JOURNAL OF BACTERIOLOGY, Feb. 1988, p. 736–743
0021-9193/88/020736-08$02.00/0

Vol. 170, No. 2

Isolation and Characterization of a Selenium Metabolism Mutant of *Salmonella typhimurium*

GEORGE F. KRAMER AND BRUCE N. AMES*

Department of Biochemistry, University of California, Berkeley, California 94720

Received 26 June 1987/Accepted 9 November 1987

Selenium is a constituent in *Escherichia coli* of the anaerobic enzyme formate dehydrogenase in the form of selenocysteine. Selenium is also present in the tRNA of *E. coli* in the modified base 5-methylaminomethyl-2-selenouracil (mnm^5Se^2U). The pathways of bacterial selenium metabolism are largely uncharacterized, and it is unclear whether nonspecific reactions in the sulfur metabolic pathways may be involved. We demonstrated that sulfur metabolic pathway mutants retain a wild-type pattern of selenium incorporation, indicating that selenite (SeO_3^{2-}) is metabolized entirely via selenium-specific pathways. To investigate the function of mnm^5Se^2U, we isolated a mutant which is unable to incorporate selenium into tRNA. This strain was obtained by isolating mutants lacking formate dehydrogenase activity and then screening for the inability to metabolize selenium. This phenotype is the result of a recessive mutation which appears to map in the general region of 21 min on the *Salmonella typhimurium* chromosome. A mutation in this gene, *selA*, thus has a pleiotropic effect of eliminating selenium incorporation into both protein and tRNA. The *selA* mutant appears to be blocked in a step of selenium metabolism after reduction, such as in the actual selenium insertion process. We showed that the absence of selenium incorporation into suppressor tRNA reduces the efficiency of suppression of nonsense codons in certain contexts and when wobble base pairing is required. Thus, one function of mnm^5Se^2U in tRNA may be in codon-anticodon interactions.

Selenium is a specific constituent of certain macromolecules in many microorganisms (33, 34, 36). In *Escherichia coli*, the enzyme formate dehydrogenase (FDH) contains selenium (15, 17). This enzyme exists in two different forms (19). Anaerobic growth in the presence of nitrate results in the induction of the nitrate reductase-linked form (FDH_N), which includes a 110-kilodalton selenium-containing subunit (15). Growth in the absence of an exogenous electron acceptor results in the induction of the hydrogenase-linked form (FDH_H), which includes an 80-kilodalton selenium-containing subunit (15). The selenium appears to be incorporated in the form of selenocysteine, which serves as a catalytically active redox center (36). These two forms of FDH can be distinguished on the basis of their ability to utilize artificial electron acceptors: FDH_N is able to reduce phenazine methosulfate but not benzyl viologen (BV), whereas FDH_H is able to reduce BV but not phenazine methosulfate (15, 19).

Selenium is also incorporated into specific tRNA species in *E. coli* (40, 42). Isoaccepting species of lysine and glutamate tRNA contain selenium (40). The selenium is present in tRNA in the modified base 5-methylaminomethyl-2-selenouracil (mnm^5Se^2U) (40, 42). This incorporation is believed to be specific for selenium due to the inability of vast excesses of sulfur in the culture medium to have a major effect on the selenium content of the tRNA (40). The sulfur analog of the selenium base, mnm^5S^2U, is found in the first (wobble) position of the anticodon of *E. coli* lysine, glutamate, and glutamine tRNAs (18). The selenium base, mnm^5Se^2U, is also apparently located in the anticodon, indicating that these tRNAs exist as a mixed population, a fraction of which contain mnm^5S^2U and the rest of which contain mnm^5Se^2U (40, 41; W.-M. Ching, Fed. Proc. **42**:2238, 1983). mnm^5S^2U has been reported to function in influencing codon-anticodon interactions (21). It has also been shown that purified tRNAs containing either mnm^5Se^2U or mnm^5S^2U have different abilities to recognize codons in which wobble base pairing is required (12; A. Wittwer and W.-M. Ching, Fed. Proc. **44**:1798, 1985). The function of tRNA base modification in position 1 of the anticodon may thus be to fine-tune interactions with the codon (20).

The pathways of selenium metabolism in bacteria are largely uncharacterized. The metabolism of selenium would appear to require (i) transport of an oxidized selenium salt such as selenite into the cell, (ii) reduction of selenite into a biologically active form, and (iii) incorporation into FDH and tRNA. Sulfur and selenium are chemically similar (30), and nonspecific substitution of selenium for sulfur by some sulfur-utilizing enzymes has been demonstrated (8, 39). The actual incorporation of selenium into macromolecules must require a high degree of specificity to discriminate between selenium and sulfur (33, 36). However, it is unclear whether the steps of selenium metabolism preceding incorporation are specific for selenium or whether nonspecific reactions in the sulfur metabolic pathways are involved. Selenite-specific transport mechanisms have been characterized in both *Salmonella typhimurium* and *E. coli* (8, 9). However, other findings indicate that sulfate and selenite may share a common transporter in *E. coli* (24). The investigators suggest that nonspecific selenite transport may be advantageous in preventing toxic amounts of selenite from entering the cell (24). The present study was initiated to characterize selenium metabolism and investigate the function of mnm^5Se^2U in the tRNA of *S. typhimurium*. Our results indicate that selenium is metabolized entirely by specific pathways. We isolated a *selA* mutant which is unable to incorporate selenium into tRNA or protein. The effects of the *selA* mutation on the efficiency of suppression by suppressor tRNA supports the involvement of mnm^5Se^2U in codon-anticodon interactions.

(A preliminary account of this investigation has been

* Corresponding author.

presented [Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, K-132, p. 215].)

MATERIALS AND METHODS

Bacterial strains and F plasmids. The bacterial strains and F plasmids used in this study are described in Tables 1 and 2. Strain S90C and the F plasmids were provided by J. Miller.

