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Sequence determination of the 3' terminal T_1 oligonucleotide of 18S ribosomal RNA.

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ABSTRACT

We have reexamined the primary structure of the 3' terminal oligonucleotide of 18S RNA from chicken fibroblasts and have shown, contrary to previously published results that this extremity G-A-U-C-A-U-U-A_{OH} is identical to that of the rabbit, drosophila and bombyx. Furthermore the electrophoretic mobility and composition of the 3' terminal oligonucleotides of 18S RNA from rat and human cells are similar to that of other RNAs and show that the identity of structure for this region of 18S RNA extends to include all tested species between yeast and man. This finding reveals a marked degree of evolutionary constraint on the structure of this region.

INTRODUCTION

Investigation of the primary structure of the 3' terminal T1 oligonucleotide in eukaryotic cells from the rabbit (1), drosophila (2), yeast (2), mouse (3) and bombyx (4) has revealed in each case the sequence G-A-U-C-A-U-U-A_{OH}. In contrast, a limited analysis of the primary structure of the homologous RNA oligonucleotide from the chicken (5) has suggested that its sequence is different, containing one extra U residue and two additional C residues. Since the evolutionary constancy of the extremity of 18S RNA suggests a special role for this region, the apparently anomalous sequence found in the chicken prompted us to make a further analysis of its nucleotide sequence. In addition we have added in this study to the list of species investigated by analysing the 3' terminal extremities of 18S RNA of rat ($78A_1$) and human cells (HeLa).

MATERIALS AND METHODS

The source of all materials used in this study has been described previously (3). Chick embryo fibroblasts were prepared from ten day old white Leghorn embryos. They were labelled during their first passage in culture by incubation for 36 hours with ^{32}P (25 μC/ml) in Eagle's minimum essential medium (MEM) supplemented with 10% dialysed calf serum. $78A_1$ and HeLa cells were cultured as monolayers in MEM supplemented with 10% calf

serum and labelled in the same way as chicken embryo fibroblasts. P^{32} labelled RNA was prepared as previously described (6). $3'T_1$ oligonucleotides were isolated by the diagonal procedure of Dahlberg (7) and purified by homochromatography on DEAE cellulose (8). Pancreatic ribonuclease digestion, partial snake venom (SVP) and spleen phosphodiesterase (SPD) hydrolyses were performed as previously described (3, 9).

RESULTS

Isolation of the 3' terminal T_1 oligonucleotide.

Following extraction of ^{32}P labelled RNA from cells it was fractionated by sedimentation on a 5%-30% sucrose gradient. The 18S RNA fractions were then hydrolysed with T_1 RNase and separated by electrophoresis in 7% Formic acid on DEAE paper using the method of Dahlberg (7). As shown by the three bidimensional separations reproduced in Fig. 1 a single oligonucleotide A in each 18S RNA was unaffected in its mobility by treatment with phosphatase and thus could be distinguished from the remaining oligonucleotides, all of which lost the terminal phosphorus in position 3'.

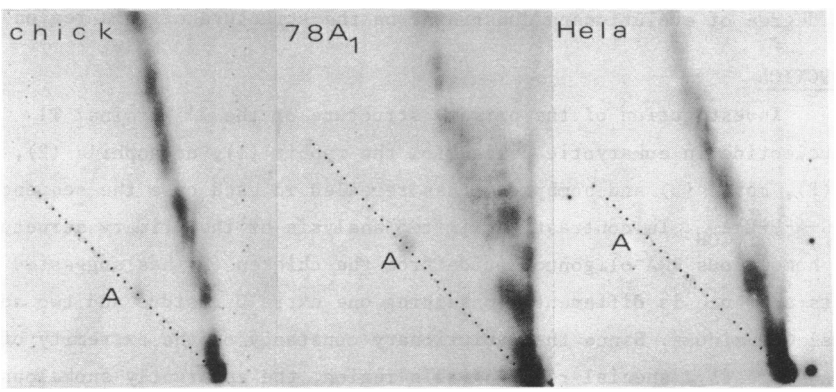

Figure 1. Isolation of 3' terminal oligonucleotides by the diagonal procedure : 18S RNA was fully digested with T_1 ribonuclease and fractionated by ionophoresis on DEAE cellulose paper in 7% formic acid. After phosphatase treatment directly on the paper, separation in the second dimension was carried out at right angles to the just one, under the same conditions. The dashed line (diagonal) joins the origine in the first dimension to the position of the blue dye marker after the second dimension.

This oligonucleotide A corresponds to the 3' terminal oligonucleotide of the RNAs studied (assuming that phosphatase digestion was effective). The mobility of this oligonucleotide relative to that of coloured markers was identical for each of the three species and also identical to that of the

3' terminal oligonucleotide of 18S RNA from L5178Y mouse cells (3). The presumed 3' terminal oligonucleotides from avian, rat and human cells were recovered and tested by alkaline digestion for the absence of a 3' Gp residue to confirm that these oligonucleotides were indeed 3' terminal oligonucleotide. From these results a further purification was necessary before satisfactory sequence data could be obtained. This purification was achieved by chromatography on DEAE plate with a 3% 30' homomixture (Fig 2).

