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Regulation of Nitrogen Utilization in *hisT* Mutants of *Salmonella typhimurium*

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Mutations in the *hisT* gene of *Salmonella typhimurium* alter pseudouridine synthetase I, the enzyme that modifies two uridines in the anticodon loop of numerous transfer ribonucleic acid species. We have examined two strains carrying different *hisT* mutations for their ability to grow on a variety of nitrogen sources. The *hisT* mutants grew more rapidly than did $hisT^+$ strains with either arginine or proline as the nitrogen source and glucose as the carbon source. The *hisT* mutations were transduced into new strains to show that these growth properties were due to the *hisT* mutations. The *hisT* mutations did not influence the growth of mutants having altered glutamine synthetase regulation. Assays of the three primary ammonia-assimilatory enzymes, glutamate dehydrogenase, glutamine synthetase, and glutamate synthase, showed that glutamate synthase activities were lower in *hisT* mutants than in isogenic $hisT^+$ controls; however, the glutamate dehydrogenase activity was about threefold higher in the *hisT* strains grown in glucose-arginine medium. The results suggest that the controls for enzyme synthesis for nitrogen utilization respond either directly or indirectly to transfer ribonucleic acid species affected by the *hisT* mutation.

The *hisT* mutants of *Salmonella typhimurium* were originally selected only for their elevated levels of the histidine biosynthetic enzymes (7, 25); however, subsequent work demonstrated that the regulation of other amino acid biosynthetic pathways is also altered (4). This pleiotropic response is due to the loss of pseudouridine synthetase I, the enzyme that can modify uridine in the anticodon loop of numerous tRNA species to pseudouridine (9). The loss of this enzyme activity in the *hisT* mutants results in the undermodification of the tRNA species that normally contain these pseudouridines (27, 28).

Since the consequences of *hisT* mutations on tRNA structure have been well documented, the *hisT* mutants have been valuable tools for investigating the role of undermodified tRNA species in the regulation of biosynthetic pathways (4, 6). We have been particularly interested in defining the components which regulate the ammonia assimilatory enzymes, glutamate dehydrogenase (EC 1.4.1.4), glutamine synthetase (EC 6.3.1.2), and glutamate synthase (EC 1.4.1.13). Specifically, we wanted to determine whether the control of any of these enzymes is altered by the *hisT* mutation. Thus, we have examined the growth and physiological properties of two *hisT* mutants of *S. typhimurium* and have found that they grow more rapidly with certain nitrogen sources and have different glutamate dehydrogenase and glutamate synthase levels when compared with a $hisT^+$ control.

MATERIALS AND METHODS

Bacterial strains. The *S. typhimurium* LT-2 strains used in this study are described in Table 1. Additional information regarding certain strain constructions is described below.

Chemicals. Tetracycline hydrochloride was obtained from Sigma Chemical Co. (St. Louis, Mo.). L-Arginyl-arginine was obtained from Vega-Fox Biochemicals (Tuscon, Ariz.). All other reagents were reagent grade and commercially available.

Media and growth conditions. Luria broth and glucose-ammonia media were prepared as described previously (1). $(NH_4)_2SO_4$ was omitted, and the amino acids were substituted at a concentration of 20 mM when they were used as sole nitrogen sources (i.e., glucose-arginine and glucose-proline) (3). Tetracycline sensitivity was scored on Luria broth or glucose-ammonia plus tetracycline (50 μg/ml) medium.

Cells for growth curves and enzyme assays were grown in Luria broth medium overnight at 37°C and diluted 1:100 into glucose-ammonia medium. Cells to be eventually grown in glucose-arginine or glucose-proline medium were grown as described above, except the glucose-ammonia media included the respective amino acid at a concentration of 20 mM. The cultures were incubated at 37°C until they reached a turbidity of 100 ± 5 Klett units as measured by a Klett-Summerson colorimeter with a blue (no. 42) filter. The cells were harvested by centrifugation at 12,100 × *g* for 10 min, washed twice with 4°C 0.85% NaCl, and suspended in 1/10 volume of 4°C 0.85% NaCl. These cells were used to inoculate the appropriate growth media such that the initial turbidity was about 10 Klett units.

