THE PRIMARY STRUCTURE OF YEAST INITIATOR TRANSFER RIBONUCLEIC ACID

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SUMMARY

The nucleotide sequence of a yeast formylatable methionine transfer RNA, which had previously been shown to initiate the synthesis of proteins in eukaryotic systems, has been deduced. This tRNA contains a total of 75 nucleotide units, including twelve modified nucleosides. The sequence G-T-ψ-C- common to all tRNAs which are active in protein synthesis and which have been sequenced to date, is absent in this tRNA and is replaced by G-A-U-C-.

Initiation of protein biosynthesis utilizes a specific methionine tRNA (1). Bacteria, chloroplasts and mitochondria use N-formylmethionyl-tRNA (fMet-tRNA^{fMet}) whereas the cytoplasmic protein synthesizing systems of eukaryotes use a specific methionyl tRNA without formylation (2-10). The eukaryotic initiator tRNAs from various sources have properties similar to the bacterial tRNA in that they can be aminoacylated by E. coli methionyl-tRNA synthetase and in many cases can be subsequently formylated by E. coli methionyl-tRNA transformylase (11-13).

It was shown previously that baker's yeast contained at least two species of methionine tRNA (12, 13). One of these species was purified and shown to initiate the synthesis of proteins in E. coli and of hemoglobin in cell free extracts of rabbit reticulocytes (3, 12, 13). Because this tRNA could be converted to formyl methionyl-tRNA by extracts of E. coli, it was designated as formylatable methionine tRNA (tRNA$_F^{Met}$). As a part of our studies on the structure-function relationships of yeast tRNA$_F^{Met}$, we had previously reported on the 5'- and 3'-terminal sequences, and of the sequences around the anticodon of this tRNA, and compared them to those

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of E. coli $tRNA^{fMet}$ (13, 14). Here we report briefly on the total nucleotide sequence of yeast $tRNA_F^{Met}$. Details of this work will be published elsewhere.

MATERIALS and METHODS

Baker's yeast tRNA was either prepared according to Holley (15) or purchased from Schwarz BioResearch Inc. $tRNA_F^{Met}$ was purified using three steps of column chromatography: (1) chromatography on benzolyated DEAE-cellulose (16), which separates $tRNA_F^{Met}$ from $tRNA_M^{Met}$; (2) chromatography on DEAE-Sephadex A-50 (17); and (3) finally, by chromatography on DEAE-Sephadex A-25 (18). The tRNA preparation used in this work accepted 1780 pmoles of methionine per O. D. $_{260}$ unit.

The structural work followed the general principles used previously (19). Conditions for complete digestion of tRNA by T_1-RNase or pancreatic RNase were as described previously (13). Large oligonucleotides necessary for obtaining overlaps of nucleotide sequences were obtained by partial digestion with T_1-RNase (20). Additional overlaps of nucleotide sequences were also obtained by specific cleavage of large fragments or of tRNA at the site occupied by 7MeG (21).

Oligonucleotides and large fragments were purified and characterized as in earlier publications (13, 19). In order to simplify the isolation and analysis of large fragments, in particular those arising from the 5'- and 3'-terminii, $tRNA_F^{Met}$ used for partial T_1-RNase digestion was labeled with ^{32}P- at the 5'-end and ^{3}H- at the 3'-end. Labeling of the 5'-end used dephosphorylation of the 5'-phosphate with E. coli alkaline phosphatase and subsequent phosphorylation of the 5'-hydroxyl group using T_4-polynucleotide kinase and γ-^{32}P-ATP (22). The 5'-^{32}P-labeled tRNA was purified by electrophoresis on 12% polyacrylamide gel in 6M urea. Labeling of the 3'-end used brief treatment of the tRNA with snake venom phosphodiesterase to remove part of the 3'-terminal C-C-A, followed by repair of the 3'-end by E. coli tRNA pyrophosphorylase in the presence of ^{12}C-CTP and ^{3}H-ATP (23)

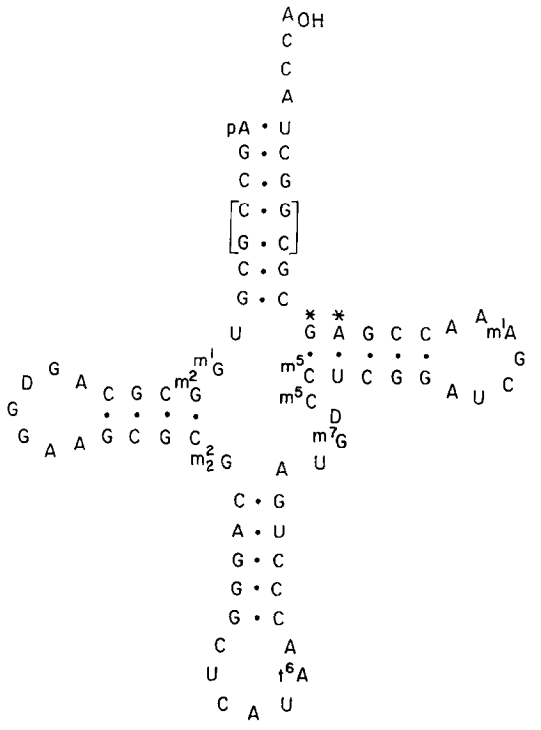

Fig. 1 Nucleotide sequence of yeast $tRNA_F^{Met}$ in the cloverleaf form. The order of nucleotides shown within parentheses have not been established.

