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Characterisation of the Promoters for the *ompA* Gene which Encodes a Major Outer Membrane Protein of *Escherichia coli*

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Summary. The regulatory region of the *ompA* gene from *Escherichia coli* has been characterized by biochemical and genetic approaches. Two overlapping promoters, P1 and P2, organized in that order with respect to the *ompA* coding sequence, were identified and it was found that *ompA* possesses an unusually long leader region. Both P1 and P2 were active in an in vitro transcription system although S1 mapping analysis of the *ompA* mRNA made in vivo showed that P2 was mainly responsible for transcription of the gene. Confirmation of this was obtained by studying down-promoter mutants of *ompA* cloned in pSC101. These mutants were classified into two groups, deletions and insertions. The deletions, which were caused by the IS102 insertion element found in pSC101 removed the −35 regions of both P1 and P2. However, since P2 was distally situated with respect to the IS element it was less extensively damaged and it is proposed that the residual P2 sequence is responsible for the low level of expression observed. In addition to an IS102 insertion in the promoter region four IS1 insertion mutants were characterized. These had integrated at different positions in the *ompA* leader region and were all incompletely polar.

Introduction

The OmpA protein is one of the major proteins of the outer membrane of *Escherichia coli* K12 (for a review see Osborn and Wu 1980). The 35,000 dalton polypeptide (Chen et al. 1980) spans the membrane (Enderman et al. 1978) and has several biological functions: it stabilizes the mating aggregates formed during F-dependent conjugation (Schweizer and Henning 1977; Skurray et al. 1974; Van Alphen et al. 1977) and is required, in combination with the lipoprotein (Braun 1975), for the structural integrity of the outer membrane and the generation of normal cell shape (Sonntag et al. 1978). Its non-physiological functions include a role in the uptake of a colicin (Chai and Foulds 1974) and the ability to serve as the receptor for several bacteriophages (Datta et al. 1977; Van Alphen et al. 1977).

In recent years various aspects of the OmpA system have been elucidated using the cloned *ompA* gene (Henning et al. 1979a; Beck and Bremer 1980; Movva et al. 1980a). These include a preliminary characterisation of the functional domains of the OmpA protein (Cole et al. 1982a) and the identification of some features required for membrane assembly (Bremer et al. 1982). One of the less well understood aspects is the regulation of expression of the *ompA* gene. Since the OmpA protein is present in $2–3 \times 10^5$ copies per cell its gene must be highly expressed. As a first step towards understanding this process we have characterised the promoter for the *ompA* gene using biochemical and genetic approaches.

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Materials and Methods

Bacteria, Plasmids and Growth Conditions. The *E. coli* K12 strain UH100 (*galK, gltA, ompA, pyrD, recA, rpsL, supE, thi, trp*; Bremer et al. 1980) was the host for all plasmids. The plasmids used and their derivatives are listed in Table 1. Cultures were grown at 37° C in L broth supplemented with tetracycline (10 μg/μl) or ampicillin (20 μg/μl), as appropriate.

General Nucleic Acid Techniques. The preparation of plasmid DNA, the isolation and 5′-end labelling of restriction fragments were recently described (Bremer et al. 1980; Cole et al. 1982b). DNA sequencing was performed according to Maxam and Gilbert (1980).

DNAseI Protection Experiments. These were performed as outlined by Galas and Schmitz (1979) but with the modifications of Talkington and Pero (1979). Restriction fragments labelled at one 5′-end were incubated in 20 μl of RNA polymerase binding buffer (Seeburg and Schaller 1975) containing $CaCl_2$ (2 mM) with RNA polymerase (2 U) for 10 min at 37° C before treatment with DNAseI (100 μg/ml). The reaction was terminated after 1 min by the addition of 20 μl stop solution (100 mM EDTA, 1 M NH_4 acetate, 20 μg tRNA). After precipitating with ethanol, the fragments were resuspended in formamide buffer (Maxam and Gilbert 1980) and heated for 2 min at 90° C. Reaction products were separated on a 10% polyacrylamide gel containing 8 M urea.

In vitro Transcription. The reaction mixtures contained a suitable DNA template (0.5 pmol), RNA polymerase (2 U), ATP, GTP, UTP (all at 100 μM), [α-^{32}P]CTP (10 μM; 400 Ci/mmol) and RNA polymerase binding buffer. After 10 min incubation at 37° C the mixture (20 μl) was processed as described by Jaurin et al. (1981). Transcripts were separated on a 5% polyacrylamide gel containing 8 M urea and visualised by autoradiography.

