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trans-Recessive Mutation in the First Structural Gene of the Histidine Operon That Results in Constitutive Expression of the Operon

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The first enzyme for histidine biosynthesis, encoded in the *hisG* gene, is involved in regulation of expression of the histidine operon in *Salmonella typhimurium*. The studies reported here concern the question of how expression of the histidine operon is affected by a mutation in the *hisG* gene that alters the allosteric site of the first enzyme for histidine biosynthesis, rendering the enzyme completely resistant to inhibition by histidine. The intracellular concentrations of the enzymes encoded in the histidine operon in a strain carrying such a mutation on an episome and missing the chromosomal *hisG* gene are three- to fourfold higher than in a strain carrying a wild-type *hisG* gene on the episome. The histidine operon of such a strain fails to derepress in response to histidine limitation and fails to repress in response to excess histidine. Furthermore, utilizing other merodiploid strains, we demonstrate that the wild-type *hisG* gene is *trans* dominant to the mutant allele with respect to this regulatory phenomenon. Examination of the regulation of the histidine operon in strains carrying the feedback-resistant mutation in an episome and *hisT* and *hisW* mutations in the chromosome showed that the *hisG* regulatory mutation is epistatic to the *hisT* and *hisW* mutations. These data provide additional evidence that the first enzyme for histidine biosynthesis is involved in autogenous regulation of expression of the histidine operon.

Regulation of expression of the histidine operon of *Salmonella typhimurium* is not fully understood, despite intensive investigation by a number of workers over more than a decade (for reviews, see references 5 and 8). One of the most puzzling features of this system is that although a corepressor, histidyl-transfer ribonucleic acid (tRNA) has been identified (1, 15, 21-24, 26, 28, 29), no classical aporepressor has been found. The major approach that has been taken to identify the regulatory components of the system is the isolation of constitutive mutants. Such mutants were selected in minimal medium containing compounds that inhibit the growth of any strain that does not express the entire histidine operon constitutively. Mutants isolated in this manner have proven to be ones in which there is defective synthesis, maturation, or aminoacylation of $tRNA^{His}$ (1, 15, 21-24, 26, 28); none of them displays defective synthesis of any regulatory protein. This finding has led to the suggestion that such a regulatory protein may have two activities, one required for the histidine operon control function and the other required for growth of the organism in minimal medium (8). An obvious group of candidates for a protein of this type is the enzymes that catalyze the pathway for histidine biosynthesis.

Over the past few years, mounting evidence has suggested a regulatory role for the allosteric enzyme that catalyzes the first step of the histidine pathway, *N*-1,5-(5'-phosphoribosyl)-adenosine triphosphate: pyrophosphate phosphoribosyltransferase (EC 2.4.2.17, termed phosphoribosyltransferase hereafter). This evidence may be summarized as follows. (i) Some mutations that cause alteration of the allosteric properties of the enzyme also result in an altered pattern of repression by histidine and in an inability of the histidine analogue, 1,2,4-triazolalanine, to repress the histidine operon (11, 12, 14). (ii) Phosphoribosyltransferase interacts specifically, and with high affinity, with aminoacylated histidyl-tRNA (3, 13, 33). (iii) Purified phosphoribosyltransferase that is resistant to inhibition by histidine fails to bind to histidyl-tRNA in vitro (30). (iv) Phosphoribosyltransfer-

ase binds specifically to a site in or near the regulatory region of the histidine operon deoxyribonucleic acid in vitro (M. Meyers, F. Blasi, C. Bruni, R. G. Deeley, R. F. Goldberger, J. S. Kovach, M. Levinthal, K. P. Mullinix, and T. Vogel, Nucleic Acids Res., in press). (v) Phosphoribosyltransferase specifically inhibits transcription of the histidine operon in a purified system in vitro (4).

