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Histidine Regulatory Mutants in *Salmonella typhimurium*

VI. Dominance Studies

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Regulation of the histidine operon in *Salmonella typhimurium* has been shown to involve at least six separate genes (Roth, Antón & Hartman, 1966; Antón, 1968). Mutations in any one of these separate genes can destroy the ability of the cell to repress fully the histidine biosynthetic enzymes. In order to understand this regulatory mechanism and the nature of these mutations, we have studied the dominance relationships between these mutations and their wild-type alleles. Studies have been done using two F′ episomes derived from *Escherichia coli* and one derived from *Salmonella typhimurium*. Four mutant regulatory genes (*hisR*, *hisU*, *hisW*, *hisT*) are recessive to their wild-type alleles. The *hisO* mutation is dominant to its wild-type allele but affects only genes located *cis* to the *hisO* mutation.

1. Introduction

A mutation in any of six regulatory genes of *Salmonella typhimurium* causes the production of very high levels (de-repressed synthesis) of the biosynthetic enzymes encoded by the histidine operon (Roth, Antón & Hartman, 1966; Antón, 1968). These regulatory genes are: *hisO*, the operator region at the beginning of the operon; *hisS*, the structural gene for histidyl-tRNA synthetase (Roth & Ames, 1966); *hisR*, a gene which is presumed to specify histidine tRNA (Silbert, Fink & Ames, 1966); *hisT*; *hisU*; and *hisW*. The functions of the latter three genes have not been determined. Each regulatory gene is distinguishable from the others by its unique map position.

In order to test the dominance of these genes, we have used two F′episomes derived from *Escherichia coli* and have constructed a third F′ which carries the histidine region of the *S. typhimurium* chromosome.

The F′14 episome of Pittard, Loutit & Adelberg (1963) carries both the *hisU* and *hisR* genes; F′32 carries both *hisT* and *hisW* genes. Although F′32 does not carry the histidine region, it is possible to select for the addition of these *S. typhimurium* genes to the F′32 episome. The resulting episome, F′T80 has lost most of the genes previously carried by F′32 and now carries a histidine region derived from *S. typhimurium*. This episome was used to study dominance of the *hisO* regulatory element.

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2. Materials and Methods

(a) *Bacteria strains*

All strains used in this study were derived from *S. typhimurium* strain LT-2, with the exception of the two *E. coli* strains mentioned below.

Auxotrophic strains were obtained from the collection of M. Demerec. This collection is at present being maintained by K. E. Sanderson, University of Alberta, Calgary, Alberta, Canada. Strains SB562, SB563 and SB568, SB621 were obtained from Dora N. Antón. All other stocks with SB prefixes were obtained from P. E. Hartman. Strain SL863 was obtained from the collection of B. A. D. Stocker.

The *E. coli* strain AB44 (*arg, thi, lac, aroC, purC*) carrying the F′32 (*dsd*) episome was kindly donated by E. McFall (1967). The F′32 episome was transferred into the *S. typhimurium* strain SB259 (*aroD5, cys-1112*) and the resulting strain (TR11) was used as F′32 donor in contruction of all F′32-carrying strains used in this study.

The *E. coli* strain AB1206 (*thi-1, pro-2, his-4, gal-2, lac-1, str-8*/F′14) was obtained from E. A. Adelberg (Pittard *et al.*, 1963). The F′14 episome was transferred into *S. typhimurium* strain TA3 (*leu-1071, ara-9, metE338, ilvC401*) and the resulting episome-carrying strain (TA36) was used as a donor in constructing all the F′14 heterogenotes reported here. The basic multiply marked strains used are listed in Table 1. Derivatives were made by infecting these strains with the various F′ episomes.

The nomenclature of Demerec, Adelberg, Clark & Hartman (1966) was used. The following gene designations were also used: *dsd* (D-serine deaminase), *gnd* (6-phosphogluconate dehydrogenase) and *hisP* (histidine-specific permease) (Shifrin, Ames & Ferro-Luzzi Ames, 1966).

