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Negative Autoregulation of *cysB* in *Salmonella typhimurium*: In Vitro Interactions of CysB Protein with the *cysB* Promoter

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CysB protein positively regulates genes of the *Salmonella typhimurium* cysteine regulon and negatively autoregulates *cysB*. The *cysB* promoter was characterized by primer extension of cellular RNA, which gave products identifying a major in vivo transcription start site located 95 bp upstream of the *cysB* start codon and two minor sites located 9 and 10 bp downstream of the major site. Gel shift binding studies and DNase I footprinting experiments showed that CysB protein binds to the *cysB* promoter from position −10 to +36 relative to the major transcription start site. We have designated this binding site CBS-B. CysB protein inhibited transcription initiation at the *cysB* promoter in an in vitro runoff assay, indicating that *cysB* is negatively autoregulated by the binding of CysB protein to the *cysB* promoter, where it acts as a repressor. *N*-Acetyl-L-serine, an inducer of the cysteine regulon, inhibited the binding of CysB protein to the *cysB* promoter and partially reversed the ability of CysB protein to inhibit transcription initiation. These effects are in contrast to those observed in studies of positively regulated *cys* promoters, in which *N*-acetyl-L-serine stimulates binding and causes CysB protein to activate transcription initiation.

The *cysB* gene of *Salmonella typhimurium* and *Escherichia coli* encodes the peptide subunit of a 144-kDa homotetramer that acts as a transcriptional activator for the cysteine regulon, a group of at least 16 genes that function in the synthesis of L-cysteine from inorganic sulfate and in L-cystine transport (3, 18). Gene activation in the cysteine regulon requires a combination of CysB protein, either *O*-acetyl-L-serine or its derivative *N*-acetyl-L-serine (both of which serve as inducers), and sulfur limitation (15–17, 29). The requirement for sulfur limitation is due to the ability of L-cysteine to feedback inhibit serine transacetylase, the enzyme that synthesizes *O*-acetyl-L-serine (19, 20), and also results from the fact that sulfide is an anti-inducer (30). Purified CysB protein has been shown in vitro to bind to sequences immediately upstream of the −35 regions of the *S. typhimurium cysJIH* and *cysK* promoters and to activate transcription from these promoters in the presence of acetyl-L-serine (27, 29, 30).

In *cysB-lac* fusion strains of *E. coli*, in which *lac* was expressed from the *cysB* promoter, introduction of a plasmid carrying a $cysB^+$ allele reduced β-galactosidase and *lac* mRNA levels as much as 10-fold, indicating that *cysB* is negatively autoregulated (4, 14). Thus, CysB protein appears to have a dual regulatory role, first as an activator of genes of the L-cysteine biosynthetic pathway and second as a repressor of its own gene. In this report, we provide in vitro evidence for negative autoregulation by showing that CysB protein binds to the *cysB* promoter and inhibits transcription initiation.

MATERIALS AND METHODS

Plasmids and media. pTEC30 is a pT7T3 19U derivative containing the *cysB* region on a 1.7-kb *SalI-SstI* fragment obtained from an *S. typhimurium* LT2 library (26). The DNA sequence of the *cysB* coding region and the 443-bp upstream region in the LT2 strain is identical to that reported for the LT7 strain (28). Plasmid pJOK201 is a pUC19 derivative with a 509-bp *KpnI-EcoRI* insert containing the *cysJIH* promoter and the first 100 bp of the *cysJ* coding region from *S. typhimurium* LT7 (29). Medium E (40), prepared with an equimolar amount of $MgCl_2$ in place of $MgSO_4$, served as our minimal medium and was supplemented with 0.5% glucose and either 1 mM Na_2SO_4, 0.5 mM L-cystine, or 0.5 mM reduced glutathione as a sulfur source.

Analyses of in vivo transcripts. Total cellular RNA was prepared by the method of Aiba et al. (1). Primer extension experiments were performed as previously described (29) with 6 U of avian myeloblastosis virus reverse transcriptase (U.S. Biochemical Corp.), 25 μg of total RNA, and 0.1 pmol of 5′ ^{32}P-labeled synthetic oligodeoxynucleotide per 50 μl of incubation mixture. Radiolabeled products were analyzed in sequencing gels.

DNA fragments. DNA fragments used for binding, DNase I protection footprinting, and transcription runoff experiments were generated by the polymerase chain reaction (PCR) according to the directions supplied with a Gene-Amp DNA amplification kit from Perkin-Elmer/Cetus. pTEC30 and pJOK201 served as templates for *cysB* and *cysJIH* promoter fragments, respectively. Oligodeoxynucleotide primers were prepared on an Applied Biosystems model 380A automated DNA synthesizer, purified and detritylated on OPC cartridges from the same company (24), and, where indicated, 5′ labeled with [γ-^{32}P]ATP (3,000 Ci/mmol) and T4 polynucleotide kinase (22). Sequences of the oligonucleotide primers used in these studies are shown in Fig. 1. A 458-bp *BglII-PvuII* fragment containing the phage λ p_L promoter with its start site oriented 325 bp upstream of the *PvuII* end (31) was obtained from D. Steege.

