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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

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Summary. pTU100 is a hybrid plasmid constructed by cloning a 7.5 Kb EcoRI fragment (carrying the wildtype *ompA* gene) onto pSC 101 (Henning et al., 1979). This plasmid confers sensitivity to phages TuII* and K3h1 when present in an *ompA* host strain, due to the expression of the phage receptor protein II* from the plasmid *ompA*$^+$ gene. Plasmid mutants have been isolated that have become resistant to one or both of these phages. Restriction endonuclease analysis and DNA-sequencing studies in these plasmids demonstrate that a *Bam*HI site and two *Pvu*II sites are located within the *ompA* gene. *Bam*HI cuts the gene at a site corresponding to residue 227 within a total of 325 amino acid residues.

Neither the wildtype *ompA* gene nor the *Bam*HI fragment encoding the NH_2-terminal part of the protein (residues 1–227) could be transferred to a high copy number plasmid, presumably due to lethal overproduction of the protein or its NH_2-terminal fragment. However, the NH_2-terminal fragment derived from one of the *ompA* mutants of pTU100 could be transferred to the high copy number plasmid pBR322, and was expressed in the presence of the amber suppressors *supD* or *supF*. Under these conditions two new envelope proteins with apparent molecular weights of 30,000 and 24,000 were synthesized, and the cells became sensitive to phage TuII*, indicating the presence of phage receptor activity in the outer membrane. The major, 24,000 dalton protein has the molecular weight expected of a protein comprising residues 1–227 of protein II*. DNA-sequencing studies demonstrated that no termination codons are present in the DNA region immediately downstream from the *Bam*HI site at residue 227 in this hybrid plasmid, and it is therefore likely that the 24,000-dalton protein arises from the posttranslational proteolytic cleavage of a larger polypeptide. The 30,000-dalton protein is a likely candidate for such a larger polypeptide. These results also demonstrate that the 98 CO_2H-terminal residues of wildtype protein II* (resisdues 228–325) are not required either for the activity of the protein as a phage receptor or for its incorporation into the outer membrane.

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Introduction

Protein II* (Garten et al., 1975) is one of the few abundant polypeptides of the *E. coli* outer cell envelope membrane (for other such proteins and other nomenclatures see DiRienzo et al., 1978). The protein (Mr ~33,000) spans the outer membrane (Endermann et al., 1978) and is synthesized in precursor form (Sekizawa et al., 1977; Halegoua and Inouye, 1979; DiRienzo and Inouye, 1979). It can serve as a receptor for phages K3 or TuII* (van Alphen et al., 1977; Datta et al., 1977), but its physological functions are not yet well understood (van Alphen et al., 1977; Skurray et al., 1974; Schweizer and Henning, 1977; Manning et al., 1977; Sonntag et al., 1978; Lugtenberg et al., 1978), nor is it known how it is incorporated into the outer membrane or how its synthesis is regulated. To gain a better understanding of these latter processes we have recently cloned the structural gene (*ompA*) of the protein on a 7.5 Kb *Eco*RI fragment employing phage λ and plasmid pSC101 as vectors (Henning et al., 1979).

We have now localized *ompA* on the cloned *Eco*RI fragment. We report on experiments designed to facilitate sequencing of the *ompA* DNA and show that an NH_2-terminal fragment of the corresponding protein can be incorporated into the outer membrane.

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Table 1. *E. coli* strains

Strain	Origin	Genotype
UH 100	W620 (*Datta et al., 1976*)	*thi, pyr*D, *glt*A, *galK, str, trp, rec*A, *omp*A[a]
UH 200	JC 3272 (Achtman et al., 1971)	*his, trp, lys, gal, lac, str, tsx, omp*A[a]
UH 201	JC 6650 (Achtman et al., 1971)	*lac, sup*F, *omp*A[a]
UH 202	JC 6255 (Achtman et al., 1971)	*trp, lac, sup*D, *omp*A[a]

[a] All *omp*A mutants were obtained by selecting for resistance to the most extended host range mutant of phage K3, K3h1 (Manning et al., 1976). Genetic nomenclature is according to Bachmann et al. (1976). UH 100 carries a weak nonsense suppressor (ochre?), and UH 200 is suppressor free

