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Pleiotropic Effects Induced by Modification Deficiency Next to the Anticodon of tRNA from *Salmonella typhimurium* LT2

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A strain of *Salmonella typhimurium* LT2 was isolated, which harbors a mutation acting as an antisuppressor toward an amber suppressor derivative, *supF30*, of $tRNA_1^{Tyr}$. The mutant is deficient in *cis*-2-methylthioribosylzeatin[N^6-(4-hydroxyisopentenyl)-2-methylthioadenosine, ms^2io^6A], which is a modification normally present next to the anticodon (position 37) in tRNA reading codons starting with uridine. The gene *miaA*, defective in the mutant, is located close to and counterclockwise of the *purA* gene at 96 min on the chromosomal map of *S. typhimurium* with the gene order *mutL miaA purA*. Growth rate of the mutant was reduced 20 to 50%, and the effect was more pronounced in media supporting fast growth. Translational chain elongation rate at 37°C was reduced from 16 amino acids per s in the wild-type cell to 11 amino acids per s in the *miaA1* mutant in the four different growth media tested. The cellular yield in limiting glucose, glycerol, or succinate medium was reduced for the *miaA1* mutant compared with wild-type cells, with 49, 41, and 57% reductions, respectively. The *miaA1* mutation renders the cell more sensitive or resistant toward several amino acid analogs, suggesting that the deficiency in ms^2io^6A influences the regulation of several amino acid biosynthetic operons. We suggest that $tRNA^{Phe}$, lacking ms^2io^6A, translates a UUU codon in the early histidine leader sequence with lowered efficiency, leading to repression of the *his* operon.

tRNAs from eucaryotes, eubacteria, and archeabacteria contain several different modified nucleosides, which are derivatives of the four major nucleosides (3). Their formation is catalyzed by highly specific enzymes, which operate during the tRNA maturation process after the primary transcript is made. The nucleosides in the anticodon region are especially prone to being specifically modified, and many different derivatives are found in position 34 (the wobble position) and at the position next to the 3′ side of the anticodon (position 37) (3).

The function of the modified nucleosides was for a long time obscure, but recent evidence indicates that some of them in the anticodon region may play a vital role in codon-anticodon interaction. Lack of pseudouridine (Ψ) in the anticodon stem reduces the efficiency of a suppressor tRNA and the polypeptide chain elongation rate and influences the synthesis of several amino acids (5, 14, 25, 35, 45). Undermodification in the wobble position also reduces the efficiency of suppression (13, 23, 44). Deficiency in isopentenyladenosine (i^6A37), normally located next to the 3′ side of the anticodon in some tRNAs from yeast, reduces the efficiency of a suppressor tRNA (24, 29). Gefter and Russel (18) have shown in vitro that 2-methylthio-N^6-isopentenyladenosine (ms^2i^6A) is important in the binding of tRNA to the ribosome. A mutant (*miaA*, formerly called *trpX*) of *Escherichia coli* which is deficient in the synthesis of ms^2i^6A37 reduces the efficiency of some suppressor tRNAs and derepresses several operons (6, 8, 36, 46, 49). Thus, modifications in the anticodon region seem to be directly involved in codon-anticodon interaction.

Mutants which are defective in tRNA modification are desirable tools for studying translational fidelity and cellular physiology. We found that *Salmonella typhimurium* strains containing a mutation which removes the *his* attenuator (*hisO1242*) are sensitive to increased salt concentration. The reason for this salt sensitivity is not known but may be due to overproduction of *hisH* and *hisF* gene products. An amber mutation, *hisD6404*, in a gene upstream of *hisH hisF* renders the cell able to grow on high-salt medium provided that the medium contains histidine. Introducing a strong amber suppressor like *supF30* makes the cell salt sensitive. We selected salt-resistant mutants of such a strain (*hisO1242 hisD6404 supF30*). One such mutant (*miaA1*) is deficient in 2-methylthio-N^6-(4-hydroxyisopentenyl)adenosine (ms^2io^6A37) in its tRNA. tRNA from *S. typhimurium* has ms^2io^6A37 instead of ms^2i^6A37, which is present in tRNA from *E. coli* (9). Our results demonstrate that the presence of ms^2io^6A37 is very important for efficient translation, and we also show that tRNA deficient in ms^2io^6A37 induces strong pleiotropic effects on cell physiology such as reductions in growth rate, cellular yield, and polypeptide chain elongation rate in vivo and different responses to several amino acid analogs. In the accompanying paper (6), it is also shown that ms^2io^6A is important in sensing the nucleotides surrounding the codon.

MATERIALS AND METHODS

Bacteria, media, and growth conditions. The bacterial strains used were all derivatives of *S. typhimurium* LT2 (Table 1). Strain GT513, used in transductional mapping of the *miaA1* gene (cf. Table 2), was constructed by first introducing *zjg-1850*::Tn*10* by transduction with strain GT468 as the donor and strain TR5688 (*purA155 rpsL1*) as the recipient. The resulting recombinant, GT507 (*purA155 rpsL1 zjg-1850*::Tn*10*), was then used as the recipient, and *amtB211*::Mu d8 was introduced by using strain GT467 as the donor.

