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*ORIENTATION OF THE HISTIDINE OPERON IN THE SALMONELLA TYPHIMURIUM LINKAGE MAP**

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Communicated by M. Demerec, April 27, 1965

A short segment of the Salmonella chromosome, the histidine operon,[1, 2] is considered to function as a unit structure in transcription from DNA to messenger RNA.[3, 4] Organized translation of this multigenic messenger molecule is then presumed to result in the formation of each of the proteins utilized in the ten reactions specific to the pathway of L-histidine biosynthesis in Salmonella. Some features of genetic fine structure of the histidine operon have been elucidated by means of transduction tests.[1, 2] The histidine operon is a polarized structure with one end, the "operator (*hisO*) end," arbitrarily considered as the "beginning end" in this report.[1, 4] The location of the histidine operon in the bacterial linkage map has been determined from results of bacterial crosses performed utilizing either colicine-factor-mediated conjugation[5] or conjugation between *Hfr* donors and suitable recipient bacteria.[6] While the histidine operon appears to be a linear segment of a much larger linear structure, the bacterial chromosome, neither published experiments involving the genetic fine structure nor those concerned with the gross chromosomal positioning of the histidine operon has given indication of the *orientation* of the histidine operon with relation to other genes on the chromosome. Knowledge of the orientation is desirable since, for example, each operon may not function completely independently of certain neighboring operons. Furthermore, one questions whether a single one of the two DNA strands is transcribed for a considerable portion or the totality of its length, or whether transcription proceeds in a more complicated fashion.[7]

The present report describes results of colicine-factor-mediated genetic crosses which indicate that the histidine operon is oriented in a "clockwise" direction in the chromosome of Salmonella as it is customarily drawn.[6] Dr. Kenneth E. Sanderson independently has arrived at a similar conclusion, based on F-factor-mediated conjugation tests.[7]

Materials and Methods.—Table 1 lists the strain numbers and genotypes of the derivatives of *Salmonella typhimurium*, strain LT-2, used in this study. Wild-type LT-2 is streptomycin- and azide-sensitive and possesses somatic antigens 4, 5, 12 and flagellar antigens i; 1, 2. The wild type has no requirements for the exogenous growth factors shown in Table 1 and can ferment each of the sugars listed. Strains with SL stock numbers were generously provided by Dr. B. A. D. Stocker, Lister Institute for Preventive Medicine, London. Three of these strains contain genes for flagellar antigens that have been transduced into *Salmonella typhimurium* from other Salmonella species.[8, 13] Strain SB93 is derived from strain SL802; the *metG319* mutation was transduced into SL802 with P22 phage, and a methionine-requiring transductional clone, genetically identical to *metG319* in transduction tests, was isolated following penicillin selection.

TABLE 1

BACTERIAL STRAINS

Strain #	Markers													Episome
	str	*H2*	*purG*	*metG*	*05*	*his*	*H1*	*try*	*gal*	*azi*	*inl*	*metA*	*rha*	*mtl*	
SL689	*s*	*enx*	+	+	+	+	*b*	*B2*	+	*s*	+	*22*	+	+	(*colI*)
SL862	*s*	*enx*	+	+	−	*D27*	*a*	*A8*	+	*s*	+	+	+	−	(*colE1*)
SL811	*s*	*enx*	+	+	+	*A870*	*b*	*B2*	*411*	*r*	*411*	*22*	−	+	(*colE1*)
SL802	*r*	*1, 2*	*2*	+	+	*E35*	*i*	+	+	*s*	+	+	+	+	
SL803	*r*	*1, 2*	*2*	+	+	*D37*	*i*	+	+	*s*	+	+	+	+	
SB93	*r*	*1, 2*	*2*	319	+	*E35*	*i*	+	+	*s*	+	+	+	+	(*P22*)

