Aminoacyl-tRNAs from Physarum polycephalum: Patterns of Codon Recognition

DOLPH HATFIELD,1 MARY RICE,1 CATHERINE A. HESSION,2 AND PETER W. MELERA2*

Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20205,1 and Laboratory of RNA Synthesis and Regulation, Sloan-Kettering Institute for Cancer Research, Walker Laboratory, Rye, New York 105802

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Isoacceptors of Physarum polycephalum Ala-, Arg-, Glu-, Gln-, Gly-, Ile-, Leu-, Lys-, Ser-, Thr-, and Val-tRNAs were resolved by reverse-phase chromatography and isolated, and their codon recognition properties were determined in a ribosomal binding assay. Codon assignments were made to most isoacceptors, and they are summarized along with those determined in other studies from Escherichia coli, yeasts, wheat germ, hymenoptera, Xenopus, and mammals. The patterns of codon recognition by isoacceptors from P. polycephalum are more similar to those of animals than to those of plants or lower fungi.

Physarum polycephalum is a myxomycete whose natural mitotic synchrony and ease of cultivation in the laboratory have made it the subject of considerable study at the biochemical level (8). While biologically quite simple and generally considered a lower eucaryote, several aspects of the biochemistry and molecular biology of P. polycephalum are reminiscent of higher eucaryotic systems. For example, in higher eucaryotes and in P. polycephalum, the 5S genes are not associated with their rRNA gene repeat (12), whereas they are in Dictyostelium (27) and yeasts (37). Furthermore, the single-copy DNA content per haploid genome of P. polycephalum, although admittedly difficult to meaningfully compare with other systems, is an order of magnitude greater (i.e., 2.3 × 10⁶ base pairs [28]) than that found in Dictyostelium (2.1 × 10⁶ [26]), yeasts (3 × 10⁵ [19]), and Achlya (3.44 × 10⁵ base pairs [18]) and is more comparable to that found in Drosophila (1.04 × 10⁶ [26]) and sea urchins (6.1 × 10⁵ [6]). Most interestingly, P. polycephalum processes its 4-kilobase rRNA precursor in a fashion similar to higher eucaryotes in that only 50% of it is conserved during the maturation of the 26S and 19S rRNAs (28, 31).

Earlier studies conducted with P. polycephalum tRNA isolated from various growth phase mitotic cycle time points and from different time points during the starvation phase preceding sporulation have shown that although P. polycephalum is remarkably stable in tRNA isoacceptor distributions and aminoacylation levels during the growth phase (30, 32), the dramatic depletion and resynthesis of the tRNA population during the starvation phase result in quantitative alterations that are tRNA family specific rather than tRNA isoacceptor specific (29). As an extension of these studies and to help us more reasonably interpret possible changes in the tRNA population which may occur during the differentiation phase, we have resolved a number of aminoacyl-tRNAs from P. polycephalum and examined the codon recognition properties of the purified isoacceptors. The patterns of codon recognition of P. polycephalum isoacceptors are generally more like those observed in animals (i.e., hymenoptera [17] and mammals [14]) than those observed in plants (i.e., wheat germ [16]) and lower fungi (i.e., yeasts [43]).

MATERIALS AND METHODS

1⁵-N-amino acids were commercial products with the following specific activities (Ci/mmol): [¹⁵N]alanine, 43; [¹⁵N]arginine, 23.4; [¹⁵N]glutamic acid, 45; [¹⁵N]glutamine, 30; [¹⁵N]glycine, 15; [¹⁵N]isoleucine, 97.6; [¹⁵N]leucine, 171; [¹⁵N]lysine, 80.5; [¹⁵N]serine, 11; [¹⁵N]threonine, 3.4; and [¹⁵N]valine, 19.

