SALMONELLA TYPHIMURIUM-ESCHERICHIA COLI HYBRIDS FOR THE TRYPTOPHAN REGION

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ABSTRACT

Fifty-eight hybrids were analyzed for their phenotypic stability, presence and nature of cryptic *trp* alleles and by P22-mediated transduction to yield percent homologies. The hybrids fall into 5 distinguishable classes: a haploid class in which selected *E. coli* genes replace equivalent sites in the *S. typhimurium* chromosome; three merodiploid classes in which the selected *E. coli* genes are integrated at novel sites in the *S. typhimurium* chromosome—on the same transducing fragment as the female genes selected against, with or without cryptic damage to a nearby gene, or not on the same transducing fragment; and one class in which recombination has not taken place and the *E. coli* DNA is presumed to be an exogenote. The homology values are heterogeneous and do not permit an accurate determination of the relative frequency of incorporation of the integrated male genetic material. A further study of 20 hybrids indicates that genetic rearrangements can occur in the hybrids.

AMONG 58 Trp⁺ *Salmonella typhimurium*-*Escherichia coli* hybrids, five distinguishable classes of hybrids were found on the basis of phenotypic stability, presence (and genotype) or absence of a cryptic *trp* allele and homology (see review by MIDDLETON and MOJICA-A 1971): (a) Haploid hybrids in which the selected *E. coli* DNA replaces the equivalent *S. typhimurium* sites. (b) More frequent were merodiploid hybrids (RAVIN and TAKAHASHI 1970) in which the *E. coli* DNA is integrated at novel sites and is added to the *S. typhimurium* genome; three distinct classes of merodiploid hybrids were found. (c) Exogenote hybrids (BARON *et al.* 1968; BARON 1969), in which the *E. coli* DNA does not integrate into the chromosome, were found at about the same frequency as haploid hybrids.

MATERIALS AND METHODS

Bacterial strains: *S. typhimurium* and *E. coli* strains are described in Table 1. *S. typhimurium* nomenclature will be used throughout for the *trp* loci (BLUME and BALBINDER 1966). Figure 1 shows the map locations of mutations and extent of deletions in the various *S. typhimurium trp* strains used as females to generate hybrids in crosses with *E. coli* Hfr B7 *trp*⁺ (SQ792) and as recipients and donors to characterize the Trp⁺ hybrids by transduction. Strain SQ351 (*cysB403 pyrF134*) was used as recipient to determine the presence of a *trp* allele in the hybrids. SQ421 (wild type) and SQ413 (TA807, *hisF41*) were used to normalize homology data. The *S. typhi-*

FIGURE 1.—A schematic map (not drawn to scale) of the tryptophan region of *S. typhimurium* for the various *trp* strains used; the positions of point mutations are indicated by X, extent of deletions by a solid line. The map is based on BLUME and BALBINDER (1966), BAUERLE and MARGOLIN (1966) and MARGOLIN and BAUERLE (1966).

murium-E. coli hybrids, produced by a recently reported protocol from females grown on supplemented minimal salts medium and preincubated at 50°C before mating (MOJICA-A and MIDDLETON 1971), are listed in Table 2. Each hybrid was selected as stable on passage through a single cell on MA and able to yield a P22 titer of at least 5.0×10^{10} p.f.u./ml.

Phage strains: The L4 mutant (SMITH and LEVINE 1967) of *Salmonella* phage P22 was used

TABLE 1
Bacterial strains

Strain	Characteristics*	Source or reference
<i>S. typhimurium</i> LT2 strains		
SQ421	wild type	R. B. MIDDLETON
525	<i>trpA</i> 512 deletion (SO501)	BALBINDER <i>et al.</i> (1968)
518	<i>trpB</i> 223 point mutation	BLUME & BALBINDER (1966)
521	<i>trpE</i> 95 deletion	BLUME & BALBINDER (1966)
711	<i>trpE</i> 141 deletion	BLUME & BALBINDER (1966)
519	<i>trpD</i> 9 point mutation	BLUME & BALBINDER (1966)
712	<i>trpD</i> 119 deletion	P. McCANN
526	<i>trpC</i> 109 deletion	BLUME & BALBINDER (1966)
713	<i>trpABE</i> 130	BLUME & BALBINDER (1966)
714	<i>trpBED</i> 763 <i>araB</i> 9 <i>gal</i> -205	BAUERLE & MARGOLIN (1966)
715	<i>trpABEDC</i> 167	BLUME & BALBINDER (1966)†
716	<i>supX</i> 34 deletion of <i>trpABED</i> <i>leu</i> -500 <i>araB</i> 9 (PM247)	MARGOLIN & BAUERLE (1966)
717	<i>trpABEDC</i> 101(<i>chr-s</i>) <i>cysB</i> 529 (PM445)	P. MARGOLIN
413	<i>hisF</i> 41 deletion (TA807)	P. E. HARTMAN
351	<i>cysB</i> 403 <i>pyrF</i> 134 (point mutation in <i>cysB</i> , deletion in <i>pyrF</i>)	R. B. MIDDLETON
<i>E. coli</i> strain		
SQ792	Hfr B7 <i>met</i> <i>O trp pyrD purE lac . . . pabB F</i>	TAYLOR & TROTTER (1967)

* Genotype and phenotype designations follow the nomenclature rules of DEMEREC *et al.* (1966).