Preparation of extracts. Cultures were grown to 100 ± 5 Klett units, harvested by centrifugation at

TABLE 1. *Strains used in this study*

Strain	Genotype	Source/reference
JL250	*hisW3333*	Brenchley and Ingraham (2)
JL553	*hisT*$^+$	B. Ames (5)
JL554	*hisT1504*	B. Ames (5)
JL601	*aroD5 purF145*	B. Ames (5)
JL604	*hisT1501*	B. Ames (5)
JL605	*hisR1223*	B. Ames (5)
JL608	*hisW1825*	B. Ames (5)
JB1078	*hisT1504* Δ*hisODCBH*	P. E. Hartman
JB1136	*glnF986*::Tn*10*	J. Roth
JB1142	*gln-1000 zig*::Tn*10*	Selection for Tn*10* insertion near *glnA* (10, 24)
JB1162	*hisT*$^+$	Transduction of JL601 with JB553 as the donor
JB1163	*hisT1504*	Transduction of JL601 with JB554 as the donor
JB1164	*hisT1501*	Transduction of JL601 with JB604 as the donor
JB1165	*hisT*$^+$ *gln-1000 zig*::Tn*10*	Transduction of JL1162 with JB1142 as the donor
JB1166	*hisT1504 gln-1000 zig*::Tn*10*	Transduction of JB1163 with JB1142 as the donor
JB1167	*hisT1501 gln-1000 zig*::Tn*10*	Transduction of JB1164 with JB1142 as the donor
JB1168	*hisT*$^+$ *glnF986*::Tn*10*	Tetracycline-resistant transductants of JB1162 with JB1136 as the donor
JB1169	*hisT1504 glnF986*::Tn*10*	Tetracycline-resistant transductants of JB1164 with JB1136 as the donor
JB1170	*hisT1501 glnF986*::Tn*10*	Tetracycline-resistant transductants of JB1164 with JB1136 as the donor

12,100 × *g* for 10 min, washed twice with 4°C 0.85% NaCl, and concentrated 100-fold in sonicating buffer [0.1 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) (pH 7.1 at 25°C), 10 mM MnCl$_2$, and 2.0 mM dithiothreitol]. Preparation of extracts was as described previously (1).

Enzyme assays. The glutamine synthetase assay was the γ-glutamyl transferase assay (10), except that 50 mM TES buffer (pH 7.3, 25°C) was used. The glutamate synthase and glutamate dehydrogenase assays followed the rate of NADPH oxidation at 37°C as described by Dendinger et al. (10), except the 50 mM TES buffer used for the glutamate synthase assay was pH 7.4 at 25°C. The arginine transport experiments followed the procedure of Funanage et al. (12), using 1 μM [^{14}C]arginine.

The method of Lowry et al. (18) was used to determine the protein concentration in extracts, with bovine serum albumin as the standard. The specific activities of all three enzymes are reported as micromoles of product formed per minute per gram of protein. The values reported are the averages of at least three separate experiments with duplicate or triplicate assays for each experiment.

Transductions. Transductions were performed by directly plating 0.1 ml each of Luria broth-grown cells and P22 *HT*105/1 *int* phage (23) onto the selective media. Strains containing a Tn*10* element cotransducible with *glnA* were isolated as described previously (10, 24).

RESULTS

Growth properties of *hisT* mutants. The effect of the *hisT* mutation on the ability of *S. typhimurium* to use various nitrogen sources was examined by comparing the growth of strains JL553 (*hisT*$^+$) and JL554 (*hisT1504*) on media with different nitrogenous compounds. The growth of the two strains was comparable with ammonia, aspartate, cytidine, glycine, or glutamate as a sole nitrogen source. However, strain JL554 clearly grew more rapidly when either arginine or proline was the nitrogen source. These results were confirmed with growth experiments for another *hisT* mutant, JL604. Although the growth of the *hisT* mutants in glucose-ammonia medium was slightly slower than that of the control (68-min generation time versus a 60-min generation time for the *hisT*$^+$ strain), the mutants grew more rapidly than did the *hisT*$^+$ strain in glucose-arginine medium (generation time of about 4 h versus 7 to 8 h for the *hisT*$^+$ strain) and in glucose-proline medium (generation time of about 5 h versus 12 h for the *hisT*$^+$ strain).

