Nucleotide sequence of wheat germ cytoplasmic initiator methionine transfer ribonucleic acid

Hara P.Ghosh*, Kakoli Ghosh*, Mehmet Simsek† and Uttam L.RajBhandary†

*Department of Biochemistry, McMaster University, Hamilton, Ontario L8N 3Z5, Canada, and †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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ABSTRACT

The primary sequence of wheat germ initiator tRNA has been determined using in vitro labelling techniques. The sequence is: pAUCAGAGUm^{1}Gm^{2}GCGCAG CGGAAGCGUm^{2}GGψGGGCCCAUt^{6}AACCCACAGm^{7}GDm^{5}Cm^{5}CCAGGAψCGm^{1}AAACCUG*GCUCUGAUACCA$_{OH}$. As in other eukaryotic initiator tRNAs, the sequence -TψCG(A)- present in loop IV of virtually all tRNA active in protein synthesis is absent and is replaced by -AψCG-. The base pair G_2:C_{71} present in all other initiator tRNAs recognized by E. coli Met-tRNA transformylase is absent and is replaced by U_2:A_{71}. Since wheat germ initiator tRNA is not formylated by E. coli Met-tRNA transformylase this implies a possible role of the G_2:C_{71} base pair present in other initiator tRNAs in formylation of initiator tRNA species.

INTRODUCTION

Initiation of protein synthesis in prokaryotes, eukaryotes and in organelles involves a unique species of methionine tRNA. The initiator tRNA species from prokaryotes and organelles are, subsequent to aminoacylation, formylated and the fMet-tRNA is then used for initiation. In contrast, the eukaryotic cytoplasmic initiator tRNAs are used as Met-tRNAs without formylation (1). Most initiator tRNAs from eukaryotic cytoplasm can, however, be both aminoacylated and formylated by Escherichia coli enzymes (1). Initiator tRNAs from plants are an exception in that they are recognized by E. coli methionyl tRNA synthetase but they are not formylated by Met-tRNA transformylase (2-5).

The nucleotide sequences of several prokaryotic and eukaryotic initiator tRNAs are now known (6-9). An interesting finding is that prokaryotic and eukaryotic cytoplasmic initiator tRNAs have unique structural features, which distinguish between themselves as a class and also from non-initiator tRNAs. Prokaryotic initiator tRNAs lack the Watson-Crick base pair in the acceptor stem between the first nucleotide at the 5'-end and the fifth nucleotide from the 3'-end. Eukaryotic initiator tRNAs do not share this feature. The

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unusual feature of eukaryotic initiator tRNAs is that they lack the sequence -TψCG(A)- present in loop IV of all tRNAs active in protein synthesis, including prokaryotic initiator tRNAs and possess instead, the sequence -AUCG- (9,10).

Previously, we showed that initiator tRNA from wheat germ is distinct from most other eukaryotic initiator tRNAs in the following respects: (i) The wheat germ initiator tRNA can not be formylated by E.coli met-tRNA transformylase. (ii) The sequence -AUCG- present in loop IV of most eukaryotic initiator tRNAs is modified to -AU*CG- in the wheat germ tRNA (11). Here we describe the sequence analysis of this tRNA. We show that this tRNA contains the sequence -AψCG- in loop IV in place of -AUCG-. Also the base pair $G_2:C_{71}$[a] present in all eukaryotic initiator tRNAs recognized by E.coli Met-tRNA transformylase is replaced by $U_2:A_{71}$.

MATERIALS AND METHODS

The purification of wheat germ initiator tRNA has been described (11). The procedures used for sequence analysis are as described in a recent review (12).

RESULTS

1. Analysis of oligonucleotides present in complete T_1-RNase and Pancreatic RNase digests and their localization in the 5'- and 3'-terminal fragments produced by cleavage of the tRNA at m^7G

The tRNA was digested with T_1-RNase or pancreatic RNase, the oligonucleotides produced were labelled at their 5'-ends with [^{32}P], separated by two-dimensional electrophoresis (see Figures 9 and 10, Ref. 11) and their sequences were determined (12). Table I (a) lists these sequences.