Table 2. Plasmids

Plasmid	Derivation and composition
pTU 100	Wild type *ompA* gene on a 7.5 Kb *Eco*RI fragment cloned in pSC101 (Henning et al., 1979)
pTU 101	Deletion S4 in pTU 100 (remaining *ompA*$^+$)
pTU 102	*ompA* mutant (amber, allele *31*) in pTU 100
pTU 103	Deletion *721* in pTU 100 (reduced expression of *ompA*)
pTU 104	Insertion *72* in pTU 100 (reduced expression of *ompA*)
pTU 201	*Eco*RI fragment with *ompA* 31 from pTU 102 cloned in pBR 325
pTU 301	1.76 Kb *Bam*HI fragment (encoding the CO_2H-terminal part of protein II*) from pTU 102, cloned in pBR 322
pTU 302	1.67 Kb *Bam*HI fragment (encoding the NH_2-terminal part of protein II*) from pTU 102, cloned in pBR 322

Materials and Methods

Strains, Plasmids, and Culture Conditions

The strains and plasmids used are listed in Tables 1 and 2, respectively. Cells were grown at 37° C in L-broth supplemented with tetracycline (10 µg/ml) when strains harbored plasmid pSC101 derivatives (Cohen et al., 1973) and with ampicillin (20 µg/ml) when either pBR325 or pBR322 derivatives were present; pBR325 (Bolivar, 1978) and pBR322 (Bolivar et al., 1977) were donated by C.P. Hollenberg and H. Schaller, respectively. Selection for phage resistance was performed on L-broth solidified with 1.5% agar.

Plasmid DNA and Cell Envelopes

Transformation of *ompA* mutants was performed as described (Henning et al., 1979). Cleared lysates were prepared essentially as described by Clewell (1972) with modifications according to Teather et al. (1978). Plasmid DNA on a preparative scale was purified by chromatography on hydroxyapatite (Colman et al., 1978) and, when the DNA had to be pure (in our hands the latter method gave DNA in excellent yield but never truly free from chromosomal DNA), by subsequent CsCl density gradient centrifugation. Restriction endonucleases were from Boehringer (*BamHI*, *Eco*RI, *Sma*I, *Hind*III) or from New England Biolabs (*Pvu*II, *Xho*I), DNA ligase was from Miles. The conditions for endonuclease cleavage were as recommended by the manufacturers. Sizes of the fragments generated were measured using, as standard, phage λ DNA cleaved with *Hind*III (Philippsen et al., 1978; purchased from Boehringer). Analytical or preparative gel electrophoreses were performed in 0.7% agarose using 40 mM Tris-acetate, pH 7.7/l mM EDTA. DNA was extracted from macerated gels by overnight incubation at 50° C in 10 mM Tris. Cl (pH 7.4)/1 mM EDTA (about 3 vol buffer/ml gel). Upon removal of the gel by centrifugation (10 min at 25,000 *g*) the supernatant was lyophilized, taken up in a suitable volume of the same buffer, and dialyzed against it.

For DNA-sequencing endonuclease fragments were 5′-end labeled with γ-^{32}P-ATP (3,000 Ci/mMole; Amersham) and polynucleotide kinase and further processed as described by Maxam and Gilbert (1977).

Cell envelopes were prepared and analyzed by SDS polyacrylamide-gel electrophoresis as described previously (Henning et al., 1978).

Results

One of the aims of the present study was to localize *ompA* on the cloned 7.5 Kb *Eco*RI fragment of pTU100 for sequence analysis of the gene and its manipulation in vivo and in vitro. To this end we have constructed a restriction endonuclease map of pTU100, and have isolated *ompA* mutants of pTU100 that proved to be valuable for the isolation of larger quantities of DNA for sequence analysis.

Restriction Endonuclease Mapping of pTU100

The plasmid was digested with endonucleases *Eco*RI, *Bam*HI, *Hind*III, *Sma*I, *Pvu*II and *Xho*I. The fragments obtained are listed in Table 3, and from these data the map of Fig. 1 was derived.

Localisation of ompA on pTU100

Thompson and Achtman (1979) have shown that transformation of *E. coli* with linear plasmid DNA (generated by restriction endonuclease cleavage) can permit the recovery, at high frequency, of random deletions extending from the restriction sites. Using this technique, we found that *ompA* is localized to one or two *Bam*HI fragments of pTU100. Strain UH100 (an *ompA* derivative of W620 *recA*: see Table 1) was transformed with a *Sma*I digest of pTU100.

