

Location of *trpR* Mutations in the *serB-thr* Region of *Salmonella typhimurium*¹

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Tryptophan biosynthesis in *Salmonella* is controlled by at least one regulatory gene, *trpR*, which is cotransducible with *thr* genes and not with the *trp* operon. Mutations in *trpR* cause derepression of tryptophan enzyme synthesis and confer resistance to growth inhibition by 5-methyltryptophan. Nineteen *trpR* mutations were mapped with respect to *thrA* and *serB* markers by two-point (ratio) and three-point transduction tests. The results are all consistent with the site order *serB80-trpR-thrA59* on the *Salmonella* chromosome. Very low or undetectable levels of recombination between different *trpR* mutations have so far prevented the determination of fine structure in the *trpR* gene. Thirteen other 5-methyltryptophan-resistant mutants previously found not to be cotransducible with either the *trp* operon or *thrA*, and designated *trpT*, were also used in these experiments. Lack of cotransducibility with *thrA* was confirmed, and no linkage with *serB* was detected. The nature and location of *trpT* mutations remain obscure.

In both *Salmonella typhimurium* and *Escherichia coli* the biosynthesis of tryptophan is regulated through a tryptophan (*trp*) operon. The systems in both organisms seem to be identical.

On the genetic map of *S. typhimurium* the *trp* operon is located at 52.5 min (10) and includes at one end (nearest the *supX* and *cysB* genes) promoter and operator regulatory elements (1). These mediate control in *cis* over five contiguous structural genes specifying the five enzymes uniquely required for tryptophan biosynthesis.

In the presence of excess tryptophan (20 µg/ml) production of tryptophan biosynthetic enzymes is repressed. Similar repression is caused by tryptophan analogues including 5-methyltryptophan (5MT) which also inhibits cell growth. Regulatory mutations reduce or abolish the capacity for repression by tryptophan and its analogues and confer resistance to growth inhibition by the analogues; thus regulatory mutations permit varying levels of constitutive tryptophan enzyme synthesis. In prototrophs and non-*trp* auxotrophs this may lead to excess biosynthesis of tryptophan which is then excreted.

The first *trp* regulatory mutation conferring resistance to 5MT was isolated in *E. coli* by Cohen and Jacob (4). In transductions it was linked to a threonine (*thr*) gene, and unlinked to the other *trp* genes. The active (wild-type, Wt) form of this regulatory gene, now designated *trpR*⁺, was shown to be *trans* dominant over mutant forms (*trpR*). It is believed to specify an aporepressor protein (8) for which tryptophan and 5MT, or derivatives of these, function as corepressors.

Balbinder and co-workers (1) isolated four classes of *trp* regulatory mutations in *S. typhimurium* on the basis of resistance to 5MT, two of which are unlinked to the *trp* operon. The unlinked mutations included some which were cotransducible with the *thrA* gene, and were designated *trpR* by analogy with *trpR* mutations in *E. coli* K-12, and others for which no linkage relationships were determined by transduction. The latter were designated *trpT* and have no apparent equivalent in *E. coli*; their status remains uncertain at present.

For both *E. coli* K-12 and *S. typhimurium* the location of *trpR* by transduction was given previously as "closely linked to *thr*." However, the precise position with respect to the cotransducible *serB* and *thr* genes (5) was not established.

In transduction experiments with *S. typhimurium* I have located 19 *trpR* mutations be-

¹ Preliminary results of this work were reported to a joint meeting of the Genetical Society and the virus group of the Society for General Microbiology, Cambridge, England, March-April 1971.

tween *serB80* and *thrA59*. In addition, the non-cotransducibility of 13 putative *trpT* mutations with *serB* or *thrA59* (McCann, *personal communication*) was confirmed. During the progress of this work the location of *trpR* between *serB* and *thr* in *E. coli* was reported, but no data were given (12).

