HISTIDINE MUTANTS REQUIRING ADENINE: SELECTION OF MUTANTS WITH REDUCED *hisG* EXPRESSION IN *SALMONELLA TYPHIMURIUM*

H. MARK JOHNSTON AND JOHN R. ROTH

Department of Biology, University of Utah, Salt Lake City, Utah 84112

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ABSTRACT

A method is described for the selection of *Salmonella typhimurium* mutants with reduced levels of *hisG* enzyme activity. This method is based on the fact that the *hisG* enzyme catalyzes the consumption of ATP in the first step of histidine biosynthesis. Normally, this reaction is closely regulated, both by feedback inhibition and by repression of the operon. However, conditions can be set up that result in the uncontrolled use of adenine in histidine biosynthesis. Cells grown under these conditions become phenotypic adenine auxotrophs. Some revertant clones that no longer require adenine contain mutations in *hisG*, *hisE*, or the *his*-control region. The *hisG* mutations are of all types (nonsense, frameshift, missense, deletion and leaky types), and they map throughout the *hisG* gene.

THE histidine operon of *S. typhimurium* is a cluster of nine genes that code for the ten enzymes that catalyze histidine biosynthesis (Figure 1) (BRENNER and AMES 1971). In the first step of this pathway, phosphoribosyltransferase catalyzes the consumption of ATP* *via* the reaction ATP + PRPP → PR-ATP (AMES, MARTIN and GARRY 1961). This enzyme is encoded by the *hisG* gene, the most operator-proximal gene of the *his* operon (BRENNER and AMES 1971).

The *hisG* enzyme is interesting for several reasons. First, the *hisG* enzyme is sensitive to feedback-inhibition by histidine (MARTIN 1963). The kinetics of this inhibition are interesting (MARTIN 1963; MONOD, WYMAN and CHANGEUX 1965), but the precise molecular events responsible are unknown (BLASI, ALOJ and GOLDBERGER 1971). Second, the *hisG* enzyme has been implicated in the regulation of his-operon transcription (MEYERS, LEVINTHAL and GOLDBERGER 1975; ROTHMAN-DENES and MARTIN 1971), although what role, if any, it plays in this process is unclear (SCOTT, ROTH and ARTZ 1975). Finally, the *hisG* enzyme has a complex structure, being composed of six identical subunits (VOLL, APPELLA and MARTIN 1967; PARSONS and KOSHLAND 1974). Unlike other *his* genes coding for multimeric enzymes, the *hisG* gene consists of only one complementation group (LOPER *et al.* 1964).

* For abbreviations, see Figure 1.

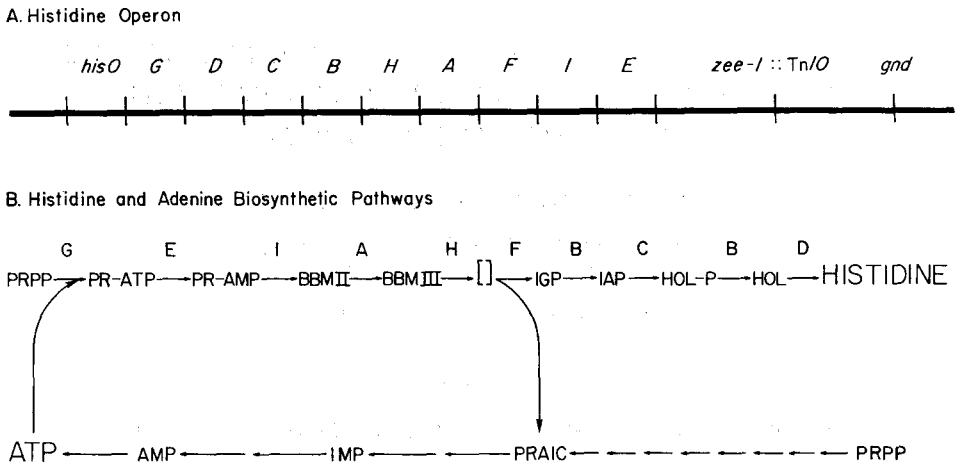

FIGURE 1.—(A) Genetic map of the histidine operon. (B) Histidine and adenine biosynthetic pathways. Abbreviations: PRPP, Phosphoribosylpyrophosphate; ATP, Adenosine triphosphate; PR-ATP, Phosphoribosyl-ATP; AMP, Adenosine monophosphate; IMP, Inosine monophosphate; PRAIC, Phosphoribosylaminoimidazole carboxamide; BBMII, (Bound Bratton-Marshall Compound), Phosphoribosylformimino-PRAIC; BBMIII, Phosphoribulosylformimino-PRAIC; [ ], unknown intermediate; IGP, Imidazole glycerol phosphate; IAP, Imidazole acetol phosphate; Hol-P, L-Histidinol phosphate; Hol, L-Histidinol.

For these reasons, we have chosen to undertake a genetic study of the *hisG* gene. In this paper, we report a method for the selection of *hisG* mutants. The selection can also be used to obtain mutants of the *his*-operon control region.

MATERIALS AND METHODS

Bacterial strains: Multiply marked strains and their sources are listed in Table 1. All are derived from *Salmonella typhimurium* strain LT2. All *his* auxotrophs, unless otherwise noted in Table 1, were obtained from P. E. HARTMAN (HARTMAN *et al.* 1971).

