ADENYLATE-RICH OLIGONUCLEOTIDES OF RIBOSOMAL AND RIBOSOMAL PRECURSOR RNA FROM HeLa CELLS

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Abstract. HeLa cell ribosomal precursor (45S) RNA has been found to contain nucleotide sequences also found in 18S RNA and others found in 28S RNA. Thus, 18S RNA and 28S RNA are readily distinguishable, whereas 45S RNA is similar to 18S and 28S RNA combined. The nucleotide sequences analysed were those resistant to the combined action of pancreatic and T1 ribonucleases.

We have studied the relationship between ribosomal precursor RNA's (45S,32S) and mature 18S and 28S ribosomal RNA's (1-3) in a way different than has hitherto been employed (4-10). Purified 18S, 28S, 32S and 45S RNA species were digested with a combination of pancreatic and T1 ribonucleases. Amongst the tetra-, penta- and hexanucleotides which survive this treatment, some were found only in 18S and others only in 28S RNA. 45S RNA had both kinds of oligonucleotides whereas 32S RNA had those found in 28S RNA. These data provide additional support for the current model of ribosomal RNA maturation in eukaryotic cells (1,3).

To isolate these oligonucleotides, the following experimental scheme was employed: (i) the appropriate radioactively labelled RNA molecules were digested with pancreatic and T1 ribonucleases; (ii) the resultant mixture of oligonucleotides was separated into size classes (isopliths) on a DEAE-Sephadex column; (iii) each size class was further fractionated into individual components by electrophoresis on polyacrylamide gels at low pH (11).

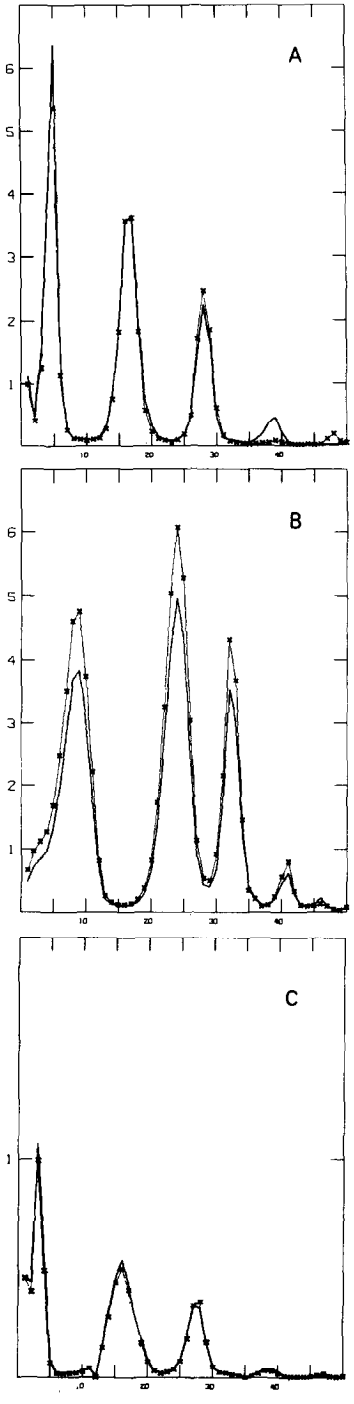

Fig. 1 Electrophoresis of ribonuclease-resistant tetranucleotides from a combined pancreatic and T1 ribonuclease digest of A. ^{3}H-18S RNA (2.3 x 10^{6} dpm) and ^{14}C-28S RNA (1.8 x 10^{6} dpm); B. ^{3}H-32S RNA (1.7 x 10^{6} dpm) and ^{14}C-18 and 28S RNA (9.3 x 10^{5} dpm); C. ^{3}H-45S RNA (1.9 x 10^{6} dpm) and ^{14}C-18 and 28S RNA (1.1 x 10^{6} dpm).

45S, 32S, 28S and 18S RNA were prepared from HeLa cells labelled with either ^{3}H or ^{14}C-adenine. Three experiments were carried out to analyse the following mixtures: (1) ^{3}H-18S RNA and ^{14}C-28S RNA; (2) ^{3}H-32S RNA and an equimolar mixture of ^{14}C-18 and 28S RNA; (3) ^{3}H-45S RNA and ^{14}C-18 and 28S RNA. In each case, individual tetra-, penta- and hexanucleotides were separated by gel electrophoresis. The electropherograms are shown in Fig. 1, 2 and 3. Characterization and quantitation of the individual peaks will be described elsewhere (12).

Amongst the tetranucleotides shown in Fig. 1A-C, the first three components show little difference in their distribution in 45S, 32S, 28S and 18S RNA. In Fig. 1A, a fourth peak is seen which occurs in 28S but not in 18S RNA. This component is also found in 32S and 45S RNA (Fig. 1B,C). A fifth peak seen in Fig. 1A is found in 18S but not in 28S RNA; however, it is not well resolved in Fig. 1B and C.

Three components only are seen to occur amongst the pentanucleotides (Fig. 2A-C). Although all occur in both 18S and 28S RNA (Fig. 2A), their quantitative distribution is markedly different. The second component is 1.9X, and the third component 3X, as frequent in 28S as in 18S RNA. In 32S RNA (Fig. 2B), the second and third components are present in slightly higher amounts than in 18S and 28S RNA. The profile for 45S RNA (Fig. 2C) closely resembles that of a mixture of 18S and 28S RNA.

