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Fine-Structure Genetic Map of the *cysB* Locus in *Salmonella typhimurium*

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A genetic map of the *cysB* region of the *Salmonella typhimurium* chromosome was constructed using bacteriophage P22-mediated transduction. Strains bearing Δ (*supX cysB*) mutations were employed to divide this regulatory locus into 12 segments containing a total of 39 single-site mutations. Twenty-five of these single-site mutations were further ordered by reciprocal three-point crosses. The results do not support the concept of multiple cistrons at *cysB* and suggest that the abortive transductants previously observed in crosses between certain *cysB* mutants were due to intracistronic complementation. The prototrophic *cys-1352* mutation, which causes the constitutive expression of the cysteine biosynthetic enzymes, was found to lie within the *cysB* region itself. It is bracketed by mutations, which lead to an inability to derepress for these enzymes and result in auxotrophy for cysteine.

Soluble sulfates, the chief source of sulfur in the environment, are reduced by two distinct enzyme systems in microogranisms (15). In the dissimilatory pathway, sulfate reduction is the final step in electron transport for energy production. The second type of microbial sulfate reduction is an assimilatory process, in which only the sulfate needed for biosynthetic purposes is reduced. The initial steps in assimilatory reduction involve the activation of sulfate, first to adenosine 5'-phosphosulfate and then to 3'-phosphoadenosine 5'-phosphosulfate. The latter compound is reduced to sulfite, which in turn is reduced further to sulfide. Sulfide then reacts with *O*-acetyl-L-serine, which is synthesized from L-serine and acetyl-coenzyme A, to form L-cysteine. The sulfur in L-cysteine can then by utilized for the biosynthesis of other intracellular metabolites which contain reduced sulfur. In *Salmonella typhimurium* and *Escherichia coli* this is normally the major method of incorporating reduced inorganic sulfur into organic molecules. The unique position of L-cysteine as a funnel for sulfur assimilation makes the regulation of its biosynthesis a key process in the proper functioning of many other metabolic pathways.

In *S. typhimurium* the genes necessary for cysteine biosynthesis are not clustered but are widely scattered on the chromosome (16). Dreyfuss and Monty (5) showed that adenosine 5'-triphosphate sulfurylase is coded for by *cysD*, adenosine-5'-phosphosulfate kinase by *cysC*, 3'-phosphoadenosine 5'-phosphosulfate reductase by *cysH*, and sulfite reductase by *cysG*, *cysI*, and *cysJ*. Mutants in *cysA* lack the active transport system for sulfate (4), and *cysE* codes for serine transacetylase, the enzyme which synthesizes *O*-acetyl-L-serine (12). The structural gene for *O*-acetylserine sulfhydrylase-A has been found to be identical to *trzA*, so named because mutants resistant to the growth-limiting effects of 1,2,4-triazole map at that locus (8). The *trzA* locus has since been redesignated *cysK*.

The *cysB* region has been characterized by a large number of pleiotropic cysteine auxotrophs which bear mutations located at a site near the *trp* operon (13). This portion of the chromosome has been thought to code for one or more elements which regulate the expression of other *cys* genes (11; H. T. Spencer, J. Collins, and K. J. Monty, Fed. Proc. **26**:677, 1967). In this communication we present a fine-structure genetic map of the *cysB* locus.

MATERIALS AND METHODS

Strains. All strains used were nonlysogenic derivatives of *S. typhimurium* LT-2 (Table 1). Strains carrying *ara-9*, *pyrF146*, *cysE2* and various *cysB* alleles were from the Demerec collection and were obtained from K. Sanderson, P. Hartman, and P. Margolin. Spontaneous *supX-cysB* deletion mutants were isolated as Leu$^+$, Cys$^-$ revertants of *leu-500 pyrF146* on medium containing L-tryptophan, L-cys-

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TABLE 1. *Designations and derivations of strains*[a]