Media: VOGEL and BONNER (1956) E medium containing 2% glucose was used as minimal medium. Supplements were added at the following concentrations: 1 mM L-histidinol (Sigma), 0.5 mM adenine, 0.05 mM thiamine, 0.1 mM L-histidine, 0.3 mM D-histidine, 1 mM 2-thiazole-DL-alanine (Cyclo), 40 mM 3-amino-1,2-triazole (Aldrich), 10 μg/ml tetracycline (Sigma). Media supplemented with adenine were also supplemented with thiamine. Amino-triazole supplemented medium also contained adenine and thiamine, (HILTON, KEARNEY and AMES 1965) Difco nutrient broth (0.8%) containing NaCl (0.5%) was used as rich medium. When necessary, rich medium was supplemented with 25 μg/ml tetracycline. Solid media contained 1.5% Difco agar.

Determination of growth on histidinol: The ability of mutants to grow on histidinol was tested by four different methods. (1) The mutant to be tested was streaked for single colonies on E plates containing 1 mM L-histidinol, or histidinol plus adenine and thiamine, incubated at the appropriate temperature and scored after one, two, and five days. (2) A soft agar overlay of the mutant to be tested was made on E plates, and a drop of 2% L-histidinol, or histidinol plus 1.35% adenine and 0.33% thiamine placed on the plate. Growth was scored after one, two, and five days of incubation at the appropriate temperature. (3) 5 μl of a 1 mM L-histidinol solution was placed on a filter disc in the middle of an E plate, and the mutant to be tested was radially streaked away from the filter disc. Growth was scored after one, two, and four days incubation

TABLE 1

Multiply marked strains

Strain	Genotype	Source
TR35	*his-712 ser-821 arg-501/F'* $_{T80}$*his+*	FINK and ROTH (unpublished)
TR47, (TR51, TR52)*	*his-612 trp-8 pur-801/F' hisD2337*	FINK and ROTH (unpublished)
TR53, (TR55)*	*his-712 ser-821 arg-501/F' hisC2383*	FINK and ROTH (unpublished)
TR75, (TR85)*	*his-712 ser-821 arg-501/F' hisB2405*	FINK and ROTH (unpublished)
TR76	*his-712 ser-821 arg-501/F' hisA2406*	FINK and ROTH (unpublished)
TR78	*his-712 ser-821 arg-501/F' hisF2408*	FINK and ROTH (unpublished)
TR79, (TR82, TR83)*	*his-712 ser-821 arg-501/F' hisI2409*	FINK and ROTH (unpublished)
TR84	*his-712 ser-821 arg-501/F' hisE2414*	FINK and ROTH (unpublished)
TG5701	*his-712 ser-821 arg-501/F' hisG2416*	R. F. GOLDBERGER
TR3376	*hisO-H2253 hisT1504*	J. SCOTT
TR4733	*hisO1242 hisB8633*	H. M. JOHNSTON (unpublished)
TR5052 thru TR5098 even numbers	*hisG8637–hisG8660 hisF645*	Ade+ revertants of *hisF645* on histidinol
TR5053 thru TR5099 odd numbers	*hisG8637–hisG8660*	Hol+ transductants into *his2226*
TR5100 thru TR5108 even numbers	*hisG8661–hisG8655 hisB8633*	Ade+ revertants of TR4733
TR5101 thru TR5109 odd numbers	*hisG8661–hisG8655*	Hol+ transductants into *his2226*
TR5329	*hisO9529 hisF645*	Ade+ revertant of *hisF645* on D-histidine
TR5548	*hisG1102 hisT1504*	This paper
TT545	*hisG8476 zee-1*::Tn*10*	This paper
TT547	*hisC117 zee-1*::Tn*10*	This paper
TT549	*hisE11 zee-1*::Tn*10*	This paper
TT550	*hisI125 zee-1*::Tn*10*	This paper
TT551	*hisF645 zee-1*::Tn*10*	This paper
TT554	*hisO1242 hisB8633 zee-1*::Tn*10*	This paper
TT598	*hisG8476 hisB8633 zee-1*::Tn*10*	This paper
TT1700	*hisG1102 hisF645 zee-1*::Tn*10*	This paper
TT1702	*hisG1102 hisE11 zee-1*::Tn*10*	This paper
TT1867	*hisG1102 hisF645*	This paper
TT1983	*hisO9529 zee-1*::Tn*10*	This paper
TT2173	*hisG8476 hisF645 zee-1*::Tn*10*	This paper
TT2221	*hisD1 hisF645 zee-1*::Tn*10*/F' $_{T80}$ *hisA-E2905*	This paper
TT2999	*hisG1102 hisB167 zee-1*::Tn*10*	This paper

* The strain in parentheses is identical to the preceding strain, except that the F'*his* episome carries a different mutation in the same gene.

at the appropriate temperature (this method was suggested by G. F. AMES. (4) Growth rates were determined by measuring OD_{650}.

Transductional crosses: The P22 mutant HT105/1, *int*$^-$ was used for all transductions; this phage carries a mutation causing high transduction frequency (SCHMIEGER 1971) and an *int*$^-$ mutation isolated by G. ROBERTS. Phage were grown as described by HOPPE *et al.* (1979). Plates were spread with 10^8 to 10^9 phage particles and approximately 2×10^8 recipient cells. Transductant colonies were picked after two to three days incubation at 37°. Transductants to be used further were purified twice selectively and once nonselectively and verified to be sensitive to P22 phage.

