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

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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*, approx. 34000. The expression of one was regulated by cyst(e)ine and the *cysB* gene product and the other by the *cpB* 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 *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*, 68000 and is composed of two identical subunits of *M*, 34000. 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 & Klopotowski, 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 λJM29, 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*cr* plasmid pKB280, the phage expressed polypeptides of *M*, 9000, 21000, 23000, 33000, 47000 and 49000 (Britton *et al.*, 1982), it was possible that the polypeptide of *M*, 33000 might be a subunit of OASL and, hence, that λJM29 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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Abbreviations: LB, Luria broth; OASL, O-acetylserine (thiol)-lyase; OASS-A, O-acetylserine sulphydrylase A.

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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 μg ampicillin ml⁻¹. Plasmid DNA was amplified by adding chloramphenicol (170 μg 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, and its concentration was determined by measurement of the A₂₆₀ assuming that an A₂₆₀ of 1.0 is equivalent to 50 μg ml⁻¹ (Davis et al., 1980).

Construction of the recombinant plasmids. Plasmid pAB15 was constructed, using DNA subcloned from the specialized λ transducing phage JJM29, 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 Daggert & Ehrlich (1979). Transformations were carried out by mixing 3.75 μg plasmid DNA with 50 μl of the competent cells (at 1·2 × 10⁹ 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 μg ml⁻¹). The plasmids were initially transformed into and checked for tetracycline resistance.

Table 1. Organisms and plasmids used

<table>
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<th>Organism/plasmid</th>
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<tr>
<td>E. coli C600</td>
<td>thr leu thi lacY</td>
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</tr>
<tr>
<td>DS410</td>
<td>minA minB thi str⁻</td>
<td>Jones-Mortimer (1968)</td>
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<td>cysB</td>
<td>Henderson et al. (1976)</td>
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<td>JM559</td>
<td>(his-gnd)⁻ fda⁺</td>
<td>JM559 and JNK55</td>
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<tr>
<td>JM1705</td>
<td>(his-gnd)⁺ fda⁻ trp::Tn10</td>
<td>Lee et al. (1982)</td>
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<tr>
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<td>cysE recA srlA::Tn10</td>
<td>Britton et al. (1982)</td>
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<td>thyA ptsI lex cysZ str⁺</td>
<td>Britton et al. (1982)</td>
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</tr>
<tr>
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<td>metB ptsP ptsH lex umgC recA srlA::Tn10</td>
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<tr>
<td>PO686</td>
<td>recA srlA::Tn10</td>
<td>P. Oliver</td>
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| λ                 | cl857 S7 xis6 min5 b515 b519 | Britton et al. (1982, 1983) |
| JNK55            | gsr⁺ ptsH⁺ lex⁺ cys⁺ cysZ⁺ b221 cl111167::Tn10 cl857 ind⁺ Oam29 | Kleckner et al. (1978) |

Plasmid pBR322 | Ap⁺ Te⁺ | Bolivar et al. (1977) |
| pAB15          | Ap⁺     | Lee et al. (1982) |
| pAB63          | Ap⁺     | This paper |
| pAB65          | Ap⁺ ptsH⁺ cys⁺ | Lee et al. (1982) |
| pAB68          | Ap⁺     | This paper |
| pAB70          | Ap⁺ ptsH⁺ | This paper |
| pAB101         | Ap⁺ ptsH⁺ cys⁺ | This paper |
Cloning of *E. coli* *cysK*

**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 x 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 10¹⁰ 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% (w/v) glycerol, to approximately 2 x 10¹⁰ minicells ml⁻¹ (assuming an A₆₀₀ 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 x 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 μl SDS-dissociating buffer and analysed by SDS-PAGE using 12–20% gradient gels (Britton et al., 1982). The 35S-labelled polypeptides were identified by fluorography as described by Britton et al. (1982). The 14C-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 μl 0-2 mM-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 mM-Tris/HCl, pH 8-0, containing 1 mM-sucrose and 1 mM-K₂EDTA, pH 8-0 (40 μl) followed 2 min later by 20 μl 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 μl 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 μl water. After centrifugation for 15 min, the supernatant solutions containing the cytoplasmic proteins were removed. The membranes were washed twice in 100 μl water and were finally resuspended in 20 μl water. Both the cytoplasmic and membrane fractions were analysed by SDS-PAGE after the addition of 20 μl 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 μg ml⁻¹) with or without cystine (100 μg ml⁻¹) at 37 °C overnight. Samples (20 mg dry mass ml⁻¹) were sonicated in an ultrasonic bath (Megasan) 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 × 7 cm) previously equilibrated with 0-01 mM-Tris/HCl, pH 7-6. A 400 ml linear concentration gradient (0-0-2 M-NaCl in 0-01 mM-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 Mₙ 34000 were pooled and dialysed against 0-01 M-potassium phosphate buffer, pH 7-6. The dialysed material was then applied to a hydroxypatite column (1 × 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 × 6 cm). OASL was eluted with 0-15 M-NaCl in 0-01 mM-Tris/HCl, pH 7-6, and further purified by gel permeation chromatography on an LKB Ultralose AcA 44 column (1-5 × 55 cm) equilibrated with 0-1 mM-Tris/HCl, pH 7-6. Fractions showing a single polypeptide of Mₙ 34000 were collected for OASL activity.

Ultracentrifugation analyses. 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 (ρ) 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-NH₄HCO₃ 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 Mₙ 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 Mₙ 26000 is the processed and active form of the bla gene product (Mₙ 28000) (Ambler & Scott, 1978).