P. polycephalum was grown, tRNA and aminoacyl-tRNA synthetases were prepared, and aminoacylation of purified tRNAs was carried out as previously described (30, 32) with the exception of tRNA⁴⁰⁰ and tRNA⁴⁰⁰⁰. Approximately 10-fold-greater aminoacylation of P. polycephalum tRNA⁴⁰⁰ and tRNA⁴⁰⁰ was obtained with rabbit reticulocyte synthetases than with P. polycephalum synthetases. Therefore, P. polycephalum [¹⁵N]glycyl-tRNA and [¹⁵N]leucyl-tRNA were prepared in the presence of rabbit reticulocyte synthetases with 0.036 M Tris-chloride (pH 7.4)–0.006 M MgCl₂–0.0036 M ATP and 0.06 M Tris-chloride (pH 7.4)–0.01 M MgCl₂–0.006 M ATP, respectively (14). [¹⁵N]labeled aminoacyl-tRNAs were resolved on a reverse-phase chromatographic column (designated RPC-5) (23) as previously described (14, 16, 17), and fractions were pooled from the developed columns and either prepared for codon recognition studies (13) or rechromatographed to achieve further resolution be-
fore being prepared for codon recognition studies. Fractions which were pooled for additional chromatography are designated by hatched areas in the figures, and fractions which were pooled for coding studies are designated by crosshatched areas. Gradients used for column chromatography are given in the legends to the figures. Ribosome binding studies were carried out by the procedure of Nirenberg and Leder (35) as given in reference 14. Trinucleoside diphosphates were the gift of M. W. Nirenberg or were prepared as previously described (15).

RESULTS

Eleven aminoacyl-tRNAs from _P. polypephalum_ were fractionated on a RPC-5 column (Fig. 1 to 4). Fractions were pooled from the developed columns and designated with a Roman numeral in the order of elution from the columns. Pooled fractions from developed columns of Ala- and Glu-tRNAs were not further chromatographed and were examined for their codon recognition properties (Fig. 1). The results of studies with Ala- and Glu-tRNAs are as follows.

Ala-tRNA eluted as a single peak from the RPC-5 column. It was divided into two fractions for coding studies. Both fractions responded to GCU, GCC, and GCA and less well to GCG.

Glu-tRNA was resolved into two major peaks. Peak I responded to GAG, and peak II responded to GAA.

Most of the major peaks observed in the first chromatographic column of Arg-, Gln-, Gly-, Ile-, Leu-, Lys-, Ser-, Thr-, and Val-tRNAs were further chromatographed (Fig. 2 to 4). Chromatography and coding studies (Fig. 2 to 4) of these aminoacyl-tRNAs are as follows.

Arg-tRNA was resolved into an initial eluting peak (I), followed by a larger eluting peak (II; see Fig. 2). Peak I was rechromatographed and found to recognize AGG. Peak II was observed to consist of at least two major isoacceptors, designated IIA and IIB, respectively. IIA responded primarily to AGA, less well to AGG, and to CGA. Further resolution of this fraction resulted in an earlier eluting shoulder (data not shown) which responded most strongly to CGA (i.e., at 0.01 M Mg2+, 2,713 cpm and 0.003 absorbance unit at 260 nm [A260 unit] were added to each ribosome binding assay, 550 cpm bound to ribosomes in the absence of template, and 1,245 cpm were stimulated over the background binding in the presence of CGA, 648 cpm in the presence of AGA, 201 cpm in the presence of AGG, and less than 100 cpm in the presence of other arginine codons). These data support the conclusion that fraction IIA contains an isoacceptor which responds to AGA and less well to AGG and an isoacceptor which recognizes CGA. The terminal eluting peak, IIB, recognizes CGU, CGC, and CGA.

Gln-tRNA was resolved into a large initial eluting peak (I) followed by two minor eluting peaks (II and III; see Fig. 2). Peak I was

![FIG. 1. Chromatography of Ala- and Glu-tRNAs and their coding responses. Columns were developed in a linear 0.45 to 0.65 M NaCl gradient, and the resulting fractions were pooled as shown by the crosshatched areas and prepared for coding studies (13). The coding studies were carried out as described in the text and are shown.](http://jb.asm.org/)

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rechromatographed and resolved into a major eluting peak (IA) and a later eluting shoulder (IB). The binding of IA, IB, and II to ribosomes was stimulated in the presence of CAG. The terminal eluting peak, III, responded most strongly to CAA and less well to CAG. Most certainly, the response of the latter fraction to CAG is due to the presence of small quantities of peaks I and II which overlap peak III during chromatography.

Gly-tRNA was resolved into a major eluting peak (I) with a trailing shoulder (II; see Fig. 2). Both I and II were rechromatographed, and each was divided into two fractions (IA and IB and IIA and IIB, respectively) for coding studies. The binding of fractions IA and IB to ribosomes was stimulated significantly in the presence of GGA. Significant stimulation of these fractions also occurred in the presence of GGU and GGC, while the binding of fraction IA was stimulated additionally in the presence of GGG. Fraction IIA responded most strongly to GGU and GGC and less well to GGA. Fraction IIB responded most strongly to GGU and GGC and weakly to GGA. These observations suggest that the major eluting peak of Gly-tRNA (fraction I) consists mostly of an isoacceptor which recognizes GGA and a minor isoacceptor which recognizes GGG.