The most striking differences in distribution between RNA molecules have been detected amongst the hexanucleotides (Fig. 3A-C). Five peaks are seen, although the first is incompletely recovered in some experiments. Of these, only the fourth occurs in 28S RNA, whereas the others occur almost exclusively in 18S RNA (Fig. 3A). The fourth peak is also the major labelled component

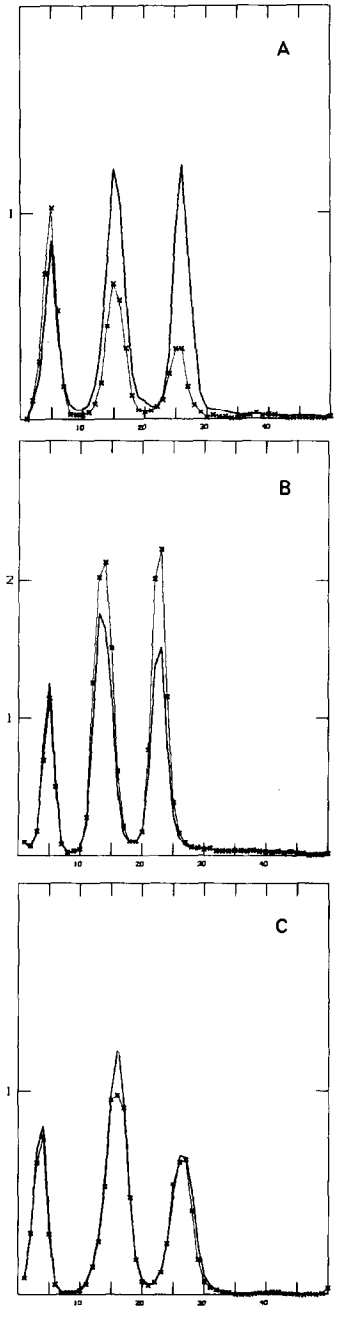

Fig. 2 Electrophoresis of ribonuclease-resistant pentanucleotides. Samples as in Fig. 1.

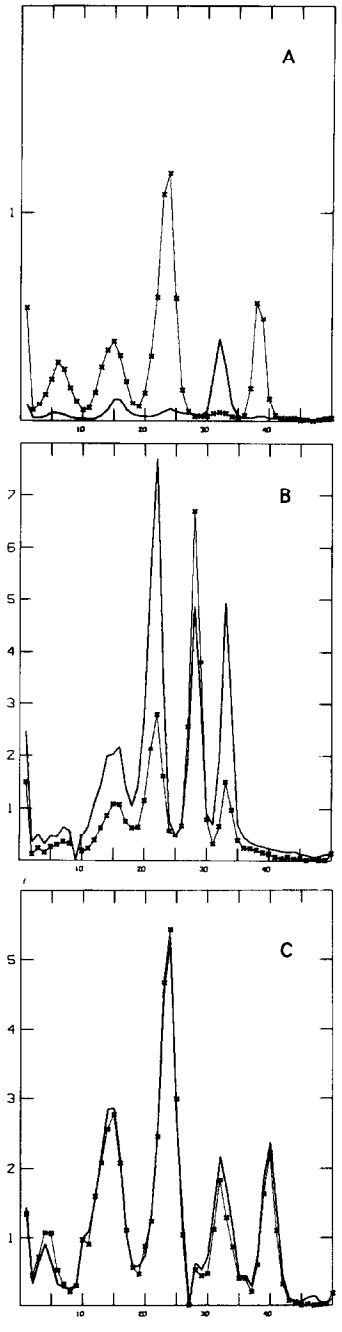

Fig. 3 Electrophoresis of ribonuclease-resistant hexanucleotides. Samples as in Fig. 1.

Fig. 1-3 Abscissa - Fraction number. Ordinate - Dpm per fraction/ Total dpm in the enzymatic digest. ^{3}H-nucleolar RNA (14) and ribosomal RNA were prepared from HeLa cells incubated at 7.5 x 10^{6} cells/ml for two hours in the presence of 2, 8-^{3}H-adenine (40 µCi/ml; 3.6 Ci/m mole). ^{14}C-ribosomal RNA was prepared from HeLa cells (5 x 10^{5}/ml) grown for 48 hr in the presence of 8-^{14}C-adenine (0.3 µCi/ml; 51 m Ci/m mole). Individual 45S, 32S, 28S and 18S RNA species were isolated by sucrose gradient centrifugation. Conditions for enzymatic digestion, DEAE-Sephadex chromatography and gel electrophoresis are described elsewhere (11,12). Oligonucleotides were eluted from gel slices in 1.5 ml 0.02M NH_4OH and counted in a scintillation counter after the addition of dioxane-based scintillator. The data, recorded directly on paper tape, was quench corrected, normalized and plotted by computer. In all figures, the origin (cathode) is on the left. ^{3}H = ——✱—— ^{14}C = ——————.

in 32S RNA (Fig. 3B). As in Fig. 1C and 2C, the profile for 45S RNA (Fig. 3C) resembles that of an equimolar mixture of 18S and 28S RNA.

The experiments are fully consistent with the current model for synthesis and maturation of ribosomal RNA in eukaryotic cells. By identifying nucleotide sequences which occur in 18S RNA but not in 28S RNA and vice versa, it has been possible to show that those occurring in 28S RNA are also found in its immediate precursor, 32S RNA. All tetra-, penta- and hexanucleotides found in 18S and 28S RNA are also found in 45S RNA. Their distribution in 45S RNA closely resembles that of an equimolar mixture of 18S and 28S RNA. No oligonucleotides were found in 45S RNA not also found in 18S or 28S RNA. This is perhaps not surprising since the radioactive label is predominantly in adenine, and apparently most adenine residues are conserved during maturation (13).

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