RESULTS

Fig. 1 shows the nucleotide sequence of yeast $tRNA_F^{Met}$ written in the cloverleaf form (24). Fig. 2 lists the large oligonucleotide fragments necessary for the derivation of this structure. The yeast $tRNA_F^{Met}$ consists of 75 nucleotides including twelve minor nucleosides, which are 2 moles each of D and 5MeC, and 1 mole each of 1MeG, 2MeG, 2DiMeG, t^6A, 7MeG, 1MeA, A* and G*.

The minor nucleoside designated t^6A is probably identical to N[9-(β-D-ribofuranosyl)-purin-6-yl carbamoyl] threonine (t^6A) described by Schweizer and coworkers (25). It yielded threonine and adenosine upon alkaline hydrolysis. Its ultraviolet absorption spectra at pH 1.0 and 7.0 are identical to that of t^6A (λmax pH 1.0, 276 nm; λ max pH 7.0, 269 and

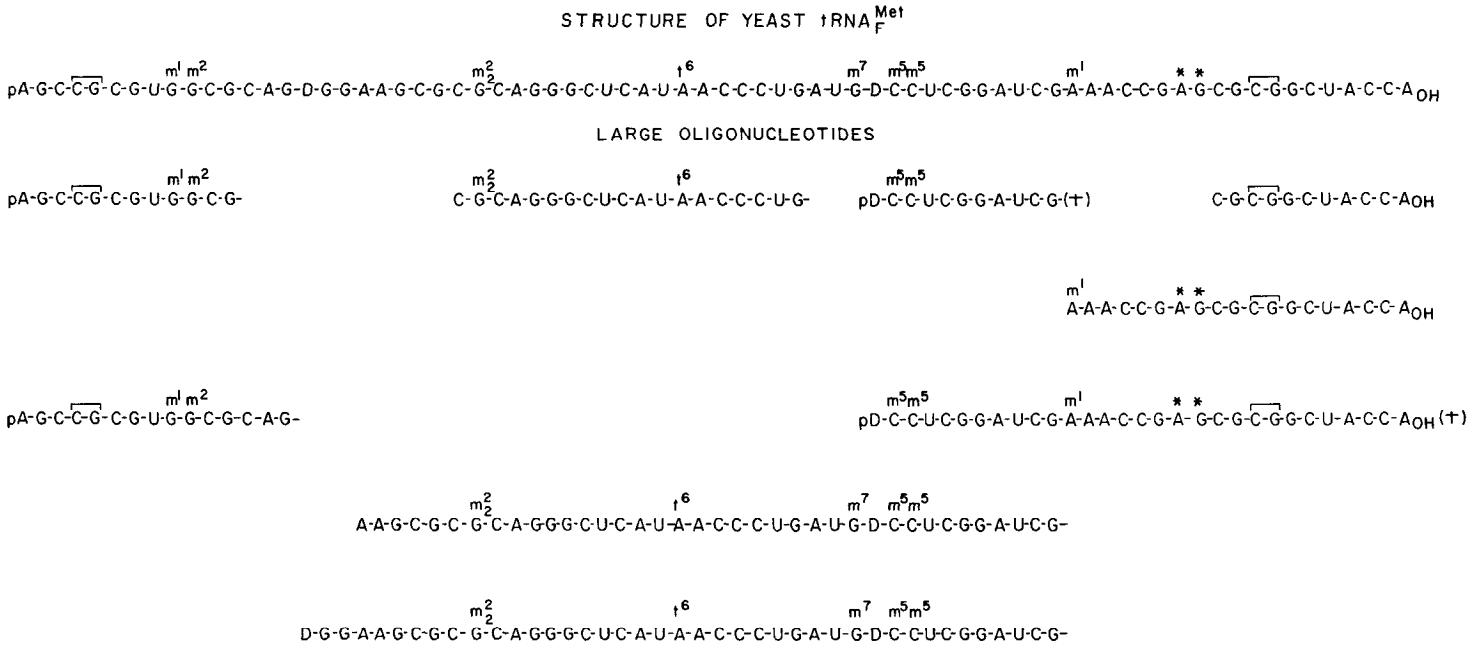

Fig. 2. The primary sequence of yeast tRNA $^{Met}_{F}$ is shown on the top. Large oligonucleotide fragments necessary for the derivation of this sequence are shown below. These large oligonucleotide fragments were obtained either from a partial T_1- digest of the tRNA or by specific cleavage of tRNA or of fragments at 7MeG (†). Each of the fragments shown was purified by chromatography on DEAE-cellulose in the presence of urea and characterized by subsequent degradation with T_1-RNase.

