# A FINE STRUCTURE MAP OF THE SALMONELLA HISTIDINE OPERATOR-PROMOTER

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ABSTRACT

Over 100 regulatory mutations linked to the histidine (*his*) operon of *S. typhimurium* have been isolated. They all map in a region estimated to be several hundred base pairs in length located at one end of the *his* operon ("the *hisO* region"). The mutations are located at sixteen recombinationally separable sites or are deletions encompassing several sites. Data obtained from pairs of reciprocal three-point tests show that "constitutive" (high enzyme levels) and "promoter-like" (low enzyme levels) *hisO* mutations are interspersed on the genetic map. In a few crosses, recombination was not observed to occur between markers shown to occupy different sites based on behavior in other recombination tests.

THE histidine operon of *Salmonella typhimurium* is comprised of nine contiguous genes with a control region (*hisO*) located at one end (HARTMAN *et al.* 1971). The location of the control region has been determined by chromosomal rearrangements (AMES, HARTMAN and JACOB 1963), polarity (AMES and HARTMAN 1963), deletion mapping (ROTH, ANTÓN and HARTMAN 1966; ATKINS and LOPER 1970), and by three-point tests (FINK, KLOPOTOWSKI and AMES 1967; HARTMAN *et al.* 1971).

This paper describes the isolation and mapping of mutations in the control region (*hisO*). Two basic types of regulatory mutations have been found to map within the *hisO region.* Mutations which cause elevated levels of *his* operon expression under conditions of repression have been designated "constitutive *hisO* mutations". Mutations of the second type, which cause reduced levels of *his* operon expression in the presence of an unlinked regulatory mutation, have been designated "promoter-like *hisO* mutations." As demonstrated in the accompanying paper, both designations are working definitions and encompass mutations causing diverse phenotypes. These *hisO* mutations have been used to construct a fine structure map of the *hisO* region by means of pairs of reciprocal three-point tests. The map of the *hisO* region contains an interspersion of constitutive and promoter-like *hisO* mutations. A preliminary report of some of this work has been presented (FANKHAUSER, ELY and HARTMAN 1971).

In the accompanying paper (ELY 1974) the *hisO* mutations are shown to affect

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a polynucleotide segment proximal to the first structural gene of the operon. The effects of these mutations on *his* operon expression are examined under various conditions of repression and derepression, and a generalized model is proposed for the structure of the *hisO* region.

## MATERIALS AND METHODS

*Bacteria, phage, and transduction tests:* Bacterial strains used in this study are listed in Table 1 in a fashion that allows discernment of strain lineages and, then, genetic backgrounds and origins. P22 mutants *int-4* (SMITH and LEVINE 1967) or *int-4 cly-2* (ELY *et al.* 1974) were used for transduction experiments performed according to the direct-plating method of ELY *et al.* (1974). Phage grown on a deletion of the *hisO* region (*hisOG203*) were used as inocula in the preparation of transducing lysates to prevent any chance of carry-over particles containing the *hisO* region.

*Media:* Difco-nutrient broth supplemented with 5 g/L NaCl was used for all strains except those containing episomes. Episome-containing strains were grown in minimal medium under conditions which would select for the presence of the episome. Minimal medium was the E medium of VOGEL and BONNER (1956) supplemented with 0.2% (low) glucose or 2% (high) glucose. Enriched minimal medium was E medium supplemented with 1.25% (v/v) nutrient broth. Amino acid supplements were added when required as follows: L-histidine, 0.1 mM; L-histidinol, 1 mM; others, 20 μg/ml. Thiamine and nicotinic acid were added at concentrations of 1 μg/ml. Solid medium was made by the addition of 1.5% agar.

Broth for growth of phage consisted of Difco-nutrient broth enriched per 100 ml with 3.5 ml 40% glucose and 1.5 ml 50-times concentrated E medium (HONG and AMES 1971).

*Qualitative techniques which estimate* his *operon enzyme levels:* Several techniques allow a rapid estimation of the degree of expression of the *his* operon and therefore identify the presence of specific *hisO* mutations. Variations in these techniques also allowed selection for and recognition of various *hisO* mutants as described below:

i) AMT (3-amino-1,2,4-triazole) inhibits the dehydratase activity of the *hisB* enzyme (HILTON, KEARNEY and AMES 1965), and sensitivity to AMT can be used to identify strains with limited expression of the *his* operon. Strains unable to derepress to approximately three times the repressed level of wild type are sensitive to AMT. AMT sensitivity was tested by streaking overnight broth cultures of test strains across a minimal high glucose plate perpendicular to a streak of 3 M AMT. Plates were incubated at 37° for 24 hours and areas of growth inhibition were compared. The size of the inhibition zone increased when the maximum level of expression of a sensitive strain decreased.

ii) TA (D,L-2-thiazolalanine) mimics L-histidine in inhibiting the feedback-sensitive *hisG* enzyme (MOYED 1961). Like AMT, sensitivity to TA can be used to identify strains with a limited expression of the *his* operon. Strains unable to derepress to a level corresponding to ten times the wild-type repressed level are sensitive to TA. Thus, strains can be resistant to AMT but sensitive to TA. TA sensitivity was tested in a manner similar to that used for AMT, except that the TA streak was made from two applications of a 0.1 M solution of TA, the limit of TA solubility in aqueous solution.

iii) A wrinkled colony morphology (VOLL 1967) is caused by elevated levels of the *hisH* and *hisF* enzymes (MURRAY and HARTMAN 1972). Colony morphology was observed with a binocular microscope by viewing the colonies either by oblique transmitted or by reflected light (Figure 1). A slightly "grainy" colony morphology occurs on high glucose plates at enzyme levels 4 to 5 times the wild-type repressed level. The degree of wrinkledness continues to increase with increasing enzyme levels until it reaches a maximum at approximately 15 times the wild-type repressed level.

iv) The inability of a strain to derepress normally was determined by transducing strain SB2700 to prototrophy with phage grown on the strain in question. SB2700 contains a regulatory mutation (*hisT1504*), a *his* promoter mutation leading to histidine auxotrophy (*hisO2321*), and

TABLE 1

*Bacterial strains*

| Strain | Genotype | Derivation or reference |
|---|---|---|
| *Salmonella typhimurium* LT-2 | | |
| | *ara-9* | P. E. HARTMAN, Stock Culture |
| | *hisD1* | HARTMAN *et al.* (1971) |
| | *hisODG63* | HARTMAN *et al.* (1971) |
| | *hisG200* | HARTMAN *et al.* (1971) |
| | *hisOG203* | AMES, HARTMAN and JACOB (1963) |
| | *hisOG1302* | 203–262 in AMES, HARTMAN and JACOB (1963) |
| DA10 | *hisR1223 hisT1501* | D. N. ANTÓN |
| SB949 | *hisO1828 hisG46 fla-2055* | SB6828 phage × SB5001 |
| SB951 | *hisO1830 hisG46 fla-2055* | SB6830 phage × SB5001 |
| SB952 | *hisO1831 hisG46 fla-2055* | SB6831 phage × SB5001 |
| SB953 | *hisO1832 hisG46 fla-2055* | SB6832 phage × SB5001 |
| SB2046 | *hisOG1302 hisT1504* | J. H. WYCHE (*his-1302* phage × TA516) |
| SB2047 | *hisO3148 fla-2055* | SB3193 phage × SB3095 |
| SB2049 | *hisO3149 fla-2055* | SB3194 phage × SB3095 |
| SB2051 | *hisO3150 fla-2055* | SB3195 phage × SB3095 |
| SB2053 | *hisO3148 hisG46 fla-2055* | SB3193 phage × SB5001 |
| SB2054 | *hisO3149 hisG46 fla-2055* | SB2049 phage × SB5001 |
| SB2055 | *hisO3150 hisG46 fla-2055* | SB2051 phage × SB5001 |
| SB2056 | *hisO3148 hisG46 hisT1504* | SB2053 phage × TA516 |
| SB2057 | *hisO3149 hisG46 hisT1504* | SB2054 phage × TA516 |
| SB2058 | *hisO3150 hisG46 hisT1504* | SB2055 phage × TA516 |
| SB2059 | *hisO2355 hisG46 fla-2055* | SB2805 phage × SB5001 |
| SB2060 | *hisO2355 hisG46 hisT1504* | SB2059 phage × TA516 |
| SB2240 | *hisO3154 fla-2055* | SB3234 phage × SB3095 |
| SB2243 | *hisO3155 fla-2055* | SB3236 phage × SB3095 |
| SB2246 | *hisO3156 fla-2055* | SB3238 phage × SB3095 |
| SB2358 | *hisO3154 hisG46 fla-2055* | SB2240 phage × SB5001 |
| SB2359 | *hisO3155 hisG46 fla-2055* | SB2243 phage × SB5001 |
| SB2360 | *hisO3156 hisG46 fla-2055* | SB2246 phage × SB5001 |
| SB2457 | *hisO1828 hisG46 hisT1504* | SB949 phage × TA516 |
| SB2469 | *hisO2672 hisG46 fla-2055* | TA1031 phage × SB5001 |
| SB2470 | *hisO2677 hisG46 fla-2055* | TA1036 phage × SB5001 |
| SB2471 | *hisO2678 hisG46 fla-2055* | TA1037 phage × SB5001 |
| SB2603 | *hisO3181 fla-2055* | SB3252 phage × SB3095 |
| SB2604 | *hisO3181 hisG46 fla-2055* | SB2603 phage × SB5001 |
| SB2606 | *hisO3181 hisG46 hisT1504* | SB2604 phage × TA516 |
| SB2609 | *hisO2666 hisG46 fla-2055* | TA1025 phage × SB5001 |
| SB2610 | *hisO2669 hisG46 fla-2055* | TA1028 phage × SB5001 |
| SB2611 | *hisO2695 hisG46 fla-2055* | TA1054 phage × SB5001 |
| SB2612 | *hisO2696 hisG46 fla-2055* | TA1055 phage × SB5001 |
| SB2613 | *hisO2705 hisG46 fla-2055* | TA1064 phage × SB5001 |
| SB2614 | *hisO2730 hisG46 fla-2055* | TA1089 phage × SB5001 |
| SB2625 | *his+ fla-2055* | *ara-9* phage × SB3095 |
| SB2665 | *hisO1242 hisO1828 fla-2055* | SB3262 phage × SB3095 |
| SB2700 | *hisO2321 hisT1504* "x" | Spontaneous in TA520 |
| SB2708 | *hisO3185 fla-2055* | SB3256 phage × SB3095 |
| SB2765 | *hisO3197 hisG46 fla-2055* | SB2771 phage × SB5001 |