One property associated with the *hisT* mutants is a wrinkled-colony morphology when grown on media containing high concentrations of certain carbon sources (i.e., 2% glucose). The wrinkling phenomenon is due to the overproduction of the *hisF* and *hisH* gene products (20). We found that the *hisT* mutant colonies were also wrinkled when grown on glucose-proline and glucose-arginine media. To determine whether the unusual colony morphology was related to high levels of histidine operon expression, strain JB1078 (which possesses a *hisT* mutation and an extensive deletion of the histidine operon, including the *hisH* gene) was grown on glucose-arginine medium supplemented with L-histidine. The resulting colonies were smooth

and still grew better than did the *hisT*$^+$ strain. Thus, the overproduction of the histidine gene products was associated with the wrinkled-colony morphology, but it was not responsible for the more rapid growth with arginine as a nitrogen source.

The growth of the *hisT* mutants was compared with that of other histidine regulatory mutants having altered tRNA processing or maturation (Table 2). The *hisR* mutants produce 50% of the wild-type amount of tRNA^{His} (5, 26), and the *hisW* mutants are defective in processing several tRNA species (2; L. Davis and L. Williams, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K107, p. 163). Both strains have wrinkled-colony morphology on 2% glucose-ammonia medium and increased levels of histidine operon expression. The *hisR* and *hisW* strains had growth rates similar to that of the wild type on glucose-arginine and glucose-proline media, showing that neither the elevated expression of histidine operon nor the altered level of tRNA^{His} could cause the better growth found for the *hisT* mutants.

Other mutants that grow better than wild-type strains on glucose-arginine medium have increased arginine transport (12, 16), and it has been suggested that the growth of *S. typhimurium* in glucose-arginine medium is limited by transport rather than arginine degradation. We examined the growth of strains JL553, JL554, and JL604 by using the dipeptide L-arginyl-arginine as the nitrogen source. All three strains grew as rapidly with 10 mM L-arginyl-arginine as with $(NH_4)_2SO_4$, suggesting that the degradation of arginine is not growth rate limited when the compound is transported by the dipeptide transport system. Results with cells grown in glucose-arginine medium showed that the *hisT* mutants had a two- to threefold increase of arginine transport as compared with the wild type (data not shown). Thus, the better growth of the *hisT* strains in glucose-arginine medium may be due, at least in part, to increased arginine transport.

TABLE 2. *Growth of hisT and other mutants having altered regulation of histidine biosynthesis*

Strain	Genotype	Growth on medium[a]: 0.4% Glucose-ammonia	2% Glucose-ammonia[b]	0.4% Glucose-arginine	0.4% Glucose-proline
JL553	*hisT*$^+$	++	S	+	+
JL554	*hisT1504*	++	W	++	++
JL604	*hisT1501*	++	W	++	++
JL605	*hisR1223*	++	W	+	+
JL608	*hisW1825*	++	W	+	+
JL250	*hisW3333*	++	W	+	+

[a] The media were as described in the text, except that the D-glucose was added at either 0.4 or 2% as noted above. Cells were spotted onto agar media, and the growth of the mutant strains was compared with the *hisT*$^+$ control after 36 to 48 h. ++, Full growth after 36 h; +, incomplete growth at 36 h and less growth than was observed for strains marked ++ even after 48 h of incubation.

[b] Colony morphology on 2.0% D-glucose-ammonia medium was smooth (S) or wrinkled (W).

Genetic analysis of *hisT* mutants. Transductions were performed to be certain that the altered phenotype of *hisT* mutants was due to the *hisT* lesion itself and not to additional mutations in these strains. The *hisT* and *purF* genes are cotransducible (7); therefore, *purF*$^+$ transductants of strain JL601 (*aroD5 purF145*) were selected and scored for the HisT$^-$ phenotype (wrinkled-colony morphology on 2% glucose-ammonia medium). The results showed that the phenotype of the two *hisT* donors (better growth on glucose-arginine and glucose-proline media) was 100% cotransducible with the *hisT* mutation (Table 3). Thus, the behavior of *hisT* mutants on alternate nitrogen sources is attributable to the *hisT* mutation itself and not to a second mutation. Isogenic strains were constructed for further experimentation by transducing strain JL601 to prototrophy (*aroD*$^+$ and *purF*$^+$) and checking for the HisT phenotype. These transductants, JB1162 (*hisT*$^+$), JB1163 (*hisT1504*), and JB1164 (*hisT1501*), were cured of phage P22 and shown to have growth properties and enzyme levels similar to those of their respective parent strains.