The *S. typhimurium* F′*his* episome is designated F′T80. The number (80) was obtained from E. A. Adelberg, and refers to his listing of F′ episomes. The letter (T) refers to the fact that the known genes carried by F′T80 are derived from *S. typhimurium*.

(b) *Bacteriophage stocks*

Transductions were performed using a non-lysogenizing mutant, L4 (Smith & Levine, 1967), of phage P22. This mutant was kindly donated by H. O. Smith. Transductant colones arising after use of this phage were found to be non-lysogenic and phage-sensitive after several single-colony isolations.

(c) *Media and cell growth*

Minimal medium is the E medium of Vogel & Bonner (1956), supplemented with 1% glucose. Auxotrophic mutants were grown on medium E supplemented with the necessary nutritional requirements at a concentration of 0·1 mM, except serine which was used at a concentration of 2 mM. Complete medium is 0·8% nutrient broth with 0·4% NaCl. All strains were grown at 37°C in a New Brunswick incubator shaker.

(d) *Episome transfer*

Episome transfer was performed on solid selective medium enriched with 0·2 ml. of nutrient broth per 30 ml. of medium. Donor strains were counter-selected by omission of nutrients required by the donor. A lawn of the donor strain (10^8 cells) was spread on the plate and a drop of the recipient auxotrophic culture (10^9 cells/ml.) added. In cases in which the presence of the episome allowed growth of the recipient strain, the area where the two strains were in contact showed confluent growth in 24 to 36 hr.

(e) *Enzyme assays*

Histidinol phosphate phosphatase and histidinol dehydrogenase were assayed by the toluenized cell procedure of Ames, Hartman & Jacob (1963), with corrections for the phosphatase as noted in Roth & Ames (1966), and a change of pH for the dehydrogenase assay from 8·3 to 8·6.

6-Phosphogluconate dehydrogenase was assayed by the toluenized cell procedure of Fink, Klopotowski & Ames (1967). The pH for this assay was changed from 8·4 to 8·6.

TABLE 1
Bacterial strains

Strain no.	Regulatory mutation	Other markers
SB109	—	*ilvC401, metE338, ara-9*
SB230	*hisW1291*	*aroD5*
SB234	*hisW1295*	*aroD5*
SB259	—	*aroD5, cysC1112*
SB268	—	*aroD5, cysA1110*, Str^r
SB562	*hisW1824*	*aroD5, purF145, metG319* Str^r
SB563	*hisW1824*	*metG319, hisE35, hisW1824*
SB621	*hisU1819*	*ilvC401, ara-9*
SL863	—	*purE801, trpA8, hisD27 mtl*
TA2	*hisR1223*	*metE338, ara-9*
TA3	—	*leu-1071, metE338, ilvC401 ara-9*
TA4	*hisO1242*	*ilvC401, metE338, ara-9*
TA6	—	*hisD2421, metE338, ilvC401 ara-9*
TA7	—	*metG319, ser-821, arg-501*
TR16	*hisR1223*	*aroD5, purF145*
TR20	*hisT1532*	*purF145*
TR35	—	*hisEIFAHBCD712,ser-821,arg-501*/F'T80 *his*$^+$
TR36	*hisT1529*	*aroD5*
TR40	*hisU1819*	*ilvC401, aro-501*
TR92	—	*purE801, trpA8, hisEIFAHB612*

3. Results

(a) *Dominance testing of* hisT *and* hisW *genes*

(i) *Properties of F'32 in* Salmonella

The region of the *Salmonella* linkage map covered by F'32 is indicated in Figure 1. This was determined by complementation tests (Roth & Fink, manuscript in preparation) in which the episome was transferred to a recipient carrying a mutant allele of the gene to be tested. Prototrophic clones resulting from such complementation were found to segregate occasional clones which again showed the recipient markers and had apparently lost the F'32 episome. Extensive tests failed to demonstrate any part of the histidine operon on F'32.