Isolation of Unlabelled RNA and S1 Mapping. RNA was purified from exponentially growing cells by extraction with hot phenol (60° C, pH 5). Hybridization of 5′-end labelled fragments (~0.1 μg) with RNA (150 μg) was performed essentially as described by Berk and Sharp (1977). After 5 h at 50° C 9 volumes of S1 nuclease buffer (0.25 M NaCl; 0.03 M Na acetate, pH 4.6;

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Table 1. Plasmids

Plasmid	Description	Reference
pTU100	pSC101 derivative bearing *ompA*$^+$	Henning et al. (1979a)
pTU104	pTU100 derivative with IS1 insertion 72	this study
pTU111	pTU100 derivative with IS1 insertion 33	this study
pTU112	pTU100 derivative with IS1 insertion 3	this study
pTU114	pTU100 derivative with IS1 insertion 8	this study
pTU115	pTU100 derivative with IS1 insertion 11	this study
pTU107	IS102 mediated deletion derivative of pTU100	this study
pTU108	IS102 mediated deletion derivative of pTU100	this study
pTU110	IS102 mediated deletion derivative of pTU100	this study
pTU113	IS102 mediated deletion derivative of pTU100	this study
pTU109	pTU100 derivative with IS102 insertion 7	this study
pTU302	pBR322 derivative bearing NH_2 terminal part of *ompA* 31 allele (an amber mutant)	Bremer et al. (1980)

1 mM $ZnSO_4$) were added and unhybridized DNA digested with S1 nuclease (~600 U). The samples were incubated for 30 min at 37° C then precipitated with ethanol, resuspended in formamide buffer and analysed on 6% polyacrylamide gels containing 8 M urea.

Isolation of Mutants Showing Reduced ompA Expression. Mutants resistant to the OmpA-specific phage TuII* (Datta et al. 1977) were isolated by plating UH100/pTU100 (5×10^8 cells) with TuII* (10^{10} P.F.U.) on L-medium containing tetracycline (10 µg/ml). After purification, suitable candidates were screened for residual OmpA production by testing with the K3 host range mutant K3h1 (Manning et al. 1976) or by a radio-immunoassay using ^{125}I-labelled anti-OmpA immunoglobulin (Henning et al. 1979b).

Synthesis of Proteins in vitro. This was performed essentially as described by Collins (1979) but with the modifications of Pratt et al. (1981). A typical reaction mixture (30 µl) contained 5 µl DNA (1.5 µg), 2 µl S30 extract (25 mg/ml protein concentration), 4.5 µl Mg acetate (0.1 M), 14 µl low molecular weight mix (according to Collins, 1979) and [^{35}S]-methionine (14 µCi). After 60 min incubation at 37° C, 5 µl pre-warmed methionine (44 mg/ml) was added and incubation was continued for a further 5 min. In those instances where the plasmid DNA was first-treated with restriction enzymes, a purification step consisting of phenol-extraction, ether-extraction and ethanol precipitation was included. The [^{35}S]-labelled proteins were then resolved by SDS-polyacrylamide gel electrophoresis (Henning et al. 1978b) and visualized by autoradiography.

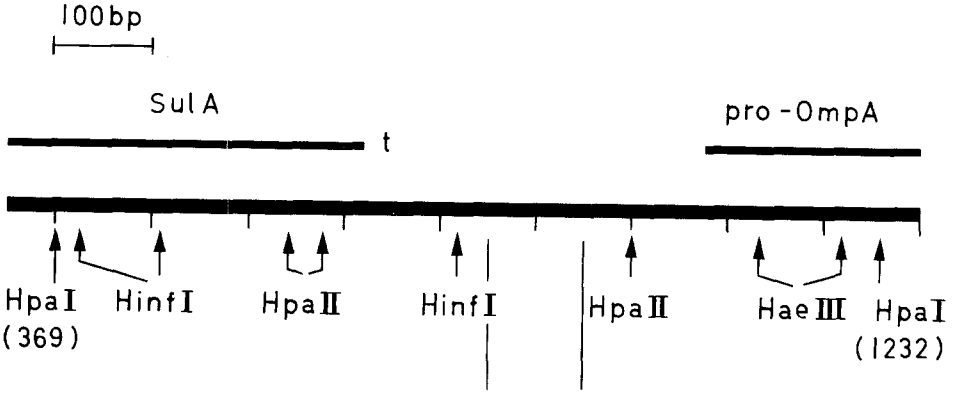
Sources of Materials. All enzymes were purchased from Boehringer Mannheim GmbH with the exception of S1 nuclease (Bethesda Research Laboratories). Isotopes were obtained from Amersham Buchler GmbH.

Results

In an earlier study (Beck and Bremer 1980) the nucleotide sequence of the *ompA* region of the *E. coli* chromosome was determined. It was, however, impossible to unambiguously identify the promoter of the *ompA* gene simply by inspecting the sequence. We have therefore performed a series of in vitro analyses with the cloned gene to achieve this and have also characterised a set of down-promoter mutants.

Location of the ompA Promoter

It was evident that the *ompA* promoter was located in a 300 base pair stretch (Fig. 1) between the terminator for the neighbouring *sulA* gene (Beck and Bremer 1980; Mizusawa and Gottesman 1982) and the beginning of the coding sequence for the pro-OmpA protein. Consequently, an ap-



820 830 840 850 860 870 880 890 900 910
AAAGATTAAA CATACCTTAT ACAAGACTTT TTTTTCATAT GCCTGACGGA GTTCACACTT GTAAGTTTTC AACTACGTTG TAGACTTTAC ATCGCCAGGG

Fig. 1. Restriction endonuclease cleavage map of the *ompA* promoter region. Parts of the coding sequence for the SulA and pro-OmpA proteins are shown. The inset shows the nucleotide sequence from positions 811 to 910, the underlined regions correspond to sites which were protected by RNA polymerase from DNAseI digestion. t denotes transcriptional terminator

