JOURNAL OF BACTERIOLOGY, Aug. 1983, p. 578–585
0021-9193/83/080578-08$02.00/0
Vol. 155, No. 2

# Cloning and Physical Mapping of the *cysB* Region of *Salmonella typhimurium*

GRAZYNA JAGURA-BURDZY† AND NICHOLAS M. KREDICH*

*Laboratories of the Howard Hughes Medical Institute at Duke University and Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710*

Received 20 December 1982/Accepted 5 May 1983

The *cysB* region of *Salmonella typhimurium* was cloned in pBR322 and localized to a 1.75-kilobase *HincII* fragment. Two-dimensional protein electropherograms showed levels of the *cysB* polypeptide chain that were several fold higher in plasmid-bearing strains than in the wild type. Fully derepressed levels of sulfite reductase and O-acetylserine sulfhydrylase in *cysB* plasmid-bearing strains were only 25% higher than in the wild type, suggesting that the product of this regulatory gene ordinarily is not a limiting factor in the expression of the cysteine regulon. The mapping of *cysB* deletions by Southern blots showed a good correlation between the genetic and the physical maps of this gene. The *supX* gene was initially cloned with *cysB* and is within 0.7 kilobase of *cysB*.

Gene expression in the cysteine regulon of *Salmonella typhimurium* and *Escherichia coli* is positively regulated by the *cysB* gene product (17, 18). *cysB* consists of a single cistron (3, 33) that codes for a polypeptide chain with a molecular weight of 39,000 in *E. coli* (25) and 34,500 in *S. typhimurium* (1). Little is known of the nature of the *cysB* protein and the mechanism by which it effects the expression of various genes in the cysteine regulon. Two other factors whose activities have not been defined, the inducer O-acetyl-L-serine and the repressor L-cysteine (or sulfide), are also involved in the genetic regulation of this pathway (17, 18).

The purification of the *cysB* protein would help considerably in the further characterization of this regulatory system. Since this protein comprises less than 0.1% of the total cell protein in *S. typhimurium* (1), we attempted to enrich cells for it by introducing the *cysB* gene into a multicopy plasmid. In this communication we report (i) the cloning in pBR322 of the *cysB* gene of *S. typhimurium*, (ii) a detailed restriction endonuclease map of the *cysB* region, and (iii) a correlation of this map with a genetic map of various *cysB* deletions.

## MATERIALS AND METHODS

**Strains and culture media.** The bacterial strains used are listed in Table 1. Minimal medium (34) contained an equimolar amount of $MgCl_2$ in place of $MgSO_4$, glucose at 5 g/liter, appropriate amino acids at 0.2 mM, and 0.1 mM L-cystine, 1 mM $Na_2SO_4$, or 1 mM glutathione as a sulfur source. LB medium and M9 medium were used as indicated previously (27). When required, ampicillin and tetracycline were present at 50 and 40 mg/liter, respectively; solid medium contained 1.5% agar.

**Enzyme assays.** The preparation of crude extracts from bacterial cells and our assay for O-acetylserine sulfhydrylase activity have been described previously (18). Sulfite reductase was assayed by the method of de Vito and Dreyfuss (5), and protein was determined by the biuret method (10) with bovine serum albumin as a standard.

**DNA purification.** For large amounts of purified plasmid DNA, bacteria were grown at 37°C in M9 medium supplemented with 0.2% Casamino Acids, and at a cell density of $6 \times 10^8$ to $8 \times 10^8$/ml, chloramphenicol was added to a final concentration of 150 mg/liter. After 12 to 16 h of further incubation, bacteria were harvested by centrifugation, and lysates were prepared as described by Kupersztoch-Portnoy et al. (19). After the addition of CsCl to a density of 1.55 g/ml and ethidium bromide to a concentration of 0.15 mg/ml, the solution was centrifuged at 40,000 rpm for 48 h at 20°C in Beckman 70 Ti rotor. The band of plasmid DNA was removed through the side of the centrifuge tube with a needle and syringe, extracted with CsCl-saturated isopropanol to remove ethidium bromide, and dialyzed exhaustively against TE buffer (20 mM Tris-hydrochloride [pH 7.4], 1 mM disodium EDTA). When necessary, DNA was precipitated at −20°C with 2 volumes of ethanol, collected by centrifugation, and dissolved in a smaller volume of TE buffer. For the preparation of chromosomal DNA, the method of Marmur (24) was used through the first ethanol precipitation step. The DNA from ca. 1 g (wet weight) of bacteria was then dissolved in 5 to 10 ml of TE buffer and further purified by CsCl density gradient centrifugation with ethidium bromide as described above for plasmid DNA purification.

**Cloning techniques.** Plasmid pBR322 DNA and either chromosomal DNA from the *hisG70* strain or

---

† Present address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-532 Warsaw, Poland.

