DITTO DELETIONS IN THE *cysC* REGION OF THE SALMONELLA CHROMOSOME¹

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PREVIOUS studies have revealed that the *cysC* region of the *Salmonella typhimurium* chromosome includes a cluster of five genes arranged in the sequence *cysC-D-H-I-J* (MIZOBUCHI, DEMEREC and GILLESPIE 1962). Genes *cysC* and *D* are adjacent to each other, as are genes *I* and *J* (Figure 1). Genes *D* and *H* are separated by a long segment of genetic material for which no functions have been found (the "silent section"). Genes *H* and *I* are separated by a similar but shorter section of the approximate length of one gene locus (DEMEREC, GILLESPIE and MIZOBUCHI 1963). The entire cluster can be carried on a single transducing fragment (MIZOBUCHI *et al.* 1962).

In the *cysC* region of strain LT-2, deletion mutations occur with an abnormally high frequency. The great majority of these deletions are extensive and are similar to one another ("ditto deletions") in that their left ends terminate in the silent section and their right ends terminate beyond the *cysJ* locus (DEMEREC 1960). Data obtained in preliminary studies on ten ditto deletions indicate that the left ends of the ditto deletions are not distributed at random along the entire silent section but, rather, are restricted to a certain portion of it (DEMEREC *et al.* 1963). The data in the present paper extend the analysis to 43 additional "ditto deletions" (Ddl). The Ddl are shown to terminate in a particular segment of the silent section. Points of termination differ slightly among independently isolated Ddl.

MATERIALS AND METHODS

Forty-three *cysC* ditto deletion mutants, five other deletion mutants (*cysC1021*, *cysCD519*, *cysHI36*, *cysHI536* and *cysI68*) and one single-site mutant (*cysD490*) were used in this study. In addition, ten ditto deletions were analyzed by DEMEREC *et al.* 1963. Each of these mutations originated in *S. typhimurium* wild-type strain LT-2. Mutants *cys-36*, *cys-68* and 13 of the ditto deletions originated spontaneously, and *cys-1021*, procured from a 2-aminopurine-treated culture, is also presumed to be of spontaneous origin. Nine ditto deletions, *cys-519*, and *cys-536* were obtained from nitrous acid-treated cultures. Eight ditto deletions and *cys-490* came from cultures treated with fast neutrons, 17 ditto deletions from X-ray treated cultures, and six ditto deletions from cultures treated with ultraviolet light. Stock cultures of these *Salmonella* mutants are now maintained by DR. KENNETH E. SANDERSON, University of Calgary, Alberta, Canada.

Minimal medium contained K₂HPO₄, 10.5g; KH₂PO₄, 4.5g; MgSO₄, 0.05g; (NH₄)₂SO₄, 1.0g;

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TABLE 1

Percentages of recombination between ditto deletions and cysCD519, D490 and C1021.

Recipients	dl- <i>CD519</i>		Donors <i>D490</i>		dl- <i>C1021</i>	
	Total colonies (donor + wild type)	Percent wild-type recombinants	Total colonies (donor + wild type)	Percent wild-type recombinants	Total colonies (donor + wild type)	Percent wild-type recombinants
Ddl- 37	2294	2.94	347	20.17	595	33.95
62	459	7.34	...	...	...	...
83	1915	10.58	266	25.94	356	40.45
85	2468	3.28	...	...	...	...
137	402	3.87	...	...	...	...
138	231	11.25	...	...	...	...
172	2805	2.74	...	...	...	...
192	2979	1.17	...	...	...	...
193	2614	8.57	413	22.76	435	48.28
305	2620	1.29	...	...	...	...
306	2393	7.44	...	...	...	...
321	2228	3.41	221	22.27	482	34.02
325	2167	5.13	...	...	...	...
326	2731	2.56	...	...	...	...
332	2228	5.59	...	...	...	...
352	2589	1.97	...	...	...	...
353	2220	1.94	...	...	...	...
359	2025	9.58	468	27.14	746	39.28
369	2509	7.61	...	...	...	...
373	2504	5.35	...	...	...	...
388	1425	8.21	...	...	...	...
395	11282	0.38	424	14.62	731	31.82
406	3327	4.24	...	...	...	...
410	4973	2.67	...	...	...	...
416	3089	1.34	325	15.69	641	28.10
422	1623	4.40	...	...	...	...
425	2353	6.46	...	...	...	...
438	2449	6.00	...	...	...	...
443	2176	1.65	...	...	...	...
444	2362	5.67	290	23.79	581	34.08
450	1403	1.92	...	...	...	...
460	2112	2.84	...	...	...	...
463	1826	6.96	...	...	...	...
464	2195	3.23	...	...	...	...
486	1880	8.30	...	...	...	...
513	1343	2.80	...	...	...	...
521	2196	7.83	...	...	...	...
526	1866	3.54	...	...	...	...
527	1451	4.14	...	...	...	...
535	1214	1.78	...	...	...	...
540	1540	5.29	...	...	...	...
548	279	2.87	...	...	...	...
549	955	10.87	...	...	...	...