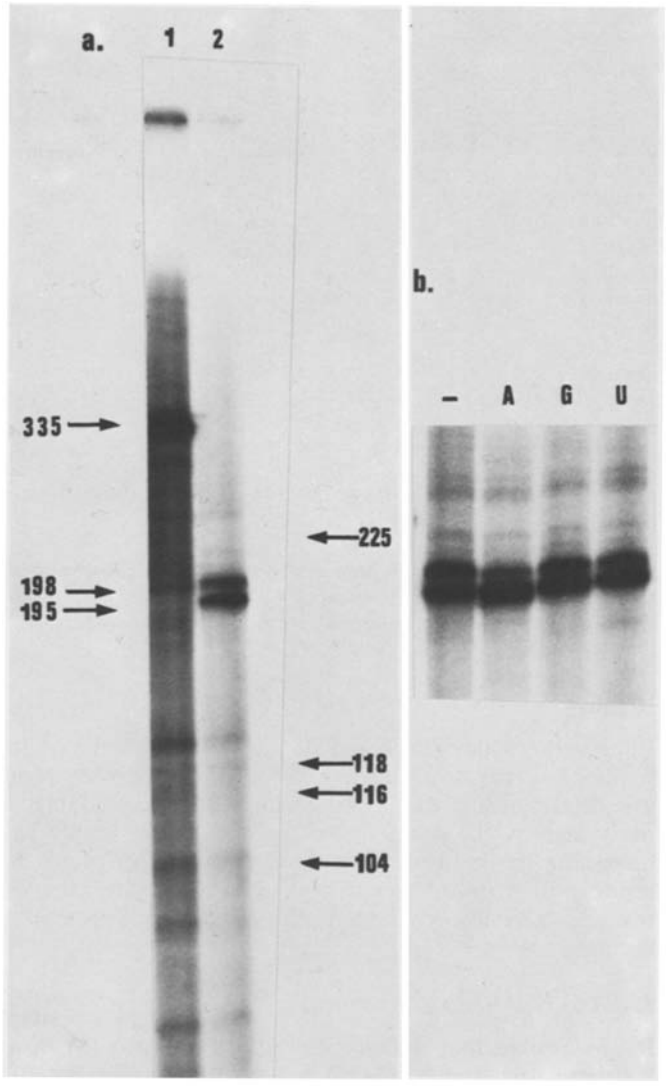


Fig. 2. a Polyacrylamide gel electrophoresis of RNAs synthesized in vitro in the presence of [α-^{32}P]CTP using the 861 base pair *Hpa*I fragment (1) or the 727 base pair *Hpa*I-*Hae*III fragment (2) as templates. The indicated size standards were RNAs synthesized from the *sulA* promoter (Cole, in preparation) and the RNA1 promoter of ColE1 (Morita and Oka 1977). **b** The effect of limiting the nucleotide concentration on transcription directed by the 727 base pair *Hpa*I-*Hae*III template. The limiting nucleotide is indicated

proximate location of the *ompA* promoter was sought by analysing restriction fragments from this intercistronic region by the DNAseI footprinting technique. RNA polymerase was used to protect a 315 base pair *Hin*fI-*Hae*III fragment, [^{32}P]-labelled at the *Hin*fI end, from the endonucleolytic activity of DNAseI. The reaction products were compared with those obtained in the absence of RNA polymerase by polyacrylamide gel electrophoresis and autoradiography. Two footprints were found (data not shown) and these corresponded to protection of the DNA between positions 830–845 and 857–885 (Fig. 1). The region between the two footprints was considerably more susceptible to DNAseI in the presence of RNA polymerase and the adenosine at position 856 was particularly vulnerable (Fig. 1).

To determine which of the footprints corresponded to the *ompA* promoter appropriate restriction fragments were isolated and used to direct RNA synthesis in an in vitro transcription system containing [α-^{32}P]CTP. When the 861 base pair *Hpa*I restriction fragment was employed a major RNA of about 335 nucleotides was produced (Fig. 2a). To unequivocally establish the direction of transcription a second template, the 451 base pair *Hin*fI-*Hpa*I fragment (Fig. 1) was utilised. This also directed the synthesis of the 335 nucleotide transcript (data not shown) thereby demonstrating that transcription proceeded into *ompA* and that the sequence upstream of the *Hin*fI site was not required. The broadness of the band corresponding to the 335 nucleotide "transcript" (Fig. 2a) suggested that it might be composed of two RNA species slightly different in size. This was confirmed when the 727 base pair *Hpa*I-*Hae*III restriction fragment (Fig. 1) was used to program RNA synthesis. Two transcripts were produced, a major species of 195 nucleotides and a minor species of 198 (Fig. 2a) as judged by comparison with suitable size standards.

By varying the concentration of a single ribonucleotide in the transcription mixture it was possible to determine the initiating nucleotides of the respective transcripts since it is known that initiation requires a higher nucleotide concentration than elongation does (Maizels 1973; Minkley and Pribnow 1973). When the ATP concentration was lowered to 5 μM the synthesis of the 198 nucleotide species, directed by the 727 base pair *Hpa*I-*Hae*III template, was significantly reduced (Fig. 2b). No effect was seen when the GTP or UTP concentrations were lowered. This indicated that the 198 nucleotide transcript initiated with adenosine and confirmation of this result was obtained when in vitro transcription was performed in the presence of [γ-^{32}P]ATP as this RNA could be terminally labelled. No effect on the synthesis of the 195 nucleotide transcript was observed when the ATP, GTP or UTP concentrations were lowered. This suggested that the transcript started with cytosine. From these findings it was concluded that the *ompA* promoter was located between nucleotides 860–890 and that transcription could initiate either with one of the adenosines at position 899 or 901 or, with one of the cytosines at position 900 and 903 (Fig. 1). This is in good agreement with the location of the second RNA polymerase binding site observed in the footprinting study.

