization Journal* 5, 773–778.

Dargent, B., Charbit, A., Hofnung, M. and Pattus, F. (1988) Effect of point mutations on the *in-vitro* pore properties of maltoporin, a protein of *Escherichia coli* outer membrane. *Journal of Molecular Biology* 201, 497–506.

Darveau, R.P., Hancock, R.E.W. and Benz, R. (1984) Chemical modification of the anion selectivity of the PhoE porin from the *E. coli* outer membrane. *Biochimica et Biophysica Acta* 774, 69–74.

Datta, D.B., Arden, B. and Henning, U. (1977) Major proteins of the *Escherichia coli* outer cell envelope membrane as bacteriophage receptors. *Journal of Bacteriology* 131, 821–829.

de Lorenzo, V., Bindereif, A., Paw, B.H. and Neilands, J.B. (1986) Aerobactin biosynthesis and transport genes of plasmid ColV-K30 in *Escherichia coli* K-12. *Journal of Bacteriology* 165, 570–578.

Elsinghorst, E.A. (1992) Epithelial cell invasion by the enterotoxigenic *E. coli tib* locus is associated with a 118 kDa outer membrane protein. In: *Abstracts of the 92nd Annual Meeting of the American Society for Microbiology, 1992*. American Society for Microbiology, Washington, DC, Abstr. B-181, p. 56.

Endermann, R., Krämer, C. and Henning, U. (1978) Major outer membrane proteins of *Escherichia coli* K-12: evidence for protein II being a transmembrane protein. *Federation of European Biochemical Societies Letters* 86, 21–24.

Ferenci, T. and Lee, K.-S. (1986) Exclusion of high-molecular-weight maltosaccharides by lipopolysaccharide O-antigen of *Escherichia coli* and *Salmonella typhimurium*. *Journal of Bacteriology* 167, 1081–1082.

Finnen, R.L., Martin, N.L., Seihnel, R.J., Woodruff, W.A., Rosok, M. and Hancock, R.E.W. (1992) Analysis of the *Pseudomonas aeruginosa* major outer membrane protein OprF by use of truncated OprF derivatives and monoclonal antibodies. *Journal of Bacteriology* 174, 4977–4985.

Francis, G., Brennan, L. and Ferenci, T. (1991a) Affinity-chromatographic purification of sixteen cysteine-substituted maltoporin variants: thiol reactivity and cross-linking in an outer membrane protein of *Escherichia coli*. *Biochimica et Biophysica Acta* 1067, 89–96.

Francis, G., Brennan, L., Stretton, S. and Ferenci, T. (1991b) Genetic mapping of starch- and Lambda-receptor sites in maltoporin: identification of substitutions causing direct and indirect effects on binding sites by cysteine mutagenesis. *Molecular Microbiology* 5, 2293–2301.

Funahara, Y. and Nikaido, H. (1980) Asymmetric localization of lipopolysaccharides on the outer membrane of *Salmonella typhimurium*. *Journal of Bacteriology* 141, 1463–1465.

Fung, J., MacAlister, T.J. and Rothfield, L.I. (1978) Role of murein lipoprotein in morphogenesis of the bacterial division septum: phenotypic similarity of

*iky*D and *lpo* mutants. *Journal of Bacteriology* 133, 1467–1471.

Gehring, K., Cheng, C.-H., Nikaido, H. and Jap, B.K. (1991) Stoichiometry of maltodextrin-binding sites in LamB, an outer membrane protein from *Escherichia coli*. *Journal of Bacteriology* 173, 1873–1878.

Gentry-Weeks, C.R., Hultsch, A.-L., Kelly, S.M., Keith, J.M. and Curtiss III, R. (1992) Cloning and sequencing of a gene encoding a 21-kilodalton outer membrane protein from *Bordetella avium* and expression of the gene in *Salmonella typhimurium*. *Journal of Bacteriology* 174, 7729–7742.

Gómez-Miguel, M.J., Moriyón, I. and López, J. (1987) *Brucella* outer membrane lipoprotein shares antigenic determinants with *Escherichia coli* Braun lipoprotein and is exposed on the cell surface. *Infection and Immunity* 55, 258–262.