The presence of m^7G in the wheat germ initiator tRNA allowed selective chemical cleavage of the RNA at this position (13). The 5'- and 3'- terminal fragments were separated by polyacrylamide gel electrophoresis, and the fragments were analyzed as described above for the intact tRNA (Table I (b)). This allowed the localization of the oligonucleotides in digests of intact tRNA to either the 5'- or 3'- terminal fragment.

2. Identification of -AψCG- among the products of T_1-RNase digestion

Previously, we showed that wheat germ initiator tRNA yielded AU*CG (11) among the products of T_1-RNase digestion whereas other eukaryotic initiator tRNAs yield AUCG (8). We have identified this U* as ψ using the following procedure. The 3'-terminal fragment produced by cleavage of tRNA at

TABLE I

Sequences of Oligonucleotides Present in Fingerprints of T_1-RNase Digest and Pancreatic RNase Digest of Wheat Germ Initiator tRNA(a) and 5'- and 3'-Terminal Fragments of the tRNA(b)

Sequence of Oligonucleotides Present in T_1-RNase Digests			Sequence of Oligonucleotides Present in Pancreatic RNase Digests		
Intact (a) tRNA	5'-Fragment (b)	3'-Fragment (b)	Intact (a) tRNA	5'-Fragment (b)	3'-Fragment (b)
pCG	+		pAC		+
pCAG	+		pGC	+	
pAG	+		pAGC	+	
pAAG	+		pGm^1AAAC		+
pUm^1Gm^2G	+		pAU	+	
			pm^1Gm^2GC	+	
pm^7GDm^5Cm^5CCAG			pt^6AAC	+	
		pDm^5Cm^5CCAG	pG*GC		+
pAψCG		+	pGU	+	
pUm_1^2G	+		pAGm^7GD	AGX†	
pψG	+		pGAU		+
pAUACCA		+	pm^2GGψ	+	
pm^1AAACUG*G		+	pGGGC	+	
pAUCAG	+		pGGAAGC	+	
pCUCUG		+	pAGAGU	+	
pCCCAUt^{6}AACCCACAG	+		AGGAψ		+

†X is a fragmented ribosyl residue produced during the β-elimination reaction used to cleave RNA chain at m^7G.

m^7G was digested with a mixture of T_1-RNase and *E.coli* alkaline phosphatase and the 3'-dephosphorylated oligonucleotides produced were separated by two-dimensional thin-layer chromatography on a cellulose plate (12). The oligonucleotide corresponding to AU*CG$_{OH}$ was further digested with T_2-RNase and the mononucleotides (Ap, U*p, Cp) produced were then labelled at the 5'-end with ^{32}P to yield [^{32}P]Xp. After treatment of this mixture with nuclease P1 to remove 3'-phosphate groups, the [^{32}P]X formed was analyzed by two-dimensional chromatography in the presence of nucleoside 5'-phosphates as markers. The only radioactive spots observed corresponded to pA, pC and pψ

(Figure 1).

3. Derivation of total sequence

The oligonucleotides listed in Table I were aligned into a unique sequence using following additional lines of evidence: (i) Analysis of sequences at the 5'- and 3'-termini of tRNA by partial nuclease P1 digestions on 5'- and 3'-[^{32}P] labelled tRNA respectively (12), (ii) analysis of large oligonucleotides present in partial pancreatic RNase digests and (iii) polyacrylamide gel electrophoretic analysis of partial nuclease digests of 5'[^{32}P] labelled tRNA (12). Figure 2 shows this sequence in the standard clover-leaf form.

