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Biochemical Characterization of an *Escherichia coli hisT* Strain

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An *Escherichia coli hisT* strain was characterized biochemically and shown to contain altered transfer ribonucleic acid and to be altered in the regulation of amino acid biosynthesis.

Although the bulk of the known variants of transfer ribonucleic acid (tRNA) are suppressors, selection for resistance to histidine analogues in *Salmonella typhimurium* has yielded a number of mutant strains altered in the structure or quantity of histidine tRNA ($tRNA^{His}$). These mutant strains fall into four classes or complementation groups: *hisR*, *hisU*, *hisW*, and *hisT*. *hisR* mutant strains contain reduced quantities of $tRNA^{His}$ as the result of an alteration of the $tRNA^{His}$ structural gene (4). *hisU* and *hisW* variants also contain reduced quantities of $tRNA^{His}$ but are believed to be altered in the maturation process of this tRNA (3). The most thoroughly characterized of these variants is the *hisT* group, which is known to contain a lesion in the structural gene for the enzyme pseudouridylate synthetase, which catalyzes the conversion of specific uridine residues to pseudouridine during maturation of some tRNA's. This function was established by sequence analysis of $tRNA^{His}$ isolated from the wild type and from *hisT* strains of *S. typhimurium* (17) and by the in vitro conversion of uridine to pseudouridine in *hisT* tRNA by extracts prepared from wild-type cells (7). The *hisT* mutation is of particular interest because it leads to alterations in tRNA's that contain pseudouridine residues in their anticodon loop. Consequently, the regulation of the enzymes

TABLE 1. *Bacterial strains used*

Strain	Relevant genotype	Source or reference
E. coli K-12		
AB2557	*ilvD188 aroC4 purF1 thi-1*	*E. coli* Genetic Stock Center
AB1369	*argE3 cysB38 his-4, proA2*	*E. coli* Genetic Stock Center
788	*trpR⁻ $trpE_{am}$9829 $trpA_{am}$9761 $lacZ_{oc}$U118*	I. Crawford
T21	*ilvD188 aroC4 purF1 thi-1 trp*	*N*-methyl-*N'*-nitroso-*N*-nitroso-guanidine mutagenesis of AB2557
T21-21	*ilvD188 aroC4 purF1 thi-1 cysB38*	T21 by transduction with AB1369 as donor
T31	*ilvD188 aroC4 purF1 thi-1 $trpE_{am}$9829 $trpA_{am}$9761*	T21-21 by transduction with AB1369 as donor
CU4	Prototroph	H. E. Umbarger
FB105	*hisT*	F. Blasi
T31-4	*ilvD188 thi-1 $trpE_{am}$9829 $trpA_{am}$9761*	T31 by transduction with CU4 as donor
T31-4-4	*thi-1 $trpE_{am}$9829 $trpA_{am}$9761*	T31-4 by transduction with CU4 as donor
T31-H	*ilvD188 thi-1 $trpE_{am}$9829 $trpA_{am}$9761 hisT*	T31 by transduction with FB105 as donor
T31-H-4	*thi-1 $trpE_{am}$9829 $trpA_{am}$9761 hisT*	T31-H by transduction with CU4 as donor
S. typhimurium		
253	*hisT*	Cortese et al. (8)
265	LT-2 wild type	Cortese et al. (8)