TABLE 1. Bacterial strains used in this study

| Strain | Description | Origin or reference |
|---|---|---|
| *E. coli* | | |
| JA199 | Δ*trpE5 leu-6 thi rbs*$^+$ *r*$^-$ *m*$_k^+$ | J. Carbon |
| NK1 | Δ*trpE5 leu-6 thi cysB rbs*$^+$ *r*$^-$ *m*$_k^+$ | This laboratory from JA199 |
| NK12 | Δ*trpE5 leu-6 thi cysB rbs*$^+$ *r*$^-$ *m*$_k^+$(pGBK1) | Transformation of NK1 |
| NK13 | Δ*trpE5 leu-6 thi cysB rbs*$^+$ *r*$^-$ *m*$_k^+$(pGBK2) | Transformation of NK1 |
| NK14 | Δ*trpE5 leu-6 thi cysB rbs*$^+$ *r*$^-$ *m*$_k^+$(pGBK3) | Transformation of NK1 |
| NK37 | Δ*trpE5 leu-6 thi cysB rbs*$^+$ *r*$^-$ *m*$_k^+$(pGBK13) | Transformation of NK1 |
| *S. typhimurium* | | |
| *hisG70* | *hisG70* | 12 |
| *cysB403* | *cysB403* | 28 |
| DU1 | *leu-409 trp* Aza$^r$ *r*$^-$ | R. Burns |
| PM247 | *leu-500 ara-9* Δ(*trp-supX34*) | 2 |
| DW48 | *cysB1352 trpA160* | 18 |
| DW353 | *leu-500 pyrF146* Δ(*supX-cysB1753*) | 3 |
| DW354 | *leu-500 pyrF146* Δ(*supX-cysB1754*) | 3 |
| DW356 | *leu-500 pyrF146* Δ(*supX-cysB1756*) | 3 |
| DW357 | *leu-500 pyrF146* Δ(*supX-cysB1757*) | 3 |
| DW360 | *leu-500 pyrF146* Δ(*trp-supX-cysB1760*) | 3 |
| DW361 | *leu-500 pyrF146* Δ(*supX-cysB1761*) | 3 |
| DW362 | *leu-500 pyrF146* Δ(*supX-cysB1762*) | 3 |
| DW363 | *leu-500 pyrF146* Δ(*supX-cysB1763*) | 3 |
| DW364 | *leu-500 pyrF146* Δ(*supX-cysB1764*) | 3 |
| DW365 | *leu-500 pyrF146* Δ(*supX-cysB1765*) | 3 |
| DW367 | *leu-500 pyrF146* Δ(*supX-cysB1767*) | 3 |
| DW377 | *leu-500 pyrF146* Δ(*trp-supX-cysB1769*) | This laboratory as a spontaneous Leu$^+$ Trp$^-$ Cys$^-$ revertant of *leu-500* |
| DW400 | *leu-500 pyrF146* Δ(*supX-cysB1767*)(pGBK3) | Transformation of DW367 |
| DW403 | *cysB1352 trpA160*(pGBK2) | Transformation of DW48 |
| DW409 | *leu-409 trp* Aza$^r$ *r*$^-$(pGBK3) | Transformation of DU1 |
| DW414 | *cysB403*(pGBK3) | Transformation of *cysB403* |

purified DNA fragments were separately digested with restriction endonucleases and then mixed at a plasmid DNA concentration of 20 μg/ml and either an equal mass of purified fragment or five times as much chromosomal DNA. Ligation with T4 DNA ligase was accomplished overnight at 4°C (4). Transformation was performed as described by Kushner (20) for *E. coli* strains and as described by Lederberg and Cohen (21) for *S. typhimurium* strains. Transformants were analyzed for plasmid DNA by the method of Meyers et al. (26).

**Restriction endonuclease analyses of DNA.** Restriction endonucleases were purchased from Bethesda Research Laboratories and used according to the recommendations of the manufacturer. Digests were electrophoresed on 0.7 to 1.5% agarose gels in Tris-acetate buffer (40 mM Tris base, 20 mM acetic acid, 2 mM disodium EDTA [pH 8.1]) at 6 V/cm for analytical vertical gels or 2 V/cm for preparative horizontal gels. Bands of DNA fragments were visualized under UV light with ethidium bromide and compared with those of fragments of known size obtained from a *Hin*dIII digest of λ DNA or a *Hae*III digest of φX174 DNA. Horizontal gels were used for the purification of DNA fragments. DNA was obtained from NaClO$_4$-solubilized gel slices by the glass fiber filter method of Yang et al. (35).

**Two-dimensional protein gels.** Overnight bacterial cultures were diluted 1/50 into 10 ml of fresh minimal medium containing 2 μCi of a mixture of $^{14}$C amino acids per ml. Cells were collected by centrifugation at late-log-phase growth and analyzed for radiolabeled *cysB* protein by a modification (1) of the two-dimensional gel technique of O'Farrell (29).

**Southern blot analyses.** Restriction endonuclease digests of genomic DNA (0.5 to 2 μg) or plasmid DNA (0.005 to 0.02 μg) were electrophoresed in 3-mm-thick agarose gels and transferred to nitrocellulose filters by the method of Southern (31). After baking for 2 h at 80°C, the filters were rinsed in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and hybridized by a modification of the method of Jeffreys and Flavell (16) to DNA that had been $^{32}$P labeled by nick translation (23). After a final wash with 0.1× SSC containing 0.1% sodium dodecyl sulfate, filters were rinsed with 3× SSC, dried, and autoradiographed by exposure to Kodak X-Omat AR film for 1 to 48 h.

