Evidence for Frameshift Mutations in the *hisH* Gene of *Escherichia coli* Causing Synthesis of a Partially Active Glutamine Amidotransferase

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ABSTRACT

Among eight strains carrying acridine-induced mutations in *hisH*, five which mapped at four different sites in the promoter-distal region of the gene showed His^+ phenotypes on media containing a purine. By complementation analysis, *hisH* enzyme was shown to be required for growth on purines. Purine-sensitive His^+ revertants of strains able to grow on purines carried second-site mutations which in one case could be shown to map in *hisG*. Strains able to grow on purines were able to grow on 2-thiazolyl-DL-alanine, too. We conclude that frameshift mutations in the promoter-distal part of the *hisH* gene of *E. coli* do not completely abolish the activity of the gene product.

FOLLOWING treatment with 9-aminoacridine (9AA) we had isolated a histidine-requiring mutant of *Escherichia coli* K12 which was able to grow on minimal medium supplemented with a purine (hypoxanthine, adenine, guanine or the corresponding nucleoside). Growth on purines of a His^- mutant had been described in *E. coli* B/r previously (LUZZATI and GUTHRIE 1955). This mutation has later been classified as *hisH* or *hisF* (SHEDLOVSKY and MAGASANIK 1962a, b), and it was leaky. In *Salmonella typhimurium*, several His^- mutants had been isolated which were able to grow on purines (HARTMAN 1956). These mutations mapped in *hisH* and in *hisF*, respectively, and were leaky, too (LOPER *et al.* 1964). Since there is excessive conversion of ATP to *N*-1-(5′-phosphoribosyl)-ATP when adenosine triphosphate phosphoribosyltransferase, the first enzyme of the histidine biosynthetic pathway, is not feedback-inhibited, purines might stimulate growth on medium lacking histidine of leaky His^- mutants by simply compensating for this ATP consumption (SHEDLOVSKY and MAGASANIK 1962a; JOHNSTON and ROTH 1979).

Leakiness is often the result of a missense mutation leading to a gene product having residual enzymatic activity. The *his* mutation mentioned above had been induced by 9AA, a mutagen known to cause mainly −1 frameshifts in runs of four or more G/C basepairs (SKOPEK and HUTCHINSON 1984). Though frameshifts completely change the sequence of codons in the promoter-distal part of the affected gene, and therefore the product of a frameshift allele should have no residual enzymatic activity, such mutations may be leaky due to some ribosomal frameshifting during translation, leading to a very low activity of the mutant gene product (ATKINS, ELSEVIERS and GORINI 1972). But, it is also conceivable that leakiness may be due either to a slow spontaneous occurrence of the chemical reaction or to a second gene coding for an enzyme which slowly carries out the reaction normally catalyzed by the enzyme affected by the mutation. For example, the enzymatic reaction catalyzed by the *hisH* enzyme can be bypassed *in vitro* by high concentrations of ammonium chloride at pH 8.8 (SMITH and AMES 1964), and SCHMID and ROTH (1983) report that T. KOHNO has found that in Salmonella the nonspecific acid phosphatase, encoded by the *phoN* gene, can substitute for *hisB* phosphatase activity.

Our mutant did not seem to be leaky. Even after incubation for 5 days on minimal agar lacking a source of histidine, growth could not be observed. To further analyze the "purine-effect" in *E. coli*, we isolated a series of acridine-induced *his* mutations in this species. In this paper we show that frameshift mutations in the promoter-distal region of the *hisH* gene of *E. coli* K12 do not completely abolish the enzymatic activity of the gene product. However, for growth on minimal medium lacking a source of histidine, strains carrying such mutations require partial inhibition of the activity of the *hisG* gene product.

MATERIALS AND METHODS

Bacterial strains: The *E. coli* K12 and *S. typhimurium* strains used are listed in Table 1.

Media: LB and R media were as described by MILLER (1972). The glucose-salts minimal medium had a pH of 7.2 and contained per liter: 14 g K_2HPO_4, 6 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.4 g $MgSO_4 \cdot 7H_2O$, 0.2 g trisodiumcitrate·$2H_2O$, 5 g glucose monohydrate, and 1 mg thiamine hydrochloride. It was supplemented as appropriate with L-arginine·HCl (120 mg; 20 mg for solid media), nicotininc acid (2 mg), L-histidine·HCl (25 mg), L-histidinol·2HCl (150 mg), L-threonine (75 mg), L-leucine (60 mg), L-proline (30

