ENTERIC HYBRIDS. II. *S. TYPHIMURIUM*-*E. COLI* HYBRIDS FOR THE *TRP-CYSB-PYRF* REGION¹

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WHEN *Salmonella typhimurium*-*Escherichia coli* hybrids are crossed by P22-mediated transduction with *S. typhimurium* auxotrophs as recipients, few wild-type recombinants generally are observed if the transducing fragment contains *E. coli* genetic material. Other chromosome regions are incorporated with a frequency close to normal (DEMEREK and OHTA 1964; DEMEREK and NEW 1965; DEMEREK 1965). DEMEREK and OHTA (1964) obtained evidence indicating that the presence of *Salmonella* genetic material on one portion of a transducing fragment assists incorporation of *E. coli* genetic material contained on the same transducing fragment. Work reported here on hybrids for the *trp-cysB-pyrF* chromosome region supports this contention. Our results also indicate that the *E. coli* and *S. typhimurium* chromosomes have diverged in two distinct fashions of potential evolutionary significance. Pyrimidine-stimulated partial auxotrophs are recovered among hybrids of the two species if crossing over occurs in the *pyrF* locus. This divergence at the intragenic level contrasts with divergence at the chromosomal level, for example, that due to inversion of the *trp-cysB-pyrF* region between the two species.

MATERIALS AND METHODS

Procedures for obtaining hybrids were those of DEMEREK and OHTA (1964). *Salmonella typhimurium* strain LT-2 mutants *pyrF134* and *pyrF146*, containing small deletions in the *pyrF* locus (YAN and DEMEREK 1965), served as recipient females, and selection was made for *pyrF*⁺. Tryptophan hybrids were prepared using deletion mutant *trpCDEB107* as recipient (DEMEREK and NEW 1965). Used as males were *Escherichia coli* K12 Hfr strains CS-101 (HfrC), AB-312, and AB-261 (Hfr P4X6); these males carry auxotrophic markers for methionine or for threonine and leucine and are *trp*⁺, *cysB*⁺ and *pyrF*⁺. CS-101 transfers *O-purE-lac-gal*; AB-261 transfers *O-proA-thr-gal-lac*; AB-312 transfers *O-str-thr-lac-gal-serA*. The particular *E. coli* and *Salmonella* parental strains from which each hybrid was derived are listed in Table 1. These hybrids are available from DR. PHILIP E. HARTMAN, Department of Biology, The Johns Hopkins University, Baltimore, Maryland.

Transduction experiments were carried out with wild-type P22 phage. Double enriched minimal medium containing 0.02% (w/v) dehydrated Difco nutrient broth was used for plating. The minimal medium contained K₂HPO₄, 10.5 g; KH₂PO₄, 4.5 g; MgSO₄, 0.05 g; (NH₄)₂SO₄, 1.0 g; sodium citrate, 0.47 g; glucose, 5.0 g; and distilled water, 1000 ml. Several strains of

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TABLE 1

S. typhimurium mutants (see Figure 1) were used as recipients and *S. typhimurium*-E. coli hybrids as donors.

Donors		Recipients										Control <i>cysC</i> -1021	Classi- fication
		<i>trp</i> cluster							<i>cysB</i>				
		<i>C</i> -109	<i>D</i> -9	<i>E</i> -230	<i>A</i> -50	-403	-529	-117	<i>prfE</i>	-134	-146		
TC <i>trpCDEB</i>													
7	261	107	6(0.2)	4(0.1)	13(0.3)	29(0.6)	126(2.8)	72(1.8)	1822(52.7)	1616(71.9)	1670(65.6)	1720	I
8	312	107	5(0.1)	3(0.1)	5(0.1)	22(0.4)	92(1.8)	51(1.1)	2006(50.9)	1648(64.4)	2002(69.1)	1954	
12	261	107	2(>0.0)	2(>0.0)	7(0.2)	10(0.2)	86(1.6)	86(1.8)	1940(47.6)	1738(65.6)	1595(53.2)	2024	
16	101	107	5(0.2)	6(0.2)	4(0.1)	14(0.4)	50(1.4)	38(1.2)	1576(55.9)	1074(58.7)	1280(61.7)	1410	
20	101	107	2(0.1)	12(0.3)	15(0.4)	12(0.2)	25(0.6)	25(0.6)	1836(52.3)	1115(48.9)	1696(65.6)	1746	
II													
1	261	107	6(0.1)	15(0.3)	31(0.7)	21(0.4)	962(19.4)	1072(23.8)	2228(57.6)	1302(51.8)	1578(55.4)	1938	II
10	312	107	13(0.3)	22(0.4)	41(0.9)	36(0.6)	1326(24.0)	1086(21.7)	2596(60.2)	1478(52.8)	2530(79.8)	2136	
13	261	107	24(0.4)	24(0.4)	37(0.6)	41(0.6)	1390(20.9)	1326(22.0)	3564(88.7)	1848(54.8)	2090(54.8)	2608	
14	261	107	4(0.2)	7(0.3)	14(0.6)	8(0.3)	475(17.8)	485(20.1)	1242(59.8)	902(66.9)	817(53.5)	1034	
15	101	107	12(0.3)	31(0.6)	43(0.9)	31(0.5)	1424(26.7)	1496(31.0)	2032(48.9)	1528(56.7)	2606(85.3)	2082	
17	101	107	11(0.2)	6(0.1)	14(0.3)	22(0.4)	1304(22.3)	1472(27.7)	2052(44.9)	1416(47.7)	1720(51.2)	2282	III
18	101	107	11(0.2)	19(0.3)	47(0.9)	38(0.6)	1511(24.3)	1210(21.4)	2740(56.4)	1634(51.8)	2404(67.3)	2418	
19	101	107	12(0.2)	7(0.1)	24(0.5)	21(0.3)	1300(21.8)	1152(21.3)	2628(56.4)	1980(65.4)	2016(58.8)	2332	
9	101	107	209(4.7)	236(4.4)	445(9.8)	431(7.6)	2530(48.4)	2212(46.7)	2120(52.0)	1756(65.3)	1972(65.8)	2026	

