GENETIC STRUCTURE OF THE *cysC* REGION OF THE SALMONELLA GENOME[1]

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THE five cysteine-controlling regions that have been identified in the *Salmonella typhimurium* genome are described in an earlier paper (MIZOBUCHI, DEMEREC, and GILLESPIE 1962). These regions are called *cysA*, *B*, *C*, *E*, and *G*, each designated by the symbol for one of the gene loci it carries. The *cysC* region, which includes a cluster of five gene loci (*cysC*, *D*, *H*, *I*, and *J*), is unusually interesting because multisite or deletion mutations occur very frequently there and because a large percentage of them are similar. This paper gives the results of a genetic analysis of region *cysC*.

MATERIALS AND METHODS

More than 400 *cysC* single-site mutants, and 95 deletion mutants, are available. All the deletion mutants and about 280 of the single-site mutants were utilized in the experiments to be described. Most of them originated in strain LT-2, either spontaneously or induced by X-rays, neutrons, ultraviolet radiation, 2-aminopurine, or nitrous acid. A few of the spontaneous mutants originated in strain LT-7.

The minimal medium contained: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4$, 0.05 g; $(NH_4)_2SO_4$, 1.0 g; sodium citrate, 0.47 g; glucose, 5.0 g; distilled water, 1000 ml. Enriched minimal medium was prepared by adding 0.01 percent dehydrated nutrient broth, and double- or triple-enriched by adding 0.02 or 0.03 percent broth powder. When the medium was supplemented with various sulfur compounds, the final concentration was 2×10^{-4} molar with respect to sulfur (SM).

Recipient bacteria for the transduction experiments were cultured overnight in nutrient broth in a shaker, infected with phage grown in the donor bacteria, and plated on a suitable medium. The multiplicity of infection was usually about five.

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The nutritional requirements of mutants of the *cysC*, *D* and *H* loci can be satisfied by either a sulfite or cysteine sulfinic acid (CSA), but those of *cysI* and *J* cannot. This distinction between mutants of different loci of the same region has proved useful in studies of frequency of recombination within the *cysC* region. When *CysI* or *J* mutants as recipients and phage from either *cysC*, *D*, or *H* mutants as donors are plated on minimal medium partially supplemented with CSA (about 25 percent of the required 2×10^{-4} SM), both donor-type and recombinant transductants can be recognized, because the donor-type colonies are considerably smaller than those of the wild-type recombinants. The donor class can also be identified by plating on medium fully supplemented with CSA and replica-plating on minimal medium. Frequency of recombination between two markers can therefore be expressed in terms of ratio of the recombinant (wild-type) class to the total of recombinant and donor classes; and thus the distances between markers can be determined more precisely than in calculations based on the value of the recombinant class alone. However, in many experiments the ratio method could not be employed, and mapping was done by the frequency of transductions.

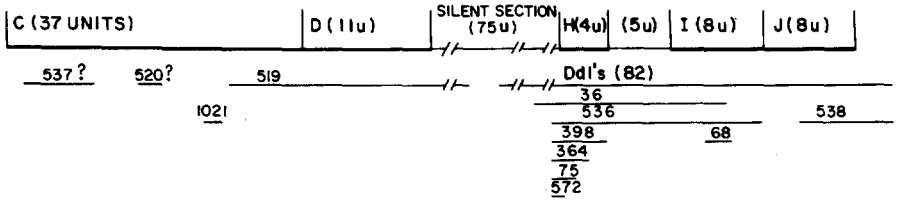
RESULTS

Properties of the cysC *region:* The work of OZEKI (1959) showed that, as far as phage PLT-22 is concerned, transduction fragments representing any particular portion of the bacterial chromosome are similar in extent. One such fragment carries the entire *cysC* region, within which the five *cys* loci are arranged in the order *C*, *D*, *H*, *I*, *J* (MIZOBUCHI *et al.* 1962). Judging by the frequencies of recombinants obtained in transduction of these various *cys* mutants with phage from the wild type, it seems probable that the *cysC* region is located near the center of the transducing fragment, because the frequency of recombination at each side of the region is high (see wild-type columns in Tables 2 and 4). Thus the fragment contains the *cysC* region at its center, and a section on either side in which genetic markers have not yet been detected.