Strains with altered glutamine synthetase regulation. Previous investigations have

TABLE 3. *Transductions with hisT mutants*[a]

Donor (genotype)	Selected phenotype (no. analyzed)	Transductants wrinkled on 2% glucose media (%)	Transductants with donor phenotype[b] (%)
JL553 (*hisT*$^+$)	PurF$^+$ (169)	0	100
JL554 (*hisT1504*)	PurF$^+$ (179)	87	87
JL604 (*hisT1501*)	PurF$^+$ (170)	81	81

[a] Log-phase cells of recipient strain JL601 *aroD5 purF145* were mixed with P22 *HT*105/1 *int* phage (10^8 to 10^9 plaque-forming units per ml) directly on the selection plate supplemented with 20 μg of L-tryptophan, L-tyrosine, and L-phenylalanine per ml.

[b] Growth was scored on glucose-arginine and glucose-proline media as described in footnote *a* of Table 2.

shown that strains with mutations affecting glutamine synthetase activity and synthesis have an altered growth response on various nitrogen sources (12, 16, 29). These effects are due to altered transport or degradation of a particular nitrogen source or both. Because the *hisT* mutations also changed nitrogen utilization, we wanted to determine what effect the combination of the *hisT* and glutamine synthetase regulatory mutations would have on the ability of the strain to utilize different nitrogen sources (Table 4). The *glnF986*:Tn*10* mutation, unlinked to the structural gene for glutamine synthetase (*glnA*), results in low levels of glutamine synthetase, thereby conferring glutamine auxotrophy (15, 21). The *glnF* gene product has not been identified, but it may be a factor which activates glutamine synthetase gene transcription. The *glnF986*::Tn*10* mutation was transduced into the wild type and the *hisT* mutants by selecting for tetracycline resistance. All Tet^r transductants were glutamine auxotrophs, demonstrating that the *hisT* mutants still require the *glnF* product for growth on glucose-ammonia media (Table 4). Furthermore, the *hisT* mutation did not overcome the glutamine requirement of the *glnF986*::Tn*10* *hisT* strain on glucose-arginine or glucose-proline medium.

The effect of another mutation that alters nitrogen utilization was examined in conjunction with the *hisT* mutation. The *gln-1000* mutation maps near *glnA*, causes a fourfold increase in glutamine synthetase levels, and has altered transport of several amino acids (12). Strains with the *gln-1000* mutation grow better on glucose-arginine and glucose-aspartate media than does an isogenic Gln$^+$ control; however, they grow more slowly on glucose-alanine and glucose-proline media and are very sensitive to D-glutamine inhibition (J. Kuchta and J. Brenchley, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K13, p. 128). To construct a *gln-1000 hisT* double mutant, a strain was selected with a Tn*10* insertion adjacent to the *glnA* gene (24). This insertion, *zig*::Tn*10*, was 15% linked with *gln-1000* by P22 *HT*105/1 *int* transduction, and it was used to transduce the *gln-1000* mutation into *hisT*$^+$ and *hisT* isogenic strains. The transductants were scored for D-glutamine sensitivity, good growth on glucose-arginine medium, and poor growth on glucose-proline medium. The results showed that 15% of the Tet^r transductants had the phenotype associated with the *gln-1000* mutation regardless of the *hisT* allele (Table 4). Furthermore, growth studies demonstrated that the *gln-1000 hisT1504* and *gln-1000 hisT1501* strains had the 1.5-h generation time in glucose-arginine medium that is characteristic of strains with the *gln-1000* mutation rather than the 4-h generation time characteristic of the *hisT* mutations. Thus, the growth response of the *hisT* mutants on glucose-arginine and glucose-proline media was not observed in strains containing mutations affecting glutamine synthetase and nitrogen control.