TC _{pyrF}		60	101	134	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(0.1)	2(0.1)	19(1.1)	11(0.7)	6(0.4)	1006	IV
70	261	134	2158(68.6)	2010(75.3)	2436(81.4)	2106(66.9)	108(3.7)	102(3.1)	68(3.3)	75(4.2)	81(4.8)	1228	V			
80	101	134	2088(76.2)	2008(86.5)	1820(69.9)	2494(91.1)	1708(67.2)	2406(83.4)	18(1.0)	0(0.0)	0(0.0)	1063	VI			
							(small colonies)=		1256(69.4)	1032(69.5)	1160(79.3)					
162	101	146	2340(55.5)	2684(75.1)	2592(64.7)	2876(68.3)	2432(62.2)	2824(63.6)	16(0.6)	0(0.0)	0(0.0)	1632				
164	101	146	2510(68.7)	2428(78.4)	1914(55.1)	2968(81.3)	(small colonies)=		1658(59.5)	1037(42.9)	1456(64.7)	1428	V			
							2584(76.2)	2108(54.8)	10(0.4)	0(0.0)	0(0.0)					
							(small colonies)=		1632(67.6)	1341(64.0)	1524(78.1)					
wild type 1			2458	2996	2556	3204	2960	2668	2270	1478	1682	1142				
wild type 2			2726	2328	2620	2756	2546	2892	1824	1566	1460	1068				

The TC_{trp} hybrids were tested in one experiment with wild type 1 as control, and the TC_{pyrF} hybrids were tested in a second experiment with wild type 2 as control. The number of colonies on two plates is listed preceding, in parentheses, the relative incorporation frequency. The relative incorporation frequency is obtained as follows: (1) Normalization for the transduction competence of recipients =

$$\frac{\# \text{ of transductants in } cysC1021 (\times) \text{ wild type}}{\# \text{ of transductants in each mutant } (\times) \text{ wild type,}}$$

(2) Normalization for the titer of phages:

$$\frac{\# \text{ of transductants in } cysC1021 (\times) \text{ wild type}}{\# \text{ of transductants in } cysC1021 (\times) \text{ each phage,}}$$

(3) Normalization for the number of transductants under idealized conditions is calculated as (1) $\times$ (2) $\times$ the number of transductants actually found (listed in Table), (4) The relative incorporation frequency:

$$(3) \times 100$$

Number of transductants in *cysC1021* ($\times$) wild type

The relative incorporation frequency gives an indication of percent homology. All colonies resembled wild type in phenotype except where a distinct class of small colonies was noted; these are separately indicated in the Table.

S. typhimurium mutant in the tryptophan transducing fragment (SANDERSON and DEMEREC 1965) were used as recipient bacteria in the transduction tests. A deletion mutant, *cysC1021* (DEMEREK, GILLESPIE, and MIZOBUCHI 1963), served as a marker to normalize the transducing titer of each phage preparation. Wild-type *S. typhimurium* strain LT-2 was used as donor to normalize for transduction competence of recipient bacteria. Donor phages (multiplicity about 5 to 10) and 0.1 ml recipient bacteria (approximately 2×10^8) were spread together on duplicate double enriched minimal agar plates and incubated at 37°C for 48 to 72 hours before recombinant colonies were scored.