## RESULTS

**Isolation of plasmids carrying the *S. typhimurium cysB* gene.** Initial efforts at cloning the *S. typhimurium cysB* gene in the *Eco*RI site of pBR322 were unsuccessful. Since the corresponding *E. coli* gene is known to function in *S. typhimurium* (13), we anticipated that there might be enough DNA sequence homology between the *cysB* genes of these two organisms to observe hybridization. We used as a probe a previously constructed plasmid, pJOH1 (J. Ostrowski, unpublished data), which consists of a 3-kilobase (kb) *Sal*I-*Eco*RI fragment of *E. coli* DNA bearing the *cysB* gene inserted into the 3.7-kb *Sal*I-*Eco*RI segment of pBR322. Purified pJOH1 was radiolabeled with $^{32}$P by nick translation and hybridized under stringent conditions to Southern blots of genomic DNA from the *S. typhimurium hisG70* (*cysB*$^+$) and DW353 (Δ*cysB*) strains that had been digested with various restriction endonucleases. Hybridization was noted, which was about 10% as intense as that obtained with a control sample of *E. coli* DNA. Differences in fragment sizes between the DNAs of the *hisG70* and DW353 strains indicated that the *E. coli* probe was in fact hybridizing to *S. typhimurium cysB* DNA. Of the five restriction enzymes tested (*Ava*I, *Bam*HI, *Eco* RI, *Hin*dIII, and *Sal*I), only *Sal*I gave fragments of less than 10 kb, and this enzyme was selected for cloning of the *S. typhimurium cysB* gene.

Genomic DNAs from the *hisG70* strain and pBR322 were digested separately with *Sal*I, and after ligation, the mixture was used to transform *E. coli* NK1 (*cysB*) to cysteine prototrophy. A single Cys$^+$, ampicillin-resistant (Ap$^r$), tetracycline-sensitive (Tet$^s$) colony was obtained, which was found to carry a 15.7-kb plasmid consisting of pBR322 and three *Sal*I fragments of 1.15, 2.7, and 7.5 kb. This plasmid, designated pGBK1, was found to transform NK1 to both Cys$^+$ and Ap$^r$ with high efficiency and was presumed to carry the *cysB* gene of *S. typhimurium*.

Restriction endonuclease digestion of purified *Sal*I fragments and of pGBK1 itself showed that the 7.5-kb fragment lies between the 1.15- and 2.7-kb fragments with the orientation shown in Fig. 1. Purified pGBK1 was used as a probe for Southern blots of *Sal*I-digested genomic DNA from the *hisG70* and DW353 strains. The expected three hybridization bands were found for *hisG70* DNA, but in the case of DW353 DNA, only the 1.15-kb fragment and a new fragment of about 9 kb were noted. Since the *supX-cysB* deletion in DW353 leads to the loss of both the 2.7- and 7.5-kb *Sal*I fragments, it seems most likely that they are contingous in the genome and that their presence in pGBK1 is due to incomplete *Sal*I digestion of the genomic DNA used in the construction of this plasmid. Other data (see below) indicate that the 1.15-kb *Sal*I fragment is not contiguous with the 7.5-kb fragment in genomic DNA.

Purified *Sal*I fragments from pGBK1 were ligated with *Sal*I-digested pBR322, and these mixtures were then used to transform NK1. Cys$^+$ transformants were obtained only from ligation mixtures containing the 2.7-kb fragment. Restriction analyses of six such transformants confirmed that each carried pBR322 with the 2.7-kb *Sal*I insert, and in five transformants the orientation of this fragment relative to pBR322 was found to be identical to that of pGBK1. One such plasmid was chosen and designated pGBK2. The plasmid containing the 2.7-kb *Sal*I fragment with the opposite orientation was designated pGBK3 (Fig. 1).

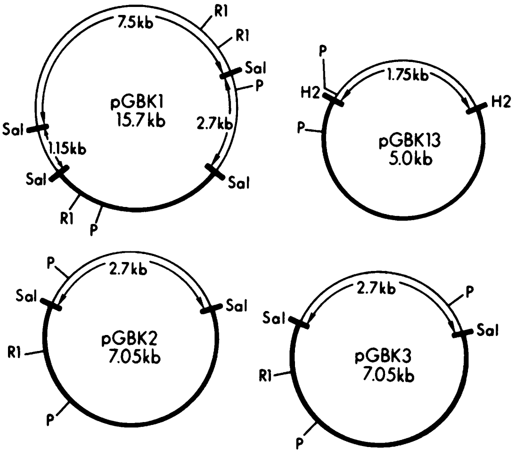

FIG. 1. Plasmids pGBK1, pGBK2, pGBK3, and pGBK13. The pBR322 portion of each plasmid is represented by a bold line. Certain restriction sites are given to indicate the orientation of fragments. Abbreviations: R1, *Eco*RI; Sal, *Sal*I; P, *Pst*I; H2, *Hin*cII.

