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Effects of the *hisT* Mutation of *Salmonella typhimurium* on Translation Elongation Rate

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The *hisT* mutation in *Salmonella typhimurium* which results in loss of pseudouridine base modifications in the anticodon regions of many tRNAs was shown to reduce the rate of protein synthesis in vivo by about 20 to 25% as compared with that measured in *hisT*$^+$ strains. Reduced protein synthesis rate occurred predominantly at the level of translation rather than transcription. Increased sensitivity of *hisT* mutants to growth inhibition by antibiotics that inhibit translation elongation, but not by those that inhibit translation initiation, transcription initiation, or transcription elongation, indicates that the *hisT* mutation leads to a defect in one or more of the steps in the polypeptide chain elongation mechanism. These results can account for effects of the *hisT* mutation on regulation of certain amino acid biosynthetic operons, including the *his*, *leu*, and *ilv* operons.

The *hisT* gene in *Salmonella typhimurium* codes for an enzyme, pseudouridine synthetase I, that converts uridine residues to pseudouridine in the anticodon regions of approximately half of the tRNA species in the cell (39). At least one isoaccepting tRNA species for 13 of the 20 common amino acids, including the single species of tRNA^{His}, contains a *hisT*-specified pseudouridine modification. The *hisT* mutation leads to highly derepressed, constitutive expression of the *his* operon (26) and nonrepressibility of the *leu* and *ilvGEDA* operons (9). As compared with wild-type strains, *hisT* mutants show altered sensitivity to growth inhibition by a number of amino acid analogs, suggesting widespread, pleiotropic effects of the mutation on cellular metabolism and regulation (39). On the other hand, the *hisT*-specified pseudouridine modification is dispensable for the life of the cell since amber and frameshift *hisT* mutations are not lethal (7); *hisT* mutants do grow with slightly increased generation times (26).

Evidence has indicated that *his* operon expression is regulated by translational control (2) of attenuation (21). A specific regulatory model was recently described based on the structure of the *hisO* regulatory region as revealed by DNA sequence analysis (20). The model proposed that *his* operon derepression occurs during slowed translation of a putative 16-amino acid peptide-coding sequence containing seven adjacent His codons. This model can account for the previous demonstration that *his* operon derepression in vivo is inversely correlated with the absolute amount of charged tRNA^{His} (26), since it is easy to envision how decreased levels of charged tRNA^{His} would lead to slowed translation of the His codons. Derepressed *his* operon expression in *hisT* mutants, however, cannot be explained in this way because pseudouridine-deficient tRNA^{His} is charged normally both in vivo (26) and in vitro (5).

This paper reports that the *hisT* mutation leads to a slowed rate of polypeptide chain elongation. These results can account for the effects of the *hisT* mutation on gene regulation.

MATERIALS AND METHODS

Bacterial strains, transductions, and matings. Properties of the *S. typhimurium* and *Escherichia coli* strains used in this study are summarized in Table 1. Transductions in *S. typhimurium* strains were done with phage P22 HT105/int-201 as described (36). Tetracycline-resistant transductants carrying a Tn*10* insertion were selected on nutrient agar plates containing 10 μg of tetracycline per ml. Nonlysogenic phage-free isolates were obtained after streaking for single colonies three times successively on green indicator medium (38). Since streptolydigin-sensitive (Stl^s) derivatives failed to grow on green indicator medium, transductants were purified on nutrient agar and tested for their ability to plaque phage P22. *hisT* mutants were scored by their wrinkled colony morphology (28). In all cases when *hisT*$^+$ strains and *hisT* mutants were compared, the strains were made as isogenic as possible.

Matings were done with fresh nutrient broth inocula of donor and recipient strains by spreading 0.1 ml of the recipient on a selective plate and streaking a loopful of the donor for single colonies on the plate. After growth at 37°C, transconjugants were picked and purified twice nonselectively before testing their properties. In experiments with F*lac* strains, cultures were tested at the end of the experiment for retention of the episome. Greater than 98% of the population maintained the F*lac* in all experiments.

