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# Identification of Sulfate Starvation-Regulated Genes in *Escherichia coli*: a Gene Cluster Involved in the Utilization of Taurine as a Sulfur Source

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**Genes whose expression is regulated by sulfate starvation in *Escherichia coli* were identified by generating random translational *lacZ* fusions in the chromosome with the λp*lac*Mu9 system. Nine *lacZ* fusion strains which expressed β-galactosidase after growth under sulfate starvation conditions but not after growth in the presence of sulfate were found. These included two strains with insertions in the *dmsA* and *rhsD* genes, respectively, and seven strains in which the insertions were located within a 1.8-kb region downstream of *hemB* at 8.5 minutes on the *E. coli* chromosome. Analysis of the nucleotide sequence of this region indicated the presence of four open reading frames designated *tauABCD*. Disruption of these genes resulted in the loss of the ability to utilize taurine (2-aminoethanesulfonate) as a source of sulfur but did not affect the utilization of a range of other aliphatic sulfonates as sulfur sources. The TauA protein contained a putative signal peptide for transport into the periplasm; the TauB and TauC proteins showed sequence similarity to ATP-binding proteins and membrane proteins, respectively, of ABC-type transport systems; and the TauD protein was related in sequence to a dichlorophenoxyacetic acid dioxygenase. We therefore suggest that the proteins encoded by *tauABC* constitute an uptake system for taurine and that the product of *tauD* is involved in the oxygenolytic release of sulfite from taurine. The transcription initiation site was detected 26 to 27 bp upstream of the translational start site of *tauA*. Expression of the *tauD* gene was dependent on CysB, the transcriptional activator of the cysteine regulon.**

In response to limitation for a specific nutrient, bacteria can adopt different survival strategies: they can increase the level of enzymes responsible for the assimilation of this nutrient; synthesize alternative, high-affinity proteins for this assimilation; or aim to obtain the nutrient from a broader range of compounds by expression of previously unused genes. All three possibilities need an accurate regulatory system which controls the expression of the proteins involved in the metabolism of the corresponding nutrient. For the three main nutrient elements carbon, nitrogen, and phosphorus, bacteria use all three such strategies, whereas for sulfur much less is known.

Sulfur is mainly assimilated from inorganic sulfate via the cysteine biosynthetic pathway. In *Escherichia coli* and *Salmonella typhimurium*, the corresponding genes have been characterized in detail and are regulated as the *cys* regulon (29). Maximum expression of these genes requires an active CysB protein, *O*-acetyl serine, and the absence of sulfate. In *E. coli*, the presence of excess sulfate reduces the expression of the *cys* genes to 40 to 50% of fully derepressed values, and in the presence of cysteine the expression is completely repressed (29). In several other bacteria, genes which are regulated by sulfur availability but which have not been shown to belong to the *cys* regulon have been detected (5, 17, 30, 35).

In nature, the levels of inorganic sulfate available to bacteria may be low (3), and bacteria then have to rely on organosulfur compounds such as sulfate esters, sulfamates, and sulfonates as sources of sulfur for the synthesis of proteins and certain cofactors. It has been known for a long time that members of the family *Enterobacteriaceae* can utilize different organosulfur compounds as sulfur sources for growth (29, 40). Only recently, however, have sulfur-containing compounds been systematically tested for their ability to allow growth under different conditions and with different genetic backgrounds (57, 58). *E. coli* is able to use several different alkanesulfonates as sulfur sources (40, 57), although none of the sulfonates tested so far could be utilized as a source of carbon and energy, or of carbon, energy, and sulfur, under either aerobic or anaerobic conditions (57). The utilization of alkanesulfonates has been shown to involve a part of the assimilatory sulfate reduction pathway, since sulfite reductase was required for growth with sulfonates and for production of sulfide from cysteate, taurine, or isethionate (58). Furthermore, mutants lacking serine transacetylase or the transcriptional activator CysB were unable to utilize isethionate for growth. It was therefore concluded that in *E. coli* sulfonate-sulfur enters the cysteine biosynthetic pathway at the stage of sulfite (58).

Although the genes and proteins involved in sulfonate transport and desulfonation are unknown, *E. coli* has been shown to utilize sulfate-sulfur in preference to sulfonate-sulfur when both are present (57). This implies that expression of the genes involved in sulfonate utilization does not occur in the presence of sulfate, or that sulfate directly inhibits the transport or desulfonation processes. The former has been observed for desulfonation of aliphatic and aromatic sulfonates and sulfate esters in pseudomonads (5, 6, 23).

By two-dimensional gel electrophoresis of *E. coli* extracts, we have previously identified a specific set of eight proteins which were not synthesized during growth with sulfate or cysteine and which were produced only when cells were grown

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TABLE 1. *E. coli* strains, phages, and plasmids used in this study

| Strain, phage, or plasmid | Relevant features | Reference or source |
|---|---|---|
| **Strains** | | |
| MC4100 | F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rbsR flbB5301* | 11 |
| MBM7014 | F$^-$ *araC*(Am) *araD139* Δ(*argF-lac*)*U169 trp*(Am) *mal*(Am) *rpsL150 relA1 thi supF* | G. M. Weinstock |
| MW-11 | MC4100 Φ(*tauC*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-15 | MC4100 Φ(*tauC*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-43 | MC4100 Φ(*tauC*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-61 | MC4100 Φ(*tauB*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-74 | MC4100 Φ(*tauD*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-82 | MC4100 Φ(*tauD*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-103 | MC4100 Φ(*rhsD*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-108 | MC4100 Φ(*tauD*::*lacZ*) (λp*lac*Mu9) | This study |
| MW-109 | MC4100 Φ(*dmsA*::*lacZ*) (λp*lac*Mu9) | This study |
| DH5α | *supE44* Δ*lacU169* (ϕ80 *lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1* | Laboratory collection |
| MDA4762 | *cysB trpB*::Tn*10* | 46 |
| MW-108*cysB* | MW-108 *cysB trpB*::Tn*10* | This study |
| **Phages** | | |
| λp*lac*Mu9 | *imm* λ *'lacZ lacY*$^+$ *lacA' 'ara' Xho*::*kan* Mu[*c*Its62 *ner*$^+$ *A*$^+$ *'S*] | 9 |
| λpMu507 | *c*I857 Sam7 Mu[*c*Its62 *ner*$^+$ *A*$^+$ *B*$^+$] | 9 |
| Mu*cts* | Heat-inducible phage Mu | DSMZ |
| λ8F10 | | 26 |
| λPC25 | Φ(*dmsA'-'lacZ*$^+$)*lacY*$^+$ *lacA*$^+$ | 12 |
| m13mp18/mp19 | Cloning vector; f1 origin of replication | 59 |
| **Plasmids** | | |
| pGEM7Zf− | Cloning vector; Ap$^r$ | Promega |
| pBluescript KS | Cloning vector; Ap$^r$ | Stratagene |
| pUC118/119 | Cloning vector; Ap$^r$; f1 origin of replication | 36 |
| pRS552 | *'lacZ lacY lacA*; used for construction of *tauA'-'lacZ* protein fusions; ColE1 replicon | 50 |
| pUC18ALA4 | 7.5-kb *Eco*RI fragment containing *tauABCD* cloned in *Eco*RI site of pUC18 | 37 |
| pME4100 | 3.1-kb *Bam*HI fragment from pUC18ALA4 in pUC18; *tau'CD* | This study |
| pME4101 | 3.0-kb *Bam*HI-*Hin*dIII fragment from pUC18ALA4 in pUC18; *tauABC'* | This study |
| pME4107 | Internal 1.8-kb *Bgl*II deletion in pUC18A1A4 | This study |
| pmE4120 | *Bam*HI-*Hin*dIII-digested PCR product obtained with primers lacZ-IK and JP8 from template pME4101 cloned in pBluescript KS | This study |
| pME4121 | *Bam*HI-*Cla*I fragment of pME4120 in pGEM7Zf− | This study |
| PME4131 | 0.46-kb *Bam*HI-*Eco*RI-digested PCR fragment obtained with primers JP8 and JP7 cloned in pRS552 | This study |
| pME4132 | 1.1-kb *Bam*HI-*Eco*RI fragment of pME4121 in pRS552 | This study |
| pME4134 | 172-bp *Bam*HI-*Mun*I fragment of pME4120 in pRS552 | This study |
| pME4135 | 235-bp *Bam*HI-*Eco*RI-digested PCR fragment obtained with primers JP8 and JP9 cloned in pRS552 | This study |
| pME4137 | 58-bp *Bam*HI-*Eco*RI-digested PCR fragment obtained with primers JP8 and JP10 cloned in pRS552 | This study |

