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The Cysteine Desulfurase IscS Is Required for Synthesis of All Five Thiolated Nucleosides Present in tRNA from *Salmonella enterica* Serovar Typhimurium

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Deficiency of a modified nucleoside in tRNA often mediates suppression of +1 frameshift mutations. In *Salmonella enterica* serovar Typhimurium strain TR970 (*hisC3737*), which requires histidine for growth, a potential +1 frameshifting site, CCC-CAA-UAA, exists within the frameshifting window created by insertion of a C in the *hisC* gene. This site may be suppressed by peptidyl-tRNA$^{Pro}_{cmo^5UGG}$ (cmo^{5}U is uridine-5-oxyacetic acid), making a frameshift when decoding the near-cognate codon CCC, provided that a pause occurs by, e.g., a slow entry of the tRNA$^{Gln}_{mnm^5s^2UUG}$ (mnm^{5}s^{2}U is 5-methylaminomethyl-2-thiouridine) to the CAA codon located in the A site. We selected mutants of strain TR970 that were able to grow without histidine, and one such mutant (*iscS51*) was shown to have an amino acid substitution in the L-cysteine desulfurase IscS. Moreover, the levels of all five thiolated nucleosides 2-thiocytidine, mnm^{5}s^{2}U, 5-carboxymethylaminomethyl-2-thiouridine, 4-thiouridine, and *N*-6-(4-hydroxyisopentenyl)-2-methylthioadenosine present in the tRNA of *S. enterica* were reduced in the *iscS51* mutant. In logarithmically growing cells of *Escherichia coli*, a deletion of the *iscS* gene resulted in nondetectable levels of all thiolated nucleosides in tRNA except *N*-6-(4-hydroxyisopentenyl)-2-methylthioadenosine, which was present at only 1.6% of the wild-type level. After prolonged incubation of cells in stationary phase, a 20% level of 2-thiocytidine and a 2% level of *N*-6-(4-hydroxyisopentenyl)-2-methylthioadenosine was observed, whereas no 4-thiouridine, 5-carboxymethylaminomethyl-2-thiouridine, or mnm^{5}s^{2}U was found. We attribute the frameshifting ability mediated by the *iscS51* mutation to a slow decoding of CAA by the tRNA$^{Gln}_{mnm^5s^2UUG}$ due to mnm^{5}s^{2}U deficiency. Since the growth rate of the *iscS* deletion mutant in rich medium was similar to that of a mutant (*mnmA*) lacking only mnm^{5}s^{2}U, we suggest that the major cause for the reduced growth rate of the *iscS* deletion mutant is the lack of mnm^{5}s^{2}U and 5-carboxymethylaminomethyl-2-thiouridine and not the lack of any of the other three thiolated nucleosides that are also absent in the *iscS* deletion mutant.

tRNA from all organisms contains modified nucleosides, which are derivatives of the four normal nucleosides adenosine (A), guanosine (G), uridine (U), and cytidine (C). At present, more than 80 different modified nucleosides have been characterized (21). Thiolated nucleosides are present in tRNAs from organisms belonging to the domains *Bacteria* and *Eucarya* (2), and recently they have also been identified in tRNAs from organisms belonging to the domain *Archaea* (17). At present, 10 different thiolated nucleosides have been characterized in tRNAs from different organisms, and five, 2-thiocytidine (s^{2}C), 4-thiouridine (s^{4}U), 5-methylaminomethyl-2-thiouridine (mnm^{5}s^{2}U), 5-carboxymethylaminomethyl-2-thiouridine (cmnm^{5}s^{2}U), and *N*-6-(4-hydroxyisopentenyl)-2-methylthioadenosine (ms^{2}io^{6}A), are present in tRNA from *Salmonella enterica* serovar Typhimurium. In *Escherichia coli* the same thiolated nucleosides are present, but instead of ms^{2}io^{6}A, tRNA from *E. coli* contains *N*-6-isopentyl-2-methylthioadenosine (ms^{2}i^{6}A) (7).