Media and reagents. Minimal growth medium consisted of VBC (38) salts supplemented with 0.4% glucose. Minimal glucose medium plates consisted of 1.5% agar supplemented with 2.0% glucose and VBC salts. MacConkey nitrate medium plates consisted of (per liter) 40 g of MacConkey agar base (Difco Laboratories), 20 g of KNO_3, 1 g of glycerol, 0.5 g of NaCOOH, and 0.1 g of glucose. BV dye overlay consisted of BV (1 mg/ml), NaCOOH (250 mM), KH_2PO_4 (25 mM, pH 7.0), and agar (0.75%). $H_2{}^{75}SeO_3$ (231 mCi/mg) was purchased from New England Nuclear Corp. Selenocysteine and BV were purchased from Sigma Chemical Co. Authentic standards of mnm^5Se^2U and mnm^5S^2U were generously provided by W. M. Ching (42).

Mutagenesis. Wild-type *S. typhimurium* LT2 was mutagenized with diethyl sulfate as described by Christman et al. (14).

Screen for FDH_N activity. MacConkey nitrate medium plates were prepared by a method similar to that of Barrett et al. (4), except that MacConkey agar base was used. Mutagenized cultures were diluted and spread on these plates. The plates were incubated at 37°C anaerobically in GasPak jars (BBL Microbiology Systems) for 24 h. Each plate contained approximately 500 to 1,000 colonies.

Screen for FDH_H activity. Strains to be assayed for FDH_H activity were streaked on minimal glucose medium plates and grown anaerobically overnight at 37°C. A BV agar overlay was prepared as described by Mandrand-Berthelot et al. (25). Immediately after the plates were removed from the anaerobic chamber, they were overlaid with 3 ml of the molten dye-agar mixture.

^{75}Se-gel incorporation assay. Cultures (2 ml) were grown in minimal glucose medium supplemented with 0.01 μM Na_2SeO_3, 0.01 μM Na_2MoO_4, 1% KNO_3, and 0.4 μCi of $H_2{}^{75}SeO_3$ per ml. Cysteine (1 mM) was added for growth of *cys* mutants. After growth overnight at 37°C without shaking, cells were harvested by centrifuging 1.5 ml in a Microfuge (Beckman Instruments, Inc.) for 10 min. The cells were washed once with VBC salts and suspended in 0.1 ml of VBC salts. Samples of the concentrated cells were mixed with an equal volume of 2× sample buffer (23). After being heated at 100°C for 3 min, the samples were loaded onto a 12.5% sodium dodecyl sulfate-polyacrylamide gel prepared and run as described previously (3). The gels were run for approximately 15 min after the tracking dye had run off the bottom. The gels were then stained and dried as described previously (3). Autoradiography was performed overnight at −80°C. An equal number of counts (usually 10,000 to 20,000) was loaded on each lane of the gel.

TABLE 1. Strains used

Strain	Genotype	Source
S. typhimurium		
TT172	*cysG1510*::Tn*10*	K. Sanderson
SA2853	*cys-168*	K. Sanderson
TT173	*cysJ1511*::Tn*10*	K. Sanderson
SA2443	*cysE396*	K. Sanderson
TA4325	*selA1*	This laboratory
TA4327	*selA1 cysJ1511*::Tn*10*	This laboratory
TA4328	*proA15 supG50 zbb-121*::Tn*10*	This laboratory
TA4329	*proA15 supG50 selA1 zbb-121*::Tn*10*	This laboratory
E. coli S90C	Δ(*lac-proB*) *ara strA*	J. Miller

TABLE 2. Plasmids used

F plasmid[a]	Coding position in the *lacIZ* gene	Nonsense codon	Residue in wild type
112	84	UAG	Lys
115	105	UAG	Glu
015	105	UAA	Glu
117	117	UAG	Glu
017	117	UAA	Glu
121	181	UAG	Glu
021	181	UAA	Glu

[a] The F plasmids are derived from F plasmid *del-14*, which contains the *lacZ* gene fused in frame with the *lacI* gene. Nonsense codons are present in the *lacI* portion of the fusion.

^{75}Se-labeled tRNA analysis. Cultures (200 ml) were grown without shaking in minimal glucose medium supplemented with 0.5 μM Na_2SeO_3 and 1 mCi of $H_2{}^{75}SeO_3$ per liter. Cells were harvested, and the tRNA was isolated and digested as described previously (10). Nucleosides were characterized by high-pressure liquid chromatography (HPLC). Buffers were prepared as described previously (10) except that buffer A was 0.15 M ammonium acetate (pH 7). Nucleosides from approximately 0.5 to 1.0 A_{260} unit of tRNA were resolved by gradient elution from a Supelco 5-μm C_{18} reverse-phase analytical column (250 by 4.6 mm) at room temperature with a flow rate of 1.5 ml/min. The gradient was generated by a Waters system controller. In the following description of the gradient, the first number refers to the percentage of buffer B being pumped and the second number refers to the elapsed time in minutes: 0, 0; 0, 3; 5, 10; 15, 25; 45, 35; 75, 45; 100, 50. All increases in buffer B were linear. The UV elution profile was determined with a Kratos Spectroflow 773 detector set at 313 nm. The data were analyzed by using a Hewlett-Packard 9816 work station. For runs involving labeled nucleosides, fractions were collected and the radioactivity in samples was counted in a Packard Tri-Carb liquid scintillation counter.

Selenocysteine analysis. ^{75}Se-labeled cells (4 ml) were prepared as described for the gel assay except that 1 mM cysteine was included in the growth medium. Cells were harvested, and the pellet was washed twice with STE buffer (0.1 M NaCl, 10 mM Tris [pH 8], 0.1 mM EDTA, 1 mM dithiothreitol). The cells were suspended in 230 μl of STE buffer, and 23 μl of fresh lysozyme (20 mg/ml) was added. The cells were then lysed by boiling for 40 s. After cooling, the cells were treated with KBH_4 and iodoacetamide as described by Stadtman (35) to derivatize the selenocysteine residues. After derivatization overnight, an equal volume of 12 N HCl was added. Hydrolysates were prepared by incubation at 110°C for 24 h under vacuum. After hydrolysis, the samples were lyophilized to dryness and suspended in 250 μl of sample buffer (67 mM trisodium citrate, 0.5% thiodiglycol, 0.01% caprylic acid [pH 2.2]). HPLC was performed by isocratic elution from a Whatman Partisil-10 SCX column with 20 mM sodium citrate (pH 2.5) as the buffer. Radioactivity was analyzed by use of a Radiomatic flowthrough liquid scintillation detector, and UV absorbance was monitored at 210 nm. The identification of the ^{75}Se-