DNA-binding assay. DNA binding was studied by the gel mobility shift method (10, 11). Horizontal polyacrylamide gels (5% acrylamide, 0.061% bisacrylamide) were prepared in running buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM disodium EDTA) and prerun at 10 V/cm for 30 min. Binding reactions were carried out in 20 μl of a solution containing 40 mM Tris hydrochloride (pH 8.0), 0.1 M KCl, 10 mM $MgCl_2$,

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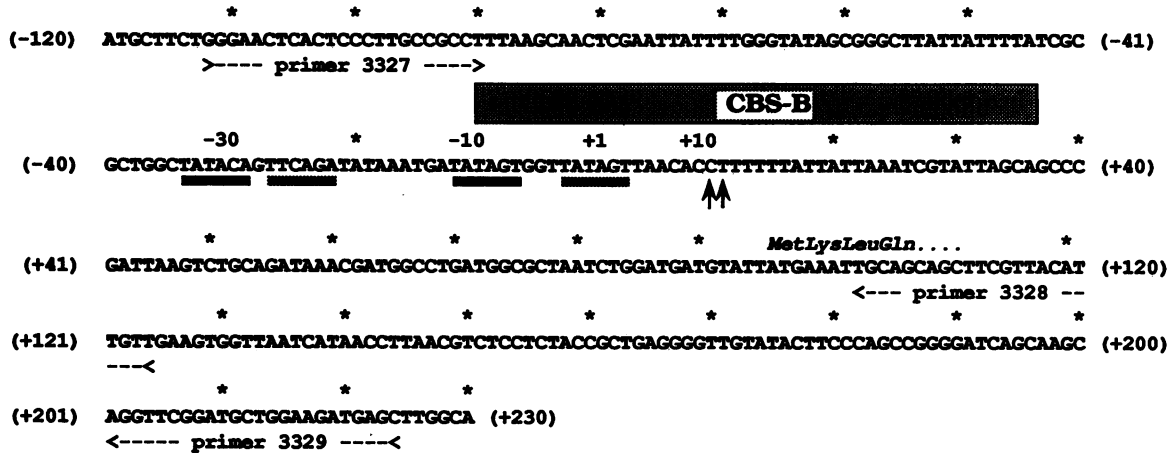

FIG. 1. DNA sequence of the *cysB* promoter region of *S. typhimurium*. Positions are numbered with reference to the major in vivo transcription start site (position +1), which was determined by primer extension analysis. Minor start sites are indicated by arrows at positions +10 and +11. Sequences corresponding to −35 and −10 regions are shown with dark underlines for the major start site and with light underlines for the minor start sites. The ATG start codon for *cysB* is at position +96. CBS-B is the binding site for CysB protein as determined by DNase I protection studies and extends from position −10 to +36. The extent and orientation of three oligonucleotide primers used in these studies are also shown.

1 mM dithiothreitol, 100 μg of nuclease-free bovine serum albumin per ml, approximately 20 ng of 5′-labeled DNA fragment (1×10^5 to 2×10^5 dpm), and various amounts of purified *S. typhimurium* CysB protein and *N*-acetyl-L-serine. DNA concentrations were estimated by determining the amount of radiolabel present in a preparation of known specific activity. A concentrated stock solution of CysB protein (approximately 0.5 mg/ml) was freshly diluted into binding buffer just before use. After incubation for 5 min at 37°C, 3 μl of loading buffer (80 mM Tris hydrochloride [pH 8.0], 0.2 M KCl, 2 mM dithiothreitol, 50% glycerol, 0.1% bromophenol blue, and 200 μg of bovine serum albumin per ml) was added with gentle mixing, and samples were loaded onto a gel with the current on. After 1 h at 10 V/cm, gels were dried, and radiolabeled DNA was visualized by radioautography.

DNase I protection footprinting. DNA-CysB protein complexes were formed by incubating a DNA fragment labeled at a single 5′ end with various amounts of purified CysB protein and *N*-acetyl-L-serine in 50 μl of a solution containing 40 mM Tris hydrochloride (pH 8.0), 0.1 M KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 100 μg of bovine serum albumin per ml for 5 min at 37°C in a 1.5-ml microcentrifuge tube. Following the addition of 2 μl of sonicated calf thymus DNA (1 mg/ml) and 5 μl of 0.1 M $CaCl_2$, digestion was initiated with 2 μl of DNase I (0.1 μg/ml). After 4 min at 37°C, the reaction was terminated with 2.5 μl of 0.25 M disodium EDTA, pH 8.0. Phenol-extracted and ethanol-precipitated products of DNA degradation were then analyzed in sequencing gels.