FIG. 2. Chromatography of Arg-, Gln-, and Gly-tRNAs and their coding responses. Columns were developed, and the resulting fractions were pooled and either used directly in coding studies (designated by crosshatched areas) or further purified by chromatography (designated by hatched areas). The initial chromatographic step is shown by the graph to the left, and subsequent steps are shown by the center graphs. Each pooled fraction is designated with a Roman numeral in the order of its elution from the column, and upon subsequent chromatography, if the eluting samples were pooled into more than one fraction, then the fractions were designated by the Roman numeral followed by A or B in the order of elution from the column. Linear NaCl gradients used for the initial chromatography of $^{3}$H-labeled aminoacyl-tRNAs and for subsequent chromatography of each fraction were: (i) Arg-tRNA, 0.45 to 0.65 M; I, 0.5 to 0.625 M; II, 0.55 to 0.7 M; (ii) Gln-tRNA, 0.475 to 0.625 M; I, 0.5 to 0.575 M; and (iii) Gly-tRNA, 0.45 to 0.65 M; I, 0.475 to 0.55 M; II, 0.5 to 0.55 M. Coding studies were carried out as described in the text and are shown in the boxes to the right.
and that the shoulder, which elutes slightly later than the major peak (fraction II), consists mostly of an isoacceptor which recognizes GGU and GGC.

Ile-tRNA was resolved into a large initial eluting peak (I), followed by two minor eluting peaks (II and III; see Fig. 3). Fraction I was rechromatographed and resolved into a large peak (IB) with a front running shoulder (IA). IA was refractionated a third time (data not shown) and found to respond primarily to AUA (i.e., at 0.02 M Mg$^{2+}$, 6,218 cpm and 0.008 A$_{260}$ unit of fraction IA were added to each ribosome binding assay; 2,290 cpm bound to ribosomes in the absence of template, and 1,480 cpm were stimulated over the background binding in the presence of AUA, 264 cpm in the presence of AUU, and -150 cpm in the presence of AUC). Fraction IB responded to AUU, AUC, and AUA (see Fig. 3). The minor peaks (II and III) also responded to AUU, AUC, and AUA.

Leu-tRNA was resolved into three closely eluting peaks (I, II, and III; see Fig. 3). The two major peaks (fractions I and II) were rechromatographed. Fraction I was then resolved into two peaks, IA and IB, and fraction II was resolved into a large initial eluting peak, designated II, followed by a minor peak. IB corresponds to peak II in the initial chromatographic run. The minor eluting peak which was resolved on the second column of fraction II corresponds to peak III in the initial chromatographic run. The fractions of Leu-tRNA were assayed for binding to ribosomes in the presence of four of the six leucine codons, UUA, UUG, CUU, and CUG. IA responded most strongly to UUG. IB and II

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**FIG. 3.** Chromatography of Ile-, Leu-, and Lys-tRNAs and their coding responses. Columns were developed, and the resulting fractions were either prepared for coding studies or were further purified and then prepared for coding studies (see the legend to Fig. 2). Linear NaCl gradients used for the initial chromatography of $^3$H-labeled aminoacyl-tRNAs and for subsequent chromatography of each fraction were: (i) Ile-tRNA, 0.45 to 0.65 M; I, 0.5 to 0.575 M; (ii) Leu-tRNA, 0.475 to 0.65 M; I, 0.5 to 0.575 M; II, 0.5 to 0.575 M; and (iii) Lys-tRNA, 0.45 to 0.65 M; I, 0.5 to 0.6 M; II, 0.525 to 0.625 M. Coding studies were carried out as described in the text and are shown in the boxes to the right.
bound strongly to ribosomes in the absence of template, and the binding of both was stimulated slightly in the presence of CUG. The binding studies with the latter two fractions support the conclusion that peak II (Fig. 3; initial chromatography of Leu-tRNA) contains at least in part an isoacceptor which recognizes CUG. Peak III responded most strongly to UUG.

Lys-tRNA was resolved into two major peaks, I and II (Fig. 3). Both I and II were rechromatographed. Peak I responded to AAG, and peak II responded to AAA and less well to AAG.