Griffiths, E., Stevenson, P. and Joyce, P. (1983) Pathogenic *Escherichia coli* express new outer membrane proteins when grown *in vivo*. *Federation of European Microbiological Societies Microbiology Letters* 16, 95–99.

Grodberg, J., Lundrigan, M.D., Toledo, D.L., Mangel, W.F. and Dunn, J.J. (1988) Complete nucleotide sequence and deduced amino acid sequence of the *ompT* gene of *Escherichia coli* K-12. *Nucleic Acids Research* 16, 1209.

Gudmundsdottir, A., Bell, P.E., Lundrigan, M.D., Bradbeer, C. and Kadner, R.J. (1989) Point mutations in a conserved region (TonB box) of *Escherichia coli* outer membrane protein BtuB affect Vitamin B_{12} transport. *Journal of Bacteriology* 171, 6526–6533.

Hancock, R.E.W. (1984) Alterations in outer membrane permeability. *Annual Review of Microbiology* 38, 237–264.

Hancock, R.E.W. (1986) Model membrane studies of porin function. In: Inouye, M. (ed.) *Bacterial Outer Membranes as Model Systems*. John Wiley & Sons, New York, pp. 187–225.

Hancock, R.E.W. (1991) Bacterial outer membranes: evolving concepts. *American Society for Microbiology News* 57, 175–182.

Hancock, R.E.W. and Bell, A. (1989) Antibiotic uptake into Gram negative bacteria. In: Jackson, G.G., Schlumberger, H.D. and Zeiler, H.J. (eds) *Perspectives in Anti-Infective Therapy*. Vieweg and Sohn, Braunshcweig, pp. 21–28.

Hancock, R.E.W. and Braun, V. (1976) Nature of the energy requirement for the irreversible adsorption of bacteriophages T1 and d80 to *Escherichia coli* K-12. *Journal of Bacteriology* 12, 409–415.

Hancock, R.E.W., Hantke, K. and Braun, V. (1976) Iron transport in *Escherichia coli* K-12: involvement of the colicin B receptor and of a citrate-inducible protein. *Journal of Bacteriology* 127, 1370–1375.

Hancock, R.E.W., Egli, C., Benz, R. and Siehnel, R.J. (1992) Overexpression in *Escherichia coli* and functional analysis of a novel PP_i-selective porin, OprO, from *Pseudomonas aeruginosa*. *Journal of Bacteriology* 174, 471–476.

Hancock, R.E.W., Karunaratne, D.N. and Bernegger-Egli, C. (1993) Molecular organization and structural role of outer membrane macromolecules. In: Ghuysen, J.-M. and Hakenbach, R. (eds) *Bacterial Cell Wall*. Elsevier Science Publishers, Amsterdam (in press).

Hannavy, K., Barr, G.C., Dorman, C.J., Adamson, J., Mazengera, L.R., Gallagher, M.P., Evans, J.S., Levine, B.A., Trayer, I.P. and Higgins, C.F. (1990) TonB protein of *Salmonella typhimurium* – a model for signal transduction between membranes. *Journal of Molecular Biology* 216, 897–910.

Hantke, K. (1976) Phage T6-colicin K receptor and nucleoside transport in *Escherichia coli*. *Federation of European Biochemical Societies Letters* 70, 109–112.

Hantke, K. (1982) Negative control of iron uptake systems in *Escherichia coli*. *Federation of European Microbiological Societies Microbiology Letters* 15, 83–86.

Hardesty, C., Ferran, C. and DiRienzo, J.M. (1991) Plasmid-mediated sucrose metabolism in *Escherichia coli*: characterization of *scrY*, the structural gene for a phosphoenolpyruvate-dependent sucrose phosphotransferase system outer membrane porin. *Journal of Bacteriology* 173, 449–456.

Harrison, J.L., Taylor, I.M., Platt, K. and O'Connor, C.D. (1992) Surface exclusion specificity of the TraT lipoprotein is determined by single alterations in a five-amino-acid region of the protein. *Molecular Microbiology* 6, 2825–2832.

Heine, H.-G., Francis, G., Lee, K.-S. and Ferenci, T. (1988) Genetic analysis of sequences in maltoporin that contribute to binding domains and pore structure. *Journal of Bacteriology* 170, 1730–1738.