The sulfur source for the synthesis of the thiolated nucleosides is cysteine (1), but cysteine is also the sulfur source for a variety of cofactors, such as biotin, lipoic acid, and thiamine (4). Although the mechanism of incorporation into the various sulfur-containing molecules has been elusive, a major advance in our understanding of this process was the identification of the NifS protein from *Azotobacter vinelandii* as a cysteine desulfurase required for the maintenance of the metallosulfur cluster in nitrogenase (30). The NifS protein splits the cysteine into alanine and elemental sulfur, and the latter is transiently bound to a specific cysteine of NifS (29). *E. coli* has a similar enzyme, IscS, and its structural gene, *iscS*, is part of a gene cluster containing nine genes (25, 28).

The IscS protein is required for the synthesis of s^{4}U in tRNA as well as the incorporation of sulfur into the thiazole ring of thiamine (15, 18). The sulfur of cysteine is transferred first to the IscS protein, thereby forming an IscS-SSH persulfide (SSH indicates a persulfide at a cysteine residue of IcsS), which in turn transfers the sulfur to the ThiI protein, forming a ThiI-SSH persulfide. In the presence of ATP-Mg, this modified protein transfers the sulfur to the tRNA, thus forming the s^{4}U in position 8 of a subset of tRNAs. Alternatively, the IscS-SSH persulfide transfers the sulfur to the ThiS protein, which catalyzes the incorporation of sulfur into the thiazole ring of thiamine. The IscS protein is also involved in the synthesis of nicotinic acid, isoleucine, valine, and other Fe-S proteins (16).

Since the IscS protein is pivotal in the formation of Fe-S clusters in proteins and in the formation of s^{4}U, the other four thiolated nucleosides present in tRNA from *S. enterica* may also require an Fe-S cluster protein. If so, thiolation of tRNA

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TABLE 1. *S. enterica* and *E. coli* strains

Strain	Genotype	Source or reference
S. enterica		
TR970	*hisO1242 hisC3737*	J. Roth
GT1948	*his-644* (deletion of the *his* operon) *serA790 lys-554*	J. Roth
GT6430	*hisO1242 hisC3737 zfh-2525*::Tn*10d*Tc	This work
GT6429	*hisO1242 hisC3737 zfh-2525*::Tn*10d*Tc *iscS51*	This work
GT6408	*hisO1242 hisC3737 zcf-2524*::Tn*10d*Tc *mnmA1*	This work
E. coli		
MW100	Wild type	Mikael Wikström
CL100	Δ*iscS*	16
TH177	*mnmA*$^+$ *fadR*::Tn*10*	This work
TH178	*mnmA1 fadR*::Tn*10*	This work

should be sensitive to the allelic state of the *iscS* gene. Indeed, the MiaB protein, which is required for the formation of the methylthio group of ms^2io^6A, is an Fe-S cluster protein (11, 12). Moreover, the formation of s^2C, which is present in only four tRNA species in bacteria, requires an active *stcA* gene, the sequence of which reveals a highly conserved C-X_1-X_2-C motif common in the thioredoxin superfamily (G. Jäger, Q. Qian, and G R. Björk, unpublished results). However, the *mnmA* (*asuE*, *trmU*) gene encodes a protein required for the thiolation of mnm^5s^2U (24), and its sequence is similar to that of the ThiI protein but does not reveal any potential Fe-S cluster.

This article addresses the question of how and to what extent an active IscS protein is required for the synthesis of the five thiolated nucleosides present in tRNA from *S. enterica*.

MATERIALS AND METHODS

Bacteria and growth conditions. The bacterial strains used were derivatives of either *S. enterica* serovar Typhimurium or *E. coli* K-12 (Table 1). As rich medium we used either Luria-Bertani (LB) (5) or NAA (Difco nutrient broth [0.8%]; Difco Laboratories, Detroit, Mich.) supplemented with the aromatic amino acids, aromatic vitamins, and adenine (8). The minimal medium was made from basal medium E (27) supplemented with 0.2% glucose and required amino acids or vitamins at the concentrations recommended earlier (8).