† Figure 2 erroneously did not show the *trp*-167 deletion extending into the *trpC* gene although all data indicate that the entire tryptophan operon is deleted (E. BALBINDER, personal communication).

TABLE 2

Properties of S. typhimurium—E. coli Trp⁺ hybrids*

Hybrid	<i>S. typhimurium</i> <i>trp</i> female	<i>E. coli trp</i> ⁺ genes selected	Percent segregation of <i>trp</i> clones†			Percent cotransduction of a cryptic <i>trp</i> allele with <i>cysE</i> §
			1‡	2	3	
CLASS I						
	SQ525 (<i>trpA512</i>)	<i>trp A</i> ⁺				33
TC10-21			0.3	0	.	0
21a			.	.	0	0
	SQ526 (<i>trpC109</i>)	<i>trpC</i> ⁺				32
55			0	0	.	0
55a			.	.	0	0
79			0	0	.	0
80			0	0	.	0
81			0	0	.	0
	SQ713 (<i>trpABE130</i>)	<i>trpABE</i> ⁺				57
34			2.0	0	.	0
35			1.6	0	.	0
35a			.	.	0	0
	SQ714 (<i>trpBED763</i>)	<i>trpBED</i> ⁺				36
52			0.5	0	.	0
52a			.	.	0	0
	SQ715 (<i>trpABEDC167</i>)	<i>trpABEDC</i> ⁺				50
45			2.8	0	.	0
45a			.	.	0	0
46			0.8	0	.	0
	SQ717 (<i>trpABEDC101</i>)	<i>trpABEDC</i> ⁺				—
47			0	0	.	0
48			0	0	.	0
49			0	0	.	—
50			0	0	.	—
50a			.	.	0	—
51			0	0	.	—
51a			.	.	0	—
CLASS II						
	SQ711 (<i>trpE141</i>)	<i>trpE</i> ⁺				26
30a			.	.	0	7.5
57a			.	.	0	11
	SQ526 (<i>trpC109</i>)	<i>trpC</i> ⁺				32
32			5.6	0	.	37
53			2.5	0	.	21
56a			.	.	0	7.3
	SQ713 (<i>trpABE130</i>)	<i>trpABE</i> ⁺				37
33			3.4	0	.	44
33a			.	.	0	23
37			0	0	.	46
75			0	0	.	36
	SQ715 (<i>trpABEDC167</i>)	<i>trpABEDC</i> ⁺				50
40a			.	.	0	2.2
41			0	0	.	56
44			0	0	.	50

TABLE 2—Continued

Hybrid	<i>S. typhimurium</i> <i>trp</i> female	<i>E. coli trp</i> ⁺ genes selected	Percent segregation of <i>trp</i> clones†			Percent cotransduction of a cryptic <i>trp</i> allele with <i>cysB</i> §
			1‡	2	3	
CLASS III						
	SQ525 (<i>trpA512</i>)	<i>trpA</i> ⁺				33
20a			.	.	0	7.5
23a			.	.	0	6.6
84			1.7	0	.	53
	SQ716 (<i>supX34</i>)	<i>trpABED</i> ⁺				—
64			0	0	.	22
65			0	0	.	22
66			0	0	.	22
67			0	0	.	22
68			0	0	.	33
69			0	0	.	33
70			0	0	.	33
71			0	0	.	33
72			0	0	.	33
CLASS IV						
	SQ525 (<i>trpA512</i>)	<i>trpA</i> ⁺				33
20			0	0	.	42
22			0	0	.	45
24			0	0	.	52
	SQ518 (<i>trpB223</i>)	<i>trpB</i> ⁺				26
29a			.	.	0	6.2
	SQ711 (<i>trpE141</i>)	<i>trpE</i> ⁺				26
30			4.7	0	.	36
57			0	0	.	30
58			1.6	0	.	27
59a			.	.	0	11
60			1.3	0	.	22
78			0	0	.	46
	SQ526 (<i>trpC109</i>)	<i>trpC</i> ⁺				32
31			4.7	0	.	27
54a			.	.	0	9.9
77			0.7	0	.	23
	SQ713 (<i>trpABE130</i>)	<i>trpABE</i> ⁺				57
36			1.7	0	.	50
36a			.	.	0	18
	SQ715 (<i>trpABEDC167</i>)	<i>trpABEDC</i> ⁺				50
42			3.1	0	.	62
43			2.2	0	.	51
44a			.	.	0	11
CLASS V						
	SQ525 (<i>trpA512</i>)	<i>trpA</i> ⁺				33
23			34	9.3	.	65
25			10	5.4	.	69
26			4	2.4	.	49
27			4	3.5	.	47
28			7.8	5.3	.	52
	SQ518 (<i>trpB223</i>)	<i>trpB</i> ⁺				26