Henriksen, A.Z. and Maeland, J.A. (1990) Antibody response to defined domains on enterobacterial outer membrane proteins in healthy persons and patients with bacteraemia. *Acta Pathologica Microbiologica et Immunologica Scandinavica* 98, 163–172.

Henriksen, A.Z., Maeland, J.A. and Brakstad, O.G. (1989) Monoclonal antibodies against three different enterobacterial outer membrane proteins. Characterization, cross-reactivity, and binding to bacteria. *Acta Pathologica Microbiologica et Immunologica Scandinavica* 97, 559–568.

Hindennach, I. and Henning, U. (1975) The major proteins of the *Escherichia coli* outer cell envelope membrane. *European Journal of Biochemistry* 59, 207–213.

Hirvas, L., Coleman, J., Koshi, P.K. and Vaara, M. (1990) Bacterial 'histone-like protein I' (HLP-I) is an outer membrane constituent. *Federation of European Biochemical Societies* 262, 123–126.

Hofnung, M. (1991) Expression of foreign polypeptides at the *Escherichia coli* cell surface. *Methods in Cell Biology* 34, 77–105.

Hofstra, H. and Dankert, J. (1980) Major outer membrane proteins: common antigens in Enterobacteriaceae species. *Journal of General Microbiology* 119, 123–131.

Hofstra, H., Van Tol, M.J.D. and Dankert, J. (1979) Immunofluorescent detection of the major outer membrane protein II in *Escherichia coli* O26:K60. *Federation of European Microbiological Societies Microbiology Letters* 6, 147–150.

Homma, H., Kobayashi, T., Chiba, N., Karasawa, K., Mizushima, H., Kudo, I., Inoue, K., Ikeda, H., Sekiguchi, M. and Nojima, S. (1984) The DNA sequence encoding *pldA* gene, the structural gene for detergent-resistant phospholipase A of *Escherichia coli*. *Journal of Biochemistry* 96, 1655–1664.

Igo, M.M., Slauch, J.M. and Silhavy, T.J. (1990) Signal transduction in bacteria: kinases that control gene expression. *New Biologist* 2, 5–9.

Inouye, M. (ed.) (1987) *Bacterial Outer Membranes as Model Systems*. John Wiley & Sons, New York.

Inouye, M., Shaw, J. and Shen, C. (1972) The assembly of a structural lipoprotein in the envelope of *Escherichia coli*. *Journal of Biological Chemistry* 247, 8154–8159.

Isberg, R.R., Voorhis, D.L. and Falkow, S. (1987) Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells. *Cell* 50, 769–778.

Jann, B. and Jann, K. (1980) SDS polyacrylamide gel electrophoresis patterns of the outer membrane proteins from *E. coli* strains of different pathogenic origin. *Federation of European Microbiological Societies Microbiology Letters* 7, 19–22.

Jeanteur, D., Lakey, J.H. and Pattus, F. (1993) The porin superfamily: diversity and common features. In: Ghuysen, J.-M. and Hakenbach, R. (eds) *Bacterial Cell Wall*. Elsevier Science Publishers, Amsterdam (in press).

Jerse, A.E. and Kaper, J.B. (1991) The *eae* gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. *Infection and Immunity* 59, 4302–4309.

Jones, C.H., Jacob-Dubuisson, F., Kuehn, D.M., Slonim, L., Striker, R. and Hultgren, S.J. (1992) Adhesin presentation in bacteria requires molecular chaperones and ushers. *Infection and Immunity* 60, 4445–4451.

Jung, J.U., Gutierrez, C. and Villarejo, M.R. (1989) Sequence of an osmotically inducible lipoprotein gene. *Journal of Bacteriology* 171, 511–520.

Kadner, R.J. (1990) Vitamin B_{12} transport in *Escherichia coli*: energy coupling between membranes. *Molecular Microbiology* 4, 2027–2033.

Kapur, V., White, D.G., Wilson, R.A. and Whittam, T.S. (1992) Outer membrane protein patterns mark clones of *Escherichia coli* O2 and O78 strains that cause avian septicemia. *Infection and Immunity* 60, 1687–1691.

Killmann, H. and Braun, V. (1992) An aspartate deletion mutation defines a binding site of the multifunctional FhuA outer membrane receptor of *Escherichia coli* K-12. *Journal of Bacteriology* 174, 3479–3486.

