MATURATION OF 5.8 S RNA IN HeLa CELLS

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1. Introduction

Vertebrate 5.8 S RNA is transcribed within the 32 S part of ribosomal precursor RNA [1–4]. The events comprising maturation of 32 S RNA into 28 S plus 5.8 S RNAs, with elimination of transcribed spacer material, are poorly characterized. Kinetic evidence [5,6] and direct chemical evidence [6] indicate that, in yeast, a transient 7 S RNA species is a precursor to 5.8 S RNA. The 7 S species is manifested by heat treatment similar to that which releases 5.8 S RNA from mature 26 S RNA (or 28 S RNA in vertebrates), suggesting that the precursor, like 5.8 S RNA itself, occurs attached to a larger structure.

Here we show that, when pure 32 S RNA from HeLa cells is subjected to brief thermal treatment, a proportion of molecules is unstable. Among the products liberated is a putative precursor to 5.8 S RNA. The precursor contains nucleotide sequences derived from both 5.8 S RNA and the transcribed spacer region of 32 S RNA. This material may be useful for further nucleotide sequence studies concerning mammalian rRNA maturation.

2. Methods and results

HeLa cells were grown in monolayers and were labelled with $^{32}PO_4$ as described previously [2,7]. Actinomycin D (4 μg/ml) was added 30 min before harvesting. This 'actinomycin chase' blocks the synthesis of new 45 S RNA, while permitting preformed 45 S RNA to be processed into lower molecular weight products, thereby allowing very pure 32 S RNA to be prepared from nucleoli, as described previously [7].

The observations leading to the present findings were as follows (fig.1). RNA, prepared from nucleoli

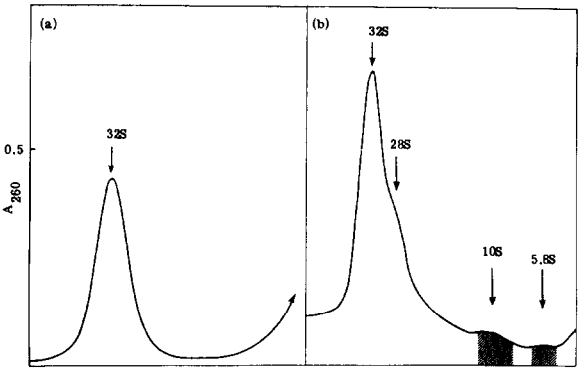

Fig.1. Sucrose gradient profiles of HeLa 32 S RNA, (a) before and (b) after heat treatment. (a) represents RNA, phenol-extracted at 20°C from nucleoli after an actinomycin chase (see text), and centrifuged (22 K, 16 h, 20°C) through a 36 ml 15–30% sucrose gradient in 'LETS' (0.1 M LiCl; 0.01 M EDTA; 0.01 M Tris–HCl (pH 7.4); 0.2% sodium dodecyl sulphate). The symmetrical 32 S peak resembles one published previously [7]. The top of the gradient, with fragmented DNA, is not included in the profile. (b) 32 S RNA, precipitated with ethanol from a gradient such as that shown in (a), was redissolved in LETS (2 ml), heated in a water bath at 60°C for 5 min, rapidly cooled and recentrifuged through a sucrose LETS gradient (10–25% 18 ml gradient was used, so the 'apparent' A_{260} is higher than in (a), though the quantity of RNA is the same). The 10 S and 5.8 S regions from gradients of ^{32}P labelled RNA were pooled separately as shown (shaded regions), carrier RNA was added and the RNAs were subjected to fingerprinting analysis (fig.2).