TABLE 1

Bacterial strains

Strain	Genotype	Source[a] or reference
A. *Escherichia coli* K12		
343/113	F⁻ *galR'18 arg-56 nad-113*	MOHN and ELLENBERGER (1977)
FP318	As 343/113, plus *hisD909*	9AA of 343/113
FP321	As 343/113, plus *hisB3086*	9AA of 343/113
FP322	As 343/113, plus *hisH3021*	9AA of 343/113
FP327	As 343/113, plus *hisH2035*	9AA of 343/113
FP328	As 343/113, plus *hisH313*	9AA of 343/113
FP330	As 343/113, plus *hisH529*	9AA of 343/113
FP331	As 343/113, plus *hisH621*	9AA of 343/113
FP476	As 343/113, plus *hisH6074*	9AA of 343/113
FP477	As 343/113, plus *hisH6018*	9AA of 343/113
FP488	As FP327, plus *hisG28*	S of FP327
FP489	As FP330, plus *hisG38*	S of FP330
FP738	As 343/113, plus *hisF23*::Tn*10*	This laboratory
FP829	As 343/113, plus *hisH6234*	ICR191 of 343/113
FP846	As FP322, plus *hisF23*::Tn*10*	FP322 × P1*FP738[b]
FP847	As FP331, plus *hisF23*::Tn*10*	FP331 × P1*FP738[b]
FP848	As FP476, plus *hisF23*::Tn*10*	FP476 × P1*FP738[b]
FP849	As FP477, plus *hisF23*::Tn*10*	FP477 × P1*FP738[b]
FP850	As FP829, plus *hisF23*::Tn*10*	FP829 × P1*FP738[b]
FP851	As FP328, plus *hisF23*::Tn*10*	FP328 × P1*FP738[b]
C600	F⁻ *thr-1 leuB6 thi-1 lacY1 tonA21 supE44*	APPLEYARD (1954)
FP399	As C600, plus Δ(*attP2H-his*)*30*	P2 eductant of C600
FP410	F' As FP399, plus FS'400 Δ*hisG410* Δ(*hisC-hisB*)*2558*	SB3360 × FP399[c]
FP411	F' As FP399, plus FS'400 Δ(*hisC-hisF*)*2605*	SB3373 × FP399[c]
FP412	F' As FP399, plus FS'400 *hisA3017*	SB3355 × FP399[c]
FP413	F' As FP399, plus FS'400 *hisG3025*	SB3362 × FP399[c]
FP414	F' As FP399, plus FS'400 Δ(*hisA-hisE*)*101 gnd? rfb?*	TS9 × FP399[c]
FP415	F' As FP1399, plus FS'400 Δ(*hisB-hisH*)*22*	TA2558 × FP399[c]
FP416	F' As FP399, plus FS'400 Δ*hisI(E)659*	SB3361 × FP399[c]
AB2463	F⁻ *thr-1 leuB6* Δ(*gpt-proA*)*62 hisG4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 rpsL31 supE44 recA13*	BACHMANN (1972), F. W. PONS (unpublished data)
FP495	F' As AB12463, plus FS'400 Δ(*hisC-hisB*)*2558*	SB3360 × AB2463[c]
B. *Salmonella typhimurium*		
TA2558	F' *his-515 met-521*/FS'400 Δ(*hisB-hisH*)*22*	P. E. HARTMAN
TS9	F' As TA2558, but FS'400 Δ(*hisA-hisE*)101 *gnd rfb*	P. E. HARTMAN
SB3355	F' *his-515 met-522 fla-1001*/FS'400 *hisA3017*	P. E. HARTMAN
SB3360	F' As SB3355, but Δ(*hisC-hisB*)*2558*	P. E. HARTMAN
SB3361	F' As SB3355, but Δ*hisI(E)659*	P. E. HARTMAN
SB3362	F' As SB3355, but *hisG3025*	P. E. HARTMAN
SB3373	F' As SB3355, but Δ(*hisC-hisF*)*2605*	P. E. HARTMAN

[a] 9AA, 9AA-induced derivative; S, spontaneous derivative; ICR191, ICR191-induced derivative; A × P1*B, result of transductional cross where B was the donor and A the recipient. A × B, result of mating where A was the donor and B the recipient.

[b] Tetracycline-resistant transductants were screened for such which were not complemented by FS'400 Δ(*hisB-hisH*)*22*.

[c] These strains were constructed to avoid complications due to restriction in complementation tests with *E. coli* recipients and Salmonella F'*his* plasmids. With FS'400 Δ(*hisC-hisB*)*2558*, the *hisG* gene was always lost during transfer when selection was for *hisD*⁺. Therefore, we have transferred this plasmid to AB2463, too. This strain carries a *hisG* mutation and thus allows for selecting for inheritance of the *hisG* gene.

mg), and inosine (60 mg), and it was solidified with Oxoid agar no. 1 (8 g; 4 g for soft agar). In modified glucose-salts minimal medium, $(NH_4)_2SO_4$ was replaced by 2 g Na_2SO_4 and 4.4 g L-glutamine. AN medium was minimal medium supplemented with arginine and nicotinic acid. ANH, ANHol, and ANI refer to AN medium supplemented with histidine, histidinol, and inosine, respectively, and AH medium was minimal medium supplemented with arginine and histidine. For selecting acridine-induced His⁺ revertants, AN medium was enriched with 2 ml 0.8% nutrient broth (Difco) per liter.

Chemicals: Unless otherwise stated, all chemicals were purchased from E. Merck, Darmstadt. L-Histidinol·2HCl, inosine, tetracycline, 9AA and ICR191 were from Serva Feinbiochemica, Heidelberg, and nicotinic acid, L-glutamine, and 2-thiazolyl-DL-alanine (TA) were from Sigma Chemie, Munich.

