Location on the *Escherichia coli* Genome of a Gene Specifying *O*-Acetylserine (Thiol)-lyase

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(Received 14 July 1983; revised 5 October 1983)

The plasmid pAB65, derived from a specialized transducing phage carrying DNA from about 52 min on the *Escherichia coli* genome, coded for two polypeptides of M_r approx. 34 000. The expression of one was regulated by cyst(e)ine and the *cysB* gene product and the other by the *cysB* gene product only. One of these polypeptides was a subunit of *O*-acetylserine (thiol)-lyase (EC 4.2.99.8); the other, associated with the *E. coli* membrane, was the N-terminus of the product of the λ *ben* gene. The pattern of peptide synthesis directed by plasmids carrying smaller DNA fragments indicated that the gene for *O*-acetylserine (thiol)-lyase was transcribed clockwise. The spectrum, amino acid composition and subunit number of the enzyme were determined. The enzyme appears homologous with the *Salmonella typhimurium* *cysK* gene product. This provides further evidence for the inversion of this region of the genome.

INTRODUCTION

Becker *et al.* (1969) have shown that *O*-acetylserine (thiol)-lyase (OASL; EC 4.2.99.8, previously known as *O*-acetylserine sulphydrylase A, OASS-A), isolated from *Salmonella typhimurium*, is a protein of M_r 68 000 and is composed of two identical subunits of M_r 34 000. The subunit of OASL is the product of the *cysK* gene. Addition of cyst(e)ine to cultures of *S. typhimurium* and *Escherichia coli* represses the synthesis of OASL (Kredich & Tomkins, 1966; Jones-Mortimer, 1968) and the expression of the cysteine biosynthetic enzymes requires a positive regulator that is specified by the *cysB*⁺ gene (Spencer *et al.*, 1967; Jones-Mortimer, 1968; Kredich, 1971; Wiater *et al.*, 1982). The *cysK* gene maps at about 52 min on the genomes of *E. coli* and of *S. typhimurium* (Fimmel & Loughlin, 1977; Wiater & Hulanicka, 1979; Hulanicka & Klopotoski, 1972; Hulanicka *et al.*, 1974).

In an attempt to refine the genetic map in this region of the *E. coli* genome we have subcloned DNA from the specialized transducing phage λ JM29, which carries the wild-type alleles of the *gsr*, *ptsH*, *iex*, *cysZ* genes and parts of the *ptsI* gene (Parra *et al.*, 1983a; Britton *et al.*, 1983). Since in UV-irradiated host cells containing the λ repressor protein expressed from the λ *clI*⁺ plasmid pKB280, the phage expressed polypeptides of M_r 9000, 21 000, 23 000, 33 000, 47 000 and 49 000 (Britton *et al.*, 1982), it was possible that the polypeptide of M_r 33 000 might be a subunit of OASL and, hence, that λ JM29 and plasmids subcloned from it also carried the *cysK* gene. We show here that OASL is specified by DNA approximately 2 kb anticlockwise from the *ptsI* gene,

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by a gene that is transcribed clockwise. We present evidence for the similarity of the *E. coli* and *S. typhimurium* enzymes. We believe the *E. coli* gene specifying OASL to be homologous to the *S. typhimurium* *cysK* gene.

METHODS

Strains and materials. The genotypes of the bacterial strains, plasmids and λ phage used are listed in Table 1. The source and grade of chemicals used are as listed by Britton *et al.* (1982). The minimal medium was that of Ashworth & Kornberg (1966).

Purification and characterization of plasmid DNA. The plasmid-containing strains were grown in 50 ml Luria broth (LB) containing 100 μ g ampicillin ml⁻¹. Plasmid DNA was amplified by adding chloramphenicol (170 μ g ml⁻¹) to the cultures and incubating them at 37 °C for 18 h. The amplified plasmid DNA was prepared as described by Birnboim & Doly (1979), followed by phenol/chloroform extraction and treatment with RNAase; it was analysed by agarose gel electrophoresis after restriction endonuclease digestion, as described by Boronat *et al.* (1982) and Britton *et al.* (1983). Plasmid DNA was stored in 10 mM-Tris/1 mM-EDTA buffer, pH 7.5, at 4 °C and its concentration was determined by measurement of the A_{260} , assuming that an A_{260} of 1.0 is equivalent to 50 μ g ml⁻¹ (Davis *et al.*, 1980).

Construction of the recombinant plasmids. Plasmid pAB15 was constructed, using DNA subcloned from the specialized λ transducing phage λ JM29, as described by Lee *et al.* (1982). Construction of plasmid pAB65 was also as described by Lee *et al.* (1982), the other plasmids were constructed as shown in Fig. 1. (The DNA fragments carried by the new plasmids are shown in Fig. 5.) The buffer systems used for the digestions were as described by Davis *et al.* (1980) and any enzymes inactivated by heating to 70 °C for 5 min. Ligation reactions were carried out by adding an equal volume of a freshly prepared solution, at pH 7.4, containing 2 mM-ATP, 20 mM-DTT, 10 mM-Tris/HCl, 10 mM-NaCl and 10 mM-MgCl₂ to the digested DNA solutions, adding 500 units T4 DNA ligase ml⁻¹ (Biolabs), and incubating at 14 °C for 18 h.