Klemm, P. (1985) Fimbrial adhesins of *Escherichia coli*. *Reviews of Infectious Diseases* 7, 321–340.

Koebnik, R. and Braun, V. (1993) Insertion derivatives containing segments of up to 16 amino acids identify surface- and periplasm-exposed regions of the FhuA outer membrane receptor of *Escherichia coli* K-12. *Journal of Bacteriology* 175, 826–839.

Konisky, J. (1979) Specific transport systems and receptor for colicins and phages. In: Inouye, M. (ed.) *Bacterial Outer Membranes*. John Wiley & Sons, New York, pp. 319–359.

Krogfelt, K.A. (1991) Bacterial adhesion: genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of *Escherichia coli*. *Reviews of Infectious Diseases* 13, 721–735.

Krogfelt, K.A., Bergmans, H. and Klemm, P. (1990) Direct evidence that the FimH protein is the mannose-specific adhesin of *Escherichia coli* Type 1 fimbriae. *Infection and Immunity* 58, 1995–1998.

Lam, C., Turnowsky, F., Schwarzinger, E. and Neruda, W. (1984) Bacteria recovered without subculture from infected human urines expressed iron-

regulated outer membrane proteins. *Federation of European Microbiological Societies Microbiology Letters* 24, 255–259.

Lazzaroni, J.-C. and Portalier, R. (1992) The *excC* gene of *Escherichia coli* K-12 required for cell envelope integrity encodes the peptidoglycan-associated lipoprotein (PAL). *Molecular Microbiology* 6, 735–742.

Leive, L., Shovlin, V.K. and Mergenhagen, S.E. (1968) Physical, chemical, and immunological properties of lipopolysaccharide released from *Escherichia coli* by ethylenediaminetetraacetate. *Journal of Biological Chemistry* 243, 6384–6391.

Lepault, J., Dargent, B., Tichelaar, W., Rosenbusch, J.P., Leonard, K. and Pattus, F. (1988) Three-dimensional reconstruction of maltoporin from electron microscopy and image processing. *European Molecular Biology Organization Journal* 7, 261–268.

Li, Z.M., Hannah, J.H., Stibitz, S., Nguyen, N.Y., Manclark, C.R. and Brennan, M.J. (1991) Cloning and sequencing of the structural gene for the porin protein of *Bordetella pertussis*. *Molecular Microbiology* 5, 1649–1656.

Loos, M. and Clas, F. (1987) Antibody-independent killing of Gram-negative bacteria via the classical pathway of complement. *Immunology Letters* 14, 203–208.

Lugtenberg, B., Meijers, J., Peters, R. and van der Hoeck, P. (1975) Electrophoretic resolution of the major outer membrane protein of *Escherichia coli* K-12 into four bands. *Federation of European Biochemical Societies Letters* 58, 254–258.

Maier, C. and Bremer, E. (1988) Pore-forming activity of the Tsx protein from the outer membrane of *Escherichia coli*. *Journal of Biological Chemistry* 263, 2493–2499.

Mangroo, D. and Gerber, G.E. (1992) Photoaffinity labeling of fatty acid-binding proteins involved in long chain fatty acid transport in *Escherichia coli*. *Journal of Biological Chemistry* 267, 17095–17101.

McNab, R.M. (1987a) Flagella. In: Neidhardt, F.C., Ingraham, J.L., Low, K.B. Magasanik, B., Schaechter, M. and Umbarger, H.E. (eds) Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 70–83.

McNab, R.M. (1987b) Motility and chemotaxis. In: Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M. and Umbarger, H.E. (eds) Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 732–759.

Medeiros, A.A., O'Brien, T.F., Rosenberg, E.Y. and Nikaido, H. (1987) Loss of OmpC porin in a strain of *Salmonella typhimurium* causes increased resistance to cephalosporins during therapy. *Journal of Infectious Diseases* 156, 751–757.

Médigue, C., Viari, A., Hénaut, A. and Danchin, A. (1991) *Escherichia coli* molecular genetic map (1500 kbp): update II. *Molecular Microbiology* 5, 2629–2640.

Melchers, F., Braun, V. and Galanos, C. (1975) The lipoprotein of the outer membrane of *Escherichia coli*: a B-lymphocyte mitogen. *Journal of Experimental Medicine* 142, 473–482.