TABLE 2—Continued

Hybrid	<i>S. typhimurium</i> <i>trp</i> female	<i>E. coli</i> <i>trp</i> ⁺ genes selected	Percent segregation of <i>trp</i> clones†			Percent cotransduction of a cryptic <i>trp</i> allele with <i>cysB</i> §
			1‡	2	3	
29			2.1	1.1		18
	SQ711 (<i>trpE141</i>)	<i>trpE</i> ⁺				26
59			0.6	0.6		34
	SQ526 (<i>trpC109</i>)	<i>trpC</i> ⁺				32
54			7.0	5.7		14
56			3.5	4.8		31
76			1.6	1.5		22
	SQ715 (<i>trpABEDC167</i>)	<i>trpABEDC</i> ⁺				50
39			21	6.7		59
40			1.9	0.9		52
UNCLASSIFIED						
	SQ713 (<i>trpABE130</i>)	<i>trpABE</i> ⁺				57
38			1.4	0.9		0
38a					1.0	0

* All hybrids resemble their female parent strains for the following characteristics: *lac*, *ara*⁺, positive reactions with *S. typhimurium* antisera and negative reaction with *E. coli* antiserum.

† The tryptophan phenotype was determined for at least 10³ colonies of each hybrid. When no *trp* segregants were found, at least 10⁴ colonies were tested. Each percent value is based on the average of 4 experiments.

‡ Column 1: 58 newly isolated hybrids after one passage through single cells on MA. Column 2: 58 hybrids after two passages through single cells; P22 preparations used for cotransduction crosses and for homology tests (Table 3) were grown on hybrids after the Column 2 test. Column 3: 20 hybrids, randomly chosen for further study and designated by suffix "a", were passed through single cells five times on MA before P22 was prepared for cotransduction crosses and for homology tests (Table 3).

§ *cysB*⁺ recombinants were selected on DEMA + TRP + URA from hybrid donors and *cysB403 pyrF134* as recipient. "0" means that no *trp* recombinants were found among at least 10³ *cysB*⁺ recombinants tested; "—" indicates that no *cysB*⁺ recombinants were produced (the donors were *cysB*).

as a vector in transduction analysis and is referred to as P22. The virulent H5 mutant of P22 was used to test the bacterial strains for sensitivity of P22.

Media: All media have been described (MOJICA-A and MIDDLETON 1971). The following abbreviations are used: ANT, anthranilic acid; DEMA, double enriched minimal agar; DNA, deoxyribonucleic acid; IND, indole; MA, minimal agar; m.o.i., multiplicity of infection; p.f.u., plaque forming units; TRP, L-tryptophan; URA, uracil.

General techniques: Phage was grown on donors (EISENSTARK 1967) for at least two growth cycles and stored over chloroform at 4°C. Sensitivity to phage was tested by cross-streaking on nutrient agar. Reactions to specific antisera were performed as described by ST. PIERRE and DEMEREC (1968). Ability to utilize various sugars as carbon and energy sources was tested by plating on MA that contained as the only carbon source the sugar to be tested. All incubations were at 37°C. A test for excretion of tryptophan or of its biosynthetic intermediates by the hybrids was performed in the same way as the cross-feeding test described by FISHER (1968) with a series of known *S. typhimurium trp* strains: *trpA512* (SQ525) for ANT, *trpC109* (SQ526) for IND, *trpABEDC167* (SQ715) for TRP. Resistance of the hybrids to the TRP analogue 5-methyl tryptophan (purchased from Sigma Chemicals) was tested as described by CORDARO and BALBINDER (1971).

Stability: The stability of the hybrids was tested in two ways that yield essentially identical results: 100–200 cells from an overnight, nutrient broth culture were plated on (a) MA and incubated for 36 hr, colonies noted, sterile TRP (final concentration 20 µg/ml) added under the

agar, and the additional colonies noted after another 36 hr were tested for Trp⁻ phenotype; (b) MA + TRP, after 36 hr incubation colonies were replicated onto MA and MA + TRP on which colonies were counted after further 36 hr incubation. A hybrid that does not show at least one *trp* clone in 10³ tested is considered to be stable.

Transduction: Transduction experiments were performed (a) on plates of selective media (BLUME and BALBINDER 1966) with the recipient strain grown to saturation and with m.o.i. of 5; or (b) spot transductions using an Accu-Drop apparatus, (Accu-Tech Corp., New York, N.Y.) with each phage preparation diluted to contain 1.0×10^{10} p.f.u./ml. (MIDDLETON 1971).

