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1-Methylguanosine Deficiency of tRNA Influences Cognate Codon Interaction and Metabolism in *Salmonella typhimurium*

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1-Methylguanosine (m^1G) is present next to the 3′ end of the anticodon (position 37) in $tRNA^{Leu}_{1,2,3}$, $tRNA^{Pro}_{1,2,3}$, and $tRNA^{Arg}_3$. A mutant of *Salmonella typhimurium* lacks m^1G in these seven tRNAs when grown at or above 37°C, as a result of a mutation (*trmD3*) in the structural gene (*trmD*) for the tRNA(m^1G37)methyltransferase. The m^1G deficiency induced 24 and 26% reductions in the growth rate and polypeptide chain elongation rate, respectively, in morpholinepropanesulfonic acid (MOPS)-glucose minimal medium at 37°C. The expression of the *leuABCD* operon is controlled by the rate with which $tRNA^{Leu}_2$ and $tRNA^{Leu}_3$ read four leucine codons in the *leu*-leader mRNA. Lack of m^1G in these tRNAs did not influence the expression of this operon, suggesting that m^1G did not influence the efficiency of $tRNA^{Leu}_{2,3}$. Since the average step time of the m^1G-deficient tRNAs was increased 3.3-fold, the results suggest that the impact of m^1G in decoding cognate codons may be tRNA dependent. The *trmD3* mutation rendered the cell more resistant or sensitive to several amino acid analogs. 3-Nitro-L-tyrosine (NT), to which the *trmD3* mutant is sensitive, was shown to be transported by the tryptophan-specific permease, and mutations in this gene (*mtr*) render the cell resistant to NT. Since the *trmD3* mutation did not affect the activity of the permease, some internal metabolic step(s), but not the uptake of the analog per se, is affected. We suggest that the *trmD3*-mediated NT sensitivity is by an abnormal translation of some mRNA(s) whose product(s) is involved in the metabolic reactions affected by the analog. Our results also suggest that tRNA modification may be a regulatory device for gene expression.

In tRNA from the three domains, *Bacteria*, *Eukarya*, and *Archaea*, 79 different modified nucleosides have been identified (23). Their formation is catalyzed by highly specific enzymes, and their importance is reflected by the fact that a substantial part (about 1%) of the genetic information in bacteria is devoted to this aspect of cellular metabolism (for a review, see reference 2).

The modified nucleosides are present in different positions of the tRNA molecule, and there is a large variety of modified nucleosides, especially in the anticodon region. One of the few modified nucleosides conserved in tRNA from all domains is 1-methylguanosine (m^1G), which is present next to the 3′ end of the anticodon (position 37) in tRNAs from *Escherichia coli* and *Salmonella typhimurium* reading CCN ($tRNA^{Pro}_{1,2,3}$) (N, any of the four major nucleosides) and CGG ($tRNA^{Arg}_3$) codons (32). $tRNA^{Leu}_{1,2,3}$ species, which read CUN codons, have an unidentified modified guanosine at position 37 (32). Mutations in the *trmD* gene in *E. coli* and in *S. typhimurium*, which is the structural gene for the tRNA(m^1G37)methyltransferase, affect the elution profiles of these leucine tRNAs (20a). Therefore, it is likely that m^1G is present also in $tRNA^{Leu}_{1,2,3}$.

A temperature-sensitive mutant of *S. typhimurium*, which lacks m^1G in its tRNAs when grown at or above 37°C, has been isolated, and the mutation is located in the *trmD* gene (4). The m^1G deficiency suppresses certain +1 frameshift mutations (4). At least in the case of $tRNA^{Pro}$, m^1G deficiency induces a frameshift by allowing the unmodified G-37 to interact with a C as a fourth base, resulting in a quadruplet translocation (19). Therefore, one specific function of m^1G is to prevent frameshifting and thereby maintain the reading frame.

Here we address the questions of how m^1G deficiency in the tRNA affects cell physiology and what role m^1G plays in the cognate tRNA-mRNA interaction. We showed that m^1G deficiency induced strong pleiotropic effects such as reduction in growth rate and polypeptide chain elongation rate (cgr_P) in vivo. Our results demonstrated that the presence of m^1G is important for efficient translation, and in a tRNA-dependent manner. Moreover, synthesis of several metabolic enzymes was affected by lack of m^1G of the *trmD3* mutant, as shown by altered responses to several amino acid analogs and enhanced ability to oxidize certain carbon sources compared with the wild-type strain.

MATERIALS AND METHODS

Bacteria and growth conditions. The bacterial strains used were all derivatives of *S. typhimurium* LT2 (Table 1). As a complex medium (NAA), we prepared Difco nutrient broth (0.8%; Difco Laboratories, Detroit, Mich.) supplied with 0.5% NaCl, adenine, tryptophan, tyrosine, phenylalanine, and the three aromatic vitamins *p*-hydroxybenzoate, 2,3-dihydroxybenzoate, and *p*-aminobenzoate. All supplements were provided at concentrations recommended by Davis et al. (13). For genetic experiments, we used medium E described by Vogel and Bonner (38). Growth rate studies were performed as described previously (3) with morpholinepropanesulfonic acid (MOPS) medium (25) supplemented with 0.4% indicated carbon sources. Rich MOPS medium was prepared as described by Neidhardt et al. (24). Following the completion of each growth rate experiment, the culture was tested for homogeneity. In no case were any fast-growing revertants observed.

Genetic procedures. Transductions with P22 HT105 (*int-201*) (29) were performed as described elsewhere (13). Rapid mapping of transposon Tn*10* insertion was performed as described by Benson and Goldman (1).

Assay of β-isopropylmalate dehydrogenase (LeuB) activity. Cells were grown at 41°C in SSA medium (10) containing 0.2% glucose and 5 μM thiamine hydrochloride. Samples were taken at different stages of the growth, and β-isopropylmalate dehydrogenase (LeuB) activity was measured as described by Searles and Calvo (30).

