The selenocysteine (Sec) tRNA population in Drosophila melanogaster is aminoacylated with serine, forms selenocysteyl-tRNA, and decodes UGA. The Km of Sec tRNA and serine tRNA for seryl-tRNA synthetase is 6.67 and 9.45 nM, respectively. Two major bands of Sec tRNA were resolved by gel electrophoresis. Both tRNAs were sequenced, and their primary structures were indistinguishable and colinear with that of the corresponding single copy gene. They are 90 nucleotides in length and contain three modified nucleosides, 5-methylcarboxymethyluridine, N9-isopentenyladenosine, and pseudouridine, at positions 34, 37, and 55, respectively. Neither form contains 1-methyladenosine at position 58 or 5-methylcarboxymethyl-2'-O-methyluridine, which are characteristically found in Sec tRNA of higher animals. We conclude that the primary structures of the two bands of Sec tRNA resolved by electrophoresis are indistinguishable by the techniques employed and that Sec tRNAs in Drosophila may exist in different conformational forms. The Sec tRNA gene maps to a single locus on chromosome 2 at position 47E or F. To our knowledge, Drosophila is the lowest eukaryote in which the Sec tRNA population has been characterized to date.

The use of UGA as a codon for selenocysteine (Sec) has been well documented in higher vertebrates (see Refs. 1–3 for reviews). The tRNAs that are responsible for inserting Sec into selenoprotein in response to UGA in protein biosynthesis have been characterized in several mammals and in Xenopus laevis (see Refs. 2 and 3 for reviews). Sec tRNAs are initially aminoacylated with serine by seryl-tRNA synthetase and are then functional by aminoacylation with serine at position 55, and 1-methyladenosine at position 58. A single copy gene for Sec tRNA has been shown to be present in the genomes of higher vertebrates as well as in other animals such as Drosophila and Caenorhabditis elegans (11).

Several studies, in addition to the present one, are beginning to elucidate the role of selenium metabolism in Drosophila. For example, Perlaky et al. (12) have shown that UGA occurs in the opening reading frame of Gld mRNA from several species of Drosophila, and these investigators have provided strong evidence that this UGA is a Sec codon. Two groups have reported a gene encoding a homologue of selenophosphate synthetase in Drosophila (13, 14). The gene product did not show any selenophosphate synthetase activity (13), although the gene has a role in imaginal disc morphogenesis (14).

The present study was initiated to expand our understanding of selenium metabolism and, in particular, selenoprotein biosynthesis in Drosophila melanogaster by analyzing the Sec tRNA population. Like its counterpart in higher vertebrates, Drosophila Sec tRNA is aminoacylated with serine, forms selenocysteyl-tRNA, decodes UGA and, therefore, is designated Sec tRNA(Sec). The Sec tRNA(Sec) population in higher vertebrates consists primarily of two isoacceptors that differ from each other by a single methyl group on the 2'-O-ribose of the nucleoside in the wobble position (4, 5), resulting in either 5-methylcarboxymethyluridine (mcm5U) or 5-methylcarboxymethyl-2'-O-methyluridine (mcm5Um). Exogenous selenium alters the levels and distributions of these isoacceptors in mammalian cells grown in culture (6), in the tissues of rats maintained on a selenium-deficient diet (4), in the tissues of selenium-deficient rats supplemented intravenously with this element (7), in Xenopus oocytes maintained in culture (8), and in mouse embryonic stem cells harboring only one functional copy of the Sec tRNA(Sec) gene (9).

The primary sequences of the major species of rat liver (4) and bovine liver (5) Sec tRNA(Sec) have been reported. They are colinear with the corresponding Sec tRNA genes from each animal (4, 10) and contain only four modified nucleosides. In addition to mcm5U or mcm5Um at position 34, both tRNAs contain N9-isopentenyladenosine (i6A) at position 37, pseudouridine (ψ) at position 55, and 1-methyladenosine at position 58. A single copy gene for Sec tRNA has been shown to be present in the genomes of higher vertebrates as well as in other animals such as Drosophila and Caenorhabditis elegans (11).