Strain	Genotype	Source or method of construction[b]
PM206	*leu-500 supX7 ara-9*	P. Margolin
PM620	*leu-500 supX83 trpA50 hisC340*	P. Margolin
DW25	*cys-1352*	*cysE2*[c] × wild type
DW50	*leu-500 pyrF146*	*leu-500 cysB529 pyrF146* × wild type
DW218	*leu-500 cysB517 pyrF146 hisC340*	PM620 × *cysB517 pyrF146*
DW219	*cysB517 pyrF146 hisC340 ara-9*	DW218 × *ara-9*
DW220	*trpA160 pyrF146 hisC340 ara-9*	DW219 × *trpA160*
DW273	*cysB27 pyrF146 hisC340 ara-9*	DW220 × *cysB27*
DW274	*cys-1352 trpA160 hisC340 ara-9*	DW220 × DW25
DW275	*cys-1352 pyrF146 hisC340 ara-9*	DW220 × DW25
DW277	*cysB517 pyrF146 hisC340 ara-9*	DW220 × *cysB517*
DW353	*leu-500 Δ(supX cysB1753) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW354	*leu-500 Δ(supX cysB1754) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW355	*leu-500 Δ(supX cysB1755) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW356	*leu-500 Δ(supX cysB1756) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW357	*leu-500 Δ(supX cysB1757) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW358	*leu-500 Δ(supX cysB1758) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW359	*leu-500 Δ(supX cysB1759) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW361	*leu-500 Δ(supX cysB1761) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW362	*leu-500 Δ(supX cysB1762) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW363	*leu-500 Δ(supX cysB1763) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW364	*leu-500 Δ(supX cysB1764) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW365	*leu-500 Δ(supX cysB1765) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW367	*leu-500 Δ(supX cysB1767) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW360	*leu-500 Δ(trp supX cysB1760) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW366	*leu-500 Δ(trp supX cysB1766) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW368	*leu-500 Δ(trp supX cysB1768) pyrF146*	DW50, spontaneous Leu$^+$ revertant
DW369	*leu-500 Δ(supX cysB1753)*	DW353 × wild type
DW370	*leu-500 Δ(supX cysB1754)*	DW354 × wild type
DW371	*leu-500 Δ(supX cysB1762)*	DW362 × wild type
DW372	*leu-500 Δ(supX cysB1763)*	DW363 × wild type
DW373	*leu-500 Δ(supX cysB1764)*	DW364 × wild type
DW374	*leu-500 Δ(supX cysB1765)*	DW365 × wild type
DW375	*leu-500 Δ(supX cysB1767)*	DW367 × wild type
DW376	*leu-500 Δ(trp supX cysB1768)*	DW368 × wild type

[a] The *trpA160 cysB* double mutants used for three-factor crosses were derived by transduction starting with a *trpA160 pyrF146* recipient as previously described (11).

[b] Strains were constructed by transduction with the recipient to the left and the donor to the right.

[c] The *cysE2* strain obtained from the Demerec collection contains the *cys-1352* allele in addition to the *cysE2* mutation. Transduction to cysteine prototrophy gives a *cysE*$^+$ recombinant carrying *cys-1352*.

tine, and uracil (*leu-500* auxotrophs are indirectly suppressed by mutation at *supX* [14]). A small fraction of these mutants were found to be Trp$^-$. The gene order on the *S. typhimurium* chromosome is *trp-supX-cysB-pyrF* (16).

Media and transductions. Complete medium for bacterial growth was 1.5% nutrient broth (Difco) supplemented with 0.1 mM L-cystine. Medium E (20) with 0.5% glucose as the carbon source was used as the standard minimal medium. When appropriate, minimal media were supplemented with one or more of the following: L-cystine, 240 μg/ml; L-tryptophan, 20 μg/ml; L-leucine, 20 μg/ml; L-histidine, 15 μg/ml; uracil, 10 μg/ml; and sodium selenate, 378 μg/ml. Solid media were prepared by adding 15 g of agar to 1 liter of liquid medium.

All transducing phage used in these studies were derived from the integration-deficient L-4 strain of P22 (17). Log-phase bacteria at 10^8 cells/ml in nutrient broth were infected with phage at a concentration of 10^8 plaque-forming units/ml and grown with aeration at 37 C for 12 to 18 h. After this time, bacterial debris was removed by centrifugation at 8,000 × *g*, and the phage suspension was stored at 4 C over chloroform. Transductions were performed by spreading 5×10^8 to 5×10^9 phage and 2×10^8 bacteria from a fully grown nutrient broth culture on selective plates. Recombinants were scored by replicating onto selective plates after 36 to 48 h.

Strains were tested for the constitutive synthesis of *O*-acetylserine sulfhydrylase-A by the method of Becker et al. (1) after growth on standard minimal medium supplemented with 1.0 mM L-cystine.

RESULTS

Deletion mapping of cysB mutants. *supX-cysB* deletion mutants proved to be

very poor recipients for transduction (data not shown); therefore, deletion mapping was performed using *pyrF*$^+$, single-site *cysB* mutants as recipients with donor phage from deletion strains containing the *pyrF146* allele. Owing to the close linkage of *pyrF* to *cysB*, a large percentage of the *cysB*$^+$ recombinants from such crosses should be *pyrF*$^-$, whereas all *cysB*$^+$ revertants of recipient strains must be *pyrF*$^+$. Thus, by scoring *cysB*$^+$ colonies for the *pyrF146* allele we were able to identify true recombinants among a large number of revertants.

