*GENETIC ANALYSES OF SALMONELLA TYPHIMURIUM × ESCHERICHIA COLI HYBRIDS**

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Bacterial hybrids derived from crosses between *Salmonella typhimurium* and *Escherichia coli* are being analyzed genetically by means of transduction experiments with Salmonella mutant strains as recipients, hybrids as donors, and phage P22 as the vector. It has been shown that when hybrid transducing fragments consist entirely of *E. coli* genetic material, there are either no transductants[1, 2] or only a few,[3, 4] whereas when transducing fragments carry only Salmonella markers, the expected numbers of transductants are found. It is evident that this finding provides a means of differentiating between *E. coli* and *S. typhimurium* regions of a hybrid chromosome; and when known genetic markers are present in a section of the genome where such regions join, it is possible to establish the relative order of the markers. Thus hybrids can serve, in much the same way as overlapping deletions, for the mapping of genes. The small numbers of transductants obtained when transducing fragments are *E. coli* in origin indicates a lack or a low order of homology between genetic materials of the two genera, and some of the experiments with hybrids enable us to obtain quantitative measurements of the degree of homology.

Materials.—A genetically well-marked region of the *S. typhimurium* chromosome was selected for these studies. It contains a cluster of 5 cysteine loci (*cysC, D, H, I, J*), all located in one transducing fragment, as well as 10 other loci, located in 9 fragments. The probable arrangement on the chromosome is: *cysG, metC, argE, serA, lys, argB,* (*cysC, D, H, I, J*), *phe, tyr,* (*purG* and *gly*).

The *cysC, D, H, I, J* cluster had been studied previously by transduction experiments with 95 deletion mutants and about 280 single-site mutants.[5] Loci *cysC* and *D* are adjacent to each other, as are *I* and *J;* but there is a long "silent section" between *D* and *H*, and a short one between *H* and *I*.

Methods.—To produce hybrids, broth cultures of *S. typhimurium* and Hfr *E. coli* were grown overnight in a shaker, and equal volumes of each were mixed in a test tube and plated on selective medium. The *S. typhimurium* parent must be a strain of LT7 that carries the mutator (*mut*) gene.[1] Our experience indicates that only a small fraction of such strains is competent.

In the transduction experiments a sample taken from an overnight broth culture of the recipient strain grown in a shaker was mixed with phage P22 grown on a hybrid (multiplicity 5 ×), and 0.1-ml aliquots were plated on an appropriate selective medium, usually minimal medium enriched with 0.02% nutrient broth powder ("double-enriched medium"). The minimal medium contained K_2HPO_4, 10.5 gm; KH_2PO_4, 4.5 gm; $MgSO_4$, 0.05 gm; $(NH_4)_2SO_4$, 1.0 gm; sodium citrate, 0.47 gm; glucose, 5.0 gm; and distilled water, 1000 ml.

Production of hybrids: Two sets of hybrids were studied in the experiments: set *A*, in which the female parent was the *S. typhimurium* double mutant *cysC-125 cysJ-109;* and set *B*, in which the female parent carried the short deletion *cysC-1021*. Both females belonged to strain LT7 and carried the *mut* gene. The female for set *B* was obtained from the strain used for set *A* by replacing the *cysD-125 J-109* markers with *dl-cysC-1021*. The male parent for both sets was *E. coli* HfrCS-101 (Cavalli), with the order of injection of markers proceeding from left to right (*cysG-metC-argE-serA* . . .).

When *cysD-125 J-109* is crossed with *E. coli* HfrCS-101 (which is cys^+), and selection is made for cys^+, it is reasonably certain that all the isolates will be hybrids carrying an *E. coli* section of chromosome at least in the region delimited by the two genes of the double-mutant parent. The only other way in which cys^+ colonies could originate would be through simultaneous reversions

at the *cysD-125* and *cysJ-109* sites. The probability of occurrence of such double mutations is extremely low, and none were observed on the control plates. Similarly, in crosses made with *cysC-1021* as the female parent, because this is a deletion mutant, all *cys*$^+$ isolates will be hybrids carrying an *E. coli* section at least within the *cysC* gene locus.