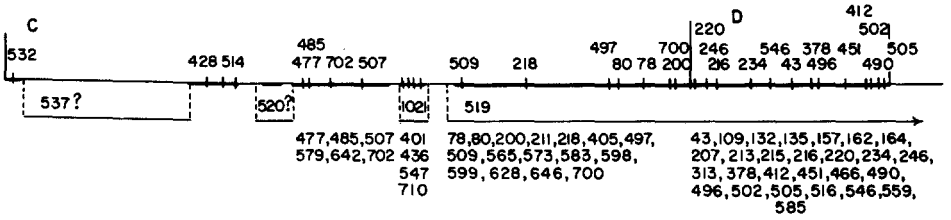
Genetic maps of the *cysC* region are shown in Figure 1. Figure 1A is a general map, indicating the approximate lengths of the five loci, and the approximate positions of deletions. B, C, and D are detailed maps of the different parts, showing the probable locations of a number of single-site mutations. By means of crosses between the single-site mutants and various deletion mutants, the single sites were first located in short sections, and then the order within each section was tentatively determined by studies of frequencies of recombination in crosses with closely located mutants (Tables 1–4).

FIGURE 1.—Genetic map of the *cysC* region, which represents a cluster of five loci: *cysC*, *D*, *H*, *I*, and *J*. The lengths of the different parts are expressed in arbitrary units derived from frequencies of recombination between appropriate markers. A: General map of the region, indicating the positions of 82 ditto and 12 ordinary deletions. B to D: Detailed maps of the various sections, showing the extents of the deletions and the approximate positions of certain single-site mutations. The map of the long silent section (C) marks the left ends of 18 ditto deletions. Deletion *–710* covers the marker *–547*.

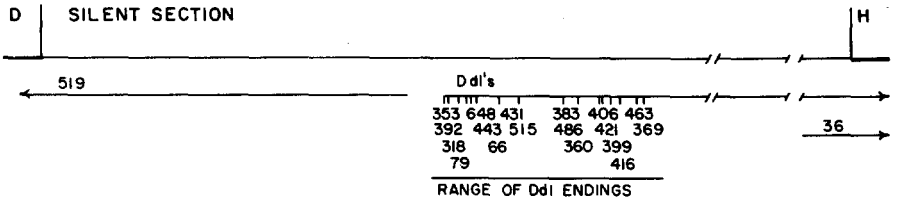
A. GENERAL MAP OF cys C REGION



B. MAP OF cysC AND D loci.



C. MAP OF SILENT SECTION



D.MAP OF cysH, I, AND J loci

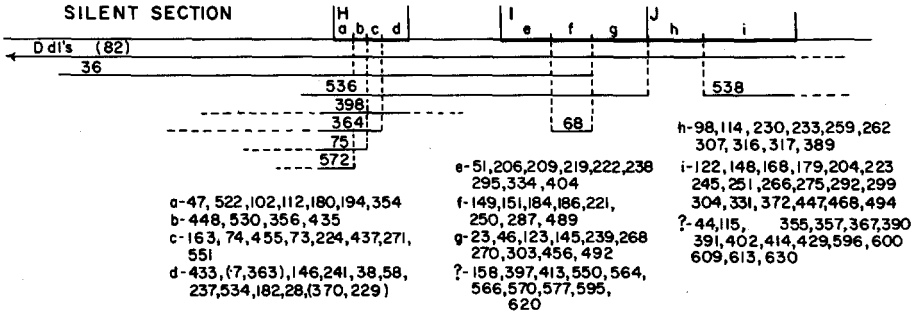


TABLE 1

Recombination between markers located on opposite sides and on the same side (figures in italics) of the silent section

Recipient	Donor				
	C–509	*D–490*	*H–522*	*I–238*	*J–299*
D–490	*171*	....	....	8205	3129
H–522	3618	4422	....	*949*	*1266*
I–295	3147	3562	*323*	*710*	*1659*
argF–50	413	638	310	718	960

Loci *C* and *D* are to the left of the section and loci *H*, *I*, and *J* to the right; *argF–50* is located in a different transducing fragment. In the body of the table are given numbers of recombinants per five Petri dishes, each plated with about 5×10^8 bacteria and 2.5×10^9 phage particles.