Genomic mapping of Δ(*supX-cysB*) strains (see below) indicated that *cysB* should be located mainly, if not entirely, between the *Pst*I and *Sst*I sites in the 2.7-kb *Sal*I fragment. The presence of *Hin*cII sites just outside the *Pst*I and *Sst*I sites prompted us to clone this 1.75-kb *Hin*cII fragment (purified from pGBK3) in pBR322, using blunt end ligation to the two *Hin*cII sites of this vector. Cys$^+$ transformants of NK1 were obtained from this ligation, and they proved to carry the expected *Hin*cII fragment. One such plasmid was designated pGBK13 and has the orientation shown in Fig. 1.

Plasmids carrying *cysB* were introduced into the $r^-$ *S. typhimurium* strain DU1 by transformation. Cleared lysates (26) from Ap$^r$ transformants were then used to transfer these plasmids with good efficiency to other *S. typhimurium* strains to study various in vivo effects.

**Demonstration of the *cysB* protein in plasmid strains.** The presence of *S. typhimurium cysB* protein in plasmid-bearing strains was confirmed by two-dimensional protein electrophoresis, the only assay yet described for this protein (1). A crude extract of $^{14}$C-labeled proteins from the Δ(*supX-cysB*) strain DW353 was compared with those of wild-type *S. typhimurium* and of DW353 carrying either pGBK1, pGBK2, or pGBK3. In the case of each plasmid-carrying strain, a *cysB* protein "spot" with a molecular weight of 34,500 and an isoelectric point of 7.1 was noted, which was not found in DW353 itself and which was identical in position to the spot in the wild type that is known to be the *cysB* polypeptide chain (1). A *cysB* protein spot also was present in extracts of NK37 (carrying pGBK13), but was absent in its parent, NK1. Extracts from strains carrying other pBR322-derived plasmids did not give this spot. The intensity of the *cysB* spot was estimated to be severalfold greater in the plasmid-carrying strains than in the wild type.

**Presence of *supX* on pGBK1.** When strain NK12 was plated on nonselective medium, 96% of the colonies were found to have lost the pGBK1 plasmid. This high rate of segregation was not observed with strains carrying pGBK2, pGBK3, or pGBK13. Similar instability has been noted for plasmids carrying the *E. coli supX* gene (J. C. Wang, personal communication), which codes for DNA topoisomerase I; and since this gene is located near *cysB*, it seemed likely that pGBK1 might carry *supX*.

*S. typhimurium* DW353 carries the *leu-500* mutation, yet is Leu$^+$ owing to its *supX* mutation. Transformation of DW353 with either pGBK1, pGBK2, or pGBK3 gave Cys$^+$ colonies, which in the case of pGBK2 and pGBK3 remained Leu$^+$. With pGBK1, however, a Cys$^+$ Leu$^-$ phenotype was obtained, indicating the presence of a *supX*$^+$ gene on this plasmid. Although we were unable to subclone the 7.5-kb *Sal*I fragment of pGBK1, other data (see below) indicate that it contains at least a portion of *supX*.

**Comparison of genetic and restriction maps of the *cysB* region.** Genetic analyses of certain *cysB* deletion strains and various point mutations have allowed the division of the *cysB* gene of *S. typhimurium* into 12 deletion segments (3). All begin at the *supX* side of *cysB* and extend various distances into *cysB*. The shortest deletion defines deletion segment I, and deletions extending successively further into *cysB* define segments II through XII. It seemed of interest to compare these genetic data with those obtained from restriction analyses of this region. Southern blots of DNA from Δ(*supX-cysB*) strains were prepared after restriction with various endonucleases, and the DNA was then hybridized to probes prepared from pGBK1, pGBK3, or purified *Sal*I fragments of pGBK1.

Analyses of the restriction patterns given by DNA from 12 different *cysB* deletion strains showed that in every case both the *Pst*I site of the 2.7-kb fragment and the *Sal*I site joining the 2.7- and 7.5-kb fragments were missing. Mapping of the *supX* deletion of the *cysB*$^+$ strain PM247 places *supX*, at least in part, in the 7.5-kb fragment and to the left of *cysB* (Fig. 2). The deletions varied in size from 0.7 kb to greater than 10.2 kb and could be grouped into three different categories according to the persistence or loss of certain restriction sites in the 2.7-kb fragment. The five deletions known from genetic analyses to extend the shortest distances into *cysB* from the *supX-cysB* junction, i.e., those defining segments I through V, were found to retain the *Hae*III site located 0.7 kb from the *Sal*I site that joins the 2.7- and 7.5-kb fragments (Fig. 2). Thus, these five deletions must include a portion of the *cysB* gene no greater than 0.35 kb, i.e., the distance between the *Hinc*II site at 0.35 kb and the *Hae*III site at 0.7 kb. DNA from those strains defining deletion segments VI, VII, VIII, and IX was found to lack this same *Hae*III site but retained the *Bgl*I site located 1.1 kb into the 2.7-kb *Sal*I fragment. DNA from strains defining the regions X and XI to XII lacked this *Bgl*I site but retained the *Sst*I site located 1.75 kb from *Sal*I (the deletion strain originally used to define the junction between segments XI and XII has been lost). The deletion in DW367 does not complement any of 38 point mutations tested (3) and yet falls short of the *Sst*I site, suggesting that this site lies beyond the *supX*-distal end of *cysB*. The deletion in DW377 seems to encompass the entire 2.7-kb *Sal*I fragment, as well as all of the 7.5-kb *Sal*I fragment. Thus, although fine detail is not yet available in the physical map, restriction analyses of DNA from these deletion strains correlate with the genetic data. They also demonstrate a close proximity of *cysB* to *supX*, since both activities are lost by the 0.7-kb deletion in DW361.