Genetic procedure. Transduction with phage P22 HT105/1 (*int-201*) (22) and mutagenesis with nitrosoguanidine (50 μg/ml) were performed as described before (8).

DNA sequencing was performed on either chromosomal DNA or PCR products following the manual of the Applied Biosystems ABI Prism Big Dye cycle sequencing ready reaction kit.

Analysis of modified nucleosides in tRNA. Bacterial strains were grown in LB medium at 37°C to about 4×10^8 to 6×10^8 cells/ml (100 to 150 Klett units). Cells were lysed, and total RNA was prepared (9), dissolved in buffer R200 (10 mM Tris-H_3PO_4 [pH 6.3], 15% ethanol, 200 mM KCl), and applied to a Nucleobond column equilibrated with the same buffer. tRNA was eluted with the same buffer except that the KCl concentration was raised to 650 mM. The tRNA was precipitated with 2.5 volumes of cold ethanol containing 1% potassium acetate, washed twice with 80% ethanol, and dried. The dried tRNA was dissolved in water, and a portion of it was degraded to nucleosides by nuclease P1, followed by treatment with bacterial alkaline phosphatase (14). The hydrolysate was analyzed by high-performance liquid chromatography (HPLC) (13).

RESULTS

Inactivation of *iscS* gene induces +1 frameshift errors. We showed earlier how hypomodification of tRNA induces +1 frameshifts (26). Relevant for this study is how hypomodified tRNA may reduce the rate of A site selection and thus give the peptidyl-tRNA a longer time to make a +1 frameshift error. The *hisC3737* mutation is a frameshift mutation which changes the *hisC*$^+$ sequence CCC-AAU-AAU to the *hisC3737* sequence CCC-CAA-UAA-U by insertion of a C (P. Chen and G. R. Björk, unpublished results; the boldface C is formally the inserted C in the mutant; the hyphens indicate the codons in the 0 frame). Thus, in the *hisC3737* mutant, the ribosome will stop translating at the stop codon UAA, thereby creating a requirement for histidine. The near-cognate $tRNA^{Pro}_{cmo^5UGG}$ normally reads CCA/CCG and CCU codons due to the presence of the cmo^5U modification in the wobble position. However, occasionally it may read the near-cognate codon CCC (19). Following translocation, such a peptidyl-$tRNA^{Pro}_{cmo^5UGG}$ is prone to shift frame when interacting with the near-cognate codon CCC, provided that a pause is induced by slow entry of the ternary complex at the A site codon (19). Accordingly, slow entry of a ternary complex containing a defective $tRNA^{Gln}_{mnm^5s^2UUG}$, which reads the A site codon CAA, may allow the error-prone peptidyl-$tRNA^{Pro}_{cmo^5UGG}$ to slip forward one nucleotide. This will reframe the ribosome into the 0 frame, and it will thereafter read codons as it does in the *hisC*$^+$ strain. Thus, among His$^+$ clones, mutations may be identified that affect the modification status of $tRNA^{Gln}_{mnm^5s^2UUG}$ in such a way that a slow entry of this tRNA at the CAA codon would mediate a +1 frameshift by the peptidyl-$tRNA^{Pro}_{cmo^5UGG}$.

In order to characterize mutations that suppress the +1 frameshift mutation *hisC3737*, we first isolated a pool of about 30,000 clones with randomly inserted Tn*10d*Tc transposons in the chromosome of strain GT1948, which contains a deletion (*his-644*) of the *his* operon. Cells of this pool were then mutagenized with nitrosoguanidine. Phage P22 was grown on this culture, and the resulting phage stock was used to transduce various Tn*10d*Tcs to the recipient strain TR970 (*hisC3737*). Among about 160,000 tetracycline-resistant (Tc^r) transductants, several His$^+$ transductants were obtained, of which one was denoted strain GT6429 (*hisC3737 zfh-2525*::Tn*10d*Tc *iscS51*).

