PHYSIOLOGICAL STUDIES OF SALMONELLA HISTIDINE OPERATOR-PROMOTER MUTANTS

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ABSTRACT

S. typhimurium hisO mutations are *cis* dominant and *trans* recessive and occur in a regulatory segment separate from but adjacent to the first structural gene of the histidine operon, *hisG*. Strains containing *hisO* mutations singly and in combination with other regulatory mutations were examined for their content of L-histidinol phosphate phosphatase when grown on limiting and on excess L-histidine. *HisO* mutations classed as "constitutive" (high enzyme levels) or as "promoter-like" (low enzyme levels) cause a variety of subphenotypes. A model is proposed that accounts for the phenotypes found as well as for the interspersion of constitutive and promoter-like mutations on the genetic map (ELY, FANKHAUSER and HARTMAN 1974). In this model we suggest that the *his* operator-promoter DNA is a functional unit that assumes alternate conformations including: (a) the classic linear duplex, active in transcription, and (b) a looped structure that is transcriptionally closed and susceptible to the binding of repressor.

THE genetic map of the *S. typhimurium* histidine operator-promoter (*hisO*) region contains an interspersion of constitutive and promoter-like mutations (ELY, FANKHAUSER and HARTMAN 1974). Here we examine the effects of these *hisO* mutations on *his* operon expression under various conditions of repression and derepression. In addition, pairs of *hisO* mutations have been constructed in the *cis* configuration and examined for interactions affecting operon expression. Our data lead us to propose a model in which the *hisO* region is assumed to exist in two alternate conformational states. One conformation is the traditional linear duplex which is open to transcription. The second is a looped structure with an unusual three-dimensional conformation and closed to transcription. We propose that *his* operon expression could involve shifts in the equilibrium between these two conformational states.

MATERIALS AND METHODS

Bacterial strains: All strains used here are derivatives of *Salmonella typhimurium* LT-2 (Table 1, as supplemented by Table 1 of ELY, FANKHAUSER and HARTMAN 1974). Important strain derivations can be traced directly in these two tables; additional information on lineages is listed elsewhere (ELY 1973).

Strain construction: SB3095 (*hisG46 fla-2055*) and its close derivatives SB5001 (*hisG46 hisD1*

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TABLE 1

Bacterial strains (also consult Table 1 in ELY, FANKHAUSER and HARTMAN 1974)

Strain	Genotype	Derivation or source
Salmonella typhimurium LT-2		
SB2048	*hisO3148 hisT1504*	SB3193 phage × TA516
SB2050	*hisO3149 hisT1504*	SB3194 phage × TA516
SB2052	*hisO3150 hisT1504*	SB3195 phage × TA516
SB2061	*hisG46 hisT1504*	*hisG46* phage × TA516
SB2231	*hisO1828 hisO3148 fla-2055*	SB3232 phage × SB3095
SB2236	*hisO2355 hisT1504*	SB2805 phage × TA516
SB2237	*hisO1242 hisO3148 fla-2055*	SB2047 phage × SB2247
SB2241	*hisO3155 hisO3148 fla-2055*	SB3235 phage × SB3095
SB2242	*hisO2321 hisO3155 fla-2055*	SB3236 phage × SB3095
SB2247	*hisO2321 hisO1242 fla-2055*	SB2812 phage × SB3095
SB2291	*hisO2321 hisO1812 fla-2055*	SB3230 phage × SB3095
SB2292	*hisO3148 hisO1812 fla-2055*	SB2047 phage × SB2291
SB2364	*hisO3150 hisO1812 fla-2055*	SB2051 phage × SB2291
SB2453	*hisO3155 hisT1504*	SB2243 phage × SB2061
SB2458	*hisO3150 hisO3155 fla-2055*	SB2051 phage × SB2242
SB2484	*his+ hisT1504*	Wrinkled recombinant from SB2047 phage × TA516
SB2617	*hisO1242 fla-2055*	TA1003 phage × SB3095
SB2618	*hisO1812 fla-2055*	TA795 phage × SB3095
SB2619	*hisO1828 fla-2055*	SB6828 phage × SB3095
SB2722	*hisO2964 fla-2055*	TA2582 phage × SB3095
SB2723	*hisO2964 hisT1504*	TA2582 phage × TA516
SB2724	*hisO2965 fla-2055*	TA2583 phage × SB3095
SB2725	*hisO2965 hisT1504*	TA2583 phage × TA516
SB2726	*hisO2966 fla-2055*	TA2584 phage × SB3095
SB2727	*hisO2966 hisT1504*	TA2584 phage × TA516
SB2783	*hisO3198 hisT1505*	SB2772 phage × TA516
SB2790	*hisG46 strB668 fla-2055*	2AP mutagenesis of SB3095
SB2812	*hisO2321 hisO1242*	TA1003 phage × SB2800
SB2846	*hisO1812 hisT1504*	TA795 phage × TA516
SB2847	*hisO1828 hisT1504*	SB6828 phage × TA516
SB3048	*his+ strB668 fla-2055*	*ara-9* phage × SB2790
SB3049	*hisO1812 strB668 fla-2055*	TA795 phage × SB2790
SB3212	*hisO2321 strB676*	Spontaneous in SB2800
SB3232	*hisO1828 hisO3148 hisT1504*	SB6828 phage × SB2056
SB3266	*hisO1828 strB668 fla-2055*	SB6828 phage × SB2790
SB3267	*hisO3150 strB668 fla-2055*	SB2051 phage × SB2790
SB3269	*hisO2965 strB668 fla-2055*	TA2583 phage × SB2790
SB3271	*hisO2964 strB668 fla-2055*	TA2582 phage × SB2790
SB3272	*hisO2355 strB668 fla-2055*	SB2805 phage × SB2790
SB3307	*hisO3155 strB668 fla-2055*	SB2243 phage × SB2790
SB3309	*hisO3148 strB668 fla-2055*	SB2047 phage × SB2790
SB3310	*hisO2966 strB668 fla-2055*	TA2584 phage × SB2790
SB3311	*hisO3149 strB668 fla-2055*	SB2049 phage × SB2790
SB3320	*hisO2965 hisO1242*	TA2583 phage × SB2812
SB3321	*hisO2966 hisO1242*	TA2584 phage × SB2812
SB3322	*hisO2965 hisO1812*	TA2583 phage × SB3230