Measurement of carbon source oxidation. Strains GT874 (*trmD*$^+$), GT875 (*trmD3*), GT2529 (*hisT*$^+$ *zej-635*::Tn*5*), and GT2530 (*hisT1504 zej-635*::Tn*5*) were grown at 37°C in NAA rich medium to between 2×10^7 and 2×10^8 cells per ml. Cells were harvested on ice, pelleted, and resuspended in the same volume of 0.9% NaCl. Samples (150 μl) were transferred to each well of an ES Microplate purchased from Biolog Inc. The Biolog MicroPlate tests the ability of a microorganism to oxidize a preselected panel of 95 different carbon compounds. Oxidation of the different carbon compounds is measured as transfer of

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TABLE 1. *S. typhimurium* strains used

Strain	Genotype	Source or reference
LT2	Wild type	John Roth
GT15	*hisG200 supK599*	This work
GT522	*miaA*$^+$	15
GT523	*miaA1*	15
GT874	*trmD*$^+$	4
GT875	*trmD3*	4
GT984	F′ *lac*$^+$/*pro-662*::Tn*10*	This work
GT985	F′ *lac*$^+$/*pro-662*::Tn*10 trmD3*	This work
GT1830	*tyrA555*::Tn*10 hisO1242 hisD3749*	This work
GT1860	*hisG6609 srl*::Tn*10 sufT621*	John Atkins
GT2529	*hisT*$^+$ *zej-635*::Tn*5*	This work
GT2530	*hisT1504 zej-635*::Tn*5*	This work
GT2872	*trmD3 sdn-1* (*mtr-1*)	This work
GT2926	*trmD3 zgh-2510*::Tn*10*	This work
GT3080	*sdn-1* (*mtr-1*)	This work
GT3110	*fels2 leuA414*(Am) r$^-$ *zgi-3717*::MudQ *argG1822*::Tn*10*	This work
GT3614	*zhb-736*::Tn*10 rpsL107*	This work
TR936	*hisO1242 hisD3018 sufB2*	John Roth
TR1457	*hisO1242 hisD3749 sufA6*	John Roth
TR1914	*hisO1242 hisC3072 sufD46*	John Roth
TR2679	*hisO1242 hisD6580 sufG70*	John Roth
TT142	*argG1822*::Tn*10*	John Roth
TT4241	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supE20*	John Roth
TT4278	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supF30*	John Roth
TT4282	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supJ60*	John Roth
TT4286	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supG50*	John Roth
TT4290	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supC80*	John Roth
TT4313	*hisO1242 hisD3749-Δ6 sufJ128*	John Roth
TT7610	*zeb-609*::Tn*10 supD10*	John Roth
TT17191	*fels2 leuA414*(Am) r$^-$ *zgi-3717*::MudQ	Ken Sanderson

electrons from NADH to a tetrazolium redox dye, which results in the formation of a purple color. After inoculation, the microplates were incubated at 37°C for 20 h, and the optical density at 620 nm (OD_{620}) was measured by a Titertek Multiscan MCC/340 reader.

Determination of sensitivity to amino acid analogs. Strains tested were grown overnight in NAA rich medium at 30°C. A sample (0.1 ml) was mixed with 2 ml of 0.5% agar in 0.9% NaCl and poured onto a plate containing medium E and 0.2% sodium citrate. Paper disks (6 mm in diameter) were placed on the surface of the plates, and 10 μl of each analog was applied to the respective disk. The plates were incubated at 37°C for 24 h before being scored. Analogs to which the two strains responded equally were DL-aspartic acid β-hydroxamate (Asn), 1,2,4-triazole (Cys), L-glutamic acid γ-hydrazide (Gln), DL-methionine hydroxamate (Gln), azaserine (Gln), L-methionine-DL-sulfoximine (Gln), 3-amino-1,2,4-triazole (His), 1,2,4-DL-triazole-3-alanine (His), 2-thiazolyl-DL-alanine (His), 4-aza-DL-leucine (Leu), *S*-2-aminoethyl-L-cysteine (Lys), β-(2-thienyl)-DL-alanine (Phe), *m*-fluoro-DL-phenylalanine (Phe), thioproline (Pro), L-thiazolidine-4-carboxylic acid (Pro), DL-5-fluorotryptophan (Trp), DL-7-azatryptophan (Trp), 5-methyl-DL-tryptophan (Trp), 3-amino-L-tyrosine (Tyr), β-DL-hydroxynorvaline (Tyr), *m*-fluoro-DL-tyrosine (Tyr), and fluoroacetic acid (acetate). All analogs were obtained from Sigma Chemical Co., St. Louis, Mo.

Tryptophan transport assay. The growth medium used was medium E (38) containing 0.2% sodium citrate. The concentration of L-phenylalanine, when used, was 1 μM. Bacteria were grown at 37°C for at least two mass doublings and were harvested at about 70 Klett units (about 3×10^8 cells per ml). After centrifugation, the cells were washed and resuspended in the same volume of medium supplemented with 80 μg of chloramphenicol per ml. The cells were incubated for 10 min at 37°C and then stored at 4°C before being used (no longer than 24 h). The specific uptake of L-[5-^{3}H]tryptophan (28 Ci/mmol; Amersham) was measured as described by Whipp and Pittard (40) at 30°C. Control filtrations were performed with boiled cells to correct for nonspecific adsorption.

RESULTS

A mutant (*trmD3*) lacking m^{1}G in its tRNAs has reduced growth rate and cgr$_p$. Strains GT874 (*trmD*$^+$) and GT875 (*trmD3*) were grown under steady-state conditions in different media at 37°C. In minimal glucose medium, a 24% reduction in growth rate for the *trmD3* mutant relative to the *trmD*$^+$ strain was observed (Table 2). A somewhat smaller reduction (16 to 18%) was observed in glycerol minimal medium and in rich MOPS medium. Only a 9% reduction was observed in acetate minimal medium. Thus, up to a specific growth rate of 0.85 (MOPS-glucose medium), the faster the growth rate, the larger the observed reduction in growth rate caused by the *trmD3* mutation.

To test whether lack of m^{1}G reduces cgr$_p$, the time required to synthesize β-galactosidase after induction of the *lac* operon was determined. By plotting the data as the square root of the difference between E_t (enzyme activity at time t) and E_0 (basal level) versus time, the time for completion of the first β-galactosidase molecule after induction can be determined (28). Using the chain length of 1,023 amino acids (aa) for the β-galactosidase subunit (21), cgr$_p$ was calculated. The *trmD3*-mediated reduction was the same in all media tested (-2.4 ± 0.1 aa/s) except in glucose minimal medium, for which the reduction was somewhat larger (-4 aa/s).

