JOURNAL OF BACTERIOLOGY, Aug. 1984, p. 570–578
0021-9193/84/080570-09$02.00/0

Vol. 159, No. 2

Escherichia coli K-12 Outer Membrane Protein (OmpA) as a Bacteriophage Receptor: Analysis of Mutant Genes Expressing Altered Proteins

RENATO MORONA,* MICHAEL KLOSE, AND ULF HENNING

Max-Planck-Institut für Biologie, D-7400 Tübingen, Federal Republic of Germany

Received 16 March 1984/Accepted 9 May 1984

The outer membrane protein OmpA of *Escherichia coli* K-12 serves as a receptor for a number of T-even-like phages. We have isolated a series of *ompA* mutants which are resistant to such phages but which still produce the OmpA protein. None of the mutants was able to either irreversibly or reversibly bind the phage with which they had been selected. Also, the OmpA protein is required for the action of colicins K and L and for the stabilization of mating aggregates in conjugation. Conjugal proficiency was unaltered in all cases. Various degrees of colicin resistance was found; however, the resistance pattern did not correlate with the phage resistance pattern. DNA sequence analyses revealed that, in the mutants, the 325-residue OmpA protein had suffered the following alterations: Gly-65→Asp, Gly-65→Arg, Glu-68→Gly, Glu-68→Lys (two isolates), Gly-70→Asp (four isolates), Gly-70→Val, Ala-Asp-Thr-Lys-107→Ala-Lys (caused by a 6-base-pair deletion), Val-110→Asp, and Gly-154→Ser. These mutants exhibited a complex pattern of resistance-sensitivity to 14 different OmpA-specific phages, suggesting that they recognize different areas of the protein. In addition to the three clusters of mutational alterations around residues 68, 110, and 154, a site around residue 25 has been predicted to be involved in conjugation and in binding of a phage and a bacteriocin (R. Freudl, and S. T. Cole, Eur. J. Biochem, 134:497–502, 1983; G. Braun and S. T. Cole, Mol. Gen. Genet, in press). These four areas are regularly spaced, being about 40 residues apart from each other. A model is suggested in which the OmpA polypeptide repeatedly traverses the outer membrane in cross-β structure, exposing the four areas to the outside.

The major outer membrane protein, OmpA, of *Escherichia coli* K-12 has been shown to function as a receptor for bacteriophages (16, 64), to be required for the action of colicins K and L (10, 17), and to be required for efficient conjugation in recipient cells (1, 61–64). The amino-terminal moiety of the 325-residue transmembrane polypeptide (19), encompassing residues 1 to about 190, is able to perform all these functions (8, 9). The interaction of the OmpA protein with other components of the outer membrane such as peptidoglycan and lipoprotein (7) has recently been reviewed by Lugtenberg and van Alphen (40).

Recent work from this laboratory has focused on correlating these functions of the protein with its structure. The *ompA* genes from several enterobacterial species have been cloned and sequenced (5, 6, 15, 22; G. Braun and S. T. Cole, Mol. Gen. Genet., in press). Comparison of the deduced amino acid sequences, including that of the *E. coli* protein (3, 49), together with functions which non-*E. coli* proteins can perform when expressed in *E. coli*, has identified areas of the protein which presumably are involved in the functions mentioned above. The comparative analysis has shown that although carboxy-terminal moieties of the OmpA proteins are rather highly conserved, their amino-terminal parts show distinct regions of considerable nonhomology. The three areas thus identified (designated by amino acid position in the *E. coli* sequence) are: region 25, which is likely to be required for the actions of colicin K and phage Ox2 and for conjugation; region 70, needed for sensitivity to colicins K and L and to phage K3; and region 110, which is needed for the action of colicin L and infection by phage K3. The interpretation of these data is that these regions are exposed on the cell surface, thereby allowing interaction with phages, colicins, and fertile donor cells. It is reassuring for this interpretation that the surface exposure of region 70 has been directly demonstrated (13).

Conclusions drawn from these comparative data are somewhat limited. The proteins can be considered multiple-step mutants of each other, and in any one gene, several often complex changes, such as deletions or insertions, are present. Therefore, to obtain a detailed understanding of the interaction of a number of OmpA-dependent phages with this protein (60), we chose to analyze a collection of phage-resistant, altered-protein, *ompA* mutants at the nucleotide sequence level.

MATERIALS AND METHODS

Bacterial strains, phages, and media. The bacterial strains used are listed in Table 1. The OmpA-specific phages (see Table 2) have been described previously (24, 42, 60). The medium used was L broth (LB [reference 47]) or nutrient broth (NB [Difco Laboratories]); the minimal medium was M9 (47). Antibiotics were used at 10 μg/ml for tetracycline and 25 μg/ml for ampicillin. Cells infected with phage M13mp8 (46) were grown in double-strength YT (47). Transductions with phages P1 or T4GT7 (66) were performed as described by Miller (47). All incubations were at 37°C.

Isolation of phage-resistant mutants producing altered OmpA proteins. Strains P530-1cII (29), P692-2eI (29), P692-13, and P692-4gII are spontaneous mutants selected for resistance to phage TuII*-6 (TuII*-6 is identical to TuII*, cf., reference 60). Strains P400-1.2, P400-2.2, P400-4.2, and P400-9.2 are independent isolates from selection to resistance to phage TuII*-46 after mutagenesis with diethyl sulfate. Strains P400-K3 and P400-M1 are spontaneous mutants selected for resistance to phages K3 and M1, respectively. The latter strains turned out to be only very partially resistant to this phage. Another *ompA* mutant, P2899 (kindly provided by J. Hackett, University of Adelaide), is an *rfa*$^+$ *zia*::Tn*5* transductant of strain P2817 (54). The remaining

* Corresponding author.

mutant alleles were obtained in *ompA* genes present on plasmids; their plasmid numbers correspond to these alleles. The plasmids pTU105 and pTU115 have been described before (13). pTU202 and pTU203 were obtained by hydroxylamine mutagenesis (35) of pTU201, which contains the complete *ompA* gene cloned into pBR325 (8), followed by transformation into strain UH201.3 and selection for resistance to phage Ox2. All mutant selections were performed by plating 2×10^7 to 2×10^8 cells together with phage, at a multiplicity of infection of 10 to 15, onto LB medium. After isolation, mutants were usually screened for their ability to grow on NB agar containing 0.5% (wt/vol) sodium deoxycholate (mutants missing the OmpA protein will not grow on this medium; however, selection for phage resistance on it is not possible, because the phage do not infect). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (38) of cell envelopes showed that, with one exception, these mutants possessed wild-type amounts of OmpA protein; strain UH201.3(pTU202) had a slightly reduced amount of OmpA protein (data not shown).