S. typhimurium × *E. coli* hybrids are not uniform. Some are unstable (probable heterogenotes); some are resistant to Salmonella phage P22; some make poor hosts for P22; and some are resistant to *coli* phage T6. These and numerous other differences are probably due to variations in the proportions of hybrid material and the areas of hybridity. Since our objective was to analyze the region of the chromosome containing the *cysC* cluster of loci, we used only *cys*$^+$ hybrids that were stable and that yielded high-titer phage.

Phage raised on the hybrids was used to transduce mutant markers of the Salmonella recipient. Six of these markers represented the five loci of the *cysC* cluster, and the others represented the other ten loci listed above, which have been shown by conjugation experiments to be located near the *cysC* cluster. In every instance the transducing fragment carried the wild-type allele of the pertinent mutant gene in the recipient. In order to avoid background noise, mutants carrying short deletions were chosen as recipients whenever available.

Results.—(1) *Hybrids as donors in transduction:* From the cross *S. typhimurium cysB-125 J-109* × *E. coli cys*$^+$, with selection on double-enriched medium, about 200 hybrids were isolated, of which 25 were found suitable for further tests. Every one of the 25 hybrid donors gave rise to a much smaller number of transductants in crosses with cysteine mutants representing the five loci of the *cysC* cluster than in crosses with certain mutants representing adjacent loci. Data of some of the crosses are given in Table 1 (*A* hybrids). These results are in full agreement with the supposition that an *E. coli* chromosomal segment behaves differently in transduction than a Salmonella segment recombining much less frequently with the Salmonella chromosome. When the mutants representing the adjacent loci are arranged in order according to whether they produce large or small numbers of transductants with the different hybrids (Table 1), the order coincides with that obtained in conjugation experiments. This finding accords with the assumption that in different hybrids the *E. coli* segments differ in length, and that consequently the hybrids can serve in a fashion similar to overlapping deletions for genetic mapping of the bacterial chromosome.

(2) *Evidence that E. coli fragments are carried by P22 phage:* The data presented in Table 1 provide good evidence that phage P22 can carry *E. coli* fragments capable of replacing mutant markers located in a Salmonella recipient. Two other lines of evidence support this interpretation. One is the presence of abortive transductants in crosses between Salmonella mutants and hybrid donors (Table 2). The numbers of abortive transductants were similar when the transducing fragments were of Salmonella origin (donor +, hybrid *A-9*, and hybrid *A-18*) and when they were *E. coli* (hybrid *A-50*), indicating that *E. coli* fragments are not discriminated against by phage P22.

The other supporting evidence was obtained by analysis of composite transducing fragments that are Salmonella in the right portion and *E. coli* in the left. Our material includes four hybrids carrying such fragments, and results obtained with two of them (from set *B*) are shown in Table 3. Experiments were also made with the mutant *phe-3* (a locus which is not involved in the hybrids) as the recipient marker, in order to normalize for the transducing competence of the phages; and with wild-type Salmonella as donor, in order to normalize for the transduction competence of the recipients. With respect to markers located in the right-hand

TABLE 1

NUMBERS OF TRANSDUCTANTS IN EXPERIMENTS WITH *S. typhimurium* MUTANTS AS RECIPIENTS AND *S. typhimurium* × *E. coli* HYBRIDS AS DONORS

Recipient→ / Donor↓	*cysG* −382	*metC* −32	*argE* −116	*serA* −13	*lys* −8	*argB* −69	*cysC* Cluster *dl-C* −537	*dl-C* −1021	*dl-CD* −519	*dl-H* −75	*dl-I* −68	*dl-J* −538	*phe* −3	*tyr* −3	*purG* −303	*gly* −1
Salmonella +	1260	555	485	446	960	740	~1400	377	~1000	1170	~1890	~1130	~3200	~2800	~2600	~3800
Hybrids																
A-18	...	85	...	129	176	126	0	7	12	12	4	13	~2000	~2400	...	...
B-33	599	732	732	205	619	305	11	4	...	...	...	5	736	1362	~2840	~1336
B-20	...	...	...	...	410	241	4	2	2	5	5	4	~1600	~1600	~1770	...
A-57	451	~1470	385	282	5	0	0	...	1	...	...	0	~3000	~3000	~2200	~3000
B-24	1320	18	92	433	8	1	0	1	1	0	2	2	~2600	~2600	~2600	...
A-45	~1430	506	326	1	3	0	0	...	0	...	...	2	~3000	~3000	~2600	~3000
B-11	541	893	96	1	4	0	0	0	0	0	0	0	0	2	25	~1277
A-50	...	122	98	2	12	0	4	2	2	2	2	12	~1600	...	...	...
A-14	...	~6000	0	1	0	0	0	...	0	...	...	2	~1500	~4800	...	~2750
A-19	...	104	0	6	19	0	0	0	...	...	0	0	12	14	56	1480
B-51	590	3	0	0	5	1	0	1	1	4	3	1	137	198	567	1663
B-74	700	8	0	0	5	0	3	1	3	1	0	1	175	1229	~1280	~1450

