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Histidine Regulation in *Salmonella typhimurium*

VIII. Mutations of the *hisT* Gene

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The *hisT* gene, one of six genes in which mutation causes derepression of the histidine operon in *Salmonella typhimurium*, is shown to code for a protein that is not essential for the growth of the bacteria. This is indicated by the characterization of particular classes of mutations in the *hisT* gene: amber mutations, frameshift mutations, and temperature-sensitive mutations that affect repression but not growth. In addition, the class of semilethal mutations was selected for but not found.

In *Salmonella typhimurium*, the level of synthesis of the enzymes of the histidine operon is affected by mutations in any of six genetic loci (3, 4, 8-10, 12). These genes are: *hisO*, the operator region at the beginning of the operon; *hisS*, the structural gene for histidyl-transfer ribonucleic acid (tRNA) synthetase; *hisR*, apparently the structural gene for $tRNA_{His}$; and *hisU*, *hisW*, and *hisT*, which recent work suggests may code for tRNA maturation enzymes. Each gene may be distinguished by its unique position in the genetic map, distant from other regulatory genes.

The object of this study was to determine if the T gene coded for a protein and, if so, whether it was an essential or nonessential protein. At the initiation of this work, *hisT* seemed to be a likely candidate for the repressor gene. Studies with partially diploid bacteria demonstrate the dominance of the wild-type *hisT* allele over the mutant allele and indicate that the *hisT* gene could code for a cytoplasmic product (8). In this paper, we report the selection and properties of some temperature-sensitive *hisT* mutants, the isolation of amber *hisT* mutants, and studies on frameshift mutagenesis of the *hisT* gene.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used were derived from *S. thyphimurium* LT-2. Strain SB560 (*aroD5 purF145*) was obtained from P. E. Hartman, and strains SB391 (F'*lac* χ82/*his* 644) and SB392 (F'*lac* U281/his 644) which carry F'lac episomes with amber mutations were constructed earlier in this laboratory (5). The suppressor strain, TA248 (*aroD5 purF145 sup500*), was obtained by mutating a culture of strain SB560 with diethylsulfate, mixing it with a culture of SB392, and then spreading the mixture on a minimal salts-lactose plate supplemented with aromatic amino acids, purines, and thiamine. Since *Salmonella* is *lac*$^-$, the only bacteria able to grow on the plate were those which not only received the mutant F'*lac* episome from strain SB392, but could also suppress the amber mutation on that episome. A number of *lac*$^+$ colonies were picked, and two which showed efficient lactose fermentation on a MacConkey indicator plate were cured of their episomes with acridine orange. The existence of the amber suppressor genes was confirmed with the method of Berkowitz et al. (5); one of the strains was called TA248 and used as a recipient for the various *hisT*$^-$ alleles.

Bacteriophage stocks. Transductions were performed by using a nonlysogenizing mutant, *int 4* [L4 of Smith and Levine (13) of phage P22]. This mutant was kindly donated by H. O. Smith. Transductant colonies arising after use of this phage were streaked out immediately. After a few serial single-colony isolations, most of the colonies were found to be phage sensitive, as determined by the pour plate method with a standard phage suspension.

Media and cell growth. Minimal medium is the E medium of Vogel and Bonner (14), supplemented with 1% glucose. Solid minimal medium is the E medium supplemented with 2% glucose and solidified with 1.5% agar. Complete medium is 0.8% nutrient broth (Difco) and 0.4% NaCl. Unless otherwise stated, liquid cultures were grown at 37 C in a New Brunswick incubator shaker.

Mutagenesis. In most cases, 0.05 ml of diethylsulfate was shaken with fully grown 2-ml cultures for 10 min at 37 C. Frameshift mutations were induced by the aza-quinacrine derivative, ICR 372 (2), which was kindly donated by H. J. Creech. Tubes containing 1 ml of minimal medium, 0.1 ml of complete medium, 10 μg of ICR 372, and about 2×10^7 cells of the wild-type strain were shaken for 18 hr at 37 C. A 0.1-ml amount of each culture was diluted in 10 ml of complete medium, and the cells were grown overnight. The fully grown cultures were then washed and subjected to selection.

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Isolation of temperature-sensitive regulatory mutants. Regulatory mutants were isolated, the *his* operons of which were repressed normally at 30 C, but derepressed at 42 C, even in the presence of excess histidine. Although regulatory mutants of the *his* operon are generally unable to grow at 42 C, such mutants will grow at this temperature when methionine is included in the medium (7). The selection of these mutants was, therefore, performed in the presence of 0.2 mM methionine.