Transformation of *E. coli* cells with plasmid DNA. Recipient strains were made competent for transformation by the method of Dagert & Ehrlich (1979). Transformations were carried out by mixing 3.75 μ g plasmid DNA with 50 μ l of the competent cells (at 1.2×10^{10} cells ml⁻¹), incubating first at 0 °C for 10 min and then at 37 °C for 5 min, diluting with 1 ml LB, and incubating at 37 °C for 1 h. The transformed cells were selected on LB plates containing ampicillin (100 μ g ml⁻¹). The plasmids were initially transformed into and checked for tetracycline

Table 1. *Organisms and plasmids used*

All genetic markers are as described by Bachmann & Low (1980) except *gsr* and *iex* (Britton *et al.*, 1982, 1983; Parra *et al.*, 1983a). In the phage and plasmid designations *cys*⁺ refers to OASL which may be the product of either the *cysK* or *cysM* gene though our evidence suggests it is the product of the *cysK* gene.

Organism/plasmid	Genotype/phenotype	Origin
<i>E. coli</i> C600	<i>thr leu thi lacY</i>	J. Reeve
DS410	<i>minA minB thi str</i> ^R	Jones-Mortimer (1968)
JM14	<i>cysB</i>	Henderson <i>et al.</i> (1976)
JM559	(<i>his-gnd</i>) ^Δ <i>fda</i> ^{ts}	JM559 and λ NK55
JM1705	(<i>his-gnd</i>) ^Δ <i>fda</i> ^{ts} <i>trp</i> ::Tn10	Lee <i>et al.</i> (1982)
PB13	<i>cysE recA srlA</i> ::Tn10	Britton <i>et al.</i> (1982)
PB15	<i>thyA ptsI iex cysZ str</i> ^R <i>recA srlA</i> ::Tn10	
PB19	<i>minA minB thi str</i> ^R <i>trp</i> ::Tn10	P1.JM1705→DS410
PB20	<i>minA minB thi str</i> ^R <i>cysB</i>	P1.JM14→PB19
FP14	<i>metB ptsI^{ts} ptsH iex umgC</i>	Britton <i>et al.</i> (1982, 1983)
FP45	<i>metB ptsI^{ts} ptsH iex umgC recA srl</i> ::Tn10	P1.PO686→FP14
PO686	<i>recA srlA</i> ::Tn10	P. Oliver
λ λ JM29	<i>cl857 S7 xis6 nin5 b515 b519</i> <i>gsr</i> ⁺ <i>ptsH</i> ⁺ <i>iex</i> ⁺ <i>cys</i> ⁺ <i>cysZ</i> ⁺	Britton <i>et al.</i> (1982, 1983)
λ NK55	<i>b221 cIII167</i> ::Tn 10 <i>cl857 ind</i> ⁺ <i>Oam29</i>	Kleckner <i>et al.</i> (1978)
Plasmid pBR322	Ap ^R Tc ^R	Bolivar <i>et al.</i> (1977)
pAB15	Ap ^R	Lee <i>et al.</i> (1982)
pAB63	Ap ^R	This paper
pAB65	Ap ^R <i>ptsH</i> ⁺ <i>cys</i> ⁺	Lee <i>et al.</i> (1982)
pAB68	Ap ^R	This paper
pAB70	Ap ^R <i>ptsH</i> ⁺	This paper
pAB101	Ap ^R <i>ptsH</i> ⁺ <i>cys</i> ⁺	This paper

Fig. 1. Construction of the various recombinant plasmids [for construction of plasmids pAB15 and pAB65 see Lee *et al.* (1982)]. Plasmids pAB15, pAB65, pAB70 and pAB101 were all shown to be *ptsH*⁺ by transformation of the *ptsH* strain FP45 to the *PtsH*⁺ phenotype. Plasmid pAB65 did not carry the *cysZ*⁺ gene as it did not transform the *cysZ* strain PB15 to a cysteine prototroph. All the plasmids were phenotypically ampicillin-resistant but tetracycline-sensitive.

sensitivity in strain C600. The plasmids were then checked for their ability to complement the *ptsH* lesion in the *recA* strain FP45.