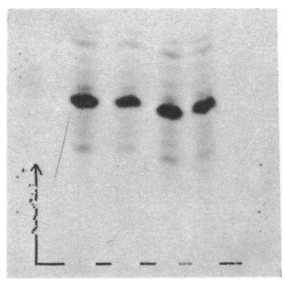

Figure 2. Purification of 3' T_1 terminal oligonucleotide by chromatography on DEAE cellulose plate with a 3% 30' hydrolysed RNA solution.

Study of the primary structure of 3' terminal oligonucleotides.

a) Pancreatic hydrolysis. Following purification the 3' terminal oligonucleotides were hydrolysed with pancreatic RNase. The hydrolysate was fractionated by electrophoresis at pH 3.5 on DEAE paper. As shown in Table 1 these three oligonucleotides had identical compositions.

TABLE I

Analysis of the 3' T_1 terminal oligonucleotide by pancreatic hydrolysis.

	Products of pancreatic digestion			Nearest Integral Number			Nucleotide composition
	A-Up	Cp	Up	A-Up	Cp	Up	
Chicken Fibroblast	18361	4645	4324	2	1	1	$(A\text{-}Up)_2,Cp,Up,N_{OH}$
$78A_1$ (rat cells)	9002	2163	2182	2	1	1	$(A\text{-}Up)_2,Cp,Up,N_{OH}$
HeLa (human cells)	52251	14878	16341	2	1	1	$(A\text{-}Up)_2,Cp,Up,N_{OH}$

The 3' terminal T_1 oligonucleotides were fully digested with pancreatic ribonuclease (1µgPan/15µgRNA), for 2 hours at 37°C. Products were fractionated by electrophoresis on DEAE cellulose paper at pH 3.5 at 10V/cm for 15 hours. After autoradiography, the radioactivity present in each spots was determined. N stands for the ultimate dephosphorylated nucleotide which cannot be determined in this case.

b) Partial hydrolysis with SPD and SVP. The complete sequence of 3'T_1 oligonucleotide of 18S RNA from chicken embryos was determined after partial hydrolysis by phosphodiesterases followed by two dimensional fractionation of the digestion products. The base composition of the products obtained after partial spleen phosphodiesterase digestion (Fig. 3A) allowed the following partial sequence to be deduced N-U-C-A-U(A,U) (Table 2). The composition of products obtained after partial snake venom phosphodiesterase digestion by pancreatic hydrolysis gave the sequence (A-U,C)A-U-U-N_{OH} (Fig. 3B, Table 3).

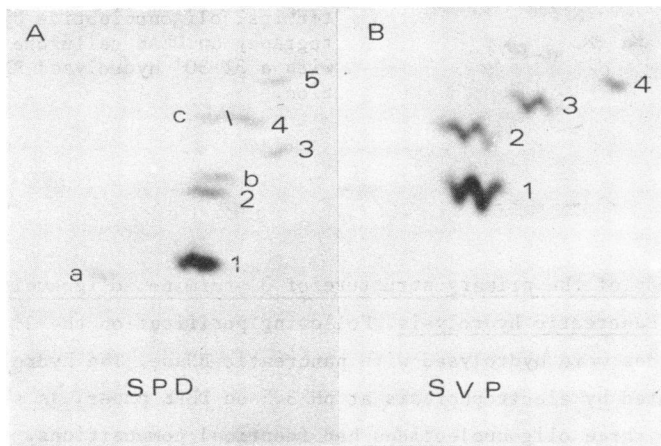

Figure 3. Radioautograph of a two dimensional fractionation of products obtained by partial exonuclease digestion of 3' terminal oligonucleotide of 18S RNA from chicken fibroblasts. A) It was digested for 20' 40' and 75' with 1 µl of spleen phosphodiesterase solution (calf spleen phosphodiesterase 2 mg/ml from Boerhinger) Spot one corresponds to the non hydrolysed oligonucleotide, while spots 2-3-4-5 correspond to the same oligonucleotide shortened at its 5' end by 1, 2, 3 and 4 residues, a b c are contaminating nucleotides. B) It was digested for 20' 40' 75' with 0,3 µl of snake venom phosphodiesterase solution (crotalus snake venom 1 mg/ml from Boerhinger). Spot one corresponds to the non hydrolysed oligonucleotide, while spots 2-3-4 correspond to the same oligonucleotide shortened at its 3' end by 1, 2 and 3 residues.

Comparison of these results allows the sequence A-U-C-A-U-U-A_{OH} to be deduced for the 3' terminal T_1 oligonucleotide of 18S RNA of chicken fibroblasts.