The results of these crosses are shown in Table 2. The end points of the deletions define 12 segments as shown in Fig. 1. Deletion segment I covers none of the mutations tested; six segments contain only one mutation each.

Mapping by three-factor crosses. The order of single-site *cysB* mutations within each deletion segment was determined by reciprocal three-point crosses, using the mutation *trpA160* in donor strains as the third factor. The transduction of a reciprocal pair in which the percentage of *cys*$^+$ *trp*$^-$ recombinants is higher should be that cross in which the *cys* site of the recipient is between *trpA* and the *cys* site of the donor. The statistical significance of each difference noted between pairs of a reciprocal cross was tested by a chi square contingency test (3). Since large numbers of *cysB*$^+$ revertants interfere with this type of mapping, only *cysB* mutants with a reversion frequency of less than 5×10^{-8} were used.

The results of these crosses are presented in Table 3 and in Fig. 1. The order derived from five crosses between mutants from different deletion segments is the same as that obtained from deletion mapping. A number of strains gave few or no recombinants in crosses with one another and could not be ordered by this technique.

Crosses between *cysB482* and either *cysB484* or *cysB938*, all located within deletion segment IX, gave no *cysB*$^+$ recombinants. Although the *cysB484* mutation leads to cysteine auxotrophy and an inability to derepress for most of the enzymes of the sulfate-reducing pathway, strains bearing this allele differ from other *cysB* auxotrophs thus far studied, since they are constitutively derepressed for *O*-acetylserine sulfhydrylase-A when grown on L-cystine (11). This enzyme was assayed in all point mutants from deletion segments VIII and IX after growth on L-cystine to determine whether this phenomenon is a general feature of mutations in this region of the *cysB* locus. Only *cysB482* and *cysB484* were found to be derepressed and thus appear to be identical both by genetic mapping and by their constitutivity for *O*-acetylserine sulfhydrylase-A.

Mapping of the constitutive mutation cys-1352. The mutation *cys-1352* is located in or near the *cysB* locus and has been characterized by its ability to cause the constitutive expression of the cysteine biosynthetic enzymes (11; H. T. Spencer, J. Collins, and K. J. Monty, Fed. Proc. **26**:677, 1967). Fine-structure mapping of *cys-1352* was hindered by the ability of strains bearing this allele to feed sulfide auxotrophs and by the fact that there is no known way to select for or against this mutation. Scoring for *cys-1352* can be accomplished, however, by its capacity to impart resistance to several inhibitors, including 1,2,4-triazole, sodium selenite, and sodium selenate (6, 7). In the course of these studies we discovered that in contrast to the former two inhibitors 2.0 mM sodium selenate in agar does not interfere with transduction, and that *cys-1352* strains give orange colonies on such media, whereas wild-type colonies appear white.

The order of the *trpA*, *pyrF*, and *cys-1352* loci was determined by three-factor crosses in which selection was for either *trpA*$^+$ or *pyrF*$^+$. Recombinants then were scored for the remaining nutritional marker and for *cys-1352*. The results presented in Table 4 reveal that *cys-1352* co-transduces with both *trpA* (46%) and *pyrF* (64%) much more frequently than these two genes co-transduce with each other (18 to 20%). In the cross DW275 $\times$ *trpA160* (donor) the rarest class of *pyr*$^+$ recombinants was *trpA*$^-$ *cys-1352*, whereas the cross DW274 $\times$ *pyrF146* (donor) gave *pyr*$^-$ *cys-1352* as the rarest class of *trp*$^+$ recombinants. In each cross the quadruple cross-over event necessary to generate the rare class of recombinants necessitates that *cys-1352* be located between *trpA* and *pyrF*, which is the gene order predicted from two-point co-transduction frequencies.