As a complex medium, 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

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TABLE 1. *S. typhimurium* strains used

Strain	Genotype	Source
LT2	Prototrophic	John Roth
GT248	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supF30 hisT1504 miaA1*	This work
GT397	*hisO1242 hisD6404 leu-414 supF30 hisT1504 zej-636*::Tn*5 mutL*::Tn*10 purA155*	This work
GT467 (EH37)	*amtB211*::Mu d8	J. Roth and D. Hillyard
GT468 (EH10)	*hisG1102 amtB(Ha26) zjg-1850*::Tn*10*	J. Roth and D. Hillyard
GT507	*purA155 rpsL1 zjg-1850*::Tn*10*	This work
GT513	*amtB211*::Mu d8 *purA155 zjg-1850*::Tn*10 rpsL1*	This work
GT518	*purA874*::Tn*10*	This work
GT522	Prototrophic, transductional pair to GT523	This work
GT523	*miaA1*	This work
GT530	*pro-662*::Tn*10*/F′ *lac*$^+$ *proAB*$^+$	This work
GT531	*pro-662*::Tn*10 miaA1*/F′ *lac*$^+$ *proAB*$^+$	This work
TR5688	*purA155 rpsL1*	John Roth
TT627	*pyrC7 rpsL1*/F′ ts114 *lac*$^+$ *zzf-20*::Tn*10*	John Roth
TT628	*pyrC7 rpsL1*/F′ ts114 *lac*$^+$ *zzf-21*::Tn*10*	John Roth
TT4427	*hisO1242 hisD6404 leu-414 zeb-618*::Tn*10 zej-636*::Tn*5*	John Roth
TT4279	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supF30 hisT1504*	John Roth
TT4278	*hisO1242 hisD6404 leu-414 zej-636*::Tn*5 supF30*	John Roth
TT273	*purA874*::Tn*10*	John Roth
GW1714	*hisG46 mutL111*::Tn*10*	G. C. Walker

were provided at concentrations recommended by Davis et al. (15). For genetic and suppression experiments, we used the medium E described by Vogel and Bonner (48). NCE medium was medium E without $(NH_4)_2SO_4$ and sodium citrate (15). Growth rate studies were performed as described earlier (4) with morpholinepropanesulfonic acid (MOPS) medium (33). Following the completion of each growth rate experiment, the culture was tested for homogeneity. In no case were any fast-growing pseudorevertants observed. With GT523 (*miaA1*) as the donor in a cross with GT518 (*purA874*::Tn*10*) only two classes of Pur$^+$ transductants were observed. One was identical to GT523, and the other was identical to GT522 (*miaA*$^+$) as judged by growth on rich medium at 42.5°C. Thus, strain GT523 (*miaA1*) does not harbor any extragenic mutation compensating for the effect of the *miaA1* mutation. The concentrations of antibiotics in rich medium were: tetracycline, 15 μg/ml; ampicillin, 30 μg/ml. In minimal medium, tetracycline was used at 2.5 μg/ml. The cellular yield in different carbon sources was determined by growing the cells during vigorous aeration (4) into stationary phase in limiting amounts of each carbon source and then measuring the optical density of the culture at 420 nm. The medium used was the MOPS minimal medium described by Neidhardt et al. (33) supplemented with different amounts of glucose, glycerol, or succinate.

Genetic techniques. Transductions were performed with a derivative of phage P22 containing the mutations HT105/I, which leads to increased frequency of generalized transduction (42), and *int-201*, which prevents formation of stable lysogens (43). Transductions were performed as previously recommended (15), and the recombinants were made phage free by streaking nonselectively on green indicator plates (11). Phage-free clones were then checked for phage sensitivity by cross-streaking against P22 H5 (clear-phage mutant) phage. Conjugal transfer of F′ plasmid was performed by printing F′ plasmid-carrying donor cells onto recipient cells directly on agar plates containing selective growth medium.

Determination of sensitivity toward different amino acid analogs. For shift-down conditions, strains GT522 (*miaA*$^+$) and GT523 (*miaA1*) were grown in rich medium overnight. 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 were placed on the surfaces of the plates, which were incubated at 37°C for 24 h before being scored.

Experiments with no shift in growth conditions were performed as described above, but NCE medium and plates containing 0.2% glycerol and 5 mM $(NH_4)_2SO_4$ were used. Additional analogs (except those shown in Table 5) to which the *miaA1* mutant is relatively more sensitive on shift-down include (analogous amino acid) L-2-acetidinecarboxylic acid (Pro), 1,2,4-triazole (Cys), S-2-aminomethylcysteine (Lys), and thioproline (Pro). Analogs tested to which both strains are equally sensitive on shift-down include azaserine (Gln), L-glutamic acid-γ-hydrazide (Gln), L-methionine-DL-sulfoximine (Gln), DL-methionine hydroxamate (Met), *m*-fluorophenylalanine (Phe), β(2-thienyl)-DL-alanine (Phe), DL-β-3-thienylalanine (Phe), 3,4-dehydro-DL-proline (Pro), thiazolidine-4-carboxylic acid (Pro), DL-7-azatryptophan (Trp), DL-5-fluorotryptophan (Trp), 5-methyl-DL-tryptophan (Trp), 3-aminotyrosine (Tyr), and *m*-fluorotyrosine (Tyr). All analogs were obtained from Sigma Chemical Co., St. Louis, Mo.

Preparation of tRNA for two-dimensional thin-layer chromatography. Cells were grown in 12.5 ml of MOPS medium containing 0.081 mM K_2SO_4 and supplemented with glucose (0.4%), histidine (0.1 mM), and leucine (0.3 mM). Radioactive sulfate (150 μCi/ml final concentration) was added, and the cells were grown from 4×10^7 to 6×10^8 cells per ml. Cells were harvested on ice and washed once with 10 mM Tris hydrochloride (pH 8.0) containing 10 mM magnesium acetate. tRNA was purified as described earlier (2), but no carrier cells were added. Digestion to nucleosides was essentially as described by Rogg et al. (38) with modifications described in detail earlier (2). The mixture was subjected to two-dimensional thin-layer chromatography with solvents as recommended by Rogg et al. (38). The first dimension was run for 18 h, allowing the front to migrate 27 to 30 cm. The nucleoside mixtures were also subjected to high-pressure liquid chromatographic analysis as described by Buck et al. (9).