Cotransduction: To test for the presence of a cryptic *trp* gene in hybrids, SQ351 (*cysB403 pyrF134*) was used as a recipient in transduction with hybrids, parent strains and SQ421 as donors. SQ351 carries a stable *cysB* mutation and a small deletion in the *pyrF* gene (INO and DEMEREC 1968) and requires URA and cysteine. CysB⁺ recombinants selected on MA + URA + TRP after 48 hr incubation were replica-plated on MA + URA, MA + URA + ANT and MA + URA + IND. For donors which yielded CysB⁺ Trp⁻ recombinants, a minimum of 6 such recombinants were chosen for further genetic analysis as recipients in transduction crosses (by Accu-Drop) with *trp* strains as donors. In addition reversion patterns of CysB⁺ Trp⁻ cotransductants to Trp⁺ were observed on DEMA + URA.

Percent homology: Percent homology refers to the frequency of recombinations between *S. typhimurium* and *E. coli* genetic material as compared with the frequency of recombination for *S. typhimurium*-*S. typhimurium* crosses. Phage is prepared on *S. typhimurium*-*E. coli* hybrids which are donors with various *S. typhimurium trp* recipients. Colony counts are normalized for both donor and recipient efficiencies which allow comparison of recombination frequencies. To normalize for donor efficiency, a recipient marker, *hisF41* (SQ413) outside the region selected for hybrid formation, was used for two reasons: *his*⁺ is injected very late by the Hfr used to generate the hybrids and the intergeneric matings were interrupted before the *his*⁺ region was injected, and recombination between the histidine, regions of *S. typhimurium* and *E. coli* is very rare or does not occur at all (ST. PIERRE and DEMEREC 1968; GARRICK-SILVERSMITH and HARTMAN 1970). All the hybrids should, therefore, carry the histidine genes of the *S. typhimurium* parent. To normalize for recipient efficiency, P22 prepared on SQ421 (wild type) was included as donor in each series of transduction crosses and the homology values for SQ421 set at 100%. Each value reported is the average of 4 experiments with 4 plates per experiment. The normalizing calculations were performed as previously described (ST. PIERRE and DEMEREC 1968; MIDDLETON and MOJICA-A 1971).

RESULTS

General properties of the hybrids: The hybrids resemble their female parent (*S. typhimurium*) strains for the following characteristics: *lac*, *ara*⁺, give positive reactions with *S. typhimurium* antisera and negative reaction with *E. coli* antiserum (MOJICA-A and MIDDLETON 1971). Their growth rates, both in minimal salts medium and nutrient broth, are indistinguishable from *S. typhimurium* wild type (SQ421).

Cross-feeding tests show that the hybrids do not excrete TRP or its precursors into the medium. Moreover, the hybrids behave like *S. typhimurium* wild type in resistance to 5-methyl tryptophan. These observations indicate that the tryptophan regulatory mechanisms of the hybrids are normal.

S. typhimurium-*E. coli* hybrids generally have a decreased ability to support phage growth. There may be some correlation between stability of the hybrid and the decreased ability to support phage growth; all the hybrids that yielded titers below 5.0×10^{10} p.f.u./ml were also very unstable (ability to segregate *trp* clones with a frequency of up to 50%) and were discarded. The titer of a P22 preparation on a hybrid on which it had been propagated was not significantly

different from that on wild type, regardless of whether the phage was titered on nutrient agar or MA, which indicates that no stable modification peculiar to the hybrid was introduced into the phage.

Classification of Trp⁺ hybrids: Hybrids were analyzed for (i) stability (unstable hybrids segregate *trp* clones at a frequency of at least 0.1%), (ii) presence or absence of a cryptic *trp* allele, (iii) nature of the cryptic *trp* allele, and (iv) genetic homology as defined by MIDDLETON and MOJICA-A (1971). By these criteria, 5 classes were identified among the original group of 58 hybrids as well as 20, indicated by suffix "a", which were chosen (from the 58) for further study.

Stability of the hybrids: Results of the stability tests are shown under "percent segregation" in Table 2: column 1 was carried out immediately after each hybrid was generated, column 2 after each hybrid had been passed twice through single cells on MA, and column 3 after each of 20 hybrids randomly chosen for further study and indicated by an "a" suffix had been passed five times through single cells. P22 was propagated on hybrids shown in columns 2 and 3 for cotransduction (Table 2) and homology (Table 3) tests. Twenty-seven of the 58 hybrids were found to be stable on the first test and remained stable for at least two years. There seems to be a tendency towards stability since of the 31 hybrids initially unstable, 18 became stable after two passages through single cells on MA and remained stable. In addition most of those hybrids (12 of Class V and 1 Unclassified) that remained unstable tended to segregate fewer *trp* clones at later testings. (Not shown are further tests which followed those shown in Table 2.) Four *trp* segregants were chosen from each unstable hybrid. Their phenotypes (utilization of ANT and IND and reversion to Trp⁺) are identical to their respective *S. typhimurium* parents.

Cryptic trp allele in Trp⁺ hybrids: To determine if the *E. coli* genetic material has integrated at the selected site, hybrids were crossed as donors with strain SQ351; the *cysB403* marker is cotransducible with the tryptophan genes. Table 2 shows the results of the cotransduction tests.