Map A shows the approximate lengths of the different parts of the *cysC* region, expressed in arbitrary units derived from frequencies of recombination, the total length of the region being 148 units. The most striking feature of the map is the long section—long, that is, in terms of recombination frequency—between loci *cysD* and *H* (Table 2). It measures about 75 units, just slightly longer than all the rest of the region; and yet no single-site mutations to auxo-

TABLE 2

Percentages of recombinants among donor and recombinant classes in crosses between ditto deletions and HI *deletions as recipients and* dl–CD–519 *and* cysD–313 *as donors. Column 5 gives data obtained in crosses with the wild type, in each of which about 6×10^8 bacteria were infected with a phage multiplicity of 5*

Recipient	Donor				
	dl–CD–519		*cysD–313*		
	Total	Percent recombinant	Total	Percent recombinant	Wild type
Ddl–66	3507	3.9	5311	38.1	827
–79	2850	3.2	3712	27.3	1000
–318	2996	2.2	5621	31.7	....
–353	8593	1.5	310	24.8	1127
–360	2189	9.4	594	34.1	1121
–383	3219	9.3	1406	31.4	....
–392	9012	1.6	2894	22.7	849
–399	1882	11.3	685	32.3	1249
–421	1662	10.8	591	34.0	983
–431	2190	5.5	166	36.2	669
–515	979	6.4	1543	37.1	980
dl–HI–36	136	45.5	206	58.2	1246
–536	2484	53.1	136	59.5	2065

The chi-square value of 1123 with ten degrees of freedom leaves no doubt that the ratio of recombinants to nonrecombinants is different for different ditto deletions crossed with *dl–CD–519*. The individual chi-square comparisons at the five percent level of significance indicate that eleven ditto deletions fall into five groups: (1) 66, 79; (2) 318; (3) 353, 392; (4) 360, 383, 399, 421; and (5) 431, 515. Recombinant percentages within each group are not significantly different from each other, but those between groups are. (We are much indebted for this analysis to Mr. Keith H. Thompson, statistician at the Brookhaven Laboratory).

trophy have been located in it, although about 400 have been found elsewhere in region *cysC*. In this sense, we call the section "genetically silent."

Of course it has been well substantiated that frequency of recombination is not always directly correlated with the assumed physical distance between markers. In the X chromosome of *Drosophila melanogaster*, for example, the frequency of recombination between *y* and *pn* is 0.8 percent, and the section between is represented on the salivary-gland-chromosome map by about 25 bands, whereas the frequency of recombination between *w* and *ec* is five times as high (four percent) within a section represented by only about 18 bands (BRIDGES and BREHME 1944). It is known, too, that certain alleles of a locus may recombine with much higher frequencies than other alleles (DEMEREC, GOLDMAN, and LAHR 1958; BALBINDER 1962). Several *cysC* and *cysH* alleles demonstrating such behavior will be mentioned later in this paper. The question therefore arises: Do the observations regarding the silent section reflect (a) simply a high frequency of recombination between certain markers; (b) the presence of a section of material, as long as the rest of the *cysC* region, that carries no genetic information; (c) the presence of a section carrying genetic information that controls properties not detectable by our methods; or (d) a combination of these possibilities? We now have evidence that eliminates (a), leaving (b), (c), and (d) to be considered. The question of the silent section will be further discussed later on in this paper.

The loci *cysC* and *cysD* appear to be adjacent to each other, as do *cysI* and *cysJ* at the other end of the region; whereas another silent section, five units long, separates *cysH* from *cysI* (Table 3).