TABLE 1. Genotype and origin of bacterial strains

S. typhimurium strain[a]	Genotype	Source or reference
TA265	Wild-type LT2	9
TA253	*hisT1504*	9
TR2241	*hisC3072* Δ(*hisGa*)*242* Δ(*proAB*)*47 serA13*/F128 *lac*$^+$	J. Roth
AZ88	*proA15*	18
TA471TT317	Δ(*hisGpeaGDCBH*)*2253 hisT1504 purF*::Tn*10*	B. Ames
AZ101	*hisT*$^+$ *proA15*	Transduction of AZ88 to Tet^r with TA471TT317 donor; eduction of *purF*::Tn*10*
AZ105	*hisT*$^+$ *proA15*/F128 *lac*$^+$	Mating of AZ101 with TR2241 donor
AZ102	*hisT1504 proA15*	Transduction of AZ88 to Tet^r with TA471TT317 donor; eduction of *purF*::Tn*10*
AZ106	*hisT1504 proA15*/F128 *lac*$^+$	Mating of AZ102 with TR2241 donor
JL2456	*amtA1 argI539 metE338* Δ(*proAB*)*47 pyrB692*::Tn*10 trp-130*	J. Ingraham
AZ81	*hisT1504* Stl^s	This work
AZ82	*hisT*$^+$ Stl^s	Transduction of AZ81 to Tet^r with *purF*::Tn*10* donor; transduction to *purF*$^+$ with TA265 donor
AZ117	*argI539 hisT*$^+$ Stl^s	Transduction of AZ82 to Tet^r with JL2456 donor; eduction of *pyrB692*::Tn*10*
AZ118	*argI539 hisT1504* Stl^s	Transduction of AZ81 to Tet^r with JL2456 donor; eduction of *pyrB692*::Tn*10*
AZ119	*argI539 hisT*$^+$ Stl^s/F128 *lac*$^+$	Mating of AZ117 with TR2241 donor
AZ120	*argI539 hisT1504* Stl^s/F128 *lac*$^+$	Mating of AZ118 with TR2241 donor

[a] All *S. typhimurium* strains were LT2 derivatives except strains TR2241 and JL2456 which were LT7 derivatives.

Media. Complex media were Difco nutrient broth (NB; Difco Laboratories, Detroit, Mich.) containing 0.5% (wt/vol) NaCl or Luria broth (27). Minimal media were the E medium of Vogel and Bonner (40) or the AB medium of Clark and Maaløe (8). Unless otherwise indicated, carbon sources were supplied at 0.4% (wt/vol). Solid media contained Difco agar at 1.5% (wt/vol).

Isolation of a streptolydigin-sensitive (Stl^s) strain of *S. typhimurium*. An overnight NB culture of strain TA253 (*hisT1504*) was diluted 1:10 into 5 ml of NB and allowed to undergo two doublings at 37°C. The culture was washed twice and suspended in 4 ml of 0.1 M citrate-NaOH buffer (pH 5.5). The suspension was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 50 μg/ml for 30 min at 37°C without aeration, washed twice with 0.1 M KH_2PO_4-NaOH buffer (pH 7.0), suspended in 5 ml of NB, and grown overnight at 37°C to allow phenotypic expression. This mutagenized NB culture was diluted 1:100 into 5 ml of fresh NB, grown to an optical density at 650 nm of about 0.1, and treated by addition of streptolydigin at 100 μg/ml followed 10 min later by addition of ampicillin at 40 μg/ml. After ampicillin counterselection for 60 min at 37°C, the culture was washed twice and suspended in 5 ml of NB and grown overnight at 37°C. The counterselection was repeated two more times before mutants sensitive to growth inhibition by 100 μg of streptolydigin per ml on nutrient agar plates were screened. Of eight Stl^s strains tested we chose one (strain AZ81) for further use. This strain grows in minimal or complex media with the same generation times as its parent; it can be used as a recipient in transductions with phage P22 and reverts to Stl^r at a frequency of about 10^{-8}. In addition to being Stl^s, strain AZ81 is also more sensitive than its parent to growth inhibition by rifampin, actinomycin D, puromycin, and novobiocin, and it fails to grow on MacConkey or green (38) plates, indicating a general permeability defect.