At 42 C, in the presence of methionine, triazole alanine-resistant mutants were selected from wild-type LT-2 as previously described (11). These strains were streaked on medium containing histidine plus methionine and incubated at 30 and 42 C, and their colony morphology was scored. Wrinkled colony morphology is an indirect but reliable means of identifying strains growing with derepressed enzyme levels; clones with repressed enzyme levels form smooth colonies. Strains carrying mutations *hisT1535*, *hisT1536*, and *hisT1537* formed wrinkled colonies at 42 C and smooth colonies at 30 C; they were saved.

Production and identification of hisT mutants. Unless otherwise stated, all *hisT* mutants were selected and identified in the following manner.

Wild-type bacteria were treated with a chemical mutagen and spread onto plates of minimal medium containing 20 mM 3-amino-1,2,4-triazole. In some cases, the cells were spread immediately after mutagenesis and, in others, after allowing the cells to grow for several generations to express the induced mutations (6).

A few crystals of 1,2,4-triazole-3-alanine were added, and colonies of bacteria resistant to the analogue were picked and purified by restreaking them on the solid minimal medium. Derepressed mutants were identified by their characteristic wrinkled morphology on this medium (11). These colonies were picked with sterile wooden applicator sticks and used to inoculate tubes containing 2.5 ml of complete medium. When the cultures reached cell densities of about 10^8 cells/ml, 2×10^7 plaque-forming units of phage were added, and the cultures were incubated until appreciable cell lysis occurred, usually 4 to 12 hr. The cultures were then shaken with a few drops of chloroform, and the resulting phage suspensions were used with no further purification.

About 10^{10} cells of strain SB560 (*aroD*$^-$ *purF*$^-$) were spread on petri plates of solid minimal medium, and about 0.05 ml of each phage suspension was dropped onto the plates. Since the *hisT* gene lies between the cotransducible *aroD* and *purF* genes, over 90% of the prototrophic transductants from this cross will carry any *hisT* gene present in the donor. *HisT* mutants were recognized by their wrinkled colony morphology. Eight to 12 donors could easily be tested on one plate. The *hisT* genes of the mutants obtained were introduced into other recipients in the same manner.

Identification of hisO, hisR, and hisS mutants. In some experiments, transducing phage which gave negative results in the *hisT* mutant screening procedure were tested for mutant *hisO*, *hisR*, and *hisS* genes. *HisO* mutants were identified by the wrinkled morphology of transductants when the deletion mutant *hisOG203* was used as a recipient. Only about 0.01 ml of each phage preparation was needed, and 16 to 20 donors could be tested on one plate. *HisR* and *hisS* mutants were mapped by using the strains *metE338* and *strB57* (which requires thiamine and nicotinic acid), respectively, as recipients. To suppress the appearance of revertants, we grew the strain *strB57* in complete medium with 50 μg of streptomycin sulfate per ml, and washed the resuspended cells in minimal medium before use.

Testing for the presence of amber suppressors. Amber suppressors were recognized by using mutant *E. coli* F'lac episomes as described earlier (5).

Enzyme assays. Histidinol phosphate phosphatase (EC 3.1.3.15) was assayed by a previously described procedure (1).

RESULTS

Mapping and properties of temperature-sensitive hisT mutants. The mutants isolated by the above methods carry mutations in or very near the *hisT* locus and show enzyme levels which are affected by growth temperature. When phage grown on these mutants is used in transduction tests with *purF145*, approximately 60% of the *pur*$^+$ recombinants show the donor temperature-sensitive phenotype (8/20 for *hisT1535*; 92/138 for *hisT1536*; 54/81 for *hisT1537*). This frequency of cotransduction with *purF145* is the same as that found previously for mutations assigned to the *hisT* locus (10).

Histidinol phosphate phosphatase levels of the three temperature-sensitive *hisT* mutants, when grown at several temperatures, are presented in Table 1. As controls, two non-temperature-sensitive mutants (*hisT1504* and *hisT1529*) and the wild-type strain (LT-2) are included.