TABLE 1—Continued

| Strain | Genotype | Derivation or reference |
|---|---|---|
| SB2766 | *hisO3198 hisG46 fla-2055* | SB2772 phage × SB5001 |
| SB2767 | *hisO3199 hisG46 fla-2055* | SB2773 phage × SB5001 |
| SB2771 | *hisO3197* | Wrinkled isolate fromSB2625 × SB3227 |
| SB2772 | *hisO3198* | Wrinkled isolate fromSB2625 × SB3228 |
| SB2773 | *hisO3199* | Wrinkled isolate fromSB2625 × SB3229 |
| SB2800 | *hisO2321* | TA520 phage × TA1003 |
| SB2801 | *hisO1202 hisG46 fla-2055* | SB6202 phage × SB5001 |
| SB2802 | *hisO1242 hisG46 fla-2055* | TA1003 phage × SB5001 |
| SB2803 | *hisO1812 hisG46 fla-2055* | TA795 phage × SB5001 |
| SB2805 | *hisO2355 fla-2055* | TA577 phage × SB3095 |
| SB2988 | *hisO2964 hisG46 fla-2055* | TA2582 phage × SB5001 |
| SB2989 | *hisO2964 hisG46 hisT1504* | SB2988 phage × TA516 |
| SB2990 | *hisO2965 hisG46 fla-2055* | TA2583 phage × SB5001 |
| SB2991 | *hisO2965 hisG46 hisT1504* | SB2990 phage × TA516 |
| SB2992 | *hisO2966 hisG46 fla-2055* | TA2584 phage × SB5001 |
| SB2993 | *hisO2966 hisG46 hisT1404* | SB2992 phage × TA516 |
| SB3095 | *hisG46 fla-2055* | Spontaneous in *hisG46* |
| SB3099 | *hisO2979 hisG46 fla-2055* | TA2597 phage × SB5001 |
| SB3100 | *hisO2979 hisG46 hisT1504* | SB3099 phage × TA516 |
| SB3193 | *hisO3148 hisT1504* | Diethylsulfate in DA10; phage × SB2046 |
| SB3194 | *hisO3149 hisT1504* | Diethylsulfate in DA10; phage × SB2046 |
| SB3195 | *hisO3150 hisT1504* | Diethylsulfate in DA10; phage × SB2046 |
| SB3224 | *hisO2321 hisO3197* | Spontaneous revertant in SB2800 |
| SB3225 | *hisO2321 hisO3198* | Spontaneous revertant in SB2800 |
| SB3226 | *hisO2321 hisO3199* | Spontaneous revertant in SB2800 |
| SB3227 | *hisO2321 hisO3197 hisT1504* | SB3224 phage × TA516 |
| SB3228 | *hisO2321 hisO3198 hisT1504* | SB3225 phage × TA516 |
| SB3229 | *hisO2321 hisO1812 hisT1504* | SB3230 phage × TA516 |
| SB3230 | *hisO2321 hisO1812* | TA975 phage × SB2800 |
| SB3231 | *hisG200 hisT1504* | *hisG200* phage × TA516 |
| SB3233 | *hisO3154 hisO3148* | Diethylsulfate mutagenesis of SB2047 |
| SB3234 | *hisO3154 hisO2321* | SB3233 phage × SB2800 |
| SB3235 | *hisO3155 hisO3148* | Diethylsulfate mutagenesis of SB2047 |
| SB3236 | *hisO3155 hisO2321* | SB3235 phage × SB2800 |
| SB3237 | *hisO3156 hisO3148* | Diethylsulfate mutagenesis of SB2047 |
| SB3238 | *hisO3156 hisO2321* | SB3237 phage × SB2800 |
| SB3252 | *hisO3181 hisT1504* "x" | Nitrosoguanidine mutagenized phage × SB2700 |
| SB3256 | *hisO3185 hisT1504* "x" | Nitrosoguanidine mutagenized phage × SB2700 |
| SB3262 | *hisO1242 hisO1828* | TA577 phage × SB949 |
| SB3273 | *hisO3185 hisG46 fla-2055* | SB2708 phage × SB5001 |
| SB3274 | *hisO3185 hisG46 hisT1504* | SB3273 phage × TA516 |
| SB3275 | *hisO2321 hisO3206* | Spontaneous revertant of SB2800 |
| SB3276 | *hisO2321 hisO3207* | Spontaneous revertant of SB2800 |
| SB3277 | *hisO2321 hisO3208* | Spontaneous revertant of SB2800 |
| SB3278 | *hisO2321 hisO3209* | Spontaneous revertant of SB2800 |
| SB3279 | *hisO2321 hisO3215* | Spontaneous revertant of SB2800 |
| SB3280 | *hisO2321 hisO3216* | Spontaneous revertant of SB2800 |
| SB3281 | *hisO2321 hisO3218* | Spontaneous revertant of SB2800 |
| SB3282 | *hisO2321 hisO3219* | Spontaneous revertant of SB2800 |
| SB3283 | *hisO2321 hisO3220* | Spontaneous revertant of SB2800 |
| SB3284 | *hisO2321 hisO3221* | Spontaneous revertant of SB2800 |

| | | |
|---|---|---|
| SB3285 | *hisO3206 fla-2055* | SB3275 phage × SB3095 |
| SB3286 | *hisO3207 fla-2055* | SB3276 phage × SB3095 |
| SB3287 | *hisO3208 fla-2055* | SB3277 phage × SB3095 |
| SB3288 | *hisO3209 fla-2055* | SB3278 phage × SB3095 |
| SB3289 | *hisO3215 fla-2055* | SB3279 phage × SB3095 |
| SB3290 | *hisO3216 fla-2055* | SB3280 phage × SB3095 |
| SB3291 | *hisO3218 fla-2055* | SB3281 phage × SB3095 |
| SB3292 | *hisO3219 fla-2055* | SB3282 phage × SB3095 |
| SB3293 | *hisO3220 fla-2055* | SB3283 phage × SB3095 |
| SB3294 | *hisO3221 fla-2055* | SB2284 phage × SB3095 |
| SB3295 | *hisO3207 hisG46 fla-2055* | SB3286 phage × SB5001 |
| SB3296 | *hisO3207 hisG46 hisT1504* | SB3295 phage × TA516 |
| SB3298 | *hisO3220 hisG46 fla-2055* | SB3293 phage × SB5001 |
| SB5001 | *hisD1 hisG46 fla-2055* | *hisD1* phage × SB3095 and penicillin selection |
| SB6828 | *hisO1828 ara-9* | Spontaneous TRA-resistant mutant in *ara-9*; phage × *hisOGD63* |
| SB6830 | *hisO1830 ara-9* | Spontaneous TRA-resistant mutant in *ara-9*; phage × *hisOGD63* |
| SB6831 | *hisO1831 ara-9* | Spontaneous TRA-resistant mutant in *ara-9*; phage × *hisOGD63* |
| SB6832 | *hisO1832 ara-9* | Spontaneous TRA-resistant mutant in *ara-9*; phage × *hisOGD63* |
| SC209 | *hisO3601 hisO1242 hisG2187* | ATKINS and LOPER (1970) |
| TA372 | *hisO1242 hisD2117* | FINK, KLOPOTOWSKI and AMES (1967) |
| TA516 | *hisD2317 hisT1504* | T. KLOPOTOWSKI (Method of FINK, KLOPOTOWSKI and AMES 1967) |
| TA520 | *hisO2321 hisT1504* | T. KLOPOTOWSKI (Method of FINK, KLOPOTOWSKI and AMES 1967) |
| TA577 | *hisO1242 hisO2355* | VOLL (1967) |
| TA795 | *hisO1812 ara-9* | ROTH, ANTÓN and HARTMAN (1966) |
| TA979 | *hisOG2639 hisT1504* | ROTH, ANTÓN and HARTMAN (1966) |
| TA1003 | *hisO1242* | G. R. FINK, cited in ROTH, ANTÓN and HARTMAN (1966) |
| TA1025–TA1091 | *hisO2666* through *hisO2732*, respectively | CHANG, STRAUS and AMES (1971) |
| TA2582 | *hisO2964* HfrK5 *serA13* | STRAUS and WYCHE (1974) |
| TA2583 | *hisO2965* HfrK5 *serA13* | STRAUS and WYCHE (1974) |
| TA2584 | *hisO2966* HfrK5 *serA13* | STRAUS and WYCHE (1974) |
| TA2590 | *hisO2972* HfrK5 *serA13* | STRAUS and WYCHE (1974) |
| TA2597 | *hisO2979* HfrK5 *serA13* | STRAUS and WYCHE (1974) |
| *Salmonella typhimurium* LT-7 | | |
| SB6202 | *hisO1202* | ROTH, ANTÓN and HARTMAN (1966) |

an unusual mutation ("x") unlinked to the histidine operon. Mutation "x" renders SB2700 sensitive to high concentrations of the *hisH* and *hisF* enzymes. Because *hisT1504* is present in this strain, the introduction of a wild-type *his* operon results in elevated levels of the *his* biosynthetic enzymes and susceptibility to the lethal effects of the "x" mutation. Therefore, in a transduction of SB2700 to His+, colonies are formed only with those transducing particles which contain a prototrophic mutation that causes a reduction in the level of the *hisH* and *hisF* gene products.