In vitro transcription assays. Transcription runoff assays were performed as described previously (29). Various amounts of CysB protein, *N*-acetyl-L-serine, and sodium sulfide were preincubated at 37°C in 20 μl of a solution containing 40 mM Tris hydrochloride, (pH 8.0), 0.1 M KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM ATP, 1 μg of template DNA per ml, 50 μg of nuclease-free *E. coli* RNA polymerase (Pharmacia-LKB Biotechnology, Inc.) per ml, and 100 μg of nuclease-free bovine serum albumin per ml. After 5 min to allow formation of initiation complexes, 2 μl was added of a solution containing 10 μM [α-^{32}P]CTP (800 Ci/mmol), 2 mM each ATP, GTP, and UTP, and 0.5 mg of sodium heparin per ml, and the reaction was allowed to proceed for another 5 min at 37°C, when it was terminated by the addition of 0.2 ml of 10 mM disodium EDTA containing 50 μg of yeast tRNA per ml. Phenol-extracted and ethanol-precipitated radiolabeled transcripts were analyzed in sequencing gels.

Other methods. *S. typhimurium* CysB protein was purified through the methyl agarose step as described previously (25) and was estimated to be 85 to 90% pure. Protein concentrations were estimated by the method of Bradford (6), with bovine serum albumin as a standard. *N*-Acetyl-L-serine was prepared by the method of Sakami and Toennies (34).

RESULTS

In vivo transcription start site. Total RNA from wild-type *S. typhimurium* LT2 grown on minimal medium containing either glutathione, sulfate, or L-cystine was used as template with reverse transcriptase to extend a 5′-labeled oligodeoxynucleotide primer complementary to nucleotides 8 to 30 of the *cysB* coding region (primer 3328 in Fig. 1). Extension products of 124, 115, and 114 nucleotides, corresponding to in vivo transcription start sites at 95, 86, and 85 nucleotides upstream of the ATG start codon, were obtained (Fig. 2). Small differences in the amounts of extension products were noted with RNA templates from cells grown on different sulfur sources (Fig. 2) but were not consistent enough in other experiments to suggest a major effect of sulfur source on *cysB* expression. Since the 124-nucleotide product constituted about 80% of the total, we consider its start site the major one in vivo and have designated it position +1 in Fig. 1. The −10 and −35 regions of this promoter are TATAGT and TATACA, respectively. The shorter extension products may be artifacts due to either premature termination of reverse transcriptase or in vivo endonucleolytic degradation, or they may correspond to minor in vivo start sites located 9 and 10 nucleotides downstream of the major start site. The latter interpretation is favored by the presence of the sequences TATAGT and TTCAGA that are appropriately positioned at the −10 and −35 regions (Fig. 1). This −10

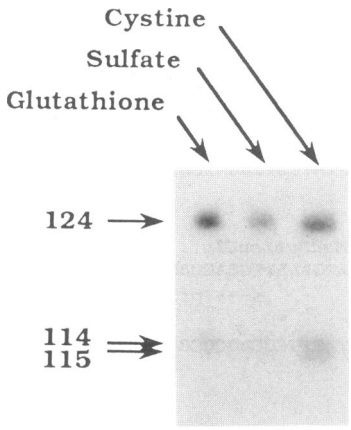

FIG. 2. Primer extension analysis of the in vivo transcription start sites for the *S. typhimurium cysB* promoter. Total RNA was isolated from the wild-type LT2 strain grown on either glutathione, sulfate, or L-cystine as a sulfur source and used as a template for extension of the 23-mer oligodeoxynucleotide primer 3328 (Fig. 1), which was 5′ labeled with ^{32}P. Product sizes were compared with DNA sequencing lanes (not shown). The numbers refer to the lengths of the products (nucleotides).

region is identical to that for the major start site and offset from it by 9 bp.

Binding of CysB protein to the *cysB* promoter region. We evaluated binding of CysB protein to the *cysB* promoter region by the gel mobility shift assay (10, 11) by using a PCR-generated, 5′-radiolabeled DNA fragment beginning at position −112 relative to the major transcription start site and extending to position +124 (generated with primers 3327 and 3328) (Fig. 1). Preincubation of this fragment with small amounts of purified CysB protein resulted in the formation of a new electrophoretic band with a mobility of 0.57 to 0.60 relative to that of free DNA (Fig. 3). With an input DNA concentration of 1 μg/ml (6.5 nM), approximately 70% of the total was complexed at 3 μg of CysB protein per ml (21 nM of tetramer) and binding was complete at 10 μg/ml. From these data a K_d of 7×10^{-9} M was calculated, which is slightly higher than the K_d of 2×10^{-9} M estimated for the binding of CysB protein to the *cysK* promoter (27). We have previously shown that nonspecific binding to DNA is minimal at these concentrations of CysB protein (27, 30). It should be noted that this K_d value is only a rough approximation and is based on several assumptions, including the accuracy of our protein estimations by the dye-ligand assay method (6) with a bovine serum albumin standard, the absence of any significant loss of binding activity during purification and storage of CysB protein, and a stoichiometry of one CysB protein tetramer per DNA molecule.

In contrast to results obtained with the *cysJIH* and *cysK* promoters, which showed that specific binding of CysB protein was stimulated by the inducer *N*-acetyl-L-serine or *O*-acetyl-L-serine (27, 30), binding to the *cysB* promoter region was inhibited by *N*-acetyl-L-serine. At a concentration of 3 μg of CysB protein per ml, the addition of 5 mM *N*-acetyl-L-serine decreased binding from about 70% of input to 20% (Fig. 3).