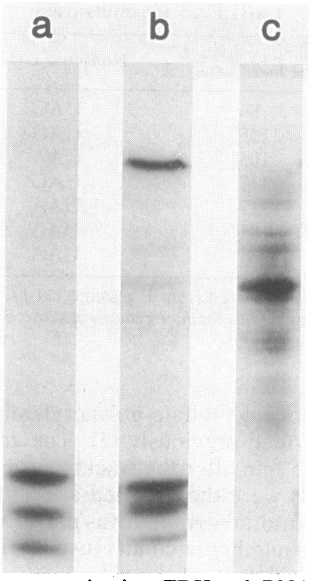

FIG. 1. ^{75}Se incorporation into FDH and tRNA. Autoradiograms of polyacrylamide gels of ^{75}Se-labeled cells showing resolution of ^{75}Se-containing macromolecules. Lanes: a, purified ^{75}Se-labeled tRNA; b, wild-type *S. typhimurium* LT2; c, *S. typhimurium* TA4325 (*selA1*). The top band in lane b corresponds to FDH_N. The bottom three bands in lanes a and b are seleno-tRNAs. The apparent differences in mobility are due to the gels being run at different times. The apparent labeling in bands other than tRNA and FDH_N generally corresponds to the heaviest Coomassie blue-staining bands and may thus represent nonspecific incorporation. The lanes were loaded with approximately the same number of counts, resulting in a greater amount of protein being loaded in the *selA1* lane (lane c).

labeled peak as the selenocysteine derivative was verified by parallel migration with an authentic standard of the derivative, which was prepared by derivatizing selenocysteine by the method of Stadtman (35).

Conjugation and β-galactosidase assay procedures. F plasmids were transferred from strain S90C to strains TA4329 (*proA supG selA1* Tn*10*) and TA4328 (*proA supG* Tn*10*) by spotting liquid cultures onto minimal glucose-tetracycline medium plates and incubating at 37°C for 40 to 48 h. The donor was in the mid-log phase, and the recipient was in the stationary phase.

Cultures (25 ml) were grown for the β-galactosidase assay in minimal glucose medium supplemented with 1 μM Na_2SeO_3 at 37°C with shaking. β-Galactosidase activity was assayed by the method of Miller (27). The results presented are the averages for two to four separate experiments.

RESULTS

Specificity of selenium metabolism in *S. typhimurium*. We developed an assay which allowed simultaneous determination of selenium incorporation into both tRNA and protein. Cells were labeled by growth in the presence of $^{75}SeO_3^{2-}$ and then run on a 12.5% sodium dodecyl sulfate-polyacrylamide slab gel. The major ^{75}Se-labeled bands corresponded to the selenium-containing subunit of FDH at a molecular mass of 110 kilodaltons and three tRNA species at lower molecular masses (Fig. 1, lane b). The identity of the lower bands at tRNA species was proved by their parallel migration with purified ^{75}Se-labeled tRNA (Fig. 1, lane a). This method was similar to that of Cox et al. (15) but allowed resolution of individual tRNA species.

The specificity of selenium metabolism was determined by testing several mutants (*cys*) lacking enzymes involved in sulfur metabolism in the gel assay. The pattern of selenium incorporation in *cysG* (TT172), *cysI* (SA2853), *cysJ* (TT173), and *cysE* (SA2443) mutants (Fig. 2) appeared to be the same as that in wild-type cells (data not shown). *O*-Acetylserine (formed by the *cysE* gene product) is a positive regulatory element in the expression of the *cys* genes (22). Thus, the requirement for any other of the enzymes from the sulfur metabolic pathway in selenium metabolism was unlikely since in the *cysE* mutant, expression of all the other *cys* genes was repressed. These results indicate that all of the steps in selenium processing were specific for selenium.

Identification of selenium-modified base in *S. typhimurium* tRNA. ^{75}Se-labeled tRNA was isolated and hydrolyzed to ribosides. HPLC analysis demonstrated the presence of one ^{75}Se-labeled nucleoside (Fig. 3A and B) which coeluted with authentic mnm^5Se^2U standard (data not shown). Selenium was incorporated into tRNA when cells were grown either anaerobically or aerobically. Therefore, as in *E. coli* (40), *S. typhimurium* tRNA contained a single selenium-modified base, mnm^5Se^2U.

Isolation of a selenium metabolism mutant. To investigate the function of mnm^5Se^2U, we attempted to isolate a mutant which was unable to incorporate selenium into tRNA. Mutations in steps of selenium metabolism such as reduction and transport would have a pleiotropic effect, blocking incorporation into either tRNA or protein. Such mutants would lack activity of both forms of FDH, FDH_H and

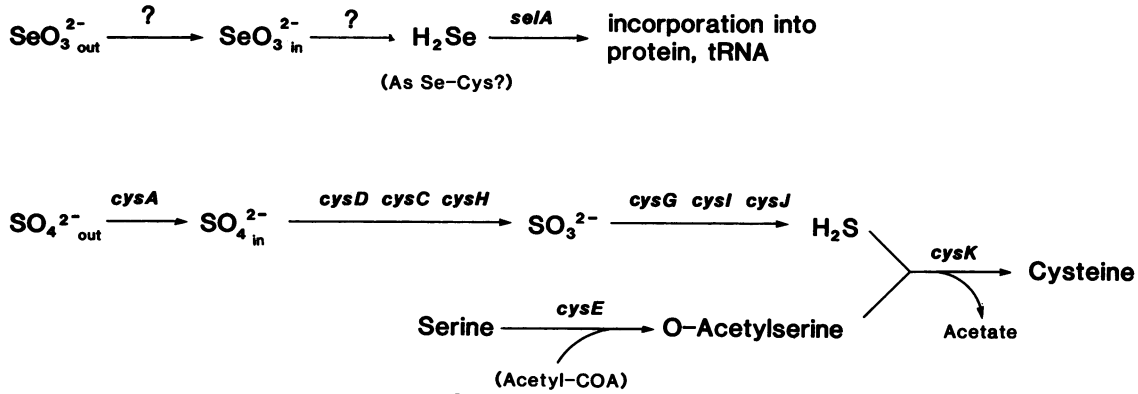

FIG. 2. Comparison of sulfur and selenium biosynthetic pathways in *S. typhimurium*. The sulfur pathway is taken from Kredich (22). The selenium pathway is based on the work of Brown and Shrift (8, 9) and results of the present study. Acetyl CoA, Acetyl coenzyme A.