*Selection of constitutive* hisO *mutations:* One series of clones containing constitutive *hisO* mutations was obtained as spontaneous His+ revertant colonies from the histidine auxotroph,

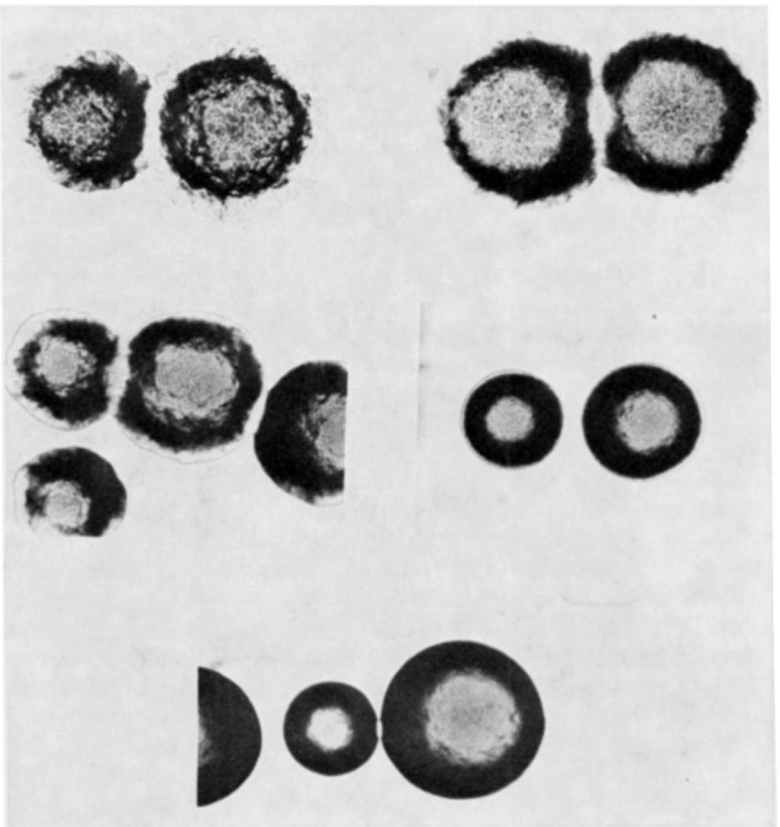

FIGURE 1.—Colony morphologies of *hisO* mutants viewed by reflected light. Colonies were streaked onto minimal high glucose plates and incubated overnight at 37°. Genotypes (and *hisB* enzyme levels in units/O.D.$_{650}$) of the strains are: Top left: *hisO1242* (38). Top right: *hisO1832* (18). Center left: *hisT1504 hisO3181* (10.8). Center right: *hisT1504 hisO3185* (5.9). Bottom: wild type (2.2). The colonies in the top two pictures are considered to have a wrinkled colony morphology. Those in the middle two pictures have a grainy colony morphology. Wild type (bottom) has a smooth colony morphology. Photographs were taken with a compound microscope.

*hisO2321* (= strains SB3224–3226, SB3275–SB3284) and separated by transduction away from the auxotrophic marker (= strains SB3285–3294). Similarly, revertants of the leaky auxotroph *hisO3148* were isolated by selection for AMT-resistant colonies after diethylsulfate mutagenesis (= strains SB3233, SB3235, and SB3237) and transduced away from the *hisO3148* marker (= strains SB2240, SB2243, SB2246).

A second series of constitutive *hisO* mutations was isolated by selection for *his* operon-linked mutations that allowed growth in the presence of both AMT and D,L-1,2,4,-triazole-3-alanine (= strains SB6202, SB6828–SB6832, TA795, TA1003, TA1025–TA1091). The basis of this method and some of the mutants are described elsewhere (ROTH, ANTÓN and HARTMAN 1966; FANKHAUSER 1971; CHANG, STRAUS and AMES 1971).

*Selection of* hisO *mutants with reduced levels of* his *enzymes:* Since constitutive *hisO* strains cannot grow on minimal medium at high temperature (VOLL 1967), it is possible to select promoter-like mutations in *hisO* strains after growth at 42°. VOLL (1967) isolated one promoter mutation by direct selection on minimal medium (= strain TA577), and an auxotrophic pro-

moter mutation was isolated on minimal medium supplemented with L-histidine (= strain TA520). Strain SC209 was isolated as a histidine auxotroph after penicillin screening (ATKINS and LOPER 1970).

Since most revertants at 42° of constitutive *hisO* mutations are not promoter-like mutants (VOLL 1967; FINK, KLOPOTOWSKI and AMES 1967), enrichment for mutations in the promoter region was achieved by a dual selection process. A culture of a doubly mutant constitutive strain with 20-fold increased enzyme levels (strain DA10 containing *hisR1223 hisT1501*, two constitutive regulatory mutations unlinked to the histidine operon) was treated with diethylsulfate and grown in minimal medium containing 10 mM AMT and 200 units/ml penicillin. DA10 contains a high enough level of histidine enzymes to grow in the presence of AMT, whereas mutants with low enzyme levels should not grow. Adenosine (20 μg/ml) was added to the medium to allow growth of other types of AMT-sensitive mutants (cf. HILTON, KEARNEY and AMES 1965; STRAUS and WYCHE 1974). After two days' incubation at 37°, cultures were plated on minimal medium and incubated at 42°. From each plate several hundred surviving colonies were suspended, and the mixed cultures used for growth of transducing phage. Strain SB2046 (*hisT1504 hisOG1302*) was used as a recipient in transduction tests since SB2046 contains a deletion of the entire operator-promoter region (*hisOG1302*) and an unlinked constitutive mutation (*hisT1504*) engendering wrinkled colony formation in *hisO*+ clones. His+ transductants of smooth colony morphology were chosen as potential promoter mutants and characterized by further tests (see RESULTS). Strains SB3193–SB3195 were procured by this technique.

Another group of promoter-like mutations was isolated by selection for prototrophic mutants hypersensitive to inhibition by the artificial feedback-inhibitor, TA (= strains TA2582–TA2597; STRAUS and WYCHE 1974).

A final series of prototrophic promoter-like mutations was isolated after treatment of transducing phage with nitrosoguanidine (WYCHE 1971) and infection of an appropriate host selective for the desired mutation (HONG and AMES 1971). Strain SB2700 was used as recipient (see *Quantitative techniques which estimate* his *operon enzyme levels*), and transductional clones were purified and tested further for genotype and phenotype (see RESULTS). Strains SB3252 and SB3256 were procured and the promoter-like mutations were transferred to standard genetic background by transduction (= strains SB2603 and SB2708).

One prototrophic promoter-like mutant was isolated as a revertant of an auxotrophic promoter mutation, *hisO2321* (= strain SB3276) and separated from *hisO2321* by transduction into another strain (= strain SB3286).

*Construction of* hisO hisG46 *double mutants:* In order to map prototrophic regulatory mutations in the *his* operon by means of reciprocal crosses, it was necessary to introduce an auxotrophic marker into the prototrophic strain for use as a counter-selective marker. Double mutants containing the missense mutation *hisG46* were constructed in combination with *hisO* mutations. Phage grown on a prototrophic strain were used to transduce strain SB5001 (*hisG46 hisD1 fla-2055*) to growth on enriched minimal high glucose plates supplemented with 1 mM histidinol (= HisD+). After two days' incubation at 37°, small colonies were picked and streaked on minimal high glucose plates. A few crystals of histidine were placed in the center of the plate and the plate was incubated at 37° for 24 hours. Small colonies which were His- gave rise to colonies only in the vicinity of the histidine and had one of two genotypes, *hisG46* or *hisO hisG46*. Streaks of *hisG46* had a large, smooth colony morphology when located near the histidine, but with increasing distance colonies were smaller and had an increasingly wrinkled colony morphology as derepression occurred. The desired recombinants, *hisO hisG46*, containing constitutive *hisO* mutations, were wrinkled through the streak, while those containing promoter-like *hisO* mutations were smooth throughout the streak. The genetic background of the *hisO hisG46* double mutants (SB5001) is isogenic (except for the *hisG46* mutation) to that of strain SB3095 in which the *hisO* single mutations are located.

## RESULTS

*Characterization of mutants:* The origins of the *hisO* mutations are described

in MATERIALS AND METHODS. The prospective *hisO* mutants were first tested for a number of characteristics to confirm their nature and to facilitate rapid identification during subsequent genetic manipulations. Mutants with a wrinkled colony morphology (see MATERIALS AND METHODS) were characterized as to the degree of wrinkledness as shown in Figure 1. To verify that they contained *hisO* mutations, phage were grown on them and used to transduce the deletion strain *hisOG203* to prototrophy. If 100% of several hundred transductants possessed a wrinkled colony morphology, the mutation causing wrinkledness was assumed to map at the beginning of the *his* operon and was designated *hisO*. If no transductants with a wrinkled colony morphology were found, the mutation causing wrinkledness was considered unlinked to the *his* operon. Instances where a transduction plate had colonies of more than one morphology did not occur, suggesting that all mutations in the *his* operon that significantly elevate enzyme levels map in the most proximal portion of the operon.

The wrinkled (constitutive) *hisO* mutations were divided into two classes by the ability of phage grown on them to transduce the histidine auxotroph SB2700 (*hisT1504 hisO2321* "x"), to prototrophy (see MATERIALS AND METHODS). Those that had an intermediate level of *his* operon expression and could not be further derepressed could transduce SB2700. Those with high levels of *his* operon expression, or those which could be derepressed to give high levels of *his* operon expression, could not transduce SB2700. Of 88 high enzyme level constitutive *hisO* mutations tested, 21 could transduce SB2700 and 67 could not. Twenty of the twenty-one mutations which could transduce SB2700 (*hisO1828*, *-2666*, *-2669*, *-2672*, *-2677*, *-2678*, *-2679*, *-2695*, *-2696*, *-2697*, *-2699*, *-2704*, *-2705*, *-2706*, *-2715*, *-2716*, *-2730*, *3219*, *-3220*, *-3221*) do not recombine with each other and map proximal to all other *hisO* mutations (see below). The remaining high enzyme mutation *hisO* which can transduce SB2700 (*hisO3198*) is a deletion mapping in the middle of the *hisO* region. This mutation is discussed in ELY (1974).

