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Genetic Analysis of *thr* Mutations in *Salmonella typhimurium*

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Previous workers divided threonine-requiring (Thr$^-$) strains of *Salmonella* into three phenotypes with mutations in four complementation groups. The mutations were deemed to define four genes in the order *thrD-C-A-B* at minute zero on the *Salmonella* linkage map. In the present study 12 of these mutants were reexamined together with eight new Thr$^-$ strains. The three phenotypes were: homoserine-requiring (Hom$^-$); Thr$^-$, feeders of Hom$^-$ strains; Thr$^-$, nonfeeders. Exact correlation between these phenotypic groups and three complementation groups was confirmed by abortive transduction. No evidence was found for intergenic complementation between mutations in Hom$^-$ strains. It is proposed that *thr* mutations define three genes rather than four and that these be renamed *thrA* (Hom$^-$), *thrB* (Thr$^-$ feeders), and *thrC* (Thr$^-$ nonfeeders) to correspond with the sequence of reactions in threonine biosynthesis. Double mutant *trpRthr* strains were used in reciprocal three-point transduction tests to establish the order of *thr* mutation sites. Although revisions were made in the classification or location of several mutations, there was an overall correlation of complementation group, phenotype, and map position. The present data provide a basis for further correlation of threonine genes and biosynthetic enzymes, and analysis of cross regulation in aspartate amino acid biosynthesis in *Salmonella*.

Although *thr* markers are often used in mapping experiments in both *Escherichia coli* and *Salmonella typhimurium* there has been little analysis of *thr* mutations themselves. Only one study has been published (9) in which threonine-requiring (Thr$^-$) mutants were divided into four groups according to nutritional requirement, syntrophism, or complementation (abortive transduction). Six mutants could use homoserine in place of threonine (Hom$^-$ mutants), and were divided into two groups *thrC* and *thrD* by apparent complementation. However, *thrD* was represented by only one mutation (*thrD16*) and all Hom$^-$ strains had moderate or high frequencies of "reversion" to prototrophy or bradytrophy on unsupplemented medium. This presumably contributed to lack of clarity in abortive transduction tests involving Hom$^-$ mutants (9). Mutations in six relatively stable Thr$^-$ mutants were clearly divided into two complementation groups, *thrA* and *thrB*. In syntrophism tests the only feeding detected was by *thrA* mutants which fed Hom$^-$ strains presumably by excretion of homoserine (see Fig. 1). Two- and three-point crosses were used to map these 12 cotransducible *thr* mutations, with *serB* as the unselected marker. The apparent order of mutations was *thrD-thrC-thrA-ThrB* (9). However, these results were tentative since data were given for only one marker in each gene. Also, the unselected *serB* marker is only about 20% cotransducible with the *thr* genes (21) which would make results of reciprocal three-point crosses difficult to interpret (1). In the present work, undertaken to clarify and extend the original mapping of *thr* mutations in *Salmonella*, the unselected prototrophic marker, *trpR582*, (about 50% cotransducible with *thr* genes [21]) was used in three-point tests instead of the more distant auxotrophic marker *serB80*.

Most work on threonine biosynthesis in bacteria has been concentrated on biochemical aspects, especially the nature of aspartokinase-homoserine dehydrogenase (AK-HSD) complexes in *E. coli* (6). Results of these studies suggest that Hom$^-$ mutants are deficient in activities of the threonine sensitive AKI-HSDI complex (3, 4, 14). This bifunctional enzyme in *E. coli* is composed of four identical polypeptides (8, 20) presumably specified by a single gene (*thrA*-previously *thrD*: J. Theze in reference 22). However, the two enzymic activities are localized in separate regions of the polypep-

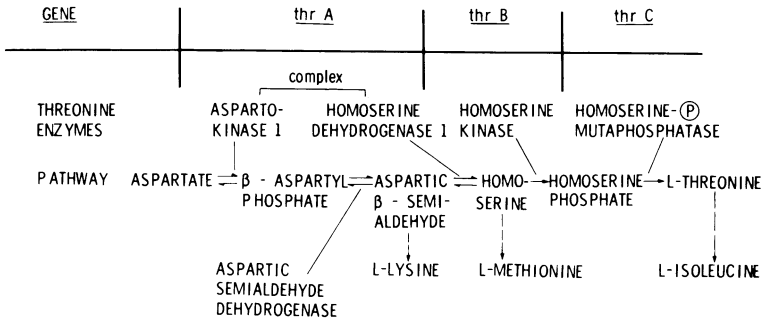


FIG. 1. *Proposed threonine biosynthetic system for E. coli K-12 and S. typhimurium LT2.*

tide chain, AKI on the amino-terminal side and HSDI on the carboxyl-terminal side (23). Indications of aggregation of AKI-HSDI activities have been found in *S. typhimurium* LT2 (3) and a similar arrangement of bifunctional polypeptides is probable. Thus, the apparent complementation between *thrC11* and *thrD16* reported by Glanville and Demerec (9) may be intragenic rather than intergenic. Genes controlling the conversion of homoserine to threonine through the enzymes homoserine kinase and threonine synthase (homoserine phosphate mutaphosphatase) in *E. coli* (5, 24) are given as *thrB* and *thrC*, respectively (22). It seems appropriate at this point to avoid differences in nomenclature between homologous genes in *E. coli* and *S. typhimurium*. Therefore, I propose to adopt for *Salmonella* the gene designations given recently for *E. coli* (22) as these are more consistent with the sequence of threonine biosynthetic reactions. Thus mutations giving a Hom⁻ phenotype probably define a single gene, *thrA*, rather than the separate genes previously designated *thrC* and *thrD* in *Salmonella*, and genes originally designated *thrA* and *thrB* (9) become *thrB* and *thrC*, respectively. The arrangement of *thr* structural genes and their probable roles in threonine biosynthesis are given in Fig. 1.