Preparation of plasmid-containing minicells. Minicells were prepared by a modification of the method described by Reeve (1979). The minicell-producing strains DS410 or PB20, containing the relevant plasmid, were grown overnight in 500 ml LB containing 10 mM-glucose, 64 µg L-methionine ml⁻¹, 2 µg thiamin ml⁻¹ and 100 µg ampicillin ml⁻¹. Cystine (100 µg ml⁻¹) was included in the growth media for strain PB20. Modifying the method of Levy (1970), the cultures were centrifuged at 650 *g* for 10 min in a Sorvall RC 5B centrifuge to remove normal cells and the supernatant fractions were centrifuged again at 16000 *g* for 10 min. The minicells were resuspended in 12 ml sulphur-free Hershey salts (Worcel & Burgi, 1974) and were purified twice through 10–30% (w/v) sucrose gradients, containing sulphur-free Hershey salts prepared as described by Reeve (1979); these centrifugations were carried out in a 6 × 38 ml swing-out rotor in an MSE Superspeed 65 preparative ultracentrifuge at 5000 *g* for 7 min. After the second sucrose gradient centrifugation, the minicells were spun at 20000 *g* for 10 min in a Sorvall RC 5B centrifuge and were resuspended in 4 ml sulphur-free Hershey salts (about 10¹¹ minicells ml⁻¹). The minicell preparations were checked for contamination with normal-sized cells by phase-contrast microscopy: on average, less than one normal cell was found per ~10⁸ minicells. The minicells were then centrifuged at 20000 *g* for 10 min and were resuspended in sulphur-free Hershey salts containing 30% (v/v) glycerol, to approximately 2 × 10¹⁰ minicells ml⁻¹ (assuming an *A*₆₀₀ of 1 is equivalent to 10¹⁰ minicells ml⁻¹) and stored at -80 °C.

Expression of plasmid-encoded proteins in minicells. Samples (100 µl) of purified minicells (2 × 10¹⁰ minicells ml⁻¹) were centrifuged in a 5412 Eppendorf microfuge for 7 min at room temperature and were resuspended in 100 µl sulphur-free Hershey salts containing 20 mM-glucose, 2 mM-cAMP, 100 µg ampicillin ml⁻¹ and 50 µg of D-cycloserine ml⁻¹ [to destroy any contaminating viable cells (Reeve, 1979)]. The samples were incubated at 37 °C for 1 h to destroy any endogenous mRNA. Labelling of the plasmid-encoded proteins was carried out by adding L-[³⁵S]methionine (final concentration 200 µCi ml⁻¹; 7.4 MBq ml⁻¹) or [U-¹⁴C]protein hydrolysate (final concentration 10 µCi ml⁻¹; 0.37 MBq ml⁻¹) and incubating for 1 h at 37 °C. The incorporation of isotope was stopped by adding either L-methionine, to a final concentration of 800 µg ml⁻¹ when ³⁵S was the isotope used, or 10 µl 1% casein hydrolysate when ¹⁴C-labelled amino acids were used, and incubating for 10 min at 37 °C. The

minicells were then centrifuged for 7 min in a 5412 Eppendorf microfuge and resuspended in 20 μ l SDS-dissociating buffer and analysed by SDS-PAGE using 12–20% gradient gels (Britton *et al.*, 1982). ^{35}S -Labelled polypeptides were identified by fluorography as described by Britton *et al.* (1982). The ^{14}C -labelled amino acid-containing polypeptides were exposed for 5 d at -80°C in contact with the X-ray film. The sizes of the polypeptides were calculated using the following marker proteins: BSA (68500), bovine γ -globulin (50000 and 23500), ovalbumin (43000), myoglobin (18500) and lysozyme (14300).

Fractionation of minicells. After labelling with isotopes, the minicells were centrifuged for 7 min in a 5412 Eppendorf microfuge and were resuspended in 40 μ l 0.2 M-Tris/HCl, pH 8.0. Spheroplasts were prepared by a slight modification of the method described by Witholt *et al.* (1976a). To the minicell suspensions was added 0.2 M-Tris/HCl, pH 8.0, containing 1 M-sucrose and 1 mM- K_2EDTA , pH 8.0 (40 μ l) followed 2 min later by 20 μ l lysozyme (1 mg ml $^{-1}$) in the sucrose buffer. After incubation at room temperature for 2 min, the minicells were exposed to a mild osmotic shock by the addition of 100 μ l deionized water to trigger lysozyme penetration of the outer membrane (Witholt *et al.*, 1976b). The minicells were then incubated at room temperature for 1 h. The spheroplasts were centrifuged as above and were burst by resuspending them in 20 μ l water. After centrifugation for 15 min, the supernatant solutions containing the cytoplasmic proteins were removed. The membranes were washed twice in 100 μ l water and were finally resuspended in 20 μ l water. Both the cytoplasmic and membrane fractions were analysed by SDS-PAGE after the addition of 20 μ l SDS-dissociating buffer; staining the gels with Coomassie blue showed reproducible differences in the protein profiles.