Binding of CysB protein to the *cysB* promoter was further characterized by DNase I footprinting experiments. CysB protein at 10 to 40 μg/ml protected the nontranscribed strand of the 236-bp DNA fragment from DNase I digestion between positions −10 and +36 relative to the major in vivo transcription start site (Fig. 4). Protection was also noted for the transcribed strand between positions −10 and +34 (data not shown). In agreement with the results of our gel shift binding experiments, protection was virtually eliminated by *N*-acetyl-L-serine at 1 and 10 mM (Fig. 4). Previous studies have demonstrated two CysB protein-binding sites, CBS-K1 and CBS-K2, in the *cysK* promoter region and a single site, CBS-J, in the *cysJIH* promoter (27). We now designate the −10 to +36 region of the *cysB* promoter CBS-B. The sizes of these four regions are similar and range between 36 and 46 bp.

In vitro transcription. In vitro transcription initiation at the *cysB* promoter was characterized in a transcription runoff

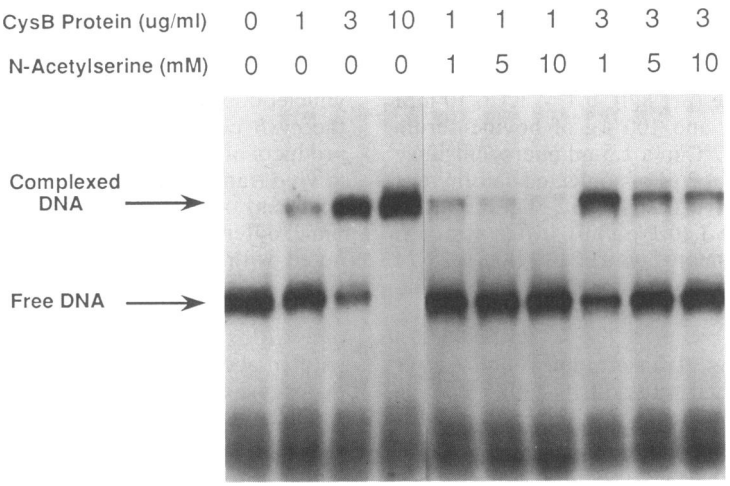

FIG. 3. Binding of CysB protein to the *cysB* promoter region in a gel mobility shift assay. The radiolabeled 236-bp DNA fragment used in this study was generated by PCR with oligonucleotide primers 3327, which was 5′ labeled, and 3328 (Fig. 1) and extended from position −112 to +124 relative to the major in vivo transcription start site. DNA (1 μg/ml) was preincubated with various amounts of purified CysB protein and *N*-acetyl-L-serine for 5 min at 37°C. Mixtures were then run in a 5% polyacrylamide gel as described in Materials and Methods, and the gel was analyzed by radioautography. Complexed and free DNA are indicated with arrows, and the radiolabel at the bottom of the gels is excess oligonucleotide primer.

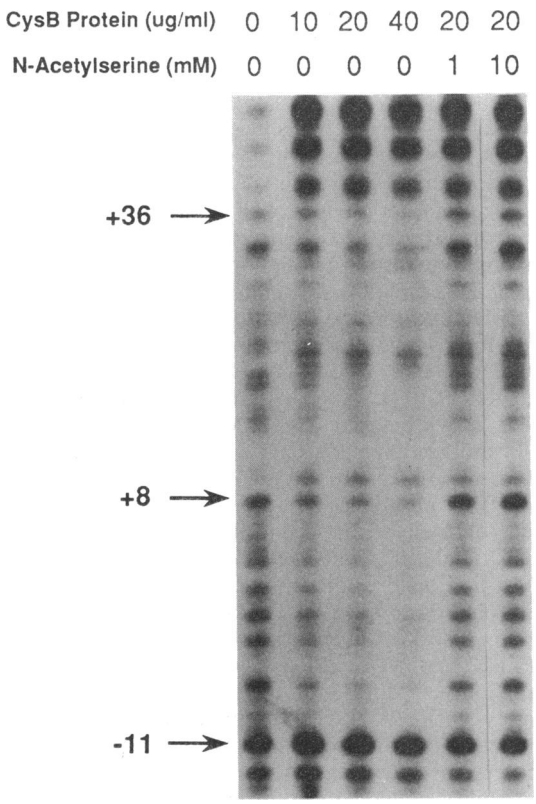

FIG. 4. DNase I protection of the nontranscribed strand of the *cysB* promoter region by CysB protein. The 236-bp DNA substrate was identical to that described in the legend to Fig. 3 and extended from position −112 to +124 relative to the major in vivo transcription start site. Following digestion with DNase I, the products were analyzed on a DNA sequencing gel as described in Materials and Methods. CysB protein and *N*-acetyl-L-serine were present as indicated. The numbers on the left refer to the position relative to the major in vivo transcription start site.

assay with a 336-bp template extending from position −112 to +224 relative to the major in vivo transcription start site (generated by PCR with primers 3327 and 3329) (Fig. 1). Products of 224 and 235 nucleotides, which correspond to initiation at the major in vivo start site and to a site at position −11, were observed (Fig. 5). The longer transcript is apparently the product of a start site that was not expressed in vivo and may be an artifact of our in vitro conditions. A minor transcription product of 176 nucleotides was also observed, which may be a premature termination product of a longer transcript. CysB protein inhibited formation of all three products by 10 to 20% at 1 μg/ml and by about 80% at 20 μg/ml. *N*-Acetyl-L-serine at 1 to 10 mM partially reversed inhibition by 20 μg of CysB protein per ml (Fig. 5). The specificity of this effect is apparent from our finding that CysB protein at 1 to 50 μg/ml, either with or without *N*-acetyl-L-serine, did not affect the formation of transcription runoff products from a DNA template containing the phage λ p_L promoter (data not shown).