MATERIALS AND METHODS

Media: Nutrient broth (0.8% Difco plus 0.4% NaCl) (N broth) or L broth (12) served as complex liquid media. Minimal agar (M agar) was made by combining equal volumes of double-strength sterile minimal salts (MS) (24) (plus supplements as required [21]) and 3% agar (Difco); single-strength minimal salts plus 0.2% glucose (MSG medium) was used as indicated.

Bacterial strains. Strains of *S. typhimurium* LT2 used are listed in Table 1. The SD strain designations are reserved for strains prepared at Dalhousie; the relevant lesion designation is often included in parentheses after the strain designation in the text. A strain carrying *thrC1001* was isolated from a Ser⁻ strain by penicillin selection in MSG medium plus 40 μg of L-serine per ml after ultraviolet (UV) irradiation (10). A P22-H5 phage-sensitive Thr⁻ recombinant was isolated on MThr agar (M agar plus 20 μg of L-threonine/ml) after transduction of the Ser⁻ Thr⁻ recipient with phage P22-L7 from a wild-type (Wt) donor. This isolate was then used as a donor for transduction with strain SD31 (*serB80*) on MThr agar to give Thr⁻ (*thrC1001*) transductants. From one of these a P22-H5 phage-sensitive strain, SD93, was obtained and used for further study. A strain carrying mutation *thrB1007* was isolated from Wt (LT2) after growth in MSG medium plus 20 μg of L-threonine per ml followed by penicillin selection in MSG medium. Strains with *trpR* mutations synthesize *trp* enzymes constitutively and are resistant to inhibition of growth by 5-methyltryptophan. They also excrete tryptophan which is detected by feeding of the deletion mutant *trpE95* (21).

Genetic procedures. Most methods have been described (21). Incubation was at 37 C. Transduction recipients were grown from single colonies in broth (5 to 10 ml) in a gyratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.) for 4 to 5 h (approximately 10^9 cells/ml). Faster growth of auxotrophs in L broth for the shorter time avoided the selection of prototrophic revertants which occurred in cultures of some Thr⁻ strains grown overnight in N broth. For full transductions equal volumes of bacteria and phage P22-L7 (approximately 10^{10} plaque-forming units [PFU]/ml) were mixed (with a multiplicity of infection of about 10), incubated for 5 to 6 min, and spread on selective media (0.2 of 0.3 ml per plate). Routine controls for each recipient culture included: transductions with phage raised previously on the recipient strain (homologous transduction), and with phage raised on the Wt strain; platings of the uninfected culture for reversions; and streaking to test nutritional phenotype. Tests for abortive transductions (11) were performed directly on M agar or on MMet agar (M agar plus 20 μg of L-methionine per ml for *thrA* [Hom⁻] recipients) as follows: plates were spread with 0.1 ml of freshly-grown recipient cultures (approximately 5×10^8 cells/ml) and allowed to dry. One drop of each donor phage preparation (approximately 10^9 PFU/ml) was placed with a Pasteur pipet on a separate sector of the prepared plate and allowed to dry. Duplicate plates with up to five donors per plate were inverted and incubated for 24 h. Unin-

TABLE 1. *Salmonella strains used*

Strain[a]	Genotype	Previous *thr* designation	Phenotype			Source[d]
			Growth[b]	Syntrophy[c] with		
				thrA11	*trpE95*	
SD1	*serB80*	—	Ser⁻	0	0	SU47 × Wt
SD91	*thrA11*	C11	Hom⁻(s)	0	0	*thrC11* (A)
SD76	*trpR582thrA11*	—	Hom⁻(s)	0	+	SD30 × *thrA11*
SD14	*serB80thrA11*	—	Ser⁻Hom⁻(s)	0	0	SU47 × *thrA11*
SD87	*thrA1008*	—	Hom⁻	0	0	SD1 × *thr-45*
SD88	*trpR582thrA1008*	—	Hom⁻	0	+	SD80 × SD87
	thrA16	D16	Hom⁻	0	0	(A)
	thrA23	B23	Hom⁻	0	0	(A)
SD78	*trpR582thrA16*	—	Hom⁻	0	+	(C)
SD2	*thrB2*	A2	Thr⁻	+	0	*thrA2cysA21* (A)
SD89	*thrB8*	A8	Thr⁻	+	0	*thrA8* (B)
SD83	*trpR582thrB8*	—	Thr⁻	+	+	(C)
	thrB9	A9	Thr⁻	+	0	(B)
SD90	*thrB10*	A10	Thr⁻	+	0	*thrA10* (B)
SD80	*trpR582thrB10*	—	Thr⁻	+	+	(C)
	thr-1007	—	Thr⁻	+	0	see text
SD98	*trpR582thr-1007*	—	Thr⁻	+	+	(C)
SD84	*thrBC12*	B12	Thr⁻	0	0	*thrB12* (B)
	thrC13	B13	[e]	0	0	(A)
SD92	*thrC29*	B29	Thr⁻	0	0	*thrB29* (A)
SD81	*trpR582thrC29*	—	Thr⁻	0	+	(C)
SD3	*thrC59*	A59	Thr⁻	0	0	SU47 (21)
SD30	*trpR582thrC59*	—	Thr⁻	0	+	(21)
	thrA1008thrC45	—45	Thr⁻	0	0	(A)
SD93	*thrC1001*	—	Thr⁻	0	0	see text
SD94	*trpR582thrC1001*	—	Thr⁻	0	+	(C)
	thrC1002	—	Thr⁻	0	0	(D)
	thrC1003	—	Thr⁻	0	0	(D)
	thrC1004	—	Thr⁻	0	0	(D)
SD95	*thrC1005*	—	Thr⁻	0	0	*trpA47Sthr*⁻ (D)
SD96	*thrC1006*	—	Thr⁻	0	0	*trpA47Sthr*⁻ (D)
SD97	*trpR582thrC1006*	—	Thr⁻	0	+	(C)
SD31	*serB80trpR582*	—	Ser⁻	0	+	(21)

[a] All SD strains were obtained by transductions with recipient strains SD1 or SD31 both of which were derived from SU47 (*serB80thrC59*) (21). All are therefore isogenic except for mutations in the *serB-thr* region.