Assay of *O*-acetylserine (thiol)-lyase. Strains were grown in minimal media supplemented with 10 mM-sodium lactate and ampicillin (100 $\mu\text{g ml}^{-1}$) with or without cystine (100 $\mu\text{g ml}^{-1}$) at 37°C overnight. Samples (20 mg dry mass ml $^{-1}$) were sonicated in an ultrasonic bath (Megason) for 10 min, centrifuged and the supernatants assayed for the production of cysteine from *O*-acetylserine and hydrogen sulphide according to the method of Fimmel & Loughlin (1977).

Purification of *O*-acetylserine (thiol)-lyase. A crude extract from strain PB13(pAB65) was prepared as described by Lee *et al.* (1982), except all buffers contained 0.1 mM-phenylmethylsulphonyl fluoride and 1 mM-benzamidine. OASL was initially purified by ion-exchange chromatography on a Whatman DE-52 DEAE-cellulose column (2.5 $\times$ 7 cm) previously equilibrated with 0.01 M-Tris/HCl, pH 7.6. A 400 ml linear concentration gradient (0–0.2 M-NaCl in 0.01 M-Tris/HCl, pH 7.6) was used to elute OASL. Fractions (4 ml) were collected and analysed by SDS-PAGE on 12–20% gradient gels; those containing a large excess of a polypeptide of M_r 34000 were pooled and dialysed against 0.01 M-potassium phosphate buffer, pH 7.6. The dialysed material was then applied to a hydroxyapatite column (1 $\times$ 6 cm) previously equilibrated with 0.01 M-potassium phosphate buffer, pH 7.6, and was eluted directly onto a second Whatman DE-52 DEAE-cellulose column (1 $\times$ 6 cm). OASL was eluted with 0.15 M-NaCl in 0.01 M-Tris/HCl, pH 7.6, and further purified by gel permeation chromatography on an LKB Ultragel AcA 44 column (1.5 $\times$ 55 cm) equilibrated with 0.1 M-Tris/HCl, pH 7.6. Fractions showing a single polypeptide of M_r 34000 were pooled and assayed for OASL activity.

Ultracentrifugation analysis. Ultracentrifugation was carried out in a Spinco Model E analytical ultracentrifuge. Sedimentation velocities were determined at 59780 r.p.m. in a single sector cell or 52640 r.p.m. in a double sector cell using Schlieren optics with continuous temperature measurement. All samples were centrifuged after exhaustive dialysis in 0.1 M sodium and potassium phosphate buffer containing 0.05 M-NaCl at pH 7.5 (ionic strength = 0.1). The partial specific volume ($\bar{v}$) of OASL was determined from the amino acid analysis as described by Lee & Timasheff (1974).

Amino acid analysis. Amino acid analyses were performed on an LKB model 4400 amino acid analyser. Samples (0.5 mg ml $^{-1}$) were exhaustively dialysed against 10 mM- NH_4HCO_3 and freeze-dried. Samples were hydrolysed as described by Lee *et al.* (1982). A sample was initially oxidized with performic acid and then hydrolysed in order to determine cysteine as cysteic acid (Perham, 1978).

RESULTS

Expression of the recombinant plasmids

Two polypeptides, of M_r 28000 and 26000, were expressed from all recombinant plasmids. These are the product of the ampicillin resistance gene, *bla*, present on pBR322 DNA (Sutcliffe, 1978). The polypeptide of M_r 26000 is the processed and active form of the *bla* gene product (M_r 28000) (Ambler & Scott, 1978).

Plasmid pAB65 expressed three polypeptides, of M_r 9000, 33000 and 49000; these were also expressed by the specialized transducing phage λ JM29 (Britton *et al.*, 1982) from which pAB65 was derived (Lee *et al.*, 1982). The polypeptides of M_r 49000 and 9000 are known to be the products of part of the *ptsI*, and of the *ptsH* genes, respectively (Britton *et al.*, 1982; Lee *et al.*, 1982). However, we suspected that the polypeptide of M_r 33000 might be a doublet. To improve the

Fig. 2. Fluorogram of the [^{35}S]methionine-labelled polypeptides expressed from the recombinant plasmids in minicells. Track 1, the ampicillin-resistant gene products, modified and unmodified, derived from plasmid pBR322; track 2, expression pattern from λJM29 as described by Britton *et al.* (1982); track 3, expression pattern of plasmid pAB65; track 4, expression pattern of plasmid pAB101; track 5, expression pattern of plasmid pAB63; track 6, expression pattern of plasmid pAB68. The gels were 12–20% gradients, electrophoresed at 4 °C. a, Enzyme I fragment; b, bEa59 fragment; c, OASL; d, bEa59 fragment; e, OASL fragment; f, β -lactamase; g, HPr (histidine-containing protein of the phosphotransferase system).

resolution of the SDS-PAGE, the analysis of the polypeptides expressed by plasmid pAB65 was carried out at 4 °C; under these conditions, not only a polypeptide of M_r 33 000 but also another of M_r 34 000 was observed (Fig. 2, track 3). The polypeptide pattern obtained after labelling with [^{14}C]amino acids appeared to be identical to that obtained after labelling with [^{35}S]methionine. Analysis of the polypeptides expressed by the specialized transducing phages at 4 °C also showed that several expressed a polypeptide of M_r 34 000, which co-electrophoresed with a polypeptide expressed from plasmid pAB65, but no polypeptide of M_r 33 000 was expressed from any phage (Fig. 2, track 2 for the expression of λJM29).