Use of the restriction endonucleases indicated in Fig. 2 allowed us also to describe the position of the *supX* side of each deletion. These endpoints were quite variable, in one case extending less than 0.1 kb into the 7.5-kb *Sal*I fragment and in two others extending all the way through this fragment. In all strains analyzed, the 1.15-kb *Sal*I fragment was present, which in the case of the large deletions in DW360 and DW377 indicates that this small fragment is not contiguous to the 7.5-kb fragment in genomic DNA and that its presence in pGBK1 is due to a multiple ligation event in the in vitro construction of the plasmid.

**Expression of the cysteine regulon in *S. typhimurium* strains carrying *cysB* plasmids.** Full expression of O-acetylserine sulfhydrylase and sulfite reductase in cells grown on a limiting sulfur source such as L-djenkolate requires a functional *cysB* gene product, and levels of these enzymes in most *cysB* strains grown under such

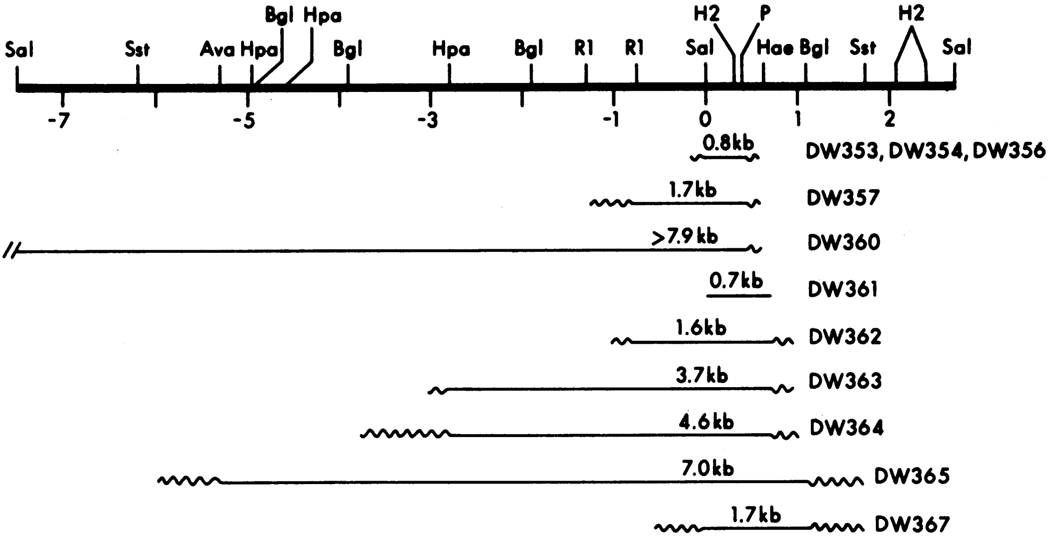

FIG. 2. Physical map of the *supX-cysB* region of *S. typhimurium* as determined from analyses of Southern blots from Δ(*supX-cysB*) strains. At the top is a restriction map of the 10.2-kb region of pGBK1 believed to exist in the genome as contiguous 7.5- and 2.7-kb *Sal*I fragments. Abbreviations: Sal, *Sal*I; Sst, *Sst*I; Ava, *Ava*I; Hpa, *Hpa*I; Bgl, *Bgl*I; R1, *Eco*RI; H2, *Hin*cII; P, *Pst*I; Hae, *Hae*III. Additional *Hae*III sites in the 2.7-kb *Sal*I fragment are present at 0.05, 0.15, 2.15, 2.35, and 2.6 kb. *Hae*III sites were not determined for the 7.2-kb *Sal*I fragment. The extent of various deletions is also shown. Unambiguous loss of DNA is indicated by a straight line which is extended as a wavy line to show the maximum possible loss. These strains define deletion segments that extend from *supX* (to the left) various distances into *cysB* (to the right). Strains DW353 to DW360 define deletion segments I to V; DW361 to DW365 define deletion segments VI to X; DW367 covers all 38 *cysB* single-site mutations tested (3).

conditions are only 1 to 2% those of the wild type (18). The introduction of pGBK3 into *cysB* strains restored *O*-acetylserine sulfhydrylase expression to normal in the case of the *cysB403* derivative DW414 and nearly to normal in the Δ(*supX-cysB*) strain DW400 (Table 2). Similar results were obtained with sulfite reductase expression (data not shown).