Ditto deletions: Of the 95 deletions available for study in the *cysC* region, 82 are very similar. Their left ends terminate in the long silent section, and since their right ends cover all the known sites of locus *J* it seems very likely that they extend beyond the locus. These deletions, constituting a fairly homogenous group and appearing to be close copies of one another, have been called "ditto" deletions (*Ddl*) to distinguish them from the 13 ordinary deletions (*dl*). In this paper the symbol for a deletion precedes the mutant symbol, for example, *Ddl–cys–66* or *dl–cys–68*. Since we are here considering cysteine mutants almost exclusively, the symbols may be abbreviated, as *Ddl–66*, *dl–68*.

All the ditto deletions complement mutations of the *cysC* and *D* loci but not those of *cysH*, *I*, or *J*, an evidence that the left ends of these deletions do not

TABLE 3

Percentages of recombinants among the donor and recombinant classes in crosses between cysI–295 *as recipient and* cysH–182 *and* dl–H–398 *as donors*

	Donor			
	cysH–182		*dl–H–398*	
Recipient	Total	Percent recombination	Total	Percent recombination
cysI–295	746	9.9	397	5.3

cover any *cysC* and *D* sites critical for the expression of gene function, but do cover the critical sites of the other three loci. They originated independently of each other in extensive experiments designed to study the capacity of different mutagens to induce deletions. Preliminary report of that study has been published (DEMEREC 1960) while a detailed report is in preparation.

Other deletions: The positions of the 13 ordinary deletions are indicated on the maps of Figure 1. Eleven of them cover several known sites, whereas two (*–537* and *–520*) do not include any site identified so far. These two are classified as deletions (with a question mark) because the mutants do not revert, either spontaneously or after treatment with potent mutagens, and also because recombination experiments with adjacent markers indicate that each of the changes involves a section of chromosome more extensive than a locus site.

Properties of the silent sections (Figure 1A and C): The results of transduction experiments made with several single-site markers and deletion mutants show that the central silent section is of considerable length. Crosses between markers located on opposite sides of the section yielded much higher frequencies of recombination than those between markers located on the same side. Data from some of these experiments are given in Table 1. Crosses (Table 2) of *cysD–313* (located near the right end of the locus), and of *dl–CD–519*, with several deletions covering loci *H*, *I*, and *J* show: (1) that *dl–CD–519* gives fewer recombinants than *cysD–313* with ditto deletions and with *dl–HI–36* and *–536;* (2) that each deletion has a characteristic recombination frequency; and (3) that crosses between *dl–CD–519* and the ditto deletions yield considerably lower recombination frequencies than those between *dl–CD–519* and *dl–HI–36* or *–536*. From this evidence we can conclude (1) that *dl–CD–519* covers the left portion of the silent section; (2) that the ditto deletions cover considerably more of the right portion of the silent section than does *dl–CD–36* or *–536*; and (3) that the left ends of the ditto deletions are close together, but not at the same point, in the silent section. Frequencies of recombination of *dl–CD–519* with *Ddl–353* and *Ddl–392* are very low, indicating that the ends of these three deletions are close together and that *dl–519*, together with either *–353* or *–392*, almost covers the entire silent section.

The last column of Table 2 gives the results of crosses between the deletion mutants and the wild type (donor.) In each of these experiments, about 6×10^8 bacteria were infected with a phage multiplicity of five. If the differences in recombination frequencies observed in the crosses between the different *Ddl* mutants and *dl–519* were due to differences in the positions of the right ends of the ditto deletions, we would expect to see a correlation between the values of columns 2 and 5. None is evident.

A comparative growth study was made, to determine whether deletion mutants are at a disadvantage. The strains tested were three ditto-deletion mutants (*Ddl–66*, *–392*, and *–421*), three deletion mutants (*H–398*, *CD–519*, and *HIJ–536*), five single-site mutants (*C–200*, *D–313*, *H–433*, *I–270*, and *J–266*), and the wild type LT–2. Overnight cultures of bacteria, grown in a shaker in broth supplemented with 2×10^{-4} SM of cysteine, were centrifuged, washed once in

saline, and resuspended in saline. An aliquot of 0.06 ml of each suspension was inoculated into 6 ml of minimal medium supplemented with 2×10^{-4} SM of cysteine, and grown in a shaker at 37°C. Growth was determined by measuring the optical density at intervals with a Klett-Summerson Photoelectric Colorimeter. The generation time for all cultures in the log phase was found to be 45 ± 5 minutes, and the saturation point at about same level. Since the silent section is almost covered by deletions *dl–CD–519* and *Ddl–392*, these results show that the section does not carry any genes affecting growth under the conditions of the experiment.

