Vol. 184, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1992 Pages 254-259

SELENOCYSTEYL-tRNAs RECOGNIZE UGA IN BETA VULGARIS, A HIGHER PLANT, AND IN GLIOCLADIUM VIRENS, A FILAMENTOUS FUNGUS

Dolph Hatfield[1], In Soon Choi[1], Sue Mischke[2] and L. D. Owens[3]

[1]Laboratory of Experimental Carcinogenesis
National Cancer Institute
National Institutes of Health
Bethesda, MD 20892

[2]Biocontrol of Plant Diseases Laboratory
[3]Plant Molecular Biology Laboratory
United States Department of Agriculture
Agricultural Research Service
Beltsville, MD 20705

Received March 3, 1992

SUMMARY: Selenocysteyl-tRNAs that decode UGA were previously identified in representatives of three of the five life kingdoms which were the monera, animal and protist kingdoms. In the present study, we show that these tRNAs also occur in representatives of the two remaining kingdoms, plants and fungi; i.e., selenocysteyl-tRNAs which code for UGA occur in *Beta vulgaris*, a higher plant, and in *Gliocladium virens*, a filamentous fungus. The fact that selenocysteyl-tRNAs are present in all five life kingdoms strongly suggests that UGA, in addition to dictating the cessation of protein synthesis, also codes for selenocysteine in the universal genetic code.

UGA is a unique codon in that it is the bearer of many different hats in the genetic code. For example, UGA serves as a termination codon in the universal genetic code (1,2), codes for tryptophan in mitochondria (3,4) and *Mycoplasma* (5,6) and is weakly read as tryptophan in *B. subtilis* (7) and presumably as tryptophan in *E. coli* (8). UGA is also a codon for selenocysteine in mammalian cells (9-11) and bacteria (12,13); thus selenocysteine is now recognized as the 21st naturally occurring amino acid in protein. More recently, UGA was reported as a cysteine codon in *Euplotes octocarinatus* (14).

Variations in codon assignments, in addition to those for UGA, have been observed in the genetic code since the code was deciphered (1,2) and shown to be universal (1,15) in the mid 1960's. For example, numerous amino acids have different codewords in mitochondria, and these vary in mitochondria in different organisms (3,4). Furthermore, some ciliates (3,4), *Mycoplasma* (16) and a yeast, *Candida cylindracea* (17), use different codewords for certain amino acids.

0006-291X/92 $1.50

Interestingly, the genetic code was shown to be universal by Nirenberg and collaborators (1,15) who demonstrated that the genetic language was the same for 20 aminoacyl-tRNAs (corresponding to the 20 [then] known amino acids in protein) from *E. coli*, *Xenopus* and guinea pigs. Most certainly, had it been known in the mid-1960's that selenocysteine was one of the 21 naturally occurring amino acids in protein, and had Marshall et al. (15) shown that selenocysteyl-tRNA from *E. coli*, *Xenopus* and guinea pigs decodes UGA, then selenocysteine would have been assigned at that time to UGA in the universal genetic code. However, since we now know that variations occur in the universal genetic language, it is necessary to show that selenocysteine tRNA is present in representatives of each of the five life kingdoms before concluding that selenocysteine belongs in the universal genetic code. Previously, a selenocysteyl-tRNA which decodes UGA or the corresponding gene was shown to be widespread in Eubacteria (18), to be ubiquitous in animals (19) and to occur in two widely divergent protists, *Thalassiosira* and *Tetrahymena* (20). In the present study, we report the presence of selenocysteyl-tRNAs which decode UGA in *Beta vulgaris*, a sugar beet and a dicot, and in *Gliocladium virens*, an ascomycete.

MATERIALS AND METHODS

B. vulgaris was grown to mid-log phase essentially as given (21) and *G. virens* was grown to mid-log phase in Neurospora minimal medium as given (22). Cells were harvested, washed in the corresponding fresh media and resuspended (approximately 3 g) in 50 ml of the same media. 2.5 mCi of $^{75}SeO_3^{2-}$ (specific activity 175 Ci $mmol^{-1}$ H_2SeO_3; obtained from Dr. Kurt Zinn of the University of Missouri Research Reactor Facility) were then added to cell cultures. After 3 hrs incubation cycloheximide (5×10^{-3} M; Sigma Chemical Co.) was added and the incubation period extended 1 additional hr. Cells were harvested, washed in fresh media to remove extracellular ^{75}Se and the packed cells stored at -80^0C until ready for use (1 to 2 days). Aminoacyl-tRNAs were extracted from labeled cells by homogenizing in 15 ml of 0.2 M NaCl, 0.005 M $Mg(CH_3COO)_2$, 0.005 M $NaCH_3COO_2$, 0.0005 M EDTA, 0.1% SDS, pH 4.5, and 15 ml of H_2O-saturated phenol. The phases were separated by centrifugation and the phenol-interphase layers were back-extracted 1 time with 15 ml of the above buffer without SDS. The aqueous phases were pooled, passed through a DE-52 column (1.5 X 4 cm) and the resulting aminoacyl-tRNAs isolated from the column as given (11). The aminoacyl-tRNA solution was adjusted to 0.4 M NaCl, fractionated on a reverse phase chromatographic (RPC-5) column, individual peaks of labeled tRNAs were isolated and responses of the tRNAs to the trinucleoside diphosphates, UGA, UGU, UGG and UAG, determined in the ribosomal binding assay of Nirenberg and Leder (23) as given (11).