Antibiotic sensitivity tests. Samples (0.1 ml) of overnight NB cultures of strains to be tested were mixed with 2.5 ml of top agar (0.6% [wt/vol] Difco agar in 0.5% [wt/vol] NaCl) held at 45°C and overlaid on plates containing the indicated media. After the top agar had solidified, a sterile paper disk (6 mm diameter) was placed on the plate and 15 μl of antibiotic solution was applied. Plates were incubated at 37°C, and the diameter of the zone of inhibition was measured after 36 h.

β-Galactosidase and *lacZ* mRNA induction kinetics. Cultures (150 ml) were grown through at least four doublings at 37°C in the indicated media to an optical density at 650 nm of about 0.3, and the *lac* operon was induced by addition of isopropyl-β-D-thiogalactopyranoside at 1.0 mM. To stop translation, 1.0-ml samples taken at brief intervals were added to 1.0 ml of ice-cold 0.1 M sodium phosphate buffer (pH 7.0) containing chloramphenicol to give a final concentration of 300 μg/ml. Before lysis and assay, samples were agitated and incubated at 37°C for 15 min to ensure assembly of β-galactosidase subunits into active tetramers. To stop transcription elongation, 1.0-ml samples were added to 1.0 ml of prewarmed (37°C) 0.1 M sodium phosphate buffer (pH 7.0) containing 5% (vol/vol) dimethyl sulfoxide and streptolydigin to give a final concentration

of 1.0 mg/ml. Streptolydigin-treated samples were incubated for 20 min with gentle shaking to allow translation of completed *lacZ* mRNA and assembly of β-galactosidase and then chilled, centrifuged to remove streptolydigin (which has a color that interferes with the β-galactosidase assay), and suspended in 2.0 ml of ice-cold 0.1 M sodium phosphate buffer (pH 7.0) containing chloramphenicol at 300 μg/ml. Cell samples were permeabilized (33) and assayed for β-galactosidase activity (27) as described. Enzyme units were given as absorbance units at 420 nm per minute and were normalized to the optical density of the samples at 650 nm.

Chemicals. Streptolydigin was the very generous gift of Joseph Grady (The Upjohn Company, Kalamazoo, Michigan). All other chemicals were obtained from commercial suppliers.

RESULTS

Reduced protein synthesis rate in a *hisT* mutant. To test whether the *hisT* mutation slows the rate of protein synthesis, we measured β-galactosidase induction lags in otherwise isogenic *hisT*$^+$ and *hisT1504* strains of *S. typhimurium*. The chain growth rate of polypeptide synthesis (cgr_p) can be estimated from the time required after induction of the *lac* operon to detect finished chains of β-galactosidase above the uninduced (basal) level. In Fig. 1, β-galactosidase activity (E_t) measured at the indicated points is plotted as a function of time (t) after addition of isopropyl-β-D-thiogalactopyranoside. Protein synthesis was stopped by sampling into chloramphenicol. The induction curves closely approximate parabolas ($E_t \propto t^2$), reflecting the mathematical relationship by which enzyme is accumulated early after induction (10, 35). The parabolic nature of the curves is maintained until the rate of decay of functional *lacZ* mRNA becomes significant (10). In the inset of Fig. 1, the data are replotted as the square root of the difference between E_t and E_0 (basal level) versus t. The square root plot converts the parabolic portions of the induction curves to straight-line functions ($\sqrt{E_t - E_0} \propto t$), which extrapolate on the abscissa at the time of completion of the first β-galactosidase subunit after induction (35). It was apparent that this time was longer in the *hisT* mutant (95 s) than in the *hisT*$^+$ strain (73 s). Based on a chain length of 1,021 amino acids for the β-galactosidase subunit (13), we calculated cgr_p values in this experiment of 10.7 and 14.0 amino acids/s for the *hisT* and *hisT*$^+$ strains, respectively. The value for the *hisT*$^+$ strain agrees closely with cgr_p values measured in wild-type *E. coli* strains under similar conditions (10, 14, 35), allowing for the revised downward estimate in chain length of the β-galactosidase subunit (13).