DISCUSSION

The wheat germ initiator tRNA contains all the features typical of eukaryotic initiator tRNAs. These include: (i) a base paired 5'-terminus pA_1:U_{72}, (ii) modified bases, m^1G, m^2G and D in positions 9, 10 and 47, respectively, (iii) a hypermodified A, t^6A, adjacent to the 3'-end of the anticodon, and (iv) the sequence AUCGm^1AAA (corresponding to nucleotide 54-60) in loop IV; the U_{55} is, however, modified to ψ_{55} in the wheat germ tRNA. The presence of ψ_{55} in the wheat germ initiator tRNA suggests that the wheat germ enzyme which converts this U_{55} to ψ_{55} does not require that the nucleotide proceding the U_{55} be a U or T.

Among the initiator tRNAs, the wheat germ tRNA is unusual in that it

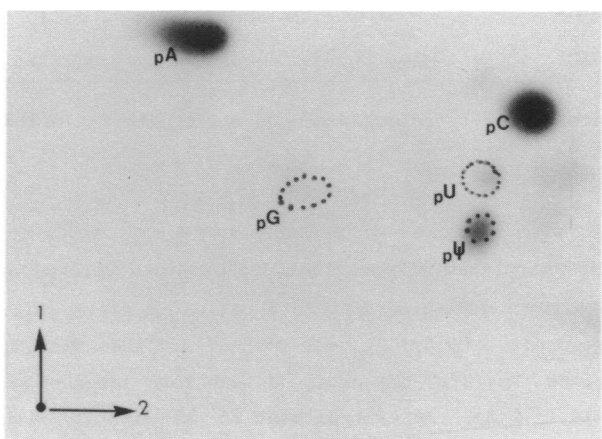

Figure 1

Autoradiograph of two-dimensional chromatogram of 5'-labelled nucleotides present in the oligonucleotide present under spot 5.

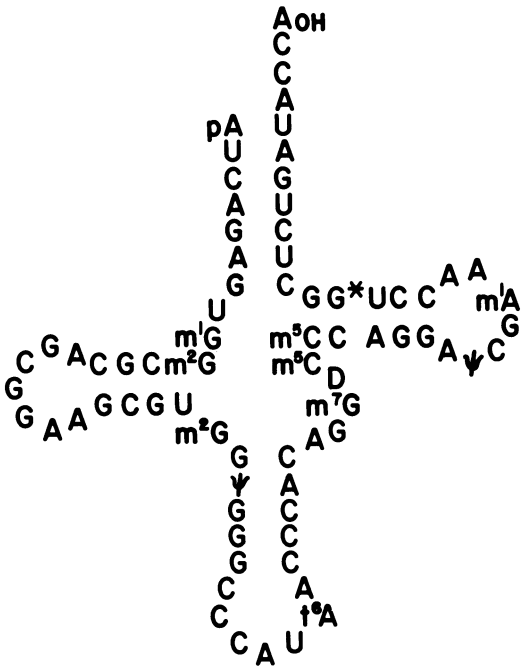

Figure 2

Nucleotide sequence of wheat germ initiator $tRNA_1^{Met}$. G* is unknown guanosine derivative.

is not recognized by the E.coli Met-tRNA transformylase. Figure 3 shows a composite structure of those tRNAs which are formylated by the E.coli enzyme. The only difference between the wheat germ tRNA and the composite structure is the presence of a U_2:A_{71} base-pair in the wheat germ tRNA instead of a G_2:C_{71} base-pair in the other tRNAs. Thus, this G_2:C_{71} base-pair may be a part of the recognition site for the E.coli Met-tRNA transformylase.

Virtually every tRNA which has been sequenced to date contains the nucleotide U before the anticodon. The only exceptions are the eukaryotic initiator tRNAs from vertebrates, from Drosophila (14) and wheat germ which contain C instead of U in this position. The possible role of this nucleotide in regulating translational initiation of prokaryotic and eukaryotic mRNAs perhaps through the formation of a "fourth base-pair" between initiator tRNA and mRNA has been discussed by Taniguchi and Weissman (15) and by Kozak and Shatkin (16).