with alternative sulfur sources, such as glutathione or ethanesulfonate (24, 38). These proteins have been named sulfate starvation-induced (SSI) proteins. By comparison of the N-terminal and internal peptide sequences of the SSI proteins with nucleotide and protein databases, three of the proteins were identified as the sulfate-binding protein, the general cystine-binding protein, and *O*-acetyl serine (thiol)lyase A (38). In enterobacteria synthesis of these proteins is known to be regulated as part of the *cys* regulon (29).

Here we describe, as an alternative to the identification of SSI proteins by two-dimensional gel electrophoresis, the isolation, characterization, and mapping of several sulfate-regulated *lacZ* fusions which have been generated with λp*lac*Mu9. The majority of these *lacZ* fusions are located in the *tauABCD* genes, which are required for the utilization of taurine as a sulfur source and whose products are thought to constitute an ABC transport system for taurine plus an enzyme involved in the desulfonation of this compound.

## MATERIALS AND METHODS

**Chemicals.** All chemicals used as sulfur sources were of the highest quality available and were obtained from Fluka (Buchs, Switzerland), except for isethionate, lanthionine, and methyl sulfate, which were purchased from Aldrich (Buchs, Switzerland). Oligonucleotides were obtained from Microsynth (Balgach, Switzerland) or Intron (Kaltbrunn, Switzerland).

**Bacterial strains and growth conditions.** Strains of *E. coli*, plasmids, and phages used in this study are listed in Table 1. All *E. coli* strains were grown at 37°C either in Luria-Bertani (LB) medium (43) or in a modified M63 medium (33) containing 64 mM potassium phosphate (pH 7.2), 33 mM $NH_4Cl$, 1% (vol/vol) sulfur-free trace elements (54), 1 mM $MgCl_2$, 0.3 mM thiamine, and 0.2% (wt/vol) glucose. Sulfur sources were added as described below to a final concentration of 250 μM. When necessary, kanamycin chloride (25 μg/ml) was added to the growth medium. Since kanamycin is commercially available only as a sulfate salt, the counterion was replaced by chloride by passage over a Dowex 1×8 column ($Cl^-$ form), elution with water, and lyophilization. Kanamycin chloride was tested for residual sulfate by ion chromatography (5) and for antibiotic activity before further use. Other antibiotics were added at the following concentrations: ampicillin, 100 μg/ml, and tetracycline, 12.5 μg/ml. Tryptophan was added at 100 μg/ml. Sulfur-limited solid media were prepared by addition of 0.6% SeaPlaque agarose (FMC BioProducts); 5-bromo-4-chloro-3-indolyl galactoside (X-Gal; 80 μg/ml) was added when necessary.

**Construction of random translational *lacZ* fusions.** Stocks of λp*lac*Mu9 were prepared in strain MC4100, whereas λpMu507 was grown in strain MBM7014 (Table 1). For the construction of *lacZ* fusion strains, MC4100 was grown to exponential phase (optical density at 600 nm [$OD_{600}$], 0.9 to 1.1) and the cells were coinfected with λp*lac*Mu9 (multiplicity of infection, approximately 0.2) and λpMu507 (multiplicity of infection, approximately 2). The cultures were incubated at room temperature without shaking for 30 min and washed three times with prewarmed LB medium. Five- and 50-fold dilutions were plated onto LB-kanamycin plates and incubated at 37°C. After overnight growth, the colonies were resuspended in LB medium with 100 mM sodium citrate and plated onto X-Gal minimal plates containing ethanesulfonate-glutathione (each 250 μM). Blue colonies were picked and tested for *lacZ* expression on X-Gal minimal plates containing either sulfate-cysteine (each 250 μM) or ethanesulfonate-glutathione (each 250 μM).

**Phage manipulations and transductions.** UV-induced excision of lambda prophages was done as follows: *lacZ* fusion strains were grown to an $OD_{600}$ of 0.4 to 0.8, washed with 10 mM $MgSO_4$, and resuspended in 0.5 volume of 10 mM $MgSO_4$. Aliquots were UV irradiated (254 nm; Sylvania) for 1 to 5 min in an open petri dish and transferred into 10 volumes of LB medium. After 4 to 6 h in the dark at 37°C, the lysate ($10^3$ to $10^4$ PFU/ml) was centrifuged to remove cell debris and dilutions were plated on a lawn of MC4100. Single plaques were picked and propagated in MC4100, thereby increasing the titer to $10^8$ to $10^{10}$ PFU/ml. Lambda lysogens were obtained by streaking out parts of a single plaque directly onto LB-kanamycin plates. λp*lac*Mu9 lysogens were tested for their sensitivity to Mu superinfection by cross-streaking against a Muc*ts* strain (Table 1) followed by incubation at 42°C (Mu induction) or at 30°C (control). Titers of phage lysates were determined by spot titration as described elsewhere (49).