Activities of the ammonia-assimilatory enzymes in *hisT* mutants. The unusual growth pattern of *hisT* mutants on various nitrogen sources suggested a possible effect on the nitrogen assimilatory enzyme levels. Therefore, the specific activities of glutamine synthetase, glutamate synthase, and glutamate dehydrogenase were assayed in cells grown on various nitrogen sources.

Because mutations that alter glutamine synthetase levels (i.e., *gln-1000*) also change the growth of the strains on alternate nitrogen sources, it is possible that the *hisT* mutation

TABLE 4. *Effects of glnF and gln-1000 mutations on the HisT phenotype*

Strain	Genotype[a]	Growth on medium[b]:			
		Glucose-ammonia	Glucose-arginine	Glucose-proline	Glucose-ammonia-D-glutamine[c]
JL553	*hisT*$^+$	++	+	+	+
JL554	*hisT1504*	++	++	++	+
JL604	*hisT1501*	++	++	++	+
JB1168	*hisT*$^+$ *glnF986*::Tn*10*	−	−	−	−
JB1169	*hisT1504 glnF986*::Tn*10*	−	−	−	−
JB1170	*hisT1501 glnF986*::Tn*10*	−	−	−	−
JB1165	*hisT*$^+$ *gln-1000 zig*::Tn*10*	++	++	±	−
JB1166	*hisT1504 gln-1000 zig*::Tn*10*	++	++	±	−
JB1167	*hisT1501 gln-1000 zig*::Tn*10*	++	++	±	−

[a] See text and Table 1 for outline of strain constructions.

[b] Media and growth conditions were as described in footnote *a* of Table 2. The designations refer to the amount of growth observed after 36 h of incubation: ++, good growth; +, growth; ±, slight growth; −, no growth.

[c] D-Glutamine was added as a supplement at 2.0 mM.

caused better growth by increasing the glutamine synthetase activity. However, the *hisT* mutants had the same glutamine synthetase activity as the wild type when grown in glucose-ammonia medium, and the activity increased similarly for strains grown in glucose-arginine medium. It is not clear whether the difference between the activities of the *hisT*$^+$ and *hisT* strains grown in glucose-proline medium is significant, but it is clear that the glutamine synthetase activities of all three strains are substantially increased above that found for glucose-ammonia-grown cells (Table 5). These results suggest that the enhanced growth of the *hisT* mutants on alternate nitrogen sources is not mediated solely via changes in glutamine synthetase regulation.

The glutamate synthase activities measured in the *hisT* strains grown in glucose-ammonia medium were two- to threefold lower than the *hisT*$^+$ control (Table 5). This reduction is independent of growth rate because the *hisT* mutants grow only slightly slower than the control in glucose-ammonia medium. It is also clear that this decrease was not alleviated by the addition of the *gln-1000* mutation. Both the control and the *hisT* mutants have even lower glutamate synthase activities when grown in glucose-arginine and glucose-proline media (Table 5). Although the cause of this decrease is unknown, one explanation is that it is related to elevated glutamate pools resulting from the degradation of arginine and proline to glutamate. These results suggest that there may be two controls for glutamate synthase, one which is altered by the *hisT* mutation and another which is dependent upon the nitrogen source (or glutamate pool). The very low values of glutamate synthase (19 and 8 U) found for the *hisT* mutants grown in glucose-arginine medium were lower than those reported for some Asm$^-$ mutants (10), but in contrast to the inability of the Asm$^-$ strains to grow on glucose-arginine medium, the *hisT* mutants grow better than the control. Thus, the results with the *hisT* mutants demonstrate that high glutamate synthase activity is not essential for growth on glucose-arginine medium and that the inability of the Asm$^-$ strains to grow is caused by another defect associated with those mutations.

The glutamate dehydrogenase activities were the same for cells grown in glucose-ammonia medium (Table 5); however, the *hisT* mutants had activities about threefold higher than that of the control when grown in glucose-ariginine medium. This increase was not related to the faster growth of the *hisT* strain in glucose-ariginine medium, because the *gln-1000* strain also grew rapidly in this medium but did not have elevated glutamate dehydrogenase activity (Table 5). To confirm the observation that high glutamate dehydrogenase activity was not needed for better growth, a *hisT* mutant (*hisT1504 gdh-51*) completely lacking glutamate dehydrogenase activity was constructed. This strain had the same growth properties as its *hisT1504 gdh*$^+$ parent (S. Rosenfeld, unpublished data).