The inhibitory effects of CysB protein on transcription initiation at the *cysB* promoter were opposite to those observed with the *cysJIH* and *cysK* promoters, where this regulatory protein, in the presence of acetyl-L-serine, acts as a transcription activator (27, 29). To illustrate this difference, we performed a transcription runoff experiment with a

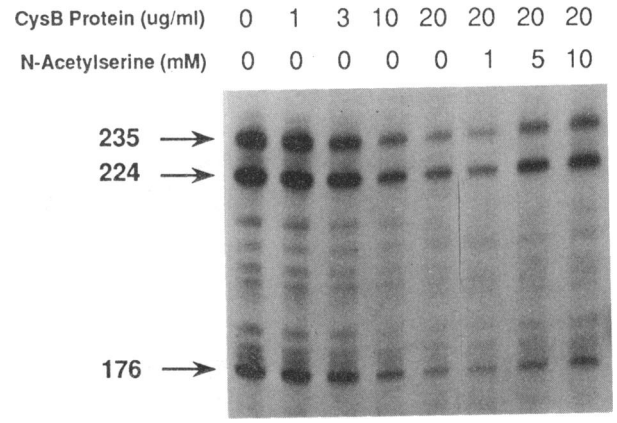

FIG. 5. Effects of CysB protein and *N*-acetyl-L-serine on transcription initiation from the *cysB* promoter in an in vitro runoff assay. Reaction mixtures contained 50 μg of RNA polymerase per ml and a 336-bp DNA template (1 μg/ml) extending from position −112 to +224 relative to the major in vivo transcription start site, which was generated by PCR with oligonucleotide primers 3327 and 3329 (Fig. 1). Mixtures were preincubated for 5 min at 37°C with CysB protein and *N*-acetyl-L-serine as indicated, and radiolabeled transcription runoff products were generated and analyzed on a sequencing gel as described in Materials and Methods. Major products of 235, 224, and 176 nucleotides are indicated.

mixture of two DNA templates, one containing the *cysB* promoter on the 336-bp fragment described above and the other containing the *cysJIH* promoter on a 315-bp PCR-generated fragment extending from position −218 to +97 relative to the major in vivo transcription start site (29). The preferred in vitro start site for *cysJIH* transcription initiation varies from one template to another in a way that has not been systematically characterized (30a). For the template used in this study, the major transcript was 90 nucleotides in length and corresponded to initiation from a minor in vivo start site (29). The results of this experiment showed that formation of the two major *cysB* transcripts was maximal in the absence of CysB protein and *N*-acetyl-L-serine, while transcription from the *cysJIH* promoter in the same reaction mixture was almost unmeasurable (Fig. 6). Addition of 20 μg of CysB protein per ml inhibited transcription from the *cysB* promoter by about 70% with no effect on the *cysJIH* promoter. The further addition of 0.3 mM or 1 mM *N*-acetyl-L-serine reversed the inhibitory effects of CysB protein on the *cysB* promoter and markedly stimulated transcription from the *cysJIH* promoter. Furthermore, sulfide, which is known to act as an anti-inducer of the cysteine regulon (17, 30), inhibited formation of the *cysJIH* transcript in the presence of 1 mM *N*-acetyl-L-serine but did not affect the *cysB* transcript (Fig. 6).

DISCUSSION

The in vitro studies reported here indicate that negative autoregulation of *cysB*, originally proposed on the basis of in vivo properties of *cysB-lac* fusion strains (4, 14), is due to the ability of CysB protein to bind to the *cysB* promoter and inhibit transcription. Autoregulation has been described for the genes of a number of prokaryote transcriptional-activator proteins (8, 32, 36) and appears to be particularly common among members of the LysR family of regulatory genes (12), of which *cysB* is a member. Other members of the LysR family for which there is evidence of autoregulation