(ii) *Dominance tests*

The wild-type *hisT*$^+$ and *hisW*$^+$ alleles carried by F'32 were found to be dominant over mutant alleles located on the chromosome. Dominance was tested by constructing strains which carried both the regulatory mutation and an auxotrophic mutation in a gene whose wild-type allele of which was known to be located on the episome. Transfer of the episome was selected by means of this auxotrophic mutation. The level of the histidine biosynthetic enzyme, histidinol phosphate phosphatase, was then assayed as an index of de-repression of the operon. The results of such assays for a number of diploids are presented in Table 2. First, the parent strain is presented, which has high enzyme levels due to the presence of a mutant regulatory gene. Next, the same strain carrying the F'32 episome is presented. For both *hisT* and *hisW* it can be seen that the material carried by the F'32 episome is able to compensate for the defect of the

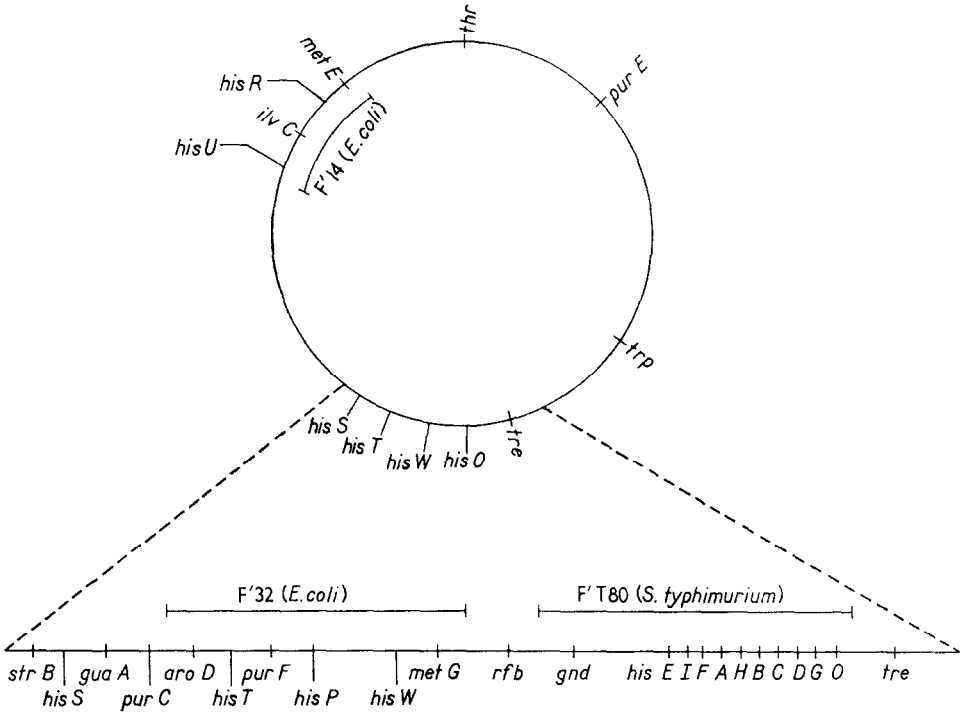

FIG. 1. The chromosomal regions carried by the various episomes are found opposite the lines labeled for these episomes. Gene loci designations are as listed in Demerec *et al.* (1966) except as noted in Materials and Methods. This *S. typhimurium* chromosomal map is based on that of Sanderson & Demerec (1965).

chromosomal regulatory mutation. Enzyme levels were also measured for segregant clones which had lost the episome. The results show that the dominance tests were in fact done on diploids and not on haploid recombinants. Thus, we infer that the wild-type alleles of *hisT* and *hisW* are dominant over the mutant alleles tested.

Two strains are listed in Table 2 as controls. One carries no regulatory mutation and the other carries a mutation in the *hisR* gene, which maps far from the region carried by F'32. In neither case does the presence of F'32 affect the function of the histidine operon.