MATERIALS AND METHODS

The designations and some characteristics of the *trp* regulatory mutants of *S. typhimurium* LT-2 used are given in Table 1; all except MTR were isolated

TABLE 1. Bacterial strains used, characterized at 37 C

Strain	<i>trp</i> Genotype	Phenotype	Re-sponse to 5MT ^a	Syn-trophy with <i>trpE95</i>
SO143	<i>R520</i>	Cys ⁻	FR	+
SO144	<i>R531</i>	Cys ⁻	FR	+
SO190	<i>R532</i>	Prototroph	FR	++
SO167	<i>R533, A47</i>	Ant ⁻	FR	0
SO294	<i>R576</i>	SO151 ^b	FR	±
SO300	<i>R582</i>	SO151	FR	±
SO311	<i>R593</i>	SO151	FR	+
SO313 ^c	<i>R595</i>	Cys ⁻	FR	+
SO328	<i>R610</i>	SO151	FR	+
SO336 ^c	<i>R618</i>	Cys ⁻ Met ⁻	FR	++
SO594	<i>R1280</i>	Cys ⁻	FR	±
SO595	<i>R1281</i>	Cys ⁻	FR	±
SO599	<i>R1285</i>	Cys ⁻	FR	±
SO606	<i>R1292</i>	Cys ⁻	FR	±
SO617	<i>R1303</i>	Cys ⁻	FR	+
SO618	<i>R1304</i>	Cys ⁻	FR	±
SO623	<i>R1309</i>	Cys ⁻	FR	±
SO396	<i>R1329</i>	Thr ⁻	FR	0
MTR	<i>R1352</i>	Cys ⁻	FR	++
SO139	<i>T542</i>	Prototroph	SR	0
SO297	<i>T579</i>	SO151	SR	0
SO298	<i>T580</i>	SO151	SR	0
SO301	<i>T583</i>	SO151	SR	0
SO303 ^c	<i>T585</i>	Cys ⁻	SR	0
SO304	<i>T586</i>	SO151	SR	0
SO305 ^c	<i>T587</i>	Cys ⁻ Met ⁻ Pro ⁻	SR	0
SO307	<i>T589</i>	SO151	SR	0
SO309	<i>T591</i>	SO151	SR	0
SO312	<i>T594</i>	SO151	SR	0
SO322	<i>T604</i>	SO151	SR	0
SO337	<i>T619</i>	SO151	SR	0
SO591	<i>T1277</i>	Cys ⁻	SR	0

^a FR = Fast growing (single colonies within 24 hr), resistant. SR = Slow growing (single colonies within 48 hr), resistant.

^b Phenotype of parental strain SO151 is: Cys⁻ Met⁻ Pro⁻ Ade⁻ Ura⁻. For explanation of phenotypic symbols see Sanderson (10); Ant⁻ = anthranilate-requiring.

^c Strains have lost one or more of the parental (SO151) markers, presumably by reversion during subculturing.

and generously provided by E. Balbinder and colleagues at Syracuse, N.Y. Studies with some of these have been published (1) and all are included in the dissertation of Peter P. McCann (Syracuse Univ., 1970). Strain MTR was isolated by R. Bauerle and obtained from P. P. McCann. The double mutant strain SU47 (*serB80, thrA59*) was kindly provided by K. E. Sanderson from the collection of the late M. Demerec; *trpE95* derives from the same collection and was provided by E. Balbinder. The poorly lysogenizing mutant, L7, of phage P22 (11) was used in all transductions, and the H5 virulent mutant of P22 was used to test the phage sensitivity of bacterial strains.

Media. Nutrient broth (1% Difco) was used as a routine complex liquid medium and was added at a final concentration of 0.01% to the defined minimal medium (MM) of Vogel and Bonner (13) solidified with 1.5% Difco agar to make an enriched minimal medium (EM); supplements were added to MM as indicated: MThr = MM plus 20 µg of L-threonine/ml; MSer = MM plus 40 µg of L-serine/ml; 5MT (Schwartz/Mann, Orangeburg, N.Y.) was always added at a final concentration of 100 µg/ml in solid media; Difco nutrient agar (23 g plus 5 g of NaCl per liter) (NA) was used as a routine complex solid medium; 0.2% glucose was included in all solid media except NA.