Attempts to use *cysB* mutants as recipients in multifactor crosses involving *cys-1352* and outside markers were unsuccessful, owing to the feeding of *cysB*$^-$ recipient bacteria by *cys-1352* recombinants. Deletion mapping proved to be a more satisfactory technique for fine-structure localization of this mutation. Donor phage from various *pyrF*$^+$ Δ(*supX cysB*) strains were used to transduce DW275 (carrying *cys-1352*) in crosses where selection was for *pyrF*$^+$ recombinants on agar plates lacking L-cystine and supplemented with L-histidine and 2.0 mM sodium selenate. Although *cys-1352* colonies were easily recognized on the original transduction plates by their orange color, their ability to feed *cysB*$^-$ strains caused *pyrF*$^+$ Δ(*supX cysB*)

TABLE 2. *Deletion mapping of cysB*[a]

Deletion segment	Recipient	Donor											
		DW353	DW354	DW356[b]	DW357[b]	DW359[b]	DW361	DW362	DW363	DW364	DW365	DW366	DW367[b]
II	*cysB14*	+	0	0	0	0	0	0	—	0	0	0	—
	cysB27	+	0	0	0	0	0	0	—	0	0	0	0
	cysB977	+	0	0	0	0	—	0	—	0	0	0	—
III	*cysB16*	+	+	0	0	0	0	0	—	0	0	0	—
	cysB40	+	+	0	0	0	0	0	—	0	0	0	—
IV	*cysB18*	+	+	+	0	0	0	0	—	0	0	0	—
	cysB24	+	+	+	0	0	0	0	—	0	0	0	—
	cysB25	+	+	+	0	0	0	0	—	0	0	0	—
	cysB70	+	+	+	0	0	0	0	—	0	0	0	—
	cysB87	+	+	+	0	0	0	0	—	0	0	0	—
	cysB88	+	+	+	0	0	0	0	—	0	0	0	—
	cysB294	+	+	+	0	—	—	0	—	0	0	—	—
	cysB434	+	+	+	0	0	0	—	—	—	—	—	—
	cysB932	+	+	+	0	—	—	0	—	0	0	—	0
V	*cysB41*	+	+	+	+	0	0	0	—	0	0	0	—
VI	*cysB403*	+	+	+	+	+	0	0	—	0	0	0	—
VII	*cysB232*	+	+	+	+	+	+	0	0	0	0	0	—
VIII	*cysB12*	+	+	+	+	+	+	+	0	0	0	0	—
	cysB529	+	+	—	+	—	—	+	0	0	0	—	—
	cysB692	+	+	—	+	—	—	+	0	0	0	—	—
	cysB697	—	+	—	+	—	—	+	0	0	0	—	—
	cysB1113	+	+	—	+	—	—	+	0	0	0		
IX	*cysB10*	—	+	—	+	—	—	+	+	0	0	—	—
	cysB15	+	+	+	+	+	+	+	+	0	0	—	—
	cysB45	+	+	+	+	+	+	+	+	0	0	—	—
	cysB400	+	+	—	+	—	—	+	+	0	0	—	—
	cysB482	+	+	+	+	+	+	+	+	0	0	—	—
	cysB484	+	+	+	+	+	+	+	+	0	0	—	—
	cysB528	+	+	—	+	—	—	+	+	0	0	—	—
	cysB913	+	+	—	+	+	—	+	+	0	0	—	—
	cysB921	—	+	—	+	—	—	+	+	0	0	—	—
	cysB936	+	+	—	+	—	—	+	+	0	0	—	—
	cysB938	+	+	—	+	—	—	+	+	0	0	—	—
	cysB948	+	+	—	+	—	—	+	+	0	0	—	—
	cysB962	+	+	—	+	—	—	+	+	0	0	—	—
X	*cysB267*	+	+	+	+	+	+	+	—	+	0	0	0
XI	*cysB661*	+	+	—	+	—	—	+	+	—	+	0	0
XII	*cysB517*	+	+	+	+	+	+	+	+	+	+	+	0

[a] Phage from different Δ(*supX cysB*) *pyrF146* donors were used to transduce *pyrF*$^+$, single-site *cysB* recipients on agar medium supplemented with uracil. Selection was for *cysB*$^+$ recombinants, and *pyrF* was scored by replica plating. The data express whether any (+) or no (0) *cysB*$^+$ *pyrF*$^-$ colonies were obtained with from three to ten transduction plates where approximately 50 such recombinants per plate are expected using a *supX*$^+$ *cysB*$^+$ *pyrF146* donor. The symbol (—) indicates the cross was not performed.

[b] Additional *cysB* deletion strains were tested: DW355 gave the same results as DW356; DW358 same as DW357; DW360 same as DW359; DW368 same as DW367.