SB3323	*hisO2966 hisO1812*	TA2584 phage × SB3230
SB3324	*hisO2964 hisO1812 hisT1504*	TA2582 phage × SB3229
SB3326	*hisO2355 hisO3155*	SB2805 phage × SB2242
SB3366	*hisO2964 hisO1812*	SB3324 phage × SB3095
TA515	*hisC2316 hisT1504*	T. KLOPOTOWSKI (Method of FINK, KLOPOTOWSKI and AMES 1967)
TM90	*hisD3911 hisR1223 ara-9 strA*	R. G. MARTIN Stock Culture

fla-2055) and SB2790 (*hisG46 fla-2055 strB668*) were chosen as a "standard" relatively isogenic genetic background. A number of *hisO* mutations were transduced into these backgrounds by selection for prototrophy or growth on histidinol (*hisD*+) via the direct-plating method (ELY, FANKHAUSER and HARTMAN 1974). Similarly, TA516 (*hisT1504 hisD2317*) and TM90 (*hisR1223 hisD3911 ara-9 strA*^{r}) served as standard genetic backgrounds for analysis of the effects of *hisT* and *hisR* regulatory mutations. Since parallel results were obtained with *hisT* and *hisR* strains (ELY 1973), only data for *hisT* are recorded in this report.

Strains containing two *hisO* mutations were constructed in a variety of ways dependent upon the phenotypic characteristics elicited by each mutation. In some cases an educated guess had to be made as to the phenotype of the double mutant. Verification was always obtained by thorough genetic analysis of the presumed double mutant. Derivations of most double mutants can be traced in Table 1 as supplemented by Table 1 of ELY, FANKHAUSER and HARTMAN (1974), while a detailed presentation may be found elsewhere (ELY 1973).

Media: Media are described in the accompanying paper (ELY, FANKHAUSER and HARTMAN 1974).

F′ matings: F′ matings were performed by streaking 0.02 ml of an unaerated, overnight culture of the donor across the center of a plate of selective medium. After the excess liquid had soaked into the agar, 0.02 ml samples of overnight cultures of recipient strains were streaked across the plate in parallel to one another and perpendicular to the donor streak. F′-merogenotes were detected in the area of intersection after 24 to 48 hours' incubation at 37°.

Histidinol phosphate phosphatase (hisB) *enzyme assays:* Bacteria were grown and toluenized extracts prepared essentially as described by WYCHE *et al.* (1974) and AMES, HARTMAN and JACOB (1963), respectively. The assay for L-histidinol phosphate phosphatase (*hisB* enzyme) activity was modified after MARTIN *et al.* (1971), as outlined in Table 2. The assay was started by the addition of histidinol phosphate and incubated at 37° for 15 minutes. Addition of 0.7 ml of color reagent stopped the reaction, and the subsequent incubation at 45° allowed for full color

TABLE 2

Histidinol phosphate phosphatase (hisB *enzyme*) *assay*

Additions	Sample (in duplicate)	Blank (in duplicate)	Histidinol phosphate Control (2 per assay)	Zero (1 per assay)
TEA buffer*	0.2 ml	0.2 ml	0.3 ml	0.3 ml
Toluenized cells	0.1 ml	0.1 ml	..	..
Histidinol phosphate†	10 μl	..	10 μl	..
	Shake to mix, incubate at 37° for 15 minutes			
Color reagent‡	0.7 ml	0.7 ml	0.7 ml	0.7 ml
	Shake to mix, incubate at 45° for 20 minutes			
	Measure absorbance at 820 nm			

* TEA buffer consists of 0.1 M triethanolamine, pH 7.5 plus 1.5 mM $MgCl_2$.