Measurements of β-galactosidase activity. Two to four independent clones of strains containing an F′ plasmid with a *lacI-lacZ* fusion with or without nonsense mutations in the

lacI part (obtained from J. Miller, Los Angeles, Calif.) were grown in medium E supplemented with glucose, histidine, and leucine at the indicated temperatures from 4×10^7 to about 2×10^8 cells per ml. Two different amounts of cells were analyzed for β-galactosidase activity as described by Miller (30) or Putnam and Koch (37). Each value is the average of two different amounts of enzyme from each culture and at least two independent clones. The values are given as percent specific activity relative to that of the F′ plasmid containing the parental *lacI-lacZ* fusion, without nonsense mutations, in the same genetic background. This eliminates general effects on growth rate by the *miaA1* mutation. Thus, the relative values shown represent a quantitative measurement of readthrough at the nonsense codons.

RESULTS

Isolation of a mutant with reduced efficiency of *supF30*-mediated suppression. *S. typhimurium* strains with a derepressed histidine operon (e.g., containing the *hisO1242* deletion, which removes the attenuator) have a wrinkled colony morphology on agar plates containing high concentrations of glucose (2%). This phenomenon is due to the high level of proteins made from the *hisH* and *hisF* genes (32). We found that such strains are unable to grow on minimal agar plates containing 0.3 M NaCl. The mechanism behind this salt sensitivity is not clear, but overproduction of proteins from genes *hisH* and *hisF* may be one reason. A nonsense mutation [*hisD6404*(Am)] in the promoter-proximal *hisD* gene exerts a polar effect on the expression of the promoter-distal genes *hisH hisF*. Therefore, such strains are able to grow on plates containing 0.3 M NaCl and histidine. If a strong translational suppressor like *supF30* is introduced, the strain becomes salt sensitive. Thus, strain TT4279 (*hisO1242 hisD6404*(Am) *leu-414*(Am) *supF30*) is salt sensitive, has a wrinkled phenotype on agar plates containing 2% glucose, and is Leu$^+$ and His$^+$. Spontaneous salt-resistant mutants of strain TT4279 were isolated (frequency, 9×10^{-5}) at 30°C on glucose minimal plates containing histidine, leucine, and 0.3 M NaCl. Some of the mutants isolated should be salt resistant owing to strong polar mutations upstream of *hisH hisF* or deletions of at least the *hisH hisF* genes. Such mutants should still be Leu$^+$ owing to the presence of an active *supF30* allele. Others having an His$^-$ Leu$^-$ phenotype may either be mutated in a gene(s) influencing the activity of the *supF30* tRNA or have lost the *supF30* allele. These two groups could be distinguished, since mutants of the former type might show some residual suppressor activity, especially at low temperature. Of 104 salt-resistant clones, one mutant (strain GT248) was isolated which was His$^{(+)}$ Leu$^{(+)}$ at 30°C but His$^-$ Leu$^-$ at 37°C. Strain GT248 was therefore a candidate to harbor an antisuppressor to *supF30* (designated *asf-1*). By using strain GT248 (*supF30 hisD6404 leu-414 asf-1*) as the donor and strain TT4427 (*hisO1242 hisD6404 leu-414 zeb-618*::Tn*10* *zej-636*::Tn*5*) as the recipient and selecting for His$^+$ transductants, we could demonstrate that the *supF30* allele was still present in strain GT248, since such transductants simultaneously become Leu$^+$ at 37°C. Thus, strain GT248 must contain an external antisuppressor (*asf*) toward *supF30*. Below we show that *asf-1* influences the synthesis of ms^2io^6A37 in a way similar to *miaA* (*trpX*) in *E. coli*. We therefore designate this new mutation of *S. typhimurium* *miaA1*.

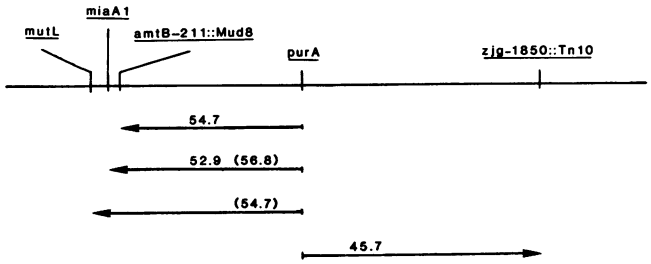

FIG. 1. Gene organization in the *miaA1* region of the *S. typhimurium* chromosome. The data are taken from Table 2, except for the cotransduction frequencies in parentheses. These are from an experiment with strain GT248 (*miaA1*) as the donor and strain GT397 (*hisO1242 hisD6404 leu-414 supF30 purA155 mutL111*::Tn*10*) as the recipient. A total of 600 Pur$^+$ transductants were scored. Mut$^+$ (donor), MiaA$^+$ (recipient), and Pur$^+$ (donor) transductants were only 0.7%, showing that the *miaA* gene is located between *mutL* and *purA*. The latter is located at min 96 of the chromosomal map of *S. typhimurium*.

TABLE 2. Transductional mapping of the *miaA1* gene[a]

miaA1	amtB211::*Mu* d8	*purA*	*zjg-1850*::*Tn10*	No.[b]	% of total
D	D	D	D	135	30
D	R	D	D	1	0.2
R	D	D	D	2	0.4
R	R	D	D	68	15.1
D	D	D	R	102	22.7
D	R	D	R	0	0
R	D	D	R	7	1.6
R	R	D	R	135	30

[a] Phage P22 was grown on strain GT248 (*miaA1*), and strain GT513 (*amtB211*::Mu d8 *purA155 zjg-1850*::Tn*10*) was used as the recipient. Pur$^+$ transductants were selected, and donor (D) and recipient (R) phenotypes were scored. The MiaA$^-$ phenotype was scored as inability to grow at 42°C on rich medium. This phenotype is easy to score provided that the recipient strain also has the *rpsL1* mutation as in strain GT513. The *rpsL* gene is located far from the *miaA* region.

[b] Total number of transductants, 450.