Mutation experiments: Bacteria growing exponentially in supplemented minimal medium were harvested by filtration (Sartorius Membranfiltergesellschaft, Göttingen, pore diameter 0.45 μm), washed with twice the original volume of minimal salts solution (minimal medium lacking glucose and thiamine hydrochloride), and resuspended at 2×10^8 colony forming units/ml in that medium. After the addition of glucose (final concentration 5 g/liter), 2 ml of the cell suspension and 20 μl of a stock solution of mutagen dissolved

TABLE 2

Characterization of acridine-induced *his* mutations by complementation mapping and determination of the growth factor requirements of the mutant strains

Recipient *his* mutation	No. of *his* mutations mapped[a]	Donor							Growth on		Mutations in *his*⁻
		SB3373 Δ*hisCBHAF*	TS9 Δ*hisAFI(E)*	SB3362 *hisG*	SB3360 Δ*hisCB*	TA2558 Δ*hisBH*	SB3355 *hisA*	SB3361 Δ*hisI(E)*	ANHol	ANI	
Type I	3 + (6)	+	+	−	NT[b]	NT	NT	NT	+	−	*G*
Type II	25 + (11)	+	+	+	NT	NT	NT	NT	−	−	*D*
Type III	116 + (13)	−	+	+	−	+	NT	NT	+	−	*C*
Type IV	14 + (5)	−	+	+	−	−	NT	NT	+	−	*B*[c]
Type Va	5	−	+	+	+	−	NT	NT	+	+	*H*
Type Vb	2 + (1)	−	+	+	+	−	NT	NT	+	−	*H*
Type VI	12 + (4)	−	−	+	+	+	−	+	+	−	*A*[d]
Type VII	1 + (3)	−	−	+	+	+	+	+	+	−	*F*
Type VIII	1 + (2)	+	−	+	NT	NT	NT	−	+	−	*I(E)*

[a] Mutations were induced by 9AA and ICR191 (figures in parentheses), respectively.
[b] NT, not tested.
[c] Tentatively classified as *hisB*; a deletion of genes *hisC* through *hisH* should show the same complementation pattern.
[d] Tentatively classified as *hisA*; a deletion of genes *hisA* and *hisF* should show the same complementation pattern.

in dimethylsulfoxide (5 mM ICR191 and 10 mM 9AA, respectively) were mixed and shaken at 37° in the dark for 2 hr.

For the isolation of mutants of strain 343/113 having acquired an additional auxotrophy, the mutagen-treated cells were filtered, washed with minimal salts solution, resuspended in a 10-fold volume of AN, and shaken at 37° for 3.5 hr to allow for segregation of pure mutant types. Then ampicillin (final concentration 40 μg/ml) was added, and shaking at 37° was continued for 2.5 hr, which resulted in a 10^4-fold decrease of the titer. Surviving bacteria were plated on LB medium and incubated over night at 37°. Auxotrophs were then isolated by replica plating on AN.

In reversion tests, appropriate dilutions of mutagen-treated cells were plated on ANH (for counts of survivors), AH (for counts of Nad⁺ reversions), and enriched AN medium (for counts of His⁺ reversions), respectively. Colonies were scored after two (survivors and Nad⁺ revertants) and three days (His⁺ revertants) of incubation at 37°, respectively.

P1 transduction: P1*vir* was grown on appropriate donor strains on R plates as described by MILLER (1972). Recipient bacteria growing exponentially in supplemented minimal medium were harvested by centrifugation and resuspended at 2×10^9/ml in a salts solution containing 5 mM $CaCl_2$ and 10 mM $MgSO_4$. Phages were added at a multiplicity of <1, and incubation of the mixture was for 20 min at 37° without shaking. When selection was for tetracycline resistance, the infected bacteria were diluted 20-fold into LB containing 40 mM citrate, and incubated without shaking for 1 hr at 37° (SILHAVY, BERMAN and ENQUIST 1984). Before spreading on selective medium, the bacteria were washed twice with a buffer containing 50 mM Tris-HCl, pH 7.5, and 100 mM NaCl, and concentrated as appropriate. Incubation of the plates was for 2 days at 37°.

Complementation tests: For testing His⁻ strains, donors and recipients growing exponentially in LB medium were cross-streaked on selective media. For testing purine-sensitive His⁺ revertants, it was necessary to avoid transfer onto the selection plates of even trace amounts of LB. Therefore, donor and recipient cultures were mixed and incubated for 30 min at 37° without shaking. Before plating on ANI, cells were washed twice with phosphate-buffered saline.

RESULTS

Auxanography of 9AA-induced mutants isolated after ampicillin-enrichment revealed that the vast majority (>60%) required histidine for growth. Therefore, in subsequent experiments all the auxotrophs identified by replica plating were transferred to ANH plates, and clones growing on this medium were then tested for growth on AN and ANI, respectively. Of 178 9AA-induced His⁻ mutants isolated in four experiments, four more (*his-313, his-529, his-621,* and *his-3021*) were able to grow on ANI. Complementation analysis of the five strains able to grow on ANI revealed that all carried *hisH* mutations. To test if there were *hisH* mutations among the mutants which were not able to grow on ANI, we mapped all of the remaining *his* mutations by complementation analysis. 9AA-induced *his* mutations predominantly mapped in *hisC*, but two more (*his-6018* and *his-6074*) mapped in *hisH*, and at least one mutation was found in each of the other *his* structural genes (Table 2 and Figure 1). Growth on inosine of strains carrying mutations *hisH313, hisH529, hisH621, hisH2035,* and *hisH3021*, respectively, was also observed on modified minimal medium containing L-glutamine as a source of nitrogen.

In a further experiment, we isolated 45 ICR191-induced His⁻ mutants (Table 2). One of these mutations (*his-6234*) mapped in *hisH*, and the strain carrying this mutation was not able to grow on inosine.