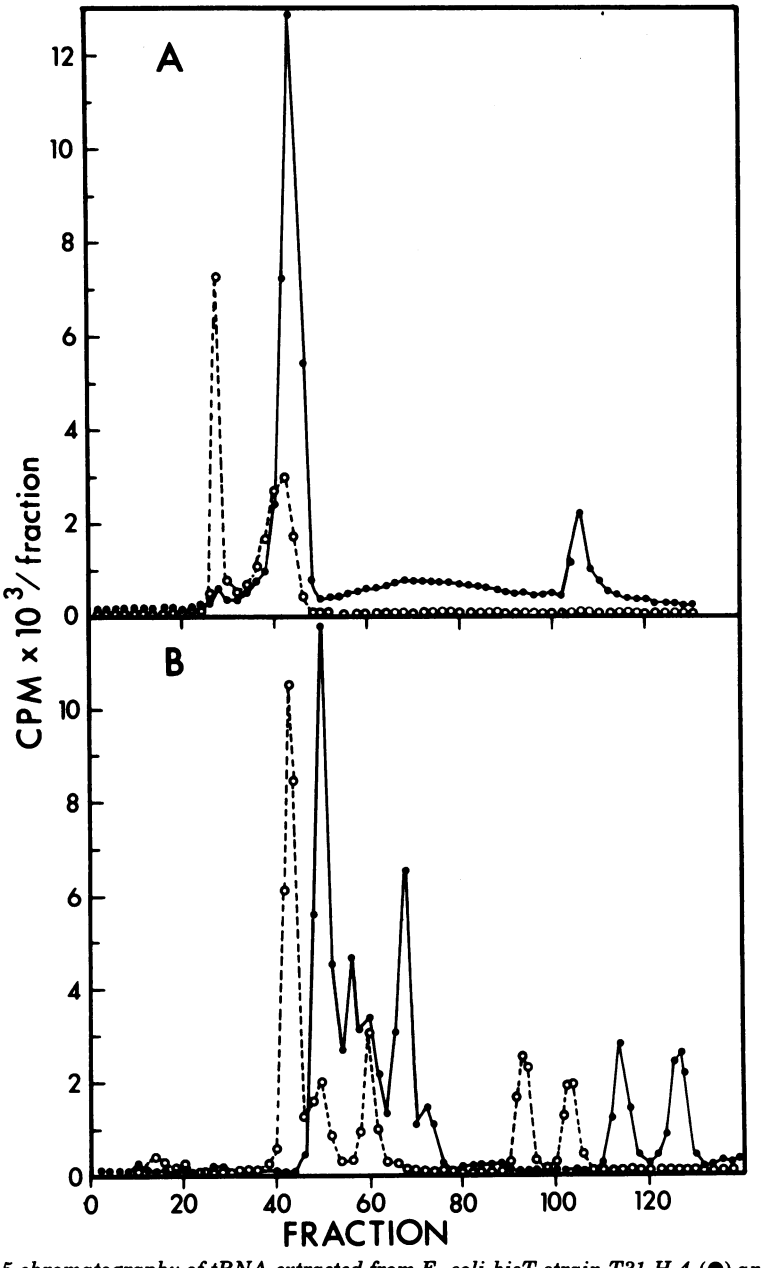

FIG. 1. *RPC-5 chromatography of tRNA extracted from E. coli hisT strain T31-H-4 (●) and E. coli wild-type tRNA (○). (A) Histidyl-tRNA profile eluted with a 0.5 to 1.5 M linear NaCl gradient. (B) Leucyl-tRNA profile eluted with a 0.5 to 1.2 M linear NaCl gradient. Wild-type tRNA was unfractioned E. coli B tRNA obtained from Plenum Chemical Co. (lot no. 74121). E. coli hisT tRNA was prepared by extraction with phenol. Aminoacylation of tRNA and reverse-phase chromatography were as described by Holmes et al. (10).*

involved in the biosynthesis of the cognate amino acids is altered in these strains (8, 15, 17). The properties of the *hisT* strains provided the first direct evidence of the involvement of tRNA molecules in cellular regulatory processes.

Among the tRNA's altered by the inactivation of pseudouridine synthetase in *hisT* mutant strains of *S. typhimurium* are $tRNA^{His}$ (17) and all five of the leucine tRNA ($tRNA^{Leu}$) species (8, 15). Co-chromatography on an RPC-5 column (Fig. 1A) of wild-type *Esche-*

richia coli tRNA with tRNA prepared from an *E. coli hisT* strain (construction of strains is outlined in Table 1) demonstrates that the histidyl-tRNA's are altered in the *E. coli hisT* strain. The elution pattern also confirms that *E. coli* contains two species of $tRNA^{His}$ (10, 19), as opposed to a single species in *S. typhimurium* (4), and that each of these species must contain pseudouridine residues. A similar experiment examining the leucyl-tRNA species (Fig. 1B) showed an alteration in all five of the leucyl-tRNA's as described previously in *S. typhimurium* (8, 15).