P1 transductions were done as previously described (33). For construction of the *cysB* strain MW-108*cysB*, a P1 lysate was prepared on strain MDA4762 (*cysB trpB*::Tn*10*) and was used to transduce strain MW-108 to tetracycline resistance. The resulting transductants were then tested for tryptophan and cysteine auxotrophy.

**Chromosomal mapping.** The chromosomal locations of sulfate-regulated *lacZ* fusions to genes of unknown function were determined by hybridization with the *E. coli* Gene Mapping Membrane (Takara Shuzo Co., Kyoto, Japan). Oligonucleotides (22 to 24-mer) derived from the determined sequences did not give detectable hybridization signals. Larger probes were therefore synthesized by incorporation of [α-$^{32}$P]dATP during PCR using a primer complementary to part of the *lacZ* gene of λp*lac*Mu9 and an upstream primer derived from the sequence of the target gene, so that each probe contained 149 bp of *lacZ* and Mu sequence plus 100 to 150 bp of the upstream gene of interest. The following primers were used: S11 (5′-cagcatcaagtccgatccacaat-3′; S15, 5′-tgcagcggtgttgttcggtatt-3′; S108, 5′-caggcgctgtttgtgaatgaagg-3′; and lacZ-IK, 5′-gttttcccagtcacgacgttgtaaaacgac-3′. The amplified DNA fragment was labelled by inclusion of 100 μCi of [α-$^{32}$P]dATP in the PCR and separated from unincorporated nucleotides by purification over a NICK-50 column (Pharmacia, Uppsala, Sweden). The Gene Mapping Membrane was prehybridized in hybridization solution according to the manufacturer's instructions at 65°C for 2 to 3 h, hybridized at the same temperature for 18 h, and washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) (twice at room temperature and once at 37°C). Autoradiography was performed at −70°C with an intensifying screen. After stripping in 0.2 N NaOH–0.1% SDS for 30 min at 37°C, the membrane could be reused at least four times.

**Enzyme assays.** β-Galactosidase activities were measured with 4-methylumbelliferyl-β-D-galactopyranoside as a substrate or by the Miller assay (33). For the former, cells were washed in reaction buffer (25 mM Tris-Cl [pH 7.5], 125 mM NaCl, 2 mM $MgCl_2$, 12 mM β-mercaptoethanol) and resuspended in 0.95 volume of reaction buffer. They were permeabilized by the addition of 0.05 volume of $CHCl_3$ and 0.05 volume of 0.1% SDS and by vigorous vortexing. β-Galactosidase was assayed with 4-methylumbelliferyl-β-D-galactopyranoside (final concentration, 80 μg/ml of reaction buffer) at 30°C for 30 or 60 min. The reaction was stopped by addition of 0.25 volume of 25% trichloroacetic acid, and cell debris was removed by centrifugation. Aliquots of the reaction mixture were transferred into a solution containing 133 mM glycine and 83 mM $Na_2CO_3$ (pH 10.7), and the amount of 4-methylumbelliferone (MUF) produced was measured spectrofluorimetrically (RF-5001 PC [Shimadzu]; $\lambda_{excitation}$ = 365 nm; $\lambda_{emission}$ = 460 nm). The linear range (0 to 800 pmol of MUF) was determined by calibration with MUF solutions of known concentrations.

**DNA manipulations.** For plasmid isolation, restriction enzyme digestion, and transformation, published procedures were used (2). To obtain lambda DNA of sufficiently high quality for direct sequencing, the protocol of Ausubel et al. (2), which included purification of phage particles by polyethylene glycol precipitation and passage over a DEAE-cellulose column, was followed.

PCR was carried out in a Perkin-Elmer Cetus DNA thermal cycler 480. Reaction mixtures consisted of 100 pmol of primers, 50 nmol of deoxynucleoside triphosphates, 2 U of Vent DNA polymerase (New England Biolabs, Schwalbach, Germany), and 1 ng of template in a final volume of 100 μl. After an initial denaturation at 95°C for 5 min, amplification was performed during 30 cycles consisting of denaturation at 95°C for 1 min, annealing at 45°C for 30 s, and extension at 72°C for 1 min.

The dideoxy chain termination method of Sanger et al. (44) was used to sequence DNA fragments cloned into pUC118 or pUC119 (59) or into M13mp18 or M13mp19 vectors (36) with an A.L.F. DNA Sequencer (Pharmacia). Deletions of various lengths were introduced by using the Takara Kilo sequence deletion kit (Takara Shuzo). Alternatively, double-stranded plasmid DNA was sequenced by using a Sequitherm cycle sequencing kit (Epicentre Technologies, Madison, Wis.) with [5-$^{32}$P]ATP-labelled primers.

**Construction of *tauA′-′lacZ* protein fusions.** For the construction of *tauA′-′lacZ* protein fusions, PCR was utilized to amplify different portions of the region upstream of *tauA* and to introduce *Bam*HI and *Eco*RI restriction sites in order to facilitate cloning in plasmid pRS552. The primers used were as follows: JP7, 5′-at**gg**aattcatatgttaagtg-3′ (bp 10179 to 10189 of the sequence deposited in the DDBJ database under accession number D64043 [see below]); JP8, 5′-tgt**ggga**t **c**cgatgaaatt-3′ (complementary to bp 192 to 210 of the sequence shown in Fig. 1); JP9, 5′-gattgagaat**tc**tacagtga-3′ (bp 10349 to 10368 of the sequence deposited under accession number D64043); JP10, 5′-attcctgaat**tc**tctgctcccat-3′ (bp 10598 to 10618 of the sequence deposited under accession number D64043); and lacZ-IK, 5′-gttttcccagtcacgacgttgtaaaacgac-3′ (bp 1340 to 1311 of the sequence deposited under accession number J01636) (the nucleotides which have been changed to introduce restriction sites are indicated in boldface). Plasmid pME4131 was constructed by digestion of the product obtained after PCR using primers JP7 and JP8 and template pUC18ALA4 with *Bam*HI and *Eco*RI followed by ligation of the resulting fragment to pRS552, which had been digested with the same enzymes. Plasmids pME4135 and pME4137 were generated in a similar way with primers JP8 and JP9 (for pME4135) and JP8 and JP10 (for pME4137). Plasmid pME4132 was made as follows: a 1.1-kb fragment was obtained by PCR using primers lacZ-IK and JP8 and pUC18ALA4 as the template, digested with *Hin*dIII and *Eco*RI, and cloned in pBluescript KS to yield pME4120. The 1.1-kb *Bam*HI-*Cla*I fragment from pME4120 was cloned in pGEM7Zf− to yield pME4121. The 1.1-kb *Bam*HI-*Eco*RI fragment of pME4121 was then cloned in the *Bam*HI-*Eco*RI sites of pRS552. Plasmid pME4134 was constructed by cloning the 175-bp *Bam*HI-*Mun*I fragment from pME4120 in pRS552.