To determine whether this promoter was also active in vivo and to precisely identify the start base the S1 mapping technique (Berk and Sharp 1978) was used to locate the 5′-end of the *ompA* mRNA. Total RNA was isolated from strains UH100/pTU100 and UH100/pTU302 and hybridized with the 181 base pair *Hin*fI-*Hpa*II fragment (Fig. 1), 5′-end labelled at the *Hpa*II site. The hybridization products were treated with S1 nuclease and the sizes of the protected DNA fragments determined by electrophoresis in parallel with the products of the Maxam and Gilbert sequencing reactions (Fig. 3). In both instances a major RNA species initiating with the cytosine at position 903 was detected and this corresponds to the major transcript seen in the in vitro system. A minor fraction of the *ompA* mRNA was found to initiate with the adenosine at position 900.

It is evident from the extent of the protection seen in Fig. 3 that plasmid pTU100 produces considerably more *ompA* mRNA than pTU302 even though there is an estimated difference in copy number of about 35 (pTU100 about 5 copies and pTU302 about 40 copies). There are two likely reasons for this discrepancy. Firstly, pTU302 carries only part of the *ompA* gene and lacks the COOH-

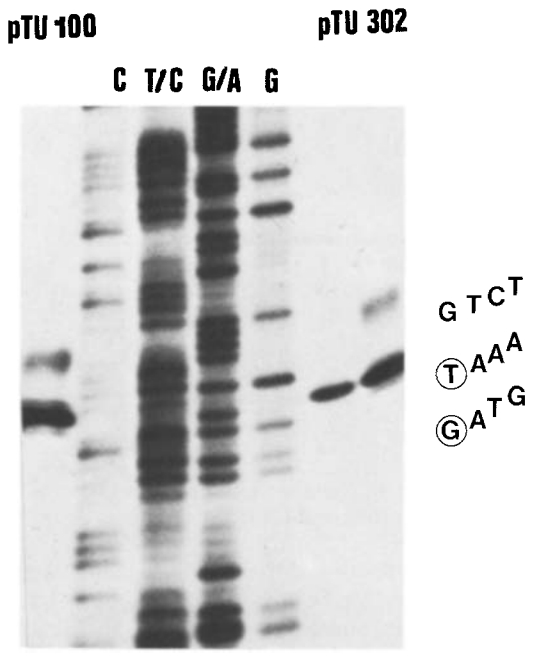


Fig. 3. S1 mapping of *ompA* mRNA synthesized in vivo by UH100/pTU100 (left) or UH100/pTU302 (right). Equal sized samples were treated in parallel, as described, then electrophoresed with the Maxam and Gilbert sequencing products. The sample volumes were pTU100 – 1 µl, pTU302 – 1 and 3.5 µl. When interpreting the results it should be remembered that the S1 nuclease products run about 1.5 nucleotides slower than the sequencing reaction products (Sollner-Webb and Reeder 1979). The sequence of the non-transcribed strand around the transcription initiation sites is shown and the bases corresponding to the 5′-termini are ringed. Extra bands are present in the T+C lane due to the reaction of hydrazine with guanosine

terminal region and the terminator. The mRNA produced from the residual gene fragment could therefore be more susceptible to degradation. Secondly, there is an in-frame amber codon early in the coding sequence (Beck and Bremer 1980; Bremer et al. 1980) which has an inhibitory effect on translational efficiency, and consequently affects the stability of the mRNA (Adhya and Gottesman 1978).

On inspection of the DNA sequence upstream from the transcriptional startpoints of *ompA* two likely promoter structures were found. These are shown in Fig. 4 where they are compared with the consensus promoter sequence (Rosenberg and Court 1979; Siebenlist et al. 1980). The −35 region of promoter 1 shows a better resemblance to the canonical −35 sequence and is located 16 bases upstream of a reasonable −10 region. There are 9 nucleotides between the invariant T in this −10 region and the less frequently used start site, the adenosine at position 899. The second promoter, P_2, also shows good homology with the consensus promoter sequence and partially overlaps P1. This situation is reminiscent of the organization of the promoters for the *gal* operon (Musso et al. 1977). The −10 region of P2 is situated 6 nucleotides upstream of the major start point for transcription of *ompA*.

Whilst this study was in progress Movva et al. (1980b) reported similar experiments with the *ompA* promoter. These workers only obtained in vitro transcripts when the reaction mixture contained 25% glycerol in contrast to our findings that extensive transcription was obtained when the glycerol concentration was 5%. Furthermore Movva et al.