We suspected that the polypeptide of M_r 34 000 might be a subunit of OASL since the *cysK* gene is known to map at about 52 min on the *E. coli* chromosome (Fimmel & Loughlin, 1977). The *cysB* minicell-producing strain PB20 was constructed by transduction to determine the effect on the expression of the polypeptide of M_r 34 000 in the absence of the positive regulator of the cysteine biosynthetic enzymes. Plasmid pAB65 was expressed in minicells derived from strain DS410 in the presence and absence of cystine ($120\ \mu\text{g ml}^{-1}$) and any polypeptides

Fig. 3. Fluorogram of the [^{35}S]methionine-labelled polypeptides expressed in the presence or absence of cystine in the *cysB*⁺ strain DS410 or in the *cysB* strain PB20 minicells. Track 1, DS410(pAB63) minicells, no cystine; track 2, DS410(pAB63) minicells plus cystine; track 3, PB20(pAB63) minicells; track 4, DS410(pAB65) minicells, no cystine; track 5, DS410(pAB65) minicells plus cystine; track 6, PB20(pAB65) minicells; track 7, DS410(pAB68) minicells, no cystine; track 8, DS410(pAB68) minicells plus cystine; track 9, DS410(pAB70) minicells, no cystine; track 10, DS410(pAB70) minicells plus cystine. a, Enzyme I fragment; b, OASL; c, bEa59 fragment; d, OASL fragment; e, OASL fragment; f, β -lactamase; g, HPr (histidine-containing protein of the phosphotransferase system).

expressed were labelled with [^{14}C]amino acids. The plasmid was also expressed in strain PB20 previously grown in the presence of cystine and then sulphur-starved. Polypeptides were again labelled with [^{14}C]amino acids. The expression of the polypeptide of M_r 34000 was depressed both in the presence of cystine in the *cysB*⁺ strain DS410 and in the *cysB* strain PB20 in the absence of cystine (Fig. 3, tracks 4, 5 and 6). The expression of the polypeptide of M_r 33000 was depressed only in the *cysB* strain PB20.

To identify the location of the genes expressing the polypeptides of M_r 34000 and 33000, several plasmids were derived from plasmid pAB65 (Fig. 1). Plasmid pAB63 expressed a polypeptide of M_r 30800 (Fig. 2, track 5) which was also repressed in the presence of cystine in minicells derived from the *cysB*⁺ strain DS410 and in minicells derived from the *cysB* strain

Table 2. Plasmid-specified *O*-acetylserine (thiol)-lyase activity

Activity was measured as described by Fimmel & Loughlin (1977). Five determinations were carried out on each extract, using different amounts of extract; activity is expressed relative to that of strain DS410(pBR322) grown in the absence of cystine. This latter formed 27 pmol cysteine (mg dry mass cell extract)⁻¹ min⁻¹.

Plasmid	Host ... Cystine in medium	Relative OASL activity		
		DS410 (<i>cysB</i> ⁺) ...	DS410 (<i>cysB</i> ⁺) +	PB20 (<i>cysB</i>) -
pBR322		100	4	ND
pAB63		100	4	7
pAB65		433	233	30
pAB68		215	4	ND
pAB70		144	15	ND
pAB101		741	348	130

ND, not done.

PB20 (Fig. 3, tracks 1, 2 and 3). This suggested that the initiation site for one of the polypeptides lay within the H1–B1 region of the cloned DNA (Fig. 5) and that its promoter was under the regulation of cysteine and the *cysB* gene product.

Plasmid pAB68 expressed two polypeptides of *M*_r 30800 and 33000 (Fig. 2, track 6). The polypeptide of *M*_r 30800 was repressed by cystine in the minicell system but the expression of the polypeptide of *M*_r 33000 was not depressed in the presence of cystine (Fig. 3, tracks 7 and 8). The difference between plasmids pAB63 and pAB68 was that plasmid pAB68 contained a piece of λ DNA, fragment λ E54.3– λ H57 (Fig. 5). This raised the possibility that the polypeptide of *M*_r 33000 might be expressed from λ DNA and that the polypeptide of *M*_r 30800 was a fragment of the polypeptide of *M*_r 34000. To answer this problem two more derivatives of plasmid pAB65 were constructed, plasmids pAB70 and pAB101 (Figs 1 and 5).