Mutants with a smooth colony morphology and a reduced expression of the *his* operon were isolated in a variety of ways (MATERIALS AND METHODS). Phage grown on these mutants were used to transduce to prototrophy strain TA979 (*hisT1504 hisOG2639*). TA979 contains a deletion of the operator-promoter region extending into the *hisG* gene and an unlinked constitutive mutation leading to wrinkled colony formation when *hisO*$^+$. Prototrophic transductants with a smooth or grainy colony morphology were considered to have mutations linked to the *his* operon. If there was a mixture of colony morphologies on the transduction plate, the linked mutation was considered to map outside of the *hisOG* deletion and to be a leaky mutation which reduced the expressed of one or more genes of the *his* operon (cf. VOLL 1967). These leaky mutations mapped in *hisB*, *hisH*, *hisA* or *hisF* (ELY 1973) and will not be discussed further here since they do not alter regulation of the histidine operon and are similar to the polar prototrophs described by VOLL (1967).

If all of several hundred colonies from the cross with TA979 had either a smooth or a grainy colony morphology, the linked mutation was designated *hisO* and was transduced into the standard SB3095 (*hisG46 fla-2055*) background and

TABLE 2

*Properties of promoter-like* hisO *mutations*

| *hisO* mutation | Inhibitor* AMT | TA | Colony morphology in SB2700 background† |
|---|---|---|---|
| *hisO+* | R | R | not viable |
| *hisO2355* | S | S | smooth |
| *hisO2965* | S | S | smooth |
| *hisO2966* | S | S | smooth |
| *hisO3148* | S | S | smooth |
| *hisO2964* | R | S | slightly grainy |
| *hisO2979* | R | S | slightly grainy |
| *hisO3149* | R | S | grainy |
| *hisO3150* | R | S | slightly grainy |
| *hisO3181* | R | S | grainy |
| *hisO3185* | R | S | grainy |

* Inhibition by aminotriazole (AMT) and by 2-thiazolealanine (TA) were measured as described in MATERIALS AND METHODS. S indicates sensitivity to the inhibitor while R indicates resistance to the inhibitor.

† The *hisO* mutations were transduced into SB2700 (*hisT1504 hisO2321* "x") and scored for colony morphology. The term grainy is used to indicate a morphology intermediate between smooth and wrinkled (Figure 1).

further characterized. Sensitivity of these strains to AMT, an inhibitor of the *hisB* enzyme, and to TA, an inhibitor of the *hisG* enzyme, is shown in Table 2. The wild-type control was resistant to both inhibitors since it could derepress to overcome the inhibitions. Each of this group of *hisO* strains was sensitive to TA, while four out of ten were sensitive to AMT (Table 2).

Phage grown on these mutants could transduce SB2700 to prototrophy, indicating that the mutations reduce the maximal expression of the *his* operon. These strains had smooth or grainy colony morphologies, which indicate low levels of operon expression (column 4, Table 2). These *hisO* mutations are referred to as "promoter-like" *hisO* mutations since they reduce operon expression.

Evidence summarized in a companion paper (ELY 1974) indicates that the *hisO* mutations are truly operator mutations and not merely defects in the first structural gene of the operon, *hisG*.

*Mapping of* hisO *mutations:* The gene order *hisO:hisG:hisD* was confirmed by a pair of crosses: (1) *hisG46* phage × *hisO1242 hisD2117* and (2) *hisO1242* phage × *hisG46 hisD1*. The crosses were performed on enriched minimal high glucose plates supplemented with 1 mM histidinol so that recombinants containing the *hisG46* marker could be recovered. The *hisD* enzyme is the only *his* biosynthetic enzyme needed for growth in medium containing histidinol. If the order of the three markers were *hisO1242:hisG46:hisD*$^-$, wild-type recombinants would be formed by a quadruple crossover when *hisG46* is the donor (Cross 1) and by a double crossover when *hisO1242* is the donor (Cross 2). Assuming that the double event is more frequent than the quadruple event, more wild-type recombinants should be formed in Cross 2 than in Cross 1. A tenfold excess of wild-type recombinants was indeed found in Cross 2. If the markers had mapped

in the order *hisG46:hisO1242:hisD*$^-$, the reverse result would have been expected. Since deletions removing *hisO* and part of *hisG* leave *hisD* intact (AMES, HARTMAN and JACOB 1963), the gene order *hisO1242:hisD:hisG46* would not be possible. Therefore, the only map order consistent with the data is *hisO1242:hisG46:hisD*. This order is in agreement with three-point tests checked by deletion mapping, and with various *hisO1242 hisG* × *hisG* crosses (FINK, KLOPOTOWSKI and AMES 1967; HARTMAN *et al.* 1971).

All other *hisO* mutations map closer to *hisO1242* than to *hisG46* (data not shown), indicating that the other *hisO* mutations map on the same side of *hisG46* as *hisO1242* does. Based on this observation, we assume the general map order *hisO:hisG46:hisD* for all subsequent mapping experiments.

*Mapping of constitutive* hisO *mutations:* Constitutive *hisO* mutations were mapped with respect to each other by means of reciprocal three-point crosses similar to those just outlined. The donor was phage grown on a strain containing one constitutive mutation, and the recipient was a strain containing a second constitutive mutation and the linked *hisG46* mutation. Recombination generating a wild-type *his* operon will give rise to smooth colonies, while those retaining a constitutive mutation will form wrinkled colonies. Thus, transductions were scored for the percentage of smooth His$^+$ colonies among the total number of recombinants. A typical cross is shown in Figure 2. If the map order is *mutation 1*:

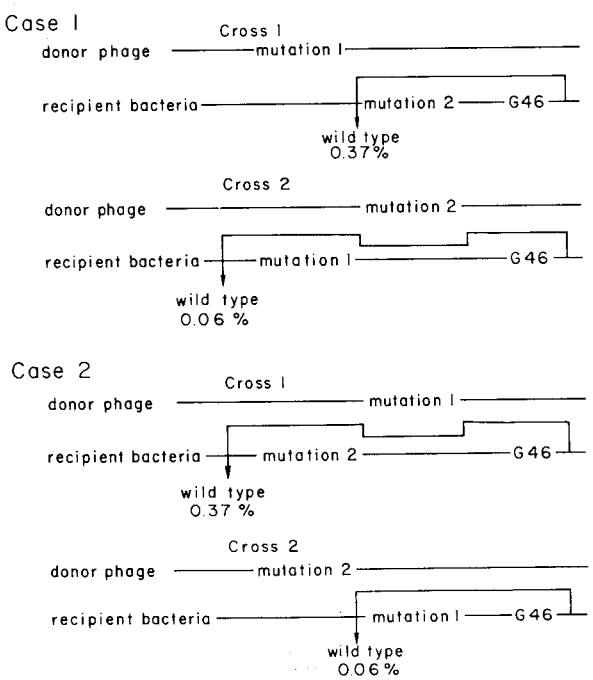


FIGURE 2.—General strategy for mapping *hisO* mutations. Cross 1 and Cross 2 refer to pairs of reciprocal three-point tests. Case 1 and Case 2 refer to two possible map orders. Data shown in the Figure support the map order shown in Case 1, but contradict the map order shown in Case 2.

*mutation 2:hisG46* as diagrammed in Case 1, smooth colonies wild type for the *his* operon can be formed by a double crossover when *mutation 1* is used as the donor. However, a quadruple crossover will be required when *mutation 1* is the recipient. Assuming that double events are more frequent than quadruple events, the frequency of smooth recombinants should be greater in Cross 1 than in Cross 2. As can be seen in Figure 2, this result was obtained. There were 0.37% smooth colonies in Cross 1 and only one-sixth as many (0.06%) in Cross 2. On the other hand, if the order of the two constitutive mutations were reversed as in Case 2, then smooth colonies would be formed by a quadruple crossover in Cross 1, and by a double crossover in Cross 2. In that case the opposite result would have been expected. Smooth colonies would have been more frequent in Cross 2 than in Cross 1. Therefore, these results are consistent only with the map order of *mutation 1:mutation 2:hisG46*.

The mapping data in the tables are presented in the form of a fraction, where the numerator is the percent smooth recombinants formed when *mutation 1* is present in the donor and *mutation 2* is present in the recipient. The denominator is the percent smooth recombinants formed in the reciprocal cross, when *mutation 2* is present in the donor. A fraction significantly greater than one indicates a presumed map order of *mutation1:mutation2:hisG46*.

Preliminary mapping of some *hisO* mutations indicated the general map locations of *hisO1202, hisO1242, hisO1812, hisO1828, hisO1830, hisO1831, hisO1832* (Figure 3). Data from selected crosses designed to determine a unique map order for these and other more recently isolated *hisO* mutations are presented in Tables 3 and 4. Beginning at the top of Table 3, the order *hisO1828:hisO3155: hisG46* and *hisO3155:hisO1812:hisG46* is clearly demonstrated by reciprocal

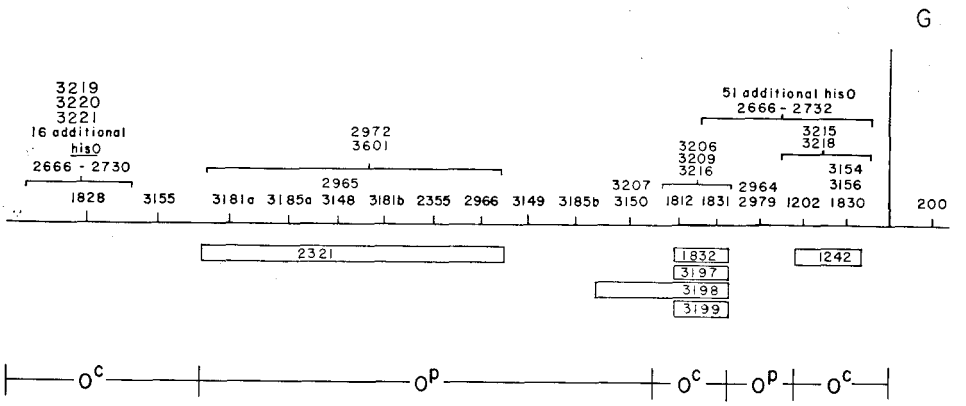


FIGURE 3.—Map of the Salmonella histidine operator-promoter region. Each number refers to an independently isolated mutation. Some mutants appear to contain two mutant sites (3181a–b, 3185a–b). Boxes indicate extent of deletion mutations. Brackets at the top of the Figure indicate approximate map position of mutations not more extensively mapped by three-point tests. The $O^c$ and $O^p$ designations at the bottom of the Figure refer to general phenotypic properties ($O^c$ = operator constitutive and $O^p$ = promoter-like) of the groups of mutations above each category as described in the text and in ELY (1974).