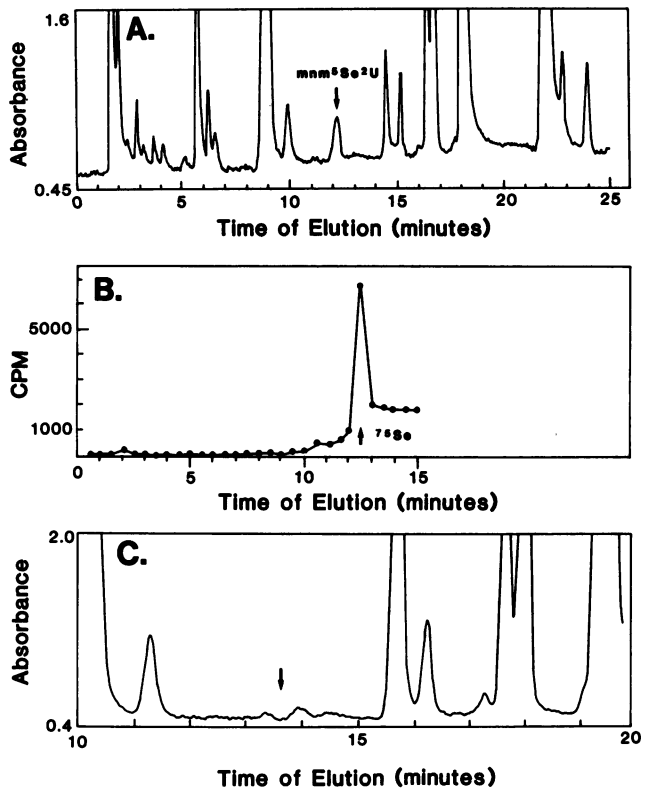

FIG. 3. HPLC resolution of tRNA modified base mnm^{5}Se^{2}U. Whole-tRNA hydrolysates of purified ^{75}Se-labeled *S. typhimurium* RNA were analyzed by reverse-phase HPLC as described in the text. (A) UV profile of wild-type tRNA; the arrow indicates the location of mnm^{5}Se^{2}U. (B) Radioactivity profile of HPLC run, showing the only ^{75}Se-labeled peak observed. (C) UV profile of hydrolysate of tRNA purified from the *selA1* mutant; no mnm^{5}Se^{2}U was detected.

FDH_N, since selenium incorporation is required for activity. FDH_N mutants were initially isolated by plating diethyl sulfate-treated cultures onto MacConkey nitrate medium plates. These plates contain a pH indicator along with 1% nitrate, so that when grown anaerobically on these plates, FDH_N mutants turn red due to the accumulation of formic acid (4). FDH_N mutants isolated by this procedure were then assayed for FDH_H activity by testing for the ability of anaerobically grown cells to reduce BV. The assay involved overlaying cells grown anaerobically on minimal glucose medium plates with top agar which contained BV (25). Cells which were unable to reduce the dye remained white, whereas those with FDH_H activity turned blue. Mutants which lacked both activities were assayed for selenium incorporation in the gel assay. Using this approach, we isolated a mutant which is unable to incorporate selenium into either tRNA or protein (Fig. 1, lane c). We propose designating this gene *selA*.

Analysis of tRNA from *selA1* mutant. HPLC analysis of tRNA hydrolysates from the *selA1* mutant demonstrated the absence of mnm^{5}Se^{2}U (Fig. 3C), confirming the results of the ^{75}Se-gel assay. tRNA from the *selA1* mutant had increased levels of mnm^{5}S^{2}U, the sulfur analog of the selenium base (data not shown). The magnitude of this increase in the mnm^{5}S^{2}U content in tRNA observed in the *selA1* mutant indicated that of the tRNAs which contained mnm^{5}Se^{2}U or mnm^{5}S^{2}U, the fraction which contained the selenium form was approximately 40% in wild-type cells. Similar conclusions have been reported for *E. coli* tRNA (41).

Characterization of *selA* function. We attempted to determine the biochemical function of the *selA* protein by assaying the ability of the *selA1* mutant to catalyze the steps in the apparent selenium metabolic pathway (Fig. 2). Initially, we assayed selenite transport in the *selA1* mutant and found it to be unchanged from that in the wild type (data not shown). We next assayed the *selA1* mutant for the ability to reduce selenite by measuring selenocysteine levels. Selenocysteine may be the activated form of reduced selenium, i.e., the source of reduced selenium utilized for incorporation into tRNA (43) and protein. Even if selenocysteine is not an intermediate in selenium processing, the ability to synthesize selenocysteine from selenite is an indication of the availability of reduced selenium. To assay for selenocysteine formation, cells were grown in the presence of $^{75}SeO_3^{2-}$, and whole-cell hydrolysates were prepared as described above. The hydrolysates were analyzed by HPLC on a strong cation-exchange column. In addition to the *selA1* mutant, a *selA1 cysJ* double mutant was also analyzed as a control for nonspecific selenite reduction via the sulfur metabolic pathway. Both the *selA1* mutant and the double mutant were able to synthesize selenocysteine (Fig. 4). The *selA* protein therefore appears to be involved in a step of selenium metabolism after selenite reduction (Fig. 2).