† 50 mM L-histidinol phosphate. Store frozen and thaw immediately prior to use.

‡ Color reagent is made immediately prior to use by mixing 6 parts 0.42% ammonium molybdate in 1 N H_2SO_4 with 1 part 10% ascorbic acid.

TABLE 3

Protein quenching in crude sonicated extracts

Strain	Crude sonic extracts 5λ	10λ	5λ + 50λ *his-515**	10λ + 50λ *his-515*
		ΔOD 820		
his+	.051	.068	—.016†	—.004‡

Strain	Toluenized cells 10λ	25λ	10λ + 50λ *his-515*§	25λ + 50λ *his-515*
		ΔOD 820		
his+	—	.033	—	.037
hisT1504	.102	—	.103	—

* Sonicated extracts prepared as described by WYCHE *et al.* (1974).
† Sample was less than the sum of the controls.
‡ 50λ of a sonic extract of *his-515*, a deletion of the entire *his* operon, was added.
§ 50λ of a toluenized cell suspension of *his-515* was added.

development. The absorbance at 820 nm was measured within a half hour on a Gilford Instruments spectrophotometer. In order to maximize sensitivity, absorbance was measured using a red filter, and the slit width was decreased by setting the "zero" at 1.00 OD rather than at 0.00 OD. All samples were run in duplicate. Activities were calculated by averaging the duplicate values and then subtracting the sum of the blank and the histidinol phosphate control from the absorbance of the complete mixture.

Toluenized cells were used for the phosphatase assay rather than crude sonic extracts since protein quenching occurs with sonicated extracts but not with toluenized cells (Table 3), and the latter procedure is more convenient.

Both assay methods have a shortcoming in that the phosphatase is subject to severe end-product inhibition. A plot of phosphatase activity *versus* OD at 650 nm for SB2484 (*hisT1504*) is shown in Figure 1 (lower curve). The deviation from linearity is due to product inhibition by histidinol, as shown in Table 4. The addition of exogeneous histidinol at concentrations generated

TABLE 4

Inhibition of histidinol phosphate phosphatase activity by histidinol

Experiment no.	Strain	Addition	ΔOD 820	Percent activity remaining
1	*hisT1504*	—	.227	100
	hisT1504	.02 μ mole hol*	.132	58
	hisT1504	.1 μ mole hol	.045	20
	hisT1504	.02 μ mole PO_4	.218	96
	hisT1504	.02 μ mole PO_4 + .02 μ mole hol	.118	52
2	*hisT1504*	—	.180	100
	hisT1504	3.9 nmole hol	.178	99
	hisT1504	7.7 nmole hol	.153	85
	hisT1504	11.6 nmole hol	.130	72
	hisT1504	15.4 nmole hol	.122	68

* hol = histidinol.

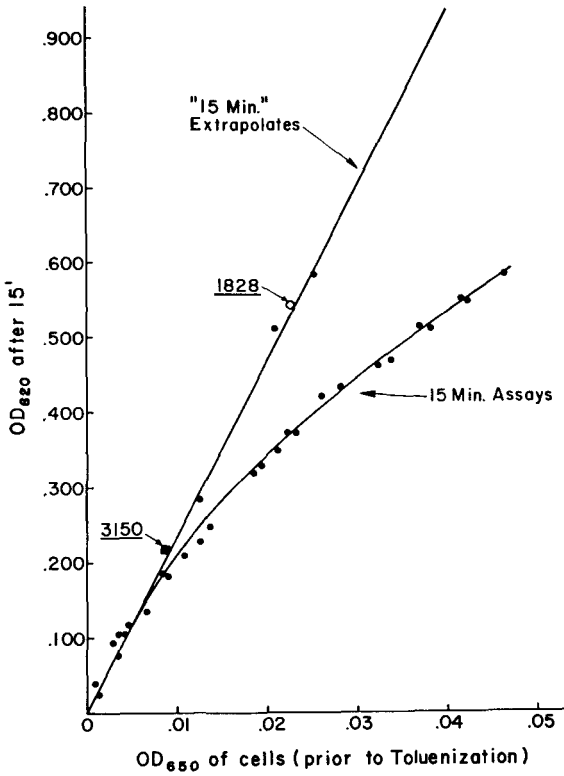

FIGURE 1.—*HisB* enzyme activity of SB2484 (*hisT1504*). The curved line represents *hisB* enzyme activities measured in toluenized cell suspensions at varying concentrations of cells. The values on the tangent to the curve represent the initial rate from the curves in Figure 2, extrapolated to 15 minutes.

in the assay causes up to 40% inhibition of activity. BRADY and HOUSTON (1973) have independently observed histidinol inhibition of phosphatase and have calculated a K_i of 52 μM.