Plasmid pAB70 expressed three polypeptides of *M*_r 49000, 32000 and 9000 (Fig. 3, track 9) confirming that the polypeptides of *M*_r 49000 and 9000 originated from the H57–H1 DNA fragment as observed by Britton *et al.* (1983). The polypeptide of *M*_r 32000 must originate from the H1–B1 DNA fragment as a polypeptide of *M*_r 30800, showing the same regulatory properties, was expressed from plasmids pAB63 and pAB68. The polypeptide of *M*_r 32000 was repressed in the presence of cystine (Fig. 3, tracks 9 and 10). The increase in the size of the polypeptide fragment (*M*_r 32000 as opposed to 30800) is probably due to a different stop codon or transcriptional termination site resulting from the H1–B1 fragment being joined to a small piece of λ DNA in pAB70 rather than to pBR322 DNA as in pAB65. The differences between plasmids pAB70 and pAB65 were that the λ E54.3– λ H57 DNA fragment was absent and that the λ H57–H1 DNA fragment was orientated in the opposite direction (Fig. 5). The λ H57–H1 DNA fragment of pAB70 was re-orientated to give plasmid pAB101, which thus differed from plasmid pAB65 only by the absence of the λ E54.3– λ H57 DNA fragment. Plasmid pAB101 expressed three polypeptides of *M*_r 49000, 34000 and 9000 (Fig. 2, track 4); the polypeptide of *M*_r 34000 was repressed in the presence of cystine (data not shown). A polypeptide of *M*_r 34000 was specified by pAB101 and a polypeptide of *M*_r 32000 by pAB70. This would only be possible if the polypeptides of *M*_r 30800 and 32000 were fragments of the polypeptide of *M*_r 34000. The transcription of these polypeptides therefore originated in the H1–B1 DNA fragment and ran into the K2–H1 DNA fragment (Fig. 5).

Determination of *O*-acetylserine (thiol)-lyase activity

The activity of OASL in plasmid-bearing strains was investigated under different conditions: (a) in the absence of cystine, (b) in the presence of cystine and (c) in the *cysB* strain PB20 (Table 2). Only two plasmids, pAB65 and pAB101, caused the expression of OASL activities higher than normal; in the absence of cystine there was a 4.3-fold increase in the case of pAB65 and a 7.4-fold increase in the case of pAB101 compared to basal levels in the absence of cystine. The

Table 3. *Amino acid composition of O-acetylserine (thiol)-lyase*

Amino acid	Composition [mol residue (mol subunit) ⁻¹] according to:†	
	This paper	Becker <i>et al.</i> (1969)
Asp	24.7	25.5
Thr*	21.0	24.0
Ser*	22.1	17.0
Glu	37.1	37.5
Pro	15.2	15.8
Gly	35.4	32.6
Ala	30.1	30.8
Cys†	0.0	0.7
Val	18.8	19.2
Met	4.4	4.9
Ile	24.5	24.1
Leu	31.3	31.6
Tyr	5.6	6.4
Phe	14.4	7.2
His	3.1	3.0
Lys	22.9	25.5
Arg	12.7	13.4
Trp	ND	5.0

ND, not determined.

* Determined from means of 24 h, 48 h and 72 h hydrolysis times.

† Determined as cysteic acid after performic acid oxidation.

‡ Determined after extrapolation to zero time.

activities of OASL in strains transformed with these plasmids, was still regulated by cystine and to a lesser extent the *cysB*⁺ product (Table 2). These findings confirmed that plasmids pAB65 and pAB101 carried a gene for OASL. The incomplete repression of OASL by cystine in strains carrying the plasmids is not in accord with the simplest forms of the positive control model. Though it may be necessary to elaborate the model, the possibility exists that the residual expression is from another *E. coli* promoter in the H1-B1 DNA fragment.

The observation that plasmids pAB65 and pAB101 increased the production of OASL also confirmed that the polypeptide of *M*_r 34000 must be the product of a structural gene for OASL. The polypeptide of *M*_r 33000 did not have any role in the activity of OASL, as shown by comparison of plasmid pAB68, which expressed the polypeptide of *M*_r 33000 but had no OASL activity, with plasmids pAB65 and pAB101.

Properties of O-acetylserine (thiol)-lyase

The OASL isolated had an intense yellow colour indicative of the pyridoxal phosphate prosthetic group and the absorption spectrum was identical with that described by Becker *et al.* (1969).

Amino acid composition. The amino acid composition (Table 3) was found to be very similar to that obtained by Becker *et al.* (1969) for the OASS-A produced by the *S. typhimurium* *cysK* gene.

Molecular weight. Three different concentrations of purified OASL were used [0.5%, 0.25% and 0.125% (w/v)] in order to calculate a *s*_{20,w}⁰ value. In each case a single and symmetrical sedimenting boundary was observed. The sedimentation velocities were calculated and corrected to their *s*_{20,w} values. They were then extrapolated to zero protein concentration yielding a value for *s*_{20,w}⁰ of 4.7S. This indicated that the native protein had an *M*_r of about 65000. A protein of *M*_r 34000 would be expected to have a sedimentation velocity of about 2.5S.