Lack of m^{1}G-37 does not affect the level of Pro-tRNA$_1^{Pro}$. tRNAs from both the *trmD*$^+$ and the *trmD3* cells were prepared, and the in vivo level of Pro-tRNA$_1^{Pro}$ was determined as described by Varshney et al. (37). Our result showed that tRNA$_1^{Pro}$ is fully aminoacylated in both the *trmD*$^+$ and the *trmD3* strains (data not shown). Thus, lack of m^{1}G does not influence the level of Pro-tRNA$_1^{Pro}$ in vivo, and therefore, the *trmD3* mutation does not affect the efficiency of translation by reducing the concentration of aminoacylated tRNA$_1^{Pro}$. Furthermore, the fact that the *trmD3* mutation does not affect the expression of *leuABCD* operon (see below) suggests that m^{1}G deficiency in tRNA$_2^{Leu}$ and tRNA$_3^{Leu}$ does not influence the charging of these tRNAs.

m^{1}G deficiency does not significantly affect the expression of the *leu* operon. The *leuABCD* operon is regulated by transcription attenuation (14, 31, 39). The rate with which the ribosome traverses the four consecutive leucine codons, CUA-CUA-

TABLE 2. Specific growth rate and rate of polypeptide synthesis in *trmD3* and *trmD*$^+$ cells[a]

Medium	Growth characteristics k[b]				cgr$_p$ (aa/s)[c]			
	k_{D^+}	k_{D3}	$k_{D3} - k_{D^+}$	$(k_{D3} - k_{D^+})/k_{D^+}$	cgr$_{pD^+}$	cgr$_{pD3}$	cgr$_{pD3}$ − cgr$_{pD^+}$	(cgr$_{pD3}$ − cgr$_{pD^+}$)/cgr$_{pD^+}$
MOPS-acetate	0.33 (±0.00)	0.30 (±0.00)	−0.03	−0.09	12.6 (±0.4)	10.1 (±0.3)	−2.5	−0.20
MOPS-glycerol	0.72 (±0.00)	0.63 (±0.01)	−0.09	−0.16	13.5 (±0.6)	11.1 (±0.2)	−2.4	−0.18
MOPS-glucose	0.85 (±0.02)	0.66 (±0.00)	−0.21	−0.24	15.5 (±0.0)	11.5 (±0.1)	−4.0	−0.26
Rich MOPS	1.54 (±0.00)	1.26 (±0.00)	−0.28	−0.18	14.6 (±0.8)	12.3 (±0.2)	−2.3	−0.16

[a] All experiments were performed at 37°C, and the data are the averages from two (GT874 and GT875) or three (GT984 and GT985) independent experiments. The variation is shown in parentheses.

[b] k, specific growth rate (=ln2/generation time [hours]). The strains used were GT874 (*trmD*$^+$ [D^+]) and GT875 (*trmD3* [*D3*]).

[c] Determined by determining the time needed to synthesize the first β-galactosidase molecule. The strains used were GT984 (*trmD*$^+$/F′ *lac*$^+$) and GT985 (*trmD3*/F′ *lac*$^+$).

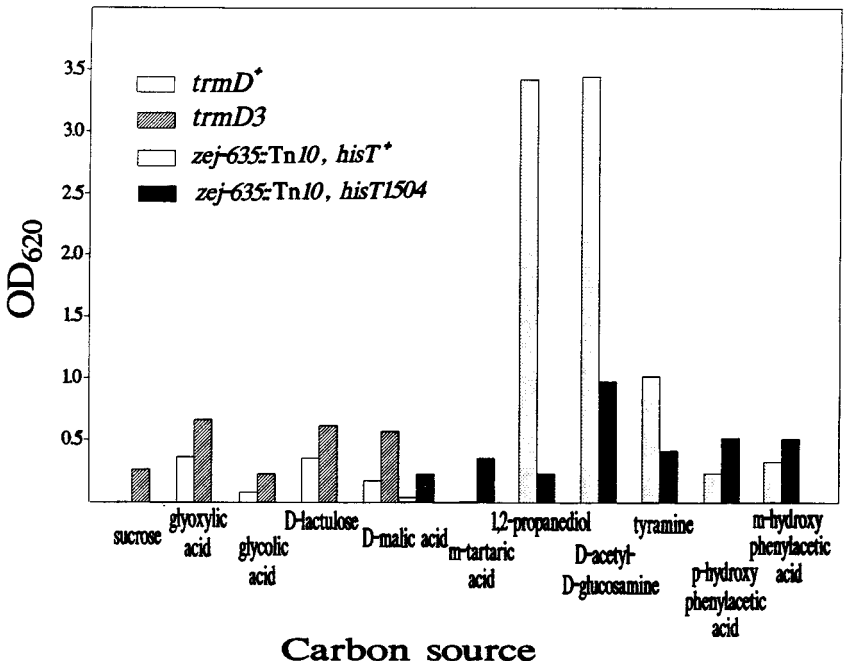

FIG. 1. Carbon source utilization in GT874 (*trmD*$^+$), GT875 (*trmD3*), GT2529 (*zej-635*::Tn*5*, *hisT*$^+$), and GT2530 (*zej-635*::Tn*5 hisT1504*) strains. The carbon sources shown are those that are oxidized significantly differently by the wild type and the mutant (the ratio OD_{620} [mutant]/OD_{620} [wild type] is below 0.5 or above 1.5). OD_{620} was measured after 20 h of incubation at 37°C, and each value is the average from two independent experiments.

CUA-CUC, in the leader transcript controls the expression of this operon (11). The CUA codon is read by $tRNA_3^{Leu}$, and the CUC codon is read by $tRNA_2^{Leu}$. Only one other codon (CCG) that is read by a m^1G-containing tRNA is present in the leader mRNA. This codon is located nine codons downstream of these four leucine codons. Therefore, the effect of m^1G on the *leuABCD* operon should be specifically due to its effect on these two leucine tRNAs. Both of these tRNAs have also Ψ in the anticodon region, $tRNA_3^{Leu}$ at positions 38 and 39 and $tRNA_2^{Leu}$ at position 38. The synthesis of these Ψs is mediated by the *hisT* gene product. According to the attenuation model by which *leuABCD* operon is regulated, derepression takes place if the ribosome is retarded at any of these four leucine control codons. cgr_p is decreased (23%) in a *hisT* mutant, which lacks Ψ-38, -39, and -40 (26), and accordingly, the *leuABCD* operon is derepressed 2.8-fold in such a mutant (27). This Ψ-mediated effect on *leuABCD* expression is due to the reduced tRNA efficiency, since the *hisT* mutation does not influence the charging of the leucine tRNAs (6). Since $tRNA_2^{Leu}$ and $tRNA_3^{Leu}$ contain m^1G at position 37 and cgr_p was even more retarded in the *trmD3* mutant (26%), we expected that the *leuABCD* operon would also be derepressed in the *trmD3* mutant. Surprisingly, the activity of isopropylmalate dehydrogenase (*leuB* gene product) in the mutant was not affected (*trmD3* activity/*trmD*$^+$ activity = 1.2 ± 0.2) (data not shown and reference 9a). Thus, whereas Ψ-deficient $tRNA_2^{Leu}$ and $tRNA_3^{Leu}$ have a decreased translational efficiency, m^1G-deficient $tRNA_2^{Leu}$ and $tRNA_3^{Leu}$ do not.