The other, shorter, silent section, located between loci *cysH* and *cysI*, is—judging by the frequencies of recombination between appropriately spaced markers—slightly longer than the *cysH* locus, but only 1/15 as long as the large silent section. No single-site mutations have been found there. Table 3 shows the frequencies of recombination between *cysI–295*, leftmost known marker of the *cysI* locus, and *cysH–182*, rightmost known marker of the *cysH* locus, and between *cysI–295* and *dl–H–398*. These data indicate that the right end of *dl–H–398* extends some distance into the short silent section.

Topography of locus cysC (Figure 1B): In terms of recombination values the *cysC* locus is 37 units long, whereas the total length of the other four *cysC* loci is only 31 units; but fewer single-site mutations have been found in *cysC* than in any of the other four loci. Of the 173 single-site mutations listed in Figure 1, 28 are in *cysC*, 29 in *D*, 32 in *H*, 40 in *I*, and 44 in *J*. This finding indicates either that the *cysC* locus has an unusually high frequency of recombination between markers, that it has an unusually low frequency of recognizable mutations, or that it contains one or more short silent sections. A study of its topography makes the last-mentioned possibility seem the most probable.

Of the 33 *cysC* mutations that have been analyzed, 28 are single site, three are deletions (*519, 710, 1021*), and two are probably deletions (*520, 537*). Deletion *519* is long, covering about 4/11 of the *cysC* locus and extending through locus *cysD* into the silent section; *dl–1021* includes *dl–710* and three known single sites (*401, 436,* and *547*); *dl–710* covers only site *547*. The probable deletions *520* and *537* fulfill the primary requirements of deletions: that is, their mutants do not revert either spontaneously or after treatment with potent mutagens (diethylsulfate, 2–aminopurine, sodium nitrite, or proflavin), and in crosses they give rise to a lower frequency of recombinants than do adjacent markers (Table 4). They do not, however, cover any of the known single-site mutations.

Both ends of deletions *1021* and *710*, and of the presumed deletions *520* and *537*, as well as the left end of deletion *519*, are located in the left two thirds of locus *cysC*, indicating a high concentration of aberrant mutations in that section of the genome. The recombination studies (Table 4) have revealed a further anomaly: the mutations are not distributed at random along the section but are grouped in clusters (*428, 514; 477, 485, 702, 507, 579, 642, 702; 401, 436, 547*). Frequencies of recombination in crosses between mutants of the same cluster are much lower than those in crosses between mutants of different clusters. It

TABLE 4

Intercrosses between mutants whose sites are in the left portion of locus cysC, *outside of* dl–519

		Donor												
Recipient	Control	+	*532*	*537*	*428*	*514*	*520*	*477*	*485*	*702*	*507*	*1021*	*401*	*436*
dl–537	0	3947	1	*0*	44	46	62	378	494	380	386	183	190	209
–428	0	2452	298	22	*9*	9	13	117	186	120	102	55	41	78
–514	0	2670	604	27	21	*0*	43	346	422	325	375	183	128	152
dl–520	0	2260	160	115	164	206	*0*	327	367	197	340	55	97	92
–477	3	2976	745	369	772	609	129	*4*	2	54	62	169	182	192
–485	2	3138	761	391	759	591	107	9	*10*	70	79	180	136	165
–702	0	3277	966	966	903	785	132	62	97	*0*	18	143	195	167
–507	0	3224	959	387	686	648	165	49	64	12	*0*	174	160	156
–642	6	2608	688	353	641	588	49	145	168	54	102	137	174	185
dl–1021	0	2678	244	178	133	312	35	121	129	103	64	*0*	0	0
–401	0	1993	420	102	147	150	12	67	75	37	56	0	*1*	*7*
–436	4	2022	411	98	225	152	11	39	55	32	66	4	28	*6*

Mutants are listed in the order of their map positions (Figure 1B). In each experiment about 1.5×10^9 bacteria and 7.5×10^9 phage particles were plated on double-enriched medium.

seems as though the clusters were separated by silent areas (or areas of high recombination frequency). Such areas are also indicated between site *532* and the *428, 514* cluster, and between the *401, 436, 547* cluster and site *509*. And it seems hardly a coincidence that the ends of the deletions are located in these areas.