A comparison of the sequence of wheat germ initiator tRNA to those of other initiator tRNAs show that similar to other eukaryotic initiators the

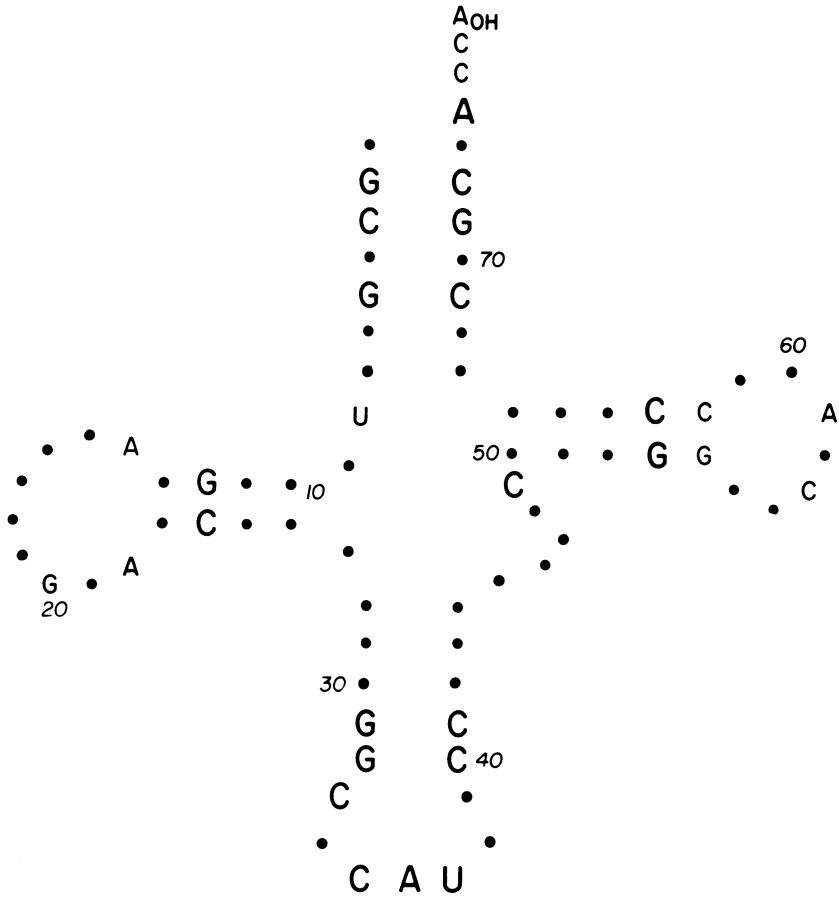

Figure 3

Composite structure of tRNA recognized by E.coli methionyl-tRNA transformylase. Nucleotides found in the same position in all tRNAs are indicated by underlined letters.

wheat germ tRNA is more homologous to eukaryotic tRNA (73-90%) than to tRNAs from prokaryotes or organelles (51-68%). Among the eukaryotic initiators, the wheat germ tRNA is more closely related to tRNAs from Drosophila and vertebrates (87-90%) than to those from fungi (73-76%).

Finally, following preliminary communication of the sequence of this tRNA (Ghosh et al., Abstracts, Cold Spring Harbor Meeting on Transfer RNA, p.6, 1978), Canaday et al., (Nucleic Acids Res. 8:999, 1980) have determined the sequence of initiator tRNA from the cytoplasm of Phaseolus vulgaris.

Except for some of the modified bases their sequence is identical to that of the wheat germ initiator tRNA. In addition Barciszewski, Joachimiak, Barciszewski, Twardowski, Rafalski and Gulewicz have purified and analyzed the initiator tRNA from yellow lupine seeds by fingerprinting of fragments present in T_1-RNase and pancreatic RNase digests. Their results suggest that the yellow lupine seed initiator tRNA is identical in sequence to that of the wheat germ initiator tRNA. Thus, as in the case of all vertebrate initiator tRNAs, the sequence of all plant initiator tRNAs may also be identical.

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Footnote

[a]The numbering system used here is as described by Gauss and Sprinzl (9).

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