PRECURSORS TO SMALL MOLECULAR WEIGHT RNA COMPONENTS

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1. Introduction

Metabolically long-lived RNA components such as tRNA and rRNA are formed from unstable larger precursors [1,2]. Baby Hamster Kidney cells (BHK-21 cells) contain four small mol. wt RNA components (sm RNA) with a metabolic half-life of 5–7 days [3]. The size of these components (D, C, A, and L) was determined to be from 145 to 235 nucleotides based on their migration in polyacrylamide gels [4]. The present paper demonstrates the presence of larger unstable precursors for some of the sm RNA components.

2. Materials and methods

Baby Hamster Kidney cells (BHK-21 cells) were grown in suspension cultures as described previously [3]. After labelling with [^{32}P]orthophosphate for 20 h and [5-^{3}H]uridine for 55–90 min, the RNA was extracted with RSB (0.01 M NaCl, 1.5 mM $MgCl_2$, 0.01 M Tris–HCl pH 7.4) and phenol containing 0.1% 8-hydroxy-quinoline [3]. RNA components were separated on polyacrylamide gels [5] and the radioactivity in the components was measured [6,7].

3. Results and discussion

Baby Hamster Kidney cells (BHK-21 cells) contain small mol. wt RNA components with a metabolic stability comparable to ribosomal RNA (rRNA) [3]. Using short periods of incubation with [5-^{3}H]uridine it is possible to demonstrate small RNA molecules with a size similar to the more stable sm RNA components (fig.1). One component migrates a little slower in polyacrylamide gels than component A and one a little slower than component C. We have designated the components pre-A and pre-C since the experiments described below show that these components most likely are precursors for A and C respectively. After about 60 min of incubation there is incorporated about equal amounts of [5-^{3}H]uridine into precursors and products. After 90 min, much less precursor than product is seen (fig.1).

One possible way to demonstrate that these components are actually precursors and not short-lived RNA molecules with no relation to the stable sm RNA components would be to stop further RNA synthesis with actinomycin D and then determine whether the

Table 1
Effect of actinomycin D on the precursor–product ratio

Component	Counts per minute	
	Labelling for 60 min	Labelling for 60 min actinomycin D 60 → 90 min
pre A	1490	0
A	1520	3050
pre A + A	3010	3050
pre C	3140	0
C	2360	5600
pre C + C	5500	5600
D	6690	6700

Experimental conditions as described in fig.1. Possible slight differences in the amounts of RNA applied to the gels were corrected for on the basis of the ^{32}P-radioactivity in the peaks.

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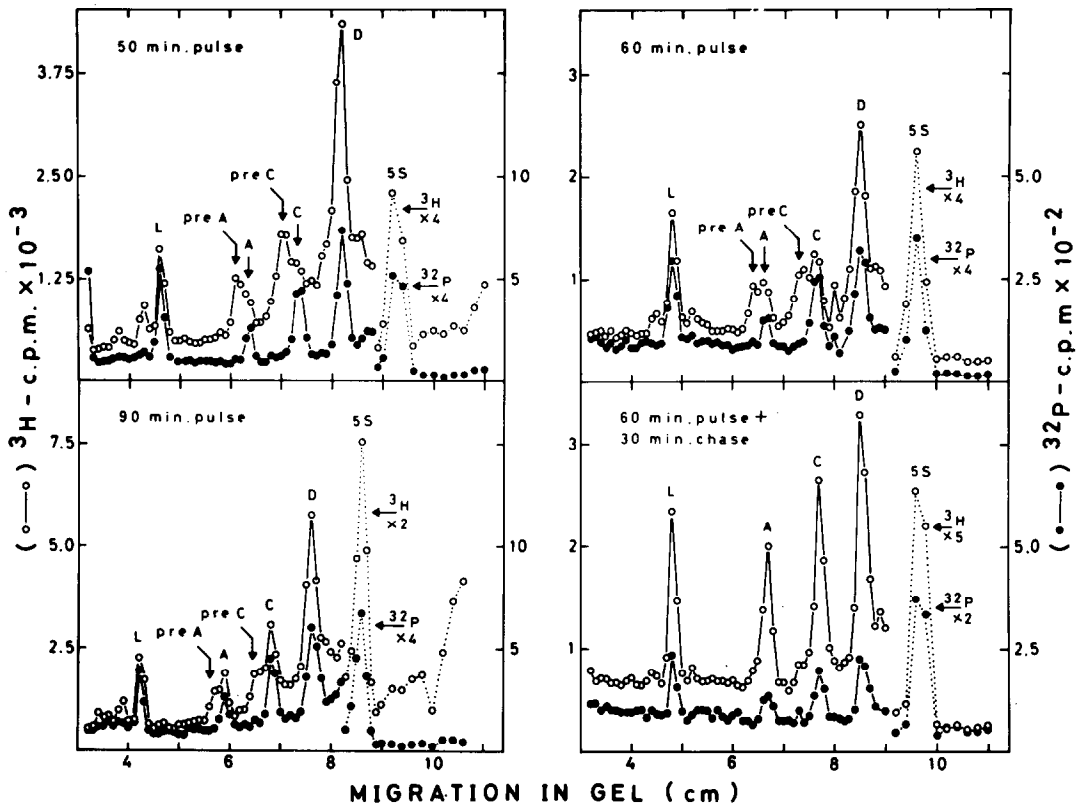

Fig.1. Relative amounts of unstable precursors after different periods of labelling and after actinomycin D treatment. 120 ml BHK-21 cells (4 × 10^5 cells/ml) were grown in suspension cultures for 20 h with 0.5 mCi [^{32}P]orthophosphate (from Risø, Denmark, 1.55 Ci/mmole). [5-^{3}H]uridine (from Amersham, England, 29 Ci/mmol) was then added and incubation continued for the time indicated. The actinomycin treatment (0.25 μg/ml) was started after 60 min labelling and continued until 90 min after addition of [5-^{3}H]uridine. The cell suspensions were immediately chilled in ice and washed once with 0.15 M NaCl RNA was extracted from the cells with RSB and phenol at 0°C. RNA was analyzed on 10% polyacrylamide gels [5].

radioactivity in the precursor peak migrates into the sm RNA component. Actinomycin D was used in a concentration of 0.25 μg/ml which has previously been found to block synthesis of rRNA and components A, C and D [3]. The results show that the radioactivity in the precursor peak can be recovered in components A and C respectively after an actinomycin D treatment (table 1).

A second possible way to demonstrate a precursor-product relationship is to grow the cells at suboptimal temperature since it has been shown that in Hela cells grown at 26–32°C, the processing of pre-tRNA to t-RNA is slowed down and this gives rise to an increase in the amounts of pre-t-RNA relative to t-RNA [8]. BHK cells were grown at 30°C and the profile of sm RNA from these cells was compared with that of control cells (fig.2). In cells grown at 30°C the amount of precursors relative to the product is much higher than in cells grown at 37°C. The ratio of cpm in precursor/cpm in product has been calculated and compared with pre-t-RNA/t-RNA (table 2). A lowering of the growth temperature has a pronounced inhibitory effect on the processing of pre A, pre C and pre-t-RNA.

In BHK cells grown at 30°C the processing of 32S to 28S rRNA is also inhibited (results not shown) in agreement with results obtained with Hela cells [9].

It is possible that there is a precursor to component D also but that this is only a few nucleotides larger than D. When RNA labelled as described in fig.1 is