Because *hisT* mutants grow more slowly than *hisT*$^+$ strains (26) and the relationship between cgr_p and bacterial growth rate has been somewhat controversial (10), we measured β-galactosidase induction lags in cultures growing at different rates (Table 2). Within experimental error, the 20 to 25% difference between cgr_p values in the *hisT*$^+$ and *hisT* strains was maintained over about a threefold range of growth rates. The reduced cgr_p in the *hisT* mutant corresponded to a similar relative reduction in growth rate under each of the three growth conditions tested. The observation that the difference in growth rates was maintained in a complex medium as well as in minimal media argues that the reduction in growth rate of the *hisT* mutant does not result from limitation of small molecule nutrients and may be a direct consequence of the reduced rate of protein synthesis.

Effect of the *hisT* mutation on transcription elongation rate. The increased lag in induction of β-galactosidase in the *hisT* mutant could result from a reduced rate of transcription rather than translation. Measurements of transcription elongation rates depend on use of antibiotic inhibitors, such as streptolydigin or actinomycin D, to which gram-negative bacteria are normally impermeable. We attempted to adapt the Tris-EDTA *E. coli* permeabilization procedure of Leive (25) for use with *S. typhimurium* but were unsuccessful in obtaining actinomycin D inhibition of transcription greater than 95%. This was insufficient for reliable estimation of transcrip-

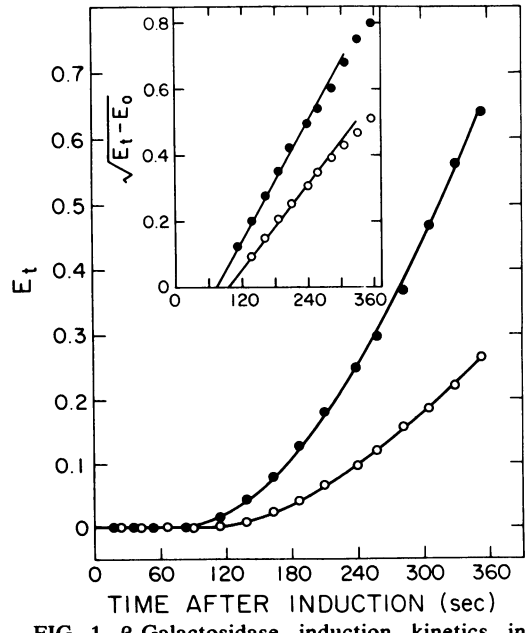

FIG. 1. β-Galactosidase induction kinetics in strains AZ105 (*hisT*$^+$; ●) and AZ106 (*hisT1504*; ○). The growth medium was AB plus glucose.

TABLE 2. β-Galactosidase induction lags in different media[a]

Growth medium	*hisT* allele	Generation time (min)	Induction lag (s)	cgr_p (amino acids/s)[b]
AB + glycerol	+	68, 64, 65	77, 73, 75	13.3, 14.0, 13.6
	−	81, 74, 76	99, 90, 102	10.3, 11.3, 10.0
AB + glucose	+	61, 57	73, 76	14.0, 13.4
	−	71, 70, 69	95, 101, 95	10.7, 10.1, 10.7
Luria broth	+	24, 25	76, 77	13.4, 13.3
	−	34, 31	91, 93	11.2, 11.0

[a] Strains used were AZ105 (*hisT*$^+$) and AZ106 (*hisT1504*). Data were obtained from lines of best fit calculated by least-squares analysis of early induction points before significant deviation from linearity. The different numbers represent data from independent experiments.

[b] Measured as described in the text.

tion elongation rates in β-galactosidase induction experiments. We therefore isolated a mutant of *S. typhimurium* that is permeable to streptolydigin (see above), an antibiotic that specifically inhibits transcription elongation by binding directly to RNA polymerase (6). With this mutant, greater than 99.9% inhibition of transcription was obtained in vivo at an external concentration of 1 mg of streptolydigin per ml (data not shown).