Misra, R. and Benson, S.A. (1989) A novel mutation, cog, which results in

production of a new porin protein (OmpG) of *Escherichia coli* K-12. *Journal of Bacteriology* 171, 4105–4111.

Mizuno, T. (1979) A novel peptidoglycan-associated lipoprotein found in the cell envelope of *Pseudomonas aeruginosa* and *Escherichia coli*. *Journal of Biochemistry* 86, 991–1000.

Mizuno, T. and Mizushima, S. (1990) Signal transduction and gene regulation through the phosphorylation of two regulatory components: the molecular basis for the osmotic regulation of the porin genes. *Molecular Microbiology* 4, 1077–1082.

Moll, A., Manning, P.A. and Timmis, K.N. (1980) Plasmid-determined resistance to serum bactericidal activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. *Infection and Immunity* 28, 359–367.

Montenegro, M.A., Bitter-Suermann, D., Timmis, J.K., Agero, M.E., Cabello, F.C., Sanyal, S.C. and Timmis, K.N. (1985) *traT* gene sequences, serum resistance and pathogenicity-related factors in clinical isolates of *Escherichia coli* and other Gram-negative bacteria. *Journal of General Microbiology* 131, 1511–1521.

Morona, R., Krämer, C. and Henning, U. (1985) Bacteriophage receptor area of outer membrane protein OmpA of *Escherichia coli* K-12. *Journal of Bacteriology* 164, 539–543.

Murphy, C.K. and Klebba, P.E. (1989) Export of FepA:PhoA fusion proteins to the outer membrane of *Escherichia coli* K-12. *Journal of Bacteriology* 171, 5894–5900.

Murphy, C.K., Kalve, V.I. and Klebba, P.E. (1990) Surface topology of the *Escherichia coli* K-12 ferric enterobactin receptor. *Journal of Bacteriology* 172, 2736–2746.

Nicolle, L.E., Ujak, E., Brunka, J. and Bryan, L.E. (1988) Immunoblot analysis of serologic response to outer membrane proteins of *Escherichia coli* in elderly individuals with urinary tract infections. *Journal of Clinical Microbiology* 26, 2087–2091.

Nikaido, H. (1992) Porins and specific channels of bacterial outer membranes. *Molecular Microbiology* 6, 435–442.

Nikaido, H. and Rosenberg, E.Y. (1983) Porin channels in *Escherichia coli*: studies with liposomes reconstituted from purified proteins. *Journal of Bacteriology* 153, 241–252.

Nikaido, H. and Rosenberg, E.Y. (1990) Cir and Fiu proteins in the outer membrane of *Escherichia coli* catalyze transport of monomeric catechols: study with β-lactam antibiotics containing catechol and analogous groups. *Journal of Bacteriology* 172, 1361–1367.

Nikaido, H. and Vaara, M. (1985) Molecular basis of bacterial outer membrane permeability. *Microbiological Reviews* 49, 1–32.

Ogata, R.T., Winters, C. and Levine, R.P. (1982) Nucleotide sequence analysis of the complement resistance gene from plasmid R100. *Journal of Bacteriology* 151, 819–827.

Overbeeke, N. and Lugtenberg, B. (1980) Major outer membrane proteins of *Escherichia coli* strains of human origin. *Journal of General Microbiology* 121, 373–380.

Overbeeke, N. and Lugtenberg, B. (1982) Recognition site for phosphorus-containing compounds and other negatively charged solutes on the PhoE protein pore of the outer membrane of *Escherichia coli* K12. *European Journal of Biochemistry* 126, 113–118.

Pagès, J.M., Pagès, C., Bernadac, A. and Prince, P. (1988) Immunological evidence for differences in the exposed regions of OmpF porins from *Escherichia coli* B and K-12. *Molecular Immunology* 25, 555–563.

Pallesen, L., Madsen, O. and Klemm, P. (1989) Regulation of the phase switch controlling expression of type 1 fimbriae in *Escherichia coli*. *Molecular Microbiology* 3, 925–931.

Parr, T.R., Poole, K., Crockford, G.W.K. and Hancock, R.E.W. (1986) Lipopolysaccharide-free *Escherichia coli* OmpF and *Pseudomonas aeruginosa* protein P porins are functionally active in lipid bilayer membranes. *Journal of Bacteriology* 165, 523–526.