TABLE 3

*Mapping of constitutive* hisO *mutations (part 1)*

| Mutation 2 | Mutation 1 *hisO1828* | *hisO3155* | *hisO1812* | *hisO3197* | *hisO3198* | *hisO3199* |
|---|---|---|---|---|---|---|
| *hisO3155* | 4.0/.78* | | | | | |
| *hisO1812* | <.007/<.006 | .82/.23 | | | | |
| *hisO3197* | <.04/<.04 | 1.3/.44 | <.04/<.04 | | | |
| *hisO3198* | <.07/<.09 | 2.0/.09 | <.04/<.05 | <.06/<.03 | | |
| *hisO3199* | <.08/<.08 | .63/.11 | <.04/<.05 | <.07/<.03 | <.03/<.03 | |
| *hisO1832* | <.04/<.15 | | <.03/<.03 | | | |
| *hisO1831* | <.04/<.06 | 1.2/.62 | .28/<.04 | <.02/<.03 | <.05/n.d. | <.03/n.d. |
| *hisO1202* | 4.4/n.d.† | 1.3/.20 | .14/.05 | .21/<.05 | .09/n.d. | .10/n.d. |
| *hisO1830* | | <.013/n.d. | | | | |
| *hisO1242* | | | | .42/n.d. | .50/n.d. | .80/n.d. |

* Values are the percent smooth recombinants formed. The numerator is from the cross: mutation 1 × mutation 2 *hisG46*. The denominator is from the reciprocal cross: mutation 2 × mutation 1 *hisG46*.

† n.d. indicates not determined.

crosses between *hisO1828* and *hisO3155* and between *hisO3155* and *hisO1812*. Therefore, we can deduce the order *hisO1828*:*hisO3155*:*hisO1812*:*hisG46* as shown in Figure 3.

*HisO3197*, *hisO3198*, *hisO3199*, and *hisO1832* fail to recombine with either *hisO1812* or *hisO1831*, and map close to *hisO1202* and *hisO1242*. In addition, *hisO3197*, *hisO3198*, and *hisO3199* map between *hisO3155* and *hisG46*. Thus, we conclude that *hisO1832*, *hisO3197*, *hisO3198*, and *hisO3199* map at the sites of *hisO1812* and *hisO1831*. Since *hisO1812* and *hisO1831* recombine with each other, they map at separate sites, and therefore *hisO3197*, *hisO3198*, *hisO3199*, and *hisO1832* are probably deletions eliminating both of these sites. The map order for these markers is shown in Figure 3.

Crosses between *hisO1812*, *hisO1831*, *hisO1202*, and *hisO1830* show significant differences between reciprocal crosses, and the data give rise to a unique inter-

TABLE 4

*Mapping of constitutive* hisO *mutations (part 2)*

| Mutation 2 | Mutation 1 *hisO1832* | *hisO1831* | *hisO1202* | *hisO1830* | *hisO3154* | *hisO3156* |
|---|---|---|---|---|---|---|
| *hisO1831* | <.03/<.03* | | | | | |
| *hisO1202* | .11/<.03 | .21/<.02 | | | | |
| *hisO1830* | .33/.10 | | .13/<.04 | | | |
| *hisO3154* | .30/n.d.† | .12/n.d. | .28/<.08 | <.04/<.02 | | |
| *hisO3156* | .27/n.d. | .25/n.d. | .15/.07 | <.03/<.02 | <.05/<.05 | |
| *hisO1242* | .54/.02 | | <.04/<.02 | <.02/<.02 | n.d./<.02 | n.d./<.02 |

* Values are the percent smooth recombinants formed. The numerator is from the cross: mutation 1 × mutation 2 *hisG46*. The denominator is from the reciprocal cross: mutation 2 × mutation 1 *hisG46*.

† n.d. indicates not determined.

pretation of map order: *hisO1812:hisO1831:hisO1202:hisO1830* (Tables 3 and 4). *HisO3154* and *hisO3156* fail to recombine with *hisO1830*, and like *hisO1830* map between *hisO1202* and *hisG46* (Table 4). Therefore, we assume that *hisO1830*, *hisO3154*, and *hisO3156* all map at the same site. The remaining mutation, *hisO1242*, appears to be a deletion covering the distal end of the *hisO* region since it fails to recombine with *hisO1202*, *hisO1830*, *hisO3154*, and *hisO3156*, while *hisO1202* does recombine with the latter three mutations (Table 4). Combining these data, we can conclude the overall map positions shown for these mutations in Figure 3.

A discrepancy with this map order is the lack of recombination between *hisO1828* and the mutants *hisO1812*, *hisO3197*, *hisO3198*, *hisO3199*, *hisO1832*, or *hisO1831* (Table 3). Furthermore, six other *hisO* mutations which are phenotypically similar to *hisO1828* (*hisO2666*, *-2677*, *-2695*, *-2696*, *-2705* and *-2730*) do not recombine with *hisO1828* nor with *hisO1831* but do recombine with *hisO3155* (data not shown). Normally, the lack of recombination between two markers indicates that the two mutations map at the same site. However, since *hisO3155* maps between *hisO1828* and *hisO1812* (Table 3), the latter mutations would appear to map at two separate sites. In addition, mapping of *hisO1828* and *hisO1812* with respect to the promoter-like *hisO* mutations places these two constitutive *hisO* mutations on opposite sides of the region where the promoter-like *hisO* mutations map (see below). Since other explanations such at the rearrangement of the data into a deletion map cannot explain these instances of non-recombination, we conclude that *hisO1828* and *hisO1812* map at separate sites and the lack of recombination is due to some other factor. Similarly, *hisO3155* and *hisO1830* fail to recombine (Table 3) although located at clearly different sites in other recombination tests (see DISCUSSION).

Additional constitutive *hisO* mutations isolated by CHANG, STRAUS and AMES (1972) or from revertants of *hisO2321*, were assigned to general regions within *hisO* by similar procedures (ELY 1973). They are denoted by brackets at the top of Figure 3.

*Mapping of promoter-like* hisO *mutations:* Pairs of promoter-like *hisO* mutations were mapped with respect to each other using reciprocal three-point crosses similar to those used for mapping the constitutive *hisO* mutations (Figure 2). The difference between the sets of crosses was that when crosses involving promoter-like *hisO* mutations were performed, the recipient was a strain with a second promoter mutation in a background containing the unlinked *hisT1504* mutation. Therefore, recombinants with a wild-type *his* operon would have wrinkled colony morphology, while the majority of the transductants would contain promoter-like *hisO* mutations and would have a smooth or slightly wrinkled colony morphology. In crosses involving *hisO3181*, and *hisO3149*, identification of wild-type colonies was more difficult since they more closely resembled donor-type recombinants. Therefore, colonies suspected of containing a wild-type *his* operon were streaked out for single colonies to confirm the colonial morphology. If there was any doubt about the phenotype of the colonies in the streaks, one was chosen and characterized by transduction experiments. In some cases, wrinkled colonies were

TABLE 5

*Mapping of promoter-like* hisO *mutations*

| Mutation 2 | Mutation 1 *hisO3181* | *hisO3148* | *hisO2965* | *hisO2355* | *hisO2966* | *hisO3149* | *hisO3150* | *hisO2964* |
|---|---|---|---|---|---|---|---|---|
| *hisO3148* | .20/.13* | | | | | | | |
| *hisO2965* | .09/.15 | <.016/<.023 | | | | | | |
| *hisO2355* | .023/.011 | .24/.08 | .24/.06 | | | | | |
| *hisO2966* | .10/.03 | <.013/<.011 | <.020/<.008 | .30/.04 | | | | |
| *hisO3149* | .74/.22 | .45/.05 | .57/.25 | 1.00/.41 | .33/.09 | | | |
| *hisO3150* | .44/<.06 | .37/.06 | .35/<.03 | .39/.05 | .43/<.03 | .34/.10 | | |
| *hisO2964* | .43/.18 | 2.9/.19 | 3.4/.35 | 1.8/.38 | 1.4/.18 | 3.2/<.03 | 3.1/.11 | |
| *hisO2979* | n.d.† | n.d. | n.d. | n.d. | n.d. | .50/<.05 | .21/.09 | <.05/<.01 |
| *hisO3185* | .16/.71 | .4/.3 | .5/.3 | .3/.3 | <.019/<.022 | .6/.5 | .04/.58 | <.04/3.7 |

* Values are the percent wrinkled recombinants formed. The numerator is from the cross: mutation 1 × mutation 2 *hisG46 hisT1504*. The denominator is from the reciprocal cross: mutation 2 × mutation 1 *hisG46 hisT1504*.

† n.d. indicates not determined.

obtained which gave rise to smooth segregants. This type of transductant, which occurs with a frequency of about 0.03%, has been shown to be a heterogenote containing two *his* operons (ELY 1973) and was not scored as a wild-type recombinant.