[b] Nutrilites required in M agar to give single colonies in 24 h at 37 C: Thr, L-threonine (20 μg/ml); Hom, L-homoserine (20 μg/ml); Ser, L-serine (40 μg/ml); Hom⁻(s), relatively slow-growing compared with other Hom⁻ strains.

[c] Stab tests (21) (a), with L-methionine (20 μg/ml) added to M agar seeded with strain SD91, (b), with Casamino Acids (Difco) (100 μg/ml) added to M agar seeded with strain *trpE95*. 0, No feeding; +, visible feeding.

[d] (A), Glanville and Demerec (9) via P.F. Smith-Keary; (B), Glanville and Demerec (9) via E. Balbinder; (C), by transduction of Thr⁻ donor to strain SD31 recipient on MThr agar; (D), Spontaneous mutant isolated from Wt (LT2) or strain *trpA47S* after penicillin selection (16).

[e] Bradytrophic, inhibited in presence of L-homoserine (20 μg/ml).

fected areas on each plate, together with Wt donor and homologous phage-infected areas served as test controls. Large prototrophic colonies within the infected areas indicated recombination between donor and recipient markers. Microscopically small colonies, more numerous than complete transductants and sometimes present without the latter, were taken to be abortive transductants. These colonies were always present in areas receiving Wt donor phage.

RESULTS

Phenotypes of the thr mutants. Nutritional phenotypes of *thr* mutants described by Glanville and Demerec (9) were largely confirmed (Table 1), but with some exceptions. The Hom⁻ mutant carrying *thrA16* (previously *D16*) appeared slightly leaky and grew as well on

MHom agar (M agar plus 20 μg of L-homoserine/ml) as on MThr agar. The previously undescribed strain *thr-45* proved to be a double mutant with one mutation apparently in *thrC* (*thrC45*), and a second mutation in *thrA* (*thrA1008*) (Table 9). A strain carrying *thrA1008* alone (SD87) was isolated from the cross SD1 (*serB80*) × *thr-45* on homoserine-supplemented medium. It was nonleaky Hom⁻ mutant with slightly faster growth than strain SD91 (*thrA11*) on MHom agar. Available stock of strain *thrC13* was bradytrophic. It grew more slowly than Wt on M agar, approached Wt on MIle agar (M agar plus 20 μg of L-isoleucine/ml) and was virtually indistinguishable from Wt on MThr agar (on which growth of Wt was slightly inhibited). However, its growth was totally inhibited by L-homoserine (20 μg/ml) even in the presence of L-isoleucine, L-methionine, or L-lysine. Bradytrophic revertants of strain SD3 (*thrC59*) showed a similar phenotype. It is unclear at present why lesions in the threonine synthase gene (Fig. 1) should cause this phenotype.

In stab tests (21) feeding of strains SD91 (*thrA11*) and SD87 (*thrA1008*) by *thrB* mutants (probably homoserine kinase-less, Fig. 1) was confirmed and was corroborated by the appearance of small Hom⁻ (*thrA*) donor colonies in transductions with *thrB* recipients. However, strain SD3 (*thr-59*), derived from strain SU47 and with a lesion originally placed in the same gene as *thr-8*, *thr-9*, and *thr-10* (i.e., *thrB* in current nomenclature) (9), did not feed *thrA* mutants. Similarly, *thrC* mutants fed neither *thrA* nor *thrB* mutants. The probable position of *thr-59* within *thrC* was confirmed by complementation and three-point transduction tests (below). The newly isolated strain *thrB1007*, also fed *thrA* mutants, but the map position of lesion *thrB1007* is anomolous (see below).

Levels of spontaneous mutability originally observed (9) were also largely confirmed except for the current stock of strain SD84 (*thr-12*). No reversion of *thr-12* has been observed, and mapping data (below) suggests it is a multisite mutation. The slight leakiness of strain *thrA16* on M agar probably contributed to its high mutation frequency and made it unusable as a transduction recipient. Similar though less frequent or delayed "reversions" were obtained with strains SD91, *thrA23*, and SD87. The term "reversions" covers secondary linked or unlinked phenotypic suppressor mutations which occur in these strains (9). Appearance of revertants of Hom⁻ mutants on solid media was delayed or inhibited by L-methionine (20 μg/ml), probably by reducing the growth rate of strains carrying *thrA* mutations (3). Some prototrophic colonies arising from Hom⁻ mutants on both M agar and MMet agar had halos of parental cell growth around them. Feeding of the parental strain SD87 (*thrA1008*) was confirmed in stab tests after single-colony isolation of these prototrophs. No feeding of strain SD90 (*thrB10*) or a strain carrying *metA55* was observed in stab tests with one such prototroph derived from strain SD87. These results suggest that this prototroph may excrete homoserine. Preliminary experiments indicate that the *thrA1008* mutation is retained and prototrophy is produced by a second mutation which may be cotransducible with *serA* (Stuttard, manuscript in preparation).