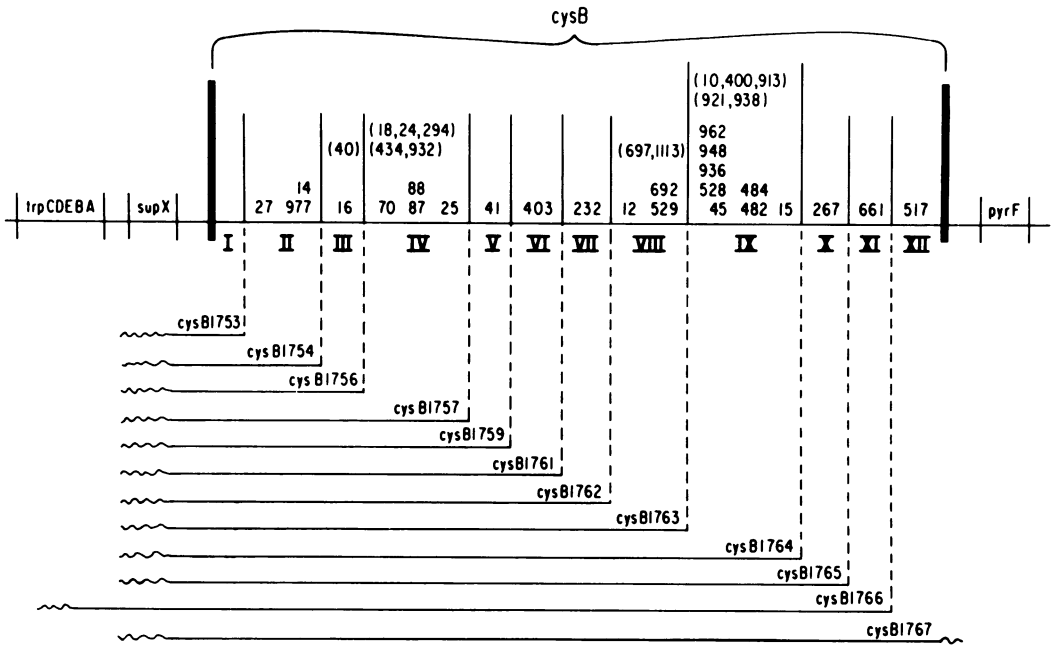

FIG. 1. *Genetic map of cysB as determined by deletion mapping and reciprocal three-point crosses. All cysB deletions extend into or through supX, and cysB1766 extends into the cluster of trp genes as well. Mutations included in parentheses were localized to a specific deletion segment but could not be ordered by three-point crosses.*

recombinants on the same plate to form white colonies which were indistinguishable from *cysB*$^+$ colonies. Therefore all white colonies were rescored in the absence of *cys-1352* colonies to verify that true *cysB*$^+$ recombinants had arisen from such crosses. Scoring for the *his* marker present in the original recipient strain insured against the possibility of random contaminants giving false positive results.

Deletions which do not cover deletion segment IX gave *pyrF*$^+$ *cysB*$^+$ colonies in these crosses, whereas deletions extending through this deletion segment gave no such recombinants (Table 5). A number of orange colonies, together with the seven *cys*$^+$ white colonies obtained using deletions *cysB1762* and *cysB1763* as donors, were cloned several times on agar lacking sodium selenate and assayed for *O*-acetylserine sulfhydrylase-A after growth on L-cystine. Each orange colony tested was found to be constitutive for this enzyme, whereas all white colonies were normally repressed.

Control experiments (Table 6) using DW273 (carrying *cysB27*) and DW277 (carrying *cysB517*) as recipients demonstrated that appreciable numbers of *pyrF*$^+$ *cysB*$^+$ recombinants could be obtained from these deletion donors in the manner predicted from our previous mapping experiments. These data indicate that *cys-1352* is located within deletion segment IX of the *cysB* locus, and we shall now refer to this allele as *cysB1352*.

DISCUSSION

In *E. coli* and *S. typhimurium* all of the cysteine biosynthetic enzymes except serine transacetylase are repressed by growth on L-cyst(e)ine or sulfide (10, 11, 20). The major form of control of serine transacetylase occurs through feedback inhibition by L-cysteine (12). Jones-Mortimer (9) and Jones-Mortimer et al. (10) studied the regulation of this biosynthetic pathway in *E. coli* and found that, in addition to sulfur starvation, the presence of *O*-acetyl-L-serine and a functional *cysB* gene are necessary for derepression. Findings identical to these have been reported for *S. typhimurium* (11; H. T. Spencer, J. Collins, and K. J. Monty, Fed. Proc. **26**:677, 1967). Thus *cysE* mutants are pleiotrophic, and when grown on sulfur-poor medium these strains can be derepressed, or "induced," only by the addition of *O*-acetyl-L-serine. When grown on a good sulfur source, such as sulfide or L-cyst(e)ine, neither wild-type nor *cysE* strains can be "induced" by exogenous *O*-acetyl-L-serine.