The activity observed in toluenized extracts can be corrected for histidinol inhibition if the initial rate of the reaction is linear before significant amounts of histidinol are generated. Initial rates were determined for several concentrations of SB2484 (*hisT1504*), and for SB2619 (*hisO1828*) and SB2051 (*hisO3150*) extracts (Figure 2). The initial rates are linear and thus can be extrapolated to give hypothetical values for activity per 15 minutes' incubation in the absence of histidinol inhibition (upper curve, Figure 1). The hypothetical values determine a tangent to the lower curve in Figure 1 which is made from actual values obtained in 15-min. assays. The hypothetical values for SB2619 and SB2051 also fall on the same tangent (upper curve, Figure 1). Since SB2619 and SB2051 have about 90% and 30% of the enzyme level of SB2484, the use of the curve for SB2484 as a standard seems justified. Therefore, the standard assay values reported in RESULTS have been corrected for histidinol inhibition by placement of the measured value on the standard curve and extrapolation to the corresponding point on the tangent. Specific activities were calculated by multiplication of the corrected value by 10 to give the activity per ml and then division by the absorbance at 650 nm to normalize for protein concentration. A unit of enzyme is defined as the formation of 1 OD at 820 nm in 15 minutes under the conditions of the assay (MARTIN *et al.* 1971).

Dehydrogenase and pyrophosphorylase assays: Preparation of cell free extracts and assay of L-histidinol dehydrogenase (*hisD* enzyme) are described elsewhere (WYCHE *et al.* 1974). Phos-

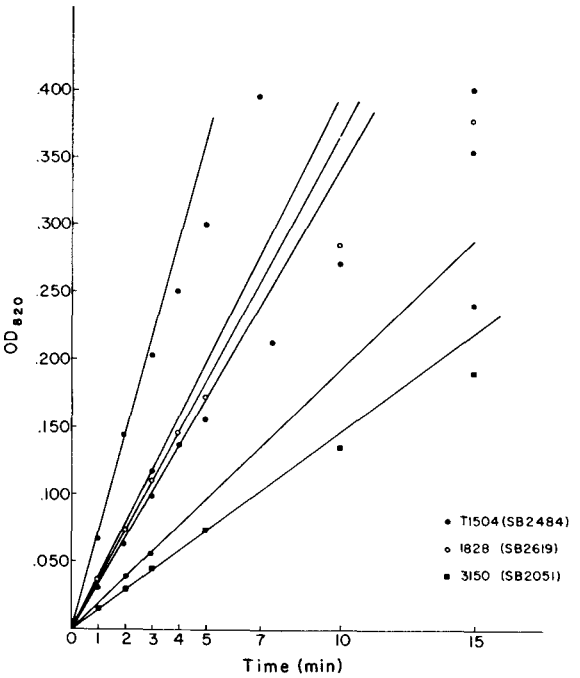

FIGURE 2.—*HisB* enzyme activity *versus* time at various cell concentrations. *HisB* assays were performed on toluenized cell suspension of SB2051 (*hisO3150*)■, SB2619 (*hisO1828*)○, and several concentrations of SB2484 (*hisT1504*)●. Lines indicate initial rates of reaction. Initial rates extrapolated to 15 minutes are plotted versus cell concentration in Figure 1.

phoribosyl-ATP pyrophosphorylase (*hisG* enzyme) was assayed by the method of VOLL, APPELLA and MARTIN (1967) and protein by the method of LOWRY *et al.* (1951) employing bovine serum albumin as a standard.

RESULTS AND DISCUSSION

HisO *mutations are operator mutations:* All of the *hisO* mutations tested are *cis* dominant and *trans* recessive. Evidence for this conclusion is presented in Tables 5 and 6. Table 5 shows that constitutive *hisO* mutations raise enzyme activity due to the chromosomal *hisD* gene located *cis*, but do not significantly change enzyme activity due to the episomal *hisG* gene located *trans*. Similarly, Table 6 shows that expression of an episomal $hisB^+$ gene is the same regardless of the level of chromosomal $hisB^+$ gene expression in strains containing chromosomal high- and low-enzyme level *hisO* mutations. Furthermore, abortive transduction tests and merodiploids of the type $hisO/F'his^+$ for auxotrophic promoter mutations *hisO2321* and *hisO3601* indicate that these mutations, too, are *trans* recessive since they do not influence the wild-type phenotype of the complementing wild-type *his* operon. These two auxotrophic *hisO* mutants essentially lack all of the histidine enzymes and complement only to the extent expected of strains lacking expression of the primary histidine promoter (ATKINS and LOPER 1970; ELY and CIESLÀ 1974). For comparison, among hundreds of mutants tested that map internally in the

TABLE 5

Cis *dominant*, trans *recessive phenotypes of* hisO *mutations in* hisO hisG46/*F'*hisD2379 *heterodiploids**

Bacterial strain carrying F' *hisD2379*	Enzyme-specific activity† *hisG*	*hisD*
hisO+ hisG46	1.6	2.7
hisO1828 hisG46	3.5	22
hisO1812 hisG46	1.6	47
hisO1831 hisG46	2.8	29
hisO1202 hisG46	1.5	35
hisO1830 hisG46	2.6	73
hisO1242 hisG46	0.5	79

* Strains were infected by contact with strain TR49, multiple auxotroph carrying an *E. coli* K12 F' containing mutation *hisD2379*. The prototrophic merozygotes were purified and grown in minimal medium to prevent loss of the episome. Strains segregate *hisG*⁻ bacteria. Since the strains are easily reconstructed, they were not preserved and are not included in Table 1.