Postle, K. (1990) TonB and the Gram-negative dilemma. *Molecular Microbiology* 4, 2019–2025.

Pressler, U., Staudenmaier, H., Zimmermann, L. and Braun, V. (1988) Genetics of the iron dicitrate transport system of *Escherichia coli*. *Journal of Bacteriology* 170, 2716–2724.

Pugsley, A.P. (1993) The complete general secretory pathway in Gram-negative bacteria. *Microbiological Reviews* 57, 50–108.

Puohiniemi, R., Karvonen, M., Vuopio-Varkila, J., Muotiala, A., Helander, I.M. and Sarvas, M. (1990) A strong antibody response to the periplasmic C-terminal domain of the OmpA protein of *Escherichia coli* is produced by immunization with purified OmpA or with whole *E. coli* or *Salmonella typhimurium* bacteria. *Infection and Immunity* 58, 1691–1696.

Rhen, M., Mäkelä, P.H. and Korhonen, T.K. (1983) P-fimbriae of *Escherichia coli* are subject to phase variation. *Federation of European Microbiological Societies Microbiology Letters* 19, 267–271.

Rice, P.A., Vayo, H.E., Tam, M.R. and Blake, M.S. (1986) Immunoglobulin G antibodies directed against protein III block killing of serum resistant *Neisseria gonorrhoeae* by immune sera. *Journal of Experimental Medicine* 164, 1735–1748.

Ried, G. and Henning, U. (1987) A unique amino acid substitution in the outer membrane protein OmpA causes conjugation deficiency in *Escherichia coli* K-12. *Federation of European Biochemical Societies Letters* 223, 387–390.

Riede, I. and Eschbach, M.-L. (1986) Evidence that TraT interacts with OmpA of *Escherichia coli*. *Federation of European Biochemical Societies Letters* 205, 241–245.

Robledo, J.A., Serrano, A. and Domingue, G.J. (1990) Outer membrane proteins of *E. coli* in the host–pathogen interaction in urinary tract infection. *Journal of Urology* 143, 386–391.

Rutz, J.M., Abdullah, T., Singh, S.P., Kalve, V.I. and Klebba, P.E. (1991) Evolution of the ferric enterobactin receptor in Gram-negative bacteria. *Journal of Bacteriology* 173, 5964–5974.

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 protein in the bacterial outer membrane. *Science* 258, 471–475.

Saint, N., De, E., Julien, S., Orange, N. and Molle, G. (1993) Ionophore properties of OmpA of *Escherichia coli*. *Biochimica et Biophysica Acta* 1145, 119–123.

Sauer, M., Hantke, K. and Braun, V. (1990) Sequence of the *fhuE* outer-membrane receptor gene of *Escherichia coli* K12 and properties of mutants. *Molecular Microbiology* 4, 427–437.

Saurin, W., Francoz, E., Martineau, P., Charbit, A., Dassa, E., Duplay, P., Gilson, E., Molla, A., Ronco, G., Szmelcman, S. and Hofnung, M. (1989) Periplasmic binding protein dependent transport system for maltose and maltodextrins: some recent studies. *Federation of European Microbiological Societies Microbiology Reviews* 63, 53–60.

Schmid, K., Ebner, R., Altenbuchner, J., Schmitt, R. and Lengeler, J.W. (1988) Plasmid-mediated sucrose metabolism in *Escherichia coli* K12: mapping of the *scr* genes of pUR400. *Molecular Microbiology* 2, 1–8.

Schöffler, H. and Braun, V. (1989) Transport across the outer membrane of *Escherichia coli* K12 via the FhuA receptor is regulated by the TonB protein of the cytoplasmic membrane. *Molecular and General Genetics* 217, 378–383.

Schülein, K., Schmid, K. and Benzl, R. (1991) The sugar-specific outer membrane channel ScrY contains functional characteristics of general diffusion pores and substrate-specific porins. *Molecular Microbiology* 5, 2233–2241.

Schuler, G.D., Altschul, S.F. and Lipman, D.J. (1991) A workbench for multiple alignment construction and analysis. *Proteins: Structure, Function, and Genetics* 9, 180–190.