Recipient genes are located close together (two plates, 4 × 10^8 bacteria, phage multiplicity 5 ×, double-enriched medium).

TABLE 2

NUMBERS OF COMPLETE (CT) AND ABORTIVE (AT) TRANSDUCTANTS WHEN THE RECIPIENT MARKERS WERE SALMONELLA *argB*, AND THE *argB*$^+$ TRANSDUCING FRAGMENTS WERE EITHER SALMONELLA OR *E. coli*

Donor ↓ / Recipient →	*argB-69* CT	*argB-69* AT	*argB-127* CT	*argB-127* AT
Salmonella *argB* +	50	108	26	120
Hybrid *A-9*	70	256	27	144
Hybrid *A-18*	141	426	48	148
E. coli argB Hybrid *A-50*	0	253	0	184

TABLE 3

FREQUENCIES OF TRANSDUCTION OF *S. typhimurium* MUTANT MARKERS LOCATED IN THE *cysC* CLUSTER

Donor ↓	Recipient →	Markers in *cysC* Cluster: *C-520*	*D-80*	*dl-CD-519*	*Ddl-7*	*dl-D-68*	*dl-J-538*	*phe-3*
Hyb. *B-22*	Observed	179	199	98	666	1022	607	1311
	Normalized	224	239	121	539	409	619	1104
	Per cent of Salm.	39	42	21	95	72	109	...
	Av. per cent		38			92		
Hyb. *B-32*	Observed	118	155	124	856	1217	664	896
	Normalized	216	273	223	1008	657	996	1104
	Per cent of Salm.	38	48	39	177	119	175	...
	Av. per cent		42			157		
Salmonella	Observed	383	398	387	590	1191	468	...
	Normalized	569	569	569	569	569	569	...

Three plates, 6 × 10^8 bacteria, phage multiplicity 5 ×, double-enriched medium.

portion of the composite fragment, the frequency of transduction between Salmonella recipients and hybrid donors is similar to that between Salmonella recipients and Salmonella donors. With respect to markers located in the left-hand portion, the frequency of transduction is about 40 per cent that obtained with Salmonella donors. An interpretation of this finding is suggested in the *Discussion*.

(3) *Quantitative tests of homology between S. typhimurium and E. coli:* We assume that the generally low frequency of incorporation of *E. coli* material into the *S. typhimurium* chromosome in the region we have examined is due to a low degree of structural homology in that region in the two bacteria. An attempt was made to give a numerical value to the degree of homology. Five hybrids that carry *E. coli* material in the transducing fragment containing the *cysC* gene cluster served as donors for transduction of three Salmonella markers spaced along the cluster. The degree of homology is expressed as percentage of incorporation of *E. coli* material into *S. typhimurium* material compared with the frequency of incorporation of Salmonella into Salmonella material. The data are given in Table 4. For the five hybrids tested, the homology values vary between 0.34 and 2.9 per cent, the average being 1.05 per cent.