Important additional evidence implicating phosphoribosyltransferase in regulation of the histidine operon would be the identification of mutations in the structural gene for this enzyme that result in constitutive expression of the operon. Patthy and Dénes (20) reported isolation of a constitutive mutant of *Escherichia coli* in which the phosphoribosyltransferase has lost its ability to be inhibited by histidine; they did not show, however, that both phenotypic alterations were due to a single mutation or that the mutation causing the regulatory defect was *trans* recessive. Rothman-Denes and Martin (25) reported isolation of a mutant in which a single mutation in the *hisG* gene caused expression of the operon to be elevated twofold, and could thus be a special case of the class of mutants proposed above.

Because of the previous findings that alterations of the allosteric site of phosphoribosyltransferase caused alterations in the kinetic pattern of repression (12, 14), we attempted to isolate mutants in which severe damage to the allosteric site of the enzyme (as judged by a high degree of resistance to inhibition by histidine) results in constitutive expression of the operon. In all previously isolated feedback-resistant mutants, the K_I of the phosphoribosyltransferase for histidine was raised by approximately one to two orders of magnitude (18, 27). Our selection, which was previously reported (11), demanded a much greater alteration in K_I. We report here results of biochemical and genetic tests that show that these mutants display a *trans*-recessive regulatory defect causing constitutive expression of the histidine operon.

MATERIALS AND METHODS

Bacterial strains. All of the strains used in the present study are listed in Table 1. The isolation of strain TG5720 was previously described by Kovach et al. (11). For the present studies, all strains that contain the F'80 *hisG1929* episome were constructed by transfer of the episome from TG5720 to the desired *his*$^-$ recipient strain, selecting for histidine prototrophy and counterselecting the donor strain by omitting adenine from the medium. All the strains used in these studies which contain the F'*his*$^+$ episome were constructed by transfer of the episome from TA24 to the desired *his*$^-$ recipient strain, selecting for histidine prototrophy and counterselecting the donor strain by omitting methionine from the medium. The relevant genotypes of the pertinent strains are given in Table 2.

Enzyme assays and substrates. Protein was determined by the method of Lowry et al. (16). The *hisB* enzyme assays were done with cells treated with toluene by the method of Ely (7). Assays for *hisG*, *hisD*, and *hisC* enzymes were performed as previously described (17). Substrates were obtained as described elsewhere (17). For the data presented in Tables 4-7, each value represents the average of assays of extracts from five separate cultures, each done in duplicate at two different protein concentrations.

Growth of bacteria. For the data presented in Table 3, the cells were grown in medium E (32) with adenine (50 μg/ml). The cells were harvested at an optical density at 700 nm of approximately 0.70, centrifuged, and washed twice with 0.05 M tris(hy-

TABLE 1. *Bacterial strains*

Strain	Genotype[a]
TA24[b]	F'*his*$^+$/*str ara9 metE338 hisR1223 hisD2420*
LT-2	*his*$^+$
PS194a	F'*his*$^+$/*hisG203*
PS194	F'80 *hisG1929/hisG203*
PS195a	F'*his*$^+$/*hisOGDCBHAF644*
PS195 (TG5721)	F'80 *hisG1929/hisOGDCBHAF644*
JS100a	F'*his*$^+$/*hisDCBHAFIE712*
JS100	F'80 *hisG1929/hisDCBHAFIE712*
PS196a[b]	F'*his*$^+$/*hisG203 hisW1824*
PS196[b]	F'80 *hisG1929/hisG203 hisW1824*
PS197a[b]	F'*his*$^+$/*hisOGDCBH2253 hisT1504*
PS197[b]	F'80*hisG1929/hisOGDCBH2253 hisT1504*
PS193a[b]	F'*his*$^+$/*hisDCBHA2604 hisT1504*
PS193[b]	F'80 *hisG1929/hisDCBHA2604 hisT1504*
TG5720[b]	F'80 *hisG1929/hisOG1302 hisW1824 pur804*[c]
TG5724[b]	F'80 *hisG1930/hisOG1302 hisW1824 pur804*
TG5725[b]	F'80 *hisG1931/hisOG1302 hisW1824 pur804*
TG5726[b]	F'80 *hisG1932/hisOG1302 hisW1824 pur804*
TG5727[b]	F'80 *hisG1933/hisOG1302 hisW1824 pur804*
TG5728[b]	F'80 *hisG1934/hisOG1302 hisW1824 pur804*