The *miaA* gene is located close to the *purA* gene at minute 96 of the *S. typhimurium* chromosome. To score the MiaA phenotype, it is necessary to have both the *supF30* and the *hisD6404* alleles present in the resulting recombinants. Therefore, conventional mapping becomes difficult to interpret. We chose an alternative approach by first placing a transposon, Tn*10*, close to the *miaA* gene and then mapping the resulting transposon insertion. Phage P22 was grown on pools of randomly inserted Tn*10* in the *S. typhimurium* LT2 chromosome, and strain GT248 was used as the recipient. Tetracycline-resistant (Tet^r) transductants were selected which had become simultaneously His$^+$ Leu$^+$ at 37°C. Such transductants are likely to have the transposon located close to the *miaA* gene and not close to the *his* operon. This was verified by transduction with the parental strain GT248 as the recipient. One of these Tn*10* insertions was located by forcing an F′ plasmid (strains TT627 and TT628), which is temperature sensitive for replication and carries a Tn*10* insertion, to integrate by means of homology at the site of the chromosomal Tn*10* insertion (12). The origin of chromosomal transfer of the resulting Hfr strain was found to be close to *purA* by interrupted mating experiments using a set of defined auxotrophic recipient strains. A genetically characterized Tn*10* insertion *zjg-1850*::Tn*10* located close to *purA* (John Roth and D. Hillyard, University of Utah, Salt Lake City) was used to show that the *miaA* gene is located close to *purA* but on the opposite side compared with

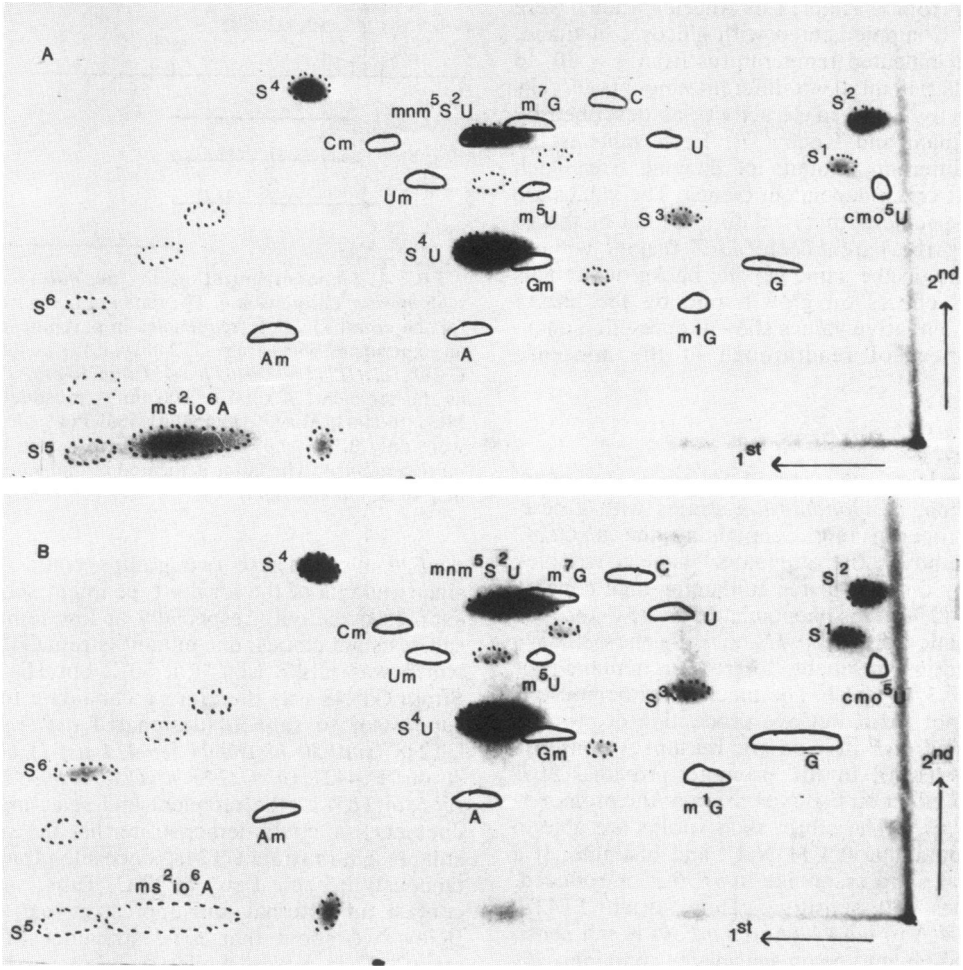

FIG. 2. Two-dimensional thin-layer chromatography of ^{35}S-labeled nucleosides from tRNA of parent strain TT4279 (A) and *miaA1* mutant strain GT248 (B) grown at 37°C. The positions of the radioactive nucleosides were visualized by autoradiography. Broken lines indicate locations of compounds where no markers were available at the time of the experiment. The chromatographic procedure was that of Rogg et al. (38). Solvent 1, isobutyric acid-isopropanol-concentrated ammonia-water (72:27.5:2.5:25, vol/vol). Solvent 2, saturated ammonium sulfate–0.1 M sodium acetate (pH 6.0)–isopropanol (79:19:2, vol/vol).

zjg-1850::Tn*10* (Table 2). Other experiments showed that the *miaA1* mutation as well as the *amtB211*::Mu d8 insertion are located between the *mutL* and *purA* genes (Fig. 1). Since *mutL* is suggested to be counterclockwise of *purA* (40), this result is consistent with a location of *miaA* counterclockwise to *purA*. Thus, the gene order in the 96-min region is *mutL miaA amtB purA zjg-1850*::Tn*10* (Fig. 1).