Figure 2 shows β-galactosidase induction kinetics with *hisT*$^+$ and *hisT1504* derivatives of the streptolydigin-sensitive strain. After induction, portions were added to streptolydigin at the indicated times and incubated an additional 20 min to allow translation of the previously synthesized mRNA. The induction lag in such samples is a measure of the time required for RNA polymerase to complete *lacZ* messenger. The extrapolation point obtained may actually be an underestimate if a significant interval exists before streptolydigin inhibits RNA synthesis. However, we were primarily interested in the relative difference in the time required to complete *lacZ* transcription in the two strains. The transcription time was about 8 s longer for the *hisT* mutant than for the *hisT*$^+$ strain (Fig. 2). Induction kinetics obtained in the same experiment by sampling into chloramphenicol were essentially the same for streptolydigin-sensitive strains (Fig. 2) as those observed for streptolydigin-resistant strains (Fig. 1, Table 2), with the *hisT* mutant requiring about 20 s longer than the *hisT*$^+$ strain to complete synthesis of β-galactosidase. Although part of this increased lag might be ascribed to a reduced rate of transcription, the results demonstrate that the primary effect of the *hisT* mutation is to slow translation rather than transcription. The 8-s increased lag in transcription elongation in the *hisT* mutant may support the possibility of a partial coupling between transcription and translation.

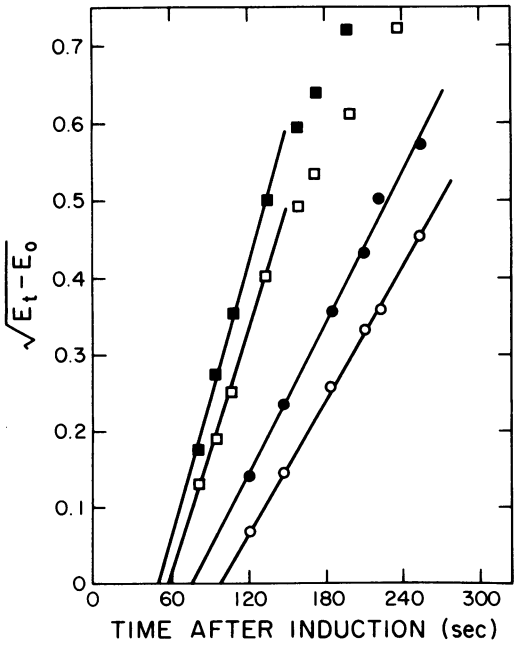

FIG. 2. Transcription time of the *lacZ* gene and translation time of *lacZ* mRNA in strains AZ119 (*hisT*$^+$; closed symbols) and AZ120 (*hisT1504*; open symbols). The growth medium was AB plus glycerol. Samples were added at the indicated times into streptolydigin (■, □) or chloramphenicol (●, ○).

Effect of the *hisT* mutation on sensitivity to growth inhibition by transcription and translation inhibitors. The defect in translation caused by the *hisT* mutation was further revealed by comparison of sensitivities of *hisT*$^+$ and *hisT* strains to growth inhibition by several antibiotic inhibitors of transcription and translation (Table 3). Growth of *hisT* mutants was selectively retarded by three antibiotics (tetracycline, chloramphenicol, and puromycin) that act at different steps in the elongation of polypeptides. In contrast, *hisT*$^+$ and *hisT* strains were equally sen-

TABLE 3. Sensitivity of *hisT*$^+$ strains and *hisT* mutants to growth inhibition by protein synthesis inhibitors