Functions of OmpA proteins. To determine the efficiency of plating by bacteriophage, cells (0.1 ml of an overnight culture) in a 3-ml soft agar overlay were spotted (2.5 μl) with a series of 10-fold dilutions of phage lysates (~10^9 PFU/ml, except TuII*-24 was 10^7 PFU/ml) and incubated for ~16 h. Colicin sensitivity was tested by placing 5 μl of a series of twofold dilutions of crude preparations (21) of colicin K or L onto a similar overlay. Colicin units per milliliter are defined as the reciprocal of the highest dilution which gave a clear zone of growth inhibition. For measuring conjugal recipient ability, donor (RMT-3, carrying pRSF2001, a kanamycin F′) and recipient strains were grown to about 2×10^8 cells per ml without shaking. They were mixed at a donor/recipient ratio of 1:10, incubated at 37°C for 30 min, and plated in appropriate dilutions onto LB plates containing kanamycin (50 μg/ml) and streptomycin (100 μg/ml). The concentration of donor or recipient cells was determined by plating a suitable dilution onto medium with one or the other antibiotic.

Phage inactivation by bacteria was measured qualitatively as described previously (16). Cells treated with chloramphenicol (100 μg/ml, 15 min, 37°C) or chloroform or both were used dependent on the phage or the bacterial strain being tested (chloramphenicol-treated cells were used for phage TuII*-46 since it was neutralized very poorly by chloroform-treated cells of strain P400). Reversible binding of phage to *ompA* mutants was determined by a competition experiment (13). A threefold excess of mutant cells were mixed with wild-type cells (strain P400), and the effect on phage absorption rate, compared with that of wild-type cells alone, was measured. The following *ompA* mutant-bacteriophage combinations were tested: P400-1.2, P400-4.2, and P2899 with TuII*-46; UH201.3(pTU202) and UH201.3(pTU203) with Ox2; P530-1cII, P692-2eI, and P692-4gII with TuII*-6; P400-K3 and P400-M1 with K3. It should be noted that P2899 and P400-M1 were tested with TuII*-46 and K3, respectively, since these two mutants are considerably sensitive to the phage used to select these mutants.

Preparation of DNA. Chromosomal DNA was prepared by the methods of Kaiser and Murray (37) or Nakamura et al. (53). For DNA from λ phage, these phage were propagated on strain C600, concentrated by precipitation with polyethylene glycol (69), and banded in a CsCl step gradient (1.5 ml of each of 56, 45, and 31.5% [wt/wt]) and then in an isopycnic

TABLE 1. Bacterial strains and plasmids[a]

Strain or plasmid	Relevant characteristics	Source (reference)
Strain		
C600	F⁻ *thi thr leu lac supE*	Laboratory collection
NM514	*hflA*	N. Murray (50)
P400	F⁻ *thi argE proA thr leu mtl xyl galK lacY rpsL supE non*	(63)
P460	P400 *ompA1*	(63)
UH201-3	*lac ompA supF recA rpsL*	(9)
JM103	F′ *traD proAB*⁺ *lacI*^{q}*ZM15* Δ(*lac-pro*) *thi rpsL endA sbcB supE hsdR*	J. Messing (45)
P2125	W1485 F⁻ *tonA pyrD*	P. Reeves (48)
RMT-3	P2125 (pRSF2001 = F′ Kan)	This work (33)
P530-1cII	P400 *non*⁺ *his ompB101 ompA2000*	(29, 58)
P692-2eI	P400 *non*⁺ *his ompB103 ompA2001*	(29, 58)
P692-13	P400 *non*⁺ *his ompB103 ompA2002*	This work
P692-4gII	P400 *non*⁺ *his ompB103 ompA2003*	This work
P400-1.2	P400 *ompA2004*	This work
P400-2.2	P400 *ompA2005*	This work
P400-4.2	P400 *rfa*? *ompA2006*	This work
P400-9.2	P400 *ompA2007*	This work
P400-M1	P400 *ompA2008*	This work
P400-K3	P400 *ompA2009*	This work
P2899	P400 *pyrD-34 zcb*::Tn*10*-43 *zia*::Tn*5 ompA725*	J. Hackett (54)
Plasmid		
pTU100	In UH201.3, complete *ompA* cloned in pSC101	(8)
pTU201	In UH201.3, *ompA* amber 31 cloned in pBR325	(8)
pTU105	In UH201.3,(pTU100 *ompA105*)	(13)
pTU115	In UH201.3,(pTU100 *ompA115*)	(13)
pTU202	In UH201.3,(pTU201 *ompA202*)	This work
pTU203	In UH201.3,(pTU201 *ompA203*)	This work

[a] Nomenclature is according to Bachmann (2).

CsCl gradient. Phage DNA was prepared by treatment with pronase and phenol (14). Plasmid DNA was prepared essentially as described recently (8).

Cloning of mutant *ompA* genes. For the purpose of cloning *ompA* genes, the phage λ vector λNM1150 (50) was used; it has an *Eco*RI site in its 434 immunity region. Initially, *Eco*RI-digested chromosomal DNA was fractionated by size on a linear 5 to 20% sucrose gradient, and those fractions containing the 7.5-kilobase *Eco*RI fragment which possesses the *ompA* gene (32) were used. This was later found to be unnecessary, and unfractionated, digested DNA was used directly for ligation into λNM1150 DNA cleaved with *Eco*RI. The ligated DNA (10 to 12°C, overnight) was packaged in vitro with strains BHB2690 and BHB2688 (34). Phages were plated on strain NM514 (50), which allows only those having inserts to form plaques (50). Recombinant phages carrying the *ompA* gene were detected by plaque hybridization (4). The probes used, labeled with [α-^{32}P]dATP by nick translation (55), were either pTU301Sh (5), coding for the carboxy-terminal moiety of the OmpA protein of *Shigella dysenteriae*, or pTU302, which codes for the amino-terminal part of this protein from *E. coli* K-12 (8). The nitrocellulose filters were hybridized by the method of Wahl et al. (65). Phages from positive plaques were retested and then purified by single-plaque isolation.

The *ompA* segment to be sequenced was subcloned into phage M13mp8 (46). Recombinant λ *ompA* DNA was digested with *Bam*HI, the fragments were separated by electrophoresis in a 1% agarose gel, and the two *Bam*HI fragments containing the *ompA* gene (8) were isolated by electroelution. They were then digested with *Hpa*I and ligated into phage M13mp8 replicative form DNA digested with *Bam*HI and *Hin*cII. After transfection of strain JM103, white plaques on 1× YT medium containing 5-bromo-4-chloro-indolyl-galactoside (40 μg/ml) and 1 mM isopropylthiogalactoside (45) were picked. The corresponding phages were propagated on the same strain, and either the replicative form DNA (36) was screened (after digestion with *Eco*RI and *Hin*dIII) for the presence of the correct-sized insert (Fig. 1) or the phage DNA was screened by a single track of the dideoxy sequencing reaction (56, 57).