Effect of *selA* mutation on suppressor tRNA function. Since mnm^{5}Se^{2}U appears to be present in position 1 of the anticodon, it could be involved in codon-anticodon interactions (36; Ching, Fed. Proc., 1983). The involvement of modified bases in the anticodon region in codon recognition has been inferred from the effect of base modification mutations on the function of suppressor tRNAs (7, 21, 37). The ochre-suppressing derivative of $tRNA^{Lys}$, coded for by *supG*, has been shown to retain full modification in position 1 of the anticodon (29). Hagervall and Björk (22) have used this suppressor to show that undermodification at the 5 position of mnm^{5}S^{2}U causes reduced readthrough of nonsense codons, i.e., affects codon recognition. These experiments were performed with the *lacI-lacZ* fusion system developed by Miller and Albertini (28). The *lacI* gene has been sequenced, and many nonsense mutations in this gene have been characterized. Several of these mutations have been transferred onto an F plasmid with an in-frame *lacI-lacZ* fusion (28). In the absence of the nonsense mutation, this fusion retains β-galactosidase (*lacZ*) activity. The introduction of a nonsense mutation into *lacI* prevents translation into the *lacZ* portion of the fusion, eliminating β-galactosidase activity. If any amino acid is inserted at the site of the nonsense mutation, such as by a suppressor tRNA, the *lacZ* portion will be translated and β-galactosidase activity will be expressed. The amount of readthrough at the nonsense codon (as measured by β-galactosidase activity) is dependent on the cognate interaction of the suppressor tRNA with the codon. Small changes in the codon-anticodon interactions of the suppressor tRNA and the nonsense codon resulting from altered base modifications can be measured by changes in β-galactosidase activity (21). We used this system to determine the effect of mnm^{5}Se^{2}U on the interaction of *supG* tRNA with nonsense codons. Strains were constructed which contained *supG* and a fusion plasmid, with or without the *selA1* mutation. β-Galactosidase activity was then measured in these strains. Thus, this experiment compared the difference in suppression efficiencies of strains which contained a population of suppressor tRNAs containing either mnm^{5}Se^{2}U or mnm^{5}S^{2}U (*selA*$^+$) with strains

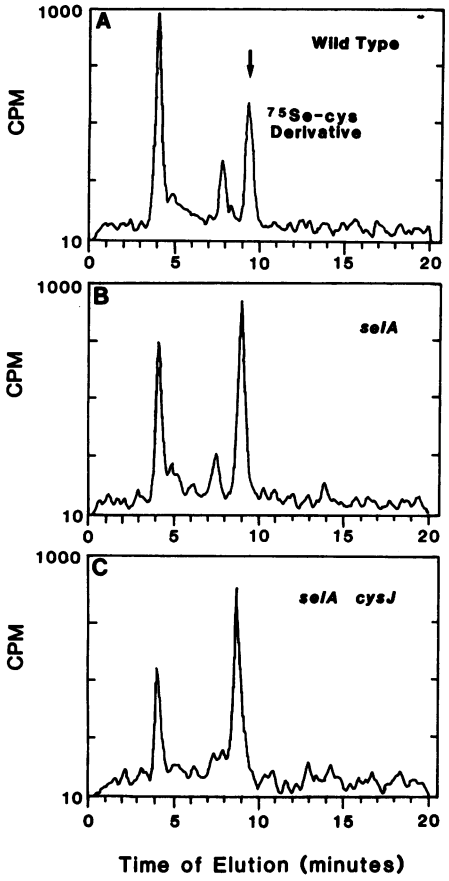

FIG. 4. Assay for ability to synthesize selenocysteine.^{75}Se-labeled cell hydrolysates were prepared and analyzed by HPLC as described in the text. Radioactivity was detected by the use of a flowthrough liquid scintillation counter. Equal amounts (20 μl) of the hydrolysates were used in all runs. The arrow indicates the position of the selenocysteine derivative. (A) Wild-type LT2; (B) *selA1* (TA4325); (C) *selA1 cysJ* (TA4327).

whose suppressor tRNAs contained only mnm^{5}S^{2}U (*selA* mutants). The results of this experiment, using several different fusion plasmids, are shown in Table 3. The results are normalized to the β-galactosidase activity obtained with a wild-type fusion plasmid (no nonsense mutation). The wild-type activity was the same in the *selA*$^+$ strains and the *selA* mutants. The absence of mnm^{5}Se^{2}U in the *selA1* mutant strains caused up to a 50% decrease in readthrough in certain contexts (Table 3). The magnitude of this effect is similar to what was observed for mutations affecting the 5 position of mnm^{5}S^{2}U (21). These results support the proposal that the selenium-modified base is involved in codon recognition.

Genetic mapping of *selA*. Hfr mapping experiments demonstrated that *selA* maps in the general region of 13 to 23 min on the *S. typhimurium* chromosome (data not shown). Several Tn*10* transposons linked to *selA* have been isolated. The Sel$^-$ phenotype can be moved into different strains by P22-mediated transduction by using these Tn*10* transposons. Deletions from these Tn*10* transposons have been generated by selecting for tetracycline sensitivity on fusaric acid medium plates (6). One class of deletion mutants had the Sel$^-$ phenotype, indicating that *selA1* is a loss-of-function mutation. Auxotrophic deletions were also generated in this manner. These mutants appeared to carry either a *pyr* mutation (*pyrC* at 23 min or *pyrD* at 21 min) or an *asp* mutation (*aspC* at 21 min) plus a *glt* mutation (*gltH* at 20 to 25 min). These results are consistent with the Hfr mapping results. However, no linkage could be demonstrated by P22 transduction with known markers in this region (*pyrD*, *aspC*, *pyrC*, and *pepN*).

Other *fdh* mutants (lacking both FDH_H and FDH_N) with mutations in this region have been isolated. Chippaux et al. (13) isolated an *fdh* mutant, *fdhB*, which was 30% linked to *aroA* at 19 min. Barrett et al. (4) isolated a class of *fdh* mutants (class 3) in which the mutations mapped close to *fdhB* by Hfr mapping but were unlinked to *aroA* and other known markers in the region. We analyzed one of the class 3 *fdh* mutants (TC170) isolated by Barrett et al. and found it to be unable to incorporate selenium into protein or tRNA (data not shown), indicating that this class of *fdh* mutants probably consists of *selA* mutants. We also analyzed two strains with large deletions in the 18- to 19-min region (TA1674 and TA1701) that were isolated in this laboratory (2) and found them to have the Fdh$^-$ mutant phenotype while still incorporating selenium into tRNA (data not shown). The deletion in these strains appears to extend through the *fdhB* gene. Thus, *fdhB* and *selA* appear to be distinct genes.

DISCUSSION

Selenium and sulfur are in the same group of elements in the periodic table and are thus chemically similar (30). The insertion of selenium into specific sites in bacterial macromolecules implies a high degree of specificity for distinguishing between selenium and sulfur (33, 34, 36). The specificity of the metabolic steps preceding the incorporation of selenium (Fig. 2) is unclear. Transport of selenite has been reported to be specific (8, 9), but this observation has also been disputed (24). We tested the ability of several *S. typhimurium cys* mutants to metabolize selenium in a ^{75}Se-gel assay. We found that all of these mutants were able to metabolize selenite normally. These results demonstrated that selenium and sulfur are metabolized by independent pathways in *S. typhimurium*. The transport and reduction of selenite and the incorporation of reduced selenium into macromolecules thus appeared to proceed entirely via a selenium-specific pathway.