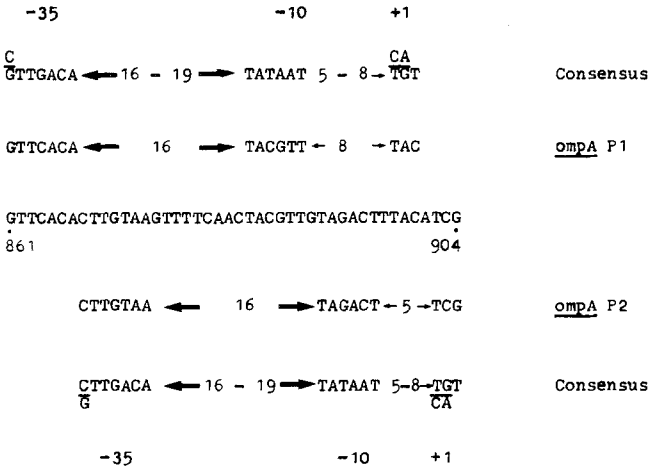


Fig. 4. Comparison of the *ompA* promoters with the consensus promoter sequence of Siebenlist et al. (1980) and Rosenberg and Court (1979)

only detected one *ompA* mRNA as opposed to the two found in this study. The reasons for these discrepancies are not known.

Isolation of Down-Promoter Mutations

To confirm our identification of the *ompA* promoter and to try to establish whether other factors were required for transcription a set of down-promoter mutants was isolated. This was achieved by selecting for derivatives of UH100/pTU100 which were resistant to phage TuII*. These were then classified into three groups by testing for the production of the OmpA protein by a radioimmunoassay. The major group (75% of the isolates) consisted of mutants that no longer produced the polypeptide whereas a second group (5%) synthesized an altered OmpA protein that could not function as the receptor for TuII*. The members of the third group (20% of the isolates) produced reduced amounts of OmpA protein and were thought to be down-promoter mutants. Some indication of the promoter strength of these mutants was obtained by testing 10 independently-isolated representatives for sensitivity to different OmpA-specific phages since these require different amounts of protein for infection to occur (Table 2; Henning et al. 1978). The cell evelopes were isolated from the mutants and compared with those of UH100/pTU100 by sodium dodecylsulphate-polyacrylamide gel electrophoresis (data not shown). It was apparent in all cases that OmpA production was reduced to roughly 2–10% of the wildtype level (Table 2).

Characterisation of the Down-Promoter Mutations

To determine the nature of the mutations causing reduced expression of *ompA* the appropriate plasmids were isolated and analysed by digestion with various restriction enzymes. This enabled the mutants to be classified into two groups (Fig. 5). Four of the mutant plasmids (pTU107, 108, 110 and 113) had incurred large deletions while a further five had apparently acquired an insertion element since they were slightly larger than pTU100. The size of the deletion was estimated as about 5.6 kilo-bases in each case and because it had one endpoint very close to the IS102 element located in the vector part of the plasmid (Ohtsubo et al.

Table 2. Properties of UH100 bearing various plasmids

Strain	Sensitivity to phages			Level of OmpA production[a] (% wildtype level)
	TuII	K3	K3h1	
UH100	–	–	–	0
UH100/pTU100	+	+	+	100
UH100/pTU104	–	+/–	+	2–5
UH100/pTU111	–	+/–	+	2–5
UH100/pTU112	–	+	+	10
UH100/pTU114	–	+	+	10
UH100/pTU115	–	+	+	10
UH100/pTU107	–	+/–	+	2–5
UH100/pTU108	–	+/–	+	2–5
UH100/pTU110	–	+/–	+	2–5
UH100/pTU113	–	+/–	+	2–5
UH100/pTU109	–	+/–	+	2–5

+/– denotes sensitivity when high moi used

[a] The OmpA concentrations should be regarded as approximations and not as exact values

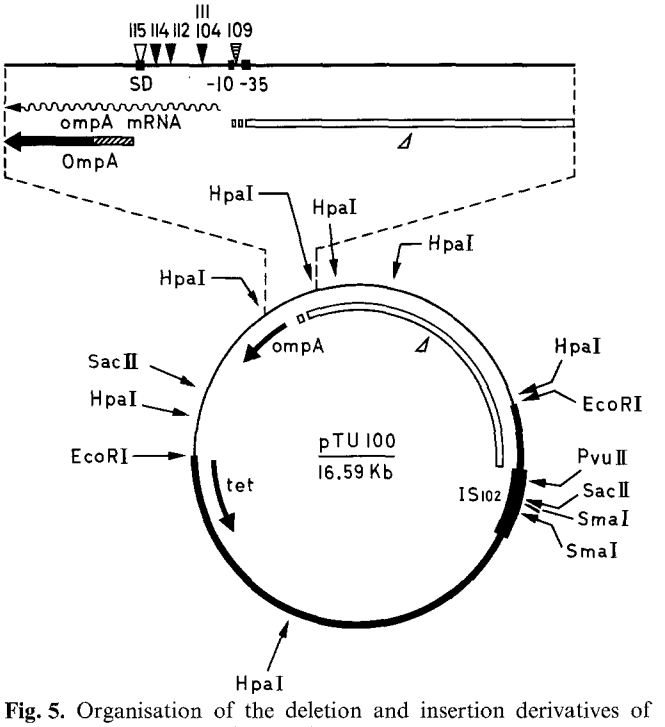


Fig. 5. Organisation of the deletion and insertion derivatives of pTU100. The extent of the deletions is shown together with the sites of insertion for IS102 (▽) and IS1 (▼). The IS1 insertion in the Shine-Dalgarno sequence (SD) has the opposite orientation (▽). Only the relevant restriction sites are indicated

1980) was thought to have been IS102 mediated. By digestion with *Hpa*I and *Pvu*II it was established that the other deletion endpoint was within the 861 base pair *Hpa*I fragment bearing the *ompA* promoter. A more precise location of the junction between IS102 and *ompA* was obtained by mapping this region by the method of Smith and Birnstiel (1976). This enabled us to define the deletion end point to within 20 base pairs since known restriction sites from the η end of IS102 (Ohtsubo et al. 1980) and the promoter region of *ompA* could be identified.