Ser-tRNA was resolved into four major peaks (Fig. 4). Each peak was rechromatographed. Peak I responded to UCU and UCA. Peak II responded to AGU and UCG and less well to UCU and UCA. Further fractionation of this peak would be required to determine if it contains isoacceptors which recognize AGU and AGC, UCG, and UCU and UCA. Peak III responded to AGU and AGC. Fraction IV was resolved into a major peak (IVA) and a fraction which eluted in the high-salt wash (IVB). IVA and IVB both responded to UCU, UCC, and UCA.

Thr-tRNA was resolved into four peaks (I, II, III, and IV, Fig. 4). Only peaks II, III, and IV were rechromatographed. Peak I did not respond to any of the threonine codons. Peak II recognized ACU, ACC, and ACA. Peak III responded most strongly to ACU, and peak IV responded most strongly to ACA and ACU.

Val-tRNA eluted as a single peak after the first and second column runs. It was divided into two fractions. Both fractions responded to all
four valine codons, GUU, GUC, GUA, and GUG. Additional fractionation would be required to determine whether this peak contains a single isoacceptor which recognizes all four valine codons (22) or multiple isoacceptors which recognize GUU, GUC, and GUA; GUG; and possibly GUA. It should be mentioned, however, that *P. polypephalum* Val-tRNA also migrates as a single eluting peak on another chromatographic column (i.e., Aminex A-28 [24]; P. Melera, unpublished data).

**DISCUSSION**

Eleven aminoacyl-tRNAs from *P. polypephalum* were resolved by RPC-5 chromatography, and codon recognition properties of the resulting isoacceptors were determined. Some *P. polypephalum* aminoacyl-tRNAs responded weakly to code words with G in the 3′ position (e.g., alanine, arginine [CGG], glycine, and serine). A serine isoacceptor responded to both UCU and UCA, and a threonine isoacceptor responded to both ACU and ACA. Responses by an isoacceptor to code words terminating only in U and A are not predicted on the basis of the wobble hypothesis (5). However, an alanine isoacceptor from yeasts was previously reported to recognize GCU and GCA (38), and we have observed a serine isoacceptor from *Xenopus laevis* which responds to UCU and UCA (D. Hatfield, unpublished data).

Use of the ribosome binding assay to determine codon recognition properties of fractionated aminoacyl-tRNAs still provides one of the simplest and most rapid means of estimating the relative abundances of isoacceptors in a tRNA population which recognize specific codons. We therefore selected for study those *P. polypephalum* aminoacyl-tRNAs which were most likely to contain different isoacceptors coding for different code words within a synonymous codon family. Since a correlation between the relative abundances of specific isoacceptors and the frequency of their cognate codons in corresponding mRNAs has been observed in *Escherichia coli* (20, 21), in the silk gland of *Bombyx mori* (4), and in rabbit (14) and human (D. Hatfield, F. Varriochio, M. Rice, and B. Forget, J. Biol. Chem., in press) reticulocytes, it is reasonable to expect that those *P. polypephalum* isoacceptors found to be present in highest levels by the codon binding assay are most likely to be those utilized most frequently by this organism in protein synthesis. We recognize, however, that the ribosome binding assay does not measure some of the other parameters of protein synthesis such as the effects of codon position in mRNA (i.e., nucleotide context [2]), the effects of secondary and tertiary structures of mRNA, or the requirements for initiation, elongation, and termination factors. Hence, the relative efficiencies of different code words as templates for given isoacceptors in the binding assay do not necessarily indicate the efficiency with which those code words are read during protein synthesis. Other experiments have shown that wobble in the anticodon of tRNA may occur to a greater extent during protein synthesis than predicted by the wobble hypothesis (5). These studies with in vitro protein synthesis systems have shown that a single leucine isoacceptor from yeasts can read all six leucine codons (44), that a two-out-of-three base code exists for some amino acids that are coded by four codons (33), and that some *E. coli* leucine isoacceptors can read leucine codons not predicted on the basis of the wobble hypothesis (10, 11). While these results are of interest and importance to the overall understanding of the translational process, their in vivo significance remains to be established, particularly since each represents an exception to the wobble hypothesis. It is not clear, therefore, that these results contradict those obtained from ribosome binding studies; they may, in fact, complement them by indicating an additional level of codon-anticodon interaction. These possibilities notwithstanding, however, in *P. polypephalum*, the most abundant glutamine isoacceptor recognizes CAG. Therefore, in accordance with the interpretation that the levels of isoacceptors in cells are adapted to their requirements for protein synthesis (4, 14, 20, 21; Hatfield et al., in press), most of the glutamine code words in *P. polypephalum* mRNA would be expected to be CAG. Similarly, few UCG, CGG, GCG, and GGG code words might be expected to occur in *P. polypephalum* mRNAs since only weak responses of isoacceptors to these codons were observed in fractionated aminoacyl-tRNAs. It is of interest to note that isoacceptors which recognize these codons may occur in relatively greater abundance in the tRNA populations of other organisms (3, 4, 14, 16, 17, 20, 21, 43; Hatfield et al., in press).