Discussion.—Transduction experiments made with phage grown on *S. typhimurium* × *E. coli* hybrids reveal that phage P22 is able to carry transducing fragments that are either *S. typhimurium*, *E. coli*, or composite (consisting partly of Salmonella and partly of *E. coli* material). Observations of abortive transduction indicate, with respect to the genetic markers studied, that phage P22 carries an *E. coli* fragment and the corresponding *S. typhimurium* fragment with

TABLE 4

QUANTITATIVE TESTS OF HOMOLOGY BETWEEN *S. typhimurium* AND *E. coli*

Recipient ↓	Donor→	Hybrids					Salm. +
		A-18	*A-24*	*A-34*	*A-54*	*A-55*	
dl-cysC-1021	Observed	14	78	0	2	10	1884
	Normalized	10.4	93.6	0	34.8	7.1	2232
	Per cent of Salm.	0.47	4.2	0	1.6	0.32	...
dl-cysH-75	Observed	32	48	11	0	15	1813
	Normalized	24.6	60	19.4	0	11.1	2232
	Per cent of Salm.	1.1	2.7	0.87	0	0.5	...
dl-cysI-68	Observed	26	58	3	2	28	3000
	Normalized	12.2	43.5	3.2	21.8	12.3	2232
	Per cent of Salm.	0.55	1.9	0.14	0.98	0.55	...
aroD-35	Observed	2727	1693	1203	117	2884	...
	Normalized	1725	1725	1725	1725	1725	...
	Av. per cent	0.7	2.9	0.34	0.86	0.46	1.05

Five plates, 1×10^9 bacteria, phage multiplicity 10 ×, double-enriched medium.

approximately the same frequency (Table 2). But integration of *E. coli* material into the *S. typhimurium* genome occurs much less frequently than the incorporation of *S. typhimurium* material, as is shown by the results of transduction studies of a section of chromosome including at least ten transducing fragments (Table 1).

It is estimated that the *S. typhimurium* genome breaks up into about a hundred transducing fragments. Thus the region studied here comprises about one tenth of the genome. Within this region, the level of frequency of integration of *E. coli* markers appears to be uniform. A quantitative study, made with five hybrids and three mutant markers located in the *cysC* region (Table 4), indicates a frequency of integration of *E. coli* genetic material into the Salmonella genome averaging about 1 per cent that of the incorporation of Salmonella genetic material.

We assume that lack of homology is responsible for failures of integration of *E. coli* material into the *S. typhimurium* chromosome. In the study made with composite transducing fragments it appeared that the Salmonella portion was incorporated with about the same frequency as if the whole fragment were Salmonella, whereas the frequency of incorporation of the *E. coli* portion was about 40 per cent that of the Salmonella portion (Table 3), or about 40 times greater than the integration of an analogous all-*E. coli* fragment. These results suggest an explanation in terms of the effect exerted by homology on the synaptic forces that bring together corresponding regions of two chromosomes. When an entire transducing fragment consists of *E. coli* material, the degree of homology with the Salmonella genome is low throughout the region—and the synaptic forces are weak, permitting only a low frequency of integration. In the case of the composite fragment, it may be surmised that synaptic forces acting between the homologous portions of the fragment and the recipient chromosome will exert a pull on the nonhomologous portions, thus increasing their frequency of integration to about 40 per cent. It may be that integration is accomplished by a copy-choice process whenever two chromosomes are in sufficiently close contact.

The most recent map of *E. coli* contains about 100 gene loci,[6] and a similar number is included in the latest *S. typhimurium* map.[7] A comparison of these maps shows a strikingly high degree of homology between the two genomes in gross genetic structure, that is, as far as the locations and functions of their genes are concerned. So far, no well-substantiated case of chromosomal rearrangement has been found. This over-all conformity presents quite a contrast to the low degree of homology

detected by our study of integration frequencies. The most plausible explanation of such divergence seems to be that determinations of gross homology are concerned with whole genes, whereas in these studies of integration we are dealing with processes that occur at a subgenic level. Thus genes may be homologous as far as transcription is concerned and yet differ in their molecular structure. The differences might originate through changes within coding triplets—changes which, because of the degeneracy of the code, do not affect the transcription of amino acids, or which modify the transcription of amino acids of nonfunctional regions of the protein, or both. According to the present view, the degeneracy of the amino acid code is so extensive that almost every amino acid can be coded by at least two different triplets. Thus two sentences (representing genes) could be composed which would transcribe exactly alike but would differ in nearly every consecutive word. If identity of words is essential for synapsis and consequent integration, then two genes represented by such sentences, although responsible for the same enzyme, would not recombine with each other.