Restriction analysis of the insertion derivatives showed that the insertional events had all occurred close to the

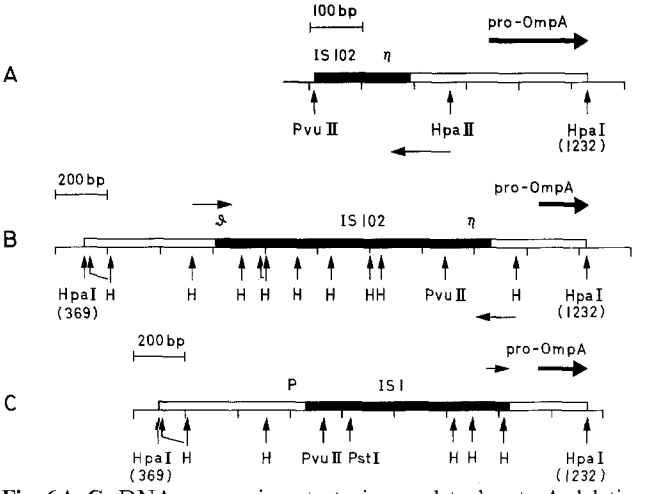


Fig. 6A–C. DNA sequencing strategies used to locate **A** deletion endpoints, **B** the IS102 insertion and **C** the IS1 insertions

ompA promoter and that two different IS elements were involved (Fig. 5). Plasmid pTU109 was about 1100 base pairs larger than pTU100 and had acquired new restriction sites for *Pvu*II, *Sma*I and *Sac*II. From these findings it was concluded that the IS102 element from the vector had transposed with a direct orientation into the *ompA* promoter region. This represents the first example of an IS102 transposition. The insertion elements in plasmids pTU111, 112, 114 and 115 were found to be about 800 base pairs long and to contain single recognition sites for *Pvu*II and *Pst*I. This indicated that they were IS1 (Ohtsubo and Ohtsubo 1978). By means of suitable double digestions it was demonstrated that the IS1 element in pTU115 had adopted the opposite orientation to those in pTU111, 112 and 114 (Fig. 5).

Sequence Analysis of Down-Promoter Mutations.

The precise locations of the deletion end points and the integration sites of the insertion elements were established by DNA sequencing using the strategies shown in Fig. 6. The sequences of the regions of interest are presented in Fig. 7.

In all four deletion mutants the η end of IS102 was found fused to the remains of the *ompA* promoter region. Each deletion had a different endpoint (Fig. 7a) and had removed the −35 regions of both promoters. In three cases the −10 regions of P1 and P2 were unimpaired but in the most extended deletion (pTU110) the −10 region of P1 had also been destroyed.

The second copy of IS102 found in pTU109 was located within the *ompA* promoter and separated the −35 region from the −10 region (Fig. 7a). A nine base pair duplication of the target sequence was found flanking the insertion element as has been reported by other workers (Ohtsubo et al. 1980). Expression of the *ompA* genes carried by pTU109 and the four deletion derivatives of pTU100 was weaker than that observed from those derivatives where IS1 had inserted near the *ompA* promoter (Table 2). Sequence analysis of these showed that all of the insertions had occurred in that part of the gene corresponding to the 5′ untranslated region of the mRNA and that the *ompA* promoter region remained undamaged. Each insertional event was accompanied by the duplication of a 9 base sequence and these are shown in Fig. 7b. The IS1 element in pTU111 was