(iii) *Absence of nonsense suppressors from F'32*

Since no amber or ochre suppressors could be detected on the F'32 episome, we conclude that suppression of the regulatory mutations tested cannot account for the results presented. This possibility was ruled out by transductional crosses using as recipient a strain which carried F'32 and a deletion (*hisGDCBH2253*) of part of the histidine region. Six nonsense mutations (1 ochre and 5 ambers) of the *hisC* gene were introduced into this strain by selection for growth on histidinol. This required no suppressor, only entry of a donor $hisD^+$ (histidinol dehydrogenase) region. In each case recombinants resulted, demonstrating that transduction was occurring. Then the crosses were repeated, selecting for growth on minimal medium, requiring entrance and suppression of the mutant *hisC* allele of the donor as well as entry of its $hisD^+$ gene. Such function could occur if a suppressor were present in the strain,

TABLE 2

Dominance tests using F'32

Locus	Regulatory mutations	Other markers	Pertinent genotype	Specific activity of phosphatase	No. of determinations
hisT	*hisT1532*	*purF145*	*T*	17·4	(2)
	hisT1532	*purF145*/F'32	F' $\frac{T^+}{T}$	3·6	(2)
	hisT1532	*purF145* (F'32 removed)	T	19·3	(1)
	hisT1529	*aroD5*	T	26·3	(2)
	hisT1529	*aroD5*/F'32	F' $\frac{T^+}{T}$	3·5	(4)
	hisT1529	*aroD5* (F'32 removed)	T	28·3	(2)
hisW	*hisW1291*	*aroD5*	W	16·1	(2)
	hisW1291	*aroD5*/F'32	F' $\frac{W^+}{W}$	3·6	(2)
	hisW1291	*aroD5* (F'32 removed)	W	18·0	(1)
	hisW1295	*aroD5*	W	11·3	(5)
	hisW1295	*aroD5*/F'32	F' $\frac{W^+}{W}$	3·4	(3)
	hisW1295	*aroD5* (F'32 removed)	W	15·8	(2)
	hisW1824	*aroD5 purF145 metG319*	W	10·3	(6)
	hisW1824	*aroD5 purF145 metG319*/F'32	F' $\frac{W^+}{W}$	4·3	(3)
	hisW1824	*aroD5 purF145 metG319* (F'32 removed)	W	10·2	(3)
Controls	*hisR1223*	*aroD5 purF145*	W^+ R	20·0	(3)
	hisR1223	*aroD5 purF145*/F'32	F' $\frac{W^+}{W^+}$ R	17·5	(3)
	hisR1223	*aroD5 purF145* (F'32 removed)	W^+ R	21·0	(4)
	none	*aroD5 cys-1112*	W^+ R^+	2·4	(2)
	none	*aroD5 cys-1112*/F'32	F' $\frac{W^+}{W^+}$ R^+	2·1	(3)
	none	*aroD5 cys-1112* (F'32 removed)	W^+ R^+	1·9	(1)

Cells were grown on minimal medium plus essential amino acids (0·5 mM) or adenine (0·5 mM) + thiamine (0·1 mM), when required.

Heterogenotes were grown under selective conditions to minimize loss of episome. Addition of amino acids or adenine to medium of heterogenotes did not alter enzyme levels appreciably. Mutants *hisW1291* and *hisW1295* were classified as *hisW* types by virtue of the dominance tests presented above and of the fact that they do not map in the *hisT* gene. Mutant *hisW1824* is an authentic *hisW* mutation mapped by Antón (1968). Histidinol phosphate phosphatase (HOL-P P'tase) was assayed as in Materials and Methods. The unit of activity is defined as the $O.D._{820}$ produced in 15 min (due to reaction of freed phosphate with molybdate–ascorbate reagent) per unit $O.D._{650}$ of the cell suspension added. In the column headed "Pertinent genotype", the genes carried by the episome are written above the line and chromosomal genes below the line. The last column indicates how many times this strain has been assayed. Each determination represents the result of assays performed in duplicate. Segregants were derived from the relevant merodiploid by selecting clones which had regained the nutritional requirements of the original strain.