Lack of m^1G affects carbon source metabolism. Levels of oxidation of carbon compounds by *trmD*$^+$ and *trmD3* cells were compared. The mutant showed major differences from the wild-type strain in its ability to oxidize 5 of the 95 carbon compounds tested (Fig. 1). It should be noted that all of these five carbon compounds were oxidized more efficiently by the mutant, which indicates that either the enzymes involved in utilizing these carbon compounds are more active (the supply of cofactors could be altered) or the amounts of such enzymes are increased as a result the *trmD3* mutation. Among these five carbon compounds, sucrose, glyoxylic acid, and D-malic acid are not normally used as carbon sources by *Salmonella* strains (16). Increased oxidation sometimes correlates with increased utilization of the carbon source measured as increase in growth rate. However, this is not true for sucrose, since neither *trmD*$^+$ nor *trmD3* cells grew on agar plates containing sucrose as the carbon source. How the *trmD3* mutation leads to the increased oxidation of these carbon sources in *Salmonella* strains is not clear.

Another tRNA modification mutant, bearing *hisT*, which has a U instead of Ψ at positions 38, 39, and 40 in many tRNAs, was also tested. In the case of *hisT*, some enzymes involved in carbon source metabolism were also affected (Fig. 1), either positively or negatively. Both *hisT* and *trmD3* mutants oxidize D-malic acid more efficiently than their wild-type parents, implying that common routes may be shared by the two different modifications in influencing the oxidation of this carbon source. Although the mechanism behind this differential carbon source metabolism caused by mutations in the *trmD* or *hisT* gene is not clear, these results suggest a global effect of tRNA modification on cell physiology.

The m^1G deficiency renders cells more resistant or sensitive to several amino acid analogs. It is known that undermodified tRNA can influence the regulation of the synthesis of several amino acids (9, 15, 36, 42). One way to screen for the possible influence of m^1G deficiency on amino acid metabolism is to determine the response to amino acid analogs (12). Of 28 different analogs to 12 of the common amino acids, the *trmD3* mutant was more resistant to 3,4-dehydro-DL-proline (Pro) and more sensitive than the *trmD*$^+$ strain to 5,5,5-trifluoro-DL-leucine (Leu), L-2-acetidine-carboxylic acid (Pro), DL-serine-hydroxamate (Ser), and 3-nitro-tyrosine (NT) (Tyr). Different responses to various analogs of the same amino acids (for

TABLE 3. Complementation of the *trmD3* phenotype toward amino acid analogs[a]

Genetic organization of the chromosomal fragment carrying the *trmD* operon on plasmids[d]:	Zone of inhibition (diam in mm)[b]								
	3,4-dehydro-DL-proline (50μg, Pro)			5,5,5-trifluoro-DL-leucine (740μg, Leu)			3-nitro-tyrosine (500μg, Tyr)		
	trmD$^+$	*trmD3*	*trmD3* relative to *trmD*$^+$[c]	*trmD*$^+$	*trmD3*	*trmD3* relative to *trmD*$^+$[c]	*trmD*$^+$	*trmD3*	*trmD3* relative to *trmD*$^+$[c]
No plasmid	31, c	21, st	R	39, st	46, st	S	22, t	23, st	S
pBR322 — Vector —	35, c	32, st	R	49, st	58, st	S	22, t	25, st	S
pMW123 (Tn*5*; Fth, S16, 21K, TrmD, L19, 16KD)	40, c	35, st	R	61, st	68, st	S	26, t	37, st	S
pBY03 (Fth, S16, 21K, TrmD, L19, 16KD)	37, c	40, c	-	58, st	57, st	-	26, t	28, t	-
pBY12 (Fth, S16, 21K, TrmD)	35, c	38, c	-	58, st	60, st	-	26, t	28, t	-

[a] Analogs of Pro (L-2-acetidine-carboxylic acid) and Ser (DL-serine-hydroxamate) have not been tested in this experiment.
[b] The indicated amount of each analog was placed on a 6-mm-diameter paper disk in the center of each agar plate. Zones were scored as c (clear), st (slightly turbid), and t (turbid). Each test was repeated two or three times, and the variation is ±2 mm.
[c] R, more resistant; S, more sensitive; −, equally sensitive.
[d] The *trmD* operon contains the genes for ribosomal protein S16, a 21-kDa protein of unknown function, the tRNA(m^1G-37)methyltransferase, and ribosomal protein L19. The transcript from the *trmD* operon starts from the S16 gene and terminates at the L19 gene. The function of the 16-kDa flanking polypeptide is unknown.

instance, the proline analogs) were observed, probably because these analogs have different targets.

To establish that this phenotype was due to a mutation in the *trmD* gene, we introduced various plasmids, containing the *trmD* operon or parts thereof, into strains GT874 (*trmD*$^+$) and GT875 (*trmD3*). The changed sensitivity of strain GT875 to different analogs was complemented by plasmids that expressed the *trmD*$^+$ gene but not by the vector (pBR322) or by plasmid pMW123, which has a disrupted *trmD* gene (Table 3). Thus, the observed responses to the amino acid analogs 3,4-dehydro-DL-proline, 5,5,5-trifluoro-DL-leucine, and NT of strain GT875 are caused by the *trmD3* mutation and not by some other unidentified mutation.

The *trmD3* mutant is slightly more sensitive than the *trmD*$^+$ strain to the leucine analog trifluoroleucine. This implies that the translational elongation rate for the m^1G-deficient $tRNA^{Leu}_{2,3}$ is unaffected, which is consistent with the normal level of β-isopropylmalate dehydrogenase in the *trmD3* mutant (see above). Deficiency of Ψ in the *hisT* mutant caused a 2.8-fold derepression of the *leuB* gene, and accordingly, this mutant is also more resistant to trifluoroleucine (data not shown and reference 12).

Isolation and genetic characterization of revertants of the *trmD3* strain that are resistant to amino acid analog NT. In an attempt to understand the mechanism by which m^1G deficiency mediates the changed sensitivity to different amino acid analogs, we isolated spontaneous suppressors. The analog chosen was NT, which gave the largest difference between the *trmD3* strain and the *trmD*$^+$ strain. On a citrate minimal plate containing 215 μg of NT per ml, the *trmD3* mutant was unable to grow at 37°C whereas *trmD*$^+$ cells grew normally. Spontaneous NT^r mutants of the *trmD3* strain were isolated at a frequency of 10^{-7}, and 28 such isolates were saved for further analysis.

In all 28 NT^r strains, the *trmD3* mutation was still present, as shown by backcross to strain GT1830 (*tyrA555*::Tn*10 hisO1242 hisD3749*) and identification of the *trmD3* mutation as being cotransducible to *tyrA* and able to suppress the *hisD3749* frameshift mutation. The NT^r revertants therefore harbor extragenic suppressors, designated *sdn-1* to *sdn-28* (*sdn* for suppressor of *trmD3*-mediated NT sensitivity), toward the *trmD3*-mediated NT sensitivity.