In order to determine the extent of the *E. coli* genome present in each hybrid, the numbers of transductants observed in different crosses were compared. The data are expressed as relative incorporation frequencies in which the titer of each hybrid phage and the transduction competence among the recipient strains are normalized. Methods used to calculate relative incorporation frequencies are described in the legend to Table 1.

RESULTS

P22 phage can jointly transduce a portion of the *Salmonella* chromosome including five tryptophan (*trpA,B,C,D,E*) loci, one cysteine (*cysB*) and one pyrimidine (*pyrF*) locus (SANDERSON and DEMEREC 1965; LEWIS M. SIEGEL, personal communication). Fourteen hybrids selected at the *trp* loci (DEMEREK and NEW 1965) and five at the *pyrF* locus were analyzed by transduction experiments to determine the extent of *E. coli* chromosome that had been integrated in each hybrid. Data are presented in Table 1, and interpretation is provided in Figure 1.

There are three classes of *trp* hybrids (TC *trp*). Class I hybrids show a low incorporation frequency (less than 3%) both for the *trp* and the *cysB* regions. *E. coli trp* and *cysB* loci are presumed to be contained in these hybrids. Members of Class II are low (relative frequency less than 1%) for *trp* and show approximately one-third of the control frequency (*Salmonella* by *Salmonella*) for *cysB*. The frequency for Class III is less than 10% for *trp* and slightly reduced (about 50%) for *cysB*. The existence of these two latter classes leads us to assume that the *E. coli* contribution ends close to *cysB* in Class II hybrids and close to *trp* in the Class III hybrid (Figure 1).

The *pyrF* hybrids (TC *pyrF*) also form three classes. The Class IV hybrid shows a low incorporation frequency (less than 1.2%) for all of the regions on the tryptophan transducing fragment and is assumed to be comprised of *E. coli* material throughout the region examined. Class V is low (less than 5%) only for *cysB* and *pyrF*. Hybrids of Class VI exhibit an unusual pattern not noted in other chromosome regions or in the other hybrids examined in this study. Class VI hybrids give a high frequency of phenotypically wild-type clones with *trp* and *cysB* recipients but none, or very few (1% or less) phenotypically wild-type recombinants with *pyrF* recipients. The vast majority of clones obtained on *pyrF* recipients are of uniform small size. Because of this peculiar behavior, the Class VI hybrids were examined further. The experiments described below indicate that hybrids of Class VI are "leaky mutants" of *pyrF* that have arisen repeatedly in these crosses of genetically dissimilar species.

The hybrids of Class VI grow slowly on minimal agar plates and grow at

FIGURE 1.—Genetic map of the tryptophan transducing fragment in *S. typhimurium* and diagram of *S. typhimurium*-*E. coli* hybrid chromosomes. It is assumed that whole, continuous blocks of *E. coli* genetic material (black) are integrated into the *S. typhimurium* chromosome (shaded) during formation of the hybrids. At the bottom of the figure are depicted map locations of *Salmonella* mutants used as recipients in transduction tests. The genetic map is based on data of BALBINDER (1962), BLUME and BALBINDER (1966), YAN and DEMEREC (1965), and L. M. SIEGEL (personal communication). The map is drawn crudely to scale. The arrow indicates the position in the *pyrF* locus that appears to contain *E. coli* genetic material in Class VI hybrids critical to the production of the uracil partial requirement; a slightly more extensive segment of *E. coli* material could be present.

normal rates on plates containing a uracil supplement (20 $\mu\text{g/ml}$). All colonies on the minimal plates are of uniform small size. No segregation of auxotrophs is detected on double enriched minimal plates (cf. AMES, HARTMAN and JACOB 1963). Three of the Class VI hybrids (TC *pyrF* 80, 162, 164) and one Class IV hybrid (TC *pyrF* 60) were used as donors in transduction tests with *pyrF* mutants (Table 2). A low frequency of large, phenotypically wild-type colonies was observed. In contrast, small colonies delayed in appearance, were observed. These required about 3 days of incubation before they could be scored, in contrast to the two days required for normal, wild-type transductional clones. In crosses with *pyrF* deletion mutants (*pyrF*134 and *pyrF*146 in Table 2), only the

TABLE 2
Numbers of large and small colonies, and incorporation frequencies in crosses
of *pyrF* hybrids with eight *Salmonella pyrF* mutant recipients