Comparison of *O*-acetylserine sulfhydrylase activities in the *cysB*$^+$ strain DU1 with those in its pGBK3 derivative DW409 showed higher levels in the latter, which had 25% more enzyme during growth on L-djenkolate and 112% more in sulfate-grown cells.

The mutation *cysB1352* is remarkable for its ability to confer constitutive expression of the cysteine regulon in cells grown on L-cystine (18). The introduction of pGBK2 into this strain lowered *O*-acetylserine levels in cells grown on L-cystine to 20% that of the parental strain and

TABLE 2. *O*-Acetylserine sulfhydrylase levels in *cysB* plasmid strains

| Strain | Pertinent genotype | *O*-Acetylserine sulfhydrylase (U/mg of protein) with following sulfur source: | | |
|---|---|---|---|---|
| | | L-Cystine | Sulfate | L-Djenkolate |
| DU1 | *cysB*$^+$ | 0.4 | 13.6 | 26.5 |
| DW409 | *cysB*$^+$ (pGBK3) | 0.5 | 28.8 | 33.2 |
| *cysB403* | *cysB* | | | <0.2 |
| DW414 | *cysB403* (pGBK3) | 0.3 | 10.7 | 28 |
| DW367 | Δ(*supX-cysB*) | <0.2 | | <0.2 |
| DW400 | Δ(*supX-cysB*) (pGBK3) | 0.5 | 13.7 | 14.3 |
| DW48 | *cysB1352* | 17 | | 35.2 |
| DW403 | *cysB1352* (pGBK2) | 3.4 | | 10.9 |

also decreased the level of enzyme in L-djenkolate-grown cells to about 30% that of the parental strain (Table 2).

## DISCUSSION

The presence of three different genomic *Sal*I fragments in pGBK1 appears to be due to both incomplete endonuclease digestion and a multiple ligation event that occurred in the construction of this plasmid. Since Southern blot analyses of DNA from wild-type *S. typhimurium* and various Δ(*supX-cysB*) strains indicate that only the 2.7- and 7.5-kb fragments are contiguous in the genome, it is this 10.2-kb portion of cloned DNA that is relevant to the study of the genetic organization of the *cysB* region.

When this DNA segment is oriented arbitrarily, as in Fig. 2, the *cysB* gene itself lies toward the right in the 2.7-kb fragment, more specifically in the 1.75-kb region bounded by *Hin*cII sites. Genetic mapping data have shown that *supX* is situated near the *trp* side of *cysB* in *S. typhimurium* (8, 22). Gene mapping by Southern blot analyses and the fact that pGBK1 carries *supX*, whereas the 2.7-kb portion of pGBK1 does not, indicate that *supX* must be located, at least in part, on the 7.5-kb *Sal*I fragment. It follows that the gene order in Fig. 2 should be *trp supX cysB*, but we have not ascertained whether the 7.5-kb fragment carries any *trp* genetic material. Previous studies of *E. coli* have shown that the *cysB* promoter is on the *trp*-proximal side (15), and if the same is true in *S. typhimurium*, *cysB* is transcribed from left to right as oriented in Fig. 2.

We have successfully cloned the purified 2.3-kb *Pst*I-*Sal*I portion of the 2.7-kb *Sal*I fragment into the *Sal*I-*Pst*I segment of pBR322 (data not shown). The transformation of NK1 with this plasmid gave Cys$^+$ colonies that grew very slowly on sulfate plates but at a normal rate on plates supplemented with L-cystine. Colonies that grew normally on sulfate plates were occasionally obtained that seemed to have arisen from more slowly growing colonies. Both slowly and rapidly growing colonies were found to carry pBR322-derived plasmids with the same 2.3-kb *Sal*I-*Pst*I insert, and transformation of NK1 with plasmid DNA from rapidly growing colonies gave rise again mostly to slowly growing colonies (data not shown). We surmise from these experiments that *cysB* is expressed, but only poorly, from the 2.3-kb *Sal*I-*Pst*I fragment and that one end of *cysB*, probably the promoter, may lie near the *Pst*I site. The rapidly growing colonies observed after transformation may be due to mutations in the host genome that improve the expression of *cys* genes in a situation in which *cysB* protein is limiting. The additional leftward 0.05 kb of DNA present in the *Hin*cII fragment of pGBK13 gives very good *cysB* gene expression, as evidenced by a normal growth rate on sulfate medium and *cysB* protein levels greater than those of the wild type as determined by two-dimensional protein gels.

The data summarized in Fig. 2 offer additional insight into the exact position of *cysB* in the 2.7-kb *Sal*I fragment. The first five *trp*-proximal, genetically defined segments of this gene (3) are included within the 0.35-kb fragment bounded by the *Hin*cII site at 0.35 kb and the *Hae*III site at 0.7 kb. Segment XII, and presumably segment XI as well, is in the fragment between the *Bgl*I site at 1.1 kb and the *Sst*I site at 1.75 kb. We estimate, therefore, that the *trp*-proximal end of *cysB* is located at 0.35 to 0.5 kb into the 2.7-kb *Sal*I fragment, perhaps just beyond the *Pst*I site, and since the 34,500-dalton *cysB* protein would require ca. 1 kb of coding sequence, that the *trp*-distal end of *cysB* is at 1.4 to 1.5 kb into this fragment.