TABLE 1. *Phosphatase (hisB) levels of temperature-sensitive hisT mutants*[a]

Strain	Enzyme level of cell grown at the indicated temp (C)			
	28	37	40	42
hisT1529	11.5	11.1	10.1	8.7
hisT1504	11.4	14.6	11.4	13.6
hisT1535	0.6	2.0	7.9	10.8
hisT1536	0.7	2.3	10.7	12.0
hisT1537	0.6	2.4	9.7	11.4
LT-2	0.6	0.9	1.8	1.7

[a] All cells are grown for approximately seven generations at the indicated temperature in minimal medium containing 0.1 mM histidine and 0.2 mM methionine. Histidinol phosphate phosphatase was assayed by the toluenized cell method of Ames, Hartman, and Jacob (1) as modified by Roth and Ames (9). The units are defined as change in optical density at 820 nm due to released PO_4 per 15 min per optical density unit at 650 nm of cells added to reaction mixture.

Figure 1 presents the time course of derepression after a temperature shift from 28 to 41 C.

Selection of amber hisT mutants. Transducing phage were used to introduce the mutant *hisT* genes into a strain carrying an amber suppressor. Amber *hisT* mutants would be suppressed by the amber suppressor and would therefore give rise to more repressed, less wrinkled colonies in the suppressor carrying strain. Of 117 *hisT* mutants examined, only two, *hisT2890* and *hisT2897*, were sufficiently suppressed in the presence of the suppressor to give less wrinkled colonies. The specific activities of histidinol phosphate phosphatase, one of the enzymes of the histidine operon, in the suppressed and unsuppressed amber *hisT* mutants, is given in Table 2. The supressor does not affect the enzyme level in *hisT*$^+$ bacteria or in *hisT1529*. The two amber *hisT* mutants were isolated from independently mutagenized clonal cultures and were suppressed to different degrees by the suppressor used. They are clearly not identical. Since amber mutations generally result in complete loss of gene function, the isolation of viable *hisT* amber mutants suggests that the *hisT* gene product is a nonessential protein. This conclusion is supported by the results of frameshift mutagenesis and of a deliberate attempt to isolate slowly growing regulatory mutants.

Frameshift mutations in histidine regulatory genes. Of 98 histidine regulatory mutants obtained after treatment of 20 independent clones of wild-type bacteria with the frameshift mutagen ICR 372, 26 were *hisT* and 61 were *hisR*. There were no *hisO* or *hisS* mutants. The remaining 11 strains are presumably mutant in the *hisU* or *hisW* regulatory genes. The mutation rate in this experiment, as judged by mutation to resistance to azetidine-2-carboxylic acid, was about 100 times the spontaneous rate. The frequency of histidine regulatory mutants obtained was increased by a similar factor.

ICR 372 produces exclusively frameshift mutations (2). Since most frameshift mutations in genes coding for proteins would be expected to result in completely nonfunctional products, we would not expect to find many frameshift mutations in genes coding for essential proteins. Indeed we did not obtain any frameshift mutants in the *hisS* gene, which codes for histidyl-tRNA synthetase, an essential protein. The fact that we did not obtain any *hisS* mutants in this experiment, even though they are a frequent product of spontaneous mutagenesis, indicates that the spontaneous contribution to the mutation rate is insignificant and that most, if not all, of the *hisT* and *hisR* mutations were ICR-induced.

The occurrence of viable strains with ICR-induced frameshift mutations in the *hisT* gene supports the conclusion that the *hisT* gene product is a nonessential protein.

Because of the low frequency of histidine operator mutants generally encountered, we cannot attach any significance to our failure to obtain any *hisO* mutants in this experiment.

Direct search for semilethal regulatory mutations. To determine which histidine regulatory genes are essential for the life of the cell, we

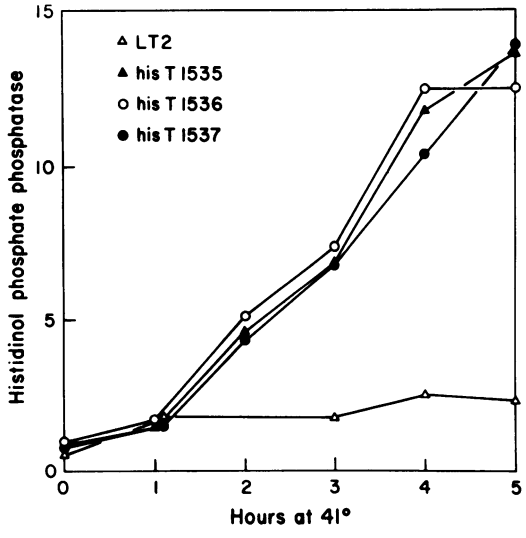

FIG. 1. *Cells were grown in minimal medium containing 0.1 mM histidine and 0.2 mM methionine. After growth at 28 C, cells were shifted at time zero to 41 C, and samples were taken periodically. Assay is as in legend to Table 1.*