Forty-two of the 58 hybrids do carry a cryptic *trp* allele cotransducible with *cysB*. TC10-49, -50 and -51 as well as the corresponding parent strain (SQ717) did not produce CysB⁺ recombinants since SQ717 is *cysB*. Chi-square tests indicate that except for TC10-23, -25, -84, the frequencies of cotransduction do not differ significantly (*P* < 5%) from those of the parent strains. Although all the "a" hybrids with a cryptic *trp* allele in the earlier test still carry it, the frequency of cotransduction of the *trp* allele with *cysB* is markedly decreased. Although the presence of a cryptic *trp* allele does not rule out the possibility of recombination having taken place, its absence almost certainly shows that recombination has occurred at the selected sites, to yield a tryptophan region indistinguishable from wild type (15 hybrids of Class I).

Nature of the cryptic trp alleles: The CysB⁺Trp⁻ recombinants were genetically analyzed in order to understand the nature of the cryptic *trp* allele. This analysis consisted of 1) Spontaneous reversion to prototrophy on DEMA+URA, 2) Growth response to ANT, IND and TRP; and 3) Recombination patterns with CysB⁺Trp⁻ cotransductants as recipients and a series of *trp* donors (Figure 1). Like the original *trp* female parents none of the CysB⁺Trp⁻ recombinants re-

TABLE 3

Transduction analysis* of S. typhimurium—E. coli Trp⁺ hybrids

Donors		<i>S. typhimurium trp</i> recipients									
Hybrids	<i>S. typhimurium trp</i> female	SQ525 (<i>trpA512</i>)	518 (<i>B223</i>)	521 (<i>E95</i>)	711 (<i>E141</i>)	519 (<i>D9</i>)	712 (<i>D119</i>)	526 (<i>C109</i>)	713 (<i>ABE</i> 130)	714 (<i>BED</i> 763)	715 (<i>ABEDC</i> 167)
CLASS I											
	SQ525	0	44	81	95	66	90	32	0	89	0
TC10-21		0	0.3	0	0	0	0	0	0	0	0.2
21a		6	65	73	106	—	78	0	0	0	0
	SQ526	48	30	34	38	20	74	0	160	35	0
55		0.2	0.4	0.1	0	0	0	0.2	0	0	0.1
55a		8	10	4	0	—	5	0	4	0	5
79		61	38	21	—	9	—	0	—	—	0.2
80		71	42	23	—	11	—	0	—	—	0.1
81		42	34	19	—	6	—	0.2	—	—	0.3
	SQ713	0	0	11	7	49	11	75	0	0	0
34		0	0.1	0	0.4	0	0.2	0	0	0	0
35		0	0	0	0	0	0	0	0.2	0	0
35a		0	129	110	63	—	62	0	0	0	0
	SQ714	11	0	0	0	22	0	27	0	0	0
52		53	40	37	34	49	46	30	55	54	70
52a		54	42	45	34	—	45	24	51	52	63
	SQ715	0	0	0	0	0	0	0	0	0	0
45		0.2	7	7	5	7	8	1	0.2	7	0.1
45a		5	11	7	6	—	0	0	0	0	0
46		0	0.1	0	0.2	0	0	0	0	0	0
	SQ717 (<i>trpABEDC101</i>)	0	0	0	0	0	0	0	0	0	0
47		0	0.1	0	0.3	0.2	0	0	0	0	2
48		0	0.1	0	0	0	0	0	0	0	0
49		0	0.1	0.1	0.2	0.1	0	0	0	0	0
50		0.3	0.1	0	0	0	0	0	0	0	2
50a		0	4	0	0	—	0	0	0	0	0
51		0	0	0	0	0	0	0	0	0	2
51a		0	0	5	0	—	0	0	0	0	0
CLASS II											
	SQ711	59	35	0	0	130	20	134	26	0	0
30a		57	38	0	0	—	0	81	19	0	0
57a		83	133	139	94	—	13	39	6	0	0
	SQ526	48	30	34	38	20	74	0	160	35	0
32		69	21	16	33	11	12	0.3	27	38	0
53		52	15	16	24	4	8	0.6	22	9	0
56a		66	143	140	92	—	128	0	14	0	0
	SQ713	0	0	11	7	49	11	75	0	0	0
33		0	0.1	0.1	0	16	0.1	3	0	0	0
33a		0	0	0	0	—	10	78	0	0	0
37		0	0	0	1	17	1	6	0.1	0	0
75		0	0.1	2	6	70	—	28	0	0	0
	SQ715	0	0	0	0	0	0	0	0	0	0
40a		0	290	115	158	—	144	0	0	0	0
41		0	0.1	0	0	0	0	0	0	0	0.1
44		0	0.1	0	0	0	0	0	0.1	0	0