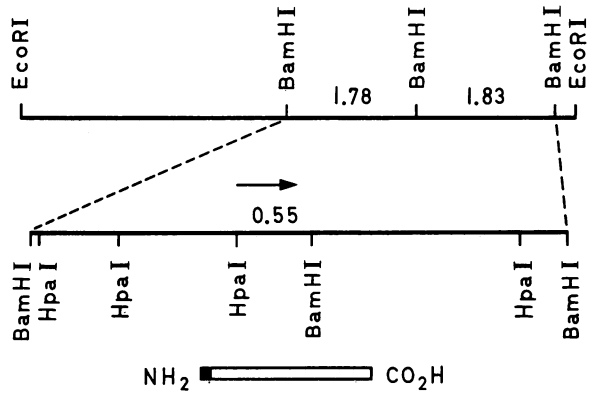

FIG. 1. Strategy for cloning and DNA sequencing. The 7.5-kilobase chromosomal *Eco*RI fragment (top line) was cleaved with *Bam*HI, the two *Bam*HI fragments (1.78 and 1.83 kilobases) were isolated, cleaved with *Hpa*I, and the 0.55-kilobase *Hpa*I-*Bam*HI fragment was ligated into phage M13mp8 (see text). Sequencing proceeded as indicated by the arrow, at the *Hpa*I site; the first codon in *ompA* corresponds to amino acid residue 46 of the OmpA protein. The black part of the bar representing the OmpA protein corresponds to the signal sequence.

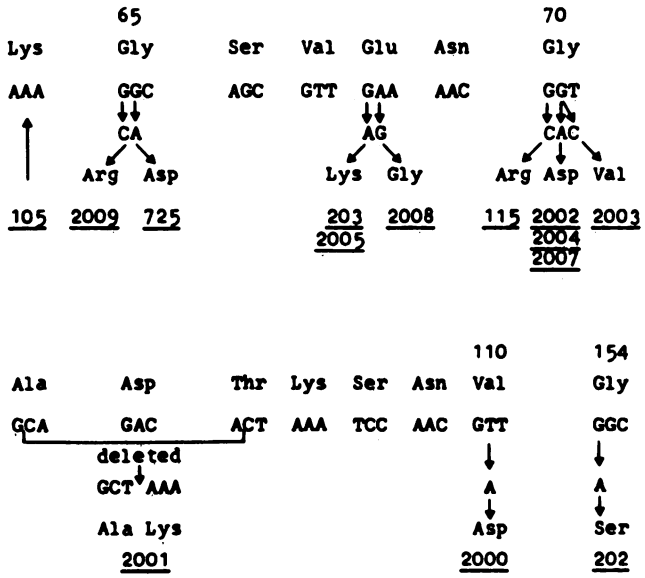

FIG. 2. Base pair alterations in *ompA* genes of phage-resistant mutants and deduced changes in OmpA primary structure. The large arrow (*ompA105*) indicates the site of the previously described 24-base-pair tandem duplication (13). Numbers above amino acid residues refer to their position in the ompA protein (11). The allele numbers are underlined.

DNA sequencing. The single-stranded DNA (18) of the *Hpa*I-*Bam*HI M13mp8 subclones was sequenced with a 15-mer universal primer (New England Biolabs) and the dideoxy chain-termination reactions (57). Gels were dried onto Whatman paper, and exposed to X-ray film (Cronex; Du Pont Co.).

Enzymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim Corp., or New England Biolabs; [α-^{32}P]dATP (600 Ci/mmol) was obtained from New England Nuclear Corp. Experimental procedures not detailed were performed essentially as described by Maniatis et al. (41).

RESULTS

Nucleotide sequence analysis of the *ompA* mutants. Previous results had shown that one apparent recognition site for phage K3 was present near amino acid position 70 (13). To locate the sites in the *ompA* gene causing phage resistance in a larger collection of altered-protein mutants, we used a cloning and sequencing strategy based on the position of region 70 as detailed above. Most mutations were chromosomal, and two such alleles were present on plasmids (pTU202 and pTU203). In the former cases, the *ompA* gene from each mutant was first cloned into phage λNM1150, and in the latter cases, the plasmids were used. The *Bam*HI fragments covering *ompA* were isolated from the relevant *Eco*RI fragment (Fig. 1). A 550-base-pair *Hpa*I-*Bam*HI restriction fragment, coding for amino acid residues 46 to 228 of OmpA, was then subcloned into phage M13mp8. DNA sequencing proceeded in the *Hpa*I-to-*Bam*HI direction (Fig. 1). With this approach, the alterations in all mutant genes could be located.

The nature of the mutational alterations and their effects on the amino acid sequence are shown in Fig. 2. These alterations were found in three areas: in region 70, in region 110, and at amino acid residue 154. Mutants having suffered the substitutions Glu-68→Lys and Gly-70→Asp were recov-

TABLE 2. Behavior of *ompA* mutants towards phages and colicins

Strain	*ompA* allele	Mutational alteration	Characteristics with bacteriophage[a]: TuII*-46	TuII*-60	TuII*-6	TuII*-24	K3	K4	K5	K3h1	Ac3	Ox3	TuII-26	Ox2	Ox4	Ox5	M1	Colicin titers[b] K	L	Class
P2899	725	Gly-65 → Asp	R	R	10^{-2}	10^{-1}	S*	S	0.2*	S	0.2*	10^{-1}	0.2*	R	10^{-2}*	R	S	256	64	IV
P400-K3	2009	Gly-65 → Arg	R	R	R	R	R	10^{-5}	10^{-4}	S	R	R	R	R	R	R	10^{-1}	32	32	I
P400-M1	2008	Glu-68 → Gly	R	R	R	R	R	R	R	10^{-6}	R	R	R	R	R	R	0.2	32	32	I
P400-2.2	2005	Glu-68 → Lys	R	R	R	R	R	R	R	R	R	R	R	R	R	R	10^{-3}	128	<8	I
UH201.3(pTU203)	203																	256	<1	
P400-1.2	2004	Gly-70 → Asp	R	R	10^{-3}	0.3	0.2	S	S	S	R	R	R	R	R	R	10^{-1}	256	64	IV
P400-9.2	2007																			
P692-13	2002																			
P400-4.2	2006	Gly-70 → Asp	R	R	R	R	R	NT	10^{-4}	S	NT	NT	R	R	NT	NT	10^{-2}	NT	NT	
P692-4gII	2003	Gly-70 → Val	R	R	R	R	R	R	R	0.05	R	R	R	R	R	R	10^{-2}	256	32	I
UH201.3(pTU115)	115	Gly-70 → Arg	R	R	R	R	R	R	R	10^{-1}(*t*)	R	R	R	R	R	R	10^{-3}	32	16	I
UH201.3(pTU105)	105	—[c]	R	R	R	R	R	R	R	S	R	R	R	R	R	R	10^{-1}	256	2	I
P692-2eI	2001	—[d]	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	256	2	II
P530-1cII	2000	Val-110 → Asp	R	R	R	R	R	R	R	S	R	R	R	10^{-1}	10^{-1}	10^{-2}	10^{-1}	256	<1	II
UH201.3(pTU202)	202	Gly-154 → Ser	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	64	<1	III
UH101(λ *ompA*-Sh)		—[e]	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S[e]	1	

[a] The efficiency of plating was measured (see text) in comparison to strain P400 for most strains; UH 201.3(pTU201) was the reference strain for testing *ompA* alleles located on plasmids. S, efficiency of plating of 1; R, complete resistance to the highest concentration of phages used; * (in body of table) reduced phage size; t, turbid plaques; NT, not tested; numbers indicate efficiency of plating.