Table 3. Restriction endonuclease fragments from pTU 100

	Enzyme(s)	Fragments generated (Kb)
1	*Eco*RI	9.09, 7.5
2	*Sma*I	11.75, 4.56, 0.28
3	*Eco*RI + *Sma*I	7.55, 4.2, 3.3, 1.26, 0.28
4	*Pvu*II	9.48, 3.7, 2.9, 0.5
5	*Eco*RI + *Pvu*II	7.88, 2.9, 2.5, 1.6, 1.2, 0.5
6	*Eco*RI + *Pvu*II + *Sma* I	7.55, 2.5, 2.1, 1.6, 1.2, 0.8, 0.5, 0.28, (0.05)
7	*Bam*HI	12.45, 1.76, 1.67, 0.7
8	*Eco*RI + *Bam*HI	8.72, 3.76, 1.76, 1.67, 0.37, 0.33
9	*Bam*HI + *Pvu*II	7.5, 3.7, 1.63, 1.29, 0.7, 0.49, (0.01)
10	*Hind*III or *Xho*I	No cleavage sites in the cloned DNA

Experiment 5 defines the *Pvu*II sites at positions 1.6 and 5.0 Kb, the *Pvu*II site at 2.1 Kb was derived from experiment 6. The order of the two *Bam*HI sites (cleavage 8) was obtained from experiment 9. The underlined fragments originate exclusively from the pSC101 moiety. The small fragments in brackets (positions 2.09–2.1 and 8.71–8.76 on the map of Fig. 1) could not be seen under the analytical conditions used. The 1.29 Kb fragment in experiment 9 was stained considerably more with ethidium bromide than the 1.63 Kb fragment, and the former represents two fragments of the same size

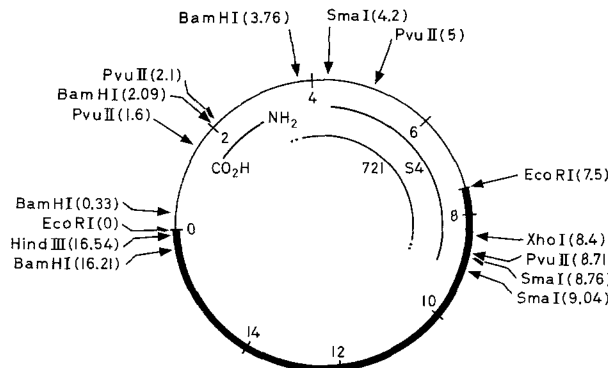

Fig. 1. Restriction endonuclease cleavage map of hybrid plasmid pTU100 (16.59 Kb). The pSC101 part of the map (heavy line) is from D. Tu and S.N. Cohen (manuscript in preparation, see also Meacock and Cohen, 1979). The extent of deletions in plasmids pTU101 (deletion S4) and pTU103 (deletion 721) (see text) is indicated by the corresponding lines, and the orientation of the gene *ompA* (CO_2H—NH_2) follows from the data of Fig. 2. The numbers in brackets are map positions in Kb. DNA sequencing has shown that the *Pvu*II site at 2.1 Kb actually consists of two such sites in very close proximity (see Fig. 2)

About two-thirds of the tetracycline resistant clones were resistant to phages TuII* and K3h1, while the rest were sensitive to both phages (and must therefore still carry the *ompA*$^+$ gene). Restriction endonuclease analysis of plasmid DNA from five of the phage-

Table 4. Restriction endonuclease fragments from deletion palsmid pTU101

Plasmid	Enzyme(s)	Fragments generated
pTU100	*Eco*RI	9.09, 7.5
pTU100	*Sma*I	11.75, 4.56, 0.28
pTU101	*Eco*RI or *Sma*I	11.75
pTU100	*Eco*RI + *Sma*I	7.55, 4.2, 3.3, 1.26, 0.28
pTU101	*Eco*RI + *Sma*I	7.55, 4.2
pTU100	*Bam*HI + *Sma*I	7.18, 4.56, 1.76, 1.67, 0.7, 0.44, 0.28
pTU101	*Bam*HI + *Sma*I	7.18, 1.76, 1.67, 0.7, 0.44

sensitive clones demonstrated that the plasmids had undergone deletion events which always removed the *Pvu*II and *Eco*RI sites at 5 and 7.5 Kb, respectively, but leaving the *BamH*I site at 3.76 Kb intact (see Fig. 1). One of the plasmids (pTU101) was analyzed in more detail. Restriction endonuclease fragments of this plasmid are presented in Table 4, and it was concluded that pTU101 contains a deletion (S4, since generated by *Sma*I), the extent of which is shown in Fig. 1. Since *ompA*$^+$ is expressed by pTU101 as well as by pTU100, the *ompA* gene must have remained intact and must therefore lie between 0 and 4.2 Kb (Fig. 1).