Patterns of codon recognition by isoacceptor aminoacyl-tRNAs within a synonymous codon set have also been characterized in the tRNA populations of *E. coli* (3, 42, 43), yeasts (42, 43), wheat germ (16), hymenoptera (17), *Xenopus* (unpublished data), and mammals (3, 14). Comparison of the results of these studies and those from *P. polypephalum* demonstrates the occurrence of many evolutionary changes in patterns of codon recognition (Fig. 5). Several isoacceptors recognize code words with adenosine and guanosine and also, in some cases, uridine in the 3′ position (e.g., see patterns of codon recognition of *E. coli* Gly-, Lys-, Ala-, Ser-, and Val-tRNAs). Often these isoacceptors recognize one codon less well than the other(s) in the ribosom-
al binding assay. This difference in the pattern of codon recognition is due to the fact that uridine in the wobble position is modified in most isoacceptors, and the modification may restrict wobble (1). The sizes of symbols shown in Fig. 5 reflect the relative efficiencies with which code words terminating in adenosine and guanosine and, in some cases, uridine serve as templates for the corresponding isoacceptors in the binding assay. For example, GAG is a more efficient template than GAA for glutamic acid isoacceptor from wheat germ, and AGA is a more efficient template than AGG for an arginine isoacceptor from E. coli. Furthermore, GCA and GCG serve equally well, but better than GCU, as templates for an alanine isoacceptor from E. coli (The relative efficiencies with which trinucleotidyl diphosphates serve as templates for aminoacyl-tRNAs in the binding assay shown in Fig. 5 do not necessarily reflect their efficiencies as templates in mRNA during protein synthesis (see above). However, evolutionary changes in patterns of codon recognition by isoacceptors from different organisms are detected by such variations in ribosome binding and are therefore shown.).

A survey of the patterns of codon recognition between different organisms (Fig. 5) demonstrates that the largest number of changes has occurred between procaryotes and eucaryotes. In procaryotes, amino acids coded by four or more code words may have isoacceptors recognizing codons terminating in A, G, and U and in U and C (e.g., alanine, serine, and valine). Such isoacceptors contain uridine-5-oxy-acetic acid, which recognizes A, G, and U, and contain G,
which recognizes U and C in the wobble position of their anticodons (9). In eucaryotes, the corresponding isoacceptors recognize codons terminating in U, C, and A, in G, or in U, C, A, and G. These isoacceptors contain I, which recognizes U, C, A (5), and possibly G (22), and contain C, which recognizes G in the wobble position of their anticodons. In general, microorganisms (E. coli and yeasts) and plants have more isoacceptors recognizing code words terminating in A and G than do the other organisms.

The isoacceptors of *P. polycephalum* display patterns of codon recognition which are more similar to those observed in hymenoptera, *Xenopus*, and mammals than to those observed in wheat germ and yeasts. For example, a glutamic acid isoacceptor from *P. polycephalum* and animals recognizes GAA, while a second isoacceptor recognizes GAG. On the other hand, a glutamic acid isoacceptor from wheat germ responds to GAG, while a second isoacceptor responds to both GAG and GAA. In yeasts, a glutamic acid isoacceptor responds to GAA, a second isoacceptor to GAG, and a third to both GAA and GAG. Additionally, an arginine isoacceptor from *P. polycephalum* and animals responds to AGG, whereas in wheat germ, a single arginine isoacceptor responds to AGG and AGA. An arginine isoacceptor which responded most strongly to AGA and less well to AGG was also observed in the tRNA population of *P. polycephalum*. A serine isoacceptor which responded to UCU and UCA was found in the tRNA populations of *P. polycephalum* and *Xenopus*. In general, the patterns of codon recognition of other isoacceptors from *P. polycephalum* are more similar to those of higher plants and animals than to those of procaryotes and lower fungi. Hence, the overall patterns of codon recognition displayed by *P. polycephalum* tRNAs, in addition to those properties mentioned above, serve as a further example at the molecular level of this biologically simple organism's higher eucaryotic characteristics.

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LITERATURE CITED


CODON RECOGNITION IN *P. POLYCEPHALUM* 1021