To map the locus of these *sdn* suppressors, we first placed a Tn*10* transposon close to one of them. Phage P22 was grown on a pool of cells with Tn*10* randomly inserted in the *S. typhimurium* LT2 chromosome, and NT^r mutant GT2872 (*trmD3 sdn-1*) was used as a recipient. Tetracycline-resistant transductants were selected and screened for NT sensitivity at 37°C. One such NT-sensitive clone, which retained the *trmD3* mutation, was isolated. This Tn*10* insertion, *zgh-2510*::Tn*10*, is about 60% linked to all *sdn* mutations. The location of this Tn*10* was mapped by selecting for loss of tetracycline resistance by using a set of Mud-P22 lysogens that are located at different positions on the *Salmonella* genome (1). This method enabled us to localize the *zgh-2510*::Tn*10* insertion to the interval of 65 to 68.5 min on the linkage map (data not shown). The *sdn-1* mutation was localized more precisely by using phage P22 harboring different transposon insertions in the 65- to 68.5-min region of the chromosome. A 3% cotransduction was found between a Tn*10* in the *argG* gene (*argG1822*::Tn*10*) and *sdn-1*. A three-factor cross experiment showed that the *sdn* gene was located on the opposite side of *argG* compared with *zgi-3717*::MudQ (Fig. 2).

***sdn* is likely the *Salmonella* counterpart of the *E. coli mtr* gene, which codes for the tryptophan-specific permease.** In *S. typhimurium*, the *sdn* gene is 12.5% cotransduced with *argG*, indicating a physical distance of 17 kb between these two genes. In *E. coli*, the *mtr* gene (structural gene for the trypto-

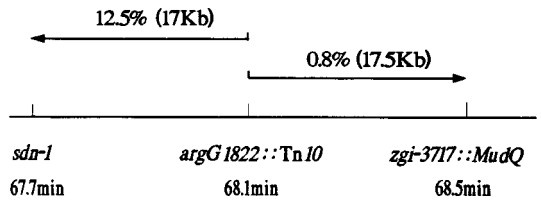

FIG. 2. Genetic organization of the *sdn-1* region.

phan-specific permease) is 60 to 80% cotransduced with the *argG* gene by P1 transduction, which corresponds to a distance of 7 to 15 kb (20, 22). Thus, the *Salmonella sdn* gene and the *E. coli mtr* gene are located at similar distances from the *argG* gene.

The wild-type *mtr* gene is positively regulated by extragenously supplied phenylalanine, and accordingly, an *mtr* mutant grown in phenylalanine fails to show enhanced transport of tryptophan (40). Thus, *mtr* mutants are resistant to the tryptophan analog 5-methyltryptophan in the presence of phenylalanine (20). Similarly, the *sdn-1* mutant was also shown to be resistant to 5-methyltryptophan under the same condition (data not shown). The Mtr-mediated tryptophan uptake was 5.5-fold less in the *sdn-1* mutant than in the wild-type strain (Fig. 3). This response is the same as that in the *mtr* mutant of *E. coli* (40). Even when phenylalanine was added to the growth medium, the level of tryptophan uptake in the *sdn-1* mutant was nearly undetectable, although it was strongly stimulated in the wild-type strain (Fig. 3). Thus, gene localization, resistance to 5-methyltryptophan, and reduced tryptophan uptake strongly suggest that the *sdn* gene is the *Salmonella* counterpart of the *E. coli mtr* gene. Since addition of tryptophan to the NT plate reversed completely the *trmD3*-mediated sensitivity to NT whereas addition of tyrosine did not (Table 4), it is likely that the analog NT is transported by this tryptophan-specific permease Mtr (Sdn) but not by the tyrosine-specific permease TyrP, which is located at min 42 on the *E. coli* chromosome (41). Therefore, the *trmD3*-mediated sensitivity to NT is reversed by mutations in the *sdn* gene most likely because such mutations greatly reduce the uptake of this analog.

***trmD3*-mediated sensitivity to NT is not due to an increased uptake of this analog.** One possible mechanism for the *trmD3*-mediated hypersensitivity to NT may be that the *trmD3* mutation increases the uptake of NT due to a derepressed *mtr* (*sdn*) gene. If so, the *mtr*-mediated tryptophan uptake should be increased in the *trmD3* mutant. However, we found that the transport of [^{3}H]tryptophan was not significantly greater in the *trmD3* mutant than in the wild-type strain (Fig. 3). This was also true in the presence of phenylalanine, which stimulated the uptake of tryptophan to the same extent in both *trmD*$^+$ and *trmD3* cells (Fig. 3). Note that the *trmD*$^+$ cells remained resistant to NT even in the presence of phenylalanine (Table 4), although phenylalanine increased the tryptophan uptake threefold (Fig. 3). These results suggest that an increased activity of NT transport mediated by the *mtr* (*sdn*)-encoded permease in the *trmD3* mutant does not cause its sensitivity to the analog NT.

Serine or adenine renders the wild-type cells sensitive to NT. The amino acid analog NT is chemically similar to tyrosine but transported by the tryptophan-specific permease. Although the *trmD3* mutant is sensitive to NT, it responded similarly to *trmD*$^+$ cells to three other tyrosine (NT, β-DL-hydroxynorvaline, and *m*-fluoro-DL-tyrosine) and three tryptophan (DL-5-fluorotryptophan, DL-7-azatryptophan, and 5-methyltryptophan) analogs. Furthermore, all 28 NT^r revertants analyzed were mutated in the *mtr* (*sdn*) gene, and none had a mutation in the *trpR* gene, which encodes the tryptophan repressor. Thus, derepressed synthesis of tryptophan within the cell does not counteract the NT-sensitive phenotype of the *trmD3* mutant. These results indicate that neither tyrosine nor tryptophan synthesis is severely affected in the *trmD3* mutant.