This interpretation is supported by results of molecular *in vitro* hybridization of DNA carried out by two different techniques. Schildkraut, Marmur, and Doty[8] did not detect any hybridization between *E. coli* K12 and *S. typhimurium* DNA, and McCarthy and Bolton,[9] by means of a much more sensitive technique, found such hybridization to be only about 70 per cent.

In the 12 hybrids listed in Table 1, the *E. coli* sections extend different distances from the *cysC* cluster and therefore can serve, like overlapping deletions, to determine the order of genetic markers located in this region. The order of the five loci of the *cysC* cluster was already known,[5] and the orientation of the cluster within the region has been ascertained in this analysis of hybrids. The relative positions of *lys-arg* and the *phe-tyr* are not known, because appropriate hybrids for their testing are not yet available; but the order of the other markers, as shown in this table, has been adequately demonstrated. It can be noted that more of the *E. coli* sections extend to the left of the *cysC* cluster, which is to be expected because the direction of entry of HfrCS-101 markers is from left to right.

Summary.—An analysis has been made, by transduction experiments with *Salmonella typhimurium* mutants and *S. typhimurium* × *Escherichia coli* hybrids, of a region covering about one tenth of the bacterial genome (about ten transducing fragments). Phage P22 can carry, without discrimination, fragments that are wholly Salmonella in origin, wholly *E. coli*, or a composite of both. Genetic material from *E. coli* fragments is incorporated into Salmonella recipient chromosomes with a frequency about 1 per cent that of the integration of material from Salmonella fragments. It is proposed that a low degree of homology between the two bacterial genomes, at the molecular level, is responsible for the low frequency of integration.

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*HOMOLOGY BETWEEN RNA FROM ROUS SARCOMA VIRUS AND DNA FROM ROUS SARCOMA VIRUS-INFECTED CELLS**

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Recently, it has been suggested[1-4] that the provirus of Rous sarcoma virus-infected cells is composed of DNA and that therefore the life cycle of Rous sarcoma virus (RSV) involves transfer of information from the RNA of the genome of the infecting virus to DNA of the provirus and from the DNA of the provirus to RNA of the progeny virus. This hypothesis is based mainly upon the results of experiments with inhibitors of nucleic acid synthesis.

The isolated virions of RSV contain RNA as their sole nucleic acid component.[5-7] The role of RNA as the genetic material of RSV is supported by the recent report of the isolation from Rous sarcoma cells of an infectious nucleic acid which is sensitive to destruction by RNase and not by DNase.[8] In further support of this role of RNA is the observation that stoppage of DNA synthesis in Rous sarcoma cells by FUDR or by amethopterin does not prevent production of new RSV.[3, 9-11]

However, prevention of DNA synthesis in cells soon after they have been exposed to RSV does prevent infection and initiation of virus production.[3, 4, 12, 13] This inhibition, by amethopterin, FUDR, BUDR, or cytosine arabanoside, is specifically prevented by the presence in the medium of the appropriate deoxyribonucleoside. Therefore, although the virion of RSV does not contain DNA and DNA synthesis is not needed for virus production after it has begun, DNA synthesis is needed for the initiation of virus production (establishment of the provirus).

Treatment of virus-producing Rous sarcoma cells with actinomycin D prevents production of infectious RSV[1, 11, 14, 15] and of virus nucleic acid.[3] Since actinomycin D prevents DNA-directed RNA synthesis,[16] this result is in agreement with the hypothesis that the provirus of RSV is DNA.

This hypothesis also most simply explains the other results and leads to the prediction that Rous sarcoma virus-infected cells contain some DNA not found in parallel uninfected cells, and that this new DNA should be homologous with RNA from RSV.

In this paper experiments are presented which confirm this prediction by showing that labeled RNA from RSV has homology with DNA from RSV-infected cells, but not with DNA from parallel uninfected cells. Two different techniques for confirming the presence of homology have been used. The first depends upon the fact that a specific RNA-DNA complex is resistant to degradation by RNase[17, 18] and can be trapped on a nitrocellulose filter.[19, 20] The second depends upon the fact