The mapping data (Table 5) are presented in the form of a fraction, where the numerator is the percent wrinkled recombinants formed when *mutation 1* is present in the donor. The denominator is the percent wrinkled recombinants formed in the reciprocal cross, when *mutation 2* is present the donor. A fraction significantly greater than 1 indicates an order of *mutation 1*:*mutation 2*:*hisG46*, while a fraction significantly less than 1 indicates an order of *mutation 2*:*mutation 1*:*hisG46*.

Beginning at the top of Table 5, we see that *hisO3181* cannot be positioned with respect to *hisO3148* or *hisO2965*. In both cases, recombination occurs, yet there is less than a twofold difference in recombination frequencies. This situation will be discussed in more detail below. *HisO3148* and *hisO2965* do not recombine and therefore are presumed to map at the same site. The order *hisO3181*:*hisO2355*:*hisG46* is suggested by crosses between *hisO3181* and *hisO2355*. However, recombination frequencies are tenfold lower in these crosses than in other crosses involving either marker. Crosses between *hisO2355* and *hisO3148* indicate the order *hisO3148*:*hisO2355*:*hisG46*. Similarly, *hisO2355* maps between *hisO2965* and *hisG46*. Therefore, we conclude the order (*hisO3148*, *hisO2965*):*hisO2355*:*hisG46*, as shown in Figure 3.

The data involving *hisO2966* present a paradox. *HisO2966* clearly maps between *hisG46* and either *hisO2355* or *his3181*. However, it does not recombine with either *hisO3148* or *hisO2965* although these markers map on the opposite side of *hisO2355*. We conclude that *hisO3148* and *hisO2966* map at separate sites despite the lack of detectable recombination between them (see DISCUSSION). Summarizing the mapping data presented, we have the map order (*hisO3148*, *hisO2965*):*hisO2355*:*hisO2966*:*hisG46*, as shown in Figure 3, with the position of *his O3181* with respect to *hisO3148* and *hisO2965* not determined.

The data from the next crosses, involving *hisO3149*, *hisO3150*, *hisO2964*, and *hisO2979* (Table 5), yield a unique interpretation of map order. *HisO3149* maps between *hisO2966* and *hisO3150*, while *hisO3150* maps between *hisO3149* and *hisO2964*. The recombination frequencies between *hisO2964* and most of the other markers is about tenfold higher than that found for other crosses, perhaps due to the specific nature of *hisO2964*. However, the data indicate that *hisO2964* maps between *hisO3150* and *hisG46*. The position of *hisO2964* with respect to the constitutive *hisO* mutations will be discussed below. An additional mutation, *hisO2979*, does not recombine with *hisO2964* and appears to map at the same site (<.01/<.05). Likewise, *hisO3207* does not recombine with *hisO3150* (<.05/<.05) and is phenotypically similar to *hisO3150*. Therefore, *hisO3207* is presumed to map at the same site as *hisO3150*. Thus the map order for these mutations is presumed to be *hisO2966*:*hisO3149*:(*hisO3150*, *hisO3207*):(*hisO2964*, *hisO2979*):*hisG46*, as shown in Figure 3.

The remaining mutation listed in Table 5, *hisO3185*, has unusual mapping

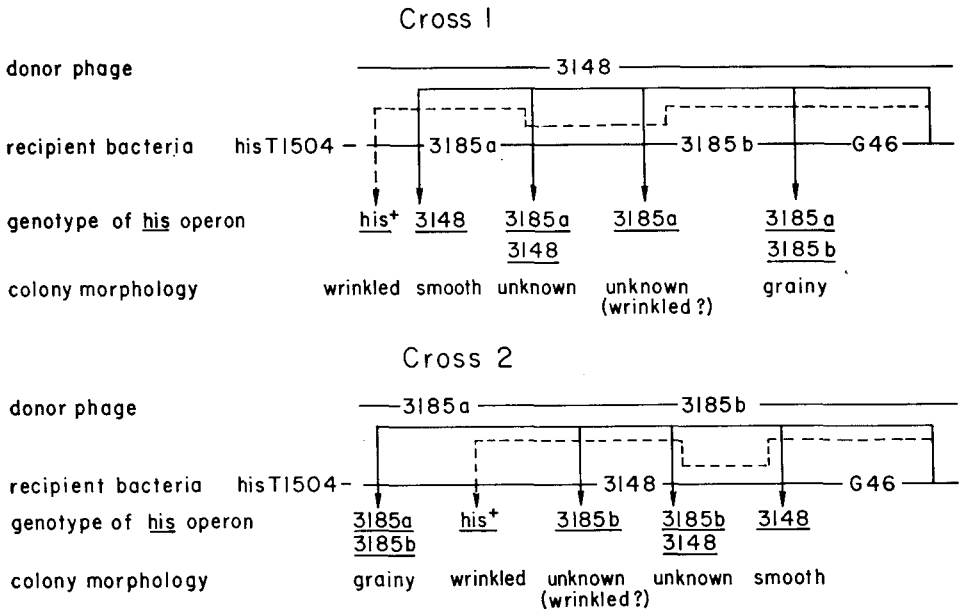


FIGURE 4.—Mapping of *hisO3185* and *hisO3148*. Cross 1 and Cross 2 indicate pairs of reciprocal three-point tests. In both cases, four crossovers would be required to form a wild-type recombinant. Three additional non-parental combinations of markers are possible but are not shown since each also would require four crossovers.

properties. Crosses with *hisO3181*, *hisO3150*, and *hisO2964* place those markers between *hisO3185* and *hisG46*. However, no map order can be deduced from crosses with the remaining markers in Table 5. Since recombination occurs between markers such as *hisO3148* and *hisO3185*, but with the same frequency in the reciprocal crosses, some additional factor(s) must be involved. Both *hisO3181* and *hisO3185* arose from NG mutagenesis (MATERIALS AND METHODS), and NG frequently causes closely linked multiple mutations in the *his* operon (HARTMAN *et al.* 1971). The unusual mapping properties of *hisO3185* can be explained if *hisO3185* consists of two component mutations, one mapping between *hisO3149* and *hisO3150*, and another mapping to the left of *hisO3148*. Thus, the order would be: *hisO3185a*:(*hisO3148*, *hisO2965*):*hisO2355*:*hisO2966*:*hisO3149*:*hisO3185b*:(*hisO3150*, *hisO3207*), as shown in Figure 3. Crosses between *hisO3185* and *hisO3148*, assuming these map positions, are diagrammed in Figure 4. The formation of wrinkled $His^+$ recombinants occurred with a frequency of 0.4% in Cross 1 and 0.3% in Cross 2. The excess of wrinkled recombinants can be explained if each of the two components of *hisO3185* has little or no effect on *his* operon expression unless the other component is present. In that case, each cross would give rise to wrinkled colonies with a genotype of *hisT1504 hisO3185a* or *hisT1504 hisO3185b*. The expected frequencies of wrinkled colonies would be approximately the same for the two crosses and in good agreement with the experimental results. Six wrinkled recombinants from each cross were

assayed for *hisB* enzyme levels in an effort to detect the presence of mutations affecting wild-type expression. In none of the twelve tests was there a significant deviation from the wild-type enzyme level in the same genetic background (data not shown). If component mutations of *hisO3185* are present in these strains as we believe, they have no effect on *his* operon expression when present singly.

The results of crosses between *hisO3181* and *hisO3148* or *hisO2965* can be explained by a situation analogous to that described for crosses with *hisO3185*. In this case, the component mutations would flank *hisO3148* and *hisO2965* with the order: *hisO3181a*:(*hisO3148*, *hisO2695*):*hisO3181b*:*hisO2355*, as shown in Figure 3.

The relative positions of *hisO3181a* and *hisO3185a* are more difficult to deduce. Crosses between the two markers suggest the order *hisO3185*:*hisO3181*:*hisG46*. However, if both *hisO3181* and *hisO3185* consist of two mutations, the interpretation of these crosses is more complex. Instead of being three-factor crosses, these crosses would be five-factor crosses with sixteen possible combinations of markers. Since we do not know what phenotype to expect for most of these combinations, no interpretation of the data can be made. However, a clue to the relative positions of *hisO3181a* and *hisO3185a* comes from the crosses with *hisO2966*. The lack of recombination in crosses with *hisO2966* seems to be specific for the site where *hisO3148* and *hisO2965* map. Since *hisO3185* does not recombine with *hisO2966*, while *hisO3181* does, it is reasonable to assume that one component of *hisO3185* maps close to *hisO3148* while both components of *hisO3181* are farther away. Therefore the map order would be: *hisO3181a*:*hisO3185a*:(*hisO3148*, *hisO2965*):*hisO3181b* as shown in Figure 3.

The prototrophic promoter-like *hisO* mutations discussed above were mapped with respect to *hisO2321* by infecting the auxotroph TA520 (*hisT1504 hisO2321*) with phage grown on strains containing a *hisO* mutation and *hisG46*. Prototrophic recombinants with a wild-type *his* operon had a wrinkled colony morphology and were scored as a percentage of the total prototrophic recombinants. The presence of the auxotrophic *hisG46* allele in the transducing phage reduces the total number of transductants about fiftyfold but has little effect on the number of wrinkled recombinants. Thus, a recombination frequency of 0.5 would be roughly equivalent to a recombination frequency of 0.01 in the absence of the *hisG46* mutation. As can be seen in Table 6, *hisO3181*, *risO3148*, *hisO2965*, *hisO2355*, and *hisO2966* do not recombine with *hisO2321* and consequently are presumed to lie within the area of the deletion. In confirmation of previous reports, (ATKINS and LOPER 1971; STRAUS and WYCHE 1974) we find that *hisO3061* and *hisO2972* also fail to recombine with *hisO2321* (data not shown). *HisO3149*, *hisO3150*, and *hisO2964* do recombine with *hisO2321* and are presumed to map outside the area of the deletion. The map positions for the promoter-like *hisO* mutations are indicated in Figure 3.