To gain further information about the mechanism by which NT affects the bacterial cell, we scanned all other amino acids and nucleosides for the ability to rescue the *trmD3* mutant on NT plates. None of these metabolites had such a capacity, but interestingly, serine or adenine rendered the wild-type cells sensitive to NT, reminiscent of the *trmD3*-mediated NT sensi-

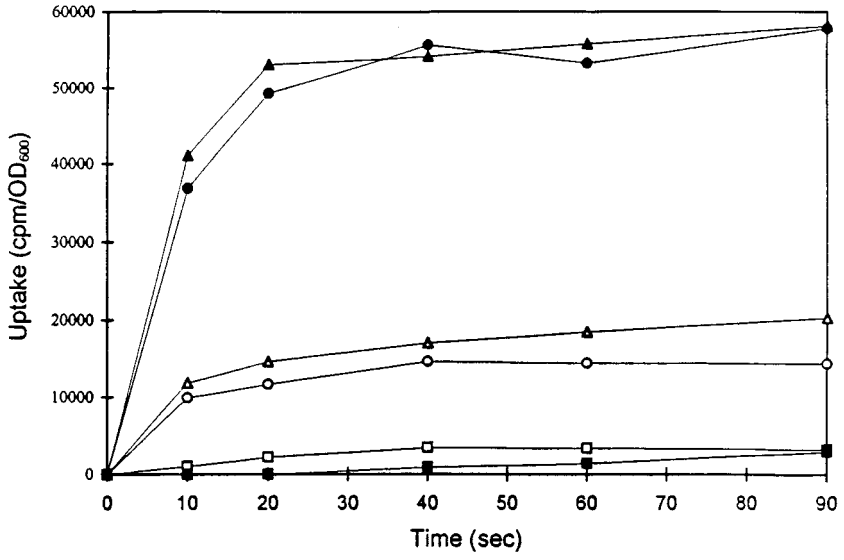

FIG. 3. Uptake of tryptophan by the tryptophan-specific transport system of strains GT874 (*trmD*$^+$), GT875 (*trmD3*), and GT3080 (*sdn-1*). Cells were grown at 37°C in minimal citrate medium (open symbols) and the same medium supplemented with L-phenylalanine (closed symbols). Symbols: ○ and ●, *trmD*$^+$ strain; △ and ▲, *trmD3* strain; □ and ■, *sdn-1* strain.

TABLE 4. Effects of additional amino acids or nucleosides on the sensitivity of cells to NT

Strain	Growth on minimal citrate plate supplemented with[a]:													
	No addition	NT	NT + Tyr	NT + Phe	NT + Trp	Ade	NT + Ade	NT + Ade + B1	Ser	NT + Ser	NT + Ser + Gly	NT + B1	NT + Gly	NT + B1 + Gly
GT847 (*trmD*$^+$)	+	+	+	+	+	+	−	+	+	−	+[b]	+	+	+
GT875 (*trmD3*)	+	−	−	−	+	+	−	−	+	−	−[b]	−	−	−
GT3080 (*sdn-1*)	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] All supplements were added at concentrations recommended by Davis et al. (13) except that the concentration of NT was 225 μg/ml. The plates were incubated at 37°C for 24 h, and the results were scored as + (growth) and − (no growth). B1, thiamine.

[b] After 24 h, no growth was observed for both *trmD*$^+$ and *trmD3* strains. After 48 h (data shown), growth was observed for the *trmD*$^+$ strain but not for the *trmD3* mutant.

tivity (Table 4). It is known that adenine at high concentration can cause thiamine starvation (7). When thiamine was supplied to the plate containing adenine and NT, the *trmD*$^+$ cells grew well. Thus, in *trmD*$^+$ cells, adenine most likely enhanced the toxic effect of NT by imposing a thiamine starvation. Addition of serine also renders the wild-type cells sensitive to NT (Table 4). Glycine could partially reverse the sensitivity to serine plus NT of the *trmD*$^+$ strain. However, addition of no metabolites other than tryptophan, including thiamine and glycine, could suppress the *trmD3*-mediated sensitivity to NT (Table 4).

Several but not all mutations known to induce translational errors also mediate sensitivity to NT. One property mediated by m^{1}G-deficient tRNAs is their ability to suppress certain +1 frameshift mutations (4, 19). Therefore, it was of interest to investigate if other mutations known to induce translational errors also imposed an increased sensitivity to NT. Indeed, several other frameshift suppressors (*sufA6*, *-B2*, *-D46*, *-G70*, and *-J128*) and nonsense suppressors (*supC80*, *-D10*, *-E20*, *-F30*, *-G50*, *-J60*, and *-K599*) also induce, to different degrees, sensitivity to NT (data not shown). However, *sufT621*, which is a derivative of tRNA$_2^{Arg}$ inducing +1 frameshifting at a CCG(U) sequence (35), did not. These results suggest that not all mutations inducing aberrant translation also mediate sensitivity to NT. If there were a correlation between induction of translational errors and NT sensitivity, one would expect that mutations (e.g., *miaA1* and *rpsL107* [5, 34]) that increase translational fidelity would be more resistant to NT than the wild type. However, this is not the case. Both of these mutants were even more sensitive to the analog (data not shown). Also, the *hisT1504* mutation, which decreases the cgr$_p$ (26) similarly to both the *trmD3* and the *miaA1* mutations, showed the same response as the wild type. We therefore conclude that the *trmD3*-mediated sensitivity to NT is not due to a general induction of the synthesis of aberrant proteins but rather is due to a defect induced by aberrant translation on some parts of the intermediary metabolism that are also affected by NT.

DISCUSSION

In *Salmonella* strains, m^{1}G is present in all three tRNA^{Pro} species (CCN codons), in three of the five tRNA^{Leu} species (CUN codons), and in one of the tRNA^{Arg} species (CGG codons). Therefore, 20% of the codons in *lacZ* are read by these tRNAs. Assuming that the reduced cgr$_p$ (from 16 aa/s in *trmD*$^+$ cells to 12 aa/s in *trmD3* cells in MOPS-glucose medium) is due only to the reduced translational efficiency of this subset of tRNAs, the average step time for these tRNA species is increased 3.3-fold. The *hisT* mutant, which lacks the enzyme that converts uridine to pseudouridine in the anticodon region (positions 38, 39, and 40), has a 23% reduction in cgr$_p$ (26). This modification is present in 17 of 40 tRNA chains sequenced (32), and accordingly, 41% of the codons in *lacZ* are read by tRNAs having this modification. Thus, the average step time of these tRNAs was increased 1.8-fold by Ψ deficiency, i.e., much less than by m^{1}G. However, whereas the *hisT* mutation derepresses the *leu* operon 2.8-fold and renders the cells resistant to leucine analogs (12, 27), we did not observe any increased expression of the leucine operon in the *trmD3* mutant compared with the wild type. Furthermore, the *trmD3* mutant was not resistant to leucine analogs. Apparently, the increased step time of tRNA$_{2,3}^{Leu}$ lacking Ψ in the anticodon region is enough to derepress the *leu* operon severalfold, whereas m^{1}G-37 deficiency of the same tRNA$_{2,3}^{Leu}$ leads to an unchanged expression, implying an unaffected step time for these leucine tRNAs. However, in these experiments, a very specific codon context, four consecutive leucine codons, was used to monitor the activities of these tRNAs. Still, these results suggest that whereas Ψ in tRNA$_{2,3}^{Leu}$ affects the rate of decoding CUA and CUC codons, m^{1}G does not. The 3.3-fold increase in the average step time caused by m^{1}G deficiency is probably due to the other five tRNA species. If so, lack of m^{1}G causes a larger (3.8-fold) increase of the average step time of tRNA$_{1,2,3}^{Pro}$, tRNA$_3^{Arg}$, and tRNA$_1^{Leu}$.