Methods. The methods of preparation and assay of phage lysates and the method of transduction have been described by Clowes (3) and Blume and Balbinder (2). When used in crosses, analogue resistance markers were always unselected. Auxotrophic and some prototrophic transductants were characterized on appropriate media after single colony isolation on NA.

Presumptive tryptophan excretion by *trpR* strains and recombinants was detected by syntrophy with *trpE95*; about 10⁸ cells of *trpE95* grown in broth were incorporated into MM and strains to be tested were stabbed into this medium. Halos of *trpE95* growth around stabs indicated cross-feeding after 24 hr of incubation, usually at 37 C. Control strains lacking a *trpR* mutation and *trpT* mutants did not cross feed *trpE95*, whereas lysogenic cells bearing *trpR* or *trpR*⁺ produced halos of lysis with or without feeding, respectively. This test was very efficient for scoring transductants and for identifying nonlysogenic clones when required.

RESULTS

Linkage of *trpR* mutations to *serB80* and *thrA59*. As a control, and for verification of linkage between *serB80* and *thrA59*, strain SU47 (*serB80, thrA59*) was used as the recipient in transductions with donor P22 (L7) phage grown on Wt *Salmonella* LT-2. The results show that the cotransduction to prototrophy of both markers occurred with a frequency of about 20% when *serB80* was non-selective (Table 2). This is in agreement with previous results (5, 9). However, when *thrA59* was nonselective this frequency was approxi-

TABLE 2. Linkage of *serB* to *thrA*
Transduction: SU47 (*serB80 thrA59*) × wild type

Donor		+		+	
Recipient		1	2	3	
		<i>serB80</i>		<i>thrA59</i>	
		17%		35%	
Selective medium ^a	Transductants				Linkage (% cross-over in regions 1-3)
	No. per plate ^b	No. tested	Phenotypes ^c		
			P	A	
MThr	53	209	74	135	35
MSer	56	168	29	139	17
EMSer	76	64	64	0	22
	large				
	275	64	0	64	
	small				

^a See Materials and Methods.
^b Approximately 1.7×10^7 infected bacteria per plate.
^c P = Prototrophs requiring crossovers in regions 1-3. A = Unselected auxotrophs (crossovers in regions 1-2 or 2-3).

mately doubled. This may reflect a differential survival of Ser⁻ recombinants compared with Thr⁻ against prototrophs on the selective media; or more probably, an inequality in the distances of the markers from respective ends of P22 transducing fragments (with region 1 larger than region 3 in Table 2). It should be noted that on broth-enriched media (EMSer) large colonies were all prototrophs, small colonies were auxotrophs, and in subsequent experiments the total proportion of small colonies per plate was counted as the proportion of unselected auxotrophs within which the proportions of *trpR* and *trpR*⁺ recombinants were scored. Slight leakiness of the *serB80* mutation allowed excessive growth of SU47 on EMThr. Consequently selection of Thr^{+/−} transductants was made on media (MThr) without broth enrichment.

Similar experiments constituting three-point test transductions with all 19 *trpR* donors are described in Tables 3 and 4. Included in Table 3 are the possible relative orders of *serB80*, *thrA59*, and *trpR* mutations and the regions where crossovers must occur to give each transductant phenotype for each order of mutation sites. In crosses with all donors (Table 4) except *trpR520* (which was scored on MSer) two distinct classes of recombinant colonies (25–500/plate) were counted on EMSer plates (*thrA59* being the contraselective marker). Large colonies comprised 12 to 40% (20–30% in 12 crosses) and, with few exceptions in only