Complementation tests and genetic mapping: Complementation tests were performed using F'*his* episomes carrying different *his* mutations (listed in Table 1). The F'*his*+ episome is of *E. coli* origin; mutants were selected and characterized by G. FINK and J. ROTH (unpublished results). Complementation tests were performed by spot tests selecting for transfer of the F'*his*− episome into the new *his* mutant. Mapping crosses were performed as described by HOPPE *et al.* (1979).

Reversion tests: An overnight nutrient broth culture of the mutant to be tested was plated (0.1 ml) on E medium supplemented with 0.005 mM L-histidine. Crystals of N-methyl-N'-nitro-N-nitrosoguanidine (NG) (Aldrich) were placed on one plate; another plate was spotted with a drop of 1 mg per ml solution of ICR191 (gift of H. J. CREECH, Institute of Cancer Research) ICR191 plates were incubated in the dark. The plates were scored for His+ revertants after two to five days at 37°.

Determination of polarity: Constitutive *his* mutants, such as *hisT*, exhibit a wrinkled colony morphology on plates containing 2% glucose due to the overproduction of the *hisF* and *hisH* enzymes (MURRAY and HARTMAN 1972). However, *hisT* mutants that also possess a polar mutation in the *his* operon have the normal smooth colony morphology (FINK, KLOPOTOWSKI and AMES 1967). We transduced each of the *hisG* mutations into a strain carrying a *hisG-hisD* deletion and a *hisT* mutation (TR3376) selecting *hisD*+, and scored the colony morphology of these transductants. Wrinkled transductant colonies indicated that the *hisG* mutation was nonpolar. Most of the polar *hisG* mutants yield both rough and smooth His+ revertants in a *hisT*− background; the smooth revertant colonies are due to the presence of a suppressor mutation in these revertants. This is behavior expected of nonsense and some frameshift mutations.

RESULTS

Rationale of the selection method: The first step of histidine biosynthesis is the consumption of ATP to form PR-ATP (Figure 1). This step is normally closely regulated, primarily by feedback inhibition of phosphoribosyltransferase (*hisG* enzyme) activity (MARTIN 1963), but also by repression of the operon (BRENNER and AMES 1971). However, conditions can be set up under which the *hisG* enzyme is released from feedback inhibition and the operon is derepressed. Cells grown under these conditions become phenotypic adenine auxotrophs, presumably due to a depletion of the adenine pool caused by the uncontrolled use of ATP in histidine biosynthesis. Since this phenotype is due to the action of the *hisG* enzyme, a mutation that renders the phosphoribosyltransferase inactive should halt the adenine drain and relieve the cells' adenine requirement. We believe this to be the basis of the selection method.

There are two ways to cause cells to grow with derepressed levels of *hisG* enzyme released from feedback inhibition. One way is to limit the cells' histidine supply in order to cause a low intracellular histidine concentration. This can be done by growing a histidine auxotroph on a poor histidine source. Operon derepression and release of *hisG* enzyme from feedback inhibition can also be caused by mutation. Mutants of *hisG* are available that produce phosphoribosyltransferase resistant to feedback inhibition (SHEPPARD 1964); also available are numerous regulatory mutants that express the *his* operon at a high level (ROTH, ANTON and HARTMAN 1966). Using these mutants, strains that contain derepressed levels of feedback resistant *hisG* enzyme have been constructed and found to be adenine auxotrophs. Under both of these conditions *hisG* mutants can be selected. Below we describe the two conditions that lead to adenine auxotrophy.

Condition I: A his *auxotrophy plated on a poor histidine source:* The last step in histidine biosynthesis is the conversion of histidinol (Hol) to histidine (His), catalyzed by histidinol dehydrogenase, the product of the *hisD* gene (Figure 1). A *his* mutant is able to grow on histidinol provided that it possesses a functional *hisD* gene. Since histidinol is a poor histidine source, presumably due to its inefficient transport into the cell, *his*$^-$ cells growing on histidinol grow under conditions of histidine starvation. According to the rationale of the selection method (above), one might expect that a *his* mutant plated on histidinol would require adenine.

We found that a number of *his* mutants that possess a functional *hisD* gene fail to grow on histidinol at 30°, but are normal for growth on histidinol at 37° or 42°. That is, these mutants are cold sensitive for growth on histidinol (Table 2). We believe that the temperature dependence of this phenotype is due to the less efficient transport of histidinol at low temperature (see DISCUSSION). However, not all *his* mutants have this phenotype. Table 2 shows that only mutants with blocks in any of the steps 3 through 9 of the histidine pathway (strains with mutations in *hisI*, *A*, *F*, *H*, *B*, or *hisC*) are cold sensitive on histidinol; strains defective in carrying out either of the first two steps of the pathway (mutants of *hisG* or *hisE*) grow normally on histidinol at 30°. (*hisD* mutants, of course, fail to grow on histidinol under any condition.) In order to eliminate any unknown strain differences, we constructed an isogenic set of these *his* mutants and obtained the same results. Therefore, the growth defect of these