The whole translation elongation cycle is composed of several steps, of which the first is the selection step of the binding of the ternary complex (EF-Tu–GTP–aminoacyl-tRNA) to the ribosomal A site. We have previously also shown that whereas m^{1}G deficiency affects the selection of proline and arginine tRNAs (1.9- to 7.1-fold reduction was observed), it does not affect the selection of tRNA$_{2,3}^{Leu}$ (18, 22a). These results support the suggestion presented above that m^{1}G-37 in tRNA$_2^{Leu}$ and tRNA$_3^{Leu}$ has no impact on the abilities of these two tRNAs to read cognate codons, which implies that the quantitative impact of m^{1}G on the cognate codon interaction is tRNA dependent.

Compared with the wild-type strain, the *trmD3* mutant has a changed sensitivity to five different amino acid analogs. In an attempt to dissect the mechanism of how the *trmD3* mutation affects the metabolism of these amino acids, we isolated extragenic suppressors (*sdn*) that reversed the *trmD3*-mediated sensitivity to one of these analogs, NT. We showed that *sdn* likely is the structural gene for the tryptophan-specific permease and that its counterpart in *E. coli* is the *mtr* gene. This gene has previously not been identified in *Salmonella* strains. Since we observed no significant increase in the activity of tryptophan-specific transport in the *trmD3* mutant, the *trmD3*-induced NT sensitivity is not caused by a derepressed transport of the analog. Therefore, some internal metabolic step(s) is affected by the *trmD3* mutation.

In an attempt to understand the mechanism by which an undermodified tRNA can induce sensitivity to NT, we noticed that even the wild-type cells were sensitive to this analog if adenine or serine was added. Each of these metabolites is

known to influence several pathways, including thiamine biosynthesis and C_1 metabolism, respectively. However, thiamine and glycine, which suppressed the effects imposed on *trmD*$^+$ cells by serine and adenine, respectively, did not suppress the *trmD3*-mediated sensitivity to NT. Thus, serine- or adenine-mediated NT sensitivity may be different from that mediated by the *trmD3* mutation. Alternatively, whereas adenine or serine affects only one or a few specific steps in some metabolic pathways, the *trmD3* mutation may affect several steps in such a way that supplementation of those metabolites is insufficient to suppress the *trmD3*-mediated defect. A clear demonstration of the mechanism behind these phenomena requires the characterization of suppressor mutations in those pathways in which m^1G exerts its effect. The characterization of one class of suppressors (*sdn*) as being defective in the uptake of the analog allows us to construct strains containing two or more copies of the *sdn* gene, which will facilitate the isolation of suppressors in the genes of interest.

The *trmD3* mutant oxidizes several carbon sources more efficiently than the wild-type strain. This finding suggests that some of the enzymes involved in the metabolism of the carbon compounds are derepressed in this mutant. Alteration in carbon oxidation has also been observed in an *miaA* mutant (33), which is deficient in ms^2i^6A in its tRNAs. All three mutations, *trmD3*, *hisT1504*, and *miaA*, enhance or decrease the metabolism of certain carbon compounds in a complex pattern; some of the mutations affect similarly the oxidation of the same carbon compound (e.g., D-malic acid in the case of *trmD3* and *hisT1504* [Fig. 1] and sucrose and lactulose in the case of *trmD3* and *miaA* [33]), whereas in several cases, the different mutations affect the oxidation of a unique carbon compound. Still, these results together with the *trmD3*-mediated responses to several amino acid analogs show that tRNA modification may influence, by a yet unknown mechanism but most likely by an effect on translation, specifically the level of enzymes in the central and intermediary metabolic pathways and thereby exert a regulatory role in cellular metabolism as has been suggested earlier (8, 17).

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REFERENCES

1. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mud-P22 prophages. J. Bacteriol. **174:**1673–1681.
2. **Björk, G. R.** 1995. Biosynthesis and function of modified nucleosides, p. 165–205. *In* D. Söll and U. L. RajBhandary (ed.), tRNA: structure, biosynthesis, and function. American Society for Microbiology, Washington, D.C.
3. **Björk, G. R., and F. C. Neidhardt.** 1975. Physiological and biochemical studies on the function of 5-methyluridine in the transfer ribonucleic acid of *Escherichia coli*. J. Bacteriol. **124:**99–111.
4. **Björk, G. R., P. M. Wikström, and A. S. Byström.** 1989. Prevention of translational frameshifting by the modified nucleoside 1-methylguanosine. Science **244:**986–989.
5. **Bouadloun, F., T. Srichaiyo, L. A. Isaksson, and G. R. Björk.** 1986. Influence of modification next to the anticodon in tRNA on codon context sensitivity of translational suppression and accuracy. J. Bacteriol. **166:**1022–1027.
6. **Brenner, M., and B. N. Ames.** 1972. Histidine regulation in *Salmonella typhimurium*. IX. Histidine transfer ribonucleic acid of the regulatory mutants. J. Biol. Chem. **247:**1080–1088.
7. **Brook, M. S., and B. Magasanik.** 1954. The metabolism of purines in *Aerobacter aerogenes*: a study of purineless mutants. J. Bacteriol. **68:**727–733.
8. **Buck, M., and B. N. Ames.** 1984. A modified nucleotide in tRNA as a possible regulator of aerobiosis: synthesis of cis-2-methyl-thioribosylzeatin in the tRNA of *Salmonella*. Cell **36:**523–531.
9. **Buck, M., and E. Griffiths.** 1982. Iron mediated methylthiolation of tRNA as a regulator of operon expression in *Escherichia coli*. Nucleic Acids Res. **10:**2609–2624.

9a. **Calvo, J. M.** Personal communication.