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**EXPERIMENTAL PROCEDURES**

**Materials**—Wild type D. melanogaster adult flies, embryos, and the Drosophila cell line (Schneider Line 2 (designated SL2)) were obtained from Dr. C. Wu at the National Institutes of Health, and the 1st, 2nd, and 3rd instar larvae and pupae were obtained by growing Drosophila. 75SeO42− (specific activity 190 Ci/mmol as H2SeO4) was obtained from...
was purchased from Bioneer, Inc. (Seoul, Korea) and used as primer for linkerTM 1800 cross-linker and was hybridized with QuikHyb RHybrid-method of random-primed labeling (24), and hybridization and detection of the hybridized fragment were carried out by the procedures of Langer-Safer et al. (25) and Schmidt et al. (26).

Transfer RNA Sequencing and Modified Base Identification—Puriﬁed Sec tRNAs were sequenced as described previously (4, 19), and modiﬁed nucleosides were determined by combined liquid chromatography/electrospray ionization mass spectrometry (LC/MS) as follows: tRNAs were digested totally to nucleosides using nuclease P1, phosphodiesterase I, and bacterial alkaline phosphatase as described (27), and the digest was injected directly into the liquid chromatograph without prior cleanup. Electrospray ionization LC/MS was conducted on a Micromass Platform II mass spectrometer interfaced to a Hewlett-Packard 1090 liquid chromatograph. Chromatography was conducted on a 250 x 2 mm LC-18S column with 20 x 2 mm LC-18 precolumn (Supelco, Inc., Bellefonte, PA). Phosphate was fractionated using an ammonium acetate-acetonitrile gradient as described (28), except that the ammonium acetate concentration was increased to 3 M NaCl-buffer A, washing the column in this buffer until the A260 units dropped below 1.0, and purifying Sec tRNA[Ser]Sec by two successive runs over the RPC-5 column as described (4). Sec tRNA[Ser]Sec was identiﬁed by the procedure of Forshhammer et al. (22) as described above. Smaller quantities of Sec tRNA were obtained from total tRNA for aminoacylation and coding studies by passing 3500 A260 units of tRNA over an RPC-5 column in 0.5 M NaCl-buffer A, washing the column in this buffer until the A260 units dropped below 1.0, and purifying Sec tRNA[Ser]Sec by two successive runs over the RPC-5 column as described (4). Sec tRNA[Ser]Sec was identiﬁed in all column fractions by northern hybridization (see “Experimental Procedures”). Ser tRNA was identiﬁed from the BD-cellulose column (4, 19) and further puriﬁed as described above for tRNA[Ser]Sec, and the single species of tRNAser that decoded UGA in a ribosomal binding assay, and that is aminoacylated with serine, that speciﬁcally and efﬁciently decodes UGA in a ribosomal binding assay as shown in the following “Results.” Ser tRNA[Ser]Sec was aminoacylated with [3H]serine in the presence of Drosophila aminoacyl-tRNA synthetases. The resulting [3H]seryl-tRNA was fractionated on a RPC-5 column as shown in Fig. 1. The bulk of the labeled tRNA eluted from the column in fractions 36–60, whereas a minor peak eluted in fractions 72–75. Because vertebrate Sec tRNAs are more hydrophobic than the corresponding Ser tRNAs and they represent from about 1 to 5% of the Ser tRNA population (reviewed in Refs. 2 and 3), we suspected that the minor later eluting peak represented the Sec tRNA population. To determine if this latter peak is Sec tRNA, column fractions were tested for their ability to hybridize to a 188-base pair fragment encoding the Drosophila Sec tRNA gene (11) by northern hybridization. The analysis conﬁrmed that the minor later eluting peak represented the Sec tRNA population in Drosophila (see Fig. 1). The Sec tRNA[Ser]Sec population represents 0.54% of the Ser tRNA population in Drosophila, which is lower than that found in mammalian cells and tissues (2, 3).

Codon Recognition Properties—To obtain sufﬁcient quantities of Sec tRNA[Ser]Sec for determining its codon recognition properties, it was separated from the bulk of other tRNAs, including tRNA, as described under “Experimental Procedures.” Sec tRNA[Ser]Sec was aminoacylated with [3H]serine and chromatographed on a RPC-5 column, and the resulting single, homogenous peak of [3H]seryl-tRNA[Ser]Sec, recognized UGA but not UGU, UGC, or UGG, which are codons with a degenerate base in the 3’-position, or the serine codons UCU, UCG, or AGU (data not shown).