[a] Order of structural genes in the histidine operon (starting from the operator end) is: *O, G, D, C, B, H, A, F, I, E.*

[b] Genes called *hisR*, *hisW*, and *hisT* are unlinked to the histidine operon. Mutations in these genes ordinarily result in constitutive expression of the histidine operon because of a defect in synthesis of the corepressor, histidyl-tRNA.

[c] See reference 11.

TABLE 2. *Genotypes of strains employed*

Strain	Episome	Chromosome
PS194a	*hisG*$^+$	*hisG*$^-$[a]
PS194	*hisG*$_{FR}$[b]	*hisG*$^-$[a]
JS100	*hisG*$_{FR}$[b]	*hisG*$^+$
JS100a	*hisG*$^+$	*hisG*$^+$
PS195	*hisG*$_{FR}$[b]	*hisG*$^-$[c]
PS195a	*hisG*$^+$	*hisG*$^-$[c]
PS197a	*hisG*$^+$	*hisG*$^-$ *hisT*[d]
PS197	*hisG*$_{FR}$[b]	*hisG*$^-$ *hisT*[d]
PS196a	*hisG*$^+$	*hisG*$^-$ *hisW*[a]
PS196	*hisG*$_{FR}$[b]	*hisG*$^-$ *hisW*[a]
PS193a	*hisG*$^+$	*hisG*$^+$ *hisT*
PS193	*hisG*$_{FR}$[b]	*hisG*$^+$ *hisT*
TG5721	*hisG*$_{FR}$[b]	*hisG*$^-$[c]

[a] A chromosomal deletion extends through the regulatory region of the histidine operon and ends in the *hisG* gene.

[b] *hisG* gene on the episome carries the mutation, *hisG1929*, which renders the *G* enzyme resistant to feedback inhibition by histidine.

[c] A chromosomal deletion extends through the regulatory region of the histidine operon, through the *hisG*, *hisD*, *hisC*, *hisB*, *hisH*, and *hisA* genes, and ends in the *hisF* gene.

[d] A chromosomal deletion extends through the regulatory region of the histidine operon, through the *hisG*, *hisD*, *hisC*, and *hisB* genes, and ends in the *hisH* gene.

TABLE 3. *Assays for phosphoribosyltransferase activity and inhibition by histidine*[a]

Strain	Phosphoribosyltransferase activity		% Inhibition
	No histidine	10^{-2} M histidine	
Wild type[b]	0.225	0.095	68
TG5720	0.200	0.210	0
TG5724	0.195	0.175	10
TG5725	0.155	0.170	0
TG5726	0.205	0.210	0
TG5727	0.190	0.200	0
TG5728	0.210	0.165	21

[a] Cultures were grown and assayed as described in Materials and Methods. Activity is expressed as the change in the optical density at 290 nm in 4 min, using the standard assay conditions (17).

[b] Purified wild-type phosphoribosyltransferase (0.1 μg of enzyme) was employed as a control for activity and inhibition by histidine. The enzyme was isolated by the method of Parsons and Koshland (19).

droxymethyl)aminomethane-hydrochloride buffer, pH 8.0. The cells were then suspended in 3.5 ml of the same buffer. Extracts were prepared from these suspensions with a French pressure cell (American Instrument Co.) at 6,000 lb/in^2, clarified by centrifugation, and assayed. For the data presented in Tables 4–7, the cells were grown in medium E (32), and then subcultured in medium E supplemented with histidine (0.1 mM) for two to three generations. The cells were harvested at an optical density at 700 nm of approximately 0.45, and extracts were prepared as described above.