10. **Calvo, J. M., M. Freundlich, and H. E. Umbarger.** 1969. Regulation of branched-chain amino acid biosynthesis in *Salmonella typhimurium*: isolation of regulatory mutants. J. Bacteriol. **97:**1272–1282.
11. **Carter, P. W., D. L. Weiss, H. L. Weith, and J. M. Calvo.** 1985. Mutations that convert the four leucine codons of the *Salmonella typhimurium leu* leader to four threonine codons. J. Bacteriol. **162:**943–949.
12. **Cortese, R., R. Landsberg, R. A. Haar, H. E. Umbarger, and B. N. Ames.** 1974. Pleiotropy of *hisT* mutants blocked in pseudouridine synthesis in tRNA: leucine and isoleucine-valine operons. Proc. Natl. Acad. Sci. USA **71:**1857–1861.
13. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Gemmill, R. M., S. R. Wessler, E. B. Keller, and J. M. Calvo.** 1979. *leu* operon of *Salmonella typhimurium* is controlled by an attenuation mechanism. Proc. Natl. Acad. Sci. USA **76:**4941–4945.
15. **Gowrishankar, J., and J. Pittard.** 1982. Regulation of phenylalanine biosynthesis in *Escherichia coli* K-12: control of transcription of the *pheA* operon. J. Bacteriol. **150:**1130–1137.
16. **Gutnick, D., J. M. Calvo, T. Klopotowski, and B. N. Ames.** 1969. Compounds which serve as the sole source of carbon or nitrogen for *Salmonella typhimurium* LT-2. J. Bacteriol. **100:**215–219.
17. **Hagervall, T. G., and G. R. Björk.** 1984. Undermodification in the first position of the anticodon of *supG*-tRNA reduces translational efficiency. Mol. Gen. Genet. **196:**194–200.
18. **Hagervall, T. G., B. Esberg, J. Li, T. M. F. Tuohy, J. F. Atkins, J. F. Curran, and G. R. Björk.** 1993. Functional aspects of three modified nucleosides, Ψ, ms^2io^6A, and m^1G, present in the anticodon loop of tRNA, p. 67–78. *In* K. H. Nierhaus (ed.), The translational apparatus. Plenum Press, New York.
19. **Hagervall, T. G., T. M. Tuohy, J. F. Atkins, and G. R. Björk.** 1993. Deficiency of 1-methylguanosine in tRNA from *Salmonella typhimurium* induces frameshifting by quadruplet translocation. J. Mol. Biol. **232:**756–765.
20. **Hiraga, S., K. Ito, T. Matsuyama, H. Ozaki, and T. Yura.** 1968. 5-Methyltryptophan-resistant mutations linked with the *argG* marker in *Escherichia coli*. J. Bacteriol. **96:**1880–1881.

20a. **Hjalmarsson, K. J., P. M. Wikström, and G. R. Björk.** Unpublished data.

21. **Kalnins, A., K. Otto, U. Rüther, and B. Müller-Hill.** 1983. Sequence of the *lacZ* gene of *Escherichia coli*. EMBO J. **2:**593–597.
22. **Kitakawa, M., E. R. Dabbs, and K. Isono.** 1979. Genes coding for ribosomal protein S15, L21, and L27 map near *argG* in *Escherichia coli*. J. Bacteriol. **138:**832–838.

22a. **Li, J., J. Curran, and G. R. Björk.** Unpublished data.

23. **Limbach, P. A., P. F. Crain, and J. A. McCloskey.** 1994. Summary: the modified nucleosides of RNA. Nucleic Acids Res. **22:**2183–2196.
24. **Neidhardt, F. C., P. L. Bloch, S. Pedersen, and S. Reeh.** 1977. Chemical measurements of steady-state levels of ten aminoacyltransfer ribonucleic acid synthetases in *Escherichia coli*. J. Bacteriol. **129:**378–387.
25. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
26. **Palmer, D. T., P. H. Blum, and S. W. Artz.** 1983. Effects of the *hisT* mutation of *Salmonella typhimurium* on translation elongation rate. J. Bacteriol. **153:** 357–363.
27. **Rizzino, A. A., R. S. Bresalier, and M. Freundlich.** 1974. Derepressed levels of the isoleucine-valine and leucine enzymes in *hisT1504*, a strain of *Salmonella typhimurium* with altered leucine transfer ribonucleic acid. J. Bacteriol. **117:**449–455.
28. **Schleif, R., W. Hess, S. Finkelstein, and D. Ellis.** 1973. Induction kinetics of the L-arabinose operon of *Escherichia coli*. J. Bacteriol. **115:**9–14.
29. **Schmieger, H.** 1972. Phage P22-mutant with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.
30. **Searles, L. L., and J. M. Calvo.** 1988. Permeabilized cell and radiochemical assays for β-isopropylmalate dehydrogenase. Methods Enzymol. **166:**225–229.
31. **Searles, L. L., S. R. Wessler, and J. M. Calvo.** 1983. Transcription attenuation is the major mechanism by which the *leu* operon of *Salmonella typhimurium* is controlled. J. Mol. Biol. **162:**377–394.
32. **Steinberg, S., A. Misch, and M. Sprinzl.** 1993. Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res. **21:**3011–3015.
33. **Tsui, H.-C. T., H.-C. E. Leung, and M. E. Winkler.** 1994. Characterization of broadly pleiotropic phenotypes caused by an *hfg* insertion mutation in *Escherichia coli* K-12. Mol. Microbiol. **13:**35–49.
34. **Tubulekas, I., R. H. Buckingham, and D. Hughes.** 1991. Mutant ribosomes can generate dominant kirromycin resistance. J. Bacteriol. **173:**3635–3643.
35. **Tuohy, T. M. F., Z. Li, J. F. Atkins, and M. P. Deutscher.** 1994. A functional mutant of $tRNA_2^{Arg}$ with ten extra nucleotides in its TFC arm. J. Mol. Biol.

235:1369–1376.
36. **Turnbough, C. L., Jr., R. J. Neill, R. Landsberg, and B. N. Ames.** 1979. Pseudouridylation of tRNAs and its role in regulation in *Salmonella typhimurium*. J. Biol. Chem. **254:**5111–5119.
37. **Varshney, U., C. P. Lee, and U. L. RajBhandary.** 1991. Direct analysis of aminoacylation levels of tRNAs *in vivo*. J. Biol. Chem. **266:**24712–24718.
38. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
39. **Wessler, S. R., and J. M. Calvo.** 1981. Control of *leu* operon expression in *Escherichia coli* by a transcription attenuation mechanism. J. Mol. Biol. **149:** 579–597.
40. **Whipp, M. J., and A. J. Pittard.** 1977. Regulation of aromatic amino acid transport systems in *Escherichia coli* K-12. J. Bacteriol. **132:**453–461.
41. **Wookey, P. J., J. Pittard, S. M. Forrest, and B. E. Davidson.** 1984. Cloning of the *tyrP* gene and further characterization of the tyrosine-specific transport system in *Escherichia coli* K-12. J. Bacteriol. **160:**169–174.
42. **Yanofsky, C., and L. Soll.** 1977. Mutations affecting $tRNA^{Trp}$ and its charging and their effect on regulation of transcription termination at the attenuator of the tryptophan operon. J. Mol. Biol. **113:**663–677.