Schweizer, M., Hindennach, I., Garten, W. and Henning, U. (1978) Major proteins of the *Escherichia coli* outer cell envelope membrane interaction of protein II with lipopolysaccharide. *European Journal of Biochemistry* 82, 211–217.

Sciortino, C.V. and Finkelstein, R.A. (1983) *Vibrio cholerae* expresses iron-regulated outer membrane proteins *in vivo*. *Infection and Immunity* 42, 990–996.

Skurray, R.A., Hancock, R.E.W. and Reeves, P. (1974) Con-mutants: class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein and defective in conjugation and adsorption of a bacteriophage. *Journal of Bacteriology* 119, 726–735.

Smelcman, S. and Hofnung, M. (1975) Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. *Journal of Bacteriology* 124, 112–118.

Sonntag, I., Schwarz, H., Hirota, Y. and Henning, U. (1978) Cell envelope and shape of *Escherichia coli*: multiple mutants missing the outer membrane lipoprotein and other major outer membrane proteins. *Journal of Bacteriology* 136, 280–285.

Strittmatter, W. and Galanos, C. (1987) Characterization of protein co-extracted together with LPS in *Escherichia coli*, *Salmonella minnesota* and *Yersinia enterocolitica*. *Microbial Pathogenesis* 2, 29–36.

Sugawara, E. and Nikaido, H. (1992) Pore-forming activity of OmpA protein of *Escherichia coli*. *Journal of Biological Chemistry* 267, 2507–2511.

Sugimura, K. (1988) Mutant isolation and cloning of the gene encoding protease VII from *Escherichia coli*. *Biochemical and Biophysical Research Communications* 153, 753–759.

Sukupolvi, S. and O'Connor, C.D. (1990) TraT lipoprotein, a plasmid-specified mediator of interactions between Gram-negative bacteria and their environment. *Microbiological Reviews* 54, 331–341.

Sultzer, B.M. and Goodman, G.W. (1976) Endotoxin protein: a B-cell mitogen and polyclonal activator of C3H/HeJ lymphocytes. *Journal of Experimental Medicine* 144, 821–827.

Sutcliffe, J., Blumenthal, R., Walter, A. and Foulds, J. (1983) *Escherichia coli* outer membrane protein K is a porin. *Journal of Bacteriology* 156, 867–872.

Suzuki, H., Nishimura, Y., Yasuda, S., Nishimura, M., Yamada, M. and Hirota, Y. (1978) Murein-lipoprotein of *Escherichia coli*: a protein involved in the stabilization of bacterial cell envelope. *Molecular and General Genetics* 167, 1–9.

Taplits, M. and Michael, J.G. (1979) Immune response to *Escherichia coli* B surface antigens. *Infection and Immunity* 25, 943–945.

Tommassen, J., Koster, M. and Overduin, P. (1987) Molecular analysis of the promoter region of the *Escherichia coli* K-12 *phoE* gene. Identification of an element, upstream from the promoter, required for efficient expression of PhoE protein. *Journal of Molecular Biology* 198, 633–641.

van Alphen, L., van Kempen-De Troye, F. and Zanen, H.C. (1983) Characterization of cell envelope proteins and lipopolysaccharides of *Escherichia coli* isolates from patients with neonatal meningitis. *Federation of European Microbiological Societies Microbiology Letters* 16, 261–267.

van der Ley, P., de Graaff, P. and Tommassen, J. (1986a) Shielding of *Escherichia coli* outer membrane proteins as receptors for bacteriophages and colicins by O-antigenic chains of lipopolysaccharide. *Journal of Bacteriology* 168, 449–451.

van der Ley, P., Kuipers, O., Tommassen, J. and Lugtenberg, B. (1986b) O-antigenic chains of lipopolysaccharide prevent binding of antibody molecules to an outer membrane pore protein in Enterobacteriaceae. *Microbial Pathogenesis* 1, 43–49.

Verhoef, C., Benz, R., Poon, A.P.W. and Tommassen, J. (1987) New pore protein produced in cells lysogenic for *Escherichia coli* phage HK253*hrk*. *European Journal of Biochemistry* 164, 141–145.

Virji, M. and Heckels, J.E. (1989) Location of a blocking epitope on outer-membrane protein III of *Neisseria gonorrhoeae* by synthetic peptide analysis. *Journal of General Microbiology* 135, 1895–1899.