Kinetic experiments. Cells were grown in 2 liters of medium E (32) with glucose at 0.5%, in a 4-liter flask. The cultures were aerated vigorously in a New Brunswick rotary shaker at 37 C. At an optical density at 700 nm of approximately 0.30, the growth was slowed by the addition of 3-amino-1,2,4-triazole (aminotriazole, 0.08 mM, Aldrich Chemical Co.). Aminotriazole inhibits imidazole glycerol phosphate dehydratase, the enzyme catalyzing step seven of the histidine pathway. Because aminotriazole also inhibits a step of purine biosynthesis (9), adenine (50 μg/ml, Cyclo Chemical Co.) was added to the culture medium. Samples (80 to 100 ml) were withdrawn periodically before the addition of aminotriazole, for 1 h after the addition of aminotriazole, and for 30 min after the addition of L-histidine (1 mM). Each sample was treated as described above and assayed for protein concentration and for the activity of the *hisC* enzyme.

RESULTS

The studies reported here concern the question of how expression of the histidine operon may be affected by a mutation in the *hisG* gene that renders the first enzyme for histidine biosynthesis highly resistant to inhibition by histidine. The method we used to select mutant strains (11) yielded 16 independently isolated strains in which mutation in the *hisG* gene resulted in the production of phosphoribosyltransferase that was enzymatically active but extremely resistant to inhibition by histidine. The data in Table 3 show assays for phosphoribosyltransferase activity in extracts of six of these strains. Of the six strains assayed (see Table 3), there was between 0 and 21% inhibition of phosphoribosyltransferase activity by histidine at a concentration (10^{-2} M) 500-fold higher than the K_I of the wild-type enzyme.

Experiments were performed to examine expression of the histidine operon in mutant strains PS194 and PS194a. In these strains the regulatory region of the chromosomal operon and part of the *hisG* gene has been deleted, and the remainder of the operon is not expressed. However, both of these strains contain an episome carrying the histidine operon. In PS194a, this episomal operon is wild type; in PS194 this episome carries a mutation in the *hisG* gene that renders the phosphoribosyltransferase resistant to inhibition by histidine. Expression of the episomal operons of the two mutant strains was compared to that of the wild type under

conditions of histidine excess and histidine limitation. The results of such experiments are shown in Table 4. The first two lines of the table show that growth of the wild type under conditions of histidine limitation results in a 20-fold increase in the intracellular concentrations of the enzymes specified by the histidine operon. The next two lines show that the wild-type episomal operon of strain PS194a derepresses to approximately the same degree when the organism is grown under conditions of limiting histidine. It should be noted, however, that the basal enzyme levels found in this organism grown on excess histidine are almost threefold higher than those found under the same conditions in the wild type, an effect that has been attributed to the multiple copies of episome per cell in the merodiploid strain. For comparison with other episome-bearing strains, we take the enzyme levels of PS194a grown in the presence of excess histidine as equal to 1. The last two lines of Table 4 show that in strain PS194, which carries in its episome the feedback-resistant mutation, *hisG1929*, the enzyme levels are about fourfold higher than in PS194a under the same conditions (growth on excess histidine). Furthermore, growth on limiting histidine does not cause any change in expression of the episomal operon of the feedback-resistant strain. The growth rates of the two mutant strains were the same on excess histidine (doubling time 48 min) and on limiting histidine (doubling time 210 min). Thus, expression of the histidine operon of strain PS194, which carries the feedback-resistant mutation *hisG1929*, is constitutive.

Having shown that the histidine operon of the episome carrying the *hisG1929* mutation is expressed constitutively, at a level fourfold higher than is the wild-type episomal operon, we examined the question of whether this effect was recessive or dominant, *cis* or *trans*. We studied the effect of a functional chromosomal *hisG* gene on expression of the episome carrying the constitutive mutation. The results of such experiments are shown in Table 5. The first two lines of the table show the enzyme levels in two different strains carrying an episome with the wild-type histidine operon. One of the these strains, JS100a, has a functional *hisG* gene on the chromosome, whereas the other strain, PS195a, does not. It should be noted that in both strains the *hisD*, *hisC*, and *hisB* genes are missing from the chromosome. The enzyme levels of these two strains were averaged and taken as equal to 1.0, as indicated in the last column of Table 5. The third line of the table shows, once again, the elevated level of expression of the episome carrying the *hisG1929* mutation (strain PS195). The fourth line of the table shows that this elevation is corrected by the presence of a functional wild-type *hisG* gene on the chromosome (strain JS100). Thus, the constitutive phenotype of the *hisG1929* mutation is *trans* recessive.