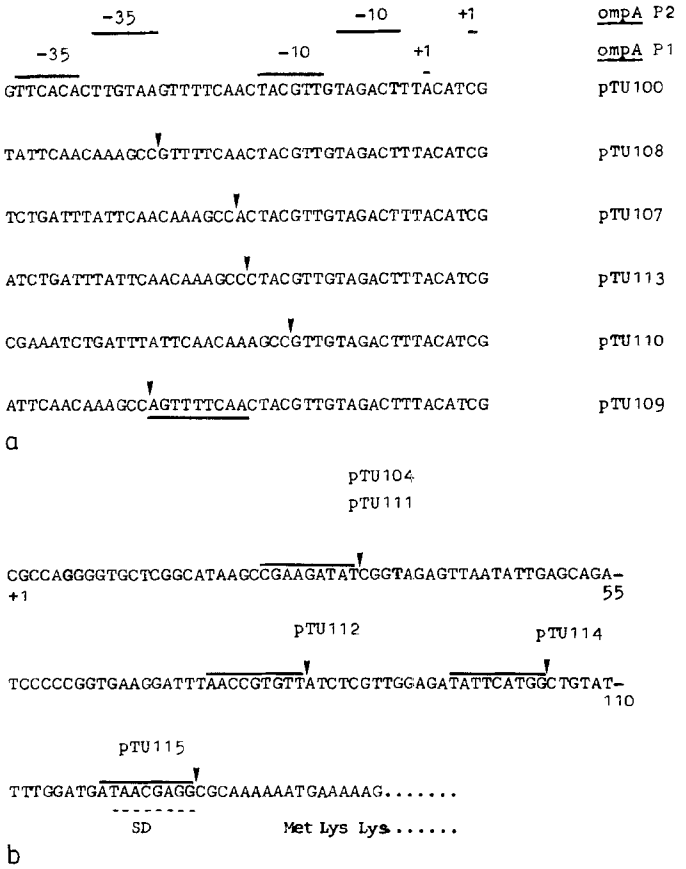


Fig. 7. a The nucleotide sequences of the IS102-mediated deletion end-points and the IS102 insertion found in pTU109. The junction between IS102 and *ompA* is indicated (▼) and the 9 base pair repeated sequence in pTU109 is underlined. **b** The nucleotide sequence of the *ompA* leader region showing the insertion points (▼) of the 4 IS1 insertions described in this study plus the previously-described IS1 insertion in pTU104. The 9 base pair repeats are overlined and the ribosome binding site (SD) is underlined (---)

found to have inserted at the same position and with the same polarity as the previously described IS1 insertion in pTU104 (Beck and Bremer 1980). In contrast, the IS1 element in pTU115 has integrated with the opposite orientation very close to the ribosome binding site of *ompA* (Fig. 7b). All of the sequences flanking the IS1 insertion points show modest homology to the ends of IS1 as described by Calos and Miller (1980).

Is ompA Being Expressed from an External Promoter?

In every case where IS102 has reduced *ompA* expression the promoter region has been extensively altered. This raised the possibility that expression resulted from transcription initiating at a promoter within the insertion element. IS102 contains one large open reading frame, encoding a protein of 224 amino acids (or 307 depending on the choice of initiation codons) aligned with the same polarity as *ompA* (Bernardi and Bernardi 1981). It was, therefore, conceivable that the promoter for this hypothetical gene was reading through *ompA*. To test this possibility the appropriate plasmids were cut with *Sac*II, thereby disconnecting *ompA* from the open reading frame in IS102 (Fig. 5) and used to direct protein synthesis in a cell-free system containing [^{35}S]methionine. Analysis of the resultant labelled polypeptides by polyacrylamide gel electrophoresis and autoradiography (Fig. 8) showed that the pro-OmpA protein was still synthesized. From this finding one can conclude that either the residual *ompA* promoter sequence is still active or, there is a promoter within IS102 distal to the *Sac*II site although no promoters have been detected in this region (Bernardi and Bernardi 1981). It should be pointed out that the levels of pro-OmpA protein produced by the mutants in vitro are several-fold higher than those seen in vivo and this may reflect the lack of competition for RNA polymerase which occurs in vivo.

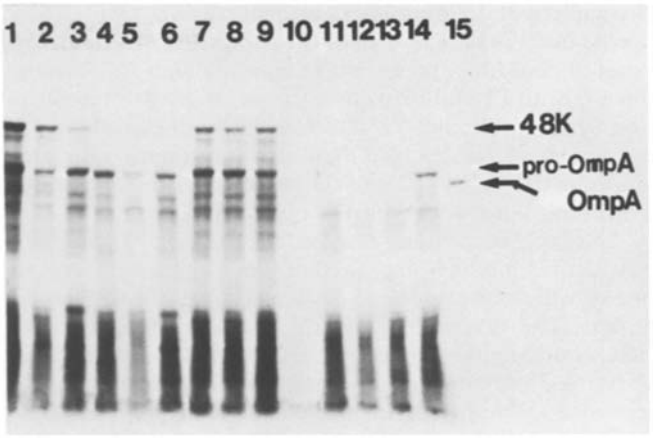


Fig. 8. Sodium dodecylsulphate-polyacrylamide gel electrophoresis of [^{35}S]-labelled proteins synthesized in a cell free system programmed by: Plasmids pTU100, 109, 108, lanes 1–3, respectively; *Sac*II digests of pTU100, 109, 108, lanes 4–6; plasmids pTU112, 114, 115, lanes 7–9, respectively; *Pst*I digests of pTU112, 114, 115, 100 lanes 11–14. Proteins made in the absence of DNA and in minicells containing pTU100 are shown in lanes 10 and 15, respectively. The positions of the pro-OmpA protein, the OmpA protein and an unidentified protein of approximate molecular weight, 48,000 (48 K) are indicated. Note the absence of processing in vitro. These experiments map the gene for the 48 K protein to the region between *ompA* and the *Eco*RI site in Fig. 5

Transcription Initiating at the OmpA Promoter Reads Through IS1

IS1 insertions, in both orientations, have a highly polar effect on expression of bacterial operons in vivo. Expression of *galK* is reduced to 1% of the wildtype level by IS1 and this polarity is *rho*-mediated (Das et al. 1977). Our finding that insertion of IS1 into the *ompA* leader region was incompletely polar in vivo suggested that transcription initiating at the *ompA* promoter reads through the element. This was confirmed by an in vitro protein synthesis analysis using the appropriate plasmids, linearized with *Pst*I, to direct polypeptide production (Fig. 8). This restriction enzyme cuts once within IS1 but not in *ompA*. The pro-OmpA protein was only produced when the IS1 containing templates were not cleaved with *Pst*I, in contrast to the control, pTU100, which was unaffected by this treatment (Fig. 8). The amount of protein produced by the mutants in vitro was again higher than that made in vivo.