TABLE 2

Phenotype of his *mutants*

Strain	Relevant genotype	Growth at 30° on Minimal	Minimal +Hol	Minimal +Hol+Ade	Growth at 42° on Minimal +His	Minimal +Hol
TT545†	*hisG*$^-$	—	+(155)*	+	+	+(53)
TT549	*hisE*$^-$	—	+(155)	+	+	+(50)
TT550	*hisI*$^-$	—	—(690)	+	+	+(100)
hisA294	*hisA*$^-$	—	—(780)	+	+	+
TT551	*hisF*$^-$	—	—(530)	+	+	+(51)
hisH107	*hisH*$^-$	—	—(495)	+	+	+
TT554	*hisB*$^-$	—	—(700)	+	+	+(75)
TT547	*hisC*$^-$	—	—(480)	+	+	+
LT2	*his*+	+	+(55)	+	+	+(56)
TR5054	*hisG*$^-$ *hisF*$^-$	—	+(190)	+	+	+
TR5100	*hisG*$^-$ *hisB*$^-$	—	+(115)	+	+	+
TT2173	*hisG*$^-$ *hisF*$^-$	—	+(90)	+	+	+
TT598	*hisG*$^-$ *hisB*$^-$	—	+(95)	+	+	+

Growth was determined on plates as described in MATERIALS AND METHODS. Similar results were obtained for a number of other *his* mutants. The results were the same regardless of the type of mutant tested (missense, nonsense, etc.) Media used are minimal E medium supplemented as indicated with histidinol (Hol) at 1 mM, with histidine (His) at 0.1 mM or with adenine at 0.2 mM.

* The numbers in parentheses are doubling times in minutes.

† TT545–TT554 are isogenic, except for the his mutations.

strains must be caused by the particular *his* mutation, and not by some other mutation in these strains. The failure of these *his* mutants to grow on histidinol at 30° is due to an adenine requirement. All of these mutants will grow at 30° on histidinol medium supplemented with 0.5 mM adenine (Ade) (Table 2). Apparently, blocks in the *hisG* or *hisE* steps of the biosynthetic pathway prevent the adenine drain and do not lead to adenine auxotrophy.

When one of these *his* mutants (cold sensitive on Hol) is plated on histidinol at 30°, revertant clones arise at a high frequency. For example, when the deletion mutant *hisF645* is plated on histidinol at 30°, revertant colonies (Ade^+) arise at a frequency of 5.2×10^{-6} per cell plated. We found that 23% (22/97) of these Ade^+ phenotypic revertants carry a new *hisG* mutation, in addition to the *hisF645* deletion already present in these strains. We determined this by complementation tests and genetic mapping. We also obtained similar results for Ade^+ revertants of a *hisB* mutant. It appears, then, that the inability of certain *his* mutants to grow on Hol at 30° can be suppressed by a *hisG* mutation. To confirm this, we constructed *hisG hisF* and *hisG hisB* double mutants, using mutations already existing in our collection. As expected, these strains were no longer cold sensitive for growth on histidinol (Table 2, lines 12 and 13).

To further confirm that the loss of phosphoribosyltransferase activity is responsible for the suppression of the cold-sensitive (Ade^-) phenotype, we used the histidine analogue 2-thiazolealanine (TA) to inhibit *hisG* enzyme. TA is a false feedback inhibitor of phosphoribosyltransferase. In the presence of 1 mM TA, a concentration sufficient to inhibit the *hisG* enzyme (SHEPPARD 1964), all *his* mutants (except $hisD^-$) are able to grow on histidinol at 30° without adenine supplementation.

The data in Table 2 show that *hisE* mutants, as well as *hisG* mutants are able to grow on histidinol at low temperature because, we believe, the second step of histidine biosynthesis, which is catalyzed by the *hisE* enzyme, is required to drive the phosphoribosyltransferase-catalyzed reaction to completion. The equilibrium of the first step is known to favor the reactants, ATP and PRPP (AMES, MARTIN and GARRY 1961; MARTIN 1963). Presumably, this reaction goes to completion only when coupled with the second step of histidine biosynthesis. We propose that mutants of *hisE* do not require adenine when plated on histidinol at 30° since the consumption of ATP requires an active *hisE* enzyme. Our selection method, therefore, should also yield *hisE* mutants. We obtained only *hisG* mutants in our initial experiment. We did, however, recover *hisE* mutants under special conditions (see below).

Results similar to those reported above were found for *his* auxotrophs growing on D-histidine, whose poor uptake also results in histidine starvation (KRAJEWSKA-GRYNKIEWICZ, WALCZAK and KLOPOTOWSKI 1971). We found that *hisF* mutants fail to grow on 0.3 mM D-His at any temperature, while isogenic *hisG hisF* double mutants are able to grow under these conditions. Furthermore, *hisF* mutants can grow on plates containing D-histidine supplemented with 0.5 mM adenine.

When *hisF645* or *hisB8633* was plated on 0.3 mM D-His, revertant clones (Ade$^+$) arose at a high frequency. From each strain, 50 of these Ade$^+$ clones (in ten independent groups) were picked and tested for the presence of a new *his* mutation by complementation tests with various F′*his*$^-$ episomes. Three of these revertants had acquired a new *his* mutation, in addition to the parental (*hisB* or *hisF*) defect. One revertant of *hisF645* contained a new *hisIE* mutation, one had acquired a new His$^-$ mutation in the control region (*hisO*) (determined by complementation and genetic mapping), and one revertant of *hisB8633* contained a new *hisG* mutation.