Phage P22 was grown on strain GT6429 and used to transduce the *zfh-2525*::Tn*10d*Tc insertion to the parental strain TR970 (*hisC3737*). The results showed that the His$^+$-inducing mutation (*iscS51*) was 97% linked to the Tn*10d*Tc transposon. By using primers complementary to sequences within the Tn*10d*Tc transposon, DNA sequences on both sides of the transposon were determined. Comparing these sequences with the DNA sequence of the *S. enterica* chromosome established that the transposon was inserted in the STM2545 gene, which is the gene immediately upstream of the *isc* operon. We also noticed that, compared to the parent strain, the mutant strain GT6429 (*hisC3737 zfh-2525*::Tn*10d*Tc *iscS51*) grew well on rich medium plates but poorly on glucose minimal plates containing histidine. Growth on histidine plates was stimulated by the addition of thiamine, indicating a potential defect in the synthesis of thiamine in the mutant.

It was demonstrated earlier that inactivation of the *iscS* gene results in a requirement of thiamine for growth (16, 23). This fact and the close linkage between the mutation and the Tn*10d*Tc insertion in the STM2545 gene, which is very close to *iscS*, prompted us to determine the sequence of the *iscS* gene in the mutant. Indeed, the *iscS51* mutation caused a C-to-T

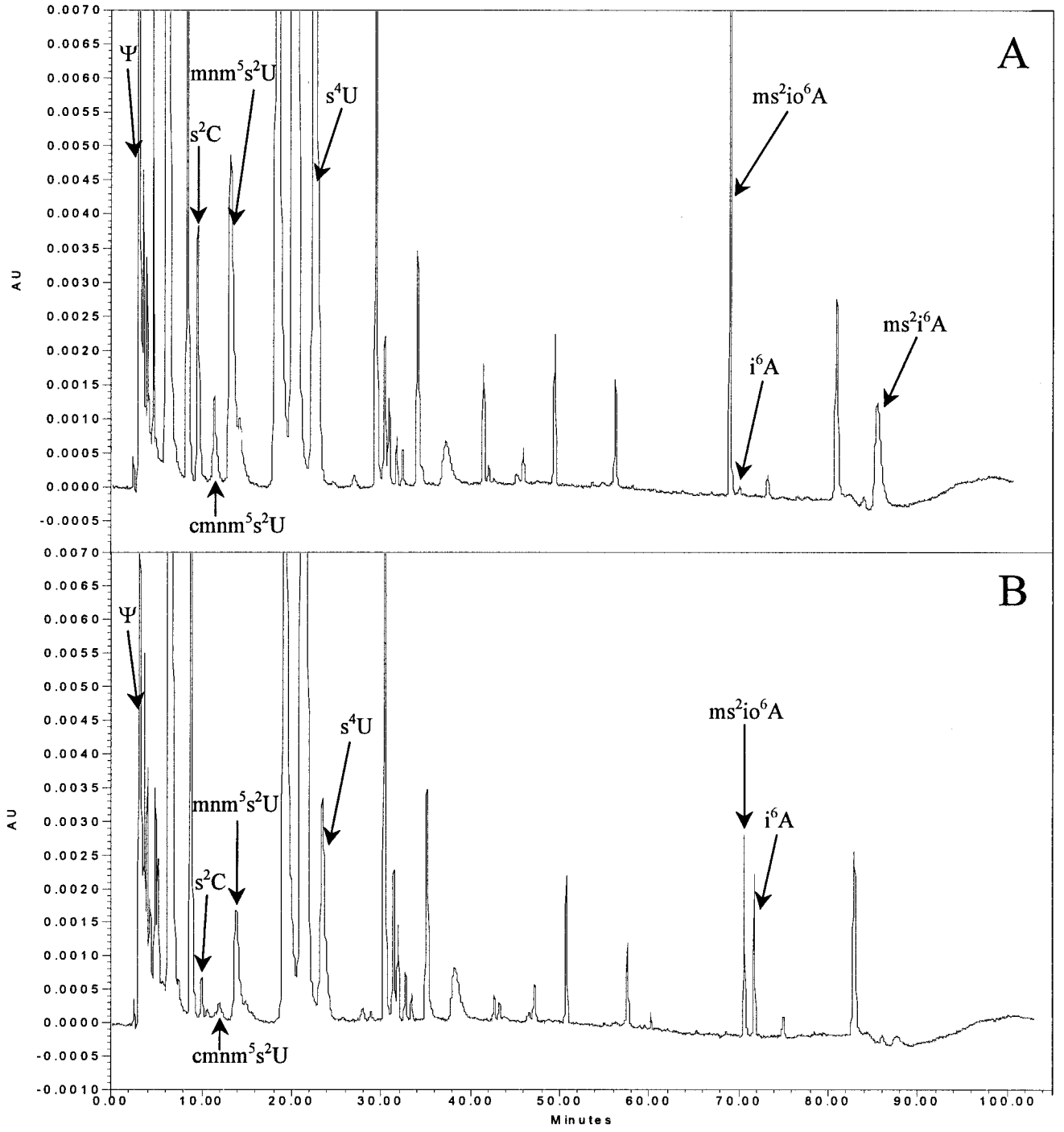

FIG. 1. HPLC chromatograms of tRNA hydrolysates from the wild-type (A) and *iscS51* mutant (B) strains. The nucleosides were monitored at 295 nm to maximize the detection of all thiolated nucleosides. mnm^5s^2U, $cmnm^5s^2U$, s^2C, ms^2io^6A, s^4U, ms^2i^6A, pseudouridine (Ψ), and *N*-6-isopentenyladenosine (i^6A) were identified by comparing UV spectra with published spectra (13). For s^2C, the molecular weight of the protonated form was determined by mass spectrometry (Jäger et al., unpublished data). AU, absorbance units.

base substitution, resulting in an amino acid change from Ala327 to Val327 in the IscS protein. Thus, this amino acid substitution in the IscS protein induced a deficiency of thiamine and suppression of the +1 frameshift mutation *hisC3737*.