Identification of Sec tRNA with Total tRNA—To identify the Sec tRNA population within total tRNA, tRNA from Drosophila embryos was aminoacylated with [3H]serine in the presence of Drosophila aminoacyl-tRNA synthetases. The resulting [3H]seryl-tRNA was fractionated on a RPC-5 column as shown in Fig. 1. The bulk of the labeled tRNA eluted from the column in fractions 36–60, whereas a minor peak eluted in fractions 72–75. Because vertebrate Sec tRNAs are more hydrophobic than the corresponding Ser tRNAs and they represent from about 1 to 5% of the Ser tRNA population (reviewed in Refs. 2 and 3), we suspected that the minor later eluting peak represented the Sec tRNA population. To determine if this latter peak is Sec tRNA, column fractions were tested for their ability to hybridize to a 188-base pair fragment encoding the Drosophila Sec tRNA gene (11) by northern hybridization. The analysis conﬁrmed that the minor later eluting peak represented the Sec tRNA population in Drosophila (see Fig. 1). The Sec tRNA[Ser]Sec population represents 0.54% of the Ser tRNA population in Drosophila, which is lower than that found in mammalian cells and tissues (2, 3).

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Identification of Selenocysteyl-tRNA[Ser]Sec—The data presented above show that Drosophila embryos contain a tRNA that is aminoacylated with serine, that speciﬁcally and efﬁciently decodes UGA in a ribosomal binding assay, and that hybridizes to the previously identiﬁed gene for Sec tRNA[Ser]Sec from this organism (11). To obtain direct evidence that Drosophila contain selenocysteyl-tRNA[Ser]Sec, we utilized the Drosophila cell line, SL2 (29). SL2 cells were labeled with 75Se, and the resulting labeled tRNA was extracted and chromatographed on a RPC-5 column (Fig. 2). Two 75Se-labeled tRNA isoacceptors were observed, a minor front-running peak followed by a major later eluting peak. Both 75Se-labeled tRNAs recognized UGA in a ribosomal binding assay as shown in the following “Results.” The major eluting peak was deacylated, and the 75Se-labeled material was identiﬁed as Sec (data not shown). Because this tRNA is aminoacylated with serine and forms sel-
enocysteyl-tRNA, it can be designated Sec tRNA\[Ser\]Sec like its counterpart in higher vertebrates.

Aminoacylation and Kinetic Studies—

Sec tRNA\[Ser\]Sec purified by RPC-5 chromatography (see "Experimental Procedures") was used for aminoacylation and kinetic studies. The extent of aminoacylation of tRNA\[Ser\]Sec in the presence of either Drosophila or mammalian aminoacyl-tRNA synthetases was determined over a wide range of tRNA\[Ser\]Sec concentrations (data not shown). After 25 min of incubation, the extent of attachment of serine to tRNA\[Ser\]Sec was indistinguishable with homologous or heterologous synthetase. However, the rate of aminoacylation of Sec tRNA\[Ser\]Sec with serine was much faster with Drosophila than with mammalian synthetase (see Fig. 3). The \(K_m\) of Drosophila Sec tRNA\[Ser\]Sec and Ser tRNASer for the homologous seryl-tRNA synthetase was determined. The \(K_m\) for Sec tRNA\[Ser\]Sec was 6.67 nM and for Ser tRNASer it was 9.45 nM. The \(K_m\) for Ser tRNASer, which decodes UCU, UCC, and UCA, from bovine liver for rabbit reticulocyte seryl-tRNA synthetase was determined as a control and found to be 3.03 nM.