276 nm). At alkaline pH the λ max is also identical to that described for t^6A (270, 279 and 300 nm), but the relative extinction at 300 nm compared to 270 and 279 nm is pH dependent; at pH > 13, the extinction coefficient at 300 nm being 1.5-1.7 times that at 270 or 279 nm.

The structure of the minor nucleosides designated as A* and G* have not been established. Complete T_1-RNase digestion of yeast $tRNA_F^{Met}$ yielded the dinucleotide A*pG*p, which was spectrally similar to ApGp (λ max at pH 7.0 was 256 nm). A*pG*p has the following properties: (1) It behaved like a tetranucleotide on DEAE-cellulose columns in the presence of 7M urea, suggesting the presence of ring substitution(s) carrying negative charges in either A* or G* or both. (2) It yielded adenine and guanine in almost equimolar amounts upon treatment with N hydrochloric acid at 100° for 1 hour. (3) It was almost totally resistant to hydrolysis with T_2-RNase, with N sodium hydroxide for twenty-four hours at room temperature or with snake venom phosphodiesterase after removal of 3'-phosphate. It is, therefore, assumed that the ribose moiety attached to A* is 2'O-alkylated ribose.

The number of fragments characterized to date do not allow us to assign an unambiguous sequence in the acceptor stem region enclosed within parentheses (Fig. 1). This leaves open the possibility that the 5'- and 3'-terminal sequences could be A-G-C-G-C-C-G and C-G-G-C-G-C-U-A-C-C-A_{OH} rather than as shown.

DISCUSSION

The most significant result from this work is the finding that yeast $tRNA_F^{Met}$ lacks the sequence G-T-ψ-C- present in all tRNAs whose sequences are known to date and which are active in protein synthesis (26). $tRNA^{Gly}$ from S. epidermidis is the only other tRNA lacking this sequence and this tRNA is known to be inactive in protein synthesis (27). If the replacement of G-T-ψ-C- by G-A-U-C- has any specific biological function in yeast

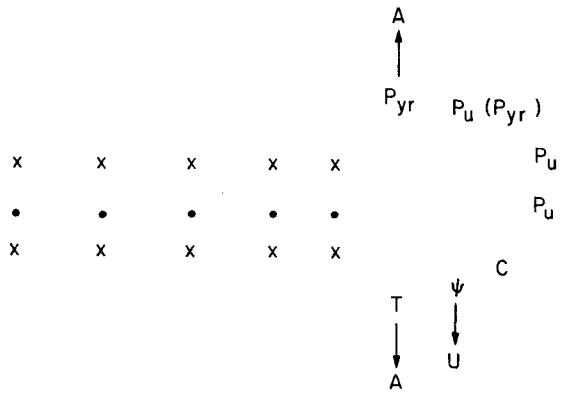

Fig. 3 Sequence of T-ψ-C- loop in tRNAs. Arrows indicate the major difference between yeast $tRNA_F^{Met}$ and other tRNAs.

$tRNA_F^{Met}$, two important possibilities must be considered: (1) the ability of this tRNA to initiate protein biosynthesis as methionyl-tRNA without formylation (3), and/or (2) the inability of this tRNA to insert methionine internally in a protein (7, 14). An answer to this question will require further structural studies on other eukaryotic and bacterial initiator tRNAs (28). Such studies with wheat germ and rabbit reticulocyte initiator tRNAs are now in progress.

Another major difference between yeast $tRNA_F^{Met}$ and other tRNAs is also located in the T-ψ-C- loop. In contrast to other tRNAs in which the last nucleoside of this loop is invariably a pyrimidine (26), the corresponding nucleoside in yeast $tRNA_F^{Met}$ is adenosine. Thus, the main structural variation between yeast $tRNA_F^{Met}$ and other tRNAs lies in the fact that both the first and the last nucleosides of the T-ψ-C- loop which are always pyrimidines in other tRNAs are purines in yeast $tRNA_F^{Met}$ (Fig. 3). In spite of these differences, however, both the size of the T-ψ-C- loop (seven nucleosides) and that of the T-ψ-C- stem (five base pairs) has remained the same in yeast $tRNA_F^{Met}$ as in other tRNAs. Although any generalizations at this stage must be tentative, it is worth considering the possibility that a change from T→A in the first nucleoside of T-ψ-C- loop

requires a corresponding change in the last nucleoside of this loop from a pyrimidine (for instance, uridine, which could potentially form a Watson-Crick type of base-pair with A) to a purine or vice versa. This would suggest that for its normal biological function, the structure of a tRNA contains constraints within it which do not allow either a T-ψ-C- loop consisting of only five nucleosides or a T-ψ-C- stem consisting of six base pairs.

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