The introduction of the *gln-1000* mutation into the *hisT* mutant eliminated the elevated glutamate dehydrogenase activities (Table 5). This is in contrast to the previous results with glutamate synthase in which the *gln-1000* mutation had no influence on the reduced glutamate synthase activities (Table 5).

DISCUSSION

The possible role for tRNA in controlling nitrogen utilization was examined by using strains harboring *hisT* mutations. Several distinct differences were found. The *hisT* mutants grow

TABLE 5. *Glutamine synthetase, glutamate synthase, and glutamate dehydrogenase activities in hisT strains*[a]

Strain	Enzyme activity[b]								
	Glutamine synthetase			Glutamate synthase			Glutamate dehydrogenase		
	Glu-amm	Glu-arg	Glu-pro	Glu-amm	Glu-arg	Glu-pro	Glu-amm	Glu-arg	Glu-pro
JB1162 *hisT*$^+$	71	371	702	209	52	34	782	793	271
JB1163 *hisT1504*	70	408	384	88	19	26	752	2,493	557
JB1164 *hisT1501*	68	467	456	97	8	27	760	2,070	536
JB1165 *hisT*$^+$ *gln-1000 zig*::Tn*10*	236	424	ND	154	ND	ND	604	510	ND
JB1166 *hisT1504 gln-1000 zig*::Tn*10*	227	530	ND	50	ND	ND	697	590	ND
JB1167 *hisT1501 gln-1000 zig*::Tn*10*	184	335	ND	51	ND	ND	675	591	ND

[a] Cells were grown in glucose minimal salts containing the indicated nitrogen sources at 20 mM. Glu, Glucose; amm, $(NH_4)_2SO_4$; arg, arginine; pro, proline; ND, not determined.

[b] Enzyme activity is expressed as micromoles of product formed per minute per gram of protein.

more rapidly than the *hisT*$^+$ strains on glucose-arginine and glucose-proline media, suggesting that the regulation of the transport or degradation (or both) of these amino acids is elevated. In addition, the *hisT* mutants have altered control of glutamate synthase and glutamate dehydrogenase. Although these differences in growth and enzyme levels are independent and separate, the change in regulation for each is caused by the mutation in the *hisT* gene. It is unknown whether the differences are caused directly by an altered tRNA changing the regulation of these genes or if some responses are due to the pleiotropic nature of the *hisT* mutation. For example, the *hisT* mutants have altered levels of various amino acid biosynthetic pathways and amino acid transport components and may influence messenger RNA translation (4; J. Roth, personal communication). These responses and others may indirectly change regulation of the ammonia assimilatory pathways. If the changes are a direct consequence of the non-pseudouridine-modified tRNA, then these results suggest a role for some tRNA species in regulating arginine and proline utilization as well as glutamate dehydrogenase and glutamate synthase production.

Current models involving tRNA in the control of gene expression suggest that the amino-acylated tRNA is necessary for normal translation of a leader RNA preceding an attenuator region (14). It is unknown whether the genes for glutamate and glutamine biosynthesis share this regulatory feature. The finding that glutamate dehydrogenase activities for the *hisT* mutants were normal for cells grown in glucose-ammonia medium but elevated for cells grown in glucose-arginine medium indicates that an altered tRNA may only influence regulation during certain growth conditions. This could be similar to the situation for the tryptophan operon in which both repression and attenuation controls are present, and the effect of de-attenuation is revealed only when tryptophan is limiting. If there is an attenuation-like control for glutamate dehydrogenase, then the elimination of the elevated activity by the *gln-1000* mutation suggests that this gene product is pertinent to the regulation. Although the decreased glutamate synthase activity is not explained by the current models for attenuation (14), it is possible that modifications of the model could account for the results. Alternatively, it is possible that because the ammonia assimilatory enzymes have a role in both amino acid biosynthesis and ammonia utilization, they are subject to novel controls that have not been predicted by studies of other biosynthetic pathways.