† Bacteria were grown to log phase in minimal medium supplemented with 1 mM L-histidine and the *hisG* and *hisD* enzymes were assayed as described in MATERIALS AND METHODS.

his operon only one (*hisD984*) is as strongly polar as these two mutations (GREEB, ATKINS and LOPER 1971; HARTMAN *et al.* 1971).

Although truly definitive data are lacking, *hisO* mutations appear to reside in a unique polynucleotide sequence and not in the first structural gene of the operon, *hisG*. This conclusion is based on the apparent identity of *hisG* enzyme obtained from *hisO+* with that obtained from *hisO1242*, or *hisO3156*—two *hisO* mutations mapping closest to *hisG* (ELY, FANKHAUSER and HARTMAN 1974), as demonstrated by: (a) specific activity of homogenous pure enzyme, (b) feedback inhibition by L-histidine, (c) molecular weights of native enzyme determined in gel filtration and subunits determined by SDS disc gel electrophoresis, (d)

TABLE 6

Cis *dominant*, trans *recessive phenotype of* hisO *mutations in* hisO hisG46/*F'*his+ *and /F'*BH2405 *heterodiploids**

Bacterial strain carrying F'	Episome F'*his*+	F' *BH2405* *hisB* specific activity	Difference (units/OD_{650})†
hisO+ hisG46	10.6	2.6	8.0
hisO1828 hisG46	26.3	17.9	8.4
hisO3148 hisG46	9.4	0.7	8.7
hisO2355 hisG46	8.4	0.5	7.9
hisO3149 hisG46	9.0	1.6	7.4
hisO3150 hisG46	15.1	6.0	9.1
hisO1812 hisG46	21.1	14.6	6.5

* Strains were infected by contact with strain TR75, a multiple auxotroph carrying an *E. coli* K12 F' containing mutation *hisBH2405*. Prototrophic merozygotes were purified and treated as described in footnote * of Table 5.

† For determination of *hisB* activity, bacteria were grown to log phase in minimal medium and assayed as described in MATERIALS AND METHODS.

Ouchterlony immunodiffusion against anti-*hisO1242* and anti-*hisO*$^+$ enzymes, (e) amino acid compositions, and (f) release of amino acids by leucine aminopeptidase from *hisO*$^+$ and *hisO1242* enzymes (S. M. PARSONS and M. LIPSKY, personal communication). Also, auxotrophic and low enzyme level *hisO* mutants revert (ELY, FANKHAUSER and HARTMAN 1974), but none gives rise among revertants to nonsense suppressors. On the other hand, auxotrophic *hisG* mutants such as *hisG46* (missense) and *hisG200* (UGA) do not appear to influence enzyme levels except that *hisG200* has a polarity expected for a mutation proximally located in a structural gene. Finally, *in vitro*, *hisO1242* DNA initiates transcription at a much higher frequency than *hisO*$^+$ DNA (KASAI 1974), suggesting the occurrence of regulation at the level of transcription rather than some sort of polarity phenomenon.

Growth of the "promoter-like" mutants is not enhanced on carbon sources such as citrate in the absence of glucose, and cyclic AMP does not influence growth of these strains (data not shown) or histidine enzyme levels of wild type grown under a variety of conditions (R. F. GOLDBERGER, personal communication). Regulation of the histidine operon thus appears to be independent of catabolite repression, and none of our low-enzyme mutants appears to have merely gained a novel repression mechanism directly related to catabolite repression.

Interspersion of effective sites in hisO: Histidinol phosphate phosphatase (*hisB* enzyme) levels for wild type and for various single and double regulatory mutants are shown in Table 7. Data for the prototype strains are shown in line 1. Columns 1 and 2 show expression of a wild-type *his* operon in the presence and absence of histidine. Columns 3 and 4 show physiological and genetic derepression of *hisO*$^+$ strains, while column 5 shows increased *his* operon expression due to increased activity of the positive control system (WYCHE *et al.* 1974).

Data for thirteen representative *hisO* mutations are arranged in Table 7 according to map order, beginning with the most proximal in line 2 (farthest from *hisG*) through the most distal in line 14 (nearest *hisG*). Exceptions are the deletion strains *hisO2321* and *hisO3198*, which are listed on lines 8 and 12 below the sites they cover (genetic map in ELY, FANKHAUSER and HARTMAN 1974). Similar data for other *hisO* mutant strains are presented in ELY (1973). Inspection of columns 1, 2, and 4 reveals that "constitutive" (repressed *hisB* enzyme levels greater than 10, e.g., *hisO1828*, *-3155*, *-1812*, *-1242*) and "promoter-like" (repressed *hisB* enzyme levels less than 6, e.g., *hisO3148*, *-2965*, *2355*, *2966*, *-3149*, *-3150*, *2964*) *hisO* mutations are interspersed in the *hisO* region.