Mutant GT248 lacks ms^{2}io^{6}A37 in its tRNA. Cells were grown in the presence of $^{35}SO_4$, and tRNA was prepared. Following digestion to nucleosides, two-dimensional thin-layer chromatographic analysis of the ^{35}S-labeled nucleosides was performed. tRNA from strain GT248 lacks a thiolated compound designated ms^{2}io^{6}A37 (Fig. 2). Similar analyses of *E. coli* tRNA and tRNA from an *E. coli miaA* mutant showed that the compound missing from tRNA of strain GT248 has similar but not identical chromatographic properties to those of ms^{2}i^{6}A37 in tRNA from *E. coli* (data not shown). Buck et al. (9) have shown by high-pressure liquid chromatographic analyses that *S. typhimurium* tRNA contains ms^{2}io^{6}A37 in its tRNA instead of ms^{2}i^{6}A37. Using their high-pressure liquid chromatographic method, we also analyzed ^{35}S-labeled nucleosides from tRNAs from strains LT2 and GT248. Similar experiments were done with *E. coli miaA* to identify ms^{2}i^{6}A37, since no marker for either ms^{2}i^{6}A37 or ms^{2}io^{6}A37 was available to us. The results indicate that the compound missing from tRNA from strain GT248 was not ms^{2}i^{6}A37 but ms^{2}io^{6}A37 (data not shown). Analyses of total unlabeled tRNA in a similar way showed that *miaA1* of *S. typhimurium* did not contain an elevated level of the intermediate ms^{2}A37 or io^{6}A37. (M. Buck, personal communication). Thus, it is likely that tRNA from the *miaA* mutant has an A37 instead of ms^{2}io^{6}A37. Since this has been shown to be true for the *miaA* mutant of *E. coli*, we have designated the mutation in strain GT248 *miaA1*.

Strain GT248 retains some suppressing activity at 30°C as judged by the His$^+$ and Leu$^+$ phenotypes at 30°C compared with 37°C (see also below). Therefore, it was of interest to determine the level of ms^{2}io^{6}A37 in tRNA from cells grown at 30°C. Table 3 shows that tRNA from strain GT248 grown at 30 and 37°C is deficient in ms^{2}io^{6}A37 to the same extent at both temperatures. Thus, the remaining suppressing activity at 30°C in strain GT248 is not due to residual synthesis of ms^{2}io^{6}A37.

A mutant lacking ms^{2}io^{6}A37 in its tRNA has reduced growth rate, polypeptide chain elongation rate, and cellular yield. Strains GT522 (*miaA*$^+$) and GT523 (*miaA1*) were grown

under steady-state conditions in different media at 37°C. In all growth media used, a large (up to 50%) reduction in growth rate for the mutant *miaA1* was observed (Table 4). It also appeared that the reduction in growth rate was larger in media supporting faster growth. To test whether lack of ms^2io^6A37 reduces the chain growth rate of polypeptides (cgr_p), the time required to produce β-galactosidase after induction of the *lac* operon from the uninduced level was determined (Fig. 3). 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, a precise time of completion of the first β-galactosidase subunit after induction can be made (Fig. 3; 41). Using the known chain length of 1,023 amino acids (26) for the β-galactosidase subunit, cgr_p was calculated. The cgr_p (16 amino acids per s) in the wild-type cell was invariant with growth rate. This was also true for the *miaA1* mutant, but the cgr_p was reduced to 11 amino acids per s.

The cellular yield was determined for the *miaA1* mutant compared with wild-type cells in limiting amounts of carbon sources. The reduction for the *miaA1* mutant was 49% in glucose, 41% in glycerol, and 57% in succinate-limiting medium (Fig. 4).

Lack of ms^2io^6A37 in tRNA induces differential effects of amino acid analogs. It is known that undermodified tRNA can influence regulation of the synthesis of amino acids (8, 21, 45, 46, 49). The presence of the *miaA1* mutation influences responses to several amino acid analogs as well as to fluoroacetic acid (Table 5). The response to the latter compound has been reported earlier (7). It should be noted that the *miaA1* mutant can be either more resistant (Asn, Met, Ser, Tyr, and Phe analogs) or more sensitive (His, Ile, Leu, Thr, and acetate analogs) to different analogs than the $miaA^+$ strain. Thus, these results suggest that the *miaA1* mutation quantitatively changes the synthesis of many amino acids.

Lack of ms^2io^6A37 reduces the activity of suppressor tRNAs. A set of suppressor strains was constructed in which all strains were *pro-688*::Tn*10* and with different allelic states of *miaA*. Into these strains a set of F′ plasmids was introduced which have the *lacI* gene fused in frame to the *lacZ* gene in such a way that a hybrid protein consisting of a part of the *lac* repressor and a part of β-galactosidase is produced. Such a hybrid protein has β-galactosidase activity. In these F′ plasmids, nonsense mutations in the *lacI* part are available (31). Thus, at these nonsense sites translation stops and little or no β-galactosidase hybrid protein is produced. However, if such an F′ plasmid is introduced into a bacterial strain containing a suppressor tRNA, translation continues beyond the nonsense mutation and enters the *lacZ* gene. Thus, the β-galactosidase activity in the strain becomes a direct measurement of readthrough at the nonsense site.

Strain GT248 (*hisD6404*(Am) *leu-414*(Am) *supF30 miaA1*) is His^+ Leu^+ at 30°C but His^- Leu^- at 37°C. To measure more quantitatively the efficiency of suppression, we measured the readthrough of UAG present in the *lacI* part of the fused *lacI-lacZ* system. Readthrough by $tRNA^{Tyr}_{UAG}$ is reduced in the *miaA1* mutant in all reading contexts at both 30 and 37°C (Table 6). This is true for the reading contexts shown in Table 6 as well as in four additional contexts (data not shown). The influence is more pronounced at 37°C compared with 30°C although, as stated above, the tRNA completely lacks ms^2io^6A37 at both temperatures.

The ochre suppressor *supC80* is allelic to the amber suppressor *supF30*, but it is only about 10% as efficient as the *supF30* amber suppressor. The deficiency in ms^2io^6A37 reduces the efficiency of *supC80*-mediated suppression to the same magnitude as that for *supF30* (data not shown). The effect is somewhat more pronounced when the ochre suppressor reads amber codons than when it reads the cognate ochre codons.