one cross, all large colonies tested (15–112 per cross) were prototrophs. The remaining colonies were small, and, again with one exception, all small colonies that were tested (32–96 per cross) were Ser⁻ auxotrophs. Within each recombinant class (prototrophs and auxotrophs) the sample of colonies that was tested to confirm the nutritional phenotype was also tested to ascertain the distribution of *trpR* and *trpR*⁺ genotypes. The proportions obtained were then extrapolated to the proportions of Ser⁺ and Ser⁻ transductants scored in each cross to give the final figures recorded in Table 4. A similar procedure was used for 11 crosses on MThr, and totals of 66 to 132 transductants were tested from each cross. In the remaining eight crosses on MThr all colonies (58–240) from one or more plates of each cross were tested. The proportions of different transductants, expressed as percentages of the totals tested, are recorded directly in Table 4, and from these the site orders can be deduced. No other significance is accorded to the results of these crosses. The relatively high proportions of auxotrophic *trpR* recombinants obtained with 18 *trpR* donors are unlikely to have arisen by quadruple crossovers required by site orders (a) and (c) (Table 3) but could arise by double crossovers with order (b). Even more significantly, the frequencies of prototrophic *trpR*⁺ recombinants (mostly less than 1%) obtained in each experiment (with the exception of *trpR576* discussed below) were lower than those of any other transductant phenotype and are thus more likely to result from quadruple crossovers required by order (b) than from double crossovers allowed by orders (a) and (c). It is concluded that the most likely order is (b): *serB80-trpR-thrA59*.

Two-point crosses. Phage-sensitive double mutant transductants with *trpR* mutations linked separately to *serB80* and to *thrA59* were isolated from the above crosses and these were used in two-point ratio tests to verify the linkage between the *trpR* markers and *serB80* or *thrA59*. The resulting distribution of *trpR* and *trpR*⁺ markers among prototrophic recombinants from crosses on EM with a Wt donor are given in Table 5. There is reasonably good agreement with results of other two-point tests in which *thrA59* or *serB80* was the recipient on EM for 5MT-resistant donors (Table 6). Included in Table 6 are results obtained with all 13 *trpT* donors, none of which gave any 5MT-resistant transductants in either cross when at least 45 colonies were scored per cross. Comparison of Tables 4, 5, and 6 reveals varying ranges of cotransduction frequencies observed for each *trpR* marker with *thrA59* or

TABLE 3. Possible relative site orders and transductant phenotypes in crosses between trpR donors and SU47 (serB80 thrA59) recipient

Diagram of cross for each site order											
(a) <i>trpR</i> + +				(b) + <i>trpR</i> +				(c) + + <i>trpR</i>			
1	2	3	4	1	2	3	4	1	2	3	4
+		<i>B80</i>	<i>A59</i>	<i>B80</i>		+	<i>A59</i>	<i>B80</i>		<i>A59</i>	+
Possible transductant phenotypes				Crossovers required to give each transductant phenotype with each site order:							
				(a)		(b)		(c)			
1. Prototroph 5MTS ^a (<i>trpR</i> ⁺)				2-4		1-2-3-4		1-3			
2. Prototroph 5MTR ^b (<i>trpR</i>)				1-4		1-4		1-4			
3. Auxotroph 5MTS											
(a) Thr ⁻				2-3		1-2		1-2			
(b) Ser ⁻				3-4		3-4		2-3			
4. Auxotroph 5MTR											
(a) Thr ⁻				1-3		1-3		1-2-3-4			
(b) Ser ⁻				1-2-3-4		2-4		2-4			

^a 5MTS = Sensitive to 5-methyltryptophan and do not feed trpE95.
^b 5MTR = Resistant to 5-methyltryptophan and feed trpE95.

serB80 markers; some (trpR531, 533) quite narrow, and others (trpR576) quite wide. The latter may be due to the temperature dependence of SO294 (trpR576) syntrophy with trpE95: at 37 C syntrophy was barely detectable, but was enhanced at 23 C. However, much of the variation is probably sampling error. No attempt is made to order the mutation sites within trpR on the present data.

Crosses between different trpR mutations. Double mutant strains bearing trpR mutations isolated from the crosses with SU47 were also used in reciprocal combinations with trpR donors to measure recombination (if any) between different trpR mutations to give prototrophic nonfeeding (trpR⁺) transductants. As yet, out of 50 combinations tested only 3 combinations of mutations—serB80 trpR520 and serB80 trpR1285 × trpR1352, and serB80 trpR1352 × trpR520—where 400, 107, and 306 transductants, respectively, were tested, have yielded any trpR⁺ colonies. Even with these no more than three such colonies were found in each cross so that no determination of relative orders could be made. This analysis is continuing.