It is known that the constitutive expression of the histidine operon observed in *hisT* and *hisW* mutant strains results from a defect in the

TABLE 4. *Constitutive effect of hisG1929 mutation*

Histidine supply	Strain	Episome	Chromosome	Enzyme sp act[a]			Fold derepression
				D	C	B	
Excess	LT-2	None	*his*$^+$	0.5	21.5	1.4	1
Limited[b]	LT-2	None	*his*$^+$	10.1	208.0	12.9	20
Excess	PS194a	*his*$^+$	*hisG203*[c]	1.4	62.0	3.7	1[d]
Limited	PS194a	*his*$^+$	*hisG203*[c]	25.8	954.0	72.2	18.0
Excess	PS194	*hisG1929*	*hisG203*[c]	4.85	225.5	13.1	3.9
Limited	PS194	*hisG1929*	*hisG203*[c]	5.0	210.8	13.9	3.6

[a] Each value represents the average of assays of extracts from five separate cultures, each done in duplicate at two different protein concentrations.

[b] In strains with a functioning pathway for histidine biosynthesis, limitation of histidine was obtained by adding to the culture medium a sufficient amount of aminotriazole to slow the growth rate by a factor of four. This compound limits endogenous synthesis of histidine by inhibiting the enzyme that catalyzes step seven of the histidine pathway.

[c] *hisG203* is a multisite mutant in which the deletion extends from outside the histidine operon, through the regulatory region, and into the *hisG* gene. The portion of the operon remaining intact is not expressed.

[d] For the purpose of comparing enzyme levels in episome-bearing strains, the levels found in a strain with a *his*$^+$ episome are taken as 1.0. The actual enzyme levels in such strains are approximately 2.75-fold higher than in a *his*$^+$ haploid strain due, apparently, to the multiplicity of episome copies per cell.

structure of $tRNA^{His}$ (6, 29). It has been proposed that the regulatory function of phosphoribosyltransferase is exerted, at least in part, by an interaction with histidyl-tRNA (3, 13, 33). It was, therefore, of interest to study regulation of the histidine operon in strains carrying the feedback-resistant *hisG* gene mutation in both *hisT* and *hisW* backgrounds. The results of such experiments are shown in Table 6. The first line of the table shows the basal level of expression of the wild-type histidine operon of an episome in a strain (PS194a) in which the chromosomal histidine operon is not expressed. This value was taken as 1. In the second line of the table is shown the 7.6-fold increase in expression of this episome caused by the presence of the chromosomal *hisW1824* mutation (strain PS196a). The third line of the table shows that when the episomal *hisG* gene carries the feedback-resistant mutation, expression of the histidine operon appears to be no longer determined by the *hisW* mutation but is, instead, limited to the 3.5-fold level characteristic of the episomal mutation alone (strain PS196). The same phenomenon is displayed by the data shown in the last two lines of the table, where strains PS197a and PS197, carrying a *hisT* mutation of the chromosome, were employed. Thus, the *hisG* regulatory mutation is epistatic to the *hisT* and *hisW* mutations.