TABLE 2. *Phosphatase (hisB) levels of hisT mutants*[a]

Strain	Specific activity
Amber mutants	
hisT2890	15.1
hisT2890 sup 500	4.0
hisT2897	15.0
hisT2897 sup 500	1.7
Controls without amber mutations	
hisT1529	16.1
hisT1529 sup 500	13.6
hisO1242	23.0
Wild-type bacteria	
LT-2	1.9
sup 500	2.0

[a] Strains carrying the amber suppressor sup 500 are isogenic with each other. The unsuppressed T mutants also comprise an isogenic series. With the exception that all cells were grown on minimal medium at 37 C, the procedures and units are the same as in Table 1.

mutagenized cultures of wild-type bacteria with diethylsulfate and selected very slowly growing histidine regulatory mutants. We used aminotriazole and triazolealanine, as described above, but we picked only the very small wrinkled colonies from the selection plates. Those colonies growing slowly on the selective medium simply because they were not sufficiently derepressed were excluded by the additional requirement that they grow very slowly on minimal medium in the absence of aminotriazole. Slow growth due to mutation of genes not closely linked to the mutated histidine regulatory genes was excluded by the requirement that transductants from the genetic mapping procedure must also be slow growers.

From 50 mutagen-treated clones, we obtained 238 slowly growing derepressed mutants. Most of these, including all 78 *hisO* and 28 *hisT* mutants isolated, yielded strains with normal growth rates upon transduction of their histidine regulatory genes into strains with relatively undamaged chromosomes. Their slow growth was attributable to unknown mutations not linked to any of the histidine regulatory genes. On the other hand, all 28 of the *hisS* mutants and 3 of the 23 *hisR* mutants grew more slowly than wild-type bacteria on minimal agar plates, even after transduction into undamaged genetic backgrounds. Eighty of the derepressed mutants obtained did not map in the *hisO*, *hisR*, *hisS*, or *hisT* regions and are presumably *hisU* or *hisW* mutants.

Since the *hisS* gene codes for the histidyl-tRNA synthetase, an essential enzyme, it is not surprising that we obtained slowly growing *hisS* mutants. About two-thirds of the *hisS* mutants selected in this experiment could have reduced affinities for histidine, since, in the presence of 0.1 mM histidine in the medium, they have wild-type growth rates and repressed histidine operons. These *hisS* mutants are functionally similar to *hisS1520*, which has been found to have a greatly increased K_m for histidine (9). The kinetic properties of the activating enzymes of some of these mutants are reported in another paper (DeLorenzo, Straus, and Ames, *in preparation*).

No slowly growing *hisO* mutants were obtained. This is consistent with the earlier observations that strains in which the *hisO* locus is deleted grow normally if supplied with histidine and that none of the nearly 100 derepressed mutants mapping in the *hisO* gene grow slowly (6; D. Fankhauser and P. E. Hartman, *personal communication*).

DISCUSSION

Several lines of evidence indicate that the *hisT* gene codes for a protein which is required for normal regulation of the histidine operon but not for growth.

(i) We isolated two independent polypeptide chain-terminating mutations of the UAG (amber) type in the *hisT* gene product. This shows that the *hisT* gene product is a protein and that this protein is not essential for the growth of the bacteria. These amber mutations are supressible by a known amber suppressor. All amber supressors that have been examined involve altered tRNA species and have been shown to work at the level of translation of the messenger RNA into protein.

(ii) We isolated *hisT* mutants which are unable to repress the histidine operon when grown at a high (40 to 42 C) temperature. Presumably, the products of these mutant *hisT* genes are inactivated at a high temperature. This property is consistent with the fact that this product is a protein.

(iii) Several frameshift type mutants were isolated in the *hisT* gene. This is also consistent with the fact that the product is a dispensible protein.

(iv) That we were unable to isolate any very slowly growing *hisT* mutants is also consistent with the fact that the *hisT* protein is not essential for growth. As a control, we could easily isolate slowly growing *hisS* mutants; this is consistent with the known function of the *hisS* gene in coding for the histidyl-tRNA synthetase, a protein essential for growth.

More recent studies indicate that the *hisT* protein is involved in the formation of pseudouridine in the histidine tRNA anticodon region (C. E. Singer, G. R. Smith, and B. N. Ames, *manuscript in preparation*) and apparently in other tRNA species as well (R. Cortese, and B. N. Ames, *unpublished data*).

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