**Primer extension analysis.** RNA was isolated from MC4100(pUC18ALA4) grown in minimal medium with sulfate or taurine as a sulfur source to an $OD_{600}$ of approximately 0.8. Isolation of total RNA and primer extension analysis were performed according to the methods of Babst et al. (4), using primer JP12 (5′-tgacgttcaccgcctgtgcctgaa-3′, complementary to bp 239 to 262 of the sequence shown in Fig. 1) and 50 to 100 μg of RNA.

**Sequence analysis.** Nucleotide and protein sequences were analyzed with the University of Wisconsin Genetics Computer Group package, version 8 (13), or with PC/Gene (Intelligenetics, Inc., Mountain View, Calif.) and compared with the EMBL database (release 43) and the SWISS-PROT database (release 31), respectively, with the program FASTA.

**Nucleotide sequence accession number.** The nucleotide sequence described here is part of the nucleotide sequence of phage 8F10 from the Kohara library (26), and it has been deposited in the DDBJ database under accession number D64043.

## RESULTS

**Isolation of sulfate-regulated *lacZ* fusions.** Random translational *lacZ* fusions in the chromosome were generated with the λp*lac*Mu9 system (9) and screened for sulfate-regulated *lacZ* expression. Nine clones which were reproducibly blue after growth with ethanesulfonate-glutathione and white after growth with sulfate-cysteine were chosen for further studies. Four clones showed higher β-galactosidase activity levels during growth with sulfate than they did during growth with ethanesulfonate, but these clones have not yet been further investigated. The nine *lacZ* fusion strains grew well with sulfate or ethanesulfonate as a single sulfur source, and we concluded that λp*lac*Mu9 had not integrated into target genes which were required for metabolism of either sulfate or ethanesulfonate.

The *lacZ* fusion strains were grown with either sulfate or ethanesulfonate, and β-galactosidase was assayed in the late exponential phase (Table 2). All strains showed expression of *lacZ* which was clearly regulated by the source of sulfur supplied for growth, being repressed in the presence of sulfate. The levels of expression varied by over 2 orders of magnitude between the different mutants. The quantitative measurements correlated well with color intensities observed during growth on X-Gal minimal plates.

**Sequence determination and chromosomal location of *lacZ* insertion sites.** The region immediately upstream of the λp*lac*Mu9 integration site in each fusion strain was isolated by

TABLE 2. Specific β-galactosidase activities of strains containing a sulfate-regulated translational *lacZ* fusion

| Fusion strain | β-Galactosidase activity (U)[a] during growth[b] with: | |
|---|---|---|
| | 250 μM sulfate | 250 μM ethanesulfonate |
| MW-11 | <1 | 8 |
| MW-15 | <1 | 25 |
| MW-43 | <1 | 453 |
| MW-61 | <1 | 24 |
| MW-74 | <1 | 5 |
| MW-82 | 13 | 62 |
| MW-103 | <1 | 56 |
| MW-108 | 12 | 938 |
| MW-109 | 4 | 26 |

[a] One unit corresponds to the production of 1 pmol of MUF per (ml · min · $OD_{600}$).

[b] Cells were harvested in the late exponential phase, and β-galactosidase activity was assayed as described in Materials and Methods.

illegitimate, UV-induced excision of the prophage (56), and the sequence at the integration site was determined. In strain MW-103, the *lacZ* gene was located in the same orientation and in the same reading frame as the target *rhsD* gene (between bp 4202 and 4203; accession no. L19084). The integration site was 1 bp downstream of the border between the core reading frame and the core extension of *rhsD* (41, 42). Until now, no function has been reported for the putative RhsD protein (42).

In strain MW-109, *lacZ* was inserted into the *dmsA* gene between bp 1809 and 1810 (accession no. J03412), but the resulting fusion was not in frame. The *dmsA* gene encodes the catalytic subunit of the dimethyl sulfoxide reductase, which catalyzes the reduction of dimethyl sulfoxide, trimethylamine *N*-oxide, and other *S*- and *N*-oxides (8, 62). Until now, this gene has been reported to be expressed only under anaerobic growth conditions (12). Strain MC4100(λPC25), which carries a transcriptional *lacZ* fusion in *dmsA* (12), was therefore tested for sulfate-regulated expression of *lacZ* under aerobic conditions. No significant difference in activities of β-galactosidase in cells grown with sulfate or ethanesulfonate was observed.

Sequence analysis of the seven remaining fusion strains showed that the insertion sites were in previously uncharacterized genes. The map positions of the insertion sites in strains MW-11, MW-15, and MW-108 were therefore determined by hybridization with the Kohara Gene Mapping Membrane. Probes specific for these three fusions all hybridized with DNA from Kohara phage 8F10, and the probes specific for strains MW-15 and MW-108 also hybridized with the DNA from the adjacent Kohara clone 6E2 (results not shown), in the region at 8.5 min of the *E. coli* chromosome. Subsequent sequence analysis of this region (see below) showed that the insertion sites for the remaining four fusion strains were also located in this region.

**Nucleotide sequence analysis of *tauABCD*.** As shown above, the majority of the insertions in our λp*lac*Mu9 mutants expressing β-galactosidase under sulfate starvation conditions were clustered in the region at 8.5 min on the chromosome, downstream of the *hemB* gene. This region is contained on plasmid pUC18ALA4 (37). The nucleotide sequence of the 3,682-bp *Nsi*I-*Hpa*I fragment immediately downstream of *hemB* was determined (Fig. 1). Analysis of the sequence revealed the presence of four open reading frames (ORFs) which were transcribed in the direction opposite to that of *hemB* and