Bacteria are assessed as sensitive (*s*) or resistant (*r*) to streptomycin (*str*) and azide (*azi*). The flagellar proteins elicited by the phase 1 locus (*H1*) and the phase 2 locus (*H2*) are listed by serotype. The presence (+) or absence (−) of somatic antigen 5, at least in part controlled by locus *05*, is noted. Mutations leading to auxotrophy or to inability to ferment a sugar are indicated by a minus sign (−) or an allele number for various mutants. The symbols refer to galactose (*gal*), histidine (*his*), inositol (*inl*), methionine (*met*), mannitol (*mtl*), purines (*pur*), rhamnose (*rha*), and tryptophan (*try*).[7] The gene loci are listed in their probable order on the circular linkage map of Salmonella.[5, 6] Where episomal genetic determinants have been added to the genetic content of *Salmonella typhimurium* strain LT-2, these are indicated by *col* for colicinogenic factors[5, 9, 10] and *P22* for phage P22.[2]

Conjugation mediated by colicinogenic factors (*colI*) (*colE1*) was performed as described by Stocker and collaborators.[5, 9] In all crosses, strain SL689 served as the donor of (*colI*).[10] The male parents, donors of chromosomal genes, contained (*colE1*), [10,11] and the recipients were non-colicinogenic for these colicines (Table 1). The recipient bacteria used in cross IV (Table 2) were passed through semisolid medium[12] immediately prior to the experiment in order to facilitate procurement of freely motile recombinant bacteria. In each of the crosses, both the (*colI*) donors and the male parents were eliminated by the presence in the minimal plating medium[2] of streptomycin and, in most crosses, also by the absence from the selective minimal plating medium of histidine or of methionine, or of both. The methionine marker selected against, *metA22*, is not closely linked with the histidine loci and, therefore, rarely transmitted with the histidine genes in colicine-mediated crosses.[5, 13] The female recipients were contraselected by the absence from plating medium of histidine; all others markers (*H2*, *purG*, *metG*, *05*, *H1*, *try*, *gal*, *azi*, *inl*, *rha*, and *mtl*) served as unselected markers. Glucose (0.2%) served as carbon source, and essential growth factors were added at a final concentration of 20 μg/ml. Because of the nature of the selection and the rarity of recombinant clones (about 10^{-7} per input male), the *his*$^+$ recombinant classes (Table 2) may contain spontaneous mutations of the female recipient to the wild-type phenotype. Individual control platings of recipient bacteria, and of SL689-male donor mixtures, showed that less than 10 per cent of the *his*$^+$ clones observed on recombination plates could have arisen by mutation.

The genetic analysis depends upon the association of the unselected markers among the selected class of recombinants. Markers essentially unlinked in colicinogenic transfer[5] (e.g., *rha* or *mtl*) were always scored among the *his*$^+$ clones to confirm the polarity of the cross (i.e., from male to female). The *azi* and *inl* markers are difficult to score and were thus omitted from the analysis. The selection plates were incubated for 48 hr at 37°C and colonies picked off with sterile toothpicks onto master plates of the same selective medium. The masters were incubated overnight and then replica-plated onto appropriately supplemented minimal media, eosin methylene blue plus 1.0 per cent galactose or mannitol, and deoxycholate plus 1 per cent rhamnose plates to define the nature of the unselected markers, i.e., whether contributions from the donor parent or contributions from the recipient parent. In crosses I and IV (Table 2), recombinant clones were streaked out on fresh plates of the selective medium for purification of individual clones. In cross I, the purified recombinants were individually tested by slide agglutination[12] for the presence or absence of somatic antigen 5. Monospecific immune serum, prepared in rabbits against heat-killed cells of the wild-type strain LT-2,[12] was exhaustively cross-adsorbed with cells of a motile transductional clone of SL861, containing flagellar antigens i; 1, 2 and lacking somatic antigen 5. In cross IV, the purified clones were tested by slide agglutination with monospecific antiflagellar immune sera supplied by Dr. L. S. Baron, Walter Reed Army Institute of Research, Washington, D. C. Where only the phase 2 antigen was detected in the recombinant clones, the recombinants were stabbed into semisolid medium[12] containing immune serum specific for phase 2. Swarms, obtained in this medium and presumedly expressing phase 1 antigen, were then inoculated into broth, grown overnight, and analyzed for flagellar antigen content by slide agglutination tests. Flagellar markers were not scored in crosses I, II, or III (Table 2).