Complementation tests. Microscopically small colonies indicative of abortive transduction were obtained at frequencies 5- to 10-fold higher than large prototrophic transductants in reciprocal crosses between *thrB* mutants and *thrC* mutants (Table 2). No abortive transductants were obtained in crosses between members of each group. Mutant *thr-1007* also had a *thrB* phenotype (Table 1) and yielded abortive transductants with all *thrC* mutants but with none of the *thrB* strains. The Hom⁻ mutants SD91 (*thrA11*), *thrA16*, *thrA23*, and SD87 (*thrA1008*) each gave abortive transductants when used as donors with *thrC* recipients. In crosses with *thrB* recipients abortive transduction was much less clear, partly because feeding allowed small colonies of donor phenotype to grow. Similar donor type transductants occurred in all crosses in which strain *thrA16* was donor, presumably because of the leakiness of the donor marker. Crosses with Hom⁻ recipients on M agar were difficult to score because of the high frequencies of prototrophic revertants (up to 10^3 per 10^8 cells plated) arising within 24 h. However, on MMet agar very few revertants of strains SD91, *thrA23* and SD87 (less than 10 per 10^8 cells plated) appeared within 24 h of plating. On MMet agar these strains gave no abortive transductants in reciprocal crosses together, nor with strain *thrA16* used as donor. The nonleaky *thrA* mutants gave abortive transductants in reciprocal crosses with *thrC* mutants and when used as recipients with *thrB* donors. From these results it is concluded that *thr* mutations fall into three distinct complementation groups (Table 2) which correspond to three distinct phenotypes.

In agreement with previous results (9) no recombination was observed between mutations *thrB8* and *thrB9*. Both of these mutations can revert, so neither is likely to be a deletion

TABLE 2. *Complementation (abortive transduction) patterns*

Recipient	*thrA*[a] 11 23 1008 16	*thrB* 2 8 9 10	*thr* −1007	*thrC* 29 59 1001-1006
thrA	0 D	+	+	+
thrB	+ D + D	0	0	+
thr-1007	+ D + D	0	0	+
thrC	+ + D	+	+	0

[a] 0, No minute colonies; +, minute colonies (abortive transductants); D, donor colonies; conditions for crosses, see text.

overlapping the other. They arose independently in strains with different genetic backgrounds (see below), and presumably represent independent mutations at the same or very close sites. Most subsequent mapping was done with only one of this pair of mutations. Similarly, mutations *thrA23* and *thrA11* did not recombine. However, different growth rates on MHom agar of strains carrying them suggest that these are different mutations. Also, mutation *thr-12* gave no recombinants with *thrB* or *thrC* mutations. Thus strain SD84 appears to carry a multisite mutation, probably a deletion covering sites in genes *thrB* and *thrC* and possibly extending into *thrA* since SD84 does not feed *thrA* mutants. All other combinations of *thr* mutations gave prototrophic recombinants in reciprocal crosses.

Three-point tests. Double mutant strains carrying *trpR582* in addition to a *thr* mutation were prepared mostly by transduction between the *thr* donor and strain SD31 (*serB80 trpR582*) on MThr agar (Table 1). These were then used in reciprocal crosses on EM agar (M agar plus 0.01% N broth) or MMet agar (for *thrA* recipients) for the ordering of mutation sites. *trp582* Was used as the unselected marker because it does not yield a nutritional deficiency and is closer than *serB* is to the *thr* genes (21). Thus, possible scoring bias owing to smaller sized auxotrophic transductants, and some ambiguities inherent in three-point tests with loosely linked markers (1) were avoided. Diagrams of the types of cross used are given in Fig. 2. For convenience of description the *tested thr* mutation is linked with $trpR^+$, the *tester* strains are double mutants, *trpR582 thr*. Site orders were derived on the assumption that recombinants requiring most crossovers are least frequent (1). Initial crosses between different *thr* recipients and phage raised on strain SD30 (*trpR582 thrC59*) revealed variations in the expression of prototrophic transductants carrying *trpR582*. Instead of the expected dense halo of feeding of *trpE95* by these transductants in stab tests, some crosses produced colonies which were only weak feeders. Recipients giving this effect included the original strains *thrA11*, *thrB8* (but not *B9*), *thrB10*, *thrC29*, and *thrC1001*. Presumably, the reduced feeding indicated lower levels of tryptophan excretion resulting from altered expression of the *trpR582* mutation in these genomes. When Thr^- strains produced by transduction of each *thr* marker into the *serB80* genome (SD91, SD89, SD90, SD92, and SD93, respectively; Table 1) were used as recipients with *trpR582* donors, no reduction in feeding of *trpE95* was seen. Thus, the modifier(s) of *trpR582* expression is not closely linked to the *thr* genes. Subsequent tests indicated that tryptophan excretion (measured by *trpE95* feeding) was reduced in all prototrophic strains prepared by transduction between strain *thrB10* and *trpR* donors, but their 5-methyl tryptophan resistance (21) was not reduced. The lesion causing this alteration of *trpR* expression in strain *thrB10* appears to be closely linked to *trpA47* in the *trp* operon (Stuttard, unpublished data).

Results of three-point transductions varied within a range of about 5 to 10% in repeat crosses (Tables 3-9). This reproducibility was adequate for the derivation of an unambiguous relative order for most of the *thr* mutations (Fig. 2). However, no definite order could be given for *thrC1002*, *1003*, and *29* because of apparent high frequencies of multiple crossovers between them (Table 7). Similarly, the position of *thrB1007* was uncertain. It appeared to be outside *thrB* and even to the right (Fig. 3) of *thrC59* (Tables 4-7). Reciprocal crosses with other *thrC* mutations gave ambiguous results possibly because *thrB1007* increases the frequency of recombination in regions adjacent to it (Table 8). The location of *thrC13* (Tables 6, 7, and 8) is tentative because leakiness and high reversion frequency of the strain carrying *thrC13* prevented its use as a recipient for reciprocal crosses. A tentative location at the extreme right (Fig. 3) of *thrC* was also derived for *thrC45*, the second mutation in addition to *thrA1008* in the original strain *thr-45*, by three-point crosses with *thrC* donor on MHom agar making *thrA1008* the unselected marker (Table