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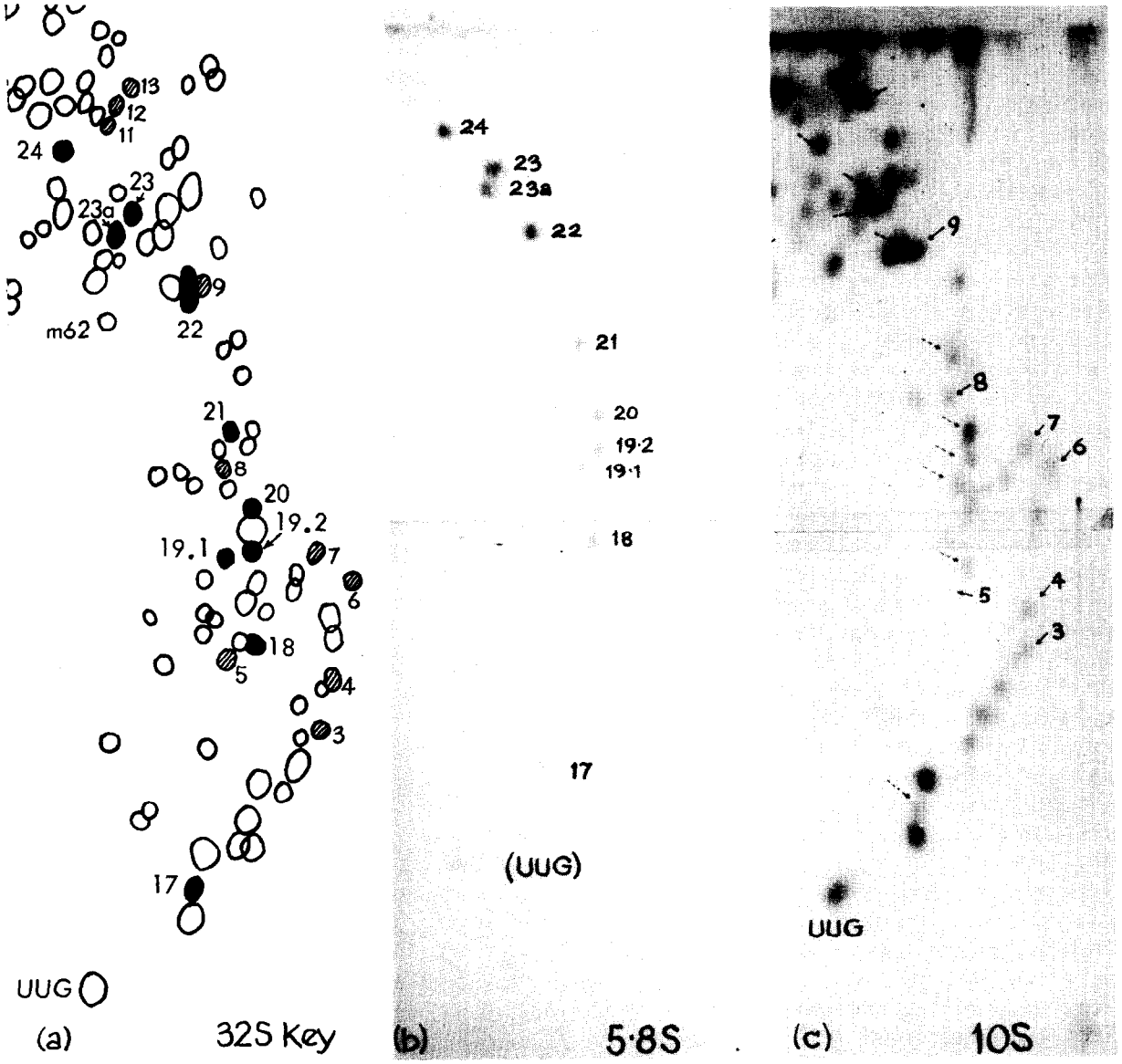

Fig. 2. Two dimensional separations of long oligonucleotides after combined T_1 RNase plus alkaline phosphatase digestion of ^{32}P-labelled RNA. The first dimension (cellulose acetate, right to left) was run for 5 h at 4.5 KV and the second dimension (DEAE paper, downwards) for 40 h at 1.2 KV. (a) Key to 32 S rRNA, adapted from [2]. Distinctive 32 S transcribed spacer products are shaded and numbered 3–13. 5.8 S products are numbered 18–24 (*HeLa 5.8 S numbering system*) and are marked black. 28 S products are marked as open circles. (b) Released 5.8 S. The pattern closely resembles plate II (a) (5.8 S) of [2] except that some weakly labelled non-specific products are present, such a U-U-G. (c) Released 10 S. The solid arrows with numbers indicate distinctive transcribed spacer products of 32 S RNA. 5.8 S products are indicated by broken arrows and can be identified by reference to (a) and (b).