Mutation *hisO1828* (line 2) is representative of a group of 20 proximally located constitutive mutations which have similar, high-enzyme levels that are decreased in the presence of an unlinked *hisT* regulatory mutation (column 1 *versus* column 4). This decrease in enzyme specific activity in the presence of *hisT* is typical of strains that cannot physiologically depress and is also observed with constitutive gluconate-6-phosphate dehydrogenase (MURRAY and KLOPOTOWSKI 1968). Thus, the decrease may be a general characteristic of constitutive genes incapable of derepression. The next mutation, *hisO3155*, elicits a high level of *his* operon expression that remains high in the presence of a *hisT* mutation.

TABLE 7

HisB *enzyme levels in wild type (line 1) and in* hisO *mutants alone and in combination with other regulatory mutations*

	Markers in addition to *hisO* and additions to growth medium*								
	1. + his	2. none	3. *hisG46*† + hol	4. *hisT1504*‡ + his	5. *strB*§ + his	6. *hisO1828*¶ + his	7. *hisO3155*¶ + his	8. *hisO1812*¶ + his	9. *hisO1242*¶ + his
	hisB specific activity (units/OD_{650})								
1. *hisO+*	1.4	2.2	26	16.3	4.6	15.6	20.0	14.0	36
2. *hisO1828*	15.6	16.4	20	9.5	18.3				42
3. *hisO3155*	19.5	21.2		21.5	23				
4. *hisO3148*	~.2‖	1.5	2.7	1.1	~.3‖	1.7	18.2	4.4	31
5. *hisO2965*	~.2‖	1.4		1.3	3.5			5.3	29
6. *hisO2355*	~.1‖	1.3	1.5	~.4‖	~.1‖	(his−)**	2.2		1.4
7. *hisO2966*	~.2‖	1.5		1.0	~.3‖			6.5	29
8. *hisO2321*	<.1‖	his−		<.1‖	~.1‖	(his+)††		~.4‖	1.1
9. *hisO3149*	1.6	2.2	6.8	7.3	2.4				
10. *hisO3150*	5.2	6.2	5.9	3.8	7.6		18	24	
11. *hisO1812*	14.0	13.3	31	16.4	41				
12. *hisO3198*	16.5			11.9					
13. *hisO2964*	1.2	1.7		4.9	2.6			17.7	
14. *hisO1242*	36	38				42			

Wild-type (line 1) and *hisO* mutants (lines 2–14) are listed in map order, except deletion mutations (lines 8, 12), which are listed below mutational sites they cover. All values for *hisB* enzyme activity are the average of two or more determinations with each determination assayed in duplicate. Standard deviations of the assay values are less than 20% except when strains with specific activities less than one unit per OD_{650} are measured, in which case the standard deviations are less than 40%. All strains, with the exception of those in column 4 and those in line 8, are isogenic outside of the markers listed (MATERIALS AND METHODS).

* 0.1 mM histidine or 1 mM histidinol were added to the growth medium where indicated.

† Physiological derepression was achieved by histidine limitation using L-histidinol as a source of histidine. Similar results were obtained by growing prototrophic strains containing *hisS1210*.

‡ Similar results were obtained using strains containing *hisR1223*.

§ A mutation in *strB* stimulates the positive control system for the *his* operon (WYCHE *et al.* 1974).

¶ strains contained the *hisO* mutation at the top of the column in a *cis* configuration with the *hisO* mutation listed on the left.

‖ The level of expression of an internal promoter (ATKINS and LOPER 1970), P2 (0.4), has been subtracted from all values less than 1.0 units/OD_{650}. P2 expression was not subtracted from values greater than 1 unit/OD_{650}; since, above this level, expression of the primary histidine promoter, P1, begins to interfere with P2 expression (ELY and CIESLÀ 1974).

** The *hisO1828 hisO2355* double mutant has not been isolated but probably requires histidine for growth (ELY 1973).

†† The *hisO2321 hisO1828* double mutant has not been constructed, but mutations similar to *hisO1828* have been separated from revertants of *hisO2321* (ELY 1973).

This phenotype is similar to the high-level constitutive mutations that map distally in *hisO* (e.g., *hisO1812*), except that strains containing *hisO3155* do not have a reduced expression when *hisO3148* is present in the *cis* configuration (line 4, columns 7 and 8).

The mutations listed on lines 4 to 10 represent a group of fourteen mutations causing at least eight different phenotypes. These mutations have been designated promoter-like *hisO* mutations since they all have low *his* operon expression in the presence of regulatory mutation *hisT* (column 4). Several of these mutations

cause hyper-repressed *his* operon expression in the presence of histidine (column 1, lines 4–7). *HisO2321* (line 8) is extreme in that it virtually eliminates *his* operon expression at the primary promoter (ELY and CIESLÀ 1974). *HisO3150* (line 10) and a similar mutation, *hisO3207* (not shown), have been designated absolute constitutives since they have a similar, intermediate level of *his* operon expressed and cannot be further depressed. These two mutants also have a decreased *his* operon expression in the presence of *hisT*, as compared to the *hisT*+ strains.