TABLE 3. Relative levels of different sulfur-containing nucleosides from strains TT4279 ($miaA^+$) and GT248 (*miaA1*)[a]

Nucleoside	Relative radioactivity (fold) at:			
	30°C		37°C	
	GT248 (*miaA1*)	TT4279 ($miaA^+$)	GT248 (*miaA1*)	TT4279 ($miaA^+$)
S^1	0.03	0.02	0.03	0.02
S^3	0.03	0.03	0.04	0.03
s^4U	1.0	1.0	1.0	1.0
mnm^5s^2U	0.20	0.19	0.19	0.22
$S^5(ms^2i^6A)$	0.01	0.02	0.03	0.01
ms^2io^6A	0.01	0.21	0.01	0.21

[a] Cells were grown at the indicated temperatures in the presence of $^{35}SO_4^{2-}$, and tRNA was prepared. After digestion to nucleosides, the mixture was subjected to two-dimensional thin-layer chromatography (cf. Fig. 2). The different nucleosides are denoted as in Fig. 2. Values are relative to the amount of radioactivity in s^4U. (25,178 cpm, GT248 at 30°C; 14,965 cpm, TT4279 at 30°C; 26,670 cpm, GT248 at 37°C; 15,882 cpm, TT4279 at 37°C.)

DISCUSSION

Using a new selection procedure, we isolated a mutant carrying an antisuppressor mutation against *supF30*. This mutation, designated *miaA1*, influences the synthesis of ms^2io^6A37 in tRNA (Fig. 2; Table 3). The gene *miaA* is located close to and counterclockwise of the *purA* gene at 96 min on the *S. typhimurium* chromosome (Fig. 1; Table 2). The deficiency in ms^2io^6A37 in tRNA reduces growth rate, polypeptide chain elongation rate, and cellular yields (Fig. 3 and 4; Table 4). Furthermore, ms^2io^6A37 deficiency influences the synthesis of several amino acids (Table 5). It was also shown that ms^2io^6A37 plays a vital role in the efficiency of translation (see the accompanying paper [6]).

No increase in suggested intermediates in the formation of ms^2io^6A37, like ms^2A37, i^6A, or io^6A37, was observed (7; data not shown). Since isopentenylation is suggested to precede the methylthiolation reaction (1), we suggest that the *miaA* gene governs the isopentenylation reaction, as has been suggested for the *miaA* gene of *E. coli* (16). Unfortunately, the *miaA* mutation of *E. coli*, earlier designated *trpX*, has so far not been precisely mapped, and therefore we cannot compare the map positions of *miaA1* and *miaA* (*trpX*). Gallagher et al. (17) have, however, isolated a Tn*10* insertion in a gene (*mia*) that has the same phenotype as *miaA* (*trpX*). The map location of *mia*::Tn*10* is similar to that of *miaA1* of *S. typhimurium*, but they did not show whether or not *mia*::Tn*10* and *miaA* (*trpX*) are mutations in the same gene.

The *miaA* (*trpX*) mutant of *E. coli* has so far not been analyzed with respect to its general physiology. We have shown that deficiency of ms^2io^6A37 in tRNA from *S. typhimurium* reduces the growth rates of cells and polypeptide chains. ms^2io^6A37 is normally present in tRNAs reading codons starting with U except $tRNA_I^{Ser}$ and $tRNA_V^{Ser}$ (3, 22). That means that 15.4% of the codons present in *lacZ* are read by tRNAs normally having ms^2io^6A37. Assuming that the reduced rate of polypeptide synthesis (from 16 amino acids per s in $miaA^+$ cells to 11 amino acids per s in *miaA1* cells) is only due to reduced translational efficiency of tRNA normally containing ms^2io^6A37, it can be calculated that the average step time for these tRNAs is increased four times.

TABLE 4. Comparison of specific growth rate and rate of polypeptide synthesis in *miaA*$^+$ and *miaA1* cells

Medium	Growth characteristics[a]				Polypeptide chain growth rate (aa/s)[b]			
	miaA$^+$ (k)	*miaA1* (k)	$k_{A1}-k_{A+}$	$\frac{k_{A1}-k_{A+}}{k_{A+}}$	*miaA*$^+$		*miaA1*	
					expt 1	expt 2	expt 1	expt 2
MOPS-acetate	0.35 (±0.01)	0.24 (±0.02)	−0.11	−0.31	15.5	15.0	10.7	9.8
MOPS-glycerol	0.67 (±0.01)	0.54 (±0.01)	−0.13	−0.19	15.5	14.8	10.7	10.7
MOPS-glucose	0.91 (±0.04)	0.64 (±0.01)	−0.27	−0.30	15.5	15.5	10.7	10.7
Rich-MOPS	1.89 (±0.00)	1.02 (±0.05)	−0.87	−0.46	15.5	16.2	10.7	10.7
Rich-MOPS-arg[c]	1.12 (±0.04)	0.66 (±0.03)	−0.46	−0.42	ND[d]	ND	ND	ND

[a] The strains used were GT522 (*miaA*$^+$) and GT523 (*miaA1*). All experiments were performed at 37°C. Determinations of specific growth rates were done twice, and the intervals are shown in parenthesis. k, Specific growth rate = ln 2.60/generation time (min).

[b] The strains used were GT530 (*miaA*$^+$ F′ *lac*) and GT531 (*miaA1* F′ *lac*$^+$). Cells were grown in the indicated media, and rates of polypeptide elongation were determined by determining the time needed to detect the first β-galactosidase molecule as described earlier (4). aa, Amino acids.

[c] This growth rate was determined between optical densities at 420 nm of 0.1 to 1.0. Below an optical density at 420 nm of 0.1, strain GT523 (*miaA1*) grew somewhat faster (k = 0.82).

[d] ND, Not done.