Independent of the exact mechanism, the changes found in the *hisT* mutants are due to the loss of the tRNA pseudouridine synthetase I activity. A recent analysis of tRNA from *S. typhimurium hisT* mutants demonstrates that at least one isoaccepting tRNA species for 14 of the 20 amino acids has an altered mobility during reverse-phase chromatography on an RPC-5 column (28). The amino acids with altered cognate tRNA are asparagine, cysteine, glutamate, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, and tyrosine. Of particular interest for this study are the $tRNA^{Glu}$ and $tRNA^{Gln}$ species. Neither the analysis by Turnbough et al. (28) nor the analysis by Lapointe et al. (17) shows any difference in the migration of the two major $tRNA^{Glu}$ species on an RPC-5 column. Additionally, the sequence analysis of the $tRNA_1^{Glu}$ and $tRNA_2^{Glu}$ from *Escherichia coli* shows that there are no pseudouridines in their anticodon loop, although there is a pseudouridine in an unusual location in the dihydrouridine loop (13). Thus, there is no evidence for a change in a major $tRNA^{Glu}$ species. However, *hisT* mutants may have two minor $tRNA^{Glu}$ isoacceptors which migrate differently from wild-type $tRNA^{Glu}$ on RPC-5 columns (28). It is unclear whether these minor changes are significant, but if $tRNA^{Glu}$ is involved in the changes observed for the *hisT* mutants then a minor species would be a candidate. In contrast to the possible minor changes for $tRNA^{Glu}$, the sequence of the anticodon loop of the $tRNA_2^{Gln}$ is almost identical to that of $tRNA^{His}$ (11), and the altered migration of $tRNA_1^{Gln}$ and $tRNA_2^{Gln}$ is strong evidence that these species are indeed undermodified in the *hisT* mutants. Thus, it is possible that either a minor $tRNA^{Glu}$ or a $tRNA^{Gln}$ species is influencing transport and glutamate dehydrogenase and glutamate synthase levels.

Other work with glutaminyl- and glutamyl-tRNA synthetase mutants of *E. coli* suggests an involvement of these tRNA species in the regulation of the ammonia regulatory enzymes. Lapointe et al. (17) found that a mutant with a thermosensitive glutamyl-tRNA synthetase grown at a partially restrictive temperature had 10-fold-derepressed levels of glutamate synthase and 50-fold-derepressed glutamine synthetase activity. Although the *hisT* mutation may affect a minor $tRNA^{Glu}$ species, we did not observe similar changes in regulation. Studies by Morgan et al. (19) revealed that the growth of a mutant with an altered glutaminyl-tRNA synthetase did not cause derepression of glutamine synthetase, but revertants with mutations in the *glnR* gene had not only elevated glutaminyl-tRNA synthetase activity but also elevated glutamine synthetase activities (8). Even though the $tRNA^{Gln}$

species are altered in the *hisT* mutants, we did not observe any changes in glutamine synthetase regulation. Thus, if there is control of glutamine synthetase mediated via tRNA, it may be more complicated than a response to the under-pseudouridylated $tRNA^{Gln}$.

Although glutamate and glutamine syntheses represent an important physiological junction between nitrogen utilization and amino acid production, little is known about the mechanism controlling expression of these biosynthetic genes. We have used the well-characterized *hisT* mutants as a tool to obtain evidence that some undermodified tRNA species affects the use of arginine and proline as nitrogen sources and the regulation of glutamate dehydrogenase and glutamate synthase. The finding that arginine transport is elevated suggests that tRNA also may function in the transport of the amino acid and is relevant to the observation of Quay et al. (22) that leucine transport may be reduced in *hisT* mutants. Further work defining the mechanism by which the *hisT* mutation affects transport would provide significant information for understanding this complex process. In addition, if the changes in the glutamate dehydrogenase and glutamate synthase activities are due directly to an altered tRNA species influencing gene expression, then these results provide the first information concerning a regulatory mechanism for their expression. To more fully understand these controls it is important to be able to select mutants directly for increased or decreased expression. We are constructing strains with the gene for β-galactosidase fused to these control regions to be used for further mutant selections to determine the mechanism by which the *hisT* mutation alters control.

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