TABLE 3

Complementation tests with F'14

Strain	Recipient	Select	Phenotype of F'14 diploids		Conclusion
SB109	*ile metE*	Ile$^+$	Ile$^+$ Met$^+$	(75/75)	*ile*, *metE* and *hisR* are on F'14
	ile metE	Met$^+$	Ile$^+$ Met$^+$	(30/30)	
	ile metE	Ile$^+$ Met$^+$	Ile$^+$ Met$^+$	(100/100)	
TA2	*met hisR*	Met$^+$	Met$^+$ HisR$^+$	(300/300)	
TR40	*hisU ilvC aro*	Ilv$^+$	Ilv$^+$ HisU$^+$ Aro	(18/18)	*hisU* is on F'14 *aro* is not on F'14
TA3	*ile metE leu*	Ile$^+$ Met$^+$	Ile$^+$ Met$^+$ Leu$^-$	(10/10)	*leu* is not on F'14
TA6	*ile metE his*	Ile$^+$ Met$^+$	Ile$^+$ Met$^+$ His$^-$	(10/10)	*his* is not on F'14
TA4	*ile metE hisO*	Ile$^+$ Met$^+$	Ile$^+$ Met$^+$; phenotypically still HisO^c		

Selection was made for the phenotypes indicated. The donor strain was TA36 (*leu-1071 ara-9 metE338 ilvC401*/F'14) for all recipients except TA3. For recipient TA3, strain TA6 carrying F'14 was used as donor. Numbers in parentheses indicate the fraction of clones tested which gave the indicated phenotypes.

either on the chromosome or on F'32. None of the nonsense mutations tested was suppressed, and it was concluded that F'32 does not carry a nonsense suppressor. The possibility that it might carry a UGA suppressor (Sambrook, Fan & Brenner, 1967) has not been ruled out, but the rarity of such mutations makes this an unlikely explanation of our results.

(b) *Dominance testing of the* hisR *and* hisU *genes*

(i) *Genes carried by F'14*

The region of the chromosome carried by F'14 is shown in Figure 1. The two experimental criteria for this designation are complementation and segregation.

(1) *Complementation.* F'14 was assumed to carry the wild-type allele of the chromosomal auxotrophic allele if the presence of F'14 in the strain could compensate for the chromosomal defect and allow growth on minimal medium.

(2) *Segregation.* Prototrophic colonies resulting from such episomal transfer were tested for their ability to give rise to auxotrophic segregants which show the requirement of the original recipient strain. These segregants could arise by episome loss. This test differentiates heterogenotes from stable recombinants. The results of some representative tests are shown in Table 3. The segregants from F'14 heterogenotes constitute 8% of the population of a nutrient broth culture. Segregant clones from the *ilvC metE*/F'14 merozygote are all *metE ilvC* and clones from *ilvC hisU*/F'14 and *metE his1223*/F'14 are *ilvC hisU* and *metE hisR1223*, respectively. These data suggest that the entire region shown under F'14 in Figure 1 is indeed carried by the episome and rules out the possibility that the prototrophic clones which appeared in the complementation test were recombinants.

(ii) *Dominance of* hisR *and* hisU

The wild-type *hisR*$^+$ and *hisU*$^+$ alleles carried by F'14 are dominant over mutant alleles located on the chromosome. Dominance was tested by analysing strains which carried the regulatory mutation and an auxotrophic mutation on the chromosome. The wild-type allele of the auxotrophic mutation was on the episome and served as a means of selecting for transfer and maintenance of the F'episome. As before, the levels of the histidine biosynthetic enzyme, histidinol phosphate phosphatase, were assayed as an index of de-repression of the operon. It can be seen in Table 4 that the heterogenotes *hisR*$^+$/*hisR* and *hisU*$^+$/*hisU* have repressed levels of the histidine biosynthetic enzymes, whereas segregants which have lost the episome, *hisR* and *hisU* haploids, have high constitutive levels. We have interpreted this to mean that the genes for *hisR*$^+$ and *hisU*$^+$ on the episome are dominant and can provide the regulatory function lacking due to *hisR* and *hisU* mutations on the chromosome. F'14 does not affect enzyme levels in a strain carrying *his01242*, a regulatory mutation mapping far from the genes carried by F'14. This shows that F'14 material does not exert a non-specific effect on function of the histidine operon.