Effect of Selenium on Drosophila Cells in Culture—

Supplementation of the medium of cultured mammalian cells (6), of Xenopus oocytes (8), or the diets of rats (4) with selenium enhances the level of the Sec tRNA\[Ser\]Sec population and causes a redistribution of the major Sec isoacceptors in cells and tissues. We therefore examined the effects of selenium on the Sec tRNA\[Ser\]Sec population in SL2 cells grown in culture (Fig. 4). SL2 cells were grown in varying levels of supplemented selenium, and the tRNA was extracted and examined by gel electrophoresis. Lane 1, which was included as a control, shows the resolution of two Sec tRNA\[Ser\]Sec forms by gel electrophoresis. Lane 2 shows a single band of Sec tRNA\[Ser\]Sec from SL2 cells that corresponds to the lower running band of Sec tRNA\[Ser\]Sec. These cells were grown in medium supplemented with nontoxic levels of sodium selenite (1 \(\mu\)M). Inter-

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**Fig. 1. Fractionation of Drosophila Sec tRNA\[Ser\]Sec.** Total Drosophila tRNA was aminoacylated with \(^{3}H\)serine in the presence of Drosophila aminoacyl-tRNA synthetases, and the resulting \(^{3}H\)seryl-tRNA was fractionated on a RPC-5 column as described under “Experimental Procedures.” Northern hybridization assays were carried out by dot blotting 5 \(\mu\)l of every other fraction onto a nitrocellulose filter and hybridizing the filter with a 188-base pair probe encoding the Drosophila Sec tRNA gene as described under “Experimental Procedures.” CPM, counts per minute.

**Fig. 2. \(^{75}Se\)-Selenocysteyl-tRNA\[Ser\]Sec.** \(^{75}Se\)-Labeled aminoacyl-tRNAs were isolated from SL2 cells (20) and fractionated on a RPC-5 column, and labeled tRNAs were pooled as shown by the hatched areas and prepared for coding studies; coding studies were carried out as described under “Experimental Procedures.” The binding of Peak I (9928 cpm and 0.029 \(A_{260}\) unit added to each reaction) and Peak II (19,307 cpm and 0.021 \(A_{260}\) unit added to each reaction) to ribosomes in the presence and absence of UGA is shown, and None indicates the binding of \(^{75}Se\)-labeled tRNA in the absence of a template. CPM, counts per minute.

**Fig. 3. Rates of aminoacylation of Sec tRNA\[Ser\]Sec.** Partial purified Drosophila Sec tRNA\[Ser\]Sec (see “Experimental Procedures”) was aminoacylated in the presence of \(^{3}H\)serine and either Drosophila or mammalian seryl-tRNA synthetase. CPM, counts per minute.
Sec tRNAs in Drosophila

fig. 4. Effect of Se on the Sec tRNA\[^{[Ser][Sec]}\] population. SL2 cells were grown in the presence of varying levels of exogenous selenium; tRNA was extracted, fractionated by gel electrophoresis, electoblotted onto a membrane, and hybridized with a probe. An autoradiogram was prepared as described under “Experimental Procedures.” Lane 1, control tRNA from young flies; lanes 2-4, tRNA from SL2 cells grown in Drosophila medium supplemented with \(10^{-6}, 5 \times 10^{-5}\), and \(10^{-4}\) selenium, respectively.

Estringly, higher levels of selenium that inhibited growth rate, and therefore are probably toxic to the cells, resulted in the appearance of the slower migrating band (lanes 3 and 4).

Primary Structures of Sec tRNAs\[^{[Ser][Sec]}\]—Total tRNA isolated from Drosophila at various stages of development, including embryonic, 1st, 2nd, and 3rd larval stages, pupae, and adult flies, manifested two bands of Sec tRNA\[^{[Ser][Sec]}\] as determined by gel electrophoresis (see Fig. 4 for adult flies). The amounts of these two bands did not appear to vary throughout development (data not shown). Transfer RNAs from both bands were isolated and purified from embryos as described under “Experimental Procedures,” and their primary structures were analyzed.