Topography of locus cysH (Figure 1D): Another locus of particular interest is *cysH*. According to the map (Figure 1A) it is the shortest of the five loci, only four units in length. It is separated from the others by the two silent sections. It is covered in its entirety by 82 ditto deletions and three of the ordinary deletions (*36, 536, 398*), and partially covered by another three (*364, 75, 572*). The right ends of these last three deletions fall within the locus, and their left ends (as well as those of *dl–36* and *dl–536*) in the long silent section, whereas *dl–398* begins in one silent section and ends in the other.

Analyses of the 32 *cysH* single-site mutations, by crosses with the overlapping deletions *398, 364, 75*, and *572*, have placed each of them in one of four sections —a, b, c, or d.

The method of mapping newly found cysteine mutants is to establish their nutritional requirements first, then cross them with markers representing the loci they might belong to. Most new mutants were readily mapped by this procedure. Three however—mutants *229, 370*, and *354*—required CSA, and therefore might have belonged to region *cysA, B*, or *C;* but intercrosses with appropriate testers produced more recombinants than crosses with the wild type. Only after crosses with the pertinent deletion mutants had been made was it possible to place the sites of these mutants in locus *cysH*. They are what we call aberrantly recombining mutants. Another similar mutant (*28*) was later identified with the same locus. The site of *354* is in section a, those of *28, 229*, and *370* in section d. Crosses between *229* and *370* produced no recombinants;

these two appear to be identical alleles, the result of mutations at the same site of the locus. The reason for the aberrant recombination frequencies is not known. The explanation proposed for the high frequencies of recombination observed between certain markers of locus *cysC* cannot be valid here, since high frequencies were observed only in crosses with *28*, *229*, *370*, or *354*, not in crosses between markers located on different sides of these mutations.

DISCUSSION

It seems probable that cysteine synthesis in Salmonella is controlled by 14 different genes (MIZOBUCHI *et al.* 1962; DREYFUSS and MONTY 1963). They are grouped on the chromosome in five clusters, which are carried independently of one another in transduciton. One of the clusters, *cysC*, includes the five gene loci *cysC*, *D*, *H*, *I*, and *J*, in that order. According to DREYFUSS and MONTY (1963), the metabolic blocks controlled by these genes are arranged as follows:

$$SO_4^{=}\text{—}D\text{—APS—}C\text{—PAPS—}H\text{—}SO_3^{=}\text{—}I \text{ and } J\text{—}S^{=}\text{—cysteine}$$

(APS = adenosine–5′phosphosulphate; PAPS = 3′–phosphoadenosine–5′phosphosulphate.) Thus it is evident that the *cysC* genes follow a pattern that is common in bacteria, whereby genes controlling related functions are clustered together. DREYFUSS and MONTY (1963) showed that the addition of cysteine to wild-type LT–2 bacteria represses coordinately the synthesis of enzymes controlled by *cysH*, *I*, and *J* loci, but does not affect the enzymes controlled by *cysC* and *D* loci. Thus it appears that there are two regulatory systems for the *cysC* region, one for *C* and *D;* and the other for *H*, *I*, *J*, loci. In that case the arrangement of *cysH*, *I*, and *J* loci on the chromosome follows the sequence of the biochemical reactions they control.