***iscS51* mutation reduces the level of all thiolated nucleosides in tRNA.** According to our model of how hypomodified tRNA induces frameshifting, we suspected that the cause of the +1 frameshift was a reduced entry rate of the ternary complex containing Gln-$tRNA^{Gln}_{mnm^5s^2UUG}$ to the CAA codon. In the sequence CCC-CAA-UAA, the CAA codon would be in the A

site when the $tRNA^{Pro}_{cmo^5UGG}$ is interacting with the CCC codon in the P site. Such slow entry of this ternary complex should stimulate the peptidyl-$tRNA^{Pro}_{cmo^5UGG}$ in the P site to frameshift (19). According to earlier results, lack of one modification of $tRNA^{Gln}_{mnm^5s^2UUG}$, ms^2io^6A, would lower the A site selection rate (26).

Therefore, we prepared tRNA from transductants differing only in the allelic state of the *iscS* gene and determined the tRNA modification pattern (Fig. 1). As expected, the *iscS51* mutation decreased the level of mnm^5s^2U but also, surprisingly, the levels of s^2C, s^4U, and ms^2io^6A (Fig. 1; Table 1). The decreased level of mnm^5s^2U explains the ability to suppress the *hisC3737* +1 frameshift mutation (26). The results also demonstrate that the IscS protein is required for synthesis of all the thiolated nucleosides present in the tRNA of *S. enterica*.

***iscS*$^+$ gene complements *iscS51*-mediated phenotypes.** Since the *iscS51* mutation was induced following nitrosoguanidine treatment, the observed suppression of the +1 frameshift mutation *hisC3737* might have been caused by a mutation other than the one found in the *iscS* gene. Therefore, we introduced plasmid piscS, which contains only the *iscS*$^+$ gene, into strain GT6429 (*hisC3737 iscS51*). Indeed, this plasmid (but not the vector) complemented the +1 frameshifting phenotype and restored all the thiolated nucleosides to the wild-type level (Table 2). We conclude that the *iscS51* mutation causes suppression of the +1 frameshift mutation *hisC3737* and the reduced level of all thiolated nucleosides in tRNA.