Initially, both purified Sec tRNAs\[^{[Ser][Sec]}\] were analyzed by the partial formamide hydrolysis method previously used to determine the structure of bovine (19) and rat Sec tRNAs\[^{[Ser][Sec]}\] (4). In short, this procedure involves partial hydrolysis of purified tRNA, 5'-end labeling of the resulting 5'-hydroxyl termini, and resolution of the labeled “ladder” on a denaturing polyacrylamide gel. A representative autoradiogram of such a gel is presented in Fig. 5. Examination of the radioactive ladders indicated that the patterns are very similar, differing mainly in the higher molecular weight region. In addition to the bands seen in the faster migrating tRNA species, the pattern seen for the slower migrating species has a large radioactive band separated by a gap in the ladder. Furthermore, several bands within the patterns show different relative intensities that are indicated by an asterisk in the figure. This signifies differences in accessibility to cleavage by formamide and typically is an indication of different conformational states of the compared molecules. It is also noteworthy that neither pattern has a single gap at the position representing the wobble nucleoside within the anticodon, as would be expected if these tRNAs contained the modified residue mcm\(^5\)Um in this position that was previously observed with one of the mammalian Sec tRNA\[^{[Ser][Sec]}\] isoacceptors (4).

Each of the radioactive bands from the acrylamide gel was excised, as were others obtained from the same digest/labeling following electrophoresis for different times to optimally resolve the ladder and finally digested to 5' 3'-nucleotide diphosphates. Digested products were resolved by ascending chromatography on polyethyleneimine cellulose to obtain the primary sequence by comparison to the known mobilities of modified and nonmodified residues (30). This analysis indicated that the primary sequence of both tRNAs was colinear with that reported for the Drosophila Sec tRNA\[^{[Ser][Sec]}\] gene (11). Only three modified residues were identified in this manner, mcm\(^5\)U at position 34, \(\tilde{\imath}\)A at position 37, and \(\psi\) at position 55, in both characterized molecules (Fig. 6). Autoradiograms of representative polyethyleneimine plates are shown for the T\(\psi\)CG loop, variable region (Fig. 6A), and the region including the anticodon (Fig. 6B). The identities of modified nucleoside diphosphates were confirmed by further digestion with nuclease P1 and two dimensional chromatography as described previously for the same modified residues (data not shown) (see Refs. 4 and 19). The modified residue content of both tRNA\[^{[Ser][Sec]}\] forms was verified by electrospray ionization LC/MS (Fig. 7).

Modified nucleoside identities were established from mass spectra and characteristic relative retention times (5, 28). In agreement with results from direct sequencing of both tRNAs, only three modified nucleosides are present in each tRNA: \(\psi\), mcm\(^5\)U, and \(\tilde{\imath}\)A.

The analyses described above failed to determine a structural explanation for the different mobilities of the two Sec tRNA species detected by electrophoresis of Drosophila embryo tRNA. Clearly, the molecules are identical for the 90 nucleotides resolved by our techniques. Furthermore, 5'-end labeling of both tRNAs and complete digestion with ribonuclease T1 resulted in labeled fragments of indistinguishable mobility, consistent with the previously reported sequence of the Drosophila Sec tRNA\[^{[Ser][Sec]}\] gene. Elongation of both tRNA\[^{[Ser][Sec]}\] forms to their 5'-ends by primer extension (see “Experimental Procedures”) revealed that each contained the same number of nucleotides in this region, suggesting that these ends of both molecules were identical. Based on the collective data, we propose that the observed differences in mobility of the Sec tRNA\[^{[Ser][Sec]}\] species in embryos and in SL2 cells when they are

Fig. 5. Partial formamide hydrolysis of purified Drosophila Sec tRNA\[^{[Ser][Sec]}\]. One \(\mu\)g of acrylamide gel-purified tRNA was hydrolyzed in denatured formamide, labeled with \(^{32}\)P, and electrophoresed in a 10% polyacrylamide gel as described previously (4). A I indicated the ladder was generated using the faster migrating tRNA species, and a 2 indicated the ladder was generated from the slower migrating tRNA. Asterisks indicate bands that display different relative intensities than the corresponding band in the ladder of the other isoacceptor.
exposed to high concentrations of selenium (Fig. 4) are because of altered conformational states of these molecules. This conclusion is supported by the observed differences in formamide cleavage susceptibility reported above. However, determination of the factors involved in the stabilization of the molecules between the conformational states will require further investigation.

Localization of Sec tRNA^[Ser][Sec] Gene on Chromosome 2—The Sec tRNA^[Ser][Sec] gene occurs in single copy in the Drosophila genome (11). A 5-kilobase BamHI fragment encoding Sec tRNA^[Ser][Sec] was used as a probe to determine the location of the gene within the genome of Drosophila. As shown in Fig. 8, the gene maps to a single site on chromosome 2 in region 47E or F.