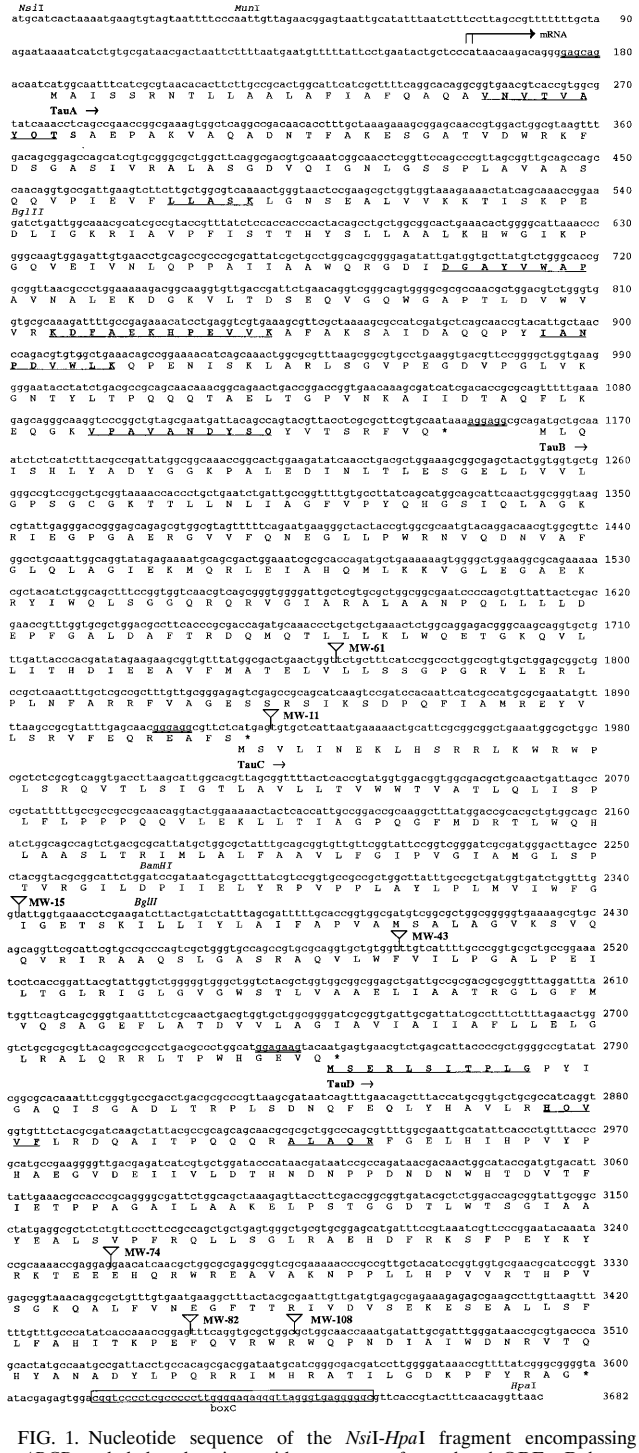


FIG. 1. Nucleotide sequence of the *Nsi*I-*Hpa*I fragment encompassing *tauABCD* and deduced amino acid sequences of translated ORFs. Relevant restriction sites are shown above the sequence. Putative ribosome binding sites are double underlined. The positions of λp*lac*Mu9 insertions are indicated (inverted triangles), and the position of box C (7) is shown. The N-terminal and internal peptide sequences of proteins Ssi1 and Ssi3 (38) are underlined and in boldface print. The transcriptional start sites are indicated by arrows.

which have been designated *tauA*, *tauB*, *tauC*, and *tauD*. All ORFs are preceded by reasonable ribosome binding sites (Fig. 1).

The protein encoded by *tauA* (bp 187 to 1154) has a typical

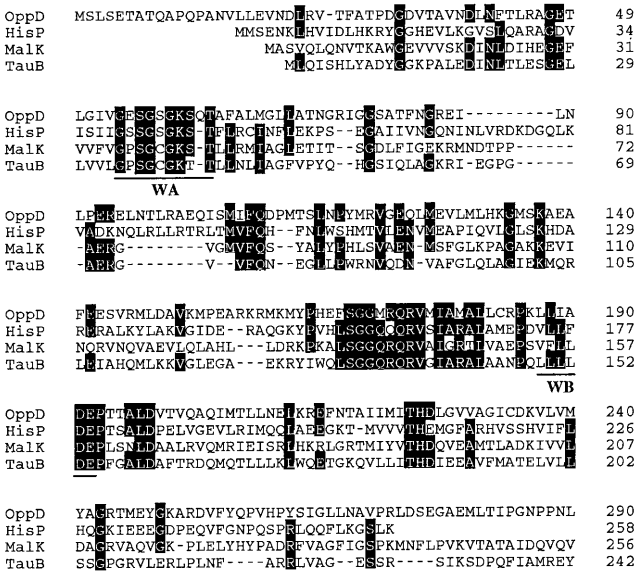


FIG. 2. PILEUP amino acid sequence alignment of TauB with the sequences of the ATP-binding proteins OppD (accession no. P04285) and HisP (accession no. P02915) from *S. typhimurium* and MalK (accession no. P02914) from *E. coli*. Residues identical in three or all of the sequences are shown in black. The Walker motifs (61) are underlined and indicated as WA and WB.

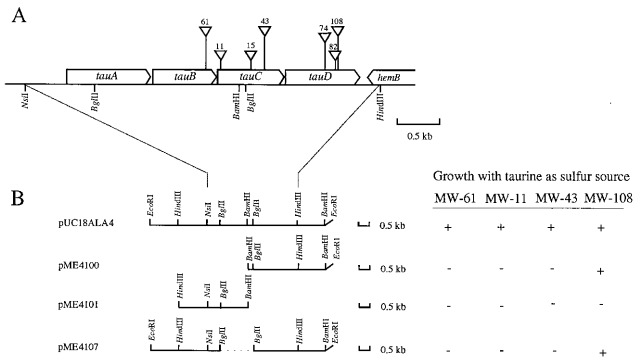


FIG. 3. (A) Organization of the *tauABCD* gene cluster and positions of λp*lac*Mu9 insertion sites. (B) Complementation of mutants MW-61, MW-11, MW-43, and MW-108 by plasmid pUC18ALA4 and derivatives thereof. +, growth; −, no growth with taurine as the sole sulfur source.

N-terminal signal sequence of 22 amino acids whose structure adheres to the −3/−1 rule of von Heijne (60). The N-terminal sequence of the mature protein is identical to the N-terminal sequence of protein Ssi1, which is one of the SSI proteins identified by two-dimensional gel electrophoresis (38). The molecular mass and pI of Ssi1 were determined to be 30.7 kDa and 7.13, respectively, which is in reasonable agreement with the calculated values of the processed TauA protein with a molecular mass of 32.0 kDa and a pI of 6.82. The sequences of six of the internal peptides obtained after tryptic digestion of Ssi1 (38) also corresponded to the derived amino acid sequence of TauA. When TauA was compared with sequences in the SWISS-PROT database, the only significant similarity found for it was with a protein encoded by an ORF of unknown function, ORFK from *Bacillus subtilis* (23.6% identity), which also has a putative signal sequence (39).

The second ORF, *tauB* (bp 1162 to 1929), encodes a protein of 255 amino acids, which has a predicted molecular mass of 28.3 kDa. TauB shows 56 to 66% similarity to ATP-binding proteins of ABC transport systems (Fig. 2), with a high degree of similarity being located in the Walker motifs, which are present in many nucleotide-binding proteins (61).

The end of *tauB* shows a 4-bp overlap with the translational start site of *tauC* (bp 1926 to 2753), which encodes a protein of 275 amino acids with a molecular mass of 29.9 kDa. TauC has a very hydrophobic character and is predicted to span the membrane six times when calculated by the algorithm of Klein et al. (25). The amino acid sequence of TauC exhibits around 25% identity with those of the membrane components of several ABC transport systems (data not shown).