In the next series of experiments we studied the dominance relationships of this epistatic effect. We used merodiploids with either a wild-type episomal *hisG* gene or a feedback-resistant episomal *hisG* gene in strains carrying the chromosomal constitutive mutation, *hisT1504*, and two different partial deletions of the chromosomal histidine operon. One of these deletion strains, *hisOGDCBH2253*, has the *hisG* gene entirely deleted from the chromosome; the other deletion strain, *hisDCBHA2604*, has an intact *hisG* gene on the chromosome. We could, therefore, examine the dominance relationship between the feedback-resistant *hisG* allele of the episome and the wild-type *hisG* gene of the chromosome. The results of these experiments are shown in Table 7. The first line of the table shows the basal level of expression of the histidine operon of an episome in a strain in which the chromosomal histidine operon is not expressed. This value was taken as 1. In the next two lines of the table

TABLE 5. *Constitutive phenotype of hisG1929 is trans recessive*[a]

Strain	Episome	Chromosome	Enzyme sp act[b]			Fold derepression
			D	C	B	
JS100a	*his*$^+$	*hisDCBHAFIE712*[c]	0.9	50.6	1.9	
PS195a	*his*$^+$	*hisOGDCBHAF644*[d]	1.3	60.1	3.5	1.0
PS195	*hisG1929*[e]	*hisOGDCBHAF644*[d]	4.7	231.2	12.6	4.4
JS100	*hisG1929*[e]	*hisDCBHAFIE712*[c]	1.2	53.7	2.8	1.0

[a] All strains were grown in the presence of excess histidine.
[b] Each value represents the average of assays of extracts from five separate cultures, each done in duplicate at two different protein concentrations.
[c] *hisG* gene is unaltered and normally expressed.
[d] Entire regulatory region and all relevant structural genes of the histidine operon are deleted.
[e] *hisG* gene mutation renders phosphoribosyltransferase feedback resistant.

TABLE 6. *hisG1929 is epistatic to hisT and hisW mutations*[a]

Strain	Episome	Chromosome	Enzyme sp act[b]			Fold derepression
			D	C	B	
PS194a	*his*$^+$	*hisG203*[c]	1.4	63.9	3.9	1
PS196a	*his*$^+$	*hisG203 hisW1824*[c]	10.2	517.2	28.5	7.6
PS196	*hisG1929*[d]	*hisG203 hisW1824*[c]	4.6	223.2	12.8	3.3
PS197a	*his*$^+$	*hisOGDCBH2253 hisT1504*[c]	16.1	712.9	40.3	11
PS197	*hisG1929*[d]	*hisOGDCBH2253 hisT1504*[c]	5.1	217.6	13.2	3.5

[a] All strains were grown in the presence of excess histidine.
[b] Each value represents the average of assays of extracts from five separate cultures, each done in duplicate at two different protein concentrations.
[c] *hisG* gene is deleted.
[d] *hisG* gene mutation renders phosphoribosyltransferase feedback resistant.

are shown the results of control experiments that demonstrate that the *hisT* mutation caused constitutive expression of a wild-type episome regardless of whether or not there was a functioning *hisG* gene on the chromosome. Thus, in strains PS193a and PS197a the *hisT* mutation caused the episomal operon to be expressed at a level approximately 11-fold higher than the wild type. The last two lines of the table demonstrate the epistatic effect of the feedback-resistant mutation in the episome when there was no *hisG* gene on the chromosome (as discussed above) and the reversal of this effect when there was a functional *hisG* gene on the chromosome. Thus, strain PS197, as we have already seen, expressed the histidine operon at a level 3.5-fold higher than wild type, despite the presence of the *hisT* mutation, whereas strain PS193 showed a reversal of the epistatic effect that can be attributed to the presence of a wild-type *hisG* gene on the chromosome, restoring expression of the histidine operon to a level 10-fold higher than wild type. We conclude that the wild-type *hisG* gene is *trans* dominant to the mutant allele with respect to allowing the *hisT* mutation to cause its usual effect on expression of the histidine operon.