The molecular weight of the active OASL molecule was calculated using the equation (Schachman, 1959):

$$M = Nf.s_{20,w}^0 \cdot \frac{1}{(1 - \bar{v}\rho)}$$

Fig. 4. Fluorogram of the [^{35}S]methionine-labelled polypeptides expressed from the recombinant plasmids pAB65 and pAB101 following fractionation into cytoplasmic and membrane components of the minicells. Track 1, expression pattern of plasmid pAB65 without fractionation; track 2, cytoplasmic polypeptides from pAB65; track 3, membrane-associated polypeptides from pAB65; track 4, cytoplasmic polypeptides from pAB101; track 5, membrane-associated polypeptides from pAB101. a, Enzyme I fragment; b, OASL; c, bEa59 fragment; d, β -lactamase; e, HPr (histidine-containing protein of the phosphotransferase system).

where the $\bar{v}$ was calculated as 0.744 from the amino acid composition. The frictional coefficient (f) was calculated assuming an f/f_0 ratio of 1.15, the ratio obtained for haemoglobin (M_r 68 500) (Alexander & Johnson, 1949). The molecular weight of OASL was found to be 64 000 indicating that it was a dimer of the polypeptide (M_r 34 000) expressed from the cloned *E. coli* DNA.

Identity of the polypeptide of M_r 33 000

Several of the plasmids were expressed in DS410-derived minicells. The resulting proteins were labelled with [^{35}S]methionine, fractionated into cytoplasmic- and membrane-associated proteins and analysed by SDS-PAGE. The processed and unprocessed products of the *bla* gene were lost in the fractionation process indicating that they were present in the periplasmic fraction as observed by Ambler & Scott (1978). The polypeptides of M_r 49 000, 34 000 and 9000 were all found to be associated with the cytoplasm (Fig. 4, tracks 2 and 4). The polypeptide of M_r 33 000 was found to be entirely membrane-associated (Fig. 4, track 3).

The difference between plasmids pAB63 and pAB68, and plasmids pAB65 and pAB101, was that plasmids pAB65 and pAB68 contained a piece of λ DNA stretching from the λ E54.3 site to the λ H57 site (Fig. 5). The plasmids carrying λ DNA also expressed a polypeptide of M_r 33 000 that had no OASL activity, as observed from plasmid pAB68, and which was membrane-

Fig. 5. Maps of λ JM29 and of *E. coli* DNA in recombinant plasmids derived from it. The DNA derived from *E. coli* is shown as a solid line and that from bacteriophage λ as an open box. The λ H57-H1 fragment in plasmid pAB70 is inverted as compared to its original orientation in plasmid pAB65. Only the relevant polypeptides expressed by the plasmids are included. The arrows show the position and direction of expression of the polypeptides of M_r 30800, 32000, 33000 and 34000. The position of the λ *ben* gene is derived from the position of bEa59 on the λ DNA map (Sanger *et al.*, 1982). The position of the gene producing OASL is deduced from the recombinant plasmids. For the construction of the plasmids see Fig. 1.

associated rather than cytoplasmic. The removal of the λ DNA from plasmid pAB65 (resulting in plasmid pAB101) caused the loss of the polypeptide of M_r 33000, confirming that it was specified by λ DNA. Examination of the λ DNA sequence reported by Sanger *et al.* (1982) from λ E54.3 to the λ attachment site, shows that it encodes part of the λ protein bEa59 (Epp, 1978), the product of the λ *ben* gene (Sumner-Smith *et al.*, 1982). Calculation of the relative molecular mass of the bEa59 fragment, from the amino acid sequence derived from the known DNA sequence, indicated that it should have an M_r of 33000. The sequence of the resulting polypeptide showed a high percentage (57%) of hydrophobic amino acids which occur in long consecutive stretches. This might cause hydrophobic domains and thus account for binding to the membrane.

The labelling of polypeptides expressed from the specialized λ transducing phages (Britton *et al.*, 1982) showed that two of the phages, λ JM29 and λ JM133, expressed a polypeptide of M_r 47000 (Fig. 2, track 2) originally thought to be the product of the *E. coli* *xap* gene (Britton *et al.*, 1982). Sumner-Smith *et al.* (1982), showed that the DNA encoding the bEa59 protein, the λ *ben* gene, contains part of the *b515* deletion. Using the λ map of Szybalski & Szybalski (1979) we calculated that the *b515* deletion in the λ *ben* gene would result in a bEa59 fragment of about M_r 45000, the loss being at the C-terminal end of the protein. This suggested that the polypeptide of M_r 47000 expressed from the two phages is the product of the partially deleted λ *ben* gene. It is known (Sumner-Smith *et al.*, 1982) and further confirmed by the λ DNA sequence data of F.