Deletion of *iscS* gene abolishes synthesis of all thiolated nucleosides in tRNA. To further verify the observation that a mutation in the *iscS* gene influenced the synthesis of all thiolated nucleosides in tRNA, we obtained an *E. coli* strain deleted for the *iscS* gene (16). This strain, CL100, was grown in LB medium at 37°C, and cells were harvested at a cell density of 4×10^8 cells/ml (100 Klett units; cells in logarithmic phase), after 24 h (cells at stationary phase), and after 32 h of incubation. tRNA was prepared, digested to nucleosides, and analyzed by HPLC. None of the thiolated nucleosides were detected in tRNA from logarithmically growing cells except a small amount of ms^2i^6A (1.6% of the wild-type level). However, tRNA from stationary-phase cells contained a small amount (20% of the wild-type level) of s^2C and a small amount of ms^2i^6A (2% of the wild-type level; Table 3). No s^4U or $cmnm^5s^2U$ could be detected. Apparently, an inefficient IscS-independent pathway exists, at least for the formation of s^2U and the methylthio group of ms^2i^6A. Clearly, a functional IscS protein is required for the efficient synthesis of all thiolated nucleosides in tRNA.

Slow growth induced by deletion of *iscS* is likely caused by the lack of mnm^5s^2U. The GT6429 (*iscS51*) mutant grows like the wild-type strain in rich medium (Table 4). This is in sharp contrast to the growth rate reduction observed for the *iscS* deletion strain of *E. coli* (Table 2), although the *iscS* deletion strain was also deficient in all thiolated nucleosides. Interestingly, the growth rate reduction caused by a mutation in the *mnmA* gene of both *E. coli* and *S. enterica* was similar to that induced by a deletion of the *iscS* gene (Table 4), and this growth reduction was correlated to the nondetectable level of (c)mnm^5s^2U. We suggest that the major cause of the growth

TABLE 2. Levels of thiolated nucleosides in tRNA from the *iscS51* mutant[a]

Strain	Relevant genotype	Plasmid[b]	Suppression[c] of *hisC3737*	Mean relative level ± SD (%)			
				s^2C (247 nm)	$cmnm^5s^2U$ (274 nm)	mnm^5s^2U (274 nm)	s^4U (330 nm)	$ms^2i(o)^6A$[d] (242 nm)
GT6430	*iscS*$^+$ *hisC3737*		No	0.135 ± 0.003 (100)	0.017 ± 0.003 (100)	0.111 ± 0.001 (100)	1.11 ± 0.02 (100)	0.230 ± 0.001 (100)
GT6429	*iscS51 hisC3737*		Yes	0.035 ± 0.002 (26)	0.006 ± 0.001 (33)	0.053 ± 0.001 (48)	0.293 ± 0.015 (26)	0.082 ± 0.004 (36)
GT6453	*iscS51 hisC3737*	pSU19	Yes	0.040 ± 0.001 (29)	0.005 ± 0.002 (31)	0.051 ± 0.006 (46)	0.272 ± 0.006 (24)	0.079 ± 0.001 (34)
GT6452	*iscS51 hisC3737*	piscS	No	0.133 ± 0.002 (99)	0.015 ± 0.001 (85)	0.133 ± 0.003 (102)	1.12 ± 0.008 (101)	0.228 ± 0.009 (99)

[a] The chromatograms were scanned at the indicated wavelengths to optimize the quantification of the five thiolated nucleosides. The levels of the various thiolated nucleosides at the indicated wavelengths are given relative to the level of ψ at 254 nm. The levels as a percentage of the wild-type level are shown in parentheses.

[b] Plasmid pSU19 (3), which is a pACYC derivative, was the vector. piscS contains only the *iscS*$^+$ gene, and its construction is described in reference 23.

[c] Ability to suppress the *hisC3737* mutation was scored as growth without histidine on agar plates containing medium E and 0.2% glucose.