The last ORF, *tauD* (bp 2750 to 3601), overlaps by 4 bp with *tauC* and encodes a protein of 283 amino acid residues with a molecular mass of 32.4 kDa and a pI of 6.76. The N-terminal sequence of TauD was identical to that obtained from Ssi3 except for the removal of the N-terminal formylmethionine residue (38). Two of the internal peptide sequences obtained after tryptic digestion of Ssi3 (38) also corresponded with the derived amino acid sequence of TauD. The TauD protein shows 29.9% sequence identity with the α-ketoglutarate-dependent 2,4-dichlorophenoxyacetate dioxygenase from *Alcaligenes eutrophus*, encoded by the *tfdA* gene (16, 52).

The intergenic region between *hemB* and *tauD* contains a motif of 43 bp which is highly conserved within the intercistronic regions of various operons in both possible orientations in *E. coli* (7, 14) and which has been proposed to play a role in transcriptional activity or stability of the mRNA (7).

The sequence of *tauABCD* was compared with the sequences upstream of the λp*lac*Mu9 insertion sites in the fusion strains. In addition to those in strains MW-108, MW-11, and MW-15, which had already been mapped in this region (see above), the *lacZ* fusions in mutants MW-43, MW-61, MW-74, and MW-82 were also found to be located in *tauABCD* (Fig. 1). In mutant MW-61, λp*lac*Mu9 had integrated in the *tauB* gene, but the *tauB-lacZ* fusion was out of frame. Three of the λp*lac*Mu9 insertion sites were located in *tauC*, but in two of the fusion strains (MW-11 and MW-15) the resulting fusions were out of frame. There were also three λp*lac*Mu9 insertions in the *tauD* gene, and again in two of the fusion strains (MW-74 and MW-82), the fusions were out of frame.

***tauABCD* are required for growth with taurine.** Since expression of the *tauABCD* genes is induced by sulfate starvation, it seemed likely that these genes encode a system that is involved in uptake and metabolism of a sulfur-containing compound. A screen of compounds that can be used as sulfur sources by strain MC4100 showed that taurine (2-aminoethanesulfonate) could not be utilized by any of the strains with insertions in the *tauBCD* genes. Sulfur sources which supported growth of strain MC4100 and the insertion mutants equally well included sulfate, *n*-alkanesulfonates ($C_2$ to $C_6$), isethionate, 2-mercaptoethanesulfonate, 2-morpholinoethanesulfonate, 3-morpholinopropanesulfonate (MOPS), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonate, piperazine-1,4-bis-2-ethanesulfonate, lanthionine, methionine, glutathione, thioglycolate, cysteine, homocysteine, cystine, and djenkolate. Transformation of the insertion mutants with plasmid pUC18ALA4, which contains an intact copy of *tauABCD*, led to restoration of growth with taurine to the levels seen with the wild-type strain. Strains MW-11, MW-43, MW-61, and MW-108 were also tested for complementation by derivatives of plasmid pUC18ALA4 (Fig. 3). Strain MW-108 could be complemented by plasmids pME4100 and pME4107, which contain a copy of *tauD* only. In pME4100, *tauD* was therefore expressed from an unidentified transcriptional start site, possibly from a promoter located on

TABLE 3. Dependence of *lacZ* expression in MW-108 on CysB

| Sulfur source (250 μM) | β-Galactosidase activity (U)[a] in strain: | |
|---|---|---|
| | MW-108 | MW-108*cysB* |
| Sulfate | <5 | NG[b] |
| Ethanesulfonate | 1,675 | NG |
| Sulfate + ethanesulfonate | 12 | NG |
| Cysteine | <5 | <5 |
| Djenkolic acid | 158 | <5 |
| Cystine | <5 | <5 |
| Lanthionine | 1,528 | <5 |
| Butanesulfonate | 2,766 | NG |
| MOPS | 1,995 | NG |

[a] One unit corresponds to the production of 1 pmol of MUF per (ml · $OD_{600}$ · min). Cells were grown in modified M63 medium with the sulfur sources indicated, and β-galactosidase activity was determined in the mid-exponential phase.

[b] NG, no growth.

the vector. The results also showed that *tauB* and *tauC* are both indispensable for growth with taurine. Strain MW-61, which has an insertion in *tauB*, could not be complemented by plasmid pME4101, which contains an intact copy of *tauB* but has deletions of *tauC* and *tauD*. This suggests that the insertion in MW-61 has a polar effect on expression of *tauC* and presumably *tauD*.

In agreement with the results obtained by Uria-Nickelsen et al. (57), the wild-type strain could not use taurine as a carbon source under aerobic conditions or as a sulfur source under anaerobic conditions (results not shown).

**Regulation of expression of *tauABCD*.** The dependence of *lacZ* expression on the sulfur source used for growth was tested in fusion strain MW-108, which has a λp*lac*Mu9 insertion in *tauD* (Fig. 1). Sulfate, cysteine, and cystine repressed β-galactosidase activity, whereas ethanesulfonate, lanthionine, butanesulfonate, and MOPS acted as derepressing substrates (Table 3). Growth with djenkolic acid as the sulfur source led to weak derepression of β-galactosidase. When a derepressing substrate (ethanesulfonate) was used with a repressing substrate (sulfate), only very low levels of expression of the *lacZ* fusion were observed (Table 3). Quantitation of *lacZ* expression throughout growth of MW-108 with ethanesulfonate showed that β-galactosidase activity reached a peak in the early exponential phase and decreased to one-third of the maximal value in the late exponential growth phase (Fig. 4). In sulfate-grown cells, only low levels of activity could be detected. Since the same sulfur source was supplied in the preculture as in the main cultures, no major change in cellular

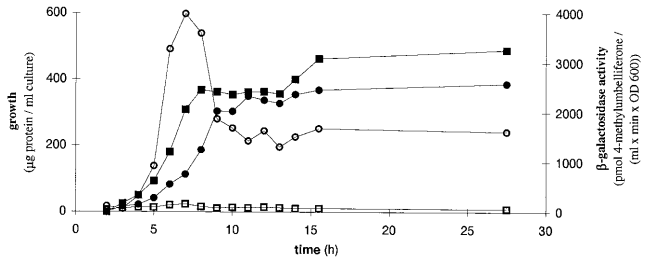


FIG. 4. Growth phase-dependent expression of *lacZ* in MW-108 during growth with 250 μM sulfate or 250 μM ethanesulfonate. Symbols represent growth with sulfate (■) or ethanesulfonate (●) and β-galactosidase activity after growth with sulfate (□) or ethanesulfonate (○).