[b] Except for those listed in the following, colicin titers were compared with those on strain P400 (K, 256; L, 32 to 64). The *ompA* alleles 2000, 2001, and 2003 (originally in an *ompB* background which itself confers resistance) were transferred into strain P2125 by P1-mediated transduction, and colicin sensitivity was determined in this strain (K, 256; L, 8 to 16). Colicin sensitivity levels of strains with *ompA* alleles on plasmids are to be compared to those on the parental strains bearing plasmids pTU201 (K, 256; L, 32), and pTU100 (K, 512; L, 16).

[c] Duplication of 24 base pairs between the codons for amino acid residues 63 and 64 (13).

[d] Deletion of 6 base pairs causing: Ala-Asp-Thr-Lys-107 → Ala-Lys

[e] OmpA protein of *S. dysenteriae* in *E. coli* (5, 15; Braun and Cole, in press).

TABLE 3. Sensitivity to phage K3: medium dependence

Medium	Strain	Sensitivity to phage[a]	
		K3	K3h1
LB agar	P400	S	S
	P400-1.2	0.2	S
NB agar	P400	R	R
	P400-1.2	R	R
NB agar + 100 mM NaCl	P400	S	S
	P400-1.2	R	S
NB agar + 10 mM $MgCl_2$	P400	R	R
	P400-1.2	R	R
NB agar + 100 mM NaCl + 10 mM $MgCl_2$	P400	S	S
	P400-1.2	0.2	S
LB agar	P400-4.2	R	S
LB agar + 10 mM $MgCl_2$	P400-4.2	R	S

[a] Sensitivity was determined as detailed in the text. S, Sensitive; R, resistant; 0.2, efficiency of plating.

ered repeatedly; all others were found only once. It is highly unlikely that other mutant sites are present in the *ompA* genes at areas that have not been sequenced. The repeated isolation, be it after mutagenesis or by spontaneous mutation, of the Glu-68→Lys and Gly-70→Asp mutants always having the same phenotype practically proves that they do not harbor additional alterations in their *ompA* genes contributing to their phenotype. Most of the amino acid substitutions confer unique phenotypes (Table 2); an exception is strain P400-4.2. Its OmpA protein has undergone a Gly-70→Asp substitution, but the strain is resistant to phage K3, in contrast to all other mutants with the same substitution (Table 2). Further testing of this mutant showed that it was resistant to phage P1, whereas all other *ompA* mutants were P1 sensitive. This suggested that the strain may harbor an *rfa* mutant site in addition to the *ompA* allele; the resistance to phage K3 possibly being due to a lipopolysaccharide (LPS)-OmpA interaction (54). Therefore, the *ompA* allele was transduced into *rfa*$^+$ strain P2125, and the phage resistance pattern of the recombinants was identical to that of the other mutants with Gly-70→Asp substitutions. The interaction of this area of the protein with LPS will be further described below.

Phenotypes of the *ompA* mutants. The functions of the altered OmpA proteins were tested as described above. The results are shown in Table 2. For comparison, the previously characterized mutants (*ompA*105 and *ompA*115 [13]) are also included.

All mutants were conjugation proficient. Several mutants showed increased resistance to colicin K alone (*ompA2008*, *ompA2009*), to colicin L alone (*ompA105*, *ompA203*, *ompA2000*, *ompA2001*, and *ompA2005*), and one (*ompA202*) showed increased resistance to both colicins. Other mutants were unaffected in this respect, although they harbored amino acid substitutions at the same position as the mutants exhibiting increased colicin resistance. In summary, substitutions in region 70 can affect the action of colicins K or L, depending on the allele; alterations in region 110 affect only colicin L, and the substitution at position 154 affects both colicins.

The phage resistance pattern is complex but interesting. In Table 2, the OmpA-specific phages have been arranged in related groups on the basis of their activity on the *ompA* mutants. By this criterion, several of the phages appear identical or closely related. The *ompA* mutants can be placed in different classes according to the phage resistance pattern. Most mutations in region 70 result in near or complete resistance to all phages (class I mutants). Class II mutants, with mutations in region 110, are sensitive to the Ox2-like phages (i.e., Ox2, Ox4, and Ox5) but are resistant to most of the other phages. The single class III mutant, resulting from an amino acid substitution at position 154, is resistant to the Ox2-like phages only. Class IV mutants are resistant to Ox2-like phages, TuII*-46, and TuII*-60. They carry the Gly-65→Asp or Gly-70→Asp substitution; these altered proteins confer a LPS-dependent phage resistance pattern (see below).

We tested the *ompA* mutants for ability to inactivate the phage with which they were selected (with the exceptions noted above). All *ompA* mutants were unable to inactivate the phage tested. Furthermore, a reversible binding (13) of these phages to the representative mutants used (see above) could not be detected. Mutants having alterations in the region 110 are very similar in their phenotype to that found for *E. coli* strains producing the *S. dysenteriae* OmpA protein (5, 15; Table 2). The *S. dysenteriae ompA* gene differs from that of *E. coli* mainly in this region (5). It has been reported (13, experiments performed by U.H.) that an *E. coli* strain expressing the *S. dysenteriae* protein can inactivate phage K3. We could not reproduce this result and believe that, for reasons that are no longer traceable, it must have been in error. In summary, we could not find any evidence that the phages tested could recognize the particular altered-protein mutants selected with these phages. Therefore, the various mutant protein sites apparently affect the binding site(s) for the phages, and for a given phage, mutational alterations at several sites of the protein lead to an unrecognizable host cell.

In contrast, the four classes of mutants were not distinguishable on the basis of their colicin resistance patterns. For example, mutants with *ompA2005* and *ompA203* have colicin resistance patterns similar to those with *ompA2000* and *ompA*2001, even though the mutants are of different classes. The simplest explanation for this is, of course, that the colicins and phages use the OmpA protein in a very different manner.

It should be noted that the results confirm and extend our previous conclusion (60) concerning a fairly remarkable property of the OmpA-specific phages, all of identical morphology and with the same receptor protein, namely that they recognize different areas of this protein.