The observed retardation in the elution from an RPC-5 column of the histidyl- and leucyl-tRNA species of an *E. coli hisT* strain indicates that the effect of this mutation on the overall structure of the tRNA's in *E. coli* is similar to the alteration in structure of tRNA's in *hisT* strains of *S. typhimurium*. Because the major species of leucyl-tRNA, $tRNA_1^{Leu}$, has an identical sequence in *E. coli* and *S. typhimurium* (1), it would be expected that the $tRNA_1^{Leu}$ species from *hisT* strains of *E. coli* and *S. typhimurium* would coelute from an RPC-5 column if the *hisT* lesion had the same effect in each of these organisms on the overall structure of these tRNA's. As can be seen in Fig. 2, the leucyl-tRNA species from *hisT* strains of *E. coli* and *S. typhimurium* did indeed coelute from an RPC-5 column. This indicates further that the effect of this *hisT* mutation in *E. coli* is identical to that in *S. typhimurium* and also that all of the leucyl-tRNA species are probably identical in these two organisms.

Mullenbach et al. (14) purified pseudouridine synthetase from baby hamster kidney cells and showed the conversion of *S. typhimurium hisT* phenylalanine tRNA ($tRNA^{Phe}$) to a product indistinguishable from *S. typhimurium* wild-type $tRNA^{Phe}$ on an RPC-5 column. This approach is an alternative means of analyzing the effect of the *hisT* mutation on *E. coli* tRNA. A wild-type extract of *S. typhimurium* should similarly convert *E. coli hisT* tRNA to the wild-type species, whereas an extract of a *S. typhimurium hisT* mutant should not alter the *E. coli hisT* tRNA. After incubation of the *E. coli hisT* tRNA with an extract of a *S. typhimurium hisT* strain (Fig. 3A), the elution pattern of the *E. coli* $tRNA^{Leu}$ remained unchanged (cf. Fig. 1B). In contrast, incubation of the mutant tRNA with a *S. typhimurium* wild-type extract (Fig. 3B) altered the elution pattern of the *E. coli hisT* $tRNA^{Leu}$ to species indistinguishable from wild-type *E. coli* $tRNA^{Leu}$.

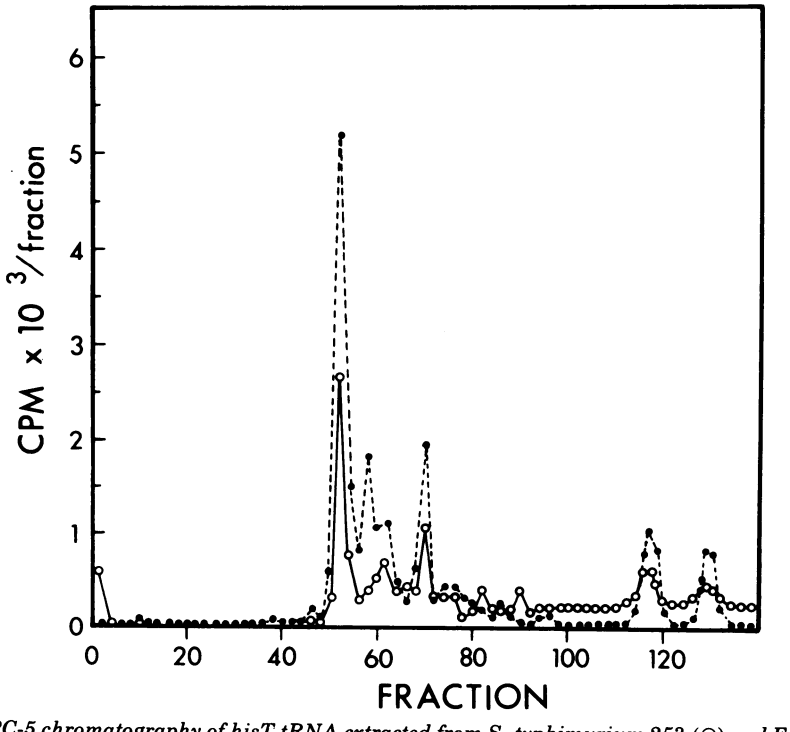

FIG. 2. *RPC-5 chromatography of hisT tRNA extracted from S. typhimurium 253* (○) *and E. coli T31-H-4* (●) *eluted with a linear 0.5 to 1.2 M NaCl gradient.*