We have previously reported that the kinetics of repression are altered in mutants affecting the allosteric site of the first enzyme in the histidine pathway (11, 12, 14). We chose, therefore, to examine the kinetics of derepression and repression in TG5721 (PS195), a strain that carries the mutation to feedback resistance on the episome and in which the entire histidine operon is deleted from the chromosome. The results obtained in these experiments confirm the finding that this strain cannot respond to histidine limitation by altering expression of its histidine operon. The results of one experiment of this type are shown in Fig. 1. The data presented here demonstrate that conditions that diminish the availability of histidine sufficiently to slow the generation time by a factor of four did not result in a change in expression of the histidine operon in this strain. Furthermore, when histidine was added back to the culture, and the growth rate increased, there was still no change in histidine operon expression. Thus, the mutation in the *hisG* gene that renders the first enzyme severely resistant to end product inhibition results in a fixed level of expression of the operon, unresponsive to the availability of histidine in the growth medium.

DISCUSSION

The method of Sheppard (27) for obtaining feedback-resistant mutants in the first structural gene of the histidine operon is easily adapted to selecting mutants with varying degrees of resistance, depending upon the concentration of thiazolalanine in the selective medium. We have been able to obtain mutants in which the phosphoribosyltransferase displays a K_I for histidine four orders of magnitude higher than that of the wild-type enzyme.

When compared with a wild-type histidine operon, a histidine operon with a feedback-resistant *hisG* mutation was found to be expressed constitutively, at a level approximately fourfold higher than the basal level. This level was not diminished by growth of the organism on excess histidine and was not further elevated by growth of the organism on limiting histidine. However, as reported previously (11), when a wild-type *hisG* gene was present on the chromosome, the episomal operon was then expressed at the wild-type basal level when the organism was grown on excess histidine and at the maximally derepressed level when the organism was grown on limiting histidine. Thus, we concluded

TABLE 7. *Epistatic effect: wild-type hisG gene is dominant to the mutant allele*

Strain	Episome	Chromosome	Enzyme sp act[a]			Fold derepression[b]
			D	C	B	
PS194a	*his*$^+$	*hisG203*[c]	1.4	63.9	3.9	1
PS197a	*his*$^+$	*hisOGDCBH2253 hisT1504*[c]	16.1	712.9	40.3	11
PS193a	*his*$^+$	*hisDCBHA2604 hisT1504*[d]	15.3	710.3	39.7	10.7
PS197	*hisG1929*[e]	*hisOGDCBH2253 hisT1504*[c]	5.1	217.6	13.2	3.5
PS193	*hisG1929*[e]	*hisDCBHA2604 hisT1504*[d]	14.7	690.3	36.8	10.3

[a] Each value represents the average of assays of extracts from five separate cultures, each done in duplicate at two different protein concentrations.

[b] All strains were grown in the presence of excess histidine.

[c] *hisG* gene is deleted.

[d] *hisG* gene is present and expressed.

[e] *hisG* gene mutation renders phosphoribosyltransferase feedback resistant.

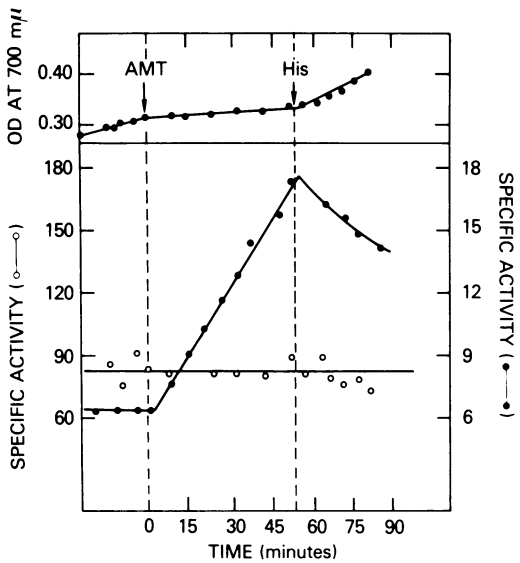

FIG. 1. *Effect of hisG feedback-resistant mutation on regulation of expression of the histidine operon. The growth curve is shown at the top (log scale). During the first part of the experiment the organisms were grown in medium E (32). When aminotriazole (AMT) was added to the culture (at 0 time), the growth rate diminished and the culture was allowed to grow at the diminished rate for approximately 50 min. Histidine (His) was then added and the growth rate returned to that characteristic of repressed cells. The specific activities of the hisC enzyme at various times during the experiments are shown in the bottom portion of the figure for the wild type (●) and for TG5721 (○). The experiment with the wild type confirms previously reported data (2) showing that the enzyme levels increase during the period of reduced growth rate and decline immediately upon the addition of histidine. The experiment with TG5721 (episome with the hisG mutation to feedback resistance and chromosome with a deletion of almost the entire histidine operon) demonstrates that the episomal histidine operon carrying the mutation to feedback resistance is not under histidine regulation.*