Recombination studies involving about 280 single-site mutants and 95 deletion mutants have revealed that the cluster of *cysC* genes is located in the central portion of the transducing fragment. Genes *C* and *D*, and also *I* and *J*, appear to be adjacent to each other. Between *D* and *H* there is a section, as long in terms of recombination as the remainder of the cluster, in which single-site mutations to auxotrophy have not been found (silent section Table 2). Another, considerably shorter, silent section separates the *H* and *I* loci (Table 3); and several very short silent sections are dispersed within the *C* locus. Of the 95 deletions, 82 (called ditto deletions) are similar, in that they include loci *H*, *I*, and *J* (Figure 1).

Properties and origin of ditto deletions: Three features characterize the ditto deletions: (1) So far they have been detected only in region *cysC*. (2) They are very similar in length, covering all known sites of loci *cysH*, *I*, and *J* and extending beyond *cysH* to the left (into the silent section) and beyond *cysJ* to the right. (3) They occur very frequently (40 percent) both among spontaneous mutations and among mutations induced by ultraviolet radiation, X-rays, fast neutrons, and sodium nitrite, but are not found among mutations induced by 2–aminopurine (DEMEREC 1960).

From the fact that ditto deletions are restricted to a certain region of the genome it may be concluded that a very specific condition is required for their origin, and that this requirement is fulfilled in the *cysC* region. Then the question arises: What properties of ditto deletions, in which they differ from other known deletions, may be responsible for their frequent occurrence? They differ strikingly from other deletions in two respects, namely, their length, extending over three loci, and the fact that both their ends are in sections having no known function (silent sections). The presence of silent sections on either side of a genetically definable section is very common in Salmonella, since every transducing fragment exhibits this characteristic; but long deletions, covering a whole genic section and extending into the silent sections flanking it, are very infrequent. It seems probable that such deletions can survive only in certain rare regions of the genome. *cysC*, then, is such a region, where long deletions not only survive but—as indicated by the growth curves of ditto-deletion mutants under the usual conditions of culturing—impose no disadvantage.

The following model is proposed to explain the origin of ditto deletions. We assume that either all or part of the silent section between *cysD* and *CysH* is a duplication of a section located to the right of the last known site in *cysJ*. Occasionally, close pairing occurs between the duplicated sections, producing a loop or ring. If such a loop or ring is formed during reproduction of the gene-string, the material within the loop will be skipped, giving rise to a deletion equivalent to that section.

Ditto deletions occur with a considerably higher frequency in strain LT–2 than in LT–7. Among 57 spontaneous *cysC* mutants of strain LT–2, 23 (40.3 percent) were due to ditto deletions, whereas in LT–7 only one *Ddl* was found among 24 spontaneous mutants. This difference may reasonably be explained by the earlier findings that most spontaneously occurring mutants of strain LT–7 are due to the presence of a mutator gene (MIYAKE 1959), which induces transition mutations (KIRCHNER 1960), known to be single-site changes (DEMEREC 1960).

Deletions similar to our ditto deletions have recently been described in the lactose region of *E. coli*, where about 30 percent of mutations were found to be long deletions (COOK and LEDERBERG 1962).

It is interesting to note that *lac*$^+$ mutations do not occur among our Salmonella auxotrophs, and that analyses of the *lac* region in Escherichia–Salmonella hybrids have led to the conclusion that *S. typhimurium* is deficient for the *lac* region of the chromosome (MIYAKE and DEMEREC 1960). It can therefore be assumed that this region is not essential to the life processes of bacterial cells—one of the conditions that we postulate to explain the origin and survival of long ditto deletions. The other condition, that the ends of such deletions are located in "silent sections," also seems to be exemplified in the COOK and LEDERBERG (1962) material.

Deletions similar to the *cysC* ditto deletions, except with regard to frequency of occurrence, have been described in one of the proline regions (MIYAKE and DEMEREC 1960) and in the *cysA* region (MIZOBUCHI *et al.* 1962). There, seven

deletions involving loci *proA* and *B* included two whose left ends were in a long section between loci *proB* and *C* and whose right ends terminated in a section so close to the end of the transducing fragment that infection of the mutants with phage grown in wild-type bacteria produced only a few recombinants. Two deletions are known in the *cysA* region, and both cover all known sites of the three complementation groups (dl–20 and –533).

