LACK OF TpψpIN LOOP IV OF A MAMMALIAN INITIATOR TRANSFER RNA

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1. Introduction

Two classes of methionine-accepting transfer RNA have been characterised from the cytoplasm of mammalian cells. As in other eukaryotic systems, one of these ($tRNA_m^{Met}$) is found to donate methionine into the internal positions of protein chains, whereas the other, the initiator ($tRNA_f^{Met}$), acts as a specific donor for the N-terminal methionine residue found in newly synthesized polypeptides [1–3]. Methionyl-$tRNA_f^{Met}$ in the cytoplasm of eukaryotes, unlike that of *E. coli* and mitochondria, does not need to be formylated before it can donate methionine into the N-terminal positions of proteins. We are attempting to relate the structure of a eukaryotic initiator tRNA to its role in the cell with the aim of explaining the differences in its behaviour from the bacterial initiator tRNA. At present we are determining the nucleotide sequence of the $tRNA_f^{Met}$ from mouse P3 myeloma cells.

Very recently the nucleotide sequence of the first eukaryotic initiator tRNA has been determined by Simsek and RajBhandary [4]. This yeast $tRNA_f^{Met}$ has a feature distinct from all other tRNA's of known sequences functioning in protein biosynthesis: the common –GpTpΨpCp– sequence found in loop IV of the cloverleaf structure is replaced in yeast $tRNA_f^{Met}$ by the sequence –GpAp–UpCp–. Now we have also found the latter sequence in a mammalian initiator tRNA replacing the common sequence confirming the notion that this region of the molecule is concerned with the special initiation role.

2. Methods and results

The mouse P3 myeloma cells have been labelled with [^{32}P]phosphate in tissue culture in this laboratory in studies on the structure of myeloma messenger RNA by Drs. G.G. Brownlee and C. Milstein. We are very grateful to them for making available to us the radioactively labelled 4 S-RNA from their cell extracts for use as our source material. In common with the $tRNA_f^{Met}$ from rabbit liver [2, 3], guinea pig liver [5], mouse ascites tumour cells [1] and yeast [4], a methionine-accepting species from these cells can be both aminoacylated with methionine by purified *E. coli* methionyl tRNA synthetase (a gift from Drs. C.J. Bruton and G. Koch) as well as formylated by the *E. coli* transformylase [6]. This property of formylation by the bacterial enzyme has been used to identify the fractionated $tRNA^{Met}$ as an initiator $tRNA_f^{Met}$. The ^{32}P-labelled mouse P3 cell $tRNA_f^{Met}$ has been purified by chromatography on reverse phase RPC-5 columns [6], and characterized by two-dimensional fractionation of the ribonuclease digestion fragments using the procedures developed by Brownlee et al. [7]. The oligonucleotide map yielded by RNAase T_1 digestion of the $tRNA_f^{Met}$ is illustrated in fig. 1. 14 oligonucleotides including two identified as the 5′-end (pApGp) and 3′-end (CpUpApCpCpA) oligonucleotides are released from the sample by RNAase T_1 digestion. This corresponds to the number which might be expected from a single species of tRNA and permits the approximate length of 75 nucleotides to be estimated from a comparison of the yields with the ends. The purity of the $tRNA_f^{Met}$ was

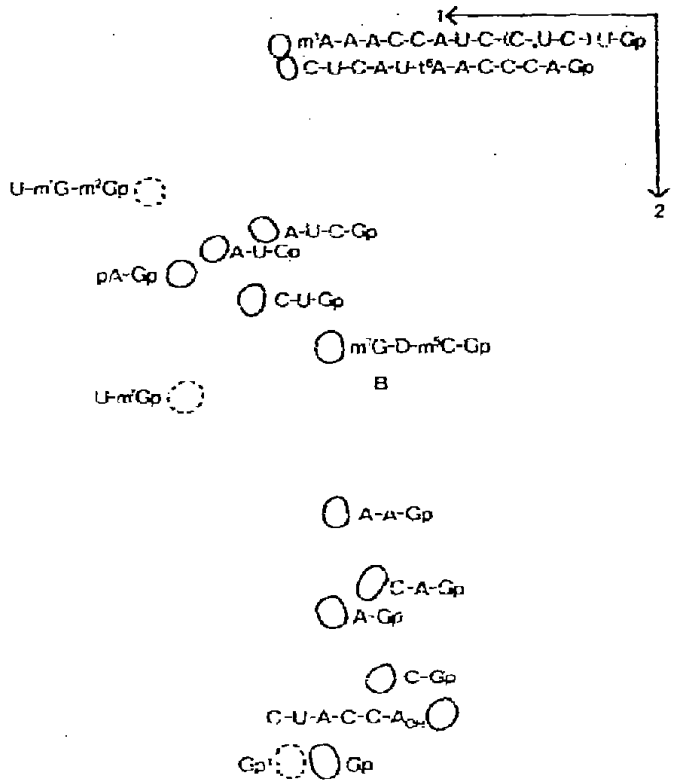

Fig. 1. Tracing of an autoradiograph of a two dimensional fingerprint of the radioactive RNAase T_2 complete digestion products of myeloma $tRNA_f^{Met}$. The arrows 1 and 2 represent electrophoresis on cellulose acetate at pH 3.5 and on DEAE-cellulose paper in 7% formic acid [7]. The origin is at the intersection of the arrows. The dotted spots represent partial molar yields. The sequences of the oligonucleotide products represented by the solid line spots are given as far as they have been determined. E is the position of the blue marker.

estimated to be approx. 80% from the level of contaminant oligonucleotides in the fingerprints.