Vuopio-Varkila, J., Karvonen, M. and Saxon, H. (1988) Protective capacity of antibodies to outer-membrane components of *Escherichia coli* in a systemic mouse peritonitis model. *Journal of Medical Microbiology* 25, 77–84.

Wandersman, C. and Delepelaire, P. (1990) TolC, an *Escherichia coli* outer membrane protein required for hemolysin secretion. *Proceedings of the National Academy of Sciences of the United States of America* 87, 4776–4780.

Weiser, J.N. and Gotschlich, E.C. (1991) Outer membrane protein A (OmpA) contributes to serum resistance and pathogenicity of *Escherichia coli* K-1. *Infection and Immunity* 59, 2252–2258.

Weiser, J.N., Blake, M.S. and Gotschlich, E.C. (1992) Antibodies to outer membrane protein A (OmpA) block bactericidal killing of *Escherichia coli*. In: *Abstracts of the 92nd Annual Meeting of the American Society for Microbiology*. American Society for Microbiology, Washington, DC, Abstr. B-175, p. 55.

Weiss, M.S., Abele, U., Weckesser, J., Welte, W., Schiltz, E. and Schulz, G.E. (1991) Molecular architecture and electrostatic properties of a bacterial porin. *Science* 254, 1627–1630.

Whitfield, C., Hancock, R.E.W. and Costerton, J.W. (1983) Outer membrane protein K of *Escherichia coli*: purification and pore-forming properties in lipid bilayer membranes. *Journal of Bacteriology* 156, 873–879.

Willetts, N. and Skurray, R. (1987) Structure and function of the F factor and mechanism of conjugation. In: Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M. and Umbarger, H.E. (eds) Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology*. American Society for Microbiology, Washington, DC, pp. 1110–1133.

Woodruff, W.A. and Hancock, R.E.W. (1989) *Pseudomonas aeruginosa* outer membrane protein F: structural role and relationship to the *Escherichia coli* OmpA protein. *Journal of Bacteriology* 171, 3304–3309.

Yamada, H. and Mizushima, S. (1980) Interaction between major outer membrane protein (0–8) and lipopolysaccharide in *Escherichia coli* K12. *European Journal of Biochemistry* 103, 209–218.

Yu, D.T., Hamachi, T., Hamachi, M. and Tribbick, G. (1991) Analysis of the molecular mimicry between HLA-B27 and a bacterial OmpA protein using synthetic peptides. *Clinical and Experimental Immunology* 85, 510–514.

Zhang, J.-J., Hamachi, M., Hamachi, T., Zhao, Y.-P. and Yu, D.T.Y. (1989) The bacterial outer membrane protein that reacts with anti-HLA-B27 antibodies is the OmpA protein. *Journal of Immunology* 143, 2955–2960.

19

Iron Acquisition Systems in *Escherichia coli*

E. GRIFFITHS
National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK

Iron and Bacterial Virulence

Spectacular advances in biochemistry, molecular biology and immunology over the past decade, together with the advent of recombinant DNA technology, have yielded powerful new techniques for studying bacterial pathogens and the diseases they cause. Although several approaches are currently being used, most investigations into bacterial virulence have been carried out with organisms grown *in vitro* under conditions that do not necessarily reflect microbial behaviour *in vivo*. That this is likely to give at best only a partial picture of bacterial characteristics associated with virulence is now increasingly recognized and more and more attention is being given to the environmentally regulated properties of bacterial pathogens (Griffiths, 1991).

One of the best understood properties of the environment encountered by pathogens in host tissues, and of its effects on bacterial characteristics and growth, concerns the availability of iron. Our understanding of the way the host normally restricts the availability of the metal has increased enormously in recent years and a considerable literature has developed on the relationship between iron and pathogenicity (Bullen and Griffiths, 1987; Crosa, 1989; Weinberg, 1989; Martinez *et al.*, 1990; Williams and Griffiths, 1992; Wooldridge and Williams, 1993). Iron is now recognized as playing a crucial role in infection. Its importance lies in the strictly limited availability of the metal in living tissues and progress made in understanding the strategies used by pathogens for acquiring iron *in vivo*, and their responses to iron restriction has provided a fresh insight into microbial pathogenicity. Much of this new understanding of what is happening as pathogenic bacteria adapt to and grow in the iron-restricted