Plasmid DNA from six of the phage resistant clones generated by *Sma*I digestion of pTU100 was also analyzed. These plasmids carried more extensive deletions in which the *BamH*I sites at 3.76 Kb, or at both 3.76 and 2.09 Kb, were removed in addition to the *Pvu*II and *Eco*RI sites at 5 and 7.5 Kb. Thus at least part of the *ompA* gene, which should comprise about 1 Kb of DNA, must be located within the 1.67 Kb *Bam*HI fragment located between 2.09 and 3.76 Kb on the map. These results also suggested that the *Bam*HI site at 2.09 Kb might be located within *ompA*.

To test this possibility, we decided to sequence the region surrounding the *Bam*HI site at 2.09 Kb. Since the amino acid sequence of protein II* is known (Chen et al., submitted), a direct comparison of the base and amino acid sequences is possible. However, to isolate a sufficient quantity of DNA for sequence analysis it was desirable to transfer the *ompA* gene from the stringently controlled pTU100 onto a high copy number plasmid. We have previously and unsuccessfully attempted to do this (Henning et al., 1979), and have speculated that the high level of expression expected from too many copies of *ompA* is lethal for the cell (Henning et al., 1979). We therefore decided to isolate mutants of pTU100 with an intact but inefficiently expressed *ompA* gene, and to use such mutants to clone *ompA* onto a high copy number plasmid.

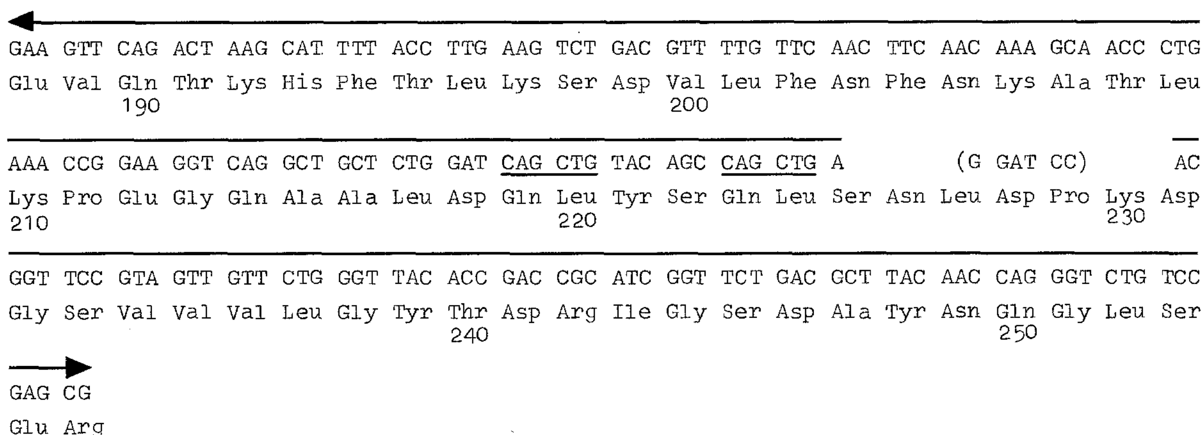

Fig. 2. Base and corresponding amino acid sequences around the *Bam*HI site at map position 2.09 Kb (cf. Fig. 1). The base sequence of the *Bam*HI site (Roberts, 1980; in brackets) has been deduced from the amino acid sequence. Base sequences for *Pvu*II sites (Roberts, 1980) are underlined. The DNA sequence did not agree, at two positions, with the amino acid sequence originally determined, and these discrepancies were resolved by protein sequencing in favor of the DNA sequence