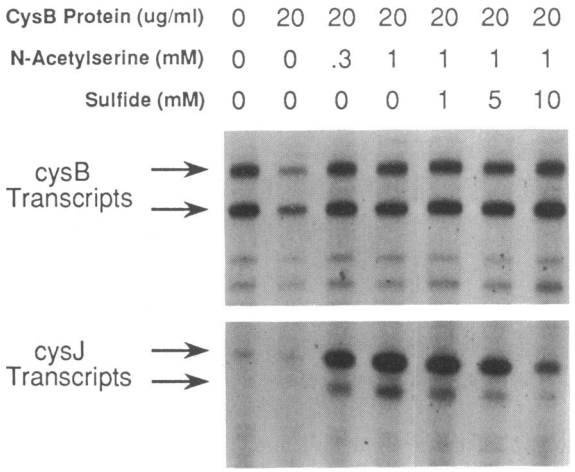

FIG. 6. Comparison of the effects of CysB protein, *N*-acetyl-L-serine, and sulfide on transcription initiation from the *cysB* and *cysJIH* promoters in an in vitro runoff assay. All reactions contained a mixture of two PCR-generated DNA templates, each at 1 μg per ml. The 336-bp *cysB* promoter template was the same as that described in the legend to Fig. 5 and extended from position −112 to +124 relative to the major in vivo transcription start site. The 315-bp *cysJIH* promoter template extended from position −218 to +97 relative to the major in vivo transcription start site (29). Mixtures were preincubated for 5 min at 37°C with CysB protein, *N*-acetyl-L-serine, and sulfide as indicated, and radiolabeled transcription runoff products were generated and analyzed on a sequencing gel as described in Materials and Methods. The middle portion of the gel has been cut away. The upper portion of the gel shows two major *cysB* runoff products of 235 and 224 nucleotides. The lower portion of the gel shows a major *cysJIH* runoff product of 90 nucleotides and a minor product of 88 nucleotides. The identity of runoff products was established in separate reactions containing single DNA templates (not shown).

include *lysR* (38), *ilvY* (41), and *oxyR* (5, 9) in *E. coli*; *metR* in both *E. coli* and *S. typhimurium* (23, 39); *nodD* in *Rhizobium* spp. (13, 33); *ampR* in *Citrobacter freundii* (21); and *nahR* in *Pseudomonas putida* (35).

For many members of the LysR family, the regulatory gene promoter is adjacent to or overlaps the promoter for a gene (or group of genes), which is positively controlled by the regulatory gene product, and the two units are transcribed divergently. In theory, this arrangement allows the binding of a protein at a single site to both activate the positively regulated promoter and repress the regulatory gene promoter. A variation of this model is found in the *ilvC-ilvY* promoter region of *E. coli*, where IlvY protein binds cooperatively to two virtually contiguous sites (41). A major requirement for a common binding site is that it must position the regulatory protein to facilitate the binding of RNA polymerase to the activated promoter while inhibiting binding to the regulatory gene promoter—presumably by a steric mechanism. *cysB* is not linked to a positively controlled gene (28), however, and the absence of a requirement for gene activation may explain certain differences between CBS-B and binding sites CBS-J (29, 30) and CBS-K1 (27), which mediate activation of the *cysJIH* and *cysK* promoters by CysB protein and acetyl-L-serine. One of the most striking differences between these sites is that acetyl-L-serine stimulates CysB protein binding to CBS-J and CBS-K1 and, as demonstrated in this report, inhibits CysB protein binding to CBS-B. The autoregulated *oxyR* gene in *E. coli* is also not linked to any of the genes for which it serves as an activator (9), but although DNase I footprints are qualitatively different with reduced and oxidized forms of highly purified OxyR protein, appreciable quantitative differences in binding to positively and negatively regulated promoters have not been observed (37).

The ability of *N*-acetyl-L-serine to inhibit the binding of CysB protein to the *cysB* promoter and to reverse inhibition of in vitro transcription initiation suggests that this phenomenon might have relevance in vivo. By altering the sensitivity of the *cysB* promoter to negative autoregulation, *N*-acetyl-L-serine, itself a signal of sulfur limitation, could serve to fine-tune *cysB* expression in response to changing conditions of sulfur availability. The results of our primer extension analyses, however, gave no indication that *cysB* mRNA levels vary between cells grown on either L-cystine, sulfate, or the limiting sulfur source glutathione. Furthermore, using a sensitive enzyme-linked immunosorbent assay for CysB protein, we have been unable to find consistent

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St  CBS-B    (-10)   TAGTGGTTATAGTTAACACCTTTTTTATTATTAAATCGTATTAGCA (+36)
Ec "CBS-B"   (-10)   TAGTGGTTATAGTTAGCACCTTTTTTATTATTAAATCGTATTAGTC (+36)
St  CBS-K2   (-117)   AAATAAGAGATGGCTTATGCTGTCTCTTATTCCATACTGATA (-76)
Ec "CBS-K2"  (-119)   GAAACAGGGGTGGCTTATGCCGCCCCTTATTCCATCTTGCAT (-80)
Consensus                              A.........TTATT..AT..T