To further characterize the *hisH* mutations, we performed reversion tests with 9AA and ICR191, respectively. Two of five strains able to grow on purines, and all of the three strains unable to do so, were induced to revert to histidine-prototrophy by acridines (Table 3), and by this criterion, at least these strains carried frameshift mutations. Alleles *hisH529* and *hisH621*, and *hisH6018* and *hisH6074*, respectively, had been isolated in the same experiment. The results of the reversion tests furthermore showed that these mutations were of independent origin.

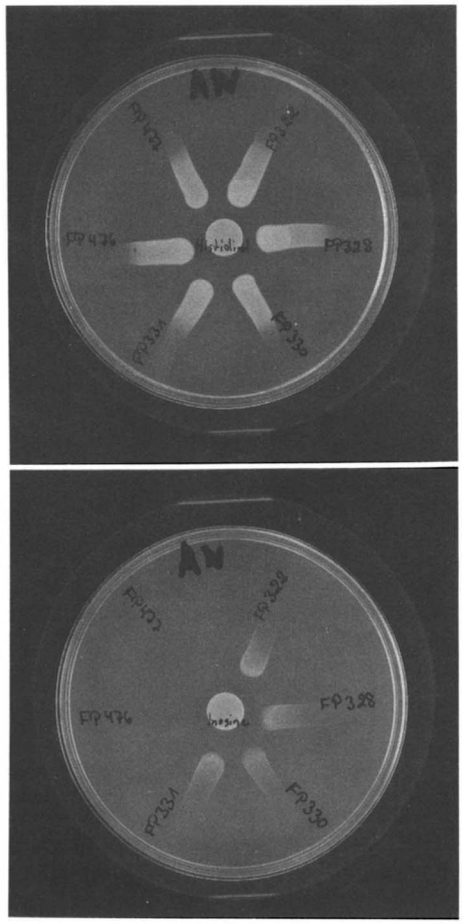

FIGURE 1.—Stimulation of growth on AN medium of strains carrying *hisH* mutations by (A) histidinol and (B) inosine. The filter discs in the middle of the AN plates contained 20 μl of solutions of 150 mg L-histidinol·2HCl/ml and 60 mg inosine/ml, respectively. Mutants to be tested were grown in ANH. Fresh overnight cultures were washed, resuspended, and diluted 10^{-1} in AN, and streaked away from the filter disc. Photographs were taken after 48 hr of incubation at 37°.

Then we constructed isogenic strains carrying different *hisH* alleles by cotransduction of the *hisH* mutations and *hisD*$^+$, using FP318 as recipient. Of 100 transductants each selected on ANHol, between 80% and 96% were His$^-$. Of the His$^-$ transductants derived from donors which were able to grow on ANI, all were able to do so, too. But, of the His$^-$ transductants derived from donors which were not able to grow on ANI, none was able to grow on this medium. Complementation tests with these His$^-$ transductants as recipients revealed that they all carried a defect *hisH* allele, but functional *hisG, D, C, B, A, F* and *I(E)* genes.

When His$^-$ strains growing exponentially in ANHol and ANH, respectively, were transferred to AN, the bacteria immediately stopped growing (data not shown). However, when the same strains growing in ANI were transferred to AN medium, bacteria went on growing, though at a reduced rate, for more than two generations (Figure 2), and hence must have been able to go on synthesizing histidine for hours even in the absence of exogenous inosine. This suggested that mutants carrying specific *hisH* alleles might be able to produce an active gene product when purines were present in excess.

To test a hypothesis designed to explain how frameshift mutations in the histidine operon might cause synthesis of an active glutamine amidotransferase only when purines were present in excess, we looked for purine-sensitive His$^+$ revertants of mutants carrying *hisH* alleles. Among several hundred spontaneous revertants of His$^-$ mutants able to grow on purines, about 2% were more or less severely inhibited on ANI, but showed normal growth when this medium was also supplemented with histidine or histidinol. However, of the acridine-induced His$^+$ revertants, and of the spontaneous His$^+$ revertants derived from His$^-$ mutants unable to grow on purines, none was inhibited on ANI plates. Though our hypothesis turned out to be wrong (and, therefore, is not worth explaining here in detail), these revertants helped to solve the problem. With revertant #38 derived from FP330, growth inhibition on ANI plates was strong enough (Figure 3A) to allow for complementation mapping of the allele responsible for purine-sensitivity. The results of these tests showed (Table 4) that purine-sensitivity was due to a mutation in *hisG*.

To test if the original *hisH* alleles were still present in the purine-sensitive His$^+$ revertants, we performed transductional crosses using two of these revertants as donors and FP318 as recipient; selection was for inheritance of *hisD*$^+$. Table 5 shows that 0.6% of the transductants were His$^-$, and were able to grow on ANI. This indicated that the genotype of purine-sensitive His$^+$ revertants was actually *hisH hisG*, and that the *hisH* mutation was leaky.