Apparent interaction of altered OmpA proteins with LPS. Puspurs et al. (54) have described *ompA*(*cr*) mutants which are resistant to phage K3 only when the mutant allele is present in an *rfa* (LPS core sugar-defective) background. The *rfa* mutants are normaly sensitive to phage K3, (24, 27), producing near normal amounts of OmpA protein (39), and the double mutants also produce near normal amounts of OmpA protein (54). An interaction between the mutant OmpA protein and the core sugars of the LPS has been postulated (54). Five of these *ompA*(*cr*) mutants were tested with all OmpA-specific phages (data not shown). Two had resistance patterns identical with the Gly-70→Asp mutants (Table 2), and the others exhibited a different pattern. One of the latter mutants (strain P2899, *ompA*725) was found to possess a Gly-65→Asp substitution in its OmpA protein (see above).

As described above, strain P400-4.2 (*ompA*2006, Gly-70→Asp) most likely displayed an LPS-dependent phage resistance pattern. This was confirmed by using phage T4GT7 to transduce the *ompA*2004 allele (also Gly-70→Asp) into an *rfa* background. The resulting transductants had acquired resistance to phage K3. Hence, amino acid substi-

tutions resulting in aspartic acid-65 or aspartic acid-70 instead of the glycine residues at these positions in the OmpA protein lead to a LPS core sugar-dependent phage resistance pattern.

The nature of LPS-dependent sensitivity or resistance toward phage K3, shown by the OmpA proteins to have undergone Gly-70→Asp substitution, was further characterized. Strains P400 (*ompA*$^+$) and P400-1.2 (*ompA2004*) were tested with phage K3 and its extended host range mutant, K3hl (42), on NB agar with and without NaCl or $MgCl_2$ or with both NaCl and $MgCl_2$. The results (Table 3) show that phage K3 was able to plate on strain P400-1.2 at a normal efficiency of plating on this medium only in the presence of 10 mM $MgCl_2$. Clearly, sensitivity to phage K3 is conditional in strains with an OmpA protein carrying aspartic acid-70 instead of glycine. The known interaction of divalent cations with LPS (59) is consistent with the LPS-OmpA interaction postulated above. Also, the addition of $MgCl_2$ to the LB agar did not restore sensitivity to K3 in strain P400-4.2 and to other *rfa ompA*(*cr*) double mutants (data not shown). This excludes the possibility that Mg^{2+} acts only on the OmpA protein. Finally, it should be noted that *rfa* mutants (*ompA*$^+$) are resistant to certain OmpA-specific phages, the pattern being dependent on the LPS defect (24; our unpublished data).

DISCUSSION

The phage-resistant *ompA* mutants analyzed possess alterations at three areas of the OmpA protein: regions 70, 110, and 154. Most of the mutational alterations we found were located at region 70 (13 of 16, including the two mutants analyzed previously [13]). For this region, direct evidence that it is exposed at the cell surface has been obtained (13). Such direct proof does not exist for the other areas. However, from DNA sequence analyses of several other enterobacterial *ompA* genes and the behavior of these gene products in *E. coli* with regard to conjugation efficiency, colicin action, and phage resistance, it is very likely indeed that region 110 is also exposed in this way (5, 6, 22; Braun and Cole, in press). In addition, the latter studies implicated a third area, region 25, to be located at the cell's surface, and it was predicted to be required for adsorption of phage Ox2, for action of colicin K, and for optimal efficiency of conjugation. Although we have so far not recovered mutants affected at this area, altered-protein *ompA* mutants have been described which are conjugation defective (26, 43, 44) and also are resistant to phage Ox2 (43, 44) and colicin K (1, 43, 44). These mutants have precisely the phenotype expected for a mutation in region 25. Some of these mutants are currently being analyzed. In summary, the simplest supposition is that the OmpA protein is exposed to the medium around amino acid residues 25, 70, 110, and 154. Consistent with this is the fact that, of the 325-residue protein, only the amino-terminal moiety encompassing residues 1 to 177 is associated with the outer membrane (8, 9, 11).

Alterations at region 70 which include an amino acid substitution not involving a change in charge can result in resistance to all phages. As we did not detect any reversible binding of the phages to such resistant cells, we assume that this area is required by all phages as part of the binding site for their long tail fibers (60). The replacement of glycine-65 or glycine-70 with aspartic acid resulted in an LPS- and medium-dependent phage sensitivity pattern (Tables 2 and 3). We interpret these results as further evidence to support the notion of this region being exposed to the cell surface and that the LPS core sugars are associated with this area. It is known that these core sugars do show some interaction with OmpA protein in vitro (62) and hence may be via region 70 (see below).

Region 110, defined by two mutants, was needed by all phages except the Ox2-like phages. This area was also required for the action of colicin L. These results correlated very well with those found for the OmpA protein of *S. dysenteriae* (5, 15; Braun and Cole, in press). The *S. dysenteriae* OmpA protein can function as a receptor for Ox2, in conjugation, and in colicin K action in *E. coli*. It differs from the *E. coli* protein mainly at region 110 (5).

The mutant proteins due to the *ompA2000* and *ompA2001* alleles were known from previous work to exhibit isoelectric points different from each other and the wild-type protein (30); the shifts observed correlate very well with the changes in charge now found. It is not yet known whether the mutant OmpA proteins affected in this area show an LPS-dependent phage resistance pattern. It is of some interest that, of all our mutant OmpA proteins, only these two exhibit a markedly altered electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gels; the *ompA*2001 polypeptide migrates faster than the wild-type protein, and the other mutant protein migrates slower (29). Similar effects have been reported by Manoil (44) for mutant OmpA proteins and by Clément et al. (12) for mutant LamB proteins (conferring resistance to phage λ).

Region 154 is defined by a single point mutation. The phenotype of the mutant is unique in that it exhibits resistance only to the Ox2-like phages. It cannot, of course, be excluded that this region may also be used by the other phages. Interestingly, regions 110 and 154 are mutually exclusive in their phage resistance patterns (Table 2) such that the Ox2-like phages do not require region 110, whereas most of the others do, and vice versa. Possibly, these two regions play the same role for the two groups of phages.

Finally, we cannot comment much on the effect of the mutational alterations on the sensitivity to the two colicins since the way in which the protein is required for their action is entirely unknown. However, since amino acid substitutions in regions of the OmpA protein which are likely to be cell surface exposed can affect colicin K or L action, this may indicate that the colicin interacts directly with the OmpA protein after initial binding to the colicin receptor.

Inspection of the amino acid sequences at the four regions thought to act as phage receptor sites revealed some remarkable homologies (Fig. 3). The four residues: histidine, threonine, glycine, and aspartic or glutamic acid, in this or a similar order, are present uninterruptedly or in close neighborhood in regions 25, 110, and 154; region 25 possesses such a sequence twice. A sequence in region 70 (Val-Glu-Asn-Gly) may also be related. The only remaining histidine residue of the protein, at position 193 (11), is not associated with the other four residues. Regions 25 and 154 exhibited additional considerable homology, and this may be related to their proposed interaction with phage Ox2. Regions 70 and 110 did not have obvious direct homologies but shared, to a large part, identical amino acid residues.