Most *cysB* auxotrophs have very low to im-

TABLE 3. *Ordering of cysB mutations within deletion segments by three-point crosses*[a]

Deletion segment	Cross		% Co-transduction between *trpA* and *cysB*		Order[b]	*P*[c]
	A	B	A as donor	B as donor		
II	*cysB977* × *cysB27*		22.7 (75)[d]	2.8 (71)	*27, 977*	<0.001
	cysB14 × *cysB27*		8.3 (410)	0.6 (354)	*27, 14*	<0.001
	cysB977 + *cysB14*		—[e]	—[e]		
III-IV	*cysB70* × *cysB16*		19.1 (451)	12.5 (345)	*16, 70*	<0.05
IV	*cysB88* × *cysB70*		20.5 (219)	13.6 (324)	*70, 88*	<0.05
	cysB87 × *cysB70*		24.8 (210)	14.5 (408)	*70, 87*	<0.01
	cysB88 × *cysB87*		—[f]	—[f]		
	cysB25 × *cysB88*		32.0 (372)	5.4 (443)	*88, 25*	<0.001
	cysB25 × *cysB87*		25.0 (444)	9.2 (338)	*87, 25*	<0.001
V-VI	*cysB403* × *cysB41*		15.9 (835)	9.8 (419)	*41, 403*	<0.01
VI-VII	*cysB232* × *cysB403*		15.5 (264)	9.7 (329)	*403, 232*	<0.05
VII-VIII	*cysB12* × *cysB232*		30.1 (123)	11.7 (103)	*232, 12*	<0.001
VIII	*cysB529* × *cysB12*		26.5 (98)	9.4 (276)	*12, 529*	<0.001
	cysB692 × *cysB12*		12.2 (41)	0 (71)	*12, 692*	<0.05
	cysB692 × *cysB529*		—[e]	—[e]		
IX	*cysB528* × *cysB45*		—[e]	—[e]		
	cysB936 × *cysB45*		—[e]	—[e]		
	cysB938 × *cysB45*		—[e]	—[e]		
	cysB948 × *cysB45*		—[e]	—[e]		
	cysB962 × *cysB45*		—[e]	—[e]		
	cysB482 × *cysB45*		19.9 (413)	10.5 (114)	*45, 482*	<0.05
	cysB484 × *cysB45*		21.9 (402)	15.3 (400)	*45, 484*	<0.05
	cysB482 × *cysB528*		12.0 (75)	0 (87)	*528, 482*	<0.01
	cysB482 × *cysB936*		22.4 (49)	4.2 (71)	*936, 482*	<0.05
	cysB482 × *cysB938*		—[f]	—[f]		
	cysB482 × *cysB948*		30.7 (101)	2.0 (29)	*948, 482*	<0.001
	cysB482 × *cysB962*		22.0 (81)	8.4 (91)	*962, 482*	<0.005
	cysB484 × *cysB482*		—[f]	—[f]		
	cysB15 × *cysB938*		28.0 (82)	1.3 (75)	*938, 15*	<0.001
	cysB15 × *cysB482*		28.6 (633)	6.4 (313)	*482, 15*	<0.001
	cysB15 × *cysB484*		15.9 (584)	7.1 (311)	*484, 15*	<0.001
XI-XII	*cysB517* × *cysB661*		13.3 (93)	2.4 (81)	*661, 517*	<0.05

[a] Selection was for *cysB*$^+$ recombinants using *trpA160 cysB*$^-$ donor and *trpA*$^+$ *cysB*$^-$ recipient strains. Transductions were performed on plates containing L-tryptophan, and the *trpA* character was determined by replica plating.

[b] The *trpA* locus is to the left.

[c] Each *P* value was calculated by a chi square contingency test (3) and expresses the probability that the differences in percentage of co-transduction between *trpA* and *cysB* noted in the reciprocal crosses were due to chance alone.

[d] Numbers in parentheses indicate total numbers of *cysB*$^+$ recombinants scored for *trpA*.

[e] Recombinants were too few to establish an order.