Donors	Recipients								Control <i>cysC</i>
	<i>pyrF</i>								
	-115	-117	-10	-140	-134	-146	-89	-1	
TC <i>pyrF</i> 60	4(0.4) 0(0.0)	7(1.0) 0(0.0)	4(0.5) 0(0.0)	2(0.3) 0(0.0)	4(0.4) 0(0.0)	7(1.0) 0(0.0)	8(1.2) 0(0.0)	7(1.0) 0(0.0)	594
80	36(2.3) 1188(74.8)	21(2.1) 850(83.4)	24(2.1) 1008(87.9)	18(2.0) 470(52.4)	0(0.0) 1012(82.1)	0(0.0) 615(61.8)	11(1.2) 657(72.4)	11(1.1) 891(88.5)	828
162	13(1.0) 596(44.2)	10(1.2) 581(67.0)	19(2.0) 702(72.3)	9(1.2) 504(66.1)	0(0.0) 720(68.8)	0(0.0) 706(83.6)	7(0.9) 704(91.3)	5(0.6) 576(67.3)	702
164	25(1.7) 1254(82.8)	13(1.3) 636(65.4)	14(1.3) 1028(94.0)	11(1.3) 698(81.5)	0(0.0) 642(54.6)	0(0.0) 692(72.9)	4(0.4) 742(85.7)	10(1.0) 705(73.4)	792
Control wild type	990	632	713	558	760	619	561	622	512

The upper numbers in each cross refer to large colonies and the lower numbers to small colonies. The actual number of colonies found on two plates is followed by the incorporation frequency, enclosed in parentheses. The incorporation frequencies were calculated as described in the legend to Table 1, and the *pyrF* markers are listed in the order of their map positions (Figure 1).

small delayed transductants appeared. Their numbers were comparable to the frequency of wild-type colonies in *Salmonella*-*Salmonella* crosses. These small transductants in both cases were identical to the parent hybrids with respect to their partial requirement for uracil and their stability upon restreaking on fresh medium. The factor determining a partial requirement for uracil thus maps within the *pyrF* gene, most probably within the region encompassed by the deletion of mutant *pyrF134* (Figure 1).

The large-colony, phenotypically wild-type transductional clones obtained with *cysB* recipients (Table 1) were reexamined in replica plating and restreaking tests on minimal medium. At least the majority of the transductional clones with hybrid donors of Classes I through V remain phenotypically wild type, but a significant percent of the clones derived from Class VI hybrids resemble the hybrid parent in exhibiting a partial requirement for uracil. Since the *cysB* and *pyrF* loci show about 51 percent cotransduction (L. M. SIEGEL, personal communication), simultaneous introduction of *cys*⁺ *pyr*^{leaky} provides further evidence that leakiness is due to a genetic lesion in the *pyrF* locus.

The above results eliminate the possibility that mutations outside of the tryptophan transducing fragment, suppressor or modifier mutations, are responsible for small colony formation. The results obtained in transduction tests with *pyrF134* and *pyrF146* and wild type as donor (Table 2) rule out the possibility that these mutants are double mutants in the *pyrF* region, containing one leaky and one stably auxotrophic mutation. In extensive tests, the slow-growing clones are stable; no mutations to fast growth on minimal medium (i.e., to wild type) have been observed.

DISCUSSION

DEMEREK and OHTA (1964) observed that genetic material homologous in a species could assist integration of genetically heterologous material. Our data, summarized in Table 3, extend this observation and also point out an apparent reciprocal effect. That is, the presence of *E. coli* genetic material on a transducing fragment decreases the frequency of integration of nearby *Salmonella* genes. If this interpretation of our results is correct, it becomes impossible to assign the

TABLE 3

Average relative incorporation frequencies for each class of hybrids, based on data in Table 1

Class of hybrid	<i>trp</i> cluster	Regions <i>cysB</i>	<i>pyrF</i>
IV	0.0	0.1	0.7
I	0.2	1.5	58.9
II	0.4	22.9	58.6
III	6.6	47.6	61.4
V	73.1	3.4	4.1
VI	72.6	67.9	66.3*

* Based on total large + small colonies.

precise origin, *E. coli* or *Salmonella*, of the genetic material near the juncture of the contributions of the two species. Our assignments in Figure 1 are thus arbitrary. Other methods, such as analyses of protein primary structure, would be necessary for such assignment. Similar observations and interpretations have been made by RAVIN and CHEN (1967) in other interspecies crosses.