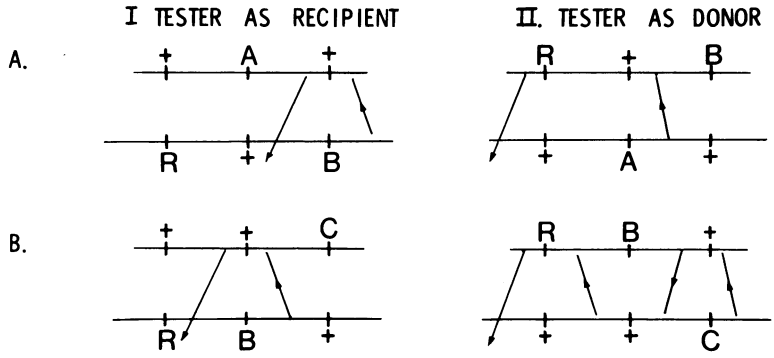


FIG. 2. *Regions for crossovers to produce prototrophic trpR582 transductants in three-point tests between thr mutations with trpR582 the unselected marker. Tester strains are trpR582thr⁻ double mutants; the tested thr mutation is linked to trpR⁺; R represents the unselected marker; A, B, and C are thr mutation sites. In A I a high proportion of transductants carry trpR582; A II and B I about 50% of transductants carry trpR582. In B II a low proportion of transductants carry trpR582.*

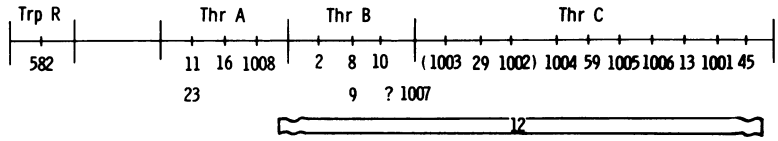


FIG. 3. *Order of thr mutation sites determined by three-point transduction tests. The position of thrB1007 and the exact extent of thr-12 are uncertain. Sites within brackets are distinct but their relative order is uncertain.*

TABLE 3. *Crosses to locate mutations within thrA with respect to trpR582*[a]

Recipient	Donor	Transductants			Site order indicated[c]
		No. tested	No. *trpR582*[b]	Nearest % *trpR582*	
trpR582thrA11 (SD76)	Wt	72	38	53	
trpR582thrA1008[d] (SD88)	Wt	80	19	24	
SD76	*thrA16*	71	35	49	*trpR582-thrA11-thrA16*
thrA11 (SD91)[d]	*trpR582thrA16* (SD78)	107	44	41	
SD88[d]	*thrA16*	24	15	63	*trpR582-thrA16-thrA1008*
thrA1008[d]	SD78	104	41	39	
SD76	*thrA1008*	24	8	33	*trpR582-thrA11-thrA1008*
thrA1008[d]	SD76	28	1	4	
SD88[d]	*thrA11*	24	21	88	
thrA23[d]	SD78	78	38	49	*trpR582-thrA23-thrA16*
thrA23[d]	SD88	160	75	47	*trpR582-thrA23-thrA1008*
SD76	*thrB9*	72	29	40	*trpR582-thrA11-thrB9*
thrB9	SD76	63	1	2	
thrB8 (SD89)	SD78	80	8	10	*trpR582-thrA16-thrB8*

[a] Prototrophs selected on EM or MMet agar; cross *thrA11* × *thrA23* gave no recombinants.
[b] Identified by feeding strain *trpE95*.
[c] See Fig. 2.
[d] MMet agar.

9). The cross with Wt donor demonstrates very close linkage (95%) between *thrA1008* and *thrC45*, two mutations at opposite ends of the *thr* genes. A majority of Hom⁻ recombinants obtained in all crosses indicates the site order *thrA1008-thrC* (*59*, *1005*, *1006*, *1001*)-*thrC45* (Table 9, order A).

DISCUSSION

The present results are in general agreement with those of Glanville and Demerec (9). How-

TABLE 4. *Reciprocal three-point crosses to locate thr mutations with respect to trpR582 and thrB8*[a]

Cross		Transductants			Site order indicated[c]
Recipient	Donor	No. tested	No. *trpR582*[b]	Nearest % *trpR582*	
trpR582thrB8 (SD83)	Wt	72	35	49	
SD83	*thrA11*	56	53	95	*trpR582-thrA11-thrB8*
thrA11 (SD91)[d]	SD83	108	34	31	
SD83	*thrA16*	52	47	90	*trpR582-thrA16-thrB8*
thrB8 (SD89)	SD78	80	8	10	
SD83	*thrA1008*	28	24	86	*trpR582-thrA1008-thrB8*
thrA1008[d]	SD83	106	44	42	
SD83	*thrB2*	37	34	92	*trpR582-thrB2-thrB8*
thrB2	SD83	120	61	51	
SD83	*thrB10*	80	51	64	*trpR582-thrB8-thrB10*
thrB10 (SD90)	SD83	119	21	18	
SD83	*thr-1007*	80	47	59	*trpR582-thrB8-thr-1007*
thr-1007	SD83	120	27	23	

[a, b, c, d] see Table 3.