The mutations listed on lines 11 and 12 represent a second group of at least nine high-level constitutive mutations which map between two groups of promoter-like mutations. *HisO3198* (line 12) is a deletion which includes the sites of *hisO3150* and *hisO1812* and causes a phenotype which includes a high enzyme level, as found in *hisO1812*, and a decreased expression in combination with *hisT1504*, as found in *hisO3150*. *HisO1812* (line 11) represents the remaining eight high-level constitutive mutations which cause similar high levels of *his* operon expression and which can be further derepressed by a mutation in *hisT*.

Closer to *hisG* are two additional promoter-like mutations, represented here by *hisO2964* (line 13). Members of this second group of promoter-like mutations have a phenotype similar to one of the promoter-like mutations (*hisO3149*) in the previous promoter-like group, but map on the opposite side of the central groups of constitutive mutations.

The remaining group, consisting of at least seven mutations mapping closest to *hisG*, is represented by the deletion *hisO1242* (line 14). These mutations cause the highest levels of constitutive *his* operon expression (20–26 units when grown in the presence of histidine—not shown in Table), with *hisO1242* the most extreme (36 units in Table 7).

A "repression loop": We have unsuccessfully attempted to design a simple linear model for the *hisO* region containing defined binding sites (overlapping or non-overlapping) for RNA polymerase, repressor, and activator. A wide variety of models utilizing varying assumptions was outlined (e.g., the probability of "up" promoter mutations, RNA chain-terminating mutations, creation of new promoters, multiple repressor binding sites, etc.). However, none of the models accounted for all the phenotypes of the *hisO* mutants or for the interactions we have observed between various pairs of *hisO* mutations. The major problem with all of these models is that changes in binding ability for RNA polymerase, repressor, and activator provide only three variables with which to account for eight different phenotypes of promoter-like mutants mapping in two separate regions and five different phenotypes of constitutive mutations mapping in three separate regions. Since these different phenotypes represent not only quantitative but also qualitative differences in response to various genetic and physiological conditions, it is obvious that three variables are insufficient to account for the observed number of phenotypes. Therefore, a region subject to structural alterations in addition to modification of binding sites is proposed. This model has been amplified and a number of additional features of the transcription process are hypothesized by KASAI (1974).

Our model assumes that the physiological variation of *his* operon expression results from the combined action of a system of negative control involving the histidyl-tRNA and perhaps other macromolecules (BRENNER and AMES 1971; GOLDBERGER and KOVACH 1972) and of a system of positive control involving the histidyl-tRNA synthetase and perhaps other macromolecules (WYCHE *et al.* 1974). Loss of the system of negative control is observed when a mutation is present in *hisT* (Table 7, column 4), while loss of the positive control system seems to have occurred in *hisO* mutants with a hyperrepressed *his* operon expression (column 1, lines 4–8). The positive control system is stimulated by physiological derepression (column 3) or by a mutation in *strB* (column 5 and WYCHE *et al.* 1974). In addition to interactions with activator and repressor, we propose that the *hisO* DNA is subject to critical structural alterations that are not truly "binding site" modifications in the sense normally supposed. That is, we suggest that the *hisO* region contains intrastrand homology that allows it to "loop out" into a transcriptionally closed form as shown diagrammatically in Figure 3. This looped form would be in equilibrium with a linear form competent in the binding of RNA polymerase and chain initiation.

In the wild type, each round of transcription may be followed by loop formation as the DNA rewinds following transcriptional opening. The loop formation would allow an opportunity for interaction with repressor when present. The basal enzyme level (1.4 units enzyme in line 1 of Table 5) reflects the probability of repressor binding (to form a more stable, inactive state) *versus* the switch to a transcriptionally competent duplex. In the absence of repressor (*hisT* column in

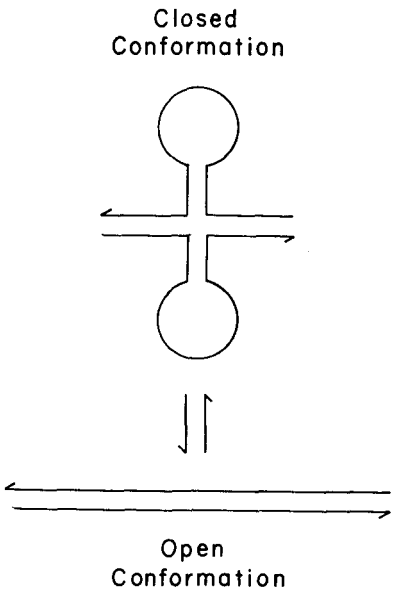

FIGURE 3.—Model of a two-conformation equilibrium for the three-dimensional structure of the *hisO* region. Closed conformation refers to the form which binds repressor; open conformation refers to the conformation which binds RNA polymerase.