St  CBS-K1   (-78) ATAACCATTATTTCCCATCAGCATATAGATATGCGAAATCCTTACTTCCCCAT (-26)
Ec "CBS-K1"  (-80) CATGTCATTATTTCCCTTCTGTATATAGATATGCTAAATCCTTACTTCCGCAT (-28)
St  CBS-J    (-79) AAACAGGTTAGTTCATTTGGTTATTT#GTTATTTCCAACCCTTCTTTAATTGT (-28)
Ec "CBS-J"   (-78) AAACAGGTTAGTCGATTTGGTTATTA#GTTATCGCTATCCCGTCTTTAATCCA (-27)
Consensus                    TTA.T.....T....AT...G.TAT....A..CC.T..TT
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FIG. 7. DNA sequence comparisons of CysB protein-binding sites CBS-B, CBS-K2, CBS-K1, and CBS-J from *S. typhimurium*. The homologous regions from *E. coli* are also included. The CBS-J, CBS-K1, and CBS-K2 sites were characterized by Monroe et al. (27). A 1-bp gap has been introduced into the CBS-J sequence to maximize its identity with CBS-K1. The sequence TTATT occurs at least once in all eight sequences.

differences between cells grown on different sulfur sources (3a). These results are consistent with those obtained with *cysB-lac* fusion strains, in which *lac* expression directed from the *cysB* promoter was not affected by the type of sulfur compound used for growth (14).

Inhibition of CysB protein binding by acetyl-L-serine has also been described for another binding site, designated CBS-K2, which is upstream of and contiguous with CBS-K1 in the *cysK* promoter (27). Deletion of CBS-K2 does not affect in vivo *cysK* promoter strength, and at present this binding site has no known function. A DNA sequence comparison of CBS-B and CBS-K2 from *S. typhimurium* and the corresponding regions from *E. coli* shows only a small region of similarity (Fig. 7). All four contain at least one copy of the sequence TTATT, which is also found in CBS-J and CBS-K1. Thus far, we have been unable to define a consensus DNA sequence that would constitute a cysteine box, and it is not known whether the TTATT sequence is important for CysB protein binding.

The ability of CysB protein to respond to acetyl-L-serine with either an increase or a decrease in affinity for different DNA sequences raises the question of whether these two types of binding occur at the same or separate regions of the protein. By analogy with certain other DNA-binding proteins, the binding domain of CysB protein is postulated to reside in a helix-turn-helix motif predicted to occur in amino acid residues 18 to 38 (7). Although no direct experimental evidence for the interaction of this region with either type of DNA sequence exists, it is of interest that a Ser→Arg change at amino acid residue 34 in the *cysB70* protein results in both a *cysB* mutant genotype and the loss of in vivo autoregulation (2, 9a).

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REFERENCES

1. **Aiba, H., S. Adhya, and B. de Crombrugghe.** 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **256**:11905–11910.
2. **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.
3. **Baptist, E. W., and N. M. Kredich.** 1977. Regulation of L-cystine transport in *Salmonella typhimurium*. J. Bacteriol. **131**:111–118.

3a. **Bastia, C., and N. M. Kredich.** Unpublished data.

4. **Bielinska, A., and D. Hulanicka.** 1986. Regulation of the *cysB* gene expression in *Escherichia coli*. Acta Biochim. Pol. **33**:133–137.
5. **Bölker, M., and R. Kahmann.** 1989. The *Escherichia coli* regulatory protein OxyR discriminates between methylated and unmethylated states of the phage Mu *mom* promoter. EMBO J. **8**:2403–2410.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **71**:248–254.
7. **Brennan, R. G., and B. W. Matthews.** 1989. The helix-turn-helix DNA binding motif. J. Biol. Chem. **264**:1903–1906.
8. **Casadaban, M. J.** 1976. Regulation of the regulatory gene for the arabinose pathway, *araC*. J. Mol. Biol. **104**:557–566.
9. **Christman, M. F., G. Storz, and B. A. Ames.** 1989. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. Proc. Natl. Acad. Sci. USA **86**:3484–3488.

9a. **Colyer, T. E., and N. M. Kredich.** 1989. Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, H 181, p. 199.