TABLE 5. *Reciprocal three-point crosses to locate thr mutations with respect to trpR582 and thrB10*[a]

Cross		Transductants			Site order indicated[c]
Recipient	Donor	No. tested	No. *trpR582*[b]	Nearest % *trpR582*	
trpR582thrB10 (SD80)	Wt	70	34	49	
SD80	*thrA11*	80	72	90	*trpR582-thrA11-thrB10*
thrA11 (SD91)[d]	SD80	110	58	53	
SD80	*thrA1008*	34	31	91	*trpR582-thrA1008-thrB10*
thrA1008[d]	SD80	116	59	51	
SD80	*thrB2*	65	55	85	*trpR582-thrB2-thrB10*
thrB2	SD80	120	60	50	
SD80	*thrB8*	80	63	79	*trpR582-thrB8-thrB10*
thrB8 (SD89)	SD80	48	13	27	
SD80	*thrB9*	36	32	89	*trpR582-thrB9-thrB10*
thrB9	SD80	60	20	33	
SD80	*thr-1007*	80	40	50	*trpR582-thrB10-thr-1007*
thr-1007	SD80	120	27	23	
SD80	*thrC1002*	46	22	48	*trpR582-thrB10-thrC1002*
thrC1002	SD80	80	13	16	
SD80	*thrC1003*	80	38	48	*trpR582-thrB10-thrC1003*
thrC1003	SD80	72	13	18	

[a, b, c, d] see Table 3.

ever, differences in fine structure mapping and classification of some mutants are apparent. The original characterizations of the lesions *thr-12*, *-13*, *-23*, and *-59* may have been erroneous, although further mutation or mislabeling could have occurred during subsequent storage and stock transfers. By using the revised nomenclature, it is clear that the current *thr-12* is a multisite mutation, presumably a deletion, covering all known sites in *thrB* and *thrC* but none in *thrA*. It remains to be seen whether or not the AKI-HSDI activities of strain SD84 (*thr-12*) are intact. Absence of feeding with *thrA* mutants suggests that homoserine is not accumulated. The Hom$^-$ mutant *thrA23* was previously classified as a Thr$^-$, nonfeeding strain while a strain carrying *thrC59* was placed in the group of Thr$^-$ feeders (originally *thrA*, now *thrB*). An example of labeling discrepancies appears in a recent list of *Salmonella* strains where a *thr* marker in strain SA991 is variously given as *thrB49* or *thrA49* (Table 1 in ref. 18), the former being the original designation (Table 1 in ref. 9). Phenotypic similarities between strain *thrC13* and bradytrophic revertants of strain SD3 (*thrC59*) suggest that the former may be an

TABLE 6. *Reciprocal three-point crosses to locate thr mutations with respect to trpR582 and thrC29*[a]

Cross		Transductants			Site order indicated[c]
Recipient	Donor	No. tested	No. *trpR582*[b]	Nearest % *trpR582*	
trpR582thrC29 (SD81)	Wt	80	40	50	
SD81	*thrB10*	72	61	85	*trpR582-thrB10-thrC29*
thrB10 (SD90)	SD81	80	35	44	
SD81	*thrC1002*	12	8	66	Indefinite
thrC1002	SD81	80	17	21	
SD81	*thrC1003*	80	50	63	Indefinite
thrC1003	SD81	96	36	38	
SD81	*thrC59*	80	42	53	*trpR582-thrC29-thrC59*
thrC59	SD81	96	14	15	
SD81	*thrC1004*	61	27	44	*trpR582-thrC29-thrC1004*
thrC1004	SD81	80	13	16	
SD81	*thr-1007*	80	44	55	*trpR582-thrC29-thr-1007*
thr-1007	SD81	80	18	23	
SD81[d]	*thrC13*	74	38	51	*trpR582-thrC29-thrC13*

[a, b, c] see Table 3.
[d] Prototrophs selected on EMHom.

TABLE 7. *Reciprocal three-point crosses to locate thr mutations with respect to trpR582 and thrC59*[a]

Cross		Transductants			Site order indicated[c]
Recipient	Donor	No. tested	No. *trpR582*[b]	Nearest % *trpR582*	
trpR582thrC59 (SD30)	Wt	109	65	60	
SD30	*thrB10*	72	53	74	*trpR582-thrB10-thrC59*
thrB10 (SD90)	SD30	110	60	55	
SD30	*thrC1002*	36	28	78	*trpR582-thrC1002-thrC59*
thrC1002	SD30	72	32	44	
SD30	*thrC1003*	117	79	68	*trpR582-thrC1003-thrC59*
thrC1003	SD30	64	34	53	
SD30	*thrC29*	120	95	79	*trpR582-thrC29-thrC59*
thrC29 (SD92)	SD30	107	53	50	
SD30	*thrC1004*	21	17	81	*trpR582-thrC1004-thrC59*
thrC1004	SD30	72	28	39	
SD30	*thrC1005*	72	30	42	*trpR582-thrC59-thrC1005*
thrC1005	SD30	64	8	13	
SD30	*thrC1001*	63	31	49	*trpR582-thrC59-thrC1001*
thrC1001 (SD93)	SD30	62	6	10	
trpR582thrC1001 (SD94)	*thrC59*	80	70	88	
SD30	*thrC1006*	141	82	58	*trpR582-thrC59-thrC1006*
thrC1006 (SD96)	SD30	107	19	18	
SD30	*thr-1007*	120	66	55	*trpR582-thrC59-thr-1007*
thr-1007	SD30	80	22	28	

[a, b, c] see Table 3.

erroneously subcultured stock of a revertant of the original strain *thr-13*.