TABLE 3—Continued

Donors		<i>S. typhimurium</i> trp recipients									
Hybrids	<i>S. typhimurium</i> trp female	SQ525 (trpA512)	518 (B223)	521 (E95)	711 (E141)	519 (D9)	712 (D119)	526 (C109)	713 (ABE 130)	714 (BED 763)	715 (ABEDC 167)
CLASS III											
	SQ525	0	44	81	95	66	90	32	0	89	0
	20a	0	0	0	0	—	0	0	0	0	0
	23a	0	27	79	120	—	157	96	0	51	0
	84	0	0.1	11	12	13	—	9	0	0	0
	SQ716 (<i>supX34</i> , <i>trpABED</i>)	0	0	0	0	0	0	52	0	0	0
	64	27	17	22	30	0	13	4	31	9	0
	65	19	12	17	30	0.5	10	3	28	6	0
	66	20	16	18	22	0	11	3	32	10	0
	67	38	29	30	41	0	13	5	31	16	0
	68	39	36	28	35	0	17	5	52	14	0
	69	43	26	29	31	0	12	5	31	11	0
	70	31	38	25	24	0	17	7	44	12	0
	71	34	27	25	30	0	14	6	43	13	0
	72	52	33	33	41	0	18	7	44	18	0
CLASS IV											
	SQ525	0	44	81	95	66	90	32	0	89	0
	20	0	71	54	105	46	64	5	0	110	0
	22	0	51	48	75	40	34	3	0	33	0
	24	0	69	59	51	48	56	3	0	54	0
	SQ518	42	0	16	31	115	17	71	0	0	0
	29a	49	0	46	73	—	32	81	0	0	0
	SQ711	59	35	0	0	130	20	134	26	0	0
	30	22	6	0	0	33	0.2	3	1	0	0
	57	18	8	0	0	84	0.3	9	4	0	0
	58	8	5	0	0	51	0.1	3	2	0	0
	59a	32	52	0	0	—	9	43	11	0	0
	60	6	3	0	0	57	0.2	4	3	0	0
	78	21	8	0	0	11	—	19	4	0	0
	SQ526	48	30	34	38	20	74	0	160	35	0
	31	60	14	14	41	5	10	0	28	39	0
	54a	107	149	121	129	—	159	0	102	80	0
	77	21	18	11	—	9	—	0	22	8	0
	SQ713	0	0	11	7	49	11	75	0	0	0
	36	0	0	0.1	1	16	1	8	0	0	0
	36a	0	0	5	4	—	74	110	0	0	0
	SQ715	0	0	0	0	0	0	0	0	0	0
	42	0	0	0	0	0	0	0	0	0	0
	43	0	0	0	0	0	0	0	0	0	0
	44a	0	0	0	0	—	0	0	0	0	0
CLASS V											
	SQ525	0	44	81	95	66	90	32	0	89	0
	23	0	97	65	82	53	75	7	0	68	0
	25	0	61	54	72	58	58	4	0	59	0
	26	0	75	65	47	62	69	3	0	67	0
	27	0	76	73	50	46	58	3	0	61	0

TABLE 3—Continued

Donors		<i>S. typhimurium trp</i> recipients									
Hybrids	<i>S. typhimurium trp</i> female	SQ525 (<i>trpA512</i>)	518 (B223)	521 (E95)	711 (E141)	519 (D9)	712 (D119)	526 (C109)	713 (ABE 130)	714 (BED 763)	715 (ABEDC 167)
	28	0	122	111	148	69	116	4	0	125	0
	SQ518	42	0	16	31	115	17	71	0	0	0
	29	16	0	3	6	13	4	2	0	0	0
	SQ711	59	35	0	0	130	20	134	26	0	0
	59	19	8	0	0	54	0.1	13	7	0	0
	SQ526	48	30	34	38	20	74	0	160	35	0
	54	39	19	13	26	6	10	0	22	8	0
	56	51	23	17	26	5	10	0	14	9	0
	76	26	15	10	—	6	—	0	22	9	0
	SQ715	0	0	0	0	0	0	0	0	0	0
	39	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0
UNCLASSIFIED											
	SQ713	0	0	11	7	49	11	75	0	0	0
	38	0	0.4	0.1	0	0.1	0.1	0.2	0	0.1	0
	38a	0	0	0	0	—	0	0	0	0	0

* Transduction values are normalized for recipient efficiencies of the *trp* strains by setting *trp*⁺ transductions from donor SQ421 (wild type) at 100% and for donor efficiencies by setting *his*⁺ transductions at 100%. "0" values indicate that no transductants were observed among 4×10^8 recipient cells; "—" means not tested.

verted to prototrophy. The cotransductants of 32 hybrids behave identically to the parent strains in growth responses and recombination patterns. Therefore of the 42 *Trp*⁺ hybrids (of Classes II, III, IV and V) which carry a cryptic *trp* allele, 32 (of Classes II, IV and V) have an allele identical to that of their *trp* female parents.