The aminoacyl moiety attached to ^{75}Se-containing tRNAs which recognize UGA were identified as follows: Unlabeled selenocysteine (1-2 μgs; reduced from selenocystine, Sigma Chemical Co.) was added to each sample of [^{75}Se]-aminoacyl-tRNA, the samples were deacylated, the resulting tRNA was removed with EtOH, and the EtOH removed with a stream of nitrogen gas. Samples were then dried in a speed vac under atmospheric conditions (which permitted selenocysteine to reoxidize to selenocystine) and redissolved in 3-4 μl H_2O (see also ref. 24). Aliquots of approximately 10,000 cpm were chromatographed on DC-Plastikfolien Kieselgel 60 (obtained from EM Separations, Gibbstown, NJ, USA) in 70% EtOH as given (24). Cochromatography of labeled samples contained approximately 5000 cpm of each of two samples. An authentic sample of ^{75}Se-selenocysteine (obtained by

deacylation of ^{75}Se-selenocysteyl-tRNA) was prepared from HL60 (human leukemia) cells as given (11). Serine and phosphoserine were commercial products, and ninhydrin spray (Nin-Sol) was obtained from Pierce Chemical Co.

RESULTS AND DISCUSSION

Cells of *B. vulgaris* and *G. virens* were grown in culture and administered ^{75}selenium to label the endogenous tRNA population. The resulting ^{75}Se-containing tRNAs were extracted from the cells and chromatographed on a reverse phase chromatographic column (see Fig. 1). The upper graph shows that *B. vulgaris* cells contain two major eluting ^{75}Se-containing isoacceptors. Column fractions containing each peak were pooled as shown and the responses of each to UGA in a

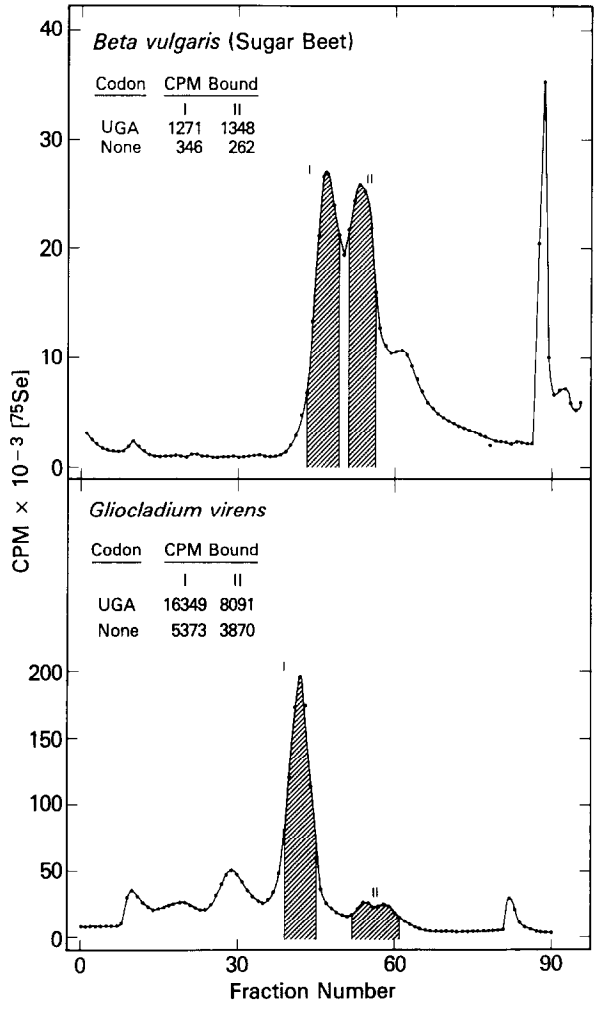

Fig. 1. Column chromatography and codon responses of selenocysteyl-tRNAs from *Beta vulgaris* and *Gliocladium virens*. Cells were grown and labeled, aminoacyl-tRNAs extracted and chromatographed, and responses of individual peaks to UGA, UGU, UGG and UAG in a ribosomal assay determined as given in Materials and Methods. Attachments of labeled aminoacyl-tRNAs to ribosomes in the presence (UGA) or absence (None) of codon are shown in the Fig. Selenocysteyl-tRNAs did not respond to the other codons assayed (data not shown).