DISCUSSION

Relative frequencies of recombination of 18 trpR mutations tested in three-point crosses with SU47 are all in agreement with the order serB80-trpR-thrA59. Results of this test with trpR576 were ambiguous. The temperature ef-

fect noted above might account for difficulties of scoring trpR576 serB80 recombinants by trpE95 syntrophy even in repeat experiments at 23 C. However, the results of two-point tests, with selection for prototrophic recombinants only, are more compatible with a location for trpR576 between serB80 and thrA59. Because each trp regulatory mutation was independently isolated (1), the absence of recombination between most of the trpR mutations when frequencies of 1 to 2% should have been detectable suggests either that all are very close together in a very small section of the serB-thr region, or that some are partially overlapping deletions of slightly varying length lying between serB80 and thrA59. Efforts to obtain a fine structure map of the trpR gene are continuing.

Of 13 putative trpT mutations examined so far, none appears to be cotransducible with serB80 or thrA59 when frequencies of 3% or lower would be detected. This seems to confirm a previous conclusion that they represent a novel class of trp regulatory mutations (1), although experiments to verify non-cotransducibility with trpA, argG, or cysG loci (locations of other trp regulatory mutations) in S. typhimurium (1; McCann, personal communication) or E. coli (7) are still in progress. The location and function of trpT thus remain obscure.

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TABLE 4. Three-point test transductions with recipient SU47 (*serB80 thrA59*) to establish the order *serB80-trpR-thrA59*

Donor <i>trpR</i> marker	Contraselective marker	Transductants (% of total scored)				Cotransduction frequencies [to nearest % (a) + (b)]	
		Prototrophs		Auxotrophs			
		<i>trpR</i> ⁺	<i>trpR</i> (a)	<i>trpR</i> ⁺	<i>trpR</i> (b)	<i>trpR</i> - <i>thrA59</i> ⁺	<i>serB80</i> ⁺ - <i>trpR</i>
520	<i>thrA</i>	<1	19	51	30	49	
	<i>serB</i>	<1	40	12	47		87
531	<i>thrA</i>	<1	20	57	22	42	
	<i>serB</i>	<1	39	28	33		72
532	<i>thrA</i>	<1	14	56	29	43	
	<i>serB</i>	<2> 1	39	22	39		77
533	<i>thrA</i>	<1	25	46	29	54	
	<i>serB</i>	<1	37	23	39		76
576	<i>thrA</i>	11	29	55	5	34	
	<i>serB</i>	12	24	26	38		62
582	<i>thrA</i>	<1	38	38	24	62	
	<i>serB</i>	<1	68	11	21		89
593	<i>thrA</i>	3	21	65	11	32	
	<i>serB</i>	1	32	34	33		65
595	<i>thrA</i>	<2	34	35	30	64	
	<i>serB</i>	<1	46	20	33		79
610	<i>thrA</i>	3	20	55	22	42	
	<i>serB</i>	8	22	22	48		70
618	<i>thrA</i>	<1	18	65	17	35	
	<i>serB</i>	<1	18	35	47		65
1280	<i>thrA</i>	<2	24	56	19	43	
	<i>serB</i>	<1	45	26	29		74
1281	<i>thrA</i>	<1	11	72	16	27	
	<i>serB</i>	3	26	22	49		75
1285	<i>thrA</i>	3	23	58	16	39	
	<i>serB</i>	3	38	25	34		72
1292	<i>thrA</i>	<1	18	61	20	38	
	<i>serB</i>	3	25	32	40		65
1303	<i>thrA</i>	<1	23	60	16	39	
	<i>serB</i>	<2	43	17	39		82
1304	<i>thrA</i>	1	18	65	16	34	
	<i>serB</i>	4	36	24	36		72
1309	<i>thrA</i>	<1	23	51	25	48	
	<i>serB</i>	4	29	13	54		83
1329	<i>thrA</i>	<1	23	61	15	38	
	<i>serB</i>	<1	39	17	43		82
1352	<i>thrA</i>	<2	25	53	21	46	
	<i>serB</i>	<1	35	16	48		83