(11) that the effect of the regulatory function of the phosphoribosyltransferase is *trans*, with the wild-type *hisG* gene dominant to the mutant allele. The findings reported here are in keeping with this previous conclusion; as shown by the data of Table 5, the constitutive phenotype of *hisG1929* is corrected by the presence of a wild-type *hisG* gene on the chromosome. Thus, we can rule out the possibility of a second mutation in the regulatory region of the operon that accounts for the regulatory defect.

It is known that the molecular basis for the derepression of the histidine operon observed in *hisT* and *hisW* mutants is a defect in the structure of $tRNA^{His}$ (6, 15, 29). The fact that the *hisG* regulatory mutation described here was found to be epistatic to the *hisT* and *hisW* mutations suggests that phosphoribosyltransferase acts as a regulatory protein in a complex formed by interaction with histidyl-tRNA or that the regulatory function of phosphoribosyltransferase overlaps that of histidyl-tRNA. This possibility is in keeping with previously reported studies on the kinetics of repression of the histidine operon (11, 12, 14) and on the high-affinity interaction of phosphoribosyltransferase with histidyl-tRNA in vitro (3, 13, 33). If one assumes that the enzyme fulfills its regulatory function as a complex formed by interaction with histidyl-tRNA, then the fact the *hisG* regulatory mutation is epistatic to *hisT* and *hisW* mutations suggests that the defective phosphoribosyltransferase produced in these mutants is unable to interact optimally with the histidyl-tRNA.

Recently, J. Scott and J. Roth (personal communication) have isolated several mutant strains in which most of the *hisG* gene is deleted but the entire promoter-operator region of the histidine operon appears to remain intact. When these strains are grown in the presence of excess histidine, they express the histidine operon at the repressed level; when they are grown under conditions of histidine limitation, they express the operon at the derepressed level. In other words, though these strains are missing almost the entire *hisG* gene, they are able to respond to changes in the availability of histidine by appropriately adjusting expression of the histidine operon. Somehow, these results of Scott and Roth must be reconciled with the findings reported here and elsewhere (3, 4, 11–14, 20, 25, 30, 33) which show that phosphoribosyltransferase does play some role in regulating expression of the histidine operon. One possibility is that an aberration of the repression process occurs only when defective phosphoribosyltransferase is present in the cell, not when the enzyme is absent. According to this view, the enzyme does not play an obligatory role in repression, but may either modify the repression system or provide a second regulatory mechanism. It is also possible that the site at which the enzyme exerts its regulatory effect is the deoxyribonucleic acid of its own structural gene. This idea was proposed by Somerville and Stetson (31) for the tryptophan operon on the basis of the finding that certain mutations in the first structural gene of the operon display a *trans*-recessive regulatory defect, whereas deletion of this gene does not prevent repression and derepression of the operon (10).

It appears likely, at the present time, that

regulation of the histidine operon involves at least a dual system of controls. Inasmuch as the mechanism of this complex regulatory system remains a mystery, it would seem wise to reserve judgment on the molecular basis for the regulatory role of phosphoribosyltransferase. It is possible that the enzyme may itself play a dual role in regulation, acting, like the *araC* protein for the arabinose operon of *E. coli*, as both positive and negative effector, depending upon the binding of one or more ligands (34). In the absence of a detailed model for control of the histidine system, we interpret the data presented here simply as additional evidence that phosphoribosyltransferase plays some direct role in autogenous regulation of the histidine operon.

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