Discussion

It is evident from this study that *ompA* can be expressed from two overlapping promoters, P1 and P2, both of which bear good resemblance to the consensus promoter sequence

(Rosenberg and Court 1979; Siebenlist et al. 1980). These are located 170 basepairs upstream of the translational start point of *ompA* in a region with unusually high A/T content (66%). Both P1 and P2 were active in an in vitro transcription system although P2 directed the synthesis of significantly more RNA. In vivo, however, transcription initiated almost exclusively at P2 and the resultant mRNA possessed a 5′-untranslated region of 134 nucleotides.

DNAseI footprinting revealed the presence of two RNA polymerase binding sites in the *ompA* regulation region, one of which corresponded to the −35 regions of the promoters. The second binding site is located in a very A/T rich stretch approximately 3 turns of the helix upstream of the −35 region of P2. This may indicate that such a sequence is important in attracting RNA polymerase to the gene thereby ensuring the constant supply of the enzyme required to maintain high levels of transcription. It should be noted that other highly expressed operons of *E. coli* such as the *lpp* gene, which codes for the lipoprotein (Nakamura and Inouye 1979) and the *unc* operon, encoding the ATP synthase (Gay and Walker 1981) also have exceptionally A/T rich sequences immediately upstream of their promoters.

The finding that the *ompA* gene is expressed from two overlapping promoters is not unique for a similar arrangement is seen in the *gal* operon of *E. coli* (Musso et al. 1977; Aiba et al. 1981). However, in the latter operon the choice of the promoter used is influenced by the growth conditions. Transcription from the *gal* P1 promoter (equivalent to *ompA* P2) is stimulated by the cAMP/CRP complex whereas transcription from *gal* P2 is repressed. Expression of *ompA* is essentially uninfluenced by growth conditions (Lugtenberg et al. 1976) and our preliminary findings suggest that the P1 promoter of *ompA* does not have a significant role in vivo although we cannot exclude that it is activated under certain circumstances.

Further support for P2 being the major promoter for *ompA* expression was provided by studying the IS102-mediated, down-promoter mutations. These have lost extensive parts of the regulatory region, including virtually all of P1, and produce the OmpA protein at about 2% of the wild type level. In each case, however, the −10 region of P2 plus between 6 and 16 of the upstream nucleotides remains intact. In no instance has the mutational event created a promoter like structure around the novel joint and it seems unlikely that transcription is initiating within IS102. We therefore believe that the residual P2 promoter sequence is still directing transcription and this is substantiated by the following facts. Firstly, there is a precedent for promoter activity in the absence of the normal −35 region: Okamoto et al. (1977) demonstrated that the fd phage promoter G3 was moderately expressed when the sequence upstream of −16 was replaced with a non-promoter sequence. Secondly, more compelling support is provided by the clustering of the five IS102 endpoints within a 13 base pair stretch between the −35 and −10 regions of P2. This strongly suggests that more extensive IS102-mediated deletions, ending in the leader region for instance, would have completely destroyed promoter activity and therefore not been detected by the selection used.

The insertion of IS1 into bacterial operons has a highly polar effect on their expression (Starlinger 1980) and this polarity is thought to be due to the *rho* dependent, premature termination of transcription (Adhya and Gottesman 1978). To date only a few examples of incompletely polar IS1 insertions have been described. These include IS1 insertions in bacteriophage λ (Kleckner 1981) which are suppressed when RNA polymerase is modified by the phage-encoded antitermination factor *N* at the *nut* sites (Greenblatt 1981). A more intriguing example is the insertion of IS1 in the ribosomal RNA operon, *rrnX* (Brewster and Morgan 1981) although this may represent a special case since transcription of the *rrn* genes is not coupled to translation. Brewster and Morgan (1981) postulated that this may be indicative of the involvement of a positive regulatory element in transcription of this operon.

The IS1 insertions in the *ompA* leader region reduce the synthesis of the OmpA protein by about 90%. This incomplete polarity suggests that transcription may initiate so frequently at the *ompA* promoter that there is insufficient *rho* factor to terminate every transcript. Alternatively, it may indicate that transcription of *ompA* is influenced by a positive regulatory factor which is antagonistic towards *rho*. A role for such a factor could be envisaged when one considers that the *ompA* gene possesses an unusually long 5′-untranslated region, with nonsense codons in all reading frames, and that extensive dyad symmetries are found within the coding sequence (Beck and Bremer 1980; Movva et al. 1980). Both of these features may have an inhibitory effect on transcription. It is apparent that if such a factor exists it is not absolutely required for transcription of *ompA* to occur thereby suggesting that it may have an enhancing effect.

To obtain a more sensitive system for studying the expression of *ompA* we are presently constructing plasmid derivatives in which *lacZ* is transcribed from the *ompA* promoter. We hope that this approach will enable us to gain more insight into the regulation of *ompA* expression and to identify any additional factors which may be involved.

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