SHEDLOVSKY and MAGASANIK (1962a, b) had shown that (1) thiazolealanine (TA), a false feedback inhibitor of the *hisG* enzyme (MARTIN 1963), stimulated growth on medium lacking a source of histidine of the leaky His$^-$ mutant of *E. coli* B/r which was able to grow on purines, and (2) a revertant of this mutant had reduced ATP phosphoribosyltransferase activity, and was inhibited on minimal medium containing adenine and TA, respectively. Since mutations in *hisG* resulted in purine-sensitive His$^+$ phenotypes of strains carrying frameshift mutations in *hisH*, we tested the effect of TA on growth on AN of strain 343/113 (*his*$^+$ wild-type), of all the strains carrying *hisH* mutations, and of the purine-sensitive revertant FP489. Figure 3B shows that only strains which were able to grow on inosine were also able "to grow on TA." This holds true for modified minimal medium, containing L-glutamine as a source of nitrogen, too. Whereas the *his*$^+$ wild-type was slightly inhibited by the same concentration of TA which stimulated growth of strains carrying leaky *hisH* mutations (the zone of growth

TABLE 3

Reversion of *hisH* mutations

Strain	Mutation	Growth on purines	Revertants per 10^8 CFU[a]					
			His$^-$ → His$^+$			*nad-113*[b] → Nad$^+$		
			Spontaneous	9AA[c]	ICR191[d]	Spontaneous	9AA	ICR191
FP328	*hisH313*	+	<1	(2)	(6)	<1	2.83×10^5	9.04×10^4
FP330	*hisH529*	+	<1	<2	<5	<1	3.27×10^5	6.39×10^4
FP331	*hisH621*	+	<1	1070	ND[e]	2	3.91×10^5	ND
FP327	*hisH2035*	+	<1	<2	<3	0.7	3.81×10^5	5.56×10^4
FP322	*hisH3021*	+	<1	1308	ND	<1	6.12×10^5	ND
FP477	*hisH6018*	−	<1	6	139	<1	4.57×10^5	7.27×10^4
FP476	*hisH6074*	−	<1	448	492	0.7	7.54×10^5	6.00×10^4
FP829	*hisH6234*	−	<1	<1	12	2	4.72×10^5	6.90×10^4

[a] < means that not a single revertant has been found, and values in parentheses indicate that only one revertant has been found.
[b] Reversion of this sensitive frameshift marker served as an internal control for successful mutagenic treatment.
[c] Survival was >50%.
[d] Survival was about 5%.
[e] ND, not determined.

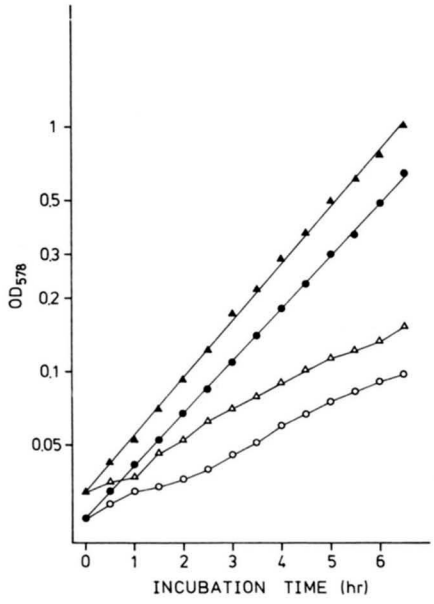

FIGURE 2.—Effect of withdrawal of inosine from the growth medium. Cultures of FP328 (circles) and FP330 (triangles) growing exponentially in ANI were harvested by filtration, washed with twice the volume of AN, and resuspended in half the original volume of this same medium. At zero time, half of this concentrated culture was diluted twofold with prewarmed AN (open symbols), and the rest was diluted twofold with prewarmed ANI medium containing 120 μg inosine/ml (closed symbols). Cultures were incubated in a waterbath-shaker at 37°, and growth was monitored by measuring the optical density of the cultures at 578 nm. At the end of the experiment, cultures were titrated on ANH and on AN plates, respectively. In cultures grown in AN, the frequencies of His$^+$ revertants were $<10^{-5}$.

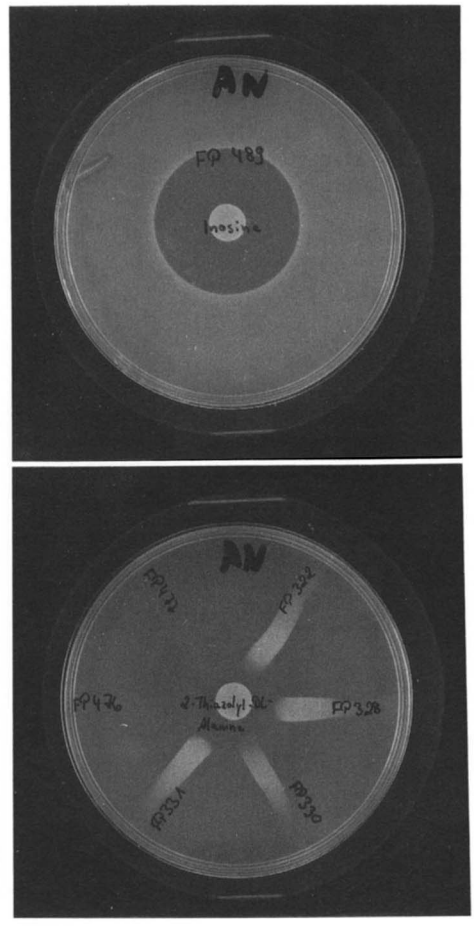

FIGURE 3.—Effect of inosine and 2-thiazolyl-DL-alanine (TA), respectively, on growth on AN of His$^+$ revertant FP489 (A) and of strains carrying *hisH* mutations (B). FP489 was grown in AN, and a soft agar overlay was made on an AN plate. Strains carrying *hisH* mutations were treated as in Figure 1. The filter discs in the middle of the plates contained 20 μl of solutions of 60 mg inosine/ml and 5 mg TA/ml, respectively. Photographs were taken after 48 hr of incubation at 37°.

inhibition was about 2.5 mm), growth of the purine-sensitive His$^+$ pseudo wild-type revertant was not influenced by this histidine analogue.