[f] No recombinants were obtained.

measurable levels of all the cysteine biosynthetic enzymes except serine transacetylase, which is always expressed constitutively. The presence of this pleiotropic phenotype in deletion mutants indicates that *cysB* codes for one or more elements of positive control in both *E. coli* and *S. typhimurium*. Merodiploid studies in *E. coli* have given results which support this conclusion (9). Although a *cysB* gene product has not yet been purified or demonstrated in vitro, the isolation of temperature-sensitive and amber mutations in *E. coli* indicates that *cysB*

TABLE 4. *Three-factor crosses with trpA160, pyrF146, and cys-1352*

Donor	Recipient[a]	Selected marker	Total no. of colonies tested	Unselected marker[b]	% of total colonies
Wild type	DW220 (*trpA160 pyrF146*)	*trp*$^+$	984	*pyr*$^+$	20.3
				pyr$^-$	79.7
		pyr$^+$	569	*trp*$^+$	18.3
				trp$^-$	81.7
pyrF146	DW274 (*trpA160 cys-1352*)	*trp*$^+$	312	*pyr*$^+$ *cys-1352*$^+$	34.6
				pyr$^+$ *cys-1352*	52.2
				pyr$^-$ *cys-1352*$^+$	11.2
				pyr$^-$ *cys-1352*	1.9
trpA160	DW275 (*pyrF146 cys-1352*)	*pyr*$^+$	412	*trp*$^+$ *cys-1352*$^+$	50.0
				trp$^+$ *cys-1352*	36.4
				trp$^-$ *cys-1352*$^+$	13.6
				trp$^-$ *cys-1352*	0

[a] All three recipients carried the *hisC340* and *ara-9* alleles, for which there was neither selection nor scoring in these crosses.

[b] Scoring for the *cys-1352* character was performed either by replicating transductants onto agar containing 2.0 mM sodium selenate or by including this agent in the original transduction plates. Since similar frequencies of recombinant classes were obtained by both techniques, the data from these experiments have been combined.

TABLE 5. *Deletion mapping of cys-1352*[a]

Donor[b]	Total *pyrF*$^+$ recombinants	Orange colonies (*cys-1352*)[c]	White colonies[d]	
			cysB$^-$	*cysB*$^+$
Wild type (*cysB*$^+$)	265	83	0	182
PM206 (*cysB*$^+$)	62	27	0	35
DW369 (*cysB1753*)	641	486	153	2
DW370 (*cysB1754*)	691	440	244	7
DW371 (*cysB1762*)	450	269	176	5
DW372 (*cysB1763*)	266	208	56	2
DW373 (*cysB1764*)	384	166	218	0
DW374 (*cysB1765*)	424	196	228	0
DW375 (*cysB1767*)	996	654	342	0
DW376 (*cysB1768*)	2668	2668	0[e]	0

[a] The recipient in all transductions was DW275 (*cys-1352 pyrF146 hisC340 ara-9*), and *pyrF*$^+$ recombinants were selected on agar supplemented with histidine and 2 mM sodium selenate.

[b] All donors are *pyrF*$^+$. The pertinent *cysB* genotype is included in parentheses after the strain designation.

[c] The orange color on sodium selenate agar is part of the phenotype of *cys-1352*.

[d] These were rescored for *cysB* on plates lacking *cys-1352* colonies, since the latter were found to feed *cysB*$^-$ strains. Most white colonies proved to be *cysB*$^-$ and presumably were of the genotype *pyrF*$^+$ Δ(*supX cysB*).

[e] Strain DW376 carries Δ(*trp supX cysB1768*), which covers all *cysB* mutations tested. Some minute white colonies did appear on these transduction plates, which upon further testing were found to be *pyrF*$^+$ *trp*$^-$ *cysB*$^-$. These recombinants probably carried the donor deletion.

TABLE 6. *Deletion mapping of DW273 (carrying cysB27) and DW277 (carrying cysB517) with various pyrF*$^+$ *cysB deletion donors*[a]

Donor[b]	Recipient	Total *pyrF*$^+$ recombinants	*pyrF*$^+$ *cysB*$^+$
Wild type (cysB$^+$)	DW273	104	40
DW369 (*cysB1753*)	DW273	416	3
DW370 (*cysB1754*)	DW273	416	0
Wild type (*cysB*$^+$)	DW277	156	104
DW369 (*cysB1753*)	DW277	260	19
DW370 (*cysB1754*)	DW277	364	25
DW371 (*cysB1762*)	DW277	346	25
DW372 (*cysB1763*)	DW277	133	30
DW373 (*cysB1764*)	DW277	126	7
DW374 (*cysB1765*)	DW277	238	13
DW375 (*cysB1767*)	DW277	746	0

[a] Selection was for *pyrF*$^+$ using *pyrF146 cysB* recipients, and recombinants were scored for *cysB* by replica plating.

[b] All donors are *pyrF*$^+$. The pertinent *cysB* allele is included in parentheses after the strain designation.

is a structural gene for at least one polypeptide chain (18).