Reconstitution of phage TuII*-6 receptor activity in vitro requires both the OmpA protein and LPS (the same is true for two other outer membrane proteins, OmpC and OmpF, and phages TuIa and TuIb, respectively [16, 62]). The lipid A component of LPS is sufficient for this reconstitution (62). Denatured OmpA protein shows conformational changes when reassociated with LPS, as judged by electrophoretic mobility and resistance to proteolytic degradation (62). These changes are assumed to reflect renaturation to the

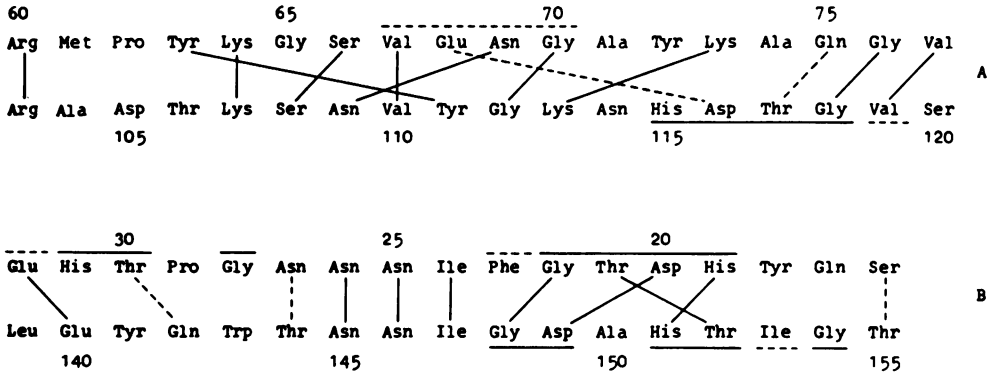

FIG. 3. Comparison of amino acid sequences at the areas of the OmpA protein proposed to be cell surface exposed. (A) comparison of regions 70 and 110; (B) comparison of regions 25 and 154. Solid lines connect identical amino acid residues, whereas dashed lines connect those with similar properties. Horizontal lines indicate regions with identical (solid lines) or possibly related residues (dashed lines) present within these areas of the protein. Additional homologies, between regions 25 and 70, 25 and 110, and 110 and 154, can be found and are centered on the over- or underlined amino acid residues.

active phage receptor. Particularly in view of another phage-receptor system, this need not be so. The receptor for phage T4 is LPS (67). This phage, however, binds to LPS only when it is of the *E. coli* B type; if it is of the K-12 type, the outer membrane protein OmpC is also required for phage inactivation (31, 68). It therefore appears that in this case the protein changes the molecular state of LPS so that it is recognizable by the phage.

The following considerations leave little, if any, doubt that, for the OmpA-specific phages, the protein is the primary recognition site. The complex, mutant-specific phage resistance pattern of the mutants described is certainly much more likely to be due to a direct effect of the protein on phage binding than by the altered proteins indirectly affecting the LPS. Furthermore, several mutant alleles (*ompA105*, *ompA115*, and *ompA2003*) have very similar phage resistance patterns, even though the type of alteration in the protein is very different in each case and is hence unlikely to have the same effect on LPS. It is also known that LPS-defective mutants lacking heptose are still sensitive to phage K3 and related phages (24, 25, 27; our unpublished data). It would be extremely difficult to understand where the type of receptor specificity observed may be located in the LPS.

The core sugars of the LPS appear to interact with region 70. Since they are not essential for the receptor activity of the wild-type protein (at least for phages TuII*-6 and K3), we suggest that, in the mutant proteins with the substitutions Gly-65→Asp or Gly-70→Asp and exhibiting an LPS-dependent phage resistance pattern, the core sugars act to provide a polypeptide conformation which allows some of the phage to recognize the mutant proteins. It remains possible, of course, that the LPS is of importance in a step later in phage adsorption rather than acting in primary recognition. Indeed, the core sugars are needed by several OmpA-specific phages for infectivity in vivo (24, 25).

In view of the absence of direct evidence for regions 25, 110, and 154 being exposed at the cellular surface, any model for the arrangement of the OmpA protein in the outer membrane must remain speculative. We nevertheless propose one because it is consistent not only with the results discussed already but also with all other available data.

It is rather striking that the four areas discussed above are very evenly spaced with about 45 amino acid residues between each region. It is known that the OmpA protein has a high content of β structure (52). This is a common feature of outer membrane proteins (51) and has been confirmed directly for the OmpF protein of *E. coli* B^E by examination of crystalline forms of this protein (23). The outer membrane was assumed to have a thickness of 4.5 nm for its apolar region (40), and 12 to 14 amino acid residues, in β conformation, would suffice to span this region. We also know that, starting around residue 177, the protein leaves the outer membrane and extends, at least to a large part, into the periplasm (11, 28). A combination of all these facts and with the assumption concerning surface exposure of four regions may lead to the arrangement displayed in Fig. 4. The protein is thought to cross the membrane eight times in an antiparallel β-sheet conformation. Although there is no evidence for the localization of the amino-terminus, we place it at the periplasmic side of the outer membrane. The areas predicted or known to be surface exposed are arbitrarily delineated by the first and last charged or polar residue in each region.

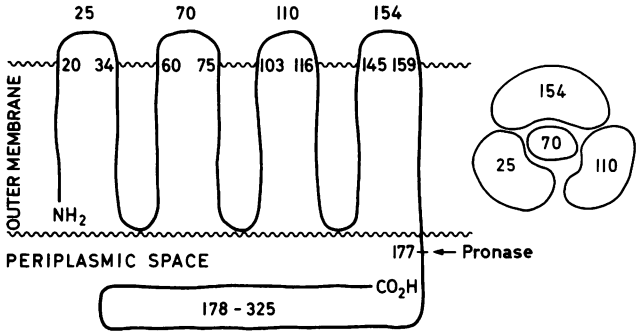

FIG. 4. Hypothetical arrangement of the OmpA protein in the outer membrane. Left, two-dimensional view. The regions exposed to the external medium are designated by amino acid residue numbers. The regions of the protein possibly spanning the outer membrane are thought to exist in a cross-β structure. The turns on the periplasmic side of the outer membrane may or may not be exposed to the periplasmic space. The pronase cleavage site shown (pronase removes the carboxy-terminal moiety of the protein when acting on cell envelopes [11, 28]) represents the transition of the membrane to the periplasmic part of the protein. Right, view of the OmpA protein from outside the cell. All eight transmembrane β chains are thought to be closely associated. For other details, see text.

Each of the eight β chains thought to be membrane embedded are at least 12 residues long (see above). The position of turns occurring on the periplasmic side of the membrane is not known. It is interesting that proline residues occur at amino acid positions 47, 86, and 133, making these regions suitable candidates for turns (49). This arrangement results in a number of charged residues being present in the membrane-spanning regions. They may easily be hidden in the interior of the protein and be involved in ion pairing (20) between the β strands. This arrangement can result in a rather compact protein structure with the four surface exposed areas close to each other (Fig. 4). Their ordering is, of course, arbitrary. Region 70 is placed central to the others since it is required by all OmpA-dependent phages, and regions 25 and 154 are drawn adjacent since both are probably needed for the Ox2-like phages.