Finally, we mapped the *hisH* mutations by transductional crosses. As can be seen in Table 6, the nonleaky *hisH* mutations formed only very few *his*$^+$ recombinants when crossed with each other, and formed fewer

TABLE 4

Complementation mapping[a] of the allele responsible for purine sensitivity

Recipient	Donor						Mutation in
	FP411 *ΔhisCBHAF*	FP414 *ΔhisAFI(E)*	FP413 *hisG*	FP495 *ΔhisCB*	FP415 *ΔhisBH*	FP410 *ΔhisG ΔhisCB*	
FP330 His$^+$ #38	+	+	−	+	+	−	*hisG*

[a] For details, see MATERIALS AND METHODS.

TABLE 5

Segregation of *hisH* alleles in transductional crosses with purine-sensitive His$^+$ revertants as donors

Donor	Recipient	Selective marker	No. of transductants analyzed	Phenotypes[a]		
				AN$^+$, ANI$^+$	AN$^+$, ANI^i	AN$^-$, ANI$^+$
FP330His$^+$ #38	FP318	*hisD*$^+$	333	44	287	2
FP327His$^+$ #28	FP318	*hisD*$^+$	200	51	148	1

Overnight cultures of *hisD*$^+$ transductants grown in ANHol were diluted 10^{-1} with phosphate-buffered saline, streaked on AN and ANI plates, respectively, and incubated at 37°.

[a] Phenotypes were classified as follows: AN$^+$, ANI$^+$ = heavy growth on both media after incubation for 24 hr; AN$^+$, ANI^i = growth on AN after 48 hr, but no, or markedly reduced, growth on ANI after the same incubation time; AN$^-$, ANI$^+$ = no growth on AN after 96 hr, but growth on ANI after 36 hr.

TABLE 6

Transductional mapping of *hisH* mutations

Recipient (genotype)	Donor (genotype)									
	his$^+$	*hisB3086*	*hisH2035*	*hisH313*	*hisH529*	*hisH621*	*hisH3021*	*hisH6018*	*hisH6074*	*hisH6234*
hisH313	6170	93	39	<1[a]	8	14	22	39	ND[b]	49
hisH529	4269	116	36	ND	<1	ND	ND	23	ND	45
hisH621	4013	77	20	ND	5	0.6[c]	<1	22	ND	43
hisH3021	3885	51	7	ND	ND	<1	<1	ND	ND	ND
hisH6018	3266	17	ND	ND	ND	ND	26	0.3[c]	0.6	<1
hisH6074	4259	21	ND	ND	ND	ND	27	0.3[c]	0.6	2
hisH6234	3108	18	26	16	ND	ND	27	0.8[c]	3	0.3[c]

Aliquots of 0.5 ml of log-phase recipient cells, resuspended at 4×10^9 colony forming units/ml in 5 mM $CaCl_2$ plus 10 mM $MgSO_4$, and 0.1 ml P1 phages grown on the donor strain indicated, were mixed in small tubes (multiplicity of infection = 0.3) and incubated without shaking for 20 min at 37°. Then 3.5 ml soft agar were added to each tube and the whole contents of a tube was poured onto an AN plate. From tubes containing phage grown on the *his*$^+$ donor, only 0.1 ml of the adsorption mixture was plated. Transductions were performed in triplicate. The figures are *his*$^+$ transductants per 10^9 plaque forming phages, and are not corrected for killing of potential transductants by infection with P1*vir*.

[a] < means that not a single *his*$^+$ recombinant has been found.

[b] ND, not determined.

[c] Arithmetic mean of two experiments.

his$^+$ recombinants with *hisB3086* than did the leaky *hisH* mutations. This indicated that they were clustered in the promotor-proximal region of the *hisH* gene. Of the leaky mutations, only *hisH621* and *hisH3021* failed to recombine with each other, and, therefore, leaky mutations mapped at four distinct sites in the promoter-distal part of the *hisH* gene.

To test if nonleakiness of *hisH6018, hisH6074* and *hisH6234* was due to a polar effect of these mutations on the promotor-distal *hisF* gene, we looked for complementation of these mutations by plasmid FS'400 Δ(*hisB-hisH*)*22* on medium containing inosine. As can be seen in Table 7, providing an additional *hisF* gene, but no *hisH* gene, did not restore growth of strains carrying nonleaky *hisH* alleles. To show that the plasmid produced a sufficient amount of *hisF* enzyme, we inactivated the *hisF* gene in six *hisH* mutants by insertion of transposon Tn*10*, and looked for complementation on ANI of these *hisH hisF* double mutations. Complementation by the plasmid lacking a functional *hisH* gene was only found with *hisH hisF* double mutants derived from strains carrying leaky *hisH* mutations (Figure 4 and Table 7). This indicated that nonleakiness of frameshift mutations *hisH6018, hisH6074* and *hisH6234*, respectively, was not due to polarity.

DISCUSSION

The pathway of histidine biosynthesis has been elucidated in *S. typhimurium*. In this pathway, the *hisH* and *hisF* enzymes catalyze the conversion of B.B.M.