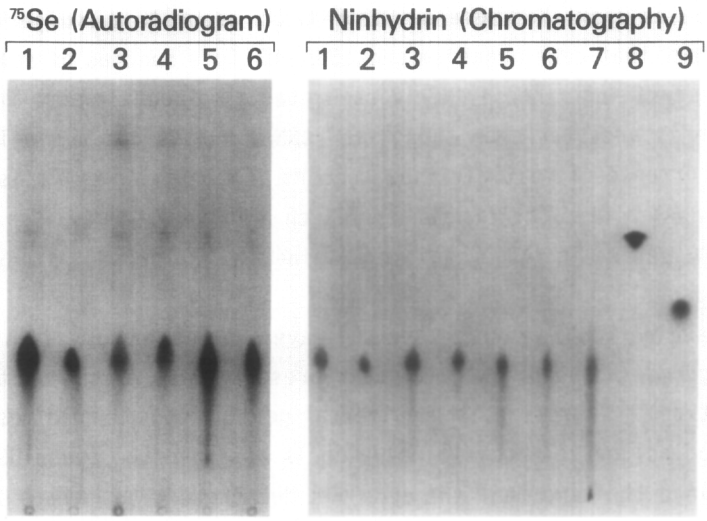

Fig. 2. Identification of selenocysteine attached to [^{75}Se]-aminoacyl-tRNAs. The left hand panel shows an autoradiogram (21 hr exposure) of the deacylated products from the ^{75}Se-containing tRNAs which recognize UGA (see Fig. 1). The components in each lane are explained in the text with the exception of lane 6 which contains equal amounts of Peak I from *B. vulgaris* and an authentic sample of ^{75}Se-selenocysteine. The right hand panel shows the corresponding chromatogram stained with ninhydrin, and the components in each lane are explained in the text. The details of the deacylation and chromatography of the resulting products are given in Materials and Methods.

ribosomal binding assay determined. Both peaks recognized UGA (see figure inset and figure legend). The smaller, latter eluting isoacceptor (in fractions 59 to 75) also responded to UGA (data not shown). Thus, there appear to be at least three isoacceptors in *B. vulgaris* which recognize UGA. In *G. virens*, the major ^{75}Se-containing peak was pooled as shown in the lower graph and it was found to recognize UGA (see figure inset and figure legend). In addition, the two later eluting minor peaks were also found to recognize UGA (see figure inset).

The ^{75}Se-containing moiety attached to the tRNAs that recognize UGA (Fig. 1) was identified as selenocysteine by deacylating the aminoacyl moiety from the tRNA in each peak and characterizing the resulting product by chromatography as shown in Fig. 2. The left hand panel in the figure shows an autoradiogram of the deacylated, labeled products, and the right hand panel shows authentic samples of selenocysteine (lanes 1-7), phosphoserine (lane 8) and serine (lane 9) stained with ninhydrin. Lanes 1-6 in the right hand panel are the same lanes as 1-6 in the left hand panel. The deacylated products from peaks I and II of *B. vulgaris* (lanes 1 and 2) and from peaks I and II of *G. virens* (lanes 3 and 4) have the same R_f in the solvent system as an authentic sample of ^{75}Se-selenocysteine (lane 5) as shown on the autoradiogram. Furthermore, these samples cochromatograph identically with authentic, unlabeled samples of selenocysteine (compare lanes 1-5 in both panels). Thus, the ^{75}Se-aminoacyl moiety attached to the tRNAs which recognize UGA in *B. vulgaris* and *G. virens* is selenocysteine.

These studies show that selenocysteyl-tRNAs occur in *B. vulgaris* and in *G. virens*. The fact that each organism contains multiple species of selenocysteine tRNAs is not surprising since higher vertebrates also contain more than a single selenocysteine isoacceptor even though the tRNAs arise from a single-copy gene (25). The occurrence of multiple isoacceptors in mammalian cells is apparently the result of RNA editing (26) and whether such a phenomenon may also account for different tRNA species in the organisms examined in the present study remains to be established.

Selenocysteine tRNAs which decode UGA have now been observed in each of the five life kingdoms including two diverse representatives of the protist kingdom (see Introduction). Therefore, the universal genetic code should most certainly be expanded to include selenocysteine which is assigned the codon UGA. AUG has been known, since the time that the code was deciphered, to have a dual role as a codon which specifies the initiation of protein synthesis as well as a codon which specifies methionine at internal positions of protein (1,2). Recognition of the role of UGA as a selenocysteine codon, in addition to its known role in specifying termination, is the first change in the universal genetic code since it was established in the mid 1960's.