Although our model is relatively primitive, we hope that it will be refined by future work, and that greater details of the topology of the OmpA protein in the outer membrane will be revealed. After this manuscript was completed, H. Vogel and F. Jähnig, using Raman spectroscopy and the isolated protein in association with pure LPS (62), completed a study on the conformation of the OmpA protein. The results show that indeed the membrane part of the protein (encompassing residues 1 to 177) consists almost entirely of cross-β structure.

ACKNOWLEDGMENTS

We acknowledge the contribution made by K. Gamon in some of the preliminary experiments described in this work. We are grateful to J. Hackett, N. E. Murray, and P. Reeves who provided bacterial and bacteriophage strains. We thank H. Vogel and F. Jähnig for allowing us to quote their unpublished data.

The financial support of the Fonds der Chemischen Industrie is much appreciated.

ADDENDUM IN PROOF

A recent report (M. D. Pierschbacher and E. Ruoslahti, Nature [London] **309**:30–33, 1984) has shown that the ability of fibronectin to bind cells can be accounted for by the tetrapeptide Arg-Gly-Asp-Ser. Its similarity to the His-Asp-Thr-Gly sequence, which is found in regions of OmpA that are thought to be involved in the binding of phages, appears to be remarkable and may not be fortuitous.

LITERATURE CITED

1. **Achtman, M., S. Schwuchow, R. Helmuth, G. Morelli, and P. A. Manning.** 1978. Cell-cell interactions in conjugating *Escherichia coli*: Con⁻ mutants and stabilization of mating aggregates. Mol. Gen. Genet. **164**:171–183.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
3. **Beck, E., and E. Bremer.** 1980. Nucleotide sequence of the gene *ompA* coding the outer membrane protein II* of *Escherichia coli* K-12. Nucleic Acids Res. **8**:3011–3027.
4. **Benton, W. D., and R. W. Davis.** 1977. Screening of λgt recombinant clones by hybridization to single plaques *in situ*. Science **196**:180–182.
5. **Braun, G., and S. T. Cole.** 1982. The nucleotide sequence coding for major outer membrane protein OmpA of *Shigella dysenteriae*. Nucleic Acids Res. **10**:2367–2376.
6. **Braun, G., and S. T. Cole.** 1983. Molecular characterization of the gene coding for major outer membrane protein OmpA from *Enterobacter aerogenes*. Eur. J. Biochem. **137**:495–500.
7. **Braun, V.** 1975. Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **415**:335–377.
8. **Bremer, E., E. Beck, I. Hindennach, I. Sonntag, and U. Henning.** 1980. Cloned structural gene (*ompA*) for an integral outer membrane protein of *Escherichia coli* K-12. Localization on hybrid plasmid pTU100 and expression of a fragment of the gene. Mol. Gen. Genet. **179**:13–20.
9. **Bremer, E., S. T. Cole, I. Hindennach, U. Henning, E. Beck, C. Kurz, and H. Schaller.** 1982. Export of a protein into the outer membrane of *Escherichia coli* K-12. Stable incorporation of the OmpA protein requires less than 193 amino-terminal amino acid residues. Eur. J. Biochem. **122**:223–231.
10. **Chai, T., and J. Foulds.** 1974. Demonstration of missing outer membrane protein in *tolG* mutants in *Escherichia coli*. J. Mol. Biol. **85**:465–474.
11. **Chen, R., W. Schmidmayr, C. Krämer, U. Chen-Schmeisser, and U. Henning.** 1980. Primary structure of major outer membrane protein II* (OmpA protein) of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **77**:4592–4596.
12. **Clément, J.-M., E. Lepouce, C. Marchal, and M. Hofnung.** 1983. Genetic study of a membrane protein: DNA sequence alterations due to 17 *lamB* point mutations affections adsorption of phage lambda. EMBO J. **2**:77–80.
13. **Cole, S. T., U. Chen-Schmeisser, I. Hindennach, and U. Henning.** 1983. Apparent bacteriophage-binding region of an *Escherichia coli* K-12 outer membrane protein. J. Bacteriol. **153**:581–587.
14. **Cole, S. T., and J. R. Guest.** 1980. Genetic and physical characterization of lambda transducing phages (λ*frdA*) containing the fumarate reductase gene of *E. coli* K-12. Mol. Gen. Genet. **178**:409–418.
15. **Cole, S. T., I. Sonntag, and U. Henning.** 1982. Cloning and expression in *Escherichia coli* K-12 of the genes for major outer membrane protein OmpA from *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Serratia marcescens*. J. Bacteriol. **149**:145–150.
16. **Datta, D. B., B. Arden, and U. Henning.** 1977. Major proteins of the *Escherichia coli* outer cell envelope membrane as bacteriophage receptors. J. Bacteriol. **131**:821–829.
17. **Davies, J. K., and P. Reeves.** 1975. Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group A. J. Bacteriol. **123**:102–117.
18. **Dente, L., G. Cesareni, and R. Cortese.** 1983. pEMBL: a new family of single stranded plasmids. Nucleic Acids Res. **11**:1645–1655.
19. **Endermann, R., C. Krämer, and U. Henning.** 1978. Major outer membrane proteins of *Escherichia coli* K-12: evidence for protein II* being a transmembrane protein. FEBS Lett. **86**:21–24.
20. **Engelman, D. M., R. Henderson, A. D. McLauchlan, and B. Wallace.** Path of the polypeptide in bacteriorhodopsin. Proc. Natl. Acad. Sci. U.S.A. **77**:2023–2027.
21. **Foulds, J.** 1972. Purification and partial characterization of a bacteriocin for *Serratia marcescens*. J. Bacteriol. **110**:1001–1009.
22. **Freudl, R., and S. T. Cole.** 1983. Cloning and molecular characterization of the *ompA* gene from *Salmonella typhimurium*. Eur. J. Biochem. **134**:497–502.
23. **Garavito, R. M., J. A. Jenkins, J. M. Neuhaus, A. P. Pugsley, and J. P. Rosenbusch.** 1982. Structural investigations of outer membrane proteins from *Escherichia coli*. Ann. Microbiol. (Paris) **133**(Suppl. A):37–41.
24. **Hancock, R. E. W., and P. Reeves.** 1975. Bacteriophage resistance in *Escherichia coli* K-12: general pattern of resistance. J. Bacteriol. **121**:983–993.
25. **Hancock, R. E. W., and P. Reeves.** 1976. Lipopolysaccharide-deficient, bacteriophage-resistant mutants of *Escherichia coli* K-12. J. Bacteriol. **127**:98–108.
26. **Havekes, L. M., and W. P. M. Hoekstra.** 1976. Characterization of a *Escherichia coli* K-12 F⁻Con⁻ mutant. J. Bacteriol. **126**:593–600.
27. **Havekes, L. M., B. J. J. Lugtenberg, and W. P. M. Hoekstra.** 1976. Conjugation deficient *E. coli* K12 F⁻ mutants with heptose-less lipopolysaccharide. Mol. Gen. Genet. **146**:43–50.
28. **Henning, U., B. Höhn, and I. Sonntag.** 1973.Cell envelope and