The majority of these revertants possessed no new *his* mutation. At least some of them probably acquired a new *dhuA* or *dhuB* lesion, which are known to cause increased levels of the histidine permease and result in more efficient transport of D-histidine (KRAJEWSKA-GRYNKIEWICZ, WALCZAK and KLOPOTOWSKI 1971). Some may also contain new *glnA* mutations, which are known to permit improved growth on D-histidine (KUSTU and MCKEREGHAN 1975). The revertants with no new *his* mutation were not characterized further.

We conclude that the selection will work on any poor histidine source. The fact that we recovered a mutant of the *his* control region is further confirmation that any mutation that results in a loss of *hisG* enzyme activity will permit growth under the selection conditions.

Condition II: Use of hisG *mutants resistant to feedback inhibition*: Strains containing fully active *hisG* enzyme can also be generated using mutations that cause the *hisG* enzyme to be resistant to inhibition by histidine (SHEPPARD 1964). Table 3 shows that the prototrophic feedback-resistant (fb^r) strain *hisG1102* does not require adenine. However, *hisG1102* (fb^r) becomes Ade$^-$ (at 42°) with the introduction of a *hisT* mutation (strain TR5548, Table 3, line 2), which causes constitutively high *his* enzyme levels due to a defect in His-tRNA modification (SINGER *et al* 1972). Apparently, repressed levels of feedback-resistant *hisG* enzyme are not sufficient to generate the adenine auxotrophy. The requirement of high temperature for the Ade$^-$ phenotype is not fully understood, but could be explained if the feedback resistant *hisG* enzyme activity of these

TABLE 3

Phenotype of strains carrying a hisG *feedback resistant mutation*

Strain	Genotype	Growth at 30° on Minimal	Growth at 30° on HIS	Growth at 42° on Minimal	Growth at 42° on HIS	Growth at 42° on HIS+ADE
	hisG1102 (fb^R)	+	+	+	+	+
TR5548	*hisG1102 hisT1504*	+	+	—	—	+
TT1867	*hisG1102 hisF645*	—	+	—	—	+
TT2999	*hisG1102 hisB167*	—	+	—	+	+
TT1702	*hisG1102 hisE11*	—	+	—	+	+

Growth of single colonies was scored on plates. Strain *hisG1102* is feedback resistant (SHEPPARD 1964). Minimal medium is the E medium of VOGEL and BONNER (1956) supplemented with histidine (HIS) or adenine (ADE) where indicated.

mutant strains is slightly cold sensitive. At lower temperatures the adenine requirement would be relieved due to the reduced *hisG* enzyme activity.

When TR5548 (*hisG1102 hisT1504*) was plated on His at 42°, revertants (Ade^+) arose at a frequency of 7.8×10^{-5} per cell plated. Revertants (800 in 100 independent groups) were picked and replicated to various media to test for a new *his* mutation. About 49% were His^-, but able to grow on histidinol (Hol^+), and, of 41 tested, all failed to complement an F'*hisG*$^-$ episome; 21% were His^-, Hol^-, and were found to contain deletions of varying lengths of the *his* operon; and 13% were His^+, but unable to fully express the *his* operon, as evidenced by their sensitivity to the histidine analog 3-amino-1,2,4-triazole (HILTON, KEARNEY and AMES 1965). All mutants in this class were found to possess a mutation mapping in the *his* control region that results in sensitivity to amino-triazole. These mutants will be described elsewhere. Presumably, these mutants survived the selection due to their decreased *hisG* enzyme levels. The remaining 17% contained no detectable new *his* mutation, and have not been further characterized.

The prototroph *hisG1102* (fb^r) also becomes Ade^- (at 42°) with the introduction of a *hisF* mutation (strain TT1867, Table 3, line 3). In this case, repressed levels of feedback-resistant *hisG* enzyme are apparently sufficient to generate the adenine auxotrophy. This is not fully understood, but could be due to the fact that the *hisF* mutation prevents recycling of the purine intermediate PRAIC (produced as a byproduct of the histidine pathway, see Figure 1), which might otherwise spare the adenine requirement. In support of this, we found that the double mutant *his G1102* (fb^r) *hisB167* (TT2999), which is able to recover some of its adenine skeleton as PRAIC, does not require adenine (Table 3, line 4). As expected, the double mutant *his G1102* (fb^r) *hisE11* does not require adenine (Table 3, line 5, since the *hisE* enzyme is required to drive the first step to completion.

When *hisG1102* (fb^r) *hisF645* (TT1867) was plated on histidine at 42°, Ade^+ revertants arose at a frequency of 4.8×10^{-7} per cell plated. Some of these Ade^+ clones contained new *hisG* mutations, as evidenced by their inability to complement an F'*hisG*$^-$ episome. We picked 350 revertants in 125 independent groups; 14 had acquired a new *hisG* mutation, and two contained deletions of the entire *his* operon. We did not obtain *hisE* mutants, but such mutants do survive the selection (see below).

Table 4 summarizes the different strains used and conditions under which the selection can be carried out.

Selection of hisE *mutants*: Strains with *hisE* mutations plated under either selection condition do not require adenine (Table 2, line 2 and Table 3, line 5). We therefore expected to find *hisE* mutants among the Ade^+ revertants of strains plated under both of our selection conditions. The fact that we recovered only *hisG* mutants under these conditions (except for the Ade^+ revertants on D-histidine) probably reflects the relative rarity of *hisE* mutants among all *his* mutants isolated (HARTMAN *et al.* 1971).