ACKNOWLEDGMENTS

The authors thank M. Nirenberg for the trinucleoside disphosphates, K. Forchhammer and August Böck for details of their procedure for identifying selenocysteine, and E. Goldman for discussions relating to the usage of UGA as a weakly suppressed codon in *E. coli*.

REFERENCES

1. Nirenberg, M., Caskey, T., Marshall, R., Brimacombe, R., Kellog, D., Doctor, B., Hatfield, D., Levin, J., Rottman, F., Pestka, S., Wilcox, M., and Anderson, F. (1966) *Cold Spring Harbor Symp. Quant. Biol.* 31, 11-24.

2. Khorana, G.H., Büchi, H., Ghosh, H., Gupta, N., Jacob, T.M., Kössel, H., Morgan, R., Narang, S.A., Ohtuska, E., and Wells, R.D. (1966) *Cold Spring Harbor Symp. Quant. Biol.* 31, 39-49.

3. Fox, T.D. (1987) *Annu. Rev. Genet.* 21, 67-96.

4. Osawa, S., Muto, A., Jukes, T.H., and Ohama, T. (1990) *Proc. Royal Soc. Lond. Ser. B Biol. Sci.* 240, 19-28.

5. Inamine, J.M., Ho, K.-C., Loechel, S., and Hu, P.-C. (1990) *J. Bacteriol.* 172, 504-506.

6. Blanchard, A. (1990) *Mole. Microbiol.* 4, 669-676.

7. Lovett, P.S., Ambulos, N.P., Mulbry, W., Noguchi, N., and Rogers, E.J. (1991) *J. Bacteriol.* 173, 1810-1812.

8. Weiner, A.M., and Weber, K. (1973) *J. Mol. Biol.* 80, 837-855.

9. Chambers, I., Frampton, J., Goldfarb, P., Affara, N. McBain, W., and Harrison, P.R. (1986) *EMBO J.* 5, 1221-1227.

10. Mullenbach, G.T., Tabrizi, A., Irvine, B.D., Bell, G.I., Tainer, G.I., and Hallewell, R. (1988) *Prot. Engin.* 2, 239-246.

11. Lee, B.J., Worland, P.J., Davis, J.N., Stadtman, T.C., and Hatfield, D. (1989) *J. Biol. Chem.* 264, 9724-9727.

12. Zinoni, F., Birkmann, A., Stadtman, T.C., and Böck, A. (1986) *Proc. Natl. Acad. Sci. USA* 84, 3156-3160.

13. Leinfelder, W., Stadtman, T.C., and Böck, A. (1989) *J. Biol. Chem.* 264, 9720-9723.

14. Meyer, F., Schmidt, H.J., Plümper, E., Hasilik, A., Mersmann, G, Meyer, H.E. Engström, A, and Heckmann, K. (1991) *Proc. Natl. Acad. Sci. USA* 88, 3758-3761.

15. Marshall, R.E., Caskey, C.T., and Nirenberg, M. (1967) *Science* 155, 820-826.

16. Oba, T., Andachi, Y., Muto, A., and Osawa, S. (1991) *Proc. Natl. Acad. Sci. USA* 88, 921-925.

17. Kawaguchi, Y., Honda, H., Taniguchi-Morimura, J., and Iwasaki, S. (1989) *Nature* 341, 164-166.

18. Heider, J., Leinfelder, W., and Böck, A. (1989) *Nucl. Acids Res.* 17, 2529-2540.

19. Lee, B.J., Rajagopalan, M., Kim, Y.S., You, K.-H., Jacobson, K.B., and Hatfield, D. (1990) *Mol. Cell. Biol.* 10, 1940-1949.

20. Hatfield, D., Lee, B.J., Price, N.M., and Stadtman, T.C. (1991) *Mole. Microbiol.* 5, 1183-1186.

21. Krens, F.A., Jamar, D., Rouwendal, G.J.A., and Hall, R.D. (1990) *Theoret. Appl. Genet.* 79, 390-396.

22. Beadle, G.W. (1945) *Physiol. Rev.* 25, 643-663.

23. Nirenberg, M., and Leder, P. (1964) *Science* 145, 1399-1407.

24. Forchhammer, K., Leinfelder, W., Boesmiller, K., Veprek, B., and Böck, A. (1991) *J. Biol. Chem.* 10, 6318-6323.

25. Hatfield, D.L., Smith, D.W.E., Lee, B.J., Worland, P.J., and Oroszlan, S. (1990) *Crit. Rev. Biochem. Mol. Biol.* 25, 71-96.

26. Diamond, A.M., Montero-Puerner, Y., Lee, B.J., and Hatfield, D. (1990) *Nucl. Acids Res.* 18, 6727.