A most unusual feature which has emerged from our analysis of the ribonuclease digestion products obtained from this species of tRNA is the complete absence of the sequence –TpΨp– in any of the fragments. In RNAase T_1 fingerprints of all our preparations –TpΨpCpGp–, the product most commonly found in RNAase T_1 digests of tRNA, is only present at contaminant level (< 10%). In addition the

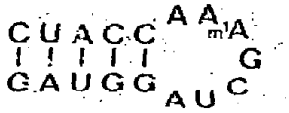

Fig. 2. Sequence of loop IV in base paired hairpin form as consistent with sequences referred to in the text.

fingerprint (not shown) of a pancreatic ribonuclease digest of this tRNA species does not reveal the presence of the mononucleotide Ψp although Ψp would be expected from the ability of pancreatic ribonuclease to cleave after Tp and Ψp were the sequence –TpΨP– in this tRNA. We have made use of thin-layer chromatography on cellulose plates, using the solvent systems described in [8], to identify the mononucleotides produced by RNAase T_2 digestion of the complete RNAase T_1 digestion products of P3 cell $tRNA_f^{Met}$, and we have not found any evidence for Tp or Ψp in this molecule.

We have also isolated partial RNAase T_1 digestion products [6] which show that the longest complete RNAase T_1 digestion fragment obtained from P3 cell $tRNA_f^{Met}$ [m¹ApApApCpCpApUpCp(Cp, UpCp)UpGp] is preceded in the molecule by the probable sequence GpApUpGpGpApUpCpGp. The part of the primary structure thereby tentatively determined can be drawn as a hairpin loop (fig. 2), not containing the anticodon from which it can be seen by comparison with known cloverleaf structures that in all probability a –GpApUpCp– replaces the –GpTpΨpCp found in other tRNA species.

3. Discussion

Simsek and RajBhandary [5] have proposed that the –GpApUpCp– sequence in the $tRNA_f^{Met}$ of yeast may be associated with its important biological role as the initiator tRNA species. They suggest that it might reflect a unique structure for this molecule which may enable methionyl-$tRNA_f^{Met}$ to act as initiator and also operate to prevent this unformylated methionyl-$tRNA_f^{Met}$ from inserting methionine into the internal positions of proteins. Our studies on the sequence of a mouse cell $tRNA_f^{Met}$ have given strikingly similar results, which indicate that this structural feature may well be unique to the cytoplasmic initiator tRNA's of eukaryotes. The only prokaryotic

$tRNA_f^{Met}$ of known primary structure is the species isolated from *E. coli* [9] and this has the normal –GpTpΨpCp– sequence in loop IV (often called TΨC-loop).

The features of the mammalian initiator tRNA which differentiate it from the *E. coli* initiator tRNA are the lack of formylation of the attached methionine during polypeptide chain initiation and the way in which it is bound to the ribosome. The recognition by the bacterial transformylase implies that the special structural feature does not dictate the specificity of formylation. Darnbrough, Hunt and Jackson [10] have recently demonstrated the existence of a complex between methionyl-$tRNA_f^{Met}$ and 40 S ribosomal subunits in rabbit reticulocyte lysates during active protein synthesis. The existence of this complex implies that the initiator tRNA has the unique ability to bind to a ribosomal subunit in the absence of the appropriate AUG initiator codon on the messenger RNA. Either this property or the lack of recognition by the elongation factor which recognises other aminoacyl-tRNA's may be a consequence of a special structural feature of the $tRNA_f^{Met}$ outlined above.

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References

[1] A.E. Smith and K.A. Marcker, Nature 226 (1970) 607.
[2] N.K. Gupta, N.K. Chatterjee, K.K. Bose, S. Bhadari and A. Chung, J. Mol. Biol. 54 (1970) 145.
[3] S.S. Kerwar, C. Spears and H. Weissbach, Biochem. Biophys. Res. Commun. 41 (1970) 78.
[4] M. Simsek and U.L. RajBhandary, Biochem. Biophys. Res. Commun. 49 (1972) 508.
[5] C.T. Caskey, B. Redfield and H. Weissback, Arch. Biochem. Biophys. 120 (1967) 119.
[6] P.W. Piper and B.F.C. Clark, to be published.
[7] G.G. Brownlee, F. Sanger and B.G. Barrell, J. Mol. Biol. 34 (1968) 379.
[8] F. Kimura-Harada, D.L. von Minden, J.A. McCloskey and S. Nishimura, Biochemistry 11 (1972) 3911.
[9] S.K. Dube, K.A. Marcker, B.F.C. Clark and S. Cory, Nature 218 (1968) 232.
[10] C. Darnbrough, T. Hunt and R.J. Jackson, Biochem. Biophys. Res. Commun. 48 (1972) 1556.