Sanger (unpublished) that the λ *ben* gene is expressed from the λ P_L promoter. The evidence from plasmids pAB65 and pAB68 indicated that the partially deleted λ *ben* gene could also be expressed from an *E. coli* promoter of the cysteine regulon, since repression of this promoter by the absence of the *cysB* gene product resulted in a decrease in the amount of the polypeptide of M_r 33000 being produced. It is probable that the polypeptide of M_r 47000 expressed by the specialized λ transducing phages λ JM29 and λ JM133 is also expressed from the same *E. coli* promoter, as, in the protein labelling experiments of Britton *et al.* (1982), the λ P_L promoter is completely shut off.

DISCUSSION

Our results show that the DNA subcloned from λ JM29, the λ E54.3-B1 fragment, encodes a gene for *O*-acetylserine (thiol)-lyase. The product of this gene, a polypeptide of M_r 34000, is controlled by the level of cysteine and a positive regulator, the *cysB* gene product. It was initially thought that the active OASL molecule may consist of two polypeptide subunits of M_r 34000 and 33000. Subsequent investigations showed that the polypeptide of M_r 33000 was expressed from λ DNA. The active OASL molecule specified by the plasmid exists as a dimer of the polypeptide of M_r 34000, from the analytical ultracentrifugation data, as was the case for *S. typhimurium* (Becker *et al.*, 1969). The *O*-acetylserine sulphydrylase A isoenzyme of *S. typhimurium* is specified by the *cysK* gene (Becker *et al.*, 1969). We have purified the OASL specified by a plasmid carrying chromosomal DNA from *E. coli* and determined its amino acid composition. The similarity of the composition of this protein to that given by Becker *et al.* (1969) for the A isoenzyme of OASL suggests to us that our plasmid-encoded enzyme is homologous with the A isoenzyme of *S. typhimurium* and thus that the cloned gene is homologous with *cysK*. If so, the *cysK* gene in *E. coli* lies between the *Kpn*I and *Bam*HI sites, labelled K2 and B1 on our map (Fig. 5) and the gene contains a *Hind*III site (H1). The properties of the polypeptide of M_r 30800 expressed from plasmids pAB63 and pAB68 show that the initiation site for the *cysK* structural gene is about 1 kb, 90% of the gene, to the left of the H1 site and that the C-terminus of the gene lies about 0.1 kb to the right of the H1 site (Fig. 5). The *cysK* promoter must also lie between the H1 and B1 sites. The observation that a λ gene, about 3.5 kb downstream, is expressed from the *cysK* promoter suggests that the promoter is very efficient and that there are no efficient transcription termination sites on the *E. coli* DNA cloned. The termination site for the mRNA transcript from the *cysK* promoter must lie in the plasmid DNA. If a rho (ρ) dependent termination site exists after *cysK* the level of ρ must be decreased in the minicells. There was no evidence that either the *ptsH* or *ptsI* genes were under the regulation of cysteine or the *cysB* product indicating they have their own promoters.

Results from this paper and from the work of Britton *et al.* (1982, 1983) show that the order of genes at about 52 min in the *E. coli* chromosome is:

52' *cysA* *gsr* *ptsI* *ptsH* *cysK* *cysZ* *lig* *dsd* 51'

The *iex* mutation described by Parra *et al.* (1983a) and Britton *et al.* (1982, 1983) may lie between the *ptsI* and *ptsH* or *ptsH* and *cysK* genes. In contrast, the gene order in *S. typhimurium* (Sanderson & Hartman, 1978) is:

52' *cysA* *cysK* *ptsH* *ptsI* *crr* *dsd* 51'

the *crr* gene of *S. typhimurium* being identical to the *E. coli* *gsr* gene (Parra *et al.*, 1983a). This supports and extends the observation by Parra *et al.* (1983b) that there is an inversion of DNA in *S. typhimurium* relative to *E. coli*. The present results indicate that *cysK* as well as *ptsH* and *ptsI* lie within the inverted region. Kingsman (1977), Kingsman & Smith (1978) and Kingsman *et al.* (1978) have shown that this region of the *S. typhimurium* genome may behave in some ways as a transposon. This may account for the differences in gene order observed.

The *cysK* promoter may prove useful for the construction of a specialized expression vector. We have demonstrated its ability to express a λ protein, a fragment of the bEa59 gene product, in plasmids pAB65 and pAB68. Only the *cysK* gene product appears to be regulated by cysteine

though the *cysB* gene product also affects the expression of other proteins. We have no satisfactory explanation for this observation.

We thank Dr F. Sanger for allowing access to his λ DNA sequence data whilst in preparation, Dr P. Johnson and Mr D. Reed for carrying out the ultracentrifugation experiments and Miss M. Mather for performing the amino acid analysis. This work was supported by the Science and Engineering Research Council, through Grant GR/B/2248.2, and was performed during the tenure of A. Boronat and F. Parra of EMBO Long-term Fellowships. We gratefully acknowledge these sources of help.

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