Isolation of ompA Mutants from pTU100

We have previously shown that TuII* resistant mutants of *E. coli* K-12 often retain a low level of protein II* (Henning et al., 1978). Such mutants usually remain sensitive to K3h1, a host-range mutant of phage K3 (Manning et al., 1976) which requires as a receptor only very low concentrations of protein II* in the outer membrane. The isolation of such mutants proved to be problematic, however. Selection for resistance to phage TuII* in strain UH100 (pTU100) yielded resistant clones at normal frequencies (10^{-6}–10^{-7}). Fifty independent mutants were however also resistant to phage K3h1 indicating complete absence of protein II*. Twenty of them were analyzed for the state of their plasmid DNA and all turned out to have lost large parts of the cloned fragment, i.e., had probably completely lost *ompA*.

To find the desired TuII* resistant, K3h1 sensitive mutants among the large number of deletion mutants the following technique was adopted which should also be applicable to related problems. The TuII* resistant mutants in UH100 (pTU100) were selected, to yield about 200 colonies per plate (about 2×10^8 cells mixed with about 10^9 pfu of TuII*), and they were replica printed (Raetz, 1975) onto filter paper and replicated onto a second plate. The master plate was overlaid with soft agar containing phage K3h1 (5×10^9 pfu). Colonies failing to regrow on this plate were picked from the replica plate and analyzed further. The replica printing technique with filter paper instead of velveteen was of critical importance; velveteen did not remove enough of the original colony so that too many cells were not exposed to the K3h1 phage in the overlay. This permitted K3h1 sensitive clones to regrow and be scored as resistant. K3h1 sensitive clones were recovered using this technique at a frequency of 0.1–1% of all TuII* resistant clones. Restriction endonuclease cleavage of the corresponding plasmid DNA's showed that 16/23 were indistinguishable from the parental DNA, and one such mutant is pTU102. The other seven plasmids had undergone more or less extensive alterations (insertions or deletions), and among these are pTU103 and pTU104.

The cloned chromosomal DNA of pTU102 was transferred, via *Eco*RI cleavage, and without difficulty, onto the high copy number plasmid pBR325. The resulting plasmid, pTU201, was used for sequencing studies.

Isolation and Sequencing of Restriction Endonuclease Fragments from pTU201

pTU201 DNA was subjected to *Bam*HI digestion. A mixture of the 1.67 and 1.76 Kb fragments (i.e., the two fragments spanning the region from 0.33 to 3.76 Kb in the restriction map, Fig. 1) was isolated by preparative agarose gel electrophoresis. The two fragments are only partially resolved on such gels, and we did not therefore attempt to separate them. The isolated DNA was ^{32}P-end-labeled and digested with *Hha*I. Electrophoresis of this digest on a 6% polyacrylamide gel yielded, as expected, four radioactive fragments. Their sizes were estimated at 500, 300, 200, and 83 bp (not shown).

DNA sequencing of the 83 bp fragment showed (Fig. 2), in one of the six possible reading frames, a base sequence corresponding to amino acid residues

from position 230 to 255 in protein II*. The 500 bp fragment contained a sequence corresponding to the amino acid residues from positions 188 to 223 (Fig. 2). In the 500 bp fragment the DNA sequence was determined from the strand complementary to the sense strand, whereas in the 83 bp fragment the sense strand was sequenced. These results showed that the two *Hha*I fragments are adjacent to each other and joined at a *Bam*HI site which must therefore lie within the DNA coding for amino acids in positions 223–230. Examination of the amino acid sequence in fact reveals this *Bam*HI site among the possible codons for the tripeptide sequence -Leu-Asp-Pro- at positions 227–229 (Fig. 2).

The DNA sequence also showed that the *Pvu*II site at map position 2.1 Kb actually consists of two such sites in very close proximity, and the orientation of the gene on pTU100 can be deduced from Fig. 2 as given in Fig. 1.