TABLE 7

Complementation on AN and ANI of nonleaky *hisH* mutations and of *hisH hisF* double mutations

Recipient (genotype)	Donor							
	FP410 *ΔhisG ΔhisCB*		FP414 *ΔhisAFI(E)*		FP415 *ΔhisBH*		FP416 *ΔhisI(E)*	
	AN	ANI	AN	ANI	AN	ANI	AN	ANI
hisH6018	NT	NT	+	+	−	−	NT	NT
hisH6074	NT	NT	+	+	−	−	NT	NT
hisH6234	NT	NT	+	+	−	−	NT	NT
hisH313 hisF23::Tn*10*	+	+	−	−	−	+	+	+
hisH621 hisF23::Tn*10*	+	+	−	−	−	+	+	+
hisH3021 hisF23::Tn*10*	+	+	−	−	−	+	+	+
hisH6018 hisF23::Tn*10*	+	+	−	−	−	−	+	+
hisH6074 hisH23:: Tn*10*	+	+	−	−	−	−	+	+
hisH6234 hisF23::Tn*10*	+	+	−	−	−	−	+	+

Donors and recipients were cross-streaked on AN and ANI plates, respectively, as described in MATERIALS AND METHODS. NT, not tested.

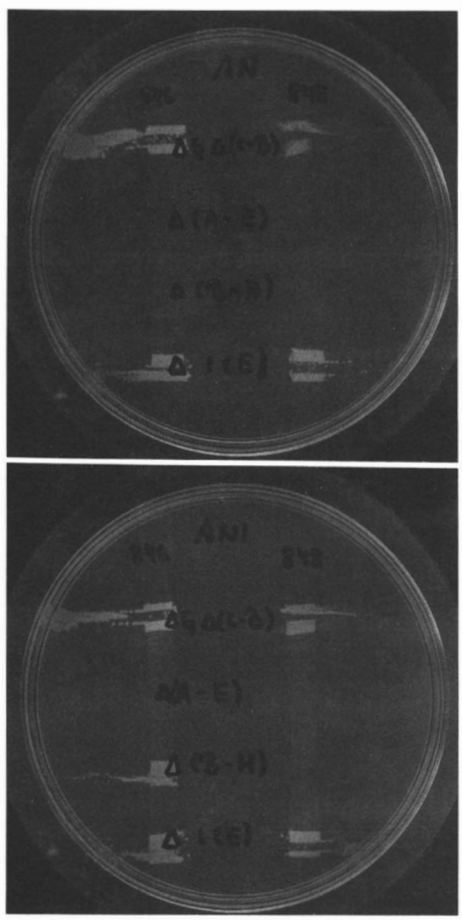

FIGURE 4.—Complementation on AN and ANI, respectively, of *hisH hisF*::Tn*10* double mutations. Donors and recipients growing exponentially in LB were cross-streaked, and the photographs were taken after 48 hr of incubation at 37°. FP846 carries the leaky *hisH3021* allele, whereas FP848 carries the nonleaky *hisH6074* allele.

III (*N*-(5′-phospho-D-ribulosylformimino)-5-amino-(5′′-phosphoribosyl)-4-imidazolylcarboxamide) to IGP (imidazoleglycerol phosphate) and AICAR (5-aminoimidazole-4-carboxamide ribotide), using glutamine as a nitrogen donor, but the intermediate in this reaction has never been isolated (MARTIN *et al.* 1971). *In vitro,* at high concentrations of ammonium salt and alkaline pH values, *hisF* enzyme alone can catalyze this reaction, and glutamine is not required (SMITH and AMES 1964). Since all *hisH* mutants which have been isolated and tested in *S. typhimurium* so far are leaky (HARTMAN 1956; LOPER *et al.* 1964), one might argue that *hisH* enzyme only permits the use of glutamine instead of free NH_3 as a nitrogen donor in a reaction catalyzed by the *hisF* enzyme, and that, therefore, even mutants carrying a deletion of the *hisH* gene be leaky, provided that the cells retain a functional *hisF* enzyme (and the medium contains enough free NH_3).

We think that this does not explain the results reported here for the following reasons.

1. Although the minimal medium used in this study contained a rather high concentration of ammonium ion (30 mM, as compared to 133 mM which was used by SMITH and AMES (1964) in the *in vitro* test), the concentration of free NH_3 should have been <1% because the pH value of the medium had been adjusted to 7.2. Furthermore, growth on inosine and TA, respectively, of strains carrying leaky *hisH* alleles was also observed on modified minimal medium, containing L-glutamine instead of $(NH_4)_2SO_4$ as a source of nitrogen.

2. All of the eight mutants carrying *hisH* alleles were complemented on AN medium by the plasmid carrying deletion *Δ(hisA-hisE)101* and, therefore, must have contained a functional *hisF* enzyme. But, only five of these mutations were leaky (*i.e.*, the mutant strains were able to grow on ANI and on AN medium supplemented with TA, respectively).

3. To save the hypothesis that *hisH* enzyme be not necessarily required for histidine biosynthesis, one might argue that nonleaky *hisH* mutations might be

polar and, therefore, reduce transcription of the promoter-distal *hisF* gene. Providing *hisF* enzyme in sufficient amounts in a complementation test should then restore growth of strains carrying such *hisH* mutations. We have tested this hypothesis by looking for complementation on ANI of (i) nonleaky *hisH* mutations, and (ii) *hisH hisF23*::Tn*10* double mutations. Non-leaky *hisH* mutations were not complemented by FS'400 Δ(*hisB-hisH*)*22*, and it was shown that this plasmid provided sufficient *hisF* enzyme for growth on ANI of leaky *hisH* mutants.