Table 4 shows the results of the growth response and recombination tests for those 10 out of 42 hybrids that showed different behavior from parent strains. Nine of the 10 hybrids (of Class III) were generated with the same parent (SQ716); they seem to carry a variety of cryptic *trp* mutations, however. Also shown in Table 4 are the results of 2 hybrids (TC10-20a and -23a) from the 20 "a" hybrids; they have a common *S. typhimurium* parent (SQ525). TC10-23a seems to be a mixed population since about half of the CysB⁺Trp⁻ recombinants behaved like the parent strain while the other half behaved differently.

Genetic homology: A total of 58 hybrids was genetically analyzed by P22-mediated transduction crosses. The normalized percent homology values are presented in Table 3. It was expected that some hybrids might show a change in homology pattern between tests.

Table 3 reveals two main types among the original group of 58 hybrids: a) the 32 hybrids of Classes I, II and III have different homology patterns from their respective parent strains and b) the 26 hybrids of Classes IV, V and Unclassified have homology patterns like their respective parent strains. Among the 20 "a" hybrids, the homology patterns of 5 hybrids remain the same as those of the

TABLE 4
Growth response and recombination patterns of some *cysB*⁺ *trp* cotransductants

Number of <i>cysB</i> ⁺ <i>trp</i> cotransductants tested	From donor strain*	Recombination pattern with <i>trp</i> donors										Probable <i>trp</i> genotypes of <i>cysB</i> ⁺ <i>trp</i> cotransductants				
		Growth response on														
		MA ⁺	MA + ANT	MA + IND	MA + TRP		SQ525 (<i>trpA</i> 512)	518 (B223)	521 (E95)	519 (D9)	712 (D119)		526 (C109)	713 (ABE 130)	714 (BED 763)	715 (ABEDC 167)
10	SQ525	—	++	++	++	—	+	+	+	+	+	—	+	—	+	A
12	TC10-84	—	—	—	++	—	—	—	—	—	—	—	—	—	+	ABEDC
16	20a	—	—	—	++	—	—	—	—	—	—	—	—	—	+	ABEDC
15	23a-1†	—	—	—	++	—	—	—	—	—	—	—	—	—	+	ABEDC
16	23a-2‡	—	+	+	++	—	+	+	+	+	+	—	+	—	+	A
10	SQ716	—	—	—	++	—	—	—	—	—	+	—	—	—	+	ABED
7	TC10-64	—	—	++	++	—	+	+	+	+	+	—	—	—	+	E
7	65	—	—	++	++	—	+	+	+	+	+	—	—	—	+	B
7	66	—	—	—	++	—	+	+	—	+	+	+	+	—	+	D
7	69	—	—	—	++	—	+	+	—	—	+	+	—	—	+	D
7	71	—	—	—	++	—	+	+	—	+	+	+	+	—	+	D
7 (of each)	67,68, 70,72	—	—	—	++	—	+	+	—	—	—	—	—	—	+	DC

* The recipient was SQ 351 (*cysB*403 *pyrF*134). TC strains are hybrids listed in Tables 2 and 3.
† All four media were supplemented with uracil.
‡ Half of *cysB*⁺ *trp* cotransductants from TC10-23a gave the "1" pattern and half the "2" pattern.

parent strains. Five hybrids now show different homology patterns from parent strains. Eight hybrids that had shown different homology patterns from parent strains remained different, but in addition the patterns now are somewhat different from those obtained with first phage preparations. One hybrid (TC10-52) did not change and one (TC10-44) "reverted" towards the pattern shown by its parent strain.

DISCUSSION

Distinguishable classes of hybrids: One might predict that after transfer by conjugation to *S. typhimurium* female cells, the alternate fates of *E. coli* DNA which bears a selected allele might yield 5 distinguishable classes of hybrids.

Class I: Selected *E. coli* DNA becomes integrated into the female chromosome to replace exactly the equivalent genetic region of the female. The resulting hybrids would be phenotypically stable, lack a cryptic allele selected against in the female parent, and have genetic homology patterns different from the female parent for the selected region.

Class II: *E. coli* DNA becomes integrated at a novel site very near the equivalent female DNA. These hybrids would be stable, carry a cryptic allele identical to the female parent, and have different homology patterns.

Class III: *E. coli* DNA becomes integrated at a novel site which involves in the recombination event the group of female genes which contains the allele selected against. These hybrids would be stable, carry a cryptic allele different from the female parent, and have different homology patterns.

Class IV: *E. coli* DNA becomes integrated at a novel location outside the transducing fragment which bears the female allele selected against. These hybrids would be stable, carry a cryptic allele identical to the female parent, and have homology patterns, in the region selected against, identical to the female parent.