Southern blot analyses have also helped to localize the *supX* gene in this 10.2-kb segment of DNA. The 0.7-kb deletion in DW361 removes the *Sal*I site at 0.0 kb and the *Hae*III site at 0.7 kb and therefore cannot extend more than about 0.05 kb into the 7.5-kb *Sal*I fragment. The *supX* genotype of DW361 means that the *cysB*-proximal end of *supX* is near this *Sal*I site and may even lie within the first 0.4 kb of the 2.7-kb fragment. Furthermore, the Δ(*supX-trp*) deletion in the *cysB*$^+$ strain PM247 removes an *Hpa*I site at −2.8 kb, but not a *Bgl*I site at −1.9 kb (Fig. 2). Therefore, *supX* extends at least 1.9 kb, the distance between *Sal*I at 0.0 and the *Bgl*I site at −1.9 kb. If the *supX*-coded topoisomerase of *S. typhimurium* is similar in subunit mass to that characterized in *E. coli* (9), ca. 3 kb of DNA would be required to code for its 110,000-dalton polypeptide chain.

The increased *cysB* copy number expected in strains carrying the relatively small (5- to 7-kb) pBR322 derivatives pGBK2, pGBK3, and pGBK13 lead to only a severalfold increase in levels of *cysB* protein, as judged by two-dimensional protein gel analyses. Recent studies indicate that *cysB* gene expression is autoregulated in *E. coli* (15), and the same phenomenon may explain the relatively modest increases in the amounts of *cysB* gene product in our plasmid strains.

The introduction of pGBK2 or pGBK3 into *cysB* strains restored the regulation of *O*-acetylserine sulfhydrylase and sulfite reductase to a nearly normal state. The 50% reduction in fully derepressed enzyme levels noted in DW400 may have been due to a nonspecific, pleiotropic effect of the *supX* character of that strain (7, 8, 11), since in the *supX*$^+$ strain DW414, enzyme regulation was perfectly normal. Of interest in this regard are recent studies showing that *E. coli supX* strains accumulate mutations affecting DNA gyrase activity and that such secondary mutations are required for normal growth (6, 30). Presumably, the excess negative DNA superhelicity caused by a *supX* mutation is detrimental to cell growth and function. It should also be noted that the *cysB* strains tested were derived from the LT2 strain of *S. typhimurium*, and the *cysB* gene that we have cloned is from the LT7 derivative *hisG70* (12). Small genetic differences between these two parental strains have been described (32) and might account for the variability of enzyme levels in our plasmid-bearing strains.

Extra *cysB* gene copies gave slight increases in *O*-acetylserine sulfhydrylase levels in a *cysB*$^+$ host, suggesting that the expression of this enzyme in the wild type is only marginally limited by the amount of *cysB* gene product. In contrast, the introduction of pGBK2 into the *cysB*$^c$ strain DW48 resulted in diminished *O*-acetylserine sulfhydrylase levels. A previous study with a *cysB*$^c$(F′ *cysB*$^+$) merodiploid strain did not show such an effect, suggesting that there is no appreciable negative control by the wild-type *cysB* gene product (14). The different results obtained here may have been due to higher ratios of *cysB*$^+$ to *cysB*$^c$ in DW403. The native *cysB* protein appears to be at least a dimer and perhaps a tetramer (1, 14), and oligomers composed of mixtures of *cysB*$^+$ subunits and *cysB*$^c$ subunits might behave more like a pure *cysB*$^+$ protein. In this case, the constitutive expression of O-acetylserine sulfhydrylase would be attenuated by a plasmid carrying *cysB*$^+$. The regulatory behavior noted in DW403 might also be due to the autoregulation of the genomic *cysB*$^c$ allele by the product of the plasmid *cysB*$^+$ allele.

Although *cysB* gene products are elevated only severalfold in strains carrying our *cysB* plasmids, these initial studies have been useful in defining the boundaries of this gene. This information should enable us to attach a high-efficiency promoter to *cysB* and eventually to create a strain with high enough *cysB* protein levels to allow easy purification. In addition, our detailed restriction map should prove to be useful in formulating strategies for sequencing the *cysB* region.

## ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of J. Burdzy and H. Kendall.

This work was supported by Public Health Service grant AM-12828 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

## LITERATURE CITED

1. **Baptist, E. W., S. G. Hallquist, and N. M. Kredich.** 1982. Identification of the *Salmonella typhimurium cysB* gene product by two-dimensional protein electrophoresis. J. Bacteriol. 151:495–499.
2. **Bauerle, R. H., and P. Margolin.** 1967. Evidence for two sites for initiation of gene expression in the tryptophan operon of *Salmonella typhimurium*. J. Mol. Biol. 26:423–436.
3. **Cheney, R. W., Jr., and N. M. Kredich.** 1975. Fine-structure genetic map of the *cysB* locus in *Salmonella typhimurium*. J. Bacteriol. 124:1273–1281.
4. **Collins, C. J., D. A. Jackson, and F. A. J. de Vries.** 1976. Biochemical construction of specific chimeric plasmids from colE1 DNA and unfractionated *Escherichia coli* DNA. Proc. Natl. Acad. Sci. U.S.A. 73:3838–3842.
5. **de Vito, P. C., and J. Dreyfuss.** 1964. Metabolic regulation of adenosine triphosphate sulfurylase in yeast. J. Bacteriol. 88:1341–1348.
6. **DiNardo, S., K. A. Voelkel, R. Sternglanz, A. E. Reynolds, and A. Wright.** 1982. *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. Cell 31:43–51.
7. **Dubnau, E., A. B. Lenny, and P. Margolin.** 1973. Nonsense mutations of the *supX* locus: further characterization of the *supX* mutant phenotype. Mol. Gen. Genet. 126:191–200.
8. **Dubnau, E., and P. Margolin.** 1972. Suppression of promoter mutations by the pleiotropic *supX* mutations. Mol. Gen. Genet. 117:91–112.
9. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. 50:879–910.
10. **Gornall, A. G., C. J. Bardawill, and M. M. David.** 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751–766.
11. **Graf, L. H., Jr., and R. O. Burns.** 1973. The *supX/leu-500* mutations and expression of the leucine operon. Mol. Gen. Genet. 126:291–301.
12. **Hartman, P. E., Z. Hartman, R. C. Stahl, and B. N. Ames.** 1971. Classification and mapping of spontaneous and induced mutations in the histidine operon of *Salmonella*. Adv. Genet. 16:1–34.
13. **Ino, I., and M. Demerec.** 1968. Enteric hybrids. II. *S. typhimurium-E. coli* hybrids for the *trp-cysB-pyrF* region. Genetics 59:167–176.
14. **Jagura, G., D. Hulanicka, and N. M. Kredich.** 1978. Analysis of merodiploids of the *cysB* region in *Salmonella typhimurium*. Mol. Gen. Genet. 165:31–38.
15. **Jagura-Burdzy, G., and D. Hulanicka.** 1981. Use of gene fusions to study expression of *cysB*, the regulatory gene of the cysteine regulon. J. Bacteriol. 147:744–751.
16. **Jeffreys, A. J., and R. A. Flavell.** 1977. The rabbit β-globin gene contains a large insert in the coding sequence. Cell 12:1097–1108.
17. **Jones-Mortimer, M. C.** 1968. Positive control of sulphate reduction in *Escherichia coli*: the nature of the pleiotropic cysteineless mutants of *E. coli* K12. Biochem. J. 110:597–602.
18. **Kredich, N. M.** 1971. Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*: effects of growth on varying sulfur sources and *O*-acetyl-L-serine on gene expression. J. Biol. Chem. 246:3474–3484.
19. **Kupersztoch-Portnoy, Y. M., M. A. Lovett, and D. R. Helinski.** 1974. Strand and site specificity of the relaxation event for the relaxation complex of the antibiotic resistance plasmid R6K. Biochemistry 13:5484–5490.
20. **Kushner, S. R.** 1978. An improved method for transformation of *E. coli* with colE1 derived plasmids, p. 17–22. *In* H. W. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland Biomedical Press, New York.
21. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. 119:1072–1074.
22. **Lenny, A. B., and P. Margolin.** 1980. Locations of the *opp* and *supX* genes of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. 143:747–752.

23. **Maniatis, T., S. G. Kee, A. Efstratiadis, and F. C. Kafatos.** 1976. Amplification and characterization of a β-globin gene synthesized *in vitro*. Cell **8**:163–182.
24. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.
25. **Mascarenhas, D. M., and M. D. Yudkin.** 1980. Identification of a positive regulatory protein in *Escherichia coli*: the product of the *cysB* gene. Mol. Gen. Genet. **177**:535–539.
26. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.
27. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 431–433. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Mizobuchi, K., M. Demerec, and D. H. Gillespie.** 1962. Cysteine mutants of *Salmonella typhimurium*. Genetics **47**:1617–1627.
29. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
30. **Pruss, G. J., S. H. Manes, and K. Drlica.** 1982. *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. Cell **31**:35–42.
31. **Southern, E.** 1979. Gel electrophoresis of restriction fragments. Methods Enzymol. **68**:152–176.
32. **Stuttard, C.** 1975. Tryptophan biosynthesis in *Salmonella typhimurium*: location in *trpB* of a genetic difference between strains LT2 and LT7. J. Bacteriol. **123**:878–887.
33. **Tully, M., and M. D. Yudkin.** 1977. Fine-structure mapping and complementation analysis of the *Escherichia coli cysB* gene. J. Bacteriol. **131**:49–56.
34. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
35. **Yang, R. C.-A., J. Lis, and R. Wu.** 1979. Elution of DNA from agarose gels after electrophoresis. Methods Enzymol. **68**:176–182.