The dominance of *hisR*$^+$ and *hisU*$^+$ over their respective mutant alleles is not complicated by the presence of a nonsense suppressor mutation on F'14, since amber mutations are not suppressed when F'14 is present in a strain.

TABLE 4

Dominance tests using F'14

Locus	Regulatory mutation	Other markers	Pertinent genotype	Specific activity of phosphatase	No. of determinations
hisR	*hisR1223*	*metE338*	R	20·3	3
	hisR1223	*metE338*/*F'14*	F' $\frac{R^+}{R}$	2·9	2
	hisR1223	*metE338* (seg. I: F'14 removed)	R	19·8	1
	hisR1223	*metE338* (seg. II: F'14 removed)	R	21·4	1
	hisR$^+$	*ilvC401 metE338*/*F'14*	F' $\frac{R^+}{R^+}$	3·2	2
hisU	*hisU1819*	*ilvC401*	U	11·7	1
	hisU1819	*ilvC401*/*F'14*	F' $\frac{U^+}{U}$	2·9	1
	hisU1819	*ilvC401* (seg. I: F'14 removed)	U	11·4	1
	hisU1819	*ilvC401* (seg. II: F'14 removed)	U	14·9	1
	hisU$^+$	*ilvC401 metE338*/*F'14*	F' $\frac{U^+}{U^+}$	3·0	1

Enzyme assay was performed by the method of Ames *et al.* (1963). Units are presented in the legend to Table 2. Segregants (seg.) were derived from the relevant merodiploids by selecting clones which had regained the methionine or isoleucine requirement. Each determination represents assays performed in quadruplicate.

(c) *Dominance testing of the* hisO *gene*

(i) *Properties of a* Salmonella *F'his* (*F'T80*)

An F'*his* has been constructed which contains the entire *S. typhimurium* histidine operon (Roth & Fink, manuscript in preparation). F'T80 was found to have the following properties:

(1) *Transfer*: the F'T80*his*$^+$ could be transferred infectively to all *his*$^-$ recipients tested when selection was made for *his*$^+$.

(2) *Loss*: the F'T80*his*$^+$ was occasionally lost from diploids and the resulting (*his*) clones were found to carry the original chromosomal lesion in the histidine operon.

(3) *Size*: the F'T80 carries the entire histidine operon and the *gnd* (6-phosphogluconate dehydrogenase) gene, both derived from *S. typhimurium*.

(ii) *Studies of the* hisO *gene*

The *hisO* mutation is dominant over its wild-type allele, but affects only those structural genes located *cis* to the *hisO* lesion. In this respect, it is similar to the operator region of the *lac* operon (Jacob & Monod, 1961) and differs from the *araC* region studied by Sheppard & Engelsberg (1967). Data leading to this conclusion are presented in Table 5.

Table 5 shows that a *hisO* mutation located on the chromosome affects only the *cis* chromosomal genes and fails to affect genes *trans* to it, carried by the episome. Line 1 presents wild-type repressed levels of the *hisD* enzyme and the *hisB* enzyme. Lines 2 to 4 present de-repressed enzyme levels caused by the *hisO1242* mutation. Lines 5 and 6 present repressed levels of these enzymes produced by episomal genes. The value for the *D* enzyme in line 6 represents the combined contributions of repressed chromosomal and episomal genes. Lines 7, 8 and 9 show that both the *hisD* and *hisB* genes are de-repressed when located *cis* to the *hisO1242* mutation, and are unaffected by *hisO* when located *trans*. Line 10 shows that the presence of an episome does not prevent de-repression of the chromosomal *hisB*$^+$ gene. Line 11 demonstrates that it is possible to de-repress genes carried by the episome. The *hisT* mutation carried by this strain is a regulatory mutation, unlinked to the histidine region.