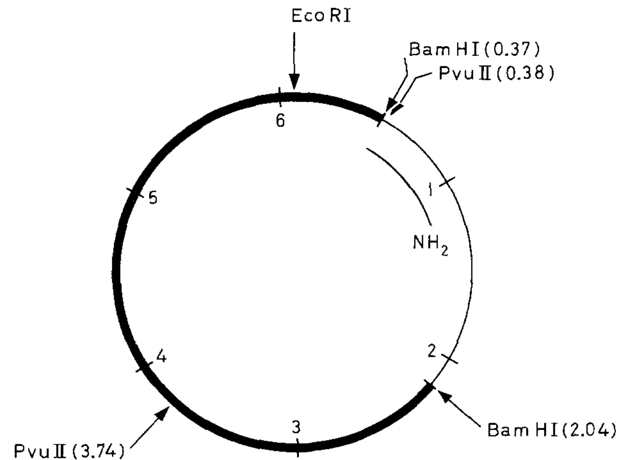

Fig. 3. Plasmid pTU302 (6.03 Kb) carrying the NH_2-terminal fragment (NH_2—) of the *ompA* gene from mutant *ompA31*. The map positions (Kb) were calculated from those of Sutcliffe (1978). The orientation of the cloned fragment was determined by cleavage with *Pvu*II. (The *Pvu*II site at 0.38 Kb consists of the two *Pvu*II sites shown in Fig. 2)

Expression of the NH_2-Terminal Fragment of Protein II^+

To facilitate sequencing of the wildtype *ompA* gene we attempted to clone separately the two *Bam*HI fragments that carry that gene. It was hoped that this would enable us to avoid the gene dosage problem that presumably had prevented the cloning of the complete gene on high copy number plasmids. pTU100 DNA was digested with *Bam*HI, and the mixture of the 1.67 and 1.76 Kb fragments, isolated electrophoretically, was ligated with *Bam*HI-cleaved high copy number plasmid pBR322. Twelve hybrid plasmids were analyzed and all 12 had incorporated the 1.76 Kb fragment encoding the CO_2H-terminus of protein II*.

The most likely explanation for this result is that the NH_2-terminal fragment, as the complete protein, is expressed at a level that is lethal for the cell. We, therefore, repeated the experiment using *Bam*HI fragments from pTU102 instead of pTU100. Fourteen hybrid plasmids (obtained from transformed clones of strain JC3272) were analyzed; six had incorporated the 1.76 Kb *Bam*HI fragment encoding the CO_2H-terminus of protein II* (e.g., pTU301) and eight had incorporated the 1.67 Kb fragment encoding the NH_2-terminus (e.g.: pTU302). The orientation of the latter fragment in pTU302 is shown in Fig. 3.

From DNA-sequencing studies using pTU102 which were carried out at the same time as the above experiments, we knew that the *ompA* gene on this plasmid carries a terminating codon of the amber type (TAG) at position 7 of the amino acid sequence (Beck and Bremer, in preparation). We therefore in-

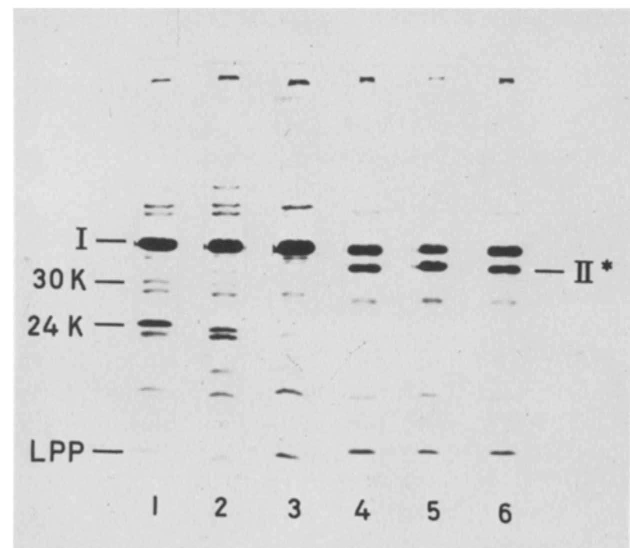

Fig. 4. SDS polyacrylamide-gel electrophoreses of cell envelopes. *1*, pTU302 in strain UH201 (combination of *supF* with the fragment of *ompA 31* encoding the NH_2-terminal part of protein II*); *2*, pTU302 in strain UH202 (combination of *supD* with the same fragment as in *1)*; *3*, pTU301 in strain UH201 (combination of *supF* with the fragment of *ompA 31* encoding the CO_2H-terminal part of the protein). The latter pattern is practically identical with those obtained from the two strains without a plasmid or from strain UH200 (suppressor free) harboring pTU302, pTU301, or pTU201. *4*, strain JC6650 (*ompA*$^+$); *5*, pTU201 in strain UH201 (combination of *supF* with the complete *ompA 31* gene); *6*, pTU201 in strain UH202 (combination of *supD* with the same gene as in *5)*. There are differences between these profiles in addition to those indicated by 30 K and 24 K; exclusively the latter two, however, have been found to be specific for the combination of pTU302 and the suppressors in three repeats with independent strain isolates. Protein I is *E. coli's* porin (e.g.: Nakae, 1976; Nikaido, 1979). LPP: outer membrane lipoprotein (Braun, 1975)