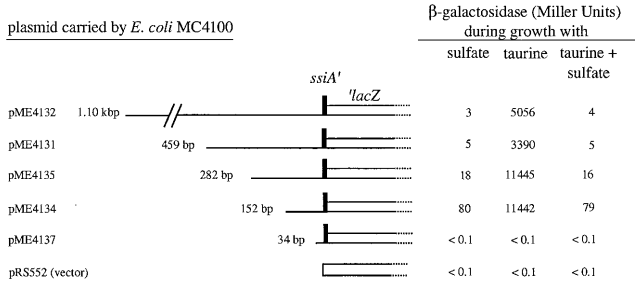


FIG. 5. β-Galactosidase activities of late-exponential-phase-grown cells of *E. coli* MC4100 carrying plasmid-encoded *tauA'-'lacZ* fusions. The numbers refer to the number of base pairs upstream of the ATG start codon of *tauA*. The resulting fusion proteins each contain the first five amino acids of TauA. Plasmids were constructed as described in Materials and Methods.

sulfur metabolism occurred during the observed period. The induction pattern thus provides evidence that in strain MW-108, expression of *lacZ* is both sulfate regulated and growth phase dependent. A similar pattern of *lacZ* expression was observed in strain MW-11, although the levels of β-galactosidase activity were much lower (data not shown).

The synthesis of many of the enzymes from the cysteine biosynthesis pathway is under positive control by the transcriptional activator CysB (29). To investigate whether CysB also regulates expression of *tauABCD*, the *cysBtrpB*::Tn*10* mutation from *E. coli* MDA4762 (46) was transferred to strain MW-108 by P1 transduction. As expected, the resulting strain, MW-108*cysB*, was unable to grow with sulfate or alkanesulfonates. Expression of *lacZ* in this strain was no longer derepressed by lanthionine or djenkolate (Table 3). It thus appears that CysB is involved in regulating the expression of *tauD* in response to sulfate starvation.

**Expression of translational *tauA'-'lacZ* fusions.** In order to determine the minimal region required for sulfate starvation-regulated expression of *tauABCD*, translational *lacZ* fusions to the *tauA* gene which contained different amounts of the region upstream of the *tauA* gene were made (Fig. 5). Expression of the resulting *tauA'-'lacZ* fusions was seen only in cells containing fusions which had 152 or more base pairs of DNA upstream of the start site of *tauA*, and which were grown in the absence of sulfate. Cells containing the fusion with only 34 bp of DNA upstream (pME4137) did not produce β-galactosidase, nor did the other strains when sulfate was present in the growth medium, even when taurine was also supplied.

**Determination of the transcription initiation site.** The transcriptional start site of *tauABCD* was determined by primer extension analysis using RNA isolated from strain MC4100 (pUC18ALA4) grown with taurine or sulfate as a sulfur source as a template for reverse transcriptase. A double band, which was absent when sulfate-grown cells were used, was visible in the reaction mixture using RNA from taurine-grown cells (Fig. 6). The transcriptional start site was determined to be 26 to 27 bp upstream of the translational start site of *tauA* (Fig. 1). Inspection of the region upstream of the transcription start site, however, revealed no sequences with significant homology to $\sigma^{70}$ or $\sigma^{54}$ promoter motifs (18, 53).

## DISCUSSION

The region of the *E. coli* chromosome downstream of *hemB* contains four genes (*tauABCD*) whose expression is regulated by sulfate starvation, as determined by measurements of *lacZ* expression in strains containing λp*lac*Mu9 insertions in *tauB*,

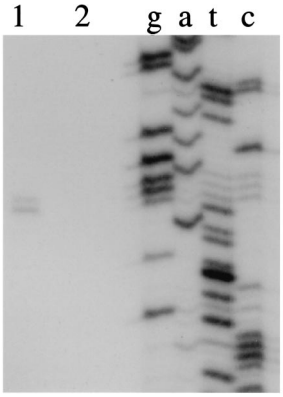


FIG. 6. Identification of the transcription start site by primer extension analysis. RNA was isolated from MC4100(pUC18ALA4) grown with taurine (lane 1) or sulfate (lane 2) as a sulfur source. A sequence ladder generated with the primer used for the primer extension is also shown.

*tauC*, and *tauD* and in cells containing plasmid-encoded *tauA'-'lacZ* fusions. The proteins encoded by *tauA* and *tauD* are identical to proteins Ssi1 and Ssi3, respectively, which have both been identified as SSI proteins by two-dimensional gel electrophoresis (38). We presume that *tauABCD* constitutes an operon, since expression of each of the individual components is regulated by sulfate starvation.

The λp*lac*Mu9 insertions in *tauB*, *tauC*, or *tauD* result in the inability to grow with taurine as a sulfur source. Taurine is a naturally occurring alkanesulfonate which has a variety of functions in animals and is involved in osmoregulation, in bile salt synthesis, in modulation of neurotransmitter and hormone release, and in modulation of $Ca^{2+}$-dependent processes (21). Despite its presence in animal tissues, taurine is not known to be metabolized by animals or by plants. The excess taurine produced by animals is excreted as such or in the form of bile salts like taurocholate. Bacteria of different genera are able to mineralize taurine either as a carbon, sulfur, nitrogen, and energy source or as a sulfur source alone (21). Cell extracts of taurine-grown *Pseudomonas aeruginosa* TAU-5 were found to contain a taurine:pyruvate transaminase which catalyzed the transamination of taurine and pyruvate, resulting in the formation of L-alanine and sulfoacetaldehyde (47). A taurine-inducible sulfoacetaldehyde sulfo-lyase activity is responsible for subsequent cleavage of sulfoacetaldehyde to acetate and sulfite (48). In *Achromobacter* spp. a similar transamination reaction with α-ketoglutarate as an acceptor and with stoichiometric production of sulfoacetaldehyde and L-glutamate was observed (55). In a second mechanism a dehydrogenase was found to be involved in the oxidative deamination of taurine to sulfoacetaldehyde in an unidentified taurine-utilizing bacterium; the sulfoacetaldehyde was subsequently converted to acetate and sulfite by a thiamine $PP_i$-dependent sulfo-lyase (27, 28). It thus appears that in general the first step in utilization of taurine is the formation of sulfoacetaldehyde, either by oxidation or by transamination. The resulting sulfoacetaldehyde is then converted to sulfite and acetate.

*E. coli* is able to utilize taurine and other alkanesulfonates only as sulfur sources and only under aerobic conditions (57; also the present paper). Strains defective in enzymes involved in the reduction of sulfate to sulfite were still able to use alkanesulfonates as sulfur sources. Strains defective in sulfite reductase, however, were unable to grow with alkanesulfonates (58), and it therefore seems likely that cleavage of the C-S bond results in the formation of sulfite and not sulfate. Oxygenative cleavage of taurine, which could be mediated by the gene product of *tauD*, is expected to produce either aminoethanol and sulfite or sulfoacetaldehyde and ammonia. At present we cannot rule out either one of these possibilities.