Plasmid pAB65 expressed three polypeptides, of Mₙ 9000, 33000 and 49000; these were also expressed by the specialized transducing phage JJM29 (Britton et al., 1982) from which pAB65 was derived (Lee et al., 1982). The polypeptides of Mₙ 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 Mₙ 33000 might be a doublet. To improve the
Cloning of *E. coli* cysK

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 AJM29 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, \(\beta\)-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 \(M_r\) 33000 but also another of \(M_r\) 34000 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 \(M_r\) 34000, which co-electrophoresed with a polypeptide expressed from plasmid pAB65, but no polypeptide of \(M_r\) 33000 was expressed from any phage (Fig. 2, track 2 for the expression of AJM29).

We suspected that the polypeptide of \(M_r\) 34000 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 \(M_r\) 34000 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 \(\mu\)g ml\(^{-1}\)) and any polypeptides
Fig. 3. Fluorogram of the [35S]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 [14C]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 [14C]amino acids. The expression of the polypeptide of M, 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, 33000 was depressed only in the cysB strain PB20.

To identify the location of the genes expressing the polypeptides of M, 34000 and 33000, several plasmids were derived from plasmid pAB65 (Fig. 1). Plasmid pAB63 expressed a polypeptide of M, 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.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Host Cystine in medium</th>
<th>DS410 (cysB^+)</th>
<th>DS410 (cysB^+)</th>
<th>PB20 (cysB)</th>
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<td>...</td>
<td>100</td>
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<td>...</td>
<td>741</td>
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<td>130</td>
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</table>

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 promotor was under the regulation of cysteine and the cysB gene product.

Plasmid pAB68 expressed two polypeptides of Mr 30800 and 33000 (Fig. 2, track 6). The polypeptide of Mr 30800 was repressed by cystine in the minicell system but the expression of the polypeptide of Mr 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 λE54.3–λH57 (Fig. 5). This raised the possibility that the polypeptide of Mr 33000 might be expressed from λ DNA and that the polypeptide of Mr 30800 was a fragment of the polypeptide of Mr 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 Mr 49000, 32000 and 9000 (Fig. 3, track 9) confirming that the polypeptides of Mr 49000 and 9000 originated from the H57–H1 DNA fragment as observed by Britton et al. (1983). The polypeptide of Mr 32000 must originate from the H1–B1 DNA fragment as a polypeptide of Mr 30800, showing the same regulatory properties, was expressed from plasmids pAB63 and pAB68. The polypeptide of Mr 32000 was repressed in the presence of cystine (Fig. 3, tracks 9 and 10). The increase in the size of the polypeptide fragment (Mr 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 λE54.3–λH57 DNA fragment was absent and that the λH57–H1 DNA fragment was orientated in the opposite direction (Fig. 5). The λH57–H1 DNA fragment of pAB70 was re-oriented to give plasmid pAB101, which thus differed from plasmid pAB65 only by the absence of the λE54.3–λH57 DNA fragment. Plasmid pAB101 expressed three polypeptides of Mr 49000, 34000 and 9000 (Fig. 2, track 4); the polypeptide of Mr 34000 was repressed in the presence of cystine (data not shown). A polypeptide of Mr 34000 was specified by pAB101 and a polypeptide of Mr 32000 by pAB70. This would only be possible if the polypeptides of Mr 30800 and 32000 were fragments of the polypeptide of Mr 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-acetylseryine (thiol)-lyase

<table>
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<td>25.5</td>
</tr>
<tr>
<td>Arg</td>
<td>12.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Trp</td>
<td>ND</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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-acetylseryine (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 $s_{20,w}$ 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 $s_{20,w}$ 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):

$$M = N_s s_{20,w} \frac{1}{(1 - v \rho)}$$
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where the \( \bar{\varepsilon} \) 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 64000 indicating that it was a dimer of the polypeptide \( (M_r, 34000) \) expressed from the cloned E. coli DNA.

Identity of the polypeptide of \( M_r, 33000 \)

Several of the plasmids were expressed in DS410-derived minicells. The resulting proteins were labelled with \([^{35}\text{S}]\text{methionine}, \text{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, 49000, 34000 \text{ and } 9000 \text{ were all found to be associated with the cytoplasm (Fig. 4, tracks 2 and 4). The polypeptide of } M_r, 33000 \text{ 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 \( \lambda \text{ DNA stretching from the } \lambda E54.3 \text{ site to the } \lambda H57 \text{ site (Fig. 5). The plasmids carrying } \lambda \text{ DNA also expressed a polypeptide of } M_r, 33000 \text{ that had no OASL activity, as observed from plasmid pAB68, and which was membrane-} \)
associated rather than cytoplasmic. The removal of the λ DNA from plasmid pAB65 (resulting in plasmid pAB101) caused the loss of the polypeptide of $M_e$, 33000, confirming that it was specified by λ DNA. Examination of the λ DNA sequence reported by Sanger et al. (1982) from λE54.3 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 $M_e$ 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, λJM29 and λJM133, expressed a polypeptide of $M_e$, 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 $M_e$, 45000, the loss being at the C-terminal end of the protein. This suggested that the polypeptide of $M_e$, 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.
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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, 33000 being produced. It is probable that the polypeptide of M, 47000 expressed by the specialized λ transducing phages λJM29 and λJM133 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 λJM29, the λE54.3–B1 fragment, encodes a gene for O-acetylserine (thiol)-lyase. The product of this gene, a polypeptide of M, 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, 34000 and 33000. Subsequent investigations showed that the polypeptide of M, 33000 was expressed from λ DNA. The active OASL molecule specified by the plasmid exists as a dimer of the polypeptide of M, 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 KpnI and BamHI sites, labelled K2 and B1 on our map (Fig. 5) and the gene contains a HindIII site (H1). The properties of the polypeptide of M, 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 cystine
though the $cysB$ gene product also affects the expression of other proteins. We have no satisfactory explanation for this observation.

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