of 'actinomycin chase' cells by phenol extraction at 20°C, revealed on sucrose gradient centrifugation a single symmetrical peak sedimenting at 32 S. However, when RNA was prepared by phenol extraction at 60°C instead of 20°C we consistently noticed that the 32 S peak contained a 28 S shoulder (also reported in [8]). We therefore examined the effect of brief heating at 60°C on 32 S RNA which had already been purified as in fig.1a. On recentrifugation, after heating at 60°C as described in the legend to fig.1, a 28 S shoulder appeared (fig.1b). Also, two small peaks appeared in approximately the 10 S and 5.8 S regions of the gradient. When heating was omitted before recentrifugation no 28 S shoulder or low molecular weight material was seen. (When the initial phenol extraction was carried out at 60°C the low mol. wt material of fig.1b was presumably released but obscured by co-sedimenting DNA fragments, resulting from the DNase digestion step in the nuclear preparation procedure (a)).

The nature of the material from various regions of the second sucrose gradient (fig.1b) was examined by fingerprinting analysis, using ^{32}P labelled RNA and combined T_1 RNase plus alkaline phosphatase digestion [10]. In such fingerprints many unique 28 S, 5.8 S and transcribed spacer oligonucleotides of 32 S RNA are directly resolved [2,7]. The main band of 32 S RNA revealed all three classes of oligonucleotides, as previously reported [2,7,11], (fig.2a, Key). The material sedimenting at approx. 5.8 S yielded a pattern resembling mature 5.8 S RNA (fig.2b), with minor non-specific contamination from some simple oligonucleotides such as U-U-G (which does not occur in fingerprints of pure 5.8 S RNA). The material sedimenting in the 10 S region revealed all of the 5.8 S oligonucleotides that are resolved in this fingerprinting system, together with several previously characterized [7] transcribed spacer oligonucleotides (fig.2c) which are recognisable by their distinctive mobilities. In particular, spacer products 3, 4, 6, 7, 8 and 9 are evident. Spacer product 5 appears to be absent. Products 11–13, near the origin of the second dimension, are difficult to identify by mobility alone. In the well resolved 'two uridylate' graticule (the lower two-thirds of the fingerprint), oligonucleotides *unique* to the major 28 S sequence are practically absent from good preparations. There is weak labelling of 28 S oligonucleotides in the upper third of the fingerprint where the autoradiographic density is high due to the sequence lengths and the compact nature of the spots. This small amount of 28 S material is probably derived from non-specific fragmentation of a few 28 S or 32 S molecules. Otherwise the major features of the 10 S preparation are the distinctive 5.8 S and 32 S transcribed spacer oligonucleotides.

3. Discussion

To account for these findings we propose that, among the molecules which sediment at 32 S after cold phenol extraction, there exists a fraction which has undergone an initial cleavage in vivo. The cleavage site is proposed to be located between the major 28 S and 5.8 S sequences in such a way that the 32 S transcribed spacer region, or a substantial proportion thereof, remains initially linked to the 5.8 S sequence. The 10 S fragment is proposed to arise from 32 S molecules in which little or no further processing has yet occurred, the 5.8 S and spacer sequences remaining covalently linked to each other. The '5.8 S' nucleolar material presumably derives from molecules which have already undergone further processing, any remaining spacer material no longer being covalently linked to the 5.8 S sequence. The 'nicked' 32 S molecules are presumably stabilized, under non-denaturing conditions, by non-covalent interactions between the major 28 S sequence and the 5.8 S sequence (and possibly, but not necessarily, the transcribed spacer sequence also).

The 10 S material is presumably analogous to the 7 S material reported in yeast [5,6]. Although our suggested S value is only approximate, it would not be surprising if the HeLa material were larger than that from yeast, because of the probably greater quantity of transcribed spacer material in HeLa (32 S) than yeast (29 S) ribosomal precursor RNA. Since only a small proportion of the HeLa 32 S molecules are thermolabile, the 10 S material has so far been obtained in only just sufficient quantity for fingerprinting analysis. If the yield could be

substantially increased the material would be of considerable value for detailed studies on the sequence relationship between the major 28 S, 5.8 S and transcribed spacer regions of 32 S RNA.

Acknowledgements

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