vestigated the effect of transferring pTU201 (carrying the complete mutant gene), pTU301 (encoding the CO_2H-terminal part of protein II*), and pTU302 (encoding the NH_2-terminal part of the protein) to two *omp*A strains, carrying the amber suppressors *sup*D or *sup*F. The combination of either suppressor with pTU201 or pTU302, but not with pTU301, resulted in regained sensitivity to phages TuII* and K3h1, indicating that the NH_2-terminal fragment of the protein is incorporated into the outer membrane. All three plasmids are unable to confer sensitivity to TuII* or K3h1 in the *omp*A derivative of strain JC3272 not harboring a nonsense suppressor. (Strain UH100 harbors an unspecified, weak nonsense suppressor and therefore pTU102, carrying the *omp*A amber allele, was originally recovered as conferring resistance to phage TuII* and sensitivity to K3h1).

Electrophoretic analyses of cell envelopes are shown in Fig. 4. Evidently, the combination of *sup*D or *sup*F with pTU302 leads to the appearance of two proteins with apparent molecular weights of 24,000 and 30,000. It will be argued below that the former, major polypeptide is a proteolytic product of the latter and that it represents a fragment of protein II* encompassing residues 1 to about 227.

Evidence for ompA Control Elements

We have analyzed several other *omp*A mutants of pTU100, and the results suggest the presence of *omp*A control elements between map positions 2.09 and 3.76 Kb, but outside *omp*A itself. Plasmid pTU103 was found to carry a deletion removing the *Eco*RI, *Pvu*II, *Sma*I, and *Bam*HI sites at positions 7.5, 5, 4.2, and 3.76 Kb, respectively (Fig. 1). Plasmid pTU104 was found to carry an insertion, and digestion with *Bam*HI showed the inserted DNA (~700 bp) to be located in the fragment between *Bam*HI sites at 2.09 and 3.76 Kb. In *omp*A strains resistant to phages TuII* and K3h1 both plasmids caused sensitivity to phage K3h1 without altering resistance to TuII*, and protein II* was no longer clearly discernible on SDS-polyacrylamide gel electrophoretograms of corresponding cell envelopes. Much reduced expression of *ompA* together with the types and localization of the alterations in the DNA strongly suggest that the structural gene itself has remained intact and that control elements (e.g., a regulatory gene or a promoter) have been impaired.

Discussion

Mutant *ompA* genes leading to reduced expression of *ompA* as well as the fragment of the wildtype gene encoding the CO_2H-terminal part of the protein could easily be cloned in plasmids subject to relaxed control, and we failed to clone either the wild type gene or its NH_2-terminal fragment in such plasmids. It thus is very likely that too high an *ompA* gene dosage effect is lethal. Since a muntant *ompA* gene carrying a terminating codon corresponding to amino acid residue 7 of the protein could be cloned in high copy number plasmids the presumed lethality certainly is not due to the presence of too many copies of *ompA* control elements (e.g., strong promoters). We therefore assume that production of too much protein II* is not tolerated. It will be of interest to determine exactly what happens under such conditions and experiments are in progress to put *ompA* under another, inducible control.

The molecular weight of the *Bam*HI generated fragment of protein II*, i.e., encompassing residues 1–227, can be calculated from the amino acid sequence to be 24,485. The nucleotide sequence of pBR322 is known (Sutcliffe, 1978) and one can thus read where, downstream from the *Bam*HI insertion site (in the orientation shown in Fig. 3) terminating codons are present. There are several TAG codons (located at positions 18, 40, 47, and 49 when the first new codon generated by the *Bam*HI insertion is designated position 1) followed by a TAA codon, not suppressible by *supD* or *supF*, at position 107. Polypeptides detectable by staining and which would correspond to termination at codons 18 and 40 have not been found. Termination at codon 107 would yield a protein of ~36,000 daltons which would not be visible on the gels because of the presence of protein I (Fig. 4). However, termination at codons 47 and 49 would produce a protein with a molecular weight of 29,900, in close agreement with the molecular weight of 30,000 observed for the minor protein produced when the NH_2-terminal fragment of the *ompA* mutant was combined with *supD* or *supF*.