*Mapping of constitutive* hisO *mutations with respect to promoter-like* hisO *mutations:* The constitutive mutations *hisO1828*, *hisO1812*, *hisO1832*, *hisO1831*, *hisO1202*, *hisO1830*, and *hisO1242* can recombine with *hisO2321* to form wild-type recombinants (data not shown). Therefore, these constitutive *hisO* muta-

TABLE 6

*Mapping* hisO2321 *with respect to other* hisO *mutations*

| Donor phage | Recipient bacteria *hisT1504 hisO2321* Percent wrinkled recombinants |
|---|---|
| *hisO3181 hisG46* | < 0.6 |
| *hisO3148 hisG46* | < 0.5 |
| *hisO2965 hisG46* | < 1.3 |
| *hisO2355 hisG46* | < 0.6 |
| *hisO2966 hisG46* | < 0.16 |
| *hisO3149 hisG46* | 36 |
| *hisO3185 hisG46* | 39 |
| *hisO3150 hisG46* | 17 |
| *hisO2964 hisG46* | 30 |

tions do not map in the region deleted by *hisO2321*. Double mutations containing *hisO2321* in combination with the constitutive mutations *hisO3154*, *hisO3155*, *hisO3156*, *hisO3197*, *hisO3198* or *hisO3199* have been isolated or constructed (MATERIALS AND METHODS and Table 1). Consequently, none of these constitutive *hisO* mutations maps in the region deleted by *hisO2321*.

The relative positions of some constitutive mutations with respect to the promoter-like mutation *hisO2355* were determined by reciprocal crosses. Table 7 shows that *hisO1812*, *hisO3197*, *hisO3198* and *hisO3199* map between *hisO2355* and *hisG46*, while *hisO1828* and *hisO3155* map to the left of *hisO2355* (Figure 3). Therefore we conclude that constitutive *hisO* mutations map on both sides of the promoter-like mutation *hisO2355*.

The relative positions of the promoter-like mutation *hisO3150* and the constitutive mutation *hisO1812* were ascertained by using phage grown on *hisO3150* to transduce the prototrophic, AMT-sensitive strain, *hisO2321 hisO1812*, to AMT resistance on enriched minimal high glucose plates containing 3 mM AMT. Wrinkled recombinants from this cross were isolated and analyzed genetically

TABLE 7

*Mapping of constitutive* hisO *mutations with respect to* hisO2355

| *hisO* mutation | *hisO2355* |
|---|---|
| *hisO1828* | .49/8.1* |
| *hisO3155* | .23/1.7 |
| *hisO1812* | .25/.06 |
| *hisO3197* | .27/.05 |
| *hisO3198* | †n.d./.13 |
| *hisO3199* | n.d./.03 |

* Values are the percent AMT-resistant recombinants formed. The numerator is from the cross: *hisO2355* × *hisO*$^c$ (constitutive *hisO* mutation) *hisG46*. The denominator is from the cross: *hisO*$^c$ × *hisO2355 hisG46*. Recombinants were scored for AMT resistance by streaking onto minimal high glucose plates containing 10 mM AMT.

† n.d. indicates not determined.

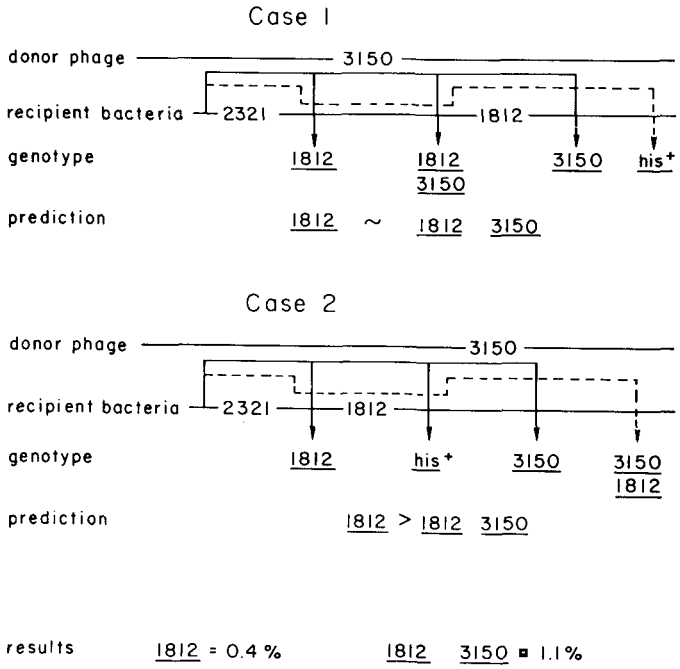


FIGURE 5.—Mapping of *hisO3150* and *hisO1812*. Case 1 and Case 2 indicate two possible map orders.

to determine their genotype. Recombinants with the genotype *hisO3150 hisO1812* outnumbered recombinants containing only *hisO1812* by a ratio of 3 to 1. If the order of the markers were *hisO2321:hisO3150:hisO1812*, as shown in Case 1 of Figure 5, recombinants with either of these genotypes would be formed by a double crossover, and their relative frequencies would depend on the relative distance between the markers. However, if the order of the markers were *hisO2321:hisO1812:hisO3150*, as shown in Case 2, the double mutant would be formed by a quadruple crossover and should be less frequent than *hisO1812* alone. Since the double mutant was more frequent than *hisO1812*, we conclude the map order *hisO2321:hisO3150:hisO1812*, as shown in Case 1.

The promoter-like mutations *hisO2964*, *hisO2979* and *hisO3150* were mapped with respect to the constitutive mutations *hisO1812*, *hisO3197*, and *hisO3198* by means of crosses of the type diagrammed in Figure 6. If the map order were *hisO2321:hisO2964:hisO1812*, as shown in Case 1, recombinants with a wild-type *his* operon would be rare since they would be formed by a quadruple crossover. However, if the map order were *hisO2321:hisO1812:hisO2964*, as shown in Case 2, recombinants with a wild-type *his* operon would be frequent compared to recombinants containing *hisO1812* or both *hisO1812* and *hisO2964*. The results of these crosses are shown in Table 8. In each cross involving *hisO2964* or *hisO2979* recombinants with a wild-type *his* operon were more numerous than the sum of the recombinants of the other two minority classes (*hisO*$^{c}$ *hisO*$^{p}$

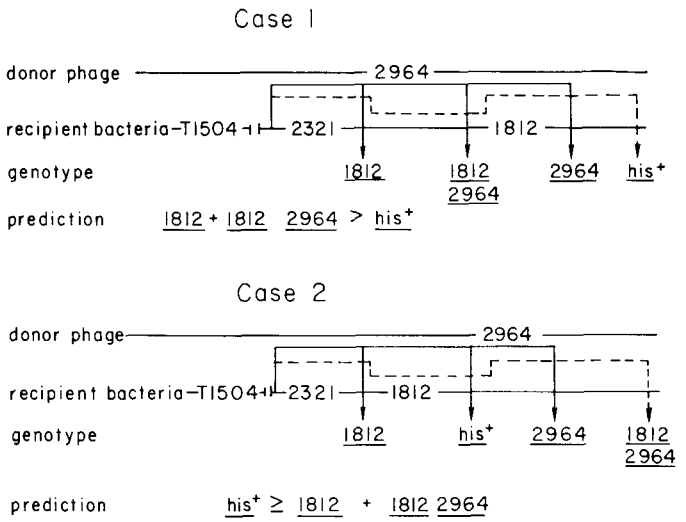


FIGURE 6.—Mapping of *hisO2964* and *hisO1812*. Case 1 and Case 2 indicate two possible map orders.

double mutants and *hisO*$^{c}$). Therefore, we conclude that *hisO1812*, *hisO3197*, and *hisO3198* map between *hisO2321* and *hisO2964* or *hisO2979*, as diagrammed in Figure 6, Case 2. This order is depicted in Figure 3. Preliminary crosses between *hisO2964* and either *hisO1242* or *hisO1830* indicate that *hisO2964* does not map in the area deleted by *hisO1242* (data not shown). Recombination frequencies were reduced 10- to 20-fold in these crosses, and recombinants were not obtained in numbers sufficient to indicate map order. However, as can be seen in the example of Figure 6, if *hisO2964* maps between *hisO2321* and the constitutive

TABLE 8

*Mapping of promoter-like* hisO *mutations with respect to constitutive* hisO *mutations*

| Cross Donor × Recipient | *His* operon recombinants* Number of recombinants | | |
|---|---|---|---|
| | *hisO*$^{+}$ | *hisO*$^{c}$ and *hisO*$^{c}$ *hisO*$^{p}$ | *hisO*$^{p}$ |
| *hisO2964* × *hisO2321 hisO1812 hisT1504* | 18 | 14 | 3600 |
| *hisO2964* × *hisO2321 hisO3197 hisT1504* | 13 | 6 | 2500 |
| *hisO2964* × *hisO2321 hisO3198 hisT1504* | 5 | 4 | 2000 |
| *hisO2979* × *hisO2321 hisO1812 hisT1504* | 16 | 10 | 3500 |
| *hisO3150* × *hisO2321 hisO3198 hisT1504* | 0 | 60† | 4800 |
| Phenotype | Wrk | Wrk | Smo |

* Recombinant genotypes were identified by using phage grown on single colony isolates of the wrinkled (Wrk) recombinants to transduce *hisG46* to prototrophy. Those phage which gave rise to recombinants with a smooth colony (Smo) morphology were considered to have been grown on a strain containing a wild-type *his* operon. Those phage which gave rise to recombinants with a wrinkled colony morphology were considered to have been grown on strains containing either a constitutive *hisO* mutation (*hisO*$^{c}$) or *hisO*$^{c}$ mutation in combination with a promoter-like *hisO* mutation (*hisO*$^{c}$ *hisO*$^{p}$ double mutant).

† Recombinants containing *hisO3198* were identified by a grainy colony morphology in contrast to the wrinkled colony morphology of recombinants containing *hisO*+.