10. **Fried, M., and D. M. Crothers.** 1981. Equilibria and kinetics of *lac* repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
11. **Garner, M. M., and A. Revzin.** 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: application to components of the *Escherichia coli* lactose operon regulatory system. Nucleic Acids Res. **9**:3047–3060.
12. **Henikoff, S., G. W. Haughn, J. M. Calvo, and J. C. Wallace.** 1988. A large family of bacterial activator proteins. Proc. Natl. Acad. Sci. USA **85**:6602–6606.
13. **Hong, G.-F., J. E. Burn, and A. W. B. Johnston.** 1988. Evidence that DNA involved in the expression of nodulation (*nod*) genes in *Rhizobium* binds to the product of the regulatory gene *nodD*. Nucleic Acids Res. **15**:9677–9689.
14. **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.
15. **Jones-Mortimer, M. C.** 1968. Positive control of sulfate reduction in *Escherichia coli*: the nature of the pleiotropic cysteineless mutants of *E. coli* K12. Biochem. J. **110**:597–602.
16. **Jones-Mortimer, M. C., J. R. Wheldrake, and C. A. Pasternak.** 1968. The control of sulphate reduction in *Escherichia coli* by *O*-acetyl-L-serine. Biochem. J. **107**:51–53.
17. **Kredich, N. M.** 1971. Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*. I. Effects of growth on varying sulfur sources and *O*-acetyl-L-serine on gene expression. J. Biol. Chem. **246**:3474–3484.
18. **Kredich, N. M.** 1987. Biosynthesis of cysteine, p. 419–428. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
19. **Kredich, N. M., M. A. Becker, and G. M. Tomkins.** 1969. Purification and characterization of cysteine synthetase, a bifunctional protein complex, from *Salmonella typhimurium*. J. Biol. Chem. **244**:2428–2439.
20. **Kredich, N. M., and G. M. Tomkins.** 1966. The enzymatic synthesis of L-cysteine in *Escherichia coli* and *Salmonella typhimurium*. J. Biol. Chem. **241**:4955–4965.
21. **Lindquist, S., F. Lindberg, and S. Normark.** 1989. Binding of the *Citrobacter freundii* AmpR regulator to a single DNA site provides both autoregulation and activation of the inducible *ampC* β-lactamase gene. J. Bacteriol. **171**:3746–3753.
22. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
23. **Maxon, M. E., B. Redfield, X.-Y. Cai, R. Shoeman, K. Fujita, W. Fisher, G. Stauffer, H. Weissbach, and N. Brot.** 1989. Regulation of methionine biosynthesis in *Escherichia coli*: effect of the MetR protein on the expression of the *metE* and *metR* genes. Proc. Natl. Acad. Sci. USA **86**:85–89.
24. **McBride, L. J., C. McCollum, S. Davidson, J. W. Efcavitch, A. Andrus, and S. J. Lombardi.** 1988. A new, reliable cartridge for the rapid purification of synthetic DNA. BioTechniques **6**:362–367.
25. **Miller, B. E., and N. M. Kredich.** 1987. Purification of the cysB protein from *Salmonella typhimurium*. J. Biol. Chem. **262**:6006–6009.
26. **Monroe, R. S., and N. M. Kredich.** 1988. Isolation of *Salmonella typhimurium cys* genes by transduction with a library of recombinant plasmids packaged in bacteriophage P22HT capsids. J. Bacteriol. **170**:42–47.
27. **Monroe, R. S., J. Ostrowski, M. M. Hryniewicz, and N. M. Kredich.** 1990. In vitro interactions of CysB protein with the *cysK* and *cysJIH* promoter regions of *Salmonella typhimurium*. J. Bacteriol. **172**:6919–6929.
28. **Ostrowski, J., G. Jagura-Burdzy, and N. M. Kredich.** 1987. DNA sequences of the *cysB* regions of *Salmonella typhimurium* and *Escherichia coli*. J. Biol. Chem. **262**:5999–6005.
29. **Ostrowski, J., and N. M. Kredich.** 1989. Molecular characterization of the *cysJIH* promoters of *Salmonella typhimurium* and

Escherichia coli: regulation by *cysB* protein and *N*-acetyl-L-serine. J. Bacteriol. **171**:130–140.

30. **Ostrowski, J., and N. M. Kredich.** 1990. In vitro interactions of CysB protein with the *cysJIH* promoter of *Salmonella typhimurium*: inhibitory effects of sulfide. J. Bacteriol. **172**:779–785.

30a. **Ostrowski, J., and N. M. Kredich.** Unpublished data.

31. **Pavco, P. A., and D. A. Steege.** 1990. Elongation by *Escherichia coli* RNA polymerase is blocked *in vivo* by a site-specific DNA binding protein. J. Biol. Chem. **265**:9960–9969.

32. **Raibaud, O., and M. Schwartz.** 1984. Positive control of transcription initiation in bacteria. Annu. Rev. Genet. **18**:173–206.

33. **Rossen, L., C. A. Shearman, A. W. B. Johnston, and J. A. Downie.** 1985. The *nodD* gene of *Rhizobium leguminosarum* is autoregulatory and in the presence of plant exudate induces the *nodA, B, C* genes. EMBO J. **4**:3369–3373.

34. **Sakami, W., and G. Toennies.** 1942. The investigation of amino acid reactions by methods of non-aqueous titrimetry. J. Biol. Chem. **144**:203–217.

35. **Schell, M. A., and E. F. Poser.** 1989. Demonstration, characterization, and mutational analysis of NahR protein binding to *nah* and *sal* promoters. J. Bacteriol. **171**:837–846.

36. **Schleif, R.** 1987. The L-arabinose operon, p. 1473–1481. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

37. **Storz, G., L. A. Tartaglia, and B. N. Ames.** 1990. Transcriptional regulation of oxidative stress-inducible genes: direct activation by oxidation. Science **248**:189–194.

38. **Stragier, P., and J. C. Patte.** 1983. Regulation of diaminopimelate decarboxylase synthesis in *Escherichia coli*. III. Nucleotide sequence and regulation of the *lysR* gene. J. Mol. Biol. **168**:333–350.

39. **Urbanowski, M. L., and G. V. Stauffer.** 1987. Regulation of the *metR* gene of *Salmonella typhimurium*. J. Bacteriol. **169**:5841–5844.

40. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.

41. **Wek, R. C., and G. W. Hatfield.** 1988. Transcriptional activation at adjacent operators in the divergent-overlapping *ilvY* and *ilvC* promoters of *Escherichia coli*. J. Mol. Biol. **203**:643–663.