To confirm the ability of *hisE* mutations to suppress the adenine requirement, we used our selection method to isolate *hisE* mutants caused by the transposable

TABLE 4

Summary of strains and conditions for the selection of hisG *mutants*

Strain	Relevant genotype	Selection condition	Conditions of plating‡	Freq. ADE+ revertants	% His⁻
hisF645	*hisF*⁻	I	1 mM Hol 30°	5.2×10^{-6}	23
TR4733	*hisB*⁻	I	1 mM Hol 30°		5
hisF645	*hisF*⁻	I	0.3 mM D-His 37°		4*
TR4733	*hisB*⁻	I	0.3 mM D-His 37°		2
TT1867	*hisG*^{fr}*hisF*⁻	II	L-His 42°	4.8×10^{-7}	18
TR5548	*hisG*^{fr}*hisT*⁻	II	L-His 42°	7.8×10^{-5}	70†

* This represents two *his* mutants of the 50 Ade+ revertants analyzed. One is a *hisO* mutant, one is *hisE* (determined by genetic mapping).

† This selection also yielded His+ promotor mutants at a frequency of 13% of the Ade+ revertants isolated (see text).

‡ Under the plating conditions, the individual strains require adenine.

drug-resistance element Tn*10* (tetracycline resistance) (KLECKNER *et al.* 1975). When Tn*10* is inserted into the Salmonella chromosome, it has the ability to cause deletion of nearby chromosomal material (KLECKNER, ROTH and BOTSTEIN 1977). Thus, *hisE* deletion mutants ought to be relatively common among the Ade+ revertants of a strain containing a Tn*10* element located near the *hisE* gene.

To test this prediction, for cells placed under selection condition I, we constructed a strain (TT551) which contains *hisF645* and a Tn*10* element located near, but outside of the operator-distal end of the *his* operon, between *hisE* and the gene for gluconate dehydrogenase, *gnd* (see Figure 1). The Tn*10* element should generate *his* deletions with high frequency. If this strain is plated on histidinol, mutants containing deletions removing *hisG* will fail to grow, since such deletions would also remove *hisD*, which is essential for growth on histidinol (see Figure 1). Therefore, *hisE* mutants should be common among the Ade+ revertants of TT551 plated on histidinol. When TT551 was plated on histidinol at 30°, a number of revertant clones (Hol+Ade+) arose that failed to complement an F′*hisE*⁻ episome (Table 5, experiment A).

In a second attempt to select *hisE* mutants under condition I, we carried out the selection on cells diploid for the *hisG* gene, but haploid for *hisE*. This strain (TT2221) carries an F′*his* episome deleted for *hisAFIE*, with a *hisD*⁻ *hisF*⁻ chromosome. The episome was maintained by selection for growth on histidinol (*hisD*+) at 42°. This strain is cold sensitive (Ade⁻) on histidinol because it does not have a functional *hisF* gene. Since it is diploid for *hisG*, a single *hisG* mutation in either copy would not relieve the adenine auxotrophy. However, since it is haploid for *hisE*, mutations in this gene should be obtained. TT2221 (*hisD*⁻ *hisF*⁻/F′*his AFIE*⁻) was plated on histidinol at 30°, and 97 Ade+ revertants (in 50 independent groups) were picked. Transducing phage were grown on these revertants and used to transduce various *his* deletions to prototrophy to test for the presence of new *his* mutations in these strains. Table 5, experiment B, shows the five classes of mutants obtained in this experiment. The new *his* mutations are all deletions, probably because TT2221 contains a chromosomal Tn*10* ele-

TABLE 5

Search for hisE *mutations among Ade+ revertants*

Extent of new *his* lesion	Number obtained Exp. A*	Exp. B†	Exp. C‡
G-	18	0	25
G- through E-	0	21	0
D- through E-	0	1	0
C- through E-	4	2	18
A- through E-	4	0	0
F- through E-	0	1	0
I- through E-	1	0	15
E-	0	0	2
None	104	72	76
Total	131	97	136

* TTT551 (*hisF645 zee*::Tn*10*) was plated on Hol at 30°. Ade+ revertants were picked and purified after two days. The genotype of these mutants was inferred from tests of their ability to complement F'*his*- episomes. All strains still harbor the parental *hisF645* mutation, except those in which this region is deleted.

† TT2221 (*hisD1 hisF645 zee-1*::Tn*10*/ F'_{T80} *hisA-E2905*) was plated on Hol at 30°. Ade+ revertants were picked, purified, and a transducing lysate grown on them. The extent of the *his* mutations in these strains was determined by testing the ability of these mutants to transduce a wild type *his* region into strains containing mutations in each of the different *his* genes. The F'*his* episome is of *E. coli* origin and shares no homology with Salmonella. It is, therefore, not able to recombine with the Salmonella *his* region (FINK and ROTH, unpublished).

‡ TT1700 (*hisG1102 hisF645 zee-1*::Tn*10*) was plated on His at 42°. Ade+ revertants were picked and purified after two days. The genotypes of these mutants were inferred from tests of their ability to complement F'*his*-episomes.

ment (between *hisE* and *gnd*) that generates these deletions. Four of these strains are sensitive to tetracycline, suggesting that at least part of the Tn*10* element was lost in the same mutational event.

