A Spectrophotometric Study of the Secondary Structure of Precursor Ribosomal Ribonucleic Acid from Krebs Ascites-Tumour Cells

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The spectrophotometric analysis of 45S precursor rRNA shows that it contains more G and C residues than does mature 28S or 18S rRNA. The helical content and the length of double-helical segments in 45S and 28S rRNA are similar.

The existence and transformations of precursor rRNA of animal cells is amply documented, but owing to their minute amount little is known about the physicochemical characteristics of these RNA species (see Attardi & Amaldi, 1970; Maden, 1971). We report the isolation and characterization by analysis of denaturation spectra (Cox, 1966, 1970) of 45S precursor rRNA from Krebs ascites-tumour cells as compared with 28S and 18S rRNA from the same source.

Experimental

Isolation of precursor rRNA

About 40 ml of packed cells from the ascitic fluid of 15–20 mice was collected by low-speed centrifugation in the cold and washed twice with cold 0.9% NaCl. The cells were disrupted by suspension in 5 vol. of water and homogenization in a glass-Teflon homogenizer. The cold lysate was mixed immediately with an equal volume of phenol saturated with 0.14 M-NaCl (pH 6.3) and RNA was extracted at 4°C for 60 min. The nuclear rRNA was extracted from the interphase layer by phenol–NaCl treatment at 45°C in the presence of sodium dodecyl sulphate as described by Hadjiolov (1966). The nuclear RNA obtained was fractionated by zonal centrifugation in the B XIV rotor at 43000 rev./min for 3 h (MSE Superspeed 65 centrifuge). A 15–50% (w/v) sucrose gradient in 0.1 M-sodium acetate–0.05 M-NaCl–0.001 M-disodium EDTA was used and the E_{260} of the collected fractions recorded. The fractions corresponding to 18S, 28S and 45S nuclear rRNA were pooled, precipitated with 2 vol. of 96% (v/v) ethanol and stored at –30°C.

Analytical methods

Absorbance measurements were made with an Unicam SP.700 recording spectrophotometer. The sample-cell holder was an electrically heated copper

block, the temperature of which was controlled to within $\pm 0.1^\circ\text{C}$ in the temperature range 25–95°C.

Sedimentation coefficients were determined with a Spinco model E analytical ultracentrifuge fitted with u.v. optics. The extinction of the solution was scanned at 4-min intervals after reaching full speed (42020 rev./min) at a temperature of 16–20°C.

Agar-gel electrophoresis of RNA was carried out as described by Tsanev & Staynov (1964) and the mobility of RNA species related to their sedimentation coefficients (Hadjiolov *et al.*, 1966).

Results and Discussion

The zonal-centrifugation profile of the nuclear RNA extracted at 45°C from the tumour cells shows four main peaks of high-molecular-weight RNA corresponding to the larger and smaller rRNA species and to 32S and 45S precursor rRNA. The fact that the two rRNA peaks in nuclear RNA are prominent indicates that this extraction technique yields a mixture of nucleolar and nuclear-sap RNA. When analysed by agar-gel electrophoresis, the peaks of 28S and 18S rRNA are regular in shape with no signs of degradation, a hazard that is hardly avoided when procedures involving prior isolation of nuclei are used. The isolation of native 45S precursor rRNA was controlled also by agar-gel electrophoresis, where it gives a single peak with a mobility corresponding to the respective RNA fraction from liver or Ehrlich ascites-tumour cells. As evidenced by agar-gel electrophoresis, cross-contamination of the separate RNA fractions studied amounts to less than 10%.

The sedimentation profile of 45S precursor rRNA shows also the homogeneity of this RNA fraction and the absence of contaminating degradation products. The estimated sedimentation coefficient, related to 28S and 18S rRNA, is 42S. Since more detailed analyses under different conditions were not carried out, we shall refer further to this precursor rRNA fraction as 45S rRNA in accordance with its common designation.

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Fig. 1. Denaturation of rRNA from Krebs ascites-tumour cells in 0.01 M-sodium phosphate buffer, pH 7.0
○, ΔE_{260} ; ●, ΔE_{280} . (a) 18S rRNA; (b) 28S rRNA; (c) 45S rRNA. The experimental points were taken from the recorded difference spectra and are corrected for changes in RNA concentration owing to thermal expansion of water.

Table 1. Secondary-structure characteristics of ribosomal ribonucleic acids from Krebs ascites-tumour cells
The heat-denaturation spectra of solutions of rRNA in 0.01 M-sodium phosphate buffer, pH 7.0, were recorded in the range 25–95°C at 5°C intervals. The characteristics of the separate rRNA species were derived according to the equations developed by Cox (1970).

Characteristics	rRNA species		
	45S	28S	18S
T_m (°C) at 260nm	57	55	49
T_m (°C) at 280nm	63	62	56
Interquartile range at 260nm (°C)	42–72	43–70	40–60
$\Delta E_{260}/\Delta E_{280}$	1.10	1.17	1.39
(A + U)/(G + C) ratio*	0.47	0.52	0.68
(G + C)/(A + U + G + C) ratio*	0.68	0.65	0.57
Average number (N) of base-pairs per double-helical segment	8	7	5
Average number of residues per hairpin loop ($2N + b$), $b = 10$	26	24	20
Estimated number of hairpin loops per molecule	530	210	130

* Helical regions.

The secondary-structure of the three nuclear rRNA species was investigated by the spectrophotometric method developed by Cox (1970). The difference spectra obtained on heating the rRNA species from

25° to 95°C were recorded and the derived heat-denaturation profiles at 260 and 280nm are represented in Fig. 1.

The secondary-structure characteristics of 45S,

28S and 18S rRNA from Krebs ascites-tumour cells were calculated according to the equations deduced by Cox (1970) and are summarized in Table 1.

The results of this study reveal that the secondary structure of 28S and 18S nuclear rRNA of Krebs ascites-tumour cells is similar to that of the respective rRNA species of reticulocytes. The characteristic properties found for 28S rRNA of rabbit reticulocytes (Cox, 1970) were T_m (the temperature at which 50% of the total increase in E is observed) 57°C (calculated from ΔE at 260nm) and T_m 68°C (calculated from ΔE at 280nm); for the double-helical parts $(A+U)/(G+C) = 0.46$, corresponding to 69% G+C. The corresponding data obtained for 18S rRNA were T_m 45°C (calculated from ΔE at 260nm) and T_m 52°C (calculated from ΔE at 280nm); for the double-helical parts $(A+U)/(G+C) = 0.75$, corresponding to 58% G+C. The number of base-pairs per double-helical segment was estimated to be 3–10 for 18S rRNA and 3–17 for 28S rRNA. The precursor 45S rRNA of Krebs ascites-tumour cells has a secondary structure closer to that of 28S rRNA. The T_m of 45S rRNA is slightly higher than that of 28S rRNA, but corresponds to a lower $(A+U)/(G+C)$ ratio. Since it is known that 45S precursor rRNA contains the complete sequences of both 28S and 18S rRNA (see Maden, 1971), our results give direct and independent evidence for the assumption that the non-conserved sequences of 45S rRNA are extremely rich in G and C residues (Amaldi & Attardi, 1968; Willems *et al.*,

1968). Further, the overall secondary structure of 45S rRNA in solution closely resembles that of 28S rRNA. Both RNA species appear to be built of numerous hairpin loops with double-helical segments containing an average of 14–16 base-paired nucleotides (see Cox, 1970). Therefore our results indicate that the specific enzymic steps involved in the processing of 45S precursor rRNA depend on local characteristics of the structure and conformation of the polynucleotide chain rather than on a peculiar secondary structure of this RNA molecule.

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