TABLE 5. Linkage of trpR to thrA59 and serB80: ratio tests with wild-type donor^a

Recipient genotype	Origin of recipient	Transductants		Linkage (nearest %)
		No. tested	No. trpR ⁺	
	Transduction on MThr			
trpR520 thrA59	SU47 × SO143	360	174	48
trpR531 thrA59	SU47 × SO144	232	104	45
trpR532 thrA59	SU47 × SO190	63	40	63
trpR533 thrA59	SU47 × SO167	300	154	51
trpR576 thrA59	SU47 × SO294	54	39	72
trpR582 thrA59	SU47 × trpR582	60	28	47
trpR593 thrA59	SU47 × SO311	48	29	60
trpR595 thrA59	SU47 × SO313	70	24	34
trpR610 thrA59	SU47 × SO328	70	45	64
trpR618 thrA59	SU47 × SO336	70	40	57
trpR1280 thrA59	SU47 × SO594	70	37	53
trpR1281 thrA59	SU47 × SO595	114	43	38
trpR1285 thrA59	SU47 × SO599	54	31	57
trpR1292 thrA59	SU47 × SO606	60	27	45
trpR1303 thrA59	SU47 × SO617	70	33	47
trpR1304 thrA59	SU47 × SO618	69	33	48
trpR1309 thrA59	SU47 × SO623	70	38	54
trpR1329 thrA59	SU47 × trpR1329	124	41	33
trpR1352 thrA59	SU47 × MTR	240	96	40
	Transduction on EMSer			
serB80 trpR520	SU47 × SO143	260	194	75
serB80 trpR531	SU47 × SO144	150	111	74
serB80 trpR532	SU47 × SO190	70	54	77
serB80 trpR533	SU47 × SO167	240	198	82
serB80 trpR576	SU47 × SO294	52	41	79
serB80 trpR582	SU47 × trpR582	60	39	65
serB80 trpR593	SU47 × SO311	79	49	62
serB80 trpR595	SU47 × SO313	70	46	66
serB80 trpR610	SU47 × SO328	60	45	75
serB80 trpR618	SU47 × SO336	35	25	71
serB80 trpR1280	SU47 × SO594	35	29	83
serB80 trpR1281	SU47 × SO595	118	85	72
serB80 trpR1285	SU47 × SO599	103	81	79
serB80 trpR1292	SU47 × SO606	70	60	86
serB80 trpR1303	SU47 × SO617	70	63	90
serB80 trpR1304	SU47 × SO618	70	56	80
serB80 trpR1309	SU47 × SO623	70	47	67
serB80 trpR1329	SU47 × trpR1329	69	52	75
serB80 trpR1352	SU47 × MTR	240	189	79

^a All crosses were plated on EM to select prototrophic recombinants. These were then sampled to determine the assortment of trpR alleles.

TABLE 6. Transductions of *trpR* donors with *thrA59* and *serB80* recipients; selecting prototrophs on EM

Donor (<i>trpR</i>)	<i>thrA59</i> as recipient		<i>serB80</i> as recipient	
	No. tested	Linkage ^a	No. tested	Linkage ^a
Wt (R ⁺)	ND ^b		102	0
520	ND		53	79
532	ND		54	61
576	ND		80	50
582	45	31	105	42
593	ND		79	63
595	ND		28	82
610	45	36	30	67
618	45	42	64	61
1280	43	53	48	83
1281	43	44	45	82
1285	41	34	40	68
1292	44	57	45	84
1303	45	40	45	82
1304	40	37	45	78
1309	36	50	44	66
<i>trpT</i> ^c	45-60 ^d	0	45-60 ^d	0

^a *trpR* transductants as nearest percent of total.
^b Not done.
^c Thirteen strains tested; none showed linkage with *thrA59* or *serB80*.
^d Per cross.

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