CONCLUSION

Pancreatic hydrolysis of the 3' terminal T_1 oligonucleotide of 18S RNA from the chicken (5) suggested that the extremity of this RNA dif-

TABLE II

Analysis of the partial spleen phosphodiesterase digestion products of the 3' terminal oligonucleotide of 18S RNA from chicken fibroblasts.

Spot Number	Base composition (count/mn)				Nearest Integral Number				Partial deduced sequence
	Cp	Ap	Gp	Up	Cp	Ap	Gp	Up	
5	—	826	—	806	—	1	—	1	N -(Ap,Up)
4	—	910	—	1970	—	1	—	2	N -U-(Ap,Up)
3	—	1351	—	1272	—	2	—	2	N -A-U-(Ap,Up)
2	1669	3515	—	3737	1	2	—	2	N -C-A-U-(Ap,Up)
1	7020	16200	—	23400	1	2	—	3	N -U-C-A-U-(Ap,Up)

Spot 1 to 5 correspond to the product of partial hydrolysis of the 3' terminal oligonucleotide with SPD. (Fig. 3A). Their base composition was determined after total snake venom phosphodiesterase. N stands for the residue in the 5' position which is not determined after SVP hydrolysis.

TABLE III

Analysis after pancreatic hydrolysis of the partial snake venom products of the 3' terminal oligonucleotide of 18S RNA from chicken fibroblasts.

Spot Number	Composition				Nearest Integral Number				Partial deduced Sequence
	A-Up	$A-U_{OH}$	Cp	Up	A-Up	$A-U_{OH}$	Cp	Up	
4	1221	—	501	—	1	—	1	—	$(A-Up,Cp)N_{OH}$
3	2812	1733	1436	—	1	1	1	—	$(A-Up,Cp)A-U_{OH}$
2	9506	—	2257	—	2	—	1	—	$(A-Up,Cp)A-U-N_{OH}$
1	19397	—	5655	3485	2	—	1	1	$(A-Up,Cp)A-U-U-N_{OH}$

Spot 1 to 4 correspond to the product of partial hydrolysis of the 3' terminal oligonucleotide by SVP (Fig. 3B). Their composition was determined after complete hydrolysis with pancreatic RNase. N represents the residue situated in the 3' position of each oligonucleotide which was not analysed after hydrolysis with pancreatic RNase.

fers from that of previously analysed 18S RNAs. The fact that this extremity probably plays an important biological role made us reconsider this question and undertake a complete analysis by partial hydrolysis with snake venom and spleen phosphodiesterase of the primary structure of the 3' ter-

minal T_1 oligonucleotide of this RNA.

The sequence deduced from these different hydrolyses is G-A-U-C-A-U-U-A_{OH}, a finding which is in conflict with the results reported by Ahmad et al. (5), but which reveals that the chicken does not differ in this respect from other previously studied species (1-4). Furthermore the electrophoretic mobility in 7% formic acid on DEAE cellulose paper) and composition (determined by pancreatic hydrolysis) of the 3' terminal T_1 oligonucleotides of 18S RNA from rat and human cells are similar to that of other RNAs and show that the identity of structure for this region of 18S RNA extends to include all tested species between yeast and man. Shine and Dalgarno have put forward two hypotheses (2, 10) concerning the role which may be played by the 3' extremity of small ribosomal RNA. Both theories are related to protein biosynthesis. The first (2), concerning the termination of protein synthesis, is based upon the possibility that the 3' terminal sequence $\overrightarrow{UUA}_{OH}$ is recognised by the nonsense codons $\overleftarrow{AAU}$, $\overleftarrow{GAU}$ and possibly $\overleftarrow{AGU}$. Although this theory has yet to be confirmed the contiguous presence in eukaryotes of the triplet $\overrightarrow{UCA}$ capable of recognising $\overleftarrow{AGU}$ provides strong support. In any event the complete structural identity of terminal oligonucleotides in 18S RNA from all eukaryotes studied so far covering the evolutionary range from yeast to man, together with the fact that the rest of the molecule shows marked species differences, strongly reinforces the concept of a special role for this extremity. The second hypothesis (10), concerning the initiation of protein synthesis, has been directly tested, using E. Coli, by Steitz and Jakes (11), who showed that the sequence CCUCC which is found between the triplets $\overrightarrow{UCA}$ and $\overrightarrow{UUA}_{OH}$ pairs with certain procaryotic mRNA nucleotides situated somewhere in front of the codon $\overrightarrow{AUG}$. The absence in eukaryotes of this sequence CCUCC among all T_1 terminal oligonucleotides of 18S RNA could conceivably, by bringing into phase the triplets $\overrightarrow{UCA}$ and $\overrightarrow{UUA}_{OH}$, increase the efficiency of termination and would imply that the process of initiation occurs in a region further from the 3' extremity.

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