[d] The values for $ms^2i(o)^6A$ are the combined values for ms^2io^6A and ms^2i^6A. A corresponding increase in i^6A was noted when the level of $ms^2i(o)^6A$ was decreased (see Fig. 1). 5-Methylaminomethyluridine was expected but not observed because it migrated in the crowded area between C and U.

TABLE 3. In-frame deletion of the *iscS* gene abolishes the synthesis of all four thiolated nucleosides in logarithmically growing cells[a]

Strain	Relevant genotype	Time of harvest	Relative level (% of ψ)			
			s^2C (247 nm)	(c)mnm^5s^2U (274 nm)	s^4U (330 nm)	$ms^2i(o)^6A$ (242 nm)
E. coli MW100	*iscS*$^+$	100 Klett units	100 (0.13)	100 (0.10)	100 (0.98)	100 (0.19)
	iscS$^+$	24 h	109	126	132	92
	iscS$^+$	32 h	106	121	130	85
E. coli CL100	Δ*iscS*	100 Klett units	<9	<0.4	<0.04	1.6
	Δ*iscS*	24 h	22	<0.4	<0.04	2.0
	Δ*iscS*	32 h	22	<0.4	<0.04	2.0

[a] Cells were grown in LB medium and harvested at 100 Klett units (4×10^8 cells) and after 24 or 32 h of incubation. tRNA was prepared, degraded to nucleosides, and analyzed by HPLC. Quantification of the various thiolated nucleosides is described in Table 2, footnote *a*, and they are expressed relative to the level in tRNA from the wild type (100%). (c)mnm^5s^2U represents the combined level of mnm^5s^2U and $cmnm^5s^2U$.

rate reduction in the *iscS* deletion strains is the deficiency of (c)mnm^5s^2U and not the lack of any other thiolated nucleoside in tRNA.

DISCUSSION

We show here that an altered IscS protein influences reading frame maintenance and results in a reduced level of all thiolated nucleosides in tRNA. Our results also demonstrate that the L-cysteine desulfurase IscS is pivotal in the synthesis of all thiolated nucleosides in tRNA of *S. enterica* serovar Typhimurium and *E. coli*.

In tRNA from logarithmically growing strain CL100 (Δ*iscS*) cells, no (c)mnm^5s^2U or s^4U was detected, whereas a low level of ms^2i^6A was apparent. Although we did not detect any s^2C, it might be present at a similarly low level, since another compound migrated very close to s^2C. Indeed, upon extended incubation of the cells in stationary phase, the presence of s^2C was apparent (Table 3), but (c)mnm^5s^2U and s^4U were still not detected. These results suggest that there is another, inefficient route to synthesizing s^2C and ms^2i^6A that is not involved in the synthesis of s^4U and (c)mnm^5s^2U.

In the synthesis of s^4U, the sulfur is delivered from IscS to the ThiI protein, which in turn transfers sulfur to the tRNA and thereby forms s^4U. The sequence of MnmA is similar to that of ThiI, suggesting that MnmA may donate sulfur to the tRNA, similar to ThiI. Thus, whereas the only route of sulfur transfer in the synthesis of (c)mnm^5s^2U and s^4U is through the IscS pathway, an alternative path to transferring sulfur in the synthesis of s^2C and ms^2i^6A exists. Alternatively, in the synthesis of these two thiolated nucleosides, a protein other than IscS may be the immediate donor of sulfur to the enzyme catalyzing the transfer of sulfur to the tRNA. In this alternative pathway, some of the other two desulfurases present in bacteria (CsdA and SufS [CsdB]) may be involved.