The *miaA1* mutant is equally deficient in ms^{2}io^{6}A37 at both 30 and 37°C (Table 3). Thus, the small suppressor activity observed at 30°C (Table 6) as well as the partial ability to grow without His and Leu at 30°C are not due to residual synthesis of ms^{2}io^{6}A37. Therefore, the decreased suppressor activity at 37°C is due to increased temperature sensitivity of the tRNA function itself due to lack of modification. Strain GT523 (*miaA1*) shows significant temperature sensitivity for growth on rich media as well as on minimal media, which may be partly due to the function(s) of some tRNA chains being temperature sensitive. The *miaA1 rpsL1* double mutant of *S. typhimurium* grows more poorly on complex media compared with the *miaA1* mutant, especially at high temperature (Table 2). Petrullo et al. (36) also observed that an *miaA* (*trpX*) *rpsL* double mutant of *E. coli* even becomes streptomycin dependent. Mutations in *miaA* or *rpsL* are known to increase the accuracy of protein synthesis (6, 20). In the case of *rpsL*, this effect has been attributed to increased loss of aminoacyl tRNA, with preference for loss of the noncognate species, at the translational GTP requiring a proofreading step(s) (39). The combination of the two mutations could lead to an even greater loss of aminoacyl tRNA during translation after GTP hydrolysis (28). This supposition is suggested by the finding that the cellular yield for strain GT523 (*miaA1*) growing on limited carbon sources is less than that for strain GT522 (*miaA*$^+$) as well as by experiments in vitro (Fig. 4; I. Diaz, M. Ehrenberg, and C. G. Kurland, Mol. Gen. Genet., in press). Since the *miaA*-dependent reduction in growth rate and cgr_p is not correlated in different media, factors other than translational step time must also be operating to determine the rate of cellular growth.

The *miaA* mutant is more sensitive to several amino acid analogs, suggesting that synthesis of these amino acids is repressed (Table 5). In fact, at 42.5°C the growth of strain GT523 (*miaA1*) is stimulated by leucine. Therefore, one reason for the reduction in growth rate may be that the pools of some amino acids are significantly decreased, leading to accumulation of uncharged tRNA. However, the decrease in growth rate is even larger in rich-MOPS medium than in MOPS-glucose medium (Table 4). This fact does not rule out that charging of tRNA as such is influenced by ms^{2}io^{6}A37. It has, however, been shown that tRNA^{Trp} and tRNA^{Tyr} lacking ms^{2}i^{6}A37 are equally well aminoacylated compared with tRNAs containing this modified nucleoside (18, 46). Furthermore, bisulfite modification of ms^{2}i^{6}A37 of tRNA^{Phe} does not affect the aminoacylation reaction (19). Taken together, these data suggest that the major effect of ms^{2}io^{6}A37 on cell physiology is through its involvement in the codon-anticodon interaction, which is also consistent with experiments in vitro (Diaz et al., in press; 18, 47).

miaA1 cells grow relatively more poorly in media supporting faster growth (Table 4). However, acetate medium is an exception, and the *miaA1* mutations made the cells grow more poorly (−31%) than in glycerol culture (−19%). *miaA1* cells were more sensitive to the acetate analog fluoroacetic acid (Table 5), as observed by Buck and Ames (7). They suggested that ms^{2}io^{6}A37 containing tRNAs may regulate some pathway(s) in the metabolism of acetate, and our growth rate studies support their suggestion. The results in Table 5 as well as the results of others (7, 8, 46, 49) demonstrate the pleiotropic effect on cell physiology of the deficiency in ms^{2}io^{6}A37. Therefore, one reason for a comparatively more reduced growth rate in rich-MOPS medium may be that too many operons are derepressed. However,

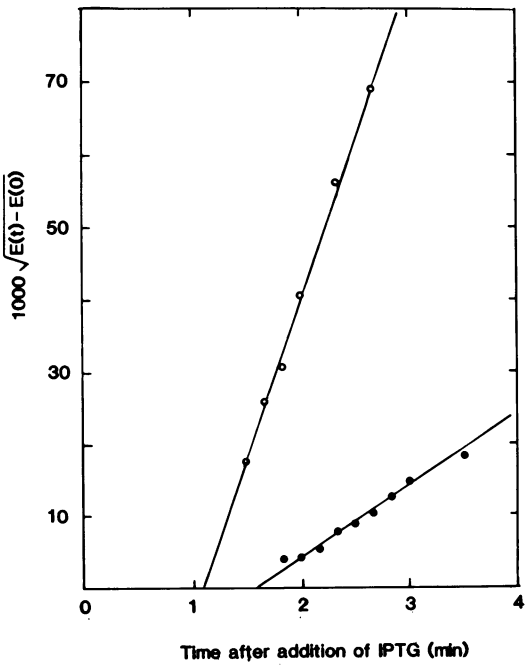

FIG. 3. Determination of in vivo polypeptide step time in glucose minimal medium. Symbols: ○, GT52 (*miaA*$^+$); ●, GT523 (*miaA1*). The activity of β-galactosidase (E_t) was corrected for the basal level (E_0) as a function of time after addition of the inducer, isopropyl β-D-thiogalactopyranoside (IPTG).