Although the mutants with λp*lac*Mu9 insertions in *tauBCD* are unable to utilize taurine as a sulfur source, they are still able to grow with a variety of other alkanesulfonates (e.g., ethanesulfonate and MOPS). We therefore expect that one or more additional systems that are responsible for the uptake and metabolism of these compounds must exist, and we are currently involved in isolating and characterizing the responsible gene or genes. It is in fact surprising that we have isolated fusions only in *tauBCD* and not in other genes which are involved in utilization of organosulfur compounds, and the reasons for this are still under investigation.

From sequence homologies and our mutant studies, it seems probable that the proteins encoded by *tauABC* constitute an ABC transport system for taurine. ABC transporters participate in the active uptake of a variety of substrates and consist of three components: an ATP-binding protein, a membrane protein, and a periplasmic solute-binding protein (20). The solute-binding protein is able to bind its cognate substrate with high affinity; this complex shows increased affinity for the corresponding membrane component and releases the substrate into a pore formed by the membrane protein, with concomitant hydrolysis of ATP by the ATP-binding protein (1). The sequences of TauB and TauC are significantly similar to those of ATP-binding proteins and membrane components, respectively, of members of the ABC transporter superfamily. According to sequence alignments, the membrane component TauC falls in cluster 1a of these transport systems (45). This cluster contains membrane proteins which have only one membrane component or two different components falling in the same cluster and which have quite a broad substrate range (45).

TauA has a putative signal sequence, indicating that it is probably located in the periplasm, and the predicted N-terminal sequence of the processed protein is identical to that of the Ssi1 protein (38). Since β-galactosidase is not enzymatically active in the periplasm, it is not surprising that we have not isolated λp*lac*Mu9 insertions in the *tauA* gene. The periplasmic location of TauA supports the assumption that TauA functions as a binding protein, although it does not have any sequence similarity to known periplasmic binding proteins.

Uptake of taurine by *E. coli* has so far been investigated only as part of the response to high osmolarity and was found to be dependent on the ProU and ProP transport systems (32). No taurine uptake was found in low-osmolarity minimal medium. Since the medium contained sulfate, it was speculated that taurine transport would be inhibited or repressed by sulfate (32). The latter is supported by our findings, but at present we cannot exclude the possibility that uptake of taurine is inhibited by sulfate.

Only three of the nine λp*lac*Mu9 insertions contain an in-frame fusion to the *lacZ* gene. In the others, +1 or −1 translational frameshifts are necessary to create an active *lacZ* fusion protein. We assume that in these strains active β-galactosidase is produced by translational initiation at internal sites or by ribosomal frameshifting, but we have not investigated this question further. The levels of β-galactosidase in strains MW-74 and MW-82, which contain an out-of-frame fusion to *tauD*, are 0.5 to 7% of those in strain MW-108, which has an in-frame fusion to *tauD*. Similar values have been found with out-of-frame fusions between *argI* and *lacZ* (15). In this case ribosomal frameshifting occurred at a frequency of 3 to 16% in the *argI* mRNA at a phenylalanine codon. The λp*lac*Mu9 in-

sertion in strain MW-11 is in the second codon of *tauC* (Fig. 1). Within 40 bp of λp*lac*Mu9 there is a stop codon in the same frame, which suggests that a −1 ribosomal frameshift should occur within this stretch. Analysis of the N-terminal sequence of the resulting fusion protein could help in determining the exact position of the frameshift. The high frequency of occurrence of out-of-frame fusions may reflect our screening strategy, in which we utilized plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) to detect β-galactosidase activity. Even very low levels of this enzyme result in a blue color on these plates. In this context it is also important to note that we used a highly sensitive β-galactosidase assay which is based on the cleavage of 4-methylumbelliferyl-β-D-galactopyranoside. The β-galactosidase values reported here are thus up to 100-fold higher than the values in Miller units obtained by the commonly used assay based on *o*-nitrophenyl-β-D-galactopyranoside hydrolysis (63). Consequently, even the derepressed β-galactosidase values in Table 2 represent low levels of expression on an absolute scale. Since the level of metabolite flow in sulfur assimilation is 2 to 3 orders of magnitude lower than that in carbon assimilation, low-level maximum expression is in accordance with the view that the proteins encoded by *tauABCD* are involved in the metabolism of sulfur sources other than sulfate or cysteine.

Of the eight proteins whose expression was previously observed by two-dimensional electrophoresis to be regulated by sulfate starvation, four have been identified as the sulfate-binding protein Sbp, the cystine-binding protein FliY, *O*-acetylserine lyase A (CysK), and alkyl-hydroperoxide reductase C22 (AhpC) (38). Two of the remaining proteins have now been identified as TauA and TauD. Thus, when starved for sulfate, *E. coli* increases the synthesis of proteins that are involved either in the biosynthesis of cysteine from sulfate or in the utilization of alternative sulfur sources such as taurine or cystine. The alkyl-hydroperoxide reductase C22 is considered a general stress protein whose synthesis is induced under a variety of different conditions (51). A role for the gene products of *dmsA* and *rhsD* in the assimilation of sulfur is unknown. An understanding of the apparent sulfate regulation of these genes will therefore require further study.

The deduced amino acid sequences of the three binding proteins Sbp, FliY, and TauA contain methionine residues but are devoid of cysteine (10, 19, 34; also the present study), whereas the proteins encoded by *tauB*, *tauC*, and *cysK* contain only one cysteine residue. This principle of economizing sulfur in proteins that are specifically expressed under sulfate limitation is also observed in the cyanobacterium *Calothrix* sp. strain PCC 7601 (31).

The expression of genes which encode the sulfate-binding protein and the cystine-binding protein is known to be regulated as part of the *cys* regulon in *S. typhimurium* (29), and *cysK* is an established member of this system in both *S. typhimurium* and *E. coli* (29). The central regulatory protein of this system is CysB, which activates transcription in the presence of *N*-acetylserine (29). The levels of the cysteine biosynthetic enzymes are maximal when a limiting sulfur source such as djenkolate is used for growth, and half of the maximum expression is observed when sulfate is used, whereas cystine and cysteine completely repress enzyme synthesis (29). In strain MW-108, the synthesis of β-galactosidase is fully repressed not only during growth with cysteine or cystine but also during growth with sulfate, indicating that expression of *tauABCD* is not regulated by CysB in the same manner as is that of the genes of the *cys* regulon (Table 3). Since *lacZ* is not expressed in strain MW-108*cysB*, it is possible that not CysB but another protein, whose synthesis is under the control of CysB, interacts with the promoter region of *tauABCD*. This hypothesis is strengthened by the fact that we were unable to find a sequence resembling the consensus CysB binding site upstream of the *tauABCD* promoter (29). Recently a novel gene, *cbl*, whose expression is regulated by CysB, was identified (22). The *cbl* gene product has 40% sequence identity to CysB and was suggested to be involved in the regulation of a second cysteine biosynthesis pathway from organic sulfur compounds (22). Cbl may thus be a candidate protein for the regulation of expression of *tauABCD*.

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