TABLE 4. Growth rate reduction is correlated to mnm^5s^2U deficiency[a]

Strain	Relevant genotype	$cmnm^5s^2U34$ and mnm^5s^2U34 (% of wild type)	Growth rate k (h^{-1}) in rich medium (% reduction in k)
S. enterica GT6409	Wild type	100	1.37
S. enterica GT6429	*iscS51*	26	1.23 (−10)
S. enterica GT6408	*mnmA1*	<0.4	0.79 (−42)
E. coli TH177	Wild type	100	1.32
E. coli CL100	Δ*iscS*	<0.4	0.62 (−53)
E. coli TH178	*mnmA1*	<0.4	0.80 (−41)

[a] Growth rates are expressed as the specific growth rate constant k, which is ln2/mass doubling time in hours. The reduction in k was calculated according to $[(k_{GT6429 \text{ or } GT6408} - k_{GT6409})/k_{GT6409}] \times 100$ (*S. enterica* strains) or $[(k_{CL100 \text{ or } TH178} - k_{TH177})/k_{TH177}] \times 100$ (*E. coli* strains).

The *iscS51* mutation was selected as a suppressor of the +1 frameshift mutation *hisC3737*. Moreover, this mutation also reduced the level of all thiolated nucleosides in tRNA, suggesting that the lack of all or one of those was the cause for the +1 frameshift suppressor phenotype. We suggest that the mnm^5s^2U deficiency in $tRNA^{Gln}_{mnm^5s^2UUG}$ causes the suppressing phenotype for the following reasons. (i) An *aroD* mutation abolishes synthesis of cmo^5U34 (6), including the one present in the $tRNA^{Pro}_{cmo^5UGG}$. The frameshifting activity mediated by the *iscS51* mutation is inhibited by the introduction of an *aroD* mutation (data not shown), demonstrating that a peptidyl-$tRNA^{Pro}_{cmo^5UGG}$ interacting with a CCC codon in the P site causes the frameshifting event (19).

There are two sites, CCC-GCG and CCC-CAA, within the 32-codon-long frameshifting window caused by the *hisC3737* mutation where such a frameshifting event can occur (P. Chen and G. R. Björk, unpublished results). Although we cannot rule out that the frameshifting occurs at the CCC-GCG site caused by slow entry of the s^4U-deficient $tRNA^{Ala}_{cmo^5UGC}$, we favor the CCC-CAA-UAA site, since we showed earlier that slow entry of the $tRNA^{Gln}_{mnm^5s^2UUG}$ to the CAA codon caused by mnm^5s^2U deficiency in this tRNA results in frameshifting (26). (ii) The selection procedure used to isolate the *iscS51* mutant also resulted in isolation of an *mnmA* mutant, which is deficient only in mnm^5s^2U (unpublished results). s^2C and ms^2io^6A are not present in any of the tRNAs that would induce a frameshifting event at these two potential frameshifting sites.

The difference in growth rate between the point mutant *iscS51* of *S. enterica* and the Δ*iscS* mutant of *E. coli* may be caused by a more severe reduction in sulfur metabolism in the Δ*iscS* strain than in the *iscS51* strain. Alternatively, it may be caused by a growth reduction caused by a lower level of the thiolated nucleosides in the deletion strain. However, we know that complete lack of s^2C does not induce any growth defects (G. Jäger, Q. Qian, and G. R. Björk, unpublished results), nor does lack of s^4U or the methylthio group of ms^2io^6A (10, 20). Therefore, the severe growth reduction caused by a deletion of the *iscS* gene compared to the slight reduction of the growth

rate mediated by the *iscS51* mutation could be due to the difference in the level of mnm^5s^2U.

A mutation in either the *S. enterica* or the *E. coli mnmA* gene abolished the synthesis of mnm^5s^2U similarly to that in the Δ*iscS* mutant of *E. coli* (Table 2). Such a mutation caused an extensive reduction of the growth rate similar to that induced by the Δ*iscS* mutation. Since the growth reduction seems to be correlated to the level of mnm^5s^2U irrespective of whether this was caused by mutations in the *iscS* gene or in the *mnmA* gene, we suggest that the major cause for the severe reduction in growth rate in rich medium by a deletion of the *iscS* gene is the reduction of the level of mnm^5s^2U. Since the IscS protein is involved in the maintenance of several Fe-S proteins, the most pivotal role with respect to the growth rate in rich medium should be the sulfur transfer involved in the synthesis of mnm^5s^2U.

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