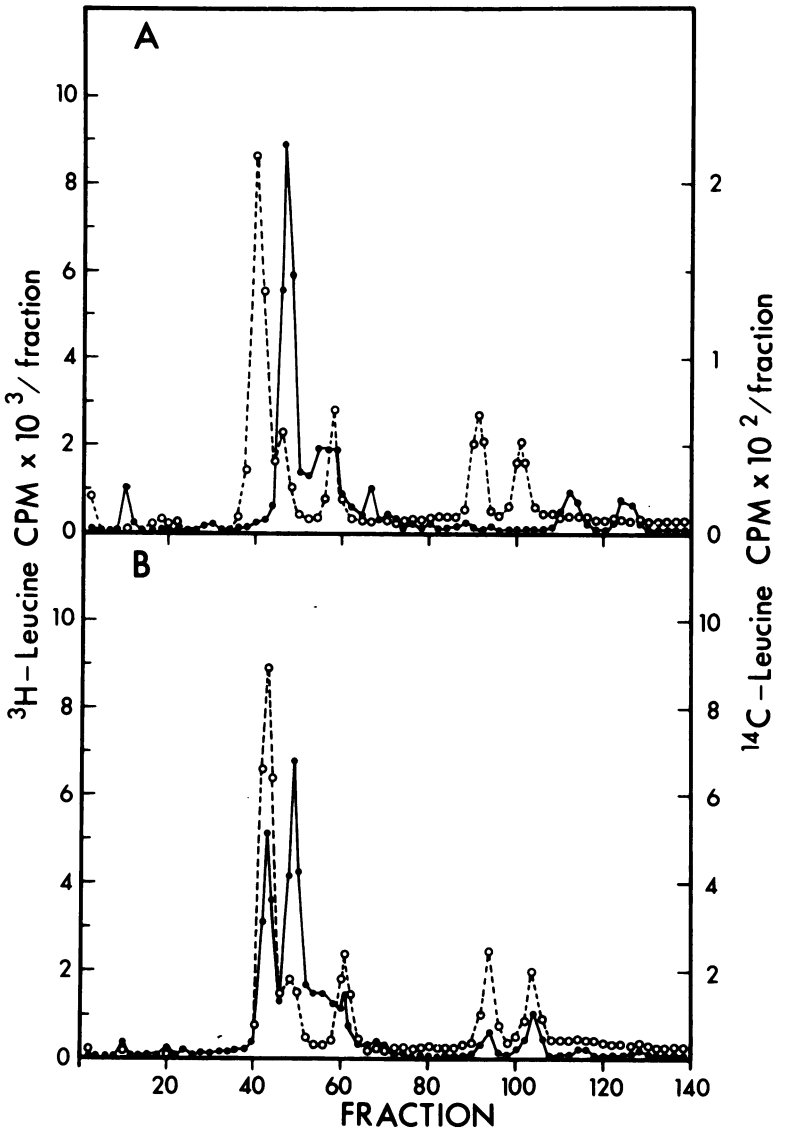

FIG. 3. *RPC-5 chromatography of tRNA extracted from E. coli hisT strain T31-H-4 incubated with extract prepared from S. typhimurium hisT strain 253 (A), or with extract prepared from S. typhimurium wild-type strain 265 (B). hisT tRNA* (●) *and wild-type tRNA* (○) *were eluted with a linear 0.5 to 1.2 M NaCl gradient.*

The *hisT* allele was originally characterized in *S. typhimurium* as conferring resistance to the histidine analogues triazolealanine and aminotriazole. This resistance is attributable to a derepression of the histidine biosynthetic operon (16). Later investigations (8, 15, 17) examining resistance to analogues of other amino acids or enzymes by direct assay established that the enzymes required for the biosynthesis of several amino acids, including isoleucine-valine and leucine, were also derepressed in *hisT* strains. Thus, the introduction of this mutation into *E. coli* allows an investigation of the role of native tRNA's and the effect of altering the structure of tRNA's on the regulation of amino acid biosynthesis in *E. coli*.