In neither of these selections did we obtain deletions lacking only *hisE*. The smallest deletion found lacked *hisI* and *hisE*. Because of this, we cannot be certain that mutation of *hisI* is not required to suppress the adenine requirement. However, the fact that *hisE*, but not *hisI*, mutants grow on histidinol at 30° makes us confident that a *hisE* defect alone is sufficient to permit growth under selection condition I.

Selection condition II will also yield *hisE* mutants under similar circumstances. When *hisG1102 his F645*, containing the Tn*10* element located between *his* and *gnd* (TT1700), is plated on histidinol at 42°, the Tn*10* element should, with a high frequency, generate *his* deletions that suppress the Ade- phenotype. However, none of these deletions can enter *hisG*, since to do so they would also have to delete *hisD*, which is essential for growth on histidinol (see Figure 1). Therefore, this selection should enrich for *hisE* deletions. Table 5, Experiment C, shows that one of the classes of Ade+ revertants obtained from this experiment contain new deletions affecting only *hisE*, confirming the expectation that *hisE* mutants survive selection condition II.

Characterization of hisG mutants: We have characterized 25 *hisG* mutations and one *hisO* mutation with respect to mutation type and map position. All of

TABLE 6

Characterization of hisG *mutations*

Mutation	Spontaneous reversion frequency*	Induced† reversion	Colony morphology in hisT⁻‡	Classification§
8637	<10	—	gr	f
8638	0	—	smo	d
8639	10–100	N	wr	m (leaky)
8640	0	—	wr	s
8641	10–100	N	wr	m (leaky)
8642	<10	—	smo	f
8643	<10	N	wr	m
8644	0	—	gr	s
8646	<10	N	wr	m
8647	<10	N	smo	n (amber)‖
8648	<10	—	gr	f
8649	0	—	wr	s
8650	<10	N	smo	n
8652	<10	—	gr	f
8654	<10	N	smo	n
8655	10–50	I	smo	f
8656	<10	N	smo	n
8657	10–40	N	smo	n (amber)‖
8658	10–100	N	smo	n
8659	<10	—	gr	f
8660	<10	N	smo	n
8661	<10	—	wr	
8663	0	N	wr	m
8664	0	—	wr	d
8665	<10	—	wr	¶
9529¶	200–400	N	—	d

* Numbers represent number of revertants per plate ($\sim 2 \times 10^8$ cells plated, see MATERIALS AND METHODS).

† (N)-induced by N-methyl-N′-nitro-N-nitrosoguanidine (NG) (—) not induced to revert by NG or ICR191; (I)-induced by ICR191.

‡ smo-smooth colony morphology. wr-wrinkled colony morphology, gr-grainy, or slightly rough colony morphology.

§ n-nonsense, m-missense, f-frameshift, s-stable, d-deletion.

‖ These mutations are suppressed by an amber suppressor.

¶ *his9529* is a deletion of the *hisO* region. It is His⁻, and was isolated as an Ade⁺ revertant of *hisF*645 on D-His.

these *hisG* mutants were obtained by plating *hisF645* or *hisB8633* on Hol at 30°. The *hisO* mutant was obtained from *hisF645* plated on D-histidine.

Before the *hisG* mutations could be characterized, they first had to be separated from the original *hisF* or *hisB* mutation present in these strains. The *hisG* mutations were transduced into *his* deletion *OGD2228*, selecting for growth on histidinol, and the transductants were screened for the presence of a wild-type *hisB* or *hisF* gene by testing their ability to complement F′*hisB*⁻ or F′*hisF*⁻ episomes. The *hisG* mutations were then classified as to mutation type based on two criteria: reversion pattern (WHITFIELD, MARTIN and AMES 1966) and polar effects on downstream genes in the operon (FINK, KLOPOTOWSKI and AMES 1967).

Table 6 summarizes the characterization of the mutations. Nonpolar mutations induced to revert by NG were classified as missense. Polar mutations induced to revert by NG were classified as nonsense. Polar mutations that reverted spontaneously, but were not induced to revert by NG or ICR191, were classified as frameshift types. One mutant was induced to revert by ICR191 and was classified as a frameshift mutant. It is apparent from Table 6 that the selection yields many types of *hisG* mutants, including "leaky" ones.

A fine-structure genetic map of the *hisG* mutations isolated under selection condition I is presented in Figure 2 of HOPPE *et al.* (1979). These mutations map in many different regions of the *hisG* gene. We conclude, therefore, that the selection method is general and yields all types of *hisG* mutants.

DISCUSSION

We have developed a method for the selection of *S. typhimurium* mutants with reduced levels of *hisG* enzyme activity. The selection is carried out under conditions that create an adenine auxotrophy due to the uncontrolled action of the *hisG* enzyme. Mutations of *hisG*, *hisE* or the *his* control region suppress the adenine requirement.

The adenine auxotrophy occurs in strains that contain high levels of fully active *hisG* enzyme. Such a situation can be brought about in two ways: (1) by plating a *his* auxotroph on a poor histidine source (such as histidinol at 30°, or D-His), which causes a low intracellular histidine concentration that leads to operon derepression and release of *hisG* enzyme from feedback inhibition; or (2) by plating a *hisG* feedback-resistant, *hisF* or *hisT* double mutant at 42° Under both conditions, Ade$^+$ revertants can be selected that contain *hisG* mutations of many different mutational types mapping throughout the *hisG* gene.