A wild-type episome does not compensate for a deletion of the operator end of the histidine region. *HisOG203* is a deletion which eliminates the *hisO* region and much of the *hisG* gene. This strain has been shown (Ames *et al.*, 1963) to lack detectable enzymic activities for the other genes of the operon, although the genetic material is demonstrably intact. Strains carrying a chromosomal *hisOG203* deletion and an F'T80 *hisD*$^-$ episome are unable to grow on minimal medium. Furthermore, when *hisOG203* carries an F'T80 *hisB*$^-$ episome, no *hisB* gene product, histidinol phosphate phosphatase, is detectable. These results demonstrate that the presence of an episomal *hisO*$^+$ allele is unable to permit function of chromosomal genes (*hisB*$^+$ and *hisD*$^+$) adjacent to the *hisOG203* deletion. The wild-type episome does not reverse the effect of a polar mutation on the chromosome. These data are presented in Table 5 (compare lines 3 and 8).

The above results can not be explained either by recombination between episome and chromosome or by episome loss. Either of these possibilities might be invoked to account for lowered levels of the products of genes carried by the episome. No recombination between episome and chromosome has been observed, although experiments were conducted which would have detected one recombinant in 10^6 cells. Episome loss is infrequent; an overnight nutrient broth culture contains less than

TABLE 5

Dominance tests of hisO1242

Line	Relevant genotype	Relative specific activity		Complete strain description
		HisD enzyme†	HisB enzyme	
1	$O^+ D^+ B^+$	1	1	LT2 (wild type)
2	$O^c D^+ B^-$	12·4	0	*hisO1242 hisBH22*
3	$O^c D^- B^+$	0	12·0(1.2)†	*hisO1242 hisDC2236*‡
4	$O^c D^+ B^+$	15·1	11·2	*hisO1242 hisF2136*
5	F′ $\frac{O^+ D^+ B^+}{O^- D^- B^-}$	1·2	1·5	*hisOGDCBHAF644*/F′T80*his*$^+$
6	F′ $\frac{O^+ D^+ B^+}{O^+ D^+ B^-}$	1·7	1·1	*hisBH22*/F′T80*his*$^+$
7	F′ $\frac{O^+ D^- B^+}{O^c D^+ B^-}$	15·0	1·7§	*hisO1242 hisBH22*/F′T80*hisD2380*§
8	F′ $\frac{O^+ D^+ B^+}{O^c D^- B^+}$	1·2	16·5(3·0)‡	*hisO1242 hisDC2236*/F′T80*his*$^+$‡
9	F′ $\frac{O^+ D^+ B^+}{O^c D^+ B^-}$	—‖	2·7	*hisO1242 hisBH22*/F′T80*his*$^+$
10	F′ $\frac{O^+ D^+ B^-}{O^c D^+ B^+}$	15·3	9·5	*hisO1242 hisF2136*/F′T80*hisB2405*
11	F′ $\frac{O^+ D^+ B^+}{O^- D^- B^-}$ (*hisT*)	13·2	14·1	*hisOGDCBH2253 hisT1529 aroD5*/F′T80*his*$^+$

All strains were grown on minimal medium plus 0·1 mM-histidine except LT2 which is grown on minimal medium alone. The *hisD* enzyme is histidinol dehydrogenase; the *hisB* enzyme is histidinol phosphate phosphatase; enzymes were assayed as described in Materials and Methods. The relevant genotype of diploid strains is indicated with episomal genes written above chromosomal genes. All enzyme activities are expressed relative to LT2 which has 1·45 units of *D* enzyme and 2·9 units of *B* enzyme. Units for *B* are defined in the legend to Table 2. The units of activity of *D* enzyme, histidinol dehydrogenase, are defined as the amount of $O.D._{520}$ produced in 20 min (by reduction of a dye mix) per $O.D._{650}$ of cell suspension added.

† Low levels of histidinol dehydrogenase are difficult to determine accurately.