Wild-type *E. coli* T31-4-4 exhibited normal levels of histidinol phosphatase activity (Table 2). However, there was a fivefold derepression of this enzyme in the *E. coli hisT* strain T31-H-4 regardless of the presence or absence of excess histidine.

The wild-type strain T31-4-4 also demonstrated (Table 2) normal repression patterns for

TABLE 2. *Effect of E. coli hisT mutation on regulation of histidine, isoleucine-valine, and leucine operons*

Strain	Medium[a]	Sp Act[b]			
		Histidinol phosphatase	Transaminase B	Threonine deaminase	Isopropylmalate synthetase
T31-4-4 (wild type)	Min	0.002	0.051	0.034	0.034
	Min + His	0.003			
	Min + Ile, Leu, Val		0.019	0.016	0.020
	L-broth	ND[c]	0.005	0.006	ND
T31-H-4 (*hisT*)	Min	0.012	0.074	0.039	0.030
	Min + His	0.010			
	Min + Ile, Leu, Val		0.053	0.036	0.050
	L-broth	ND	0.010	0.010	ND

[a] Minimal medium (Min) was M63 prepared as described by Miller (12); amino acid supplements were 1.0 mM histidine and valine, 0.4 mM isoleucine and leucine, and 0.25 mM tryptophan.

[b] Assay for histidinol phosphatase was as described by Ames et al. (2). Assays for threonine deaminase and transaminase B were as described by Kline et al. (11). Assay for isopropylmalate synthetase was as described by Burns et al. (5), except that free sulfhydryl groups generated from acetyl coenzyme A were titrated with 5,5'-dithio-bis(2-nitrobenzoic acid) as described by Morino and Snell (13). Specific activities are expressed in micromoles per minute per milligram of protein.

[c] ND, Not detectable.

TABLE 3. *Effect of E. coli hisT lesion on derepression of transaminase B and threonine deaminase*

Strain	Medium[a]	Sp act	
		Transaminase B	Threonine deaminase
T31-4	Ile, Val, Leu	0.027	0.0012
	Ile, Val, Leu	0.043	0.0022
	Ile, *Val*, Leu	0.066	0.0018
T31-H	Ile, Val, Leu	0.033	0.0020
	Ile, Val, Leu	0.084	0.0023
	Ile, *Val*, Leu	0.052	0.0033

[a] Amino acids were added as described in footnote *a* of Table 2, except for those italicized, in which case that amino acid was limiting: *Ile*, 80 μM Ile; *Val*, 80 μM glycylvaline.

the isoleucine-valine biosynthetic enzymes threonine deaminase and transaminase B and for the leucine biosynthetic enzyme isopropylmalate synthetase upon the addition of excess isoleucine, valine, and leucine to minimal media. In contrast, the level of these enzymes in the *hisT* strain T31-H-4 was unaffected by the addition of these amino acids to the media.

The *hisT* allele, however, did not confer resistance to repression of these four enzymes by growth in rich media. The derepression of histidinol phosphatase and the absence of repression on threonine deaminase, transaminase B, and isopropylmalate synthetase are identical to the regulatory effects seen in *S. typhimurium hisT* strains (8).

T31-4 is an isoleucine-valine auxotroph and T31-H is an isogenic strain containing the *hisT* mutation. The *hisT* mutation had no effect on the derepression signal for the isoleucine-valine biosynthetic operon (Table 3). Both of these strains derepressed for transaminase B upon limitation for either isoleucine or valine. Threonine deaminase also exhibits normal derepression in both of these strains, but the levels are greatly reduced due to the polarity of the *ilvD* ochre mutation on the operator distal threonine deaminase gene (6, 18).

Although the *hisT* mutation has been known in *S. typhimurium* for some time, it has not been described previously in *E. coli*. The data presented here characterize the properties of this mutation in *E. coli* and show that it exerts the same effects on both tRNA structure and the regulation of amino acid biosynthetic enzymes in *E. coli* as in *S. typhimurium*. The characterization of this mutation in *E. coli* now makes it possible to investigate the regulation of systems thought to be controlled by the "state" of tRNA in this organism.

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