Table 5) the switch occurs freely (16.3 units enzyme). An activator molecule (WYCHE *et al.* 1974; KASAI 1974) could either enhance transcription by interaction with the linear form or the linear-form-RNA polymerase complex, or it could enhance the availability of the linear form more indirectly. (For example, it could compete with repressor for binding to the loop form, but dissociate more readily). The loop model presented is perhaps equally plausible with others that could be designed. Our simple model merely serves in a generic sense as a center for further discussion. A similar model has been proposed on theoretical grounds by GIERER (1966).

A main point in favor of the model is that mutations affecting a certain phenotypic property may be brought together to define a single binding site if a simple loop forms. Also, mutations can occur which either interfere with or facilitate loop formation. Thus, *hisO* mutations can have five effects: changes in binding ability for RNA polymerase, repressor, or activator and changes enhancing or inhibiting loop formation. With this many variables, the phenotypes of all *hisO* mutants analyzed to date can be explained and interactions of pairs of *hisO* mutations in the *cis* configuration are predicted successfully by the model (a detailed discussion can be found in ELY 1973).

Evidence supporting the loop model: Two mutations, *hisO3150* and *his3198*, provide a strong argument for the involvement of a conformational equilibrium in the regulation of the *his* operon. Strains containing *hisO3150* are absolute constitutives and have lost all ability to respond to either repressor or activator (line 2, Table 7). Therefore, one must assume that both the activator and the repressor binding sites are rendered nonfunctional by the *hisO3150* mutation. If this assumption is true, then a mutation such as *hisO3198*, which deletes the region where *hisO3150* maps, should not cause a higher level of expression than *hisO3150* does. Since strains containing *hisO3198* have three times as much *hisB* activity as those containing *hisO3150*, we conclude that *hisO3198* must cause a structural alteration of the *hisO* region which can stimulate his operon expression. A similar argument applies to the properties of the *hisO3150 hisO1812* double mutant as compared to *hisO3150* and *hisO1812* alone.

Additional evidence for conformational changes of the *hisO* region comes from studies of double mutants containing the deletion *hisO1242* and a promoter-like *hisO* mutation in the *cis* configuration. *HisO2321* and *hisO2355* are able to reduce *his* operon expression in the presence of *hisO1242*, while strains containing *hisO3148*, *hisO2965*, and *hisO2966* have maximal *his* operon expression in the presence of *hisO1242* (column 9, Table 7). This paradox can be explained if one assumes that the *hisO1242* mutation prevents the formation of the transcriptionally closed loop conformation. If the repression loop could not be formed, then the presence of repressor and activator would be superfluous and maximal *his* operon expression would be obtained. Only mutations such as *hisO2321* and *hisO2355*, which appear to reduce RNA polymerase binding, would be able to reduce expression of strains containing *hisO1242*. Mutations such as *hisO3148*, *hisO2965*, and *hisO2966*, which appear to stabilize the looped conformer or to reduce activator binding, are fully corrected by *hisO1242*.

Genetic evidence that *hisO1242* causes an altered three-dimensional structure of the *hisO* region is presented in the accompanying paper (ELY, FANKHAUSER and HARTMAN 1974). When the *hisO1242* deletion is present, recombination between *hisO1828* and *hisO1812* occurs at the expected frequency. However, in the absence of *hisO1242*, wild-type recombinants are not formed in reciprocal transductional crosses between *hisO1828* and *hisO1812*, although the two markers map at widely separated sites. We can think of no explanation compatible with our data other than the supposition that *hisO1242* alters the confrontation of the entire *hisO* region. Thus, the genetic data are compatible with the physiological data, suggesting that *hisO1242* prevents formation of the looped structure.

Further support for the loop model comes from *in vitro* studies of the *his* operon. KASAI (1974) has shown that DNA obtained from ϕ80*his* transducing phage is more actively transcribed in a simple system containing *E. coli* RNA polymerase if it carries *hisO1242* than if it carries *hisO*$^+$. Since neither activator nor repressor is added in this system, we conclude that some innate characteristics of the *hisO* DNA influences transcription frequency, and a propensity for loop formation provides one explanation. Similar results have been obtained in a coupled transcription-translation system (J. BROACH, S. ARTZ and B. N. AMES, personal communication). Transducing phages containing other *hisO* mutations have been prepared and will allow additional tests for predictions based on the loop model.

Genetic studies indicate that the *hisO* region is some 200 to 500 base pairs in length (ELY, FANKHAUSER and HARTMAN 1974), and *in vitro* transcription results (KASAI 1974) so far indicate that the proposed looped structure may not be a rare conformer of DNA in solution. Therefore, it may be possible to actually visualize the *hisO* region in electron microscopy of ϕ80*his* DNA. Such experiments are in preparation. However, ultimate resolution will require extensive *in vitro* studies both on regulation and on nucleotide sequencing.

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