Lysine- and glutamate-accepting species of the tRNA of *E. coli* have been shown to contain selenium in the form of

TABLE 3. β-Galactosidase activity in *selA* mutant

Position of mutation	Sequence	% β-Galactosidase activity relative to that of F plasmid *del-14* (SE)[a]		Ratio *selA*$^+$/*selA1*
		selA$^+$	*selA1*	
Amber				
84	AUU-UAG-UCU	2.48 (0.10)	1.14 (0.02)	0.46
105	GUC-UAG-GCC	1.22 (0.10)	0.69 (0.04)	0.56
117	GCG-UAG-CGC	0.72 (0.01)	0.42 (0.05)	0.58
181	CAG-UAG-AUC	1.30 (0.04)	0.74 (0.08)	0.57
Ochre				
105	GUC-UAA-GCC	0.50 (0.07)	0.49 (0.08)	0.98
117	GCG-UAA-CGG	0.36 (0.08)	0.33 (0.07)	0.92
181	CAG-UAA-AUC	1.55 (0.15)	0.74 (0.03)	0.48

[a] The β-galactosidase activities obtained with different F plasmids in *selA*$^+$ *supG* (TA4328) and *selA1 supG* (TA4329) strains are expressed as the percentages of the activity obtained with the wild-type F plasmid *del-14*.

the modified base mnm^{5}Se^{2}U (40, 42). The selenium-containing form of the base is apparently substituted for the sulfur-containing form, mnm^{5}S^{2}U, in a fraction of these tRNA species (36, 41; Ching, Fed. Proc., 1983). We found that *S. typhimurium* tRNA, like *E. coli* tRNA, contained a single selenium-modified base, mnm^{5}Se^{2}U. The lysine-, glutamate-, and glutamine-accepting tRNA species of *E. coli* contain mnm^{5}S^{2}U in position 1 of the anticodon (18). We showed that *S. typhimurium* contained three seleno-tRNA species by using the ^{75}Se-gel assay. Since mnm^{5}Se^{2}U appears to substitute for the sulfur form of the base (41), the three seleno-tRNA species observed may have corresponded to lysine-, glutamate-, and glutamine-accepting species.

Selenium is an essential component of the bacterial enzyme FDH (17). This enzyme exists in two forms made under different growth conditions, each of which contains selenium (15). Mutations which disrupt selenium metabolism would be expected to have a pleiotropic effect, eliminating the activity of FDH_H and FDH_N in addition to preventing seleno-tRNA formation. Such a mutant was isolated by screening for mutants lacking both FDHs. Mutants of this type were tested for selenium incorporation in the ^{75}Se-gel assay. The mutant which was isolated, *selA*, does not incorporate selenium into either tRNA or protein. The *selA* gene appeared to map in the 20- to 22-min region of the *S. typhimurium* chromosome.

The *selA* protein appeared to be involved in the actual selenium insertion process. We tested the *selA1* mutant for the ability to reduce selenite by assaying for selenocysteine biosynthesis. The mutant retained the ability to synthesize selenocysteine even in the presence of a sulfite reductase mutation. The formation of selenocysteine in this double mutant demonstrated that reduced selenium is available for further processing. The *selA* protein therefore appears to act in a step of selenium metabolism after reduction, such as incorporation. The incorporation of selenium into tRNA and protein appears to proceed by reactions which are quite different. Incorporation into tRNA appears to involve exchange of sulfur for selenium (unpublished data; 41). This conclusion was based on experiments in which various *E. coli* mutants with altered biosynthesis of mnm^{5}S^{2}U were assayed for their ability to synthesize mnm^{5}Se^{2}U. Strains with mutations in the *trmC*, *trmE*, or *trmF* gene which affect modification of the base at position 5 (5, 16) contained an undermodified selenium base (unpublished data; 41). However, in an *asuE* mutant, which appears to lack thiolation of mnm^{5}S^{2}U (37), no selenium was incorporated into tRNA (unpublished data). This strain maintained selenium incorporation into FDH; therefore, seleno-tRNA does not serve as a source of reduced selenium for incorporation into protein, as has been suggested (40). The presence of sulfur thus appeared to be necessary for selenium incorporation into mnm^{5}Se^{2}U. This conclusion is in agreement with results demonstrating that this selenium incorporation in *E. coli* involves an exchange reaction with sulfur (41).

The mechanism of selenium incorporation into FDH_H has been proposed to be cotranslational (44). Selenocysteine has been found to be incorporated at sites encoded by the opal stop codon, UGA (11, 44). For FDH_H, Zinoni et al. (44) speculated that $tRNA^{Trp}$ interacts with the opal codon and that tryptophan is then converted to selenocysteine by replacement of the indole group with SeH to form selenocysteinyl-$tRNA^{Trp}$. The apparent mechanisms of incorporation of selenium into tRNA and protein are sufficiently different that it is unlikely that the *selA* protein alone could catalyze both reactions. The formation of the tRNA modified base 4-thiouracil (S^{4}U) requires two enzymes (1). The product of the *nuvC* gene appears to be involved in transferring sulfur to both S^{4}U and thiazole (31). The function of the *nuvA* protein may be to activate the specific site on tRNA for thiol transfer (1). By analogy to the *nuvC* protein, the *selA* protein may be a general selenium transfer enzyme. Other enzymes analogous to the *nuvA* protein would thus be required to direct this selenium transfer into protein or tRNA.