Antibiotic	Inhibitor of:	Zone of inhibition (diam in mm)[a]			
		TA265 (*hisT*$^+$)	TA253 (*hisT1504*)	AZ82[b] (*hisT*$^+$)	AZ81[b] (*hisT1504*)
Rifamycin (75 μg)[c]	Transcription initiation[d]	25	25	29	30
Streptolydigin (300 μg)	Transcription elongation	<6	<6	16	16
Kasugamycin (300 μg)	Translation initiation	23	23	ND[e]	ND
Tetracycline (3 μg)	Translation elongation	20	25	ND	ND
Chloramphenicol (1 μg)	Translation elongation	22	29	ND	ND
Puromycin (30 μg)	Translation elongation	<6	<6	10	13

[a] Antibiotic sensitivities were determined (see text) on C$^-$ agar plates (1) supplemented with 0.4% (wt/vol) glycerol as carbon source. The filter paper disk had a diameter of 6 mm; therefore, <6 represents no measurable zone of inhibition.

[b] Strains AZ81 and AZ82 are permeable derivatives; see the text.

[c] Amount of antibiotic on disk.

[d] Modes of action of antibiotics: rifamycin (37), streptolydigin (6), translation inhibitors (31).

[e] ND, Not determined.

sitive to growth inhibition by antibiotics that specifically inhibit transcription initiation (rifampin), transcription elongation (streptolydigin), and translation initiation (kasugamycin). It is possible that increased sensitivity of *hisT* mutants to growth inhibition by translation elongation inhibitors could result from secondary effects of the *hisT* mutation rather than direct effects at the level of tRNA-ribosome interaction. For example, membrane transport of the antibiotics might be enhanced by the *hisT* mutation. However, the structural dissimilarity among the antibiotics tested, together with selectivity for increased inhibition by translation elongation inhibitors as opposed to antibiotics with other targets in protein synthesis, makes it unlikely that these are all secondary effects. We suggest, therefore, that the *hisT* mutation leads to a defect in one or more of the steps in the translation elongation mechanism.

Lack of a differential effect by the translation initiation inhibitor kasugamycin is consistent with the finding (12, 39) that tRNA^{fMet} does not contain a pseudouridine modification catalyzed by the *hisT* enzyme. This result therefore further supports the interpretation that the decreased rate of translation in *hisT* mutants as measured in β-galactosidase induction experiments described above is the consequence specifically of reduction in the rate of polypeptide chain elongation rather than initiation.

DISCUSSION

Results described in this paper demonstrate that the *hisT* mutation in *S. typhimurium* results in a decreased rate of translation. The effect appears to occur at the level of translation elongation rather than initiation, consistent with the fact that tRNA^{fMet} is not modified by the *hisT* enzyme (12, 39). It is tempting to conclude that slowed translation rate in *hisT* mutants is a direct consequence of loss of pseudouridine modifications in the anticodon regions of the approximately 30 tRNA species that are known to be modified by the *hisT* enzyme (39). However, it is difficult to establish definitively from in vivo studies that the effect is a direct one. *hisT* mutants have been shown to display a number of alterations in cellular regulation (9, 26, 34, 39) and it is quite possible that other alterations are yet to be discovered. Conceivably, one of these perturbations might indirectly alter translation rate. For example, if the intracellular pools of translation elongation factors or GTP were significantly decreased in *hisT* mutants, this might lead to slowed translation. Preliminary results with a tRNA-dependent, DNA-directed cell-free protein-synthesizing system, however, indicate that the effect of the pseudouridine modification is a direct one. Addition to this system of purified, bulk tRNA isolated from a *hisT* mutant as compared with that from a *hisT*$^+$ strain results in a reduced rate of translation (unpublished data) concomitant with the previously reported in vitro derepression of *his* operon expression (2). Since the cell-free system contains uniform concentrations of all of the components required for protein synthesis and the only variable in such experiments is the type of tRNA added, we conclude that lack of the pseudouridine modification leads directly to slowed translation rate.