The major 24,000 dalton species produced under the same conditions most likely consists of the *Bam*HI fragment of protein II*, generated by complete proteolysis of the CO_2H-terminal extension(s). From earlier studies we know that the CO_2H-terminal part of protein II* (at least 150 residues long) is located inside the outer membrane (Schweizer et al., 1978), and the CO_2H-terminal extension in the hybrid protein should be at the same site, a location which may facilitate degradation during or after membrane incorporation. Such proteolysis during preparation of cell envelopes appears unlikely: the electrophoretic profile did not change whether envelopes were used for electrophoresis immediately or after storage for 24 h at 4° C.

Phages TuII*, K3, and the K3 host-range mutants

differ in the amounts of receptor that must be present for successful infection with the highest concentration required for TuII* (Manning et al., 1976; Henning et al., 1978). From inspections of a large number of stained SDS polyacrylamide gels from cell envelopes of wildtype and *ompA* mutant strains we estimate that TuII* can no longer infect when the concentration of protein II* decreases to less than 20–50% of the wildtype level (about 10^5 copies per cell). Sensitivity to phage TuII* together with the amount of the ~24,000-daltons fragment (cf. Fig. 4) strongly indicate, therefore, that practically all of this polypeptide is incorporated correctly into the outer membrane. We cannot make definite statements concerning the location of the larger polypeptide. It cannot be extracted from cell envelopes with Sarkosyl and it is thus most likely also associated with the outer membrane (Acthman et al., 1979). In any event the data show that the 98 amino acid residues at the CO_2H-terminus of protein II* (almost a third of the protein) are not required in vivo for its incorporation into the outer membrane. Very recently Movva et al. (1980) reported results indicating that an *ompA* protein missing a CO_2H-terminal portion (most likely the same fragment as the one we have described here) is incorporated as functional phage receptor into the outer membrane. Also, Achtman et al. (1979) noted that a 20,000-dalton amber fragment of the 25,000-dalton *tra T* protein (an outer membrane protein encoded on the F sex factor) is incorporated into the outer membrane of minicells.

Our results complement those obtained by gene fusions (e.g., Silhavy et al., 1977; Bassford et al., 1979) creating hybrid polypeptides between *E. coli* cell envelope proteins and β-glactosidase. Of particular relevance to our study are such hybrids involving another outer membrane protein, the receptor for phage λ (*lamB* protein). A hybrid protein containing, at the NH_2-terminus, about $^1/_3$ of the *lamB* protein, and at the CO_2H-terminus almost all of the 116,000 dalton β-galactosidase was partially (20–30%) found associated with the outer membrane, partially (20–30%) with the plasma membrane, and the remainder was in the soluble fraction (Silhavy et al., 1977). In this case the distribution of β-galactosidase activity was measured and if, as it is apparently the case with our (still hypothetical) hybrid protein, proteolytic breakdown had destroyed more or less of the enzymatic activity or released it in soluble form, considerably more of the *lamB* fragment may have been incorporated into the outer membrane. Proteolysis has, in fact, also been observed with another hybrid having fused the periplasmic maltose binding protein to β-galactosidase (Bassford et al., 1979). These workers also found that if the membrane protein part of these hybrids became too short they were exclusively cytoplasmic. Both proteins possess an NH_2-terminal signal sequence and this finding indicates that it is not sufficient simply to attach a signal sequence to a protein in order to export it from the cytosol, although the results of these elegant studies may be somewhat biased by the enormous size of the enzyme used. Experiments are in progress to further shorten the NH_2-terminal part of the *ompA* gene and to determine the fate of the correponding polypeptides.

It may finally be noted that substitution of the tryptophan residue in position 7 of protein II* (Endermann et al., 1978) by serine (*supD*) or tyrosine (*supF*) does not interfere with phage receptor activity or membrane incorporation of the polypeptide. Experiments not described here have shown that the same is true for glutamine specified by the amber suppressor *supE*.

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