DISCUSSION

Species representative of the three primary domains, archaea, bacteria, and eukaryotes (31), recognize the UGA triplet as the codon for Sec (32). Because Sec tRNAs^[Ser][Sec] are essential for both Sec biosynthesis and the subsequent incorporation of this amino acid into selenoproteins, we have chosen to examine tRNA^[Ser][Sec] in an eukaryotic organism that is both evolutionarily distant from mammals yet amenable to study either as a whole organism or as an established cell line. Herein, we demonstrate that Drosophila contains a Sec tRNA^[Ser][Sec], which is aminoacylated with serine, and that the seryl-tRNA^[Ser][Sec] is converted to selenocysteyl-tRNA^[Ser][Sec], which is capable of specifically recognizing UGA in a ribosomal binding assay. In this respect, this process is similar to that observed in bacteria and mammals.

Drosophila represents the lowest eukaryote from which the direct sequence analysis of a Sec tRNA^[Ser][Sec] has been determined. The sequence presented within this study is colinear with the gene sequence previously reported (11). We have detected only three modified nucleosides, $\psi$, $i^A$, and mcm$^5$U, although it remains possible that other modifications occur in a fraction of the tRNA population that is below the limits of our detection. Although we detect two distinct Sec tRNAs by electrophoresis of Drosophila tRNA derived from embryos and numerous other stages of development, only one species was detected in this manner from cultured cells. On the other hand, two peaks of selenocysteyl-tRNA^[Ser][Sec] from SL2 cells were resolved by RPC-5 chromatography when the cells were labeled with $^{75}$Se. This observation suggests the existence of multiple species of Sec tRNA^[Ser][Sec] in SL2 cells, which may differ either in base modification or in their primary sequences that might also occur in developing tissues but are below our limits of detection during sequencing procedures.

It is possible that the multiple forms of Sec tRNA^[Ser][Sec] analyzed in embryos are present in different cell types, whereas only one form is seen in SL2 cells by electrophoresis because of its clonogenic origin. Our analyses of these two forms clearly demonstrate that they do not differ because of 2'-O-methylation of mcm$^5$U, as seen in mammalian cells and tissues. This would have been evident from the partial formamide hydrolysis procedure (Fig. 5) or from modified nucleoside analysis by LC/MS (Fig. 7). As we have been unable to detect either a difference in primary sequence or base modification of these Sec tRNA^[Ser][Sec] forms, we hypothesize that the distinct electrophoretic mobilities are because of a conformational difference between these molecules. Although the basis for the stabilization might occur during the initial folding of the molecules or by association with as yet undetectable molecules, we...
common with and distinct from that already described for other eukaryotes. The general principle that this process requires a dedicated, relatively undermodified tRNA that is the site of selenocysteine synthesis from serine and decodes UGA is demonstrated for this species as well. The lack of any Sec tRNA species including either the 1-methyladenosine or mcm^5Um modifications, as well as the conformational response to selenium status described above, suggests that the study of selenium metabolism in Drosophila will yield novel insights into the evolution of the translation of selenium-containing proteins. In addition, the relatively low abundance of Sec tRNA^{[Ser][Sec]} in Drosophila (Fig. 1) as compared with higher vertebrates (2, 3) suggests that because the intracellular levels of tRNAs reflect their requirements for protein synthesis (see Refs. 2 and 3 and references therein), a lower amount of selenoprotein biosynthesis and perhaps a variety of selenoproteins synthesized occurs in flies.

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FIG. 7. Chromatogram (UV absorbance at 290 nm) from LC/MS analysis of modified nucleosides in electrophoretically separated tRNA^[Ser][Sec]_species. A, faster migrating tRNA; B, slower migrating tRNA.

FIG. 8. Chromosomal mapping of the Drosophila Sec tRNA^[Ser][Sec]_gene. A digoxigenin-labeled clone hybridized at a single site on chromosome 2 to region 47E or F as shown by the arrow. The inset shows hybridization of the probe in a second chromosome squash.