Since in transductional crosses nonleakiness was 100% linked to specific *hisH* alleles, the possibility was ruled out that leakiness of *hisH* mutations was due to a second gene coding for an enzyme which can substitute for glutamine amidotransferase. From the results reported here we therefore conclude that (1) in *E. coli* K12, and under the growth conditions used in this study, not only *hisF*, but also *hisH* enzyme is required for the conversion of B.B.M. III to IGP and AICAR, and (2) leakiness of frameshift mutations in *hisH* must be due to synthesis of a partially active glutamine amidotransferase.

In the *trpE* gene of *S. typhimurium*, some nucleotides up stream of a −1 frameshift mutation, a transversion converting the sequence ACA to AAA has been found which leads to partial restoration of synthesis of anthranilate synthetase (ATKINS, NICHOLS and THOMPSON 1983), and in phage T7, ribosomal frameshifting at a specific site is even used to produce a minor capsid protein (DUNN and STUDIER 1983). Thus, frameshifting during translation seems to be rather common, but to depend upon specific nucleotide sequences. Since translational frameshifting, just like a frameshift mutation, completely changes the sequence of amino acids in the carboxyl terminus of the protein being synthesized, for a given frameshift mutation to be leaky due to this inaccuracy of translation, there must exist a sequence prone to frameshifting at, or in the very vicinity of, the site of mutation. However, data from transductional crosses between different *hisH* mutations show that the five leaky frameshift mutations mapped at four different sites in the promotor-distal part of the gene. This makes ribosomal frameshifting an unlikely explanation for leakiness of frameshift mutations in this gene. Even if we assume that there be several sites in the *hisH* gene where ribosomal frameshifting can occur, glutamine amidotransferase must be tolerant to many different amino acid exchanges in the carboxyl-terminal part of the protein. If so, one can also imagine that a frameshift mutation in this part of the gene be leaky *per se*. This might explain why most of the *hisH* mutations isolated so far, and even frameshift mutations, are leaky.

It should be very interesting to compare wild-type and mutant *hisH* enzymes. However, little is known about this enzyme, its substrate is not commercially available, and its activity can only be assayed by measuring the decrease of B.B.M. III in the presence of excess *hisF* enzyme (MARTIN *et al.* 1971).

The result that strains carrying leaky *hisH* mutations were able to grow rather well on medium lacking a source of histidine, when the *hisG* enzyme was inhibited by TA, supported the hypothesis that the purine-effect in these mutants might be due to compensation of an "adenine drain" occurring when the activity of ATP phosphoribosyltransferase is not regulated by feedback inhibition, and AICAR, a by-product in histidine biosynthesis and an intermediate in purine biosynthesis, is not recovered (SHEDLOVSKY and MAGASANIK 1962a; JOHNSTON and ROTH 1979). However, how does purine-sensitivity of histidine-prototrophic *hisH hisG* genotypes fit into this picture? The activity of the *hisG* enzyme is regulated not only by feedback inhibition, but also competitively by binding of AMP and ADP, respectively. In the presence of histidine, this enzyme even discriminates against its substrate ATP and preferentially binds AMP and ADP (MORTON and PARSONS 1977). Purine bases and nucleosides taken up from the medium by microorganisms are readily interconverted by the purine salvage pathways (NYGAARD 1983). Uptake of inosine should, therefore, lead to an increase of AMP and ADP pools relative to the ATP pool, resulting in competitive inhibition of the *hisG* enzyme. In the purine-sensitive His^+ pseudo wild-type revertant, the mutant *hisG* enzyme seemed to be feedback-resistant. If we assume that the mutant enzyme had not only lost the ability to bind histidine, but had also acquired the new ability to discriminate against ATP and to preferentially bind AMP and ADP in the absence of histidine, we can explain both, histidine-prototrophy and purine-sensitivity of this *hisH hisG*(Fbr) genotype. Preferential binding of AMP and ADP to the *hisG*(Fbr) enzyme should regulate the flow of intermediates through the histidine biosynthetic pathway such that there should never be a depletion of the cell's ATP pool, and the organism should grow at a rate limited by the enzymatic activity of the mutant *hisH* enzyme. When purines are present in the medium, however, the increase in intracellular AMP and ADP pools should further slow down the flow of intermediates through the histidine biosynthetic pathway such that, due to the low enzymatic activity of the mutant *hisH* gene product, not enough histidine is synthesized for growth on minimal medium.

In transductional crosses with *hisH hisG*(Fbr) genotypes as donors and FP318 as recipient, 0.6% of the transductants had the genotype $hisG^+$ $hisD^+$ *hisH*, and 81.6% were *hisG*(Fbr) $hisD^+$ *hisH*. This shows that recombination between the *hisD909* site and the sites of mutations *hisG38*(Fbr) and *hisG28*(Fbr), respec-

tively, had occurred in only 0.6% of the transductants. Therefore, the vast majority, if not all of the 95 transductants which were not inhibited by inosine must have been *hisG*(Fbr) *hisD*$^+$ *hisH*$^+$ genotypes. This indicates that enough histidine was synthesized even when the activity of the *hisG*(Fbr) enzyme was competitively inhibited by AMP and ADP, provided that all the other enzymes of the histidine biosynthetic pathway were fully active.

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