Abortive transduction results showed a complete correlation between the three phenotypic groups (Hom$^-$; Thr$^-$ feeders; and Thr$^-$ nonfeeders) and three complementation groups. Although direct gene-enzyme correlations remain incomplete it is reasonable to conclude that these three groups define three structural genes, *thrA*, *thrB*, and *thrC* which specify the threonine-specific enzymes aspartokinase-homoserine dehydrogenase, homoserine kinase, and threonine synthase (homoserine phosphate mutaphosphatase), respectively (5, 14, 25). Pre-

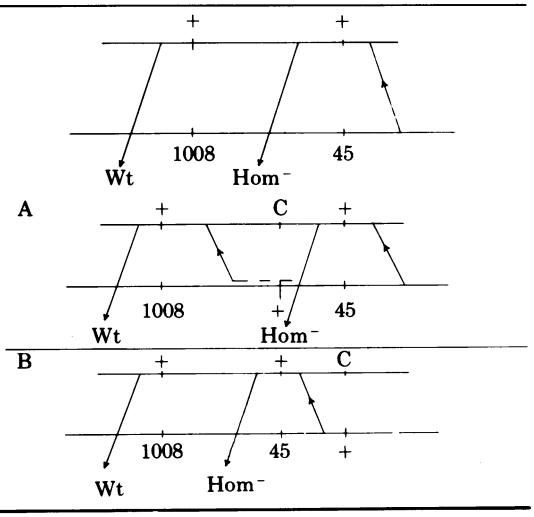
TABLE 8. *Three-point crosses to locate mutations with thrC*[a]

Cross		Transductants			Site order indicated[c]
Recipient	Donor	No. tested	No. *trpR582*[b]	Nearest % *trpR582*	
trpR582thrC1001 (SD94)	Wt	80	35	44	
SD94	*thrC1005*	79	65	82	*trpR582-thrC1005-thrC1001*
thrC1005	SD94	66	31	47	
SD94[d]	*thrC13*	80	57	71	*trpR582-thrC13-thrC1001*
trpR582thrC1006 (SD97)	Wt	77	43	56	
SD97	*thrC1001*	57	28	49	*trpR582-thrC1006-thrC1001*
thrC1001 (SD93)	SD97	80	13	16	
thrC1006 (SD96)	SD94	34	18	53	
SD97[d]	*thrC13*	40	24	60	*trpR582-thrC1006-thrC13*
SD94	*thr-1007*	80	58	73	Indefinite
thr-1007	SD94	80	24	30	
trpR582thr-1007 (SD98)	*thrC1001*	80	48	60	
thrC1001 (SD93)	SD98	80	32	40	
SD98	Wt	78	43	55	*trpR582-thr-1007-thrC1006*
SD97	*thr-1007*	78	56	72	
thr-1007	SD97	80	23	29	
SD98	*thrC1006*	80	38	48	
thrC1006 (SD96)	SD98	80	21	26	
SD98	*thrC1005*	80	55	69	*trpR582-thrC1005-thr-1007*
thrC1005	SD98	80	27	34	*trpR582-thr-1007-thrC1005*

[a, b, c] see Table 3.
[d] Prototrophs selected on EMHom agar.

TABLE 9. *Three-point crosses to locate thrC45; the recipient in all crosses was the double mutant thrA1008thrC45*

Donor	Transductants			Diagrams of crosses with alternative site orders
	No. tested	Phenotype		
		Wt	Hom⁻	
Wt	80	76	4	
thrC59	80	34	46	
thrC1005	78	19	59	
thrC1006	56	0	56	
thrC1001	69	4	65	



sumably, *thrC* mutants would accumulate homoserine phosphate (Fig. 1) which, in common with other phosphorylated pathway intermediates, would not serve as a nutrilite for *thrA* or *thrB* mutants (7). Cafferata and Freundlich (3, 4) have shown that strains C-5 (*thrA5*) and C-11 (*thrA11*) are deficient in threonine-specific homoserine dehydrogenase I activity. No analysis of their aspartokinase I activities was reported. However, given the close genetic and biochemical similarities between *S. typhimurium* LT2 and *E. coli* K-12 (17, 22) it is very likely that AKI activity in *Salmonella* also resides on the polypeptide with HSDI activity (3). In *E. coli* K-12 the native bifunctional enzyme is a tetramer (8, 20, 23). A similar arrangement in *Salmonella* could permit intragenic complementation between *thrA* mutants. However, the reported abortive transduction in crosses between strain *thr-16* and other Hom$^-$ mutants (9) was not observed. There seems no reason to place the *thr-16* lesion in a gene other than *thrA*. At present it is unknown which activity, AKI or HSDI, is deficient in strain *thrA16*, or whether any *thrA* mutants lack both activities. All three types of Hom$^-$ mutant have been isolated from *E. coli* (14) and have lesions apparently assigned to the *thrA* gene (22). The presence of three aspartokinases and two homoserine dehydrogenases with possible subunit exchange in vivo (3) further complicates complementation studies with Hom$^-$ mutants (14).

The present order of six *thr* mutation sites (Fig. 3) is similar to the approximate order given by Glanville and Demerec (9) except that the relative positions of *thrA11* and *A16* are reversed, as are those of *thrB8* (and *B9*) and *thrB10*. In addition, *thr-59*, which was not included in the provisional site order, is now located well within *thrC*. One Thr$^-$ and four Hom$^-$ mutants used by Glanville and Demerec were not obtained for the present study. However, eight new, and four previously unmapped *thr* mutations were analysed. Two of these, *thrC1002* and *thrC1003*, are very close to *thrC29*. Failure to obtain a clear minority class of recombinants in reciprocal crosses precluded the determination of an unambiguous site order for these mutations. High frequencies of multiple exchange (negative intereference) have been frequently observed in recombination studies especially with very close markers and with specific alleles (2). These effects could largely be avoided if deletion mutations were used for mapping but as yet *thr-12* is the only one available. The apparently anomolous location of *thrB1007* within *thrC* may also be referable to specific allele effects on recombination. However, more data would be required to define such effects.