Mizobuchi et al. (13) observed abortive transductants in crosses between certain *cysB* auxotrophs, from which they concluded that this locus comprises three closely linked cistrons, *cysBa*, *cysBb*, and *cysBc*. These authors proposed the order *trp-cysBa-cysBb-cysBc* from the results of three-point crosses in which they found that *cysBa18*, *cysBa24*, *cysBb14*, *cysBb87*,

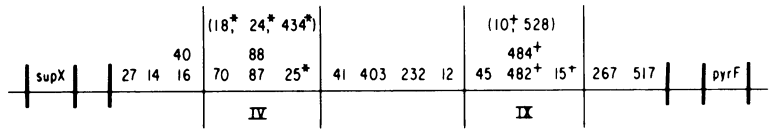

FIG. 2. *Genetic map of cysB including only those mutations previously assigned (13; M. Demerec, Stockbook, 1966) to one of the three complementation groups cysBa (*), cysBb (no mark), and cysBc (+).*

and *cysBb403* are situated between *trpD* and *cysBb12*, and that *cysBc10*, *cysBc15* and *cysBc482* are located on the *pyrF* side of *cysBb12*. Although the leakiness of all *cysB* auxotrophs on solid media has frustrated our attempts to detect abortive transductants, our studies are compatible with those previously reported for the positions of certain mutations relative to *cysB12*. The additional detail of our map, however, shows that the *cysBb* mutations tested by Mizobuchi et al. (13) are not contiguous. Furthermore, if other mutations which have been assigned to specific cistrons (M. Demerec, Stockbook, 1966) are ordered according to our map (Fig. 2), it is clear that *cysBb* mutations occur on both sides of *cysBa* mutations and on both sides of *cysBc* mutations. Unfortunately the data are insufficient to determine whether contiguity exists within the "clusters" of *cysBa* and *cysBc* mutations located in deletion segments IV and IX, respectively. Since these findings are not easily explained by the existence of three individual cistrons, each coding for its own polypeptide chain, we assume that the abortive transductants observed previously were due to intracistronic complementation.

The nutritional characteristics of *cysB* auxotrophs also have been used to assign mutations to specific cistrons. Mutants of the type *cysBb* are the mose fastidious, growing only on sulfide or L-cyst(e)ine, whereas both *cysBa* and *cysBc* mutants can utilize a number of inorganic sulfur sources, including sulfite, thiosulfate and dithionite (2, 13). Although auxotrophs of all three classes are known to have markedly reduced or immeasurable levels of all the enzymes of sulfate reduction, certain *cysB* mutants have been shown to synthesize significant amounts of several of these proteins, particularly sulfite reductase (11). These findings suggest that the nutritional dissimilarities noted among *cysB* mutants may be due to quantitative rather than qualitative differences between alleles of this genetic site. In the absence of convincing genetic evidence for the multicistronic structure of *cysB* we favor the notion that this locus is composed of a single cistron, mutations in which give rise to auxotrophs of variable "leakiness."

The presence of the *cysB1352* allele in a *cysE* strain which cannot synthesize *O*-acetyl-L-serine obviates the need for this inducer and results in the full expression of *O*-acetylserine sulfhydrylase-A and the enzymes of sulfate reduction during sulfur starvation (11). However, *cysB1352* does not lead to complete insensitivity to L-cyst(e)ine, and strains carrying this mutation can be repressed to a moderate extent for most of the enzymes of cysteine biosynthesis.

At the outset of this work we considered the possibility that the *cysB1352* allele might consist of a mutated promotor or operator region which leads to the constitutive synthesis of both the *cysB* gene product and the enzymes under the control of this locus. This model assumes that *O*-acetyl-L-serine and L-cyst(e)ine exert their regulatory effects primarily by controlling the rate of synthesis of the *cysB* gene product, rather than by interacting with this regulatory protein. Our fine-structure map indicates, however, that *cysB1352* is located within deletion segment IX of the *cysB* locus, and not at one extreme end as would be expected for an operator or promotor mutation. Furthermore, a partially constitutive auxotroph, *cysB482*, also is located in this same deletion segment, suggesting that a small internal region of this gene may be particularly susceptible to mutations leading to constitutivity.

Although these data do not eliminate the possibility that *cysB* is itself regulated, we feel that *cysB1352* probably codes for an altered gene product which can function as an element of positive control in the absence of *O*-acetyl-L-serine while retaining partial sensitivity to L-cyst(e)ine. The *cysB482* allele is of particular interest since it presumably codes for a regulatory molecule which has lost most of its ability to allow derepression of the sulfate-reducing enzymes but which permits the constitutive synthesis of *O*-acetylserine sulfhydrylase-A. If the *cysB* gene product is in fact a single polypeptide chain, these findings indicate that it must carry out multiple, nonidentical functions.

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