We have proposed that the adenine requirement observed under our conditions is due to a depletion of the cells' adenine pool caused by the uncontrolled use of ATP in histidine biosynthesis. However, it is also possible that the adenine requirement is due to the inhibition of some adenine biosynthetic enzyme by one of the histidine intermediates, many of which are structurally very similar to adenine. If this is correct, the inhibitory intermediate is likely to be the product of the *hisE* reaction, PR-AMP, since mutants of the next step, *hisI*, do not lose the adenine auxotrophy (Table 2).

The selection method also yields *hisE* mutants, under special conditions. This is consistent with the fact that *hisE* mutants do not require adenine under our conditions. We believe this is because the equilibrium of the *hisG*-catalyzed step favors the substrates (AMES, MARTIN and GARRY 1961; MARTIN 1963). The second reaction is required for irreversible destruction of ATP. A *hisE* mutant, therefore, would not carry out the consumption of ATP in the first step of histidine biosynthesis. It is equally possible that *hisE* mutants do not require adenine due to their failure to make a histidine biosynthetic intermediate inhibitory to adenine synthesis. The failure to find *hisE* mutants under normal selection conditions probably reflects the relative rarity of *hisE* mutants among all *his* mutants isolated (HARTMAN *et al.* 1971).

A large majority of the Ade$^+$ phenotypic revertants obtained by our selection method contain no new *his* mutation. These strains must possess mutations in some other gene or genes that serve to suppress the adenine requirement. It seems possible that some of these mutations could directly affect purine biosynthesis. This class of mutants was not analyzed further.

The adenine requirement of certain *his* mutants growing on histidinol is manifested only at low temperatures (Table 2). Presumably, this results from the fact that histidinol serves as a poor histidine source only at temperatures of 30° and below. We propose that this is due to the relatively inefficient transport of histidinol at 30°, compared to higher temperatures. Consistent with this hypothesis in the observation that higher histidinol concentrations relieve the adenine requirement of *his* mutants at 30°. Conversely, lower histidinol concentrations cause these *his* mutants to require adenine even at high temperatures (data not shown). Furthermore, mutants of *hisP*, a gene essential for the high affinity histidine permease, have been selected under conditions similar to those used here (AMES *et al.* 1977; G. AMES, personal communication). Presumably, these mutants transport histidinol better than the parent strain.

The adenine requirement of certain strains containing the feedback-resistant mutation *hisG1102* is manifested only at high temperatures (Table 3). This could be explained if the *hisG* enzyme activity of these mutant strains was slightly cold sensitive. At lower temperatures, the adenine requirement would be relieved due to the reduced *hisG* enzyme activity.

SHEDLOVSKY and MAGASANIK (1962a,b) obtained results similar to ours for a mutant strain of *E. coli*. These workers described a leaky *hisF* (or *hisH*) mutant that required either histidine or adenine for normal growth. Also, 2-thiazolealanine, an inhibitor of the *hisG* enzyme, was able to substitute for the adenine or histidine requirement. These results were explained as being due to a depletion of the cells' adenine pools caused by the uncontrolled use of ATP in histidine biosynthesis. Histidine or TA relieved the adenine requirement by inhibiting the first step of histidine biosynthesis and thereby halting the adenine drain. Furthermore, they were able to select a revertant strain that no longer required adenine for growth. This mutant was found to contain a new mutation in *hisG* that suppressed the adenine requirement of the *hisF* mutation.

Other workers have used conditions similar to ours to select *dhuA* and *glnA* mutants of Salmonella (KRAJEWSHA-GRYNKIEWICZ, WALCZAK and KLOPOTOWSKI 1971; KUSTU and MCKEREGHAN 1975). *dhuA* mutations allow *his* mutants to grow on D-histidine by causing an increase in histidine permease levels (KRAJEWSKA-GRYNKIEWCZ, WALCZAK and KLOPOTOWSKI 1971). Mutations of *glnA*, which result in an altered glutamine synthetase, also allow growth of *his* mutants on D-histidine (KUSTU and MCKEREGHAN 1975). The reason these mutations allow growth of *his* mutants plated on D-histidine is not fully understood. One suggested explanation is that both mutations cause increased production of L-histidine from D-histidine. Our results suggest that these mutations could relieve an adenine drain caused by high *hisG* enzyme activity. In the case of *dhuA*, increased permeation of D-histidine would lead to higher intracellular histidine

levels and result in inhibition of *hisG* enzyme activity. It is not clear how a *glnA* mutation could relieve the adenine drain.

This selection method should be useful for the isolation of *his* promotor-like mutants. When the selection is carried out in medium containing L- or D-histidine (see Table 4), mutants that have lost the ability to express the *his* operon can be recovered. A different positive selection for *his* mutants (FINK, KLOPOTOWSKI and AMES 1967) yielded no promotor-like mutants out of 121 *his* mutants isolated. Because mutations in any one of the first seven genes of the *his* operon were recovered from that particular selection method, *his*-promotor mutants would be expected to be a small proportion of all mutants isolated under these conditions. Our selection method has the advantage of reducing the target for mutation to the *hisG* gene, and the *his* control region, making promotor mutations more frequent among the mutants that survive our selection. We found that 13% of the Ade$^+$ revertants of TR5548 contain a *his* promotor-like mutation (Table 4, line 5). We are currently using this selection method to isolate a large number of *his*-promotor mutants in the hope of better understanding the role of this region in *his* operon regulation.

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