*hisO* mutation as in Case 1, it could reduce recombination in that region and consequently reduce the frequency of wrinkled recombinants. On the other hand, in *hisO2964* does not map between *hisO2321* and the constitutive *hisO* mutation, wrinkled recombinants containing the constitutive *hisO* mutation should be formed at a normal frequency. Therefore, the reduced recombination frequency suggests the order depicted in Case 1, Figure 6, and shown in Figure 3.

When *hisO3150* phage were used to transduce the slow-growing AMT-sensitive recipient SB3228 (*hisO2321 hisO3198 hisT1504*), no recombinants with a wild-type *his* operon were obtained, indicating that *hisO3150* maps in the region deleted by *hisO3198*. This map position is consistent with the fact that like *hisO3150*, *hisO3198* has reduced *his* operon expression in the presence of *hisT1504* (ELY 1974). In contrast, the deletions *hisO3197*, *hisO3199*, and *hisO1832* had an increased *his* operon expression in strains containing *hisT1504*, and thus they are depicted in Figure 3 as ending between *hisO1812* and *hisO3150*.

FINK, KLOPOTOWSKI and AMES (1967) and P. E. HARTMAN (unpublished data) in three-point tests established the map order *hisO1242:hisG2101:hisG200* for the most distal mutation in *hisO* and the two most proximal of over 50 *hisG* mutations (HARTMAN *et al.* 1971). We demonstrated the map order *hisO2964: hisG200:hisG46* by reciprocal crosses between SB2989 (*hisO2964 hisG46 hisT1504*) and SB3231 (*hisG200 hisT1504*). $His^+$ recombinants with a smooth colony morphology were formed with a frequency of 7% when *hisO2964 hisG46* was the donor, and only 3% when *hisG200* was the donor. A greater percentage of smooth recombinants when *hisO2964 hisG46* is the donor indicates the map order *hisO2964:hisG200:hisG46*, consistent with the map order depicted in Figure 3.

## DISCUSSION

*Genetic map of* hisO: In two- and three-factor bacterial crosses, marker-specific effects often are more important for the determination of recombination frequency than are the map distances between the markers. In addition, conversion events are common in short genetic regions. Consequently, three-point tests sometimes fail to give a map order (cf. LOPER *et al.* 1964; MARTIN and TALAL 1968; NORKIN 1970; CRAWFORD and PREISS 1972). We have resorted in our mapping to pairs of reciprocal three-point tests where contrasting recombination frequencies are expected to reflect the differences between double and quadruple crossover classes rather than marker-specific effects and differential conversion events. Data obtained from such pairs of reciprocal tests result in a unique genetic map of the *hisO* region (Figure 3).

In the *lac* operon, operator and promoter mutations map in clearly distinguishable regions (MILLER *et al.* 1968; BECKWITH, GRODZICKER and ARDITTI 1972; ARDITTI, GRODZICKER and BECKWITH 1973) that nevertheless interact (SMITH and SADLER 1971) in a fashion yet to be explained. In contrast, our constitutive and promoter-like *hisO* mutations are clearly interspersed. In the accompanying paper we propose a model that accounts for the distribution of mutant sites and the physiological properties of our mutants (ELY 1974).

*Failure of recombinant detection:* While the map order determined in many

reciprocal three-point tests is unambiguous, several discrepancies exist in the genetic data. Each discrepancy stems from the failure to detect recombination between two markers that are clearly located at different sites as determined by other three-point tests. The instances of non-recombination occurred between *hisO1828* and similarly located mutations *versus hisO1812* and similarly located mutations, between *hisO3155 versus hisO1830*, and between *hisO3148* and *hisO2965 versus hisO2966*. We have considered, without success, a number of explanations for this phenomenon. The negative recombination data do not fit with a "deletion map" nor do cryptic *his*$^-$ mutations seem to be present in the strains involved. Models involving chromosomal rearrangements can explain the lack of recombination in these crosses, but are contradicted by three-point test data from other crosses. Since the bulk of the three-point test data appear to be valid, we propose that the failure to detect recombinants in certain recombination tests is a consequence of some unusual structure of the *hisO* region (ELY 1974). That is, a unique structure of *hisO* DNA effects formation of *certain* heteroduplexes or results in abnormal "repair" of heteroduplexes once they are found (cf. LACKS 1970).

We have put this hypothesis to one genetic test. A situation where recombination is expected but found lacking is in the cross of *hisO1828* and *hisO1812* (Table 4), and we propose that the structures of the respective DNA's block completion of recombination in the region between these two sites. In the accompanying paper we propose that a third mutation, *hisO1242*, eliminates most, if not all, of some unique tertiary structure of *hisO* DNA (ELY 1974). Therefore, we carried out the cross SB2665 (*hisO1828 his O1242*) as the donor and SB2803 (*hisO1812 hisG46*) as recipient. As shown in Cross 1 of Figure 7, wild-type recombinants would be formed by a quadruple crossover with three restricted areas for crossing

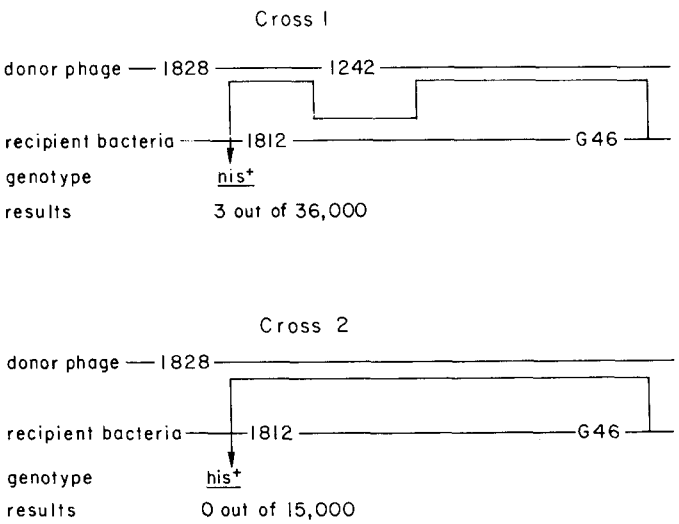


FIGURE 7.—Transduction cross of SB2803 (*hisO1812 hisG46 fla-2055*) recipient and phage grown on SB6828 (*hisO1828*) or SB2665 (*hisO1828 hisO1242*).

over. On the other hand, they would be formed by a double crossover when *hisO1242* was not present (Cross 2). The data in Figure 7 show that wild-type recombinants were formed with *hisO1242* present and not when it was absent, even though they should have been approximately ten times more frequent in the latter case. Since *hisO1242* does not map between *hisO1828* and *hisO1812*, it seems reasonable to assume that the presence of *hisO1242* enhances recombination between the latter two markers by altering some aspect of the three-dimensional structure of the entire *hisO* region.

*Size of the* hisO *region:* We estimate that the *hisO* region is some 200 to 500 base pairs in length. This estimate utilizes the recombination values of 1.7%, 2.4%, and 4.6%, between *hisG200* and *hisO3148*, *O3155* and *O1828*, respectively. Mutation *hisG200* is the second of some 40 recombinationally separable mutational sites in the *hisG* gene (HARTMAN *et al.* 1971). The *hisG* enzyme monomer has a molecular weight of 35,000 and an N-terminal methionine (VOLL and APPELLA and MARTIN 1967) so that mutation *hisG200* would be located about 30 nucleotide pairs from the proximal end of gene *G* if the recombinationally separable mutations are located randomly. In keeping with this estimate, we find recombination values of 0.5% and 0.6% between *hisG200* and two operator mutations close to *hisG*, *hisO3154* and *hisO3156*, respectively (Figure 3). We assume that the length of P22 DNA is about 40,000 base pairs (RHOADES, MACHATTIE and THOMAS 1968) and that P22 transducting particles contain the same amount of DNA as infectious particles (EBEL-TSIPIS, BOTSTEIN and FOX 1972). Application of the recombination frequencies noted above to the formula of WU (1966) allows us to calculate distances of about 250, 300 and 600 base pairs between *hisG200* and the operator mutations *hisO3148*, *-3155*, and *-1828*, respectively. Mutation *hisO1828* often shows excessive recombination (cf. Table 3) so that this estimate may be distorted toward a high value. Pronounced marker-specific effects have not been observed with the other two mutations nor with *hisG200* (data not shown). These considerations lead to our estimate of some 200 to 500 base pairs for the *hisO* region. This size is intermediate between the estimates of 100 base pairs for the *lacPO* region (MILLER *et al.* 1968) and of some 2000 base pairs for the *trpO* region (HIRAGA 1969).

*Nature of* hisO *mutations:* Although the *hisO* region is proposed to be fairly long, relatively few mutant sites have been detected. Many of our mutations would seem to be deletions. For example, neither *hisO1242* (VOLL 1967) nor *hisO2321* has been detected to revert and both map as multisite mutations. There are clusters of independently isolated non-recombining mutations at either end of the *hisO* region which could be deletion mutations. In contrast, one promoter-like mutation to histidine auxotrophy, *hisO3601*, can revert (ATKINS and LOPER 1970; FANKHAUSER 1971) as can the promoter-like mutation *hisO2355* (FANKHAUSER 1971). Strong base substitution mutagens (e.g., diethylsulfate) but not ICR191 stimulate true reversion. These mutations are located in the region we propose is critical to RNA polymerase binding and/or initiation (ELY 1974).

It appears that operator constitutive mutations can be induced by diethylsulfate (CHANG, STRAUS and AMES 1971) and nitrosoguanidine, and several pro-

motor-like mutations were detected after similar treatment (STRAUS and WYCHE 1974). A more quantitative measure of mutagen efficiency in induction of high-level operator constitutive mutations comes, however, from reversion studies on *hisO2321* and *hisO3148* (MATERIALS AND METHODS). We found that diethylsulfate was effective and ICR191 was not effective in induction of constitutive mutations in *hisO2321*. Similarly, diethylsulfate was effective and 2-aminopurine and 5-bromouracil were not effective in induction of constitutive mutations in *hisO3148*. Thus, high enzyme level constitutive mutations may be base-substitution transversions and deletions but not simple frameshifts in G/C-rich regions or transition mutations.

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