In each experiment, about 4×10^8 bacteria were infected with a phage multiplicity of 5. All crosses were carried out with five plates containing double enriched minimal medium supplemented with CSA. The cross between Ddl-395 and *cysCD519* was made with 30 plates.

sodium citrate, 0.47g; glucose, 5.0g; distilled water, 1000 ml. Double enriched minimal medium was prepared by adding dehydrated Difco nutrient broth to a concentration of 0.02% in minimal medium. This medium was supplemented with cysteine sulfinic acid (CSA) at a final concentration of 30 µg/ml.

Mutants of the *cysC*, *D* and *H* loci grow slowly on minimal medium supplemented with CSA while mutants defective in genes *cysI* and *J* are unable to grow on CSA (DEMEREK *et al.* 1963). Transduction tests using CSA nonresponsive bacteria as recipients and phage grown of *cysC*, *D*, *CD* and *H* mutants as donors give rise to two kinds of colony on double enriched minimal medium supplemented with CSA. Donor-type recombinants carrying mutant *cysC*, *D*, *CD* or *H* alleles form colonies considerably smaller than colonies composed of wild-type recombinants. Thus, two recombinant classes may be scored on a single Petri plate. Recipient bacteria for transduction tests were grown overnight at 37°C in Difco nutrient broth supplemented with cysteine (20 µg/ml) on a shaker. Wild-type P22 phage was grown on appropriate donor strains. The multiplicity of infection in transduction tests was about five.

RESULTS

DEMEREK, GILLESPIE and MIZOBUCHI (1963) performed extensive mapping experiments with the *cysC* region (see map in Figure 1). To determine the positions of the left ends of the ditto deletions (Ddl), 43 Ddl mutants were used as recipients and three mutants (*cysCD519*, *cysD490* and *cysC1021*) were used as donors in transduction tests. The data in Table 1 show that recombination frequencies are restricted to a range of 0.38 to 11.25% with *cysCD519* and that relative recombination frequencies for the three donor strains show the same pattern of order. These data indicate that the ditto deletions end within a very

TABLE 2

Replicate recombination tests between eight ditto deletions and dl-CD519

Recipients	Donor dl-CD519			$\bar{X}$	SE
	Total colonies (donor + wild type)		Percent wild-type recombinants		
Ddl-395	1530, 1326, 1516	0.13, 0.45, 0.52	0.375	0.066	
	1390, 1668, 3852	0.29, 0.60, 0.26			
Ddl-305	2841, 1902, 2087	1.09, 0.84, 1.29	1.290	0.190	
	1338, 2626, 3067	2.24, 1.37, 0.91			
Ddl-416	1681, 3131, 2389	0.95, 1.34, 1.47	1.335	0.123	
	2319, 1718, 4721	1.71, 1.16, 1.38			
Ddl-325	842, 602, 543	4.26, 5.32, 2.95	4.397	0.330	
	637, 735, 2165	3.92, 5.17, 4.76			
Ddl-540	961, 1375, 850	5.52, 5.45, 4.82	5.128	0.234	
	1035, 1381, 1153	4.73, 4.27, 5.98			
Ddl-325	842, 602, 543	4.26, 5.32, 2.95	5.593	0.349	
	735, 675, 2286	5.17, 4.44, 5.34			
Ddl-62	360, 240, 253	5.83, 4.58, 6.32	7.338	0.965	
	273, 244, 187	10.99, 6.15, 10.16			
Ddl-83	736, 726, 884	13.18, 9.23, 10.97	10.575	0.655	
	699, 667, 545	11.09, 10.91, 8.07			

Each value represents an independent test on an individual culture. Recipient bacteria of each strain were prepared as follows: a small amount of a broth culture of each Ddl mutant was inoculated into 3ml of fresh broth, and incubated overnight at 37°C in a shaker. $\bar{X}$ indicates the mean value of percent recombination; SE: the standard error of the percent recombination. METHODS were as for other experiments.

small chromosome region close to the termination of the *cysCD519* deletion. However, the various Ddl can be distinguished from one another and thus are presumed to terminate at different points.