The precise step in the translation elongation mechanism altered by the *hisT* mutation cannot yet be defined. Pseudouridine-deficient tRNA might interact abnormally with elongation factor Tu during aminoacyl-tRNA binding or at subsequent steps in the mechanism or both. It is unlikely that slowed translation results from defective interaction of pseudouridine-deficient tRNAs with aminoacyl-tRNA synthetases. Pre-

vious evidence has clearly shown that *hisT* mutants contain wild-type levels of charged $tRNA^{His}$ in vivo (26), and there is some evidence that this is also the case for charging levels of $tRNA^{Leu}$ and $tRNA^{Lys}$ (4). All of the isoaccepting species of these tRNAs are missing pseudouridine modifications in *hisT* mutants (39).

After the demonstration that *his* operon expression is regulated by an attenuator mechanism (21) involving translational control (2), DNA sequence analysis of the *his* leader region (3, 11, 20) revealed the presence of a potential 16-amino acid peptide-coding sequence containing seven adjacent His codons. The hypothetical peptide coded is: Met-Thr-Arg-Val-Gln-Phe-Lys-$(His)_7$-Pro-Asp. It has been proposed that the rate at which a ribosome translates the "regulatory" His codons influences formation of alternative RNA secondary structures and determines whether transcription termination occurs at the downstream attenuator site (20). Slowed translation would lead to derepressed *his* operon expression, unimpeded translation would lead to the repressed (basal) level. We have recently been able to show directly in vitro that translation of the predicted leader peptide amino acid sequence does, in fact, regulate attenuation (S. Artz and D. Palmer, manuscript in preparation).

The regulatory model (20) nicely accommodated earlier evidence indicating that *his* operon derepression is correlated with reduced levels of charged $tRNA^{His}$ (26). It was obvious how reduction in amount of His-$tRNA^{His}$ would slow translation of His codons. However, it was not so obvious how the *hisT* mutation would lead to derepression since charging of pseudouridine-deficient $tRNA^{His}$ was unaffected by the mutation and it had been assumed that protein synthesis also was normal (9, 26). Our results strongly support the concept that pseudouridine-deficient tRNA in *hisT* mutants leads to *his* operon derepression by slowing translation of the *hisO* peptide. The three amino acids (Gln, Phe, Lys) immediately preceding the seven His residues in the leader peptide sequence must also be inserted by tRNAs modified by the *hisT* enzyme (39). Whether slowed translation in *hisT* mutants at these earlier positions in the sequence contributes to the regulatory mechanism remains to be determined.

Constitutive expression of the *ilvGEDA* and *leu* operons in *hisT* mutants (9) may also be explained by slowed translation since regulation of both operons is presumed to occur by translational control of attenuation (22, 24, 29). The observation of altered regulation of a gene in *hisT* mutants may be diagnostic for mechanisms involving translational controls. It will be interesting to survey systematically what other genes show altered regulation in *hisT* mutants and whether such effects extend to genes other than those involved in amino acid biosynthesis. We are currently performing such a survey.

Finally, it is interesting to mention that slowed translation in *hisT* mutants appears to be accompanied by an increase in translational fidelity. The *hisT* mutation reduced aminoglycoside antibiotic suppression of a number of *his* and *lac* nonsense and missense mutations (D. T. Palmer, P. H. Blum and S. W. Artz, Fed. Proc. **40**:1750, 1981; P. Blum and S. Artz, manuscript in preparation) and resulted in impaired function of a glutamine-inserting, amber suppressor tRNA (20). In addition, so-called antisuppressor mutations in yeast have been described, and at least one of these mutations has been shown to eliminate a tRNA modification (19).

A number of years ago Gorini (15) proposed that the ribosome possesses a "recognition screen" which increases the probability of selecting the correct aminoacyl tRNA during translation. Some sort of aminoacyl tRNA discrimination mechanism must exist if, as it has been argued (23), the strength of codon-anticodon interaction alone cannot account for the observed low error frequency during translation. Hopfield (17) and Ninio (30) have devised kinetic proofreading models to account for the low error frequency, and several in vivo studies (14, 16, 32, 41) have demonstrated an inverse correlation between translation rate and translational fidelity. It seems plausible that at least one function of certain tRNA modifications, including the *hisT*-specified pseudouridine modification, may be to aid in determining an appropriate compromise between maximum efficiency (rate) and accuracy of translation.

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