Class V: *E. coli* DNA does not become integrated into the female chromosome. The maintenance, regulation and replication in hybrids of an exogenote of *E. coli* genetic material are not understood although it may behave in an episome-like fashion. Such hybrids would be unstable (segregation of female-like clones by occasional loss of the exogenote), harbor a cryptic allele identical to the female parent, and have homology patterns identical to the female parent. In one case a chromosomal deletion was required for the maintenance of an exogenote in *E. coli* (CURTISS 1968). A deletion may not be required to maintain an *E. coli* exogenote in hybrids with *S. typhimurium*, however, since hybrid TC10-29 was selected from a point mutation. Duplicated integrations are known to be phenotypically unstable in *E. coli* (SOLL and BERG 1969; FOLK and BERG 1971; LEVINTHAL and YEH 1972). Instability in Class V hybrids is probably not due to duplicated integrations which would produce homology patterns different from the female parent. Moreover, the frequency of auxotrophic segregations in unstable hybrids is at least a hundred-fold higher than the segregation of duplicated genes in *E. coli* (FOLK and BERG 1971). The *trp* segregants of all unstable hybrids are phenotypically identical to their respective *trp* female parents. Hybrids found to be stable at any stage of analysis preserve their stability through subsequent

tests, which indicates that a Trp⁺ exogenote can be integrated into the chromosome by recombination several generations after the initial mating.

It might be argued that the presence of cryptic *trp* alleles is the result of mutations by limited homology recombination (FAN 1969) in which the presence of *E. coli* genetic material in a transducing fragment causes *trp* mutations in transduction with *cysB*. It would be expected that each hybrid would yield more than one class of CysB⁺Trp⁻ recombinants which is not the case for most hybrids. It is therefore concluded that the cryptic alleles are carried by the hybrids.

Of the original group of 58 hybrids, the tests in Tables 2 and 3 assign 15 to Class I, 7 to Class II, 10 to Class III, 13 to Class IV, and 12 to Class V. Class I hybrids are exact recombinants; hybrids of Classes II, III and IV are merodiploids with the selected male genes integrated at novel sites (RAVIN and TAKAHASHI 1970), and in Class V hybrids the male genes are present as an exogenote (BARON *et al.* 1968; BARON 1969). The inconsistent behavior of the unclassified hybrid, TC10-38 (phenotypic instability, absence of cryptic *trp* allele and different homology patterns), can best be explained as due to a duplication integration similar, perhaps, to the pi factor in *S. typhimurium* (LEVINTHAL and YEH 1972).

In general, the Trp⁺ hybrids yield fewer transductants than wild type, although the patterns of homology are not the same among hybrids of the same class generated with the same female parent (Table 3). The differences can be explained if it is postulated that hybrids in the same class and from the same cross carry different lengths of integrated *E. coli* DNA. In this respect our term "class" differs from that of INO and DEMEREC (1968). We did not attempt to determine the ends of the *E. coli* genetic material in the tryptophan transducing fragment. In addition the variable state (length, exogenote or integrated, location if integrated) of the male genetic segment which bears the selected genes makes difficult an exact estimate of the low genetic homology. Since no correlation between homology values and similarities of DNA sequences has yet been determined, we feel that homology data should be used cautiously as a quantitative indicator of evolutionary divergence (DEMEREC and NEW 1965).

Since TC10-45 is haploid (Class I) and was generated from a strain (SQ715) that carries a deletion which covers the five structural genes of the tryptophan operon, the homology for the tryptophan operon between *E. coli* and *S. typhimurium* ranges from 0.2 to 8%. These values are in good agreement with those of Class III hybrids of INO and DEMEREC (1968).

The ends of the operon appear to play an important role in recombination between the two species. The probable presence of *S. typhimurium* genetic material at the operator-distal end (TC10-64 to -72) or at the operator-proximal end (TC10-52) of the tryptophan region of hybrids appears to enhance the homology values for the other tryptophan genes.

Genetic rearrangements in hybrids: 20 Trp⁺ hybrids (indicated by suffix "a" in Tables 2 and 3) were chosen from the original 58 and passed through single cells on MA five times before P22 was propagated on the expectation that further opportunities for recombination between the male genetic material and the

female chromosome might arise. The expectations were in part borne out. Eleven of the 20 hybrids remained unchanged. Eight are assigned to a different class: seven in the expected direction to a class with a lower number, and one, TC10-44a, from Class II to IV. One hybrid, TC10-38a, remains inconsistent and unclassified.

The generally lower cotransduction frequencies in "a" hybrids might indicate a greater distance between the cryptic *trp* allele and *cysB*⁺ perhaps as a result of the insertion, by recombination, of the selected male gene between *trp* and *cysB*⁺. Alternative explanations include mixed populations of transducing fragments since P22 transducing fragments may be heterogeneous (ROTH and HARTMAN 1965; ENOMOTO 1967). TC10-23a (Table 4) may represent a case of mutation by limited homology recombination (FAN 1969) since two classes of CysB⁺Trp⁻ recombinants are recovered—the parental types and a novel type. TC10-20a (Table 4) on the other hand seems to be a recombinant in which the *S. typhimurium* tryptophan genes have been involved in recombination with the *E. coli* genes since the cryptic *trp* allele is different from its parental type and only the novel type of cotransductants is recovered.

The observations with the group of 20 "a" hybrids indicate that genetic arrangements can continue to take place in the hybrids. Work in progress will hopefully yield further information on the novel sites of integration and other properties of hybrids.

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