Additional transduction experiments were performed to assist in analysis of the significance of the experimental values shown in Table 1. These tests were in part dictated by the observation of M. DEMEREC and D. H. GILLESPIE (unpublished) that secondary *cys* mutations frequently accumulate in cultures of particular *cys* mutants, including some of those used here. It appears that *cys* double mutants, with two independent mutations in the pathway of cysteine biosynthesis, have a selective advantage over the original single mutant. Populations containing significant numbers of double *cys* mutations can lead to spuriously decreased

FIGURE 1.—Location of the left ends of 53 ditto deletion mutations. The numbers in parentheses above the topmost line indicate the percent of the total map distance of the region contained in each section; for example, mutations in the *cysC* locus are located across 25% of the total genetic region. The numbers immediately below the line designate the percent of the region at various relative points (5, 10, 50, 60, and 100%) from the beginning of the *cysC* gene. Bars underneath the topmost line indicate the locations of some *cys* deletion (dl) and ditto deletion (Ddl) mutations. An enlargement of 15% of the total map, from 45 to 60%, shows the relative locations of the ends of Ddl as determined from the data of DEMEREC *et al.* (1963) and in Tables 1 and 2. Asterisks (*) follow those mutants for which the standard error of percent recombination was calculated in replicate crosses (Table 2).

transduction frequencies. Six broth cultures of each strain, originating from small inocula, were used as recipient bacteria. The donor bacterial strain was *cysCD519*. The data in Table 2 show that all values of the standard error are within 1/5 to 1/20 of the mean recombination values. The data indicate that the recombination tests give a valid estimate of recombination frequencies between the markers involved in each cross.

Additional transduction experiments were carried out to confirm the relative position of the several *cys* mutants. The results of these crosses serve to map more precisely relative positions of the left ends of ditto deletions.

Crosses were performed with the three donor strains used previously (Table 1): *cysCD519*, *D490* and *C1021*, in combination with three deletion mutant recipient strains which are not Ddl: *cysI68*, *HI36* and *HI536* (cf. DEMEREC *et al.* 1963). The latter two of these deletions extend throughout gene *H* and terminate in the "silent section". The data (Table 3) show that *HI* and *I* deletions are much more loosely linked with *C*, *D* and *CD* donor mutations than are the ditto deletions. The crosses show that the low recombination values obtained with the Ddl are not due to inadequacies in the *C-D* phage stocks.

Cotransduction was attempted with Ddl and other markers shown by SANDERSON and DEMEREC (1965) to be located nearby on the *Salmonella* chromosome. No linkage (less than 0.1% cotransduction) was detected with mutants at the *lys*, *thy*, *argB* and *phe* loci.

DISCUSSION

Crosses by the transduction technique were carried out in order to determine the relative positions of the left ends of ditto deletions (Ddl) in the *cysC* region. Replicate tests gave reproducible results. The values of the standard error in crosses of eight Ddl mutants with *cysCD519* (Table 2) are under one tenth of the mean recombination frequencies for five of the eight crosses examined. The values range from 13 to 18% in the remaining three crosses. Therefore, we assume that our data give a reliable estimate of the relative termination points of Ddl (Figure 1). The ditto deletions terminate within a restricted portion of the long "silent section" which, itself, comprises some 50.7% of the entire genetic

TABLE 3

Percentages of wild-type recombinants in crosses between dl-HI36, dl-I68, and dl-HI536 as recipients and dl-CD519, D490 and dl-C1021 as donors

Recipients	Donors					
	dl-CD519		D490		dl-C1021	
	Total colonies (donor + wild type)	Percent wild-type recombinants	Total colonies (donor + wild type)	Percent wild-type recombinants	Total colonies (donor + wild type)	Percent wild-type recombinants
dl-HI36	1617	44.11	534	56.74	170	70.00
dl-HI536	988	69.37	579	66.32	1717	73.73
dl-I68	1105	90.77	784	80.36	799	74.09

On each plate, 2×10^8 bacteria were infected with a phage multiplicity of 5. Values are the sums of colonies on five plates containing double enriched minimal medium supplemented with CSA 30 $\mu\text{g/ml}$.

region. The length of this restricted segment in which ditto deletions end corresponds to about 16% of the silent section and to about 8% of the entire *cysC* region (Figure 1). Our own data, coupled with those of DEMEREC *et al.* (1963, their Table 2), show that various Ddl end in at least ten different positions within the restricted segment.

Redundant segments of DNA probably underlie the occurrence of ditto deletions. The silent section may contain a repetition or near repetition of a nucleotide sequence located to the right of gene *cysJ*. In this case, a loop containing *cysH*, *I* and *J* may form by pairing of the segments of identical or near identical nucleotide sequence. This loop may occasionally be eliminated owing to its failure to be copied during DNA replication or, more likely, it could be crossed out by a single recombinational event resulting in a deletion and an inviable circular genetic element containing genes *cysH*, *I* and *J* (cf. THOMAS 1966).

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SUMMARY

Transduction tests were performed to determine the points of termination of 43 ditto deletions covering the *cysH*, *I* and *J* loci in the *cysC* region of the *Salmonella typhimurium* chromosome. A preliminary analysis of ten additional ditto deletions was carried out by DEMEREC, GILLESPIE and MIZOBUCHI (1963). The left end of each of the deletions is located in a restricted segment of the *cysC* "silent section" comprising about 16% of the long silent section and about 8% of the entire *cysC* region. Different deletions terminate at different positions within the restricted segment.

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