Results.—The upper portion of Table 2 summarizes the classes of recombinant

TABLE 2

RECOMBINANTS OBTAINED IN COLICINE-FACTOR-MEDIATED BACTERIAL CROSSES

Genotype	Number found	No. Crossovers Required If Histidine Genes Are Oriented: Clockwise, EIFAHBCDGO	Counterclockwise OGDCBHAFIE
Cross I: SL862 (×) SL802		Selected for *str-r his⁺ met⁺*	
his⁺	12	2	2
05⁻his⁺	10	2	4
purG2⁺ 05⁻his⁺	7	2	4
purG2⁺ his⁺	3	4	4
05⁻his⁺ tryA8	2	4	6
purG2⁺ his⁺ tryA8	1	6	6
Cross II: SL811 (×) SL802		Selected for *str-r his⁺ met⁺*	
his⁺	57	2	2
his⁺ tryB2	12	4	2
pur G2⁺ his⁺	8	2	4
purG2⁺ his⁺ tryB2	2	4	4
Cross III: SL811 (×) SL803		Selected for *str-r his⁺ met⁺*	
his⁺	36	2	2
his⁺ tryB2	16	2	4
pur G2⁺ his⁺	9	4	2
purG2⁺ his⁺ tryB2	7	4	4
Cross IV: SL811 (×) SB93		Selected for *str-r his⁺*	
his⁺	264	2	2
met G319⁺ his⁺	17	2	4
purG2⁺ his⁺	2	4	4
Total recombinants (with two or more markers from donor):			
Double crossovers		58	21
Quadruple crossovers		37	72
Sextuple crossovers		1	3

clones detected in each of four crosses. The first column lists the genotypic contribution of the donor parent found among the *his⁺* recombinants. The markers are listed in the order of their positions in the Salmonella linkage map.[5, 6, 13] All other markers scored were those of the recipient.

The second column in Table 2 lists the number of recombinants found in each genotypic class. The third and fourth columns list the number of recombinational events (crossovers) necessary to secure each recombinant class if: (1) the histidine operon is oriented in a clockwise fashion, that is, in, the order *metG-hisE-I-F-A-H-B-C-D-G-O-try* (third column), or (2) the histidine operon is oriented in a counterclockwise direction, that is, in the order *metG-hisO-G-D-C-B-H-A-F-I-E-try* (fourth column).

The recombinant classes containing only *his⁺* are uninformative since they could arise through double crossovers regardless of the orientation of the operon. The more infrequent classes of recombinants containing two or more markers from the donor, however, are pertinent to the analysis. The lower portion of Table 2 tabulates the results of this analysis. The data indicate that quadruple crossover classes are found frequently. However, if one assumes that crossover classes of higher orders are less frequent than those of lower orders, only one orientation of the operon is favored.

We conclude that the histidine operon[1, 2] of *Salmonella typhimurium* is oriented in a clockwise direction, i.e., in the order *gal-try-H1-hisO-G-D-C-B-H-A-F-I-E-metG-purG-str-metA*, in the linkage map as usually drawn.[6] The results of preliminary experiments by Stocker,[13] using the same methods as ours, also favor this

orientation; and Sanderson[7] has utilized a different methodology in reaching a conclusion in accord with ours.

* This work was supported in part by research grant AI 01650, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. We thank Drs. Kenneth E. Sanderson and M. Demerec for information regarding unpublished results, Dr. B. A. D. Stocker for supplying most of the strains used in this study as well as valuable instruction in performing colicine-factor-mediated crosses, and Dr. L. S. Baron for supplying monospecific immune sera used in the analysis of flagellar antigens.

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*INFORMATION TRANSFER IN SALMONELLA TYPHIMURIUM**

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Communicated by M. Demerec, April 27, 1965

A considerable body of data suggests that information transfer, both transcription and translation, occurs in a polarized manner. In transcription this implies that information transfer begins at one end of the operon and proceeds to the other end. The polarity of operons may be determined using either operator[1] or polarity[2] mutants, which affect the transfer of genetic information in one direction from their site. Studies of these mutants in the histidine (*his*),[2] tryptophan (*try*),[3] and leucine (*leu*)[4] operons of *Salmonella typhimurium* have indicated the polarity of these operons with respect to other loci in the P22 transducing fragment in which each operon occurs, but the transduction methods used in these studies could not reveal the orientation of these transducing fragments with respect to the entire chromosome. Our investigations, which have used F-factor-mediated conjugation, have demonstrated that on the circular genetic map of *S. typhimurium*[5] the *his* and *try* operons