‡ *hisD2236* is a strongly polar deletion having 10% residual activity for the products of distal genes. Values presented are calculated to correct for polarity. Values in parentheses are actual values obtained.

§ *hisD2380* is a non-polar mutation.

‖ Not assayed.

0·5% segregants. As an added control for such loss, the enzyme 6-phosphogluconate dehydrogenase (*gnd*) was routinely assayed in all experiments. The gene for this enzyme is carried by the F′T80 episome but is not within the histidine operon or under the control of histidine (Murray & Klopotowski, 1968). All strains without the F′T80

episome have specific activities of approximately 1·8, whereas strains carrying the episome and thus having a chromosomal copy and at least one copy of the F′T80 episome have levels of about 4·1. This indicates that strains carrying this episome have slightly more than one episome copy per chromosomal histidine region, in agreement with the results based on assays of two histidine enzymes (Table 5).

4. Discussion

It was previously shown that two of the six classes of histidine regulatory genes are involved in the production of histidyl-tRNA. The *hisS* gene is the structural gene for histidyl-tRNA synthetase (Roth & Ames, 1966). A decrease in the activity of this enzyme results in de-repression of the histidine operon. Mutations of the *hisR* gene result in a decrease in the amount of histidine-specific tRNA ($tRNA^{His}$), a consequence of which is de-repression of the histidine operon (Silbert *et al.*, 1966). This decrease in tRNA suggested that *hisR* is either a structural gene for $tRNA^{His}$ or is involved in some step in its production. These results led to the conclusion that histidyl-tRNA might be directly involved in repression of the histidine operon.

We were able to select and use *E. coli* episomes for the dominance tests because homologous genes occupy the same position on the genetic map of *E. coli* and *S. typhimurium* (Sanderson & Demerec, 1965; Taylor & Thoman, 1964). An objection which might be raised is that *E. coli* episomes were used in the dominance tests involving F′32 and F′14. This objection would have complicated the interpretation had any of the regulatory mutations proved dominant to the homologous wild-type alleles of *E. coli*. However, the finding that the *E. coli* wild-type alleles are completely dominant demonstrates that their products function normally in the *S. typhimurium* histidine regulatory mechanism. This objection is obviated in dominance tests of the *hisO* region, since an episome (F′T80) was used which carries an *S. typhimurium* histidine region, isogenic with the chromosome.

The dominance studies of the *hisO* gene indicate that this regulatory element acts only on the structural genes immediately adjacent (*cis*) to it and does not produce a diffusable cytoplasmic product. These properties of the *hisO* mutation are similar to those of the O^c mutations of the *lac* (Jacob & Monod, 1961) and *gal* (Buttin, 1963) operons and to the *oprA* and *oprB* mutations of the isoleucine–valine operons (Ramakrishnan & Adelberg, 1965). Our results give no evidence to suggest a positive control mechanism such as that reported by Sheppard & Engelsburg (1967) for the arabinose operon. It should be emphasized that the dominance studies on the operator do not show whether or not this genetic element is transcribed into the messenger RNA, or whether regulation occurs at the level of DNA or at the level of RNA. A biochemical explanation of how histidyl-tRNA is involved in repression of the histidine operon and the nature of its interaction with the operator (RNA or DNA) is still premature. However, the results presented here restrict the types of permissible theories.

Our findings rule out the possibility that the *hisU*, *hisT* and *hisW* genes are structural genes for minor non-histidine tRNA species which may be altered so as to interact with the histidine regulatory mechanism. This possibility predicts that mutations of these genes would be dominant and is contrary to the experimental results. These results also rule out the possibility that any of the regulatory genes represents a transposed duplication of the histidine operon. Such a transposed operon would have appeared dominant in the tests performed here.

Note added in proof: Another possible interpretation of the *hisU* dominance results have not been eliminated. It is possible that the two copies of the *hisR*$^+$ gene in F'14 cliploids serve to compensate for the defect caused by the chromosomal *hisU* mutation.

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