Properties and origin of other deletions: Figure 1A shows the positions of the 13 "ordinary" deletions represented among our 95 deletion mutants. It is apparent that they are not distributed randomly in the *cysC* region, since eight of them have one end located either in the long silent section (*519*, *36*, *572*, *75*, *364*, *398*, and *536*) or in its presumed duplication lying beyond *cysJ* (*538*). The fact that over 60 percent of these deletions end (or begin) in the same section as the ditto deletions makes it seem likely that a large proportion of them originate through a similar mechanism, namely, the formation of loops initiated by duplications.

The frequency of occurrence of these ordinary deletions may be due to the fact that either one or both of the duplications responsible for them are short, since it seems reasonable to expect a close correlation between frequency of pairing and the length of duplicated sections. It is also possible that frequency of pairing is influenced by the folding of the DNA string. According to this model, nonrandom distribution of duplications is responsible for the nonrandom distribution of deletions.

Nature of the silent section: The main findings and conclusions about the long silent section are as follows. (1) Frequencies of recombination between markers located to either side of this section are high, as high as the total recombination frequency within other parts of region *cysC*. (2) No mutations to auxotrophy have been detected in this section. (3) The deletion *CD–519* on the left and the ditto deletion *–392* on the right together cover at least 95 percent of the section, but mutants carrying either of these deletions do not suffer any detectable disadvantage in growth rate by comparison with strains carrying single-site *cys* mutations. This finding suggests that other genes critical under the growth conditions listed are not likely to be present in the section. (4) All 82 ditto deletions, as well as seven of 13 ordinary deletions, have one end within the section. These four conclusions, supported by experimental evidence, are supplemented by a fifth, implicit in the working hypothesis: that the silent section is able to synapse with a long section located beyond the right end of locus *cysJ* (to produce ditto deletions) and with several small sections in the *cysC* region (to produce most of the ordinary deletions).

The available information allows us to speculate about the properties of the silent section. Supposing it to be a duplication, we would expect it to be silent regardless of whether its DNA is coded or uncoded; if the section is coded, a mutation occurring within it would not be detectable because it would be covered by the normal structure of the other duplicate section. We are inclined to assume that the silent section is made up of DNA different from that of the genic parts of the region, and that the nature of the DNA is responsible for the silence and

partially responsible for the high recombination frequencies. We also think that this special DNA is not differentiated—that it carries no genetic code, and is able to synapse with similar DNA, which in our material has been detected in a large amount near the right end of the *cysC* region and in smaller amounts scattered throughout the region. Furthermore, we assume that the undifferentiated DNA is so constructed that any portion of it is homologous to any other portion and synapsis may thus occur between any of its parts. In many ways such DNA can be compared to the heterochromatic regions of chromosomes of higher organisms, particularly Drosophila, where the evidence indicates, in addition to large heterochromatic sections located in the spindle-fiber-attachment regions of all the chromosomes, a scattering of small sections throughout the chromosomal complex (HANNAH 1951). DNA composed of polynucleotide pair sequences $\frac{\text{AAA---}}{\text{TTT---}}$ or $\frac{\text{GGG---}}{\text{CCC---}}$ would fulfill all the requirements of our model.

SUMMARY

The genes controlling cysteine synthesis in *S. typhimurium*, probably 14 in number, are arranged in five independently transducing clusters. One of these clusters, region *cysC*, contains five genes arranged in the order *cysC–D–H–I–J*. *C* 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*. Studies involving 280 single-site mutants and 95 deletion mutants have shown that the single-site mutations are distributed equally among the five loci. Of the deletions, 82 are long and similar in extent (ditto deletions), covering loci *H*, *I*, and *J*. The other 13 deletions are shorter. All the ditto and seven of the ordinary deletions have one end in the longer of the two silent sections. The discussion proposes that the long silent section is a duplication of a section beyond locus *cysJ*; that the duplicated sections synapse to create a loop; and that during replication the material within the loop is excluded, producing a deletion. It is suggested that the duplicated material is nongenic, comparable to the heterochromatin found in Drosophila and other higher organisms.

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