The depression of transduction frequencies of *Salmonella* material in such composite transducing fragments occurs in our material but is absent in the four hybrids for another chromosome region studied by DEMEREC and OHTO (1964). This may be due to differing aspects of "homology" in the two chromosome regions, one a homology at the level of nucleotide sequences within genes or operons and the other to gross ordering of genes in the chromosome. In both *E. coli* and *S. typhimurium* the gene order appears to be *trp-cysB-pyrF*; however, this group of genes is inverted between the two species (SANDERSON 1965; SIGNER, BECKWITH and BRENNER 1965; K. E. SANDERSON and C. A. HALL 1968, manuscript in preparation). The *trp* operon is oriented ending toward *cysB* in both species, and the ordering of the five genes within the *trp* cluster is the same in the two species (BALBINDER 1962; SANDERSON 1965; BLUME and BALBINDER 1966; MARGOLIN and BAUERLE 1966; SIGNER, BECKWITH and BRENNER 1965; IMAMOTO, ITO and YANOFSKY 1966). While the two species may have considerable homology at the level of genetic fine-structure (cf. BALBINDER 1964; CREIGHTON *et al.* 1966), this homology may be masked by chromosomal rearrangement. Estimates of homology based on transduction tests with the Class IV hybrid (Table 1 and further experiments not shown here) give average relative incorporation frequencies for *trp*, *cysB*, and *pyrF* of <0.03, 0.1 and 0.7, respectively. These figures may reflect the presence of one end of the inversion near the *trp* region (to the "left" on the map shown in Figure 1), or they may be biased by the presence in this hybrid of *Salmonella* genetic material beyond the *pyrF* gene. The chromosomal orientation of the genes in the hybrids has not been determined directly; presumably in all hybrids except possibly Class IV, the orientation is that of the *Salmonella* recipient.

The above considerations indicate that formation of viable hybrids between divergent species may take place only in restricted regions, the more diverse the species the fewer the effective sites. This may account for the isolation of two general classes of hybrids, Class II with 8 members and one Class III hybrid, instead of a spectrum of hybrids recombinant between *trp* and *cysB*. NIKAIDO, NIKAIDO and MÄKELÄ (1966) noted that recombination in the highly divergent rough B (*rfb*) regions of otherwise reasonably homologous group B and C₁ *Salmonella* spp. occurs rarely and at only a few sites. RAVIN and CHEN (1967) have observed analogous effects in another system of interspecific crosses.

Examination of three hybrids (Class VI) indicates that incorporation of a small portion of the *E. coli pyrF* locus into *S. typhimurium* results in a partial requirement for pyrimidine. Substitution of the entire *pyrF* gene locus of *E. coli* for that of *S. typhimurium* allows formation of phenotypically prototrophic bacteria. Our data show that the factor controlling a partial uracil requirement is located in the *pyrF* gene and is not due to an extragenic suppressor. The *pyrF*

gene in *S. typhimurium* appears by complementation tests to be a single cistron, the structural gene for the decarboxylase involved in the conversion of orotidine-5'-phosphate to uridine-5'-phosphate (YAN and DEMEREC 1965).

It seems reasonable to assume that the defect in the *pyrF* hybrids arises from incompatibilities in a portion of the polypeptide chain dictated by *E. coli* material with the portion of the polypeptide chain dictated by the genetic contribution of *S. typhimurium*. Alteration of one segment of a polypeptide chain can be compensated for by alteration at another position in the same chain (HELINSKI and YANOFSKY 1963). One can guess that such alterations and compensating changes have occurred during evolution of the *pyrF* genes in *S. typhimurium* and *E. coli*. These changes lead to incompatibilities when recombination occurs between the two species. In addition to the partial incompatibility exhibited in the case of the *pyrF* hybrids described here, more extreme incompatibilities can readily be envisioned. Since selection was performed for *pyrF*⁺, totally defective *pyrF* hybrid genes would have gone undiscovered. The *pyrF* hybrids do not revert to the wild-type phenotype; the incompatibility thus probably involves more than can be corrected by a single base-pair substitution or deletion.

The *pyrF* hybrids point out how a system of single-gene isolation can be initiated, setting the stage in turn for rapid divergence of that gene. Intragenic recombinational events such as the one engendering a partial requirement for uracil may be important in other situations, for example, in the "degeneration" of hybrid progeny (see STEBBINS 1950, p. 227).

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SUMMARY

Two types of genetic incompatibilities between *E. coli* and *S. typhimurium* appear to occur in the *trp-cysB-pyrF* region of the chromosome. One type is presumed to be effected mainly at the level of the chromosome since the *trp-cysB-pyrF* region is inverted between the two species. A second type appears to reside in the inability of a hybrid enzyme, dictated by gene *pyrF*, to function normally. The first type hinders genetic exchange while the second type affects the growth rate of hybrids in the absence of pyrimidine.

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