The ability of nonsense mutations to be suppressed by mnm^{5}S^{2}U-containing tRNAs is reduced by undermodification of the base (16, 21, 37). These experiments were done with both suppressor (21, 37) and wild-type (16) tRNAs. Since mnm^{5}Se^{2}U is also found in the anticodon of tRNAs, it may also be involved in codon-anticodon interactions (12, 36). There is in vitro evidence supporting the involvement of mnm^{5}Se^{2}U in codon recognition (12; Wittwer and Ching, Fed. Proc., 1985). Using purified $tRNA^{Glu}$ from *E. coli* with either mnm^{5}Se^{2}U or mnm^{5}S^{2}U, it was found that the selenium-containing form had a significantly greater affinity for GAG codons than did the sulfur-containing form, whereas no difference was observed for the binding to GAA codons (Wittwer and Ching, Fed. Proc., 1985). Comparison of the binding of selenium-containing $tRNA^{Glu}$ from *Clostridium sticklandii* with that of sulfur-containing $tRNA^{Glu}$ from *E. coli* to GAA and GAG codons demonstrated that the sulfur-containing tRNA had a fivefold greater affinity for the GAA codon than for the GAG codon, whereas the selenium-containing form had an equal affinity for both codons (12). These results indicate that mnm^{5}Se^{2}U may function to increase wobble base pairing, i.e., increase the affinity of the modified uridine for base pairing with guanosine. We investigated the effect of mnm^{5}Se^{2}U on in vivo codon recognition of a suppressor tRNA by using the *lacI-lacZ* fusion system (28). The effect of the *selA* mutation on the ability of *supG* tRNA to suppress ochre and amber mutations in several contexts was determined. Suppression of nonsense codons has been found to be context dependent, often correlating with the adjacent 3′ base (28). Our results show that in the absence of mnm^{5}Se^{2}U, the efficiency of suppression of UAG codons by *supG* tRNAs was reduced approximately 50%. The efficiency of suppression of UAA codons was not affected by the absence of seleno-tRNA unless the codon was followed by an A (UAA-A). These results are consistent with the involvement of mnm^{5}Se^{2}U in codon recognition, especially in forming wobble base pairs. This function may suggest that the translation of mRNA species which contain a large number of codons that are read more efficiently by selenium-containing tRNAs could be regulated by selenium availability (12). It is interesting that the sequence of the selenium-containing subunit of *E. coli* FDH_H (*fdhA*) contains an unusually high percentage of lysine and glutamate codons which appear to be sensitive to the presence of mnm^{5}Se^{2}U (65% RAG plus RAA-A for Glu [R = G] and 56% for Lys [R = A]), compared with the average codon usage in *E. coli* (44% RAG plus RAA-A for Glu and 42% for Lys), as compiled by Shpaer (32).

Suppressor tRNAs which contain mnm^{5}Se^{2}U appear to be more efficient at reading UAA codons than those with mnm^{5}S^{2}U when A is the 3′ base flanking the codon. The function of the modified U at position 34 of the tRNA may be to enhance codon recognition through stacking interactions involving the middle base of the anticodon and the base 3′ to the codon (20, 32). The context effects seen in our experiments could be explained by differences in the ability of mnm^{5}Se^{2}U and mnm^{5}S^{2}U to stack in this manner. When

paired with G, the selenium-containing base may have a greater ability to stack with the 3′ base of the codon than does the sulfur-containing base. Also, when base paired with A, the selenium-containing form of the base may have an enhanced ability to stack with an A 3′ of the codon compared with the sulfur-containing form. Thus, the absence of the selenium form (in the *selA1* mutant) led to a decreased ability to suppress UAA codons with a 3′ A. When the UAA codon was followed by G or C, there appeared to be no difference between the sulfur and selenium forms in these stacking interactions. The function of mnm^5Se^2U in tRNA may be to enhance wobble base pairing and to stabilize cognate base pairs in the context of RAA-A. Such a function is consistent with the involvement of modified bases in the anticodon region in the fine-tuning of the translational process (20).

The physiological consequences of lacking mnm^5Se^2U appear to be fairly subtle. When the bacteria were grown aerobically, the only effect of the *selA1* mutation appeared to be a lack of seleno-tRNA, since selenoenzymes are expressed only anaerobically. The aerobic growth rate of the *selA1* mutant in minimal glucose medium was identical to that of strain LT2 (data not shown). We also found that the *selA1* mutant was not sensitive to oxidizing agents, amino acid analogs, or heat killing (data not shown). These results are similar to those obtained for another mutant, *trmC*, which contains altered modification of mnm^5S/Se^2U in tRNA. Like the *selA1* mutant, *trmC* mutants cause reduced efficiency of suppression by *supG* tRNA (21) yet retain a wild-type growth rate (26) and have no other reported phenotype. The effects of mnm^5Se^2U on codon interactions may be important under environmental conditions but may not be apparent under laboratory conditions. The physiological consequences of undermodification of mnm^5Se^2U and mnm^5S^2U in the anticodon thus remain to be characterized.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM19993 from the National Institutes of Health and National Cancer Institute Outstanding Investigator grant CA39910 to B.N.A. and by Public Health Service grant ES01896 from the National Institute of Environmental Health Sciences. G.F.K. was supported by Public Health Service training grant ES07075 from the National Institute of Environmental Health Sciences.