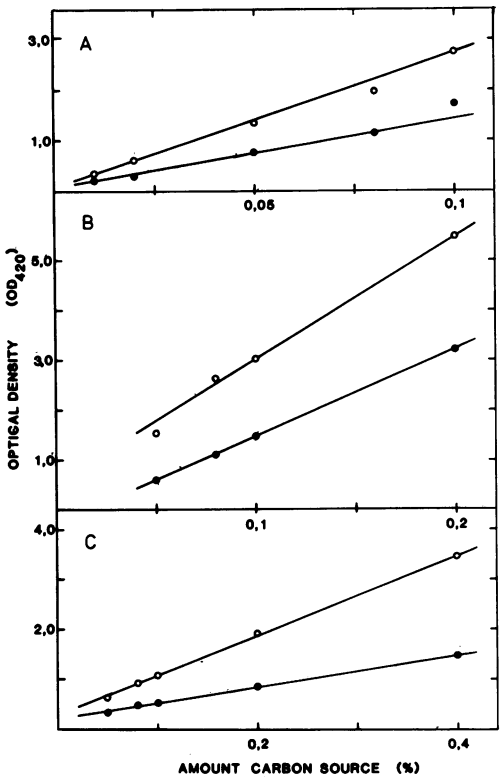

FIG. 4. Cellular yield of strains GT522 (*miaA*$^+$) and GT523 (*miaA1*) in media containing limiting amounts of glucose (A), glycerol (B), and succinate (C). Symbols: ○, GT522; ●, GT523.

our studies with analogs does not support such a suggestion. *miaA1* cells are sensitive to different histidine analogs, especially triazole-3-alanine (Table 5). This suggests that the histidine operon is repressed in the *miaA1* strain, although it is still His$^+$. The *his* operon has no identified repressor, and the leader peptide contains only one amino acid (Phe) coded for by a tRNA normally containing ms^{2}io^{6}A37 (25). This Phe codon (UUU) is the 6th of the 16 codons in the histidine leader, one codon upstream of the 7 His codons present in a row, which are postulated to sense the concentration of charged tRNA^{His} and thus affect attenuation (25). Slower translation early in the leader would lead to increased probability of forming the terminator. Thus, our results imply that tRNA^{Phe} lacking ms^{2}io^{6}A37 has a lower rate of decoding the UUU codon in the *his* leader sequence. Derepression of the histidine operon by introduction of the *hisT1504* mutation into both the *miaA1* and *miaA*$^+$ strains makes them equally resistant to the histidine analogs tested (data not shown); i.e., as expected, the *hisT1504* mutation is epistatic on the *miaA1* mutation.

In the accompanying paper (6), we show that the codon context sensitivity of tRNA might be increased by ms^{2}io^{6}A37-ms^{2}i^{6}A37 deficiency. It is therefore possible to imagine that the degree of modification might serve as a regulatory device; i.e., the ratio of unmodified tRNA to fully modified tRNA can regulate some operons by affecting the efficiency of attenuation (6, 7, 23, 45). Thus, by carefully controlling the modification of tRNA and placing modifica-

TABLE 6. Comparison of readthrough of amber codons by tRNA$^{Tyr}_{UAG}$ at different sites in *miaA*$^+$ and *miaA1* strains and at different temperatures

F-factor position	Sp act (% *lac*$^+$) at a growth temp of[a]:						
	30°C			37°C[b]			37°C/30°C[c]
	miaA$^+$	*miaA1*	*miaA1*/*miaA*$^+$	*miaA*$^+$	*miaA1*	*miaA1*/*miaA*$^+$	
84	11.9	2.9	0.24	18.5	1.3	0.07	0.28
181	25.2	8.8	0.35	50.4	3.3	0.07	0.19

[a] Specific activity is expressed relative to the specific activity of the wild-type F′ plasmid containing no nonsense mutation in the *lacI* gene. The value is the average of two independent experiments.
[b] For comparison, these data are from Bouadloun et al. (6).
[c] This ratio represents the *miaA1*–*miaA*$^+$ ratio at 37°C divided by the *miaA1*–*miaA*$^+$ ratio at 30°C.

TABLE 5. Sensitivity of *miaA*$^+$ and *miaA1* strains to amino acid analogs[a]

Analog (μg)	Analog of:	Zone of inhibition (diam in mm)[b]					
		Shift down			No shift		
		miaA$^+$	*miaA1*	*miaA1* relative to *miaA*$^+$[c]	*miaA*$^+$	*miaA1*	*miaA1* relative to *miaA*$^+$[c]
Aspartic acid β-hydroxamate (50)	Asn	20, t	<6	R	25, t	17, vt	R
2-Thiazo-DL-alanine (50)	His	17, c	42, c	S	22, c	33, c	S
Triazole-3-alanine (50)	His	<6	42, c	S	<6	38, c	S
β-Amino-1,2,4-triazole (8,400)	His	18, c	25, c	S	35, c	42, c	S
β-Chloro-L-alanine (50)	Ile	30, c	42, c	S	23, c	48, c	S
4-Aza-DL-leucine (400)	Leu	<6	23, t	S	15, c	12, c	
5,5,5-Trifluoro-DL-leucine (740)	Leu	50, c	54, c		46, c	63, c	S
L-Ethionine (100)	Met	30, t	<6	R	<6	<6	
α-Methyl-DL-methionine (50)	Met	37, c	34, t		32, c	50, c	S
L-Norleucine (50)	Met	17, c	<6	R	<6	<6	
DL-Serinehydroxamate (1,000)	Ser	30, c	21, c	R	33, c	25, c	R
DL-β-Hydroxynorvaline (50)	Thr	20, c	40, c	S	14, t	27, c	S
3-Nitro-L-tyrosine (500)	Tyr	20, t	<6	R	21, c	25, c	
p-Fluoro-DL-phenylalanine (50)	Phe	24, c	18, t	R	40, c	38, c	
Fluoroacetic acid (50)	Acetate	43, c	55, c	S	<6	<6	

[a] Additional analogs tested are listed in Material and Methods.
[b] The indicated amount (in micrograms) of each analog was placed on a 6-mm paper disk. Therefore, <6 indicates that no zone was observed. Zones were scored as: c, clear; t, turbid; and vt, very turbid.
[c] R, Resistant; S, sensitive.

tion-sensitive reading contexts in control regions like attenuators, a strong and sensitive regulatory mechanism can be envisioned. We have shown that the synthesis of tRNA-modifying enzymes is strictly regulated and several mechanisms seem to operate (10, 34). Furthermore, tRNA modification is also linked to intermediary metabolism (2, 3, 27). Thus, proper modification of tRNA is vital for the function of tRNA and the balanced synthesis of several cellular constituents.

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