Although the *thr* genes are very closely linked and appear to form a single genetic unit, the question of whether or not they form an operon remains open. As yet no operator-type or unlinked regulatory mutations have been reported although there is some evidence that isoleucyl- and threonyl-transfer ribonucleic acid synthetases may be involved in multivalent repression of threonine biosynthetic enzymes (13). No polarity mutations have yet been positively identified although they might be expected to occur in *thrA* if *thr* genes are transcribed into polygenic messenger ribonucleic acid in the order *thrA-B-C*. Strains with polarity mutations in *thrA* would have low levels of homoserine kinase and possibly threonine synthase, and should grow slowly on MHom agar. This is the phenotype of strain SD91 (*thrA11*) but not strain *thrA23*, both of which have mutations at or close to the same site. Thus, *thrA11* may be a polarity mutation although other explanations for its slow growth on MHom agar are possible. Polarity mutations may or may not give abortive transductions with mutations in adjacent genes (11).

A good genetic map is an essential part of the analysis of genetic control of a biochemical pathway. The present data and mutants provide a groundwork for analysis of threonine gene-enzyme relationships in *Salmonella*, and of wider interactions in the biosynthesis of 'aspartate' amino acids which are still unresolved (19).

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LITERATURE CITED

1. Balbinder, E. 1962. The fine structure of the loci *tryD* and *tryC* of *Salmonella typhimurium*. I. Determination of the order of mutational sites by three-point transduction tests. Genetics **47**:469-482.
2. Balbinder, E. 1962. The fine structure of loci *tryC* and *tryD* of *Salmonella typhimurium* II. Studies of reversion patterns and the behaviour of specific alleles during recombination. Genetics **47**:545-559.
3. Cafferata, R. L., and M. Freundlich. 1969. Evidence for a methionine controlled homoserine dehydrogenase in *Salmonella typhimurium*. J. Bacteriol. **97**:193-198.
4. Cafferata, R. L., and M. Freundlich. 1970. Evidence for channeling of homoserine in *Salmonella typhimurium*. Biochem. Biophys. Acta **222**:671-674.
5. Cohen, G. N., and J-C. Patte. 1963. Some aspects of the regulation of amino acid biosynthesis in a branched pathway. Cold Spring Harbor Symp. Quant. Biol. **28**:513-516.

6. Cohen, G. N. 1969. The aspartokinases and homoserine dehydrogenases of *Escherichia coli*, p. 183-231. *In* B. L. Horecker and E. R. Stadtman (ed.), Current topics in cellular regulation. Academic Press Inc., New York.
7. Davis, B. D., and E. S. Mingioli. 1953. Aromatic biosynthesis VII. Accumulation of two derivatives of shikimic acid by bacterial mutants. J. Bacteriol. **66**:129-136.
8. Falcoz-Kelly, F., J. Janin, J. C. Saari, M. Veron, P. Truffa-Bachi, and G. N. Cohen. 1972. Revised structure of aspartokinase I-homoserine dehydrogenase I of *Escherichia coli* K12. Eur. J. Biochem. **28**:507-519.
9. Glanville, E. V., and M. Demerec. 1960. Threonine, isoleucine and isoleucine-valine mutants of *Salmonella typhimurium*. Genetics **45**:1359-1374.
10. Gorini, L., and H. Kaufman. 1960. Selecting bacterial mutants by the penicillin method. Science **131**:604-605.
11. Hartman, P. E., Z. Hartman, and D. Serman. 1960. Complementation mapping by abortive transduction of histidine requiring *Salmonella* mutants J. Gen. Microbiol. **22**:354-368.
12. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage PI. Virology **1**:190-206.
13. Nass, G., K. Poralla, and H. Zähner. 1969. Effect of the antibiotic Borrelidin on the regulation of threonine biosynthetic enzymes in *E. coli*. Biochem. Biophys. Res. Commun. **34**:81-91.
14. Patte, J-C., P. Truffa-Bachi, and G. N. Cohen. 1966. The threonine-sensitive homoserine dehydrogenase and aspartokinase activities of *Escherichia coli*. I. Evidence that the two activities are carried by a single protein. Biochim. Biophys. Acta **128**:426-439.
15. Richaud, F., and G. N. Cohen. Selection of *Escherichia coli* mutants devoid of one or of both the activities carried by a multifunctional protein. Biochem. Biophys. Res. Commun. **30**:45-49.
16. Riyasaty, S., and G. W. P. Dawson. 1967. The recovery of tryptophan A auxotrophs at high frequency in a strain of *Salmonella typhimurium*. Genet. Res. **10**:127-134.
17. Sanderson, K. E. 1972. Linkage map of *Salmonella typhimurium*, Edition IV. Bacteriol. Rev. **36**:558-586.
18. Sanderson, K. E., H. Ross, L. Ziegler, and P. H. Mäkelä. 1972. F^+, Hfr, and F' strains of *Salmonella typhimurium* and *Salmonella abony*. Bacteriol. Rev. **36**:608-637.
19. Smith, D. A. 1971. S-Amino acid metabolism and its regulation in *Escherichia coli* and *Salmonella typhimurium*. Advan. Genet. **16**:141-166.
20. Starnes, W. L., P. Munk, S. B. Maul, G. N. Cunningham, D. J. Cox, and W. Shive. 1972. Threonine-sensitive aspartokinase-homoserine dehydrogenase complex, amino acid composition, molecular weight, and subunit composition of the complex. Biochemistry **11**:677-687.
21. Stuttard, C. 1972. Location of *trpR* mutations in the *serB-thr* region of *Salmonella typhimurium*. J. Bacteriol. **111**:368-374.
22. Taylor, A. L., and C. D Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.
23. Veron, M., F. Falcoz-Kelly, and G. N. Cohen. 1972. The threonine-sensitive homoserine dehydrogenase and aspartokinase activities of *Escherichia coli* K12. Eur. J. Biochem. **28**:520-527.
24. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
25. Wormser, E. H., and A. B. Pardee. 1958. Regulation of threonine biosynthesis in *Escherichia coli*. Arch. Biochem. Biophys. **78**:416-432.