LITERATURE CITED

1. **Abrell, J. W., E. E. Kaufman, and M. N. Lipsett.** 1971. The biosynthesis of 4-thiouridylate. J. Biol. Chem. **246**:294–301.
2. **Alper, M. D., and B. N. Ames.** 1975. Positive selection of mutants with deletions of the *gal-chl* region of the *Salmonella* chromosome as a screening procedure for mutagens that cause deletions. J. Bacteriol. **121**:259–266.
3. **Ames, G. F.-L.** 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs: membrane, soluble, and periplasmic fractions. J. Biol. Chem. **249**:634–644.
4. **Barrett, E. L., C. E. Jackson, H. T. Fukumoto, and G. W. Chang.** 1979. Formate dehydrogenase mutants of *Salmonella typhimurium*: a new medium for their isolation and new mutant classes. Mol. Gen. Genet. **177**:95–101.
5. **Björk, G. R., and K. Kjellin-Stråby.** 1978. *Escherichia coli* mutants with defects in the biosynthesis of 5-methylaminomethyl-2-thio-uridine or 1-methylguanosine in their tRNA. J. Bacteriol. **133**:508–517.
6. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
7. **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.
8. **Brown, T. A., and A. Shrift.** 1980. Assimilation of selenate and selenite by *Salmonella typhimurium*. Can. J. Microbiol. **26**:671–675.
9. **Brown, T. A., and A. Shrift.** 1982. Selective assimilation of selenite by *Escherichia coli*. Can. J. Microbiol. **28**:307–310.
10. **Buck, M., M. Connick, and B. N. Ames.** 1983. Complete analysis of tRNA-modified nucleosides by high-performance liquid chromatography: the 29 modified nucleosides of *Salmonella typhimurium* and *Escherichia coli* tRNA. Anal. Biochem. **129**:1–13.
11. **Chambers, I., J. Frampton, P. Goldfarb, N. Affara, W. McBain, and P. R. Harrison.** 1986. The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. EMBO J. **5**:1221–1227.
12. **Ching, W.-M., L. Tsai, and A. J. Wittwer.** 1985. Selenium-containing transfer RNAs. Curr. Top. Cell. Regul. **27**:497–507.
13. **Chippaux, M., M.-C. Pascal, and F. Casse.** 1977. Formate hydrogenlyase system in *Salmonella typhimurium*. Eur. J. Biochem. **72**:149–155.
14. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
15. **Cox, J. C., E. S. Edwards, and J. A. DeMoss.** 1981. Resolution of distinct selenium-containing formate dehydrogenases from *Escherichia coli*. J. Bacteriol. **145**:1317–1324.
16. **Elseviers, D., L. A. Petrullo, and P. J. Gallagher.** 1984. Novel *E. coli* mutants deficient in biosynthesis of 5-methylaminomethyl-2-thiouridine. Nucleic Acids Res. **12**:3521–3534.
17. **Enoch, H. G., and R. L. Lester.** 1975. The purification and properties of formate dehydrogenase and nitrate reductase from *Escherichia coli*. J. Biol. Chem. **250**:6693–6705.
18. **Gauss, D. H., and M. Sprinzl.** 1983. Compilation of tRNA sequences. Nucleic Acids Res. **11**:r1–r34.
19. **Giordano, G., C. L. Medani, M.-A. Mandrand-Berthelot, and D. H. Boxer.** 1983. Formate dehydrogenases from *Escherichia coli*. FEMS Microbiol. Lett. **17**:171–177.
20. **Grosjean, H., and H. Chantrenne.** 1980. On codon-anticodon interactions, p. 347–367. *In* F. Chapeville and A.-L. Haenni (ed.), Molecular biology, biochemistry & biophysics, vol. 32. Chemical Recognition in Biology. Springer-Verlag KG, Berlin.
21. **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.
22. **Kredich, N. M.** 1971. Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*. I. Effects of growth on varying sulfur sources and o-actyl-L-serine on gene expression. J. Biol. Chem. **246**:3474–3484.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Lindblow-Kull, C., F. J. Kull, and A. Shrift.** 1985. Single transporter for sulfate, selenate, and selenite in *Escherichia coli* K-12. J. Bacteriol. **163**:1267–1269.
25. **Mandrand-Berthelot, M.-A., M. Y. K. Wee, and B. A. Haddock.** 1978. An improved method for the identification and characterization of mutants of *Escherichia coli* deficient in formate dehydrogenase activity. FEMS Microbiol. Lett. **4**:37–40.
26. **Marinus, M. G., N. R. Morris, D. Söll, and T. C. Kwong.** 1975. Isolation and partial characterization of three *Escherichia coli* mutants with altered transfer ribonucleic acid methylases. J. Bacteriol. **122**:257–265.
27. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Miller, J. H., and A. M. Albertini.** 1983. Effects of surrounding sequence on the suppression of nonsense codons. J. Mol. Biol. **164**:59–71.
29. **Prather, N. E., B. H. Mims, and E. J. Murgola.** 1983. *supG* and *supL* in *Escherichia coli* code for mutant lysine tRNAs. Nucleic Acids Res. **11**:8283–8286.
30. **Reddy, C. C., and E. J. Massaro.** 1983. Biochemistry of selenium: a brief overview. Fundam. Appl. Toxicol. **3**:431–436.

31. **Ryals, J., R.-Y. Hsu, M. N. Lipsett, and H. Bremer.** 1982. Isolation of single-site *Escherichia coli* mutants deficient in thiamine and 4-thiouridine syntheses: identification of a *nuvC* mutant. J. Bacteriol. **151**:899–904.
32. **Shpaer, E. G.** 1986. Constraints on codon context in *Escherichia coli* genes: their possible role in modulating the efficiency of translation. J. Mol. Biol. **188**:555–564.
33. **Stadtman, T. C.** 1980. Biological functions of selenium. Trends Biochem. Sci. **5**:203–206.
34. **Stadtman, T. C.** 1983. New biologic functions—selenium-dependent nucleic acids and proteins. Fundam. Appl. Toxicol. **3**: 420–423.
35. **Stadtman, T. C.** 1984. Occurrence and characterization of selenocysteine in proteins. Methods Enzymol. **107**:576–581.
36. **Stadtman, T. C.** 1985. Specific occurrence of selenium in certain enzymes and amino acid transfer ribonucleic acids. Phosphorus Sulfur **24**:199–216.
37. **Sullivan, M. A., J. F. Cannon, F. H. Webb, and R. M. Bock.** 1985. Antisuppressor mutation in *Escherichia coli* defective in biosynthesis of 5-methylaminomethyl-2-thiouridine. J. Bacteriol. **161**:368–376.
38. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
39. **Wilson, L. G., and R. S. Bandurski.** 1958. Enzymatic reactions involving sulfate, sulfite, selenate, and molybdate. J. Biol. Chem. **233**:975–981.
40. **Wittwer, A. J.** 1983. Specific incorporation of selenium into lysine- and glutamate-accepting tRNAs from *Escherichia coli*. J. Biol. Chem. **258**:8637–8641.
41. **Wittwer, A. J., and T. C. Stadtman.** 1986. Biosynthesis of 5-methylaminomethyl-2-selenouridine, a naturally occurring nucleoside in *Escherichia coli* tRNA. Arch. Biochem. Biophys. **248**:540–550.
42. **Wittwer, A. J., L. Tsai, W.-M. Ching, and T. C. Stadtman.** 1984. Identification and synthesis of a naturally occurring selenonucleoside in bacterial tRNAs: 5-[(methylamino)methyl]-2-selenouridine. Biochemistry **23**:4650–4655.
43. **Young, P. A., and I. I. Kaiser.** 1975. Aminoacylation of *Escherichia coli* cysteine tRNA by selenocysteine. Arch. Biochem. Biophys. **171**:483–489.
44. **Zinoni, F., A. Birkmann, T. C. Stadtman, and A. Böck.** 1986. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:4650–4654.