shape of *Escherichia coli* K12. The ghost membrane. Eur. J. Biochem. **39**:27–36.
29. **Henning, U., I. Hindennach, and I. Haller.** 1976. The major proteins of the *Escherichia coli* outer cell envelope membrane: evidence for the structural gene of protein II*. FEBS Lett. **61**:46–48.
30. **Henning, U., I. Sonntag, and I. Hindennach.** 1978. Mutants (*ompA*) affecting a major outer membrane protein of *Escherichia coli* K12. Eur. J. Biochem. **92**:491–498.
31. **Henning, U., and K. Jann.** 1979. Two-component nature of bacteriophage T4 receptor activity in *Escherichia coli* K-12. J. Bacteriol. **137**:664–666.
32. **Henning, U., H.-D. Royer, R. M. Teather, I. Hindennach, and C. P. Hollenberg.** 1979. Cloning of the structural gene (*ompA*) for an integral outer membrane protein of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **76**:4360–4364.
33. **Heffron, F., P. Bedinger, J. J. Champoux, and S. Falkow.** 1977. Deletions affecting the transposition of an antibiotic resistance gene. Proc. Natl. Acad. Sci. U.S.A. **74**:702–706.
34. **Hohn, B.** 1979. *In vitro* packaging of λ and cosmid DNA. Methods Enzymol. **68**:299–309.
35. **Humphreys, G. O., G. A. Willshaw, H. R. Smith, and E. S. Anderson.** 1976. Mutagenesis of plasmid DNA with hydroxylamine: isolation of mutants of multi-copy plasmids. Mol. Gen. Genet. **145**:101–108.
36. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9**:2989–2998.
37. **Kaiser, K., and N. E. Murray.** 1979. Physical characterization of the "Rac prophage" in *E. coli* K-12. Mol. Gen. Genet. **175**:159–174.
38. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
39. **Lugtenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147**:251–262.
40. **Lugtenberg, B., and L. van Alphen.** 1983. Molecular architecture and functioning of the outer membrane of *Escherichia coli* and other gram-negative bacteria. Biochim. Biophys. Acta **737**:51–115.
41. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Manning, P. A., A. Puspurs, and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K-12: isolation of mutants with altered protein 3A by using host range mutants of bacteriophage K3. J. Bacteriol. **127**:1080–1084.
43. **Manoil, C., and J. P. Rosenbusch.** 1982. Conjugation-deficient mutants of *Escherichia coli* distinguish classes of functions of the outer membrane OmpA protein. Mol. Gen. Genet. **187**:148–156.
44. **Manoil, C.** 1983. A genetic approach to defining sites of interaction of a membrance protein with different external agents. J. Mol. Biol. **169**:507–519.
45. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
46. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double digest restriction fragments. Gene **19**:269–276.
47. **Miller, J. H. (ed.).** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Morona, R., and P. Reeves.** 1981. Molecular cloning of the *tolC* locus of *Escherichia coli* K-12 with the use of transposon Tn*10*. Mol. Gen. Genet. **184**:430–433.
49. **Movva, N. R., K. Nakamura, and M. Inouye.** 1980. Gene structure of the OmpA protein, a major surface protein of *Escherichia coli* required for cell-cell interaction. J. Mol. Biol. **143**:317–328.
50. **Murray, N. E.** 1983. Phage lambda and molecular cloning, p. 395. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
51. **Nakamura, K., D. N. Ostrovsky, I. Miyazawa, and S. Mizushima.** 1974. Infrared spectra of outer and cytoplasmic membranes of *Escherichia coli*. Biochim. Biophys. Acta **332**:329–335.
52. **Nakamura, K., and S. Mizushima.** 1976. Effects of heating in dodecyl sulfate solution on the conformation and electrophoretic mobility of isolated major outer membrane proteins from *Escherichia coli* K-12. J. Biochem. **80**:1411–1422.
53. **Nakamura, K., R. M. Pirtle, and M. Inouye.** 1979. Homology of the gene coding for outer membrane lipoprotein within various gram-negative bacteria. J. Bacteriol. **137**:595–604.
54. **Puspurs, A., P. Medon, C. Corless, J. Hackett, and P. Reeves.** 1983. A class of *ompA* mutants of *Escherichia coli* K12 affected in the interaction of OmpA protein and the core region of lipopolysaccharide. Mol. Gen. Genet. **189**:162–165.
55. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
56. **Sanger, F. A., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
57. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A.**74**:5463–5467.
58. **Sarma, V., and P. Reeves.** 1977. Genetic locus (*ompB*) affecting a major outer-membrane protein in *Escherichia coli* K-12. J. Bacteriol. **132**:23–27.
59. **Schindler, M., and M. J. Osborn.** 1979. Interaction of divalent cations and polymyxin B with lipopolysaccharide. Biochemistry **18**:4425–4430.
60. **Schwarz, H., I. Riede, I. Sonntag, and U. Henning.** 1983. Degrees of relatedness of T-even type *E. coli* phages using different or the same receptors and topology of serologically cross-reacting sites. EMBO J. **2**:375–380.
61. **Schweizer, M., and U. Henning.** 1977. Action of a major outer cell envelope membrane protein in conjugation of *Escherichia coli* K-12. J. Bacteriol. **129**:1651–1652.
62. **Schweizer, M., I. Hindennach, W. Garten, and U. Henning.** 1978. Major proteins of the *Escherichia coli* outer cell envelope membrane. Interaction of protein II* with lipopolysaccharide. Eur. J. Biochem. **82**:211–217.
63. **Skurray, R. A., R. E. W. Hancock, and R. Reeves.** 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. J. Bacteriol. **119**:726–735.
64. **Van Alphen, L., L. Havekes, and B. Lugtenberg.** 1977. Major outer membrane protein d of *Escherichia coli* K-12. Purification and *in vitro* activity of bacteriophage K3 and F-pilus mediated conjugation. FEBS Lett. **75**:285–290.
65. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextransulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.
66. **Wilson, G. G., K. K. Y. Young, G. G. Edlin, and W. Konigsberg.** 1979. High frequency generalized transduction by bacteriophage T4. Nature (London) **280**:80–82.
67. **Wilson, J. H., R. B. Luftig, and W. B. Wood.** 1970. The interaction of bacteriophage T4 tail components with a lipopolysaccharide fraction from *Escherichia coli*. J. Mol. Biol. **51**:423–434.
68. **Yu, F., and S. Mizushima.** 1982. Roles of lipopolysaccharide and outer membrane protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. J. Bacteriol. **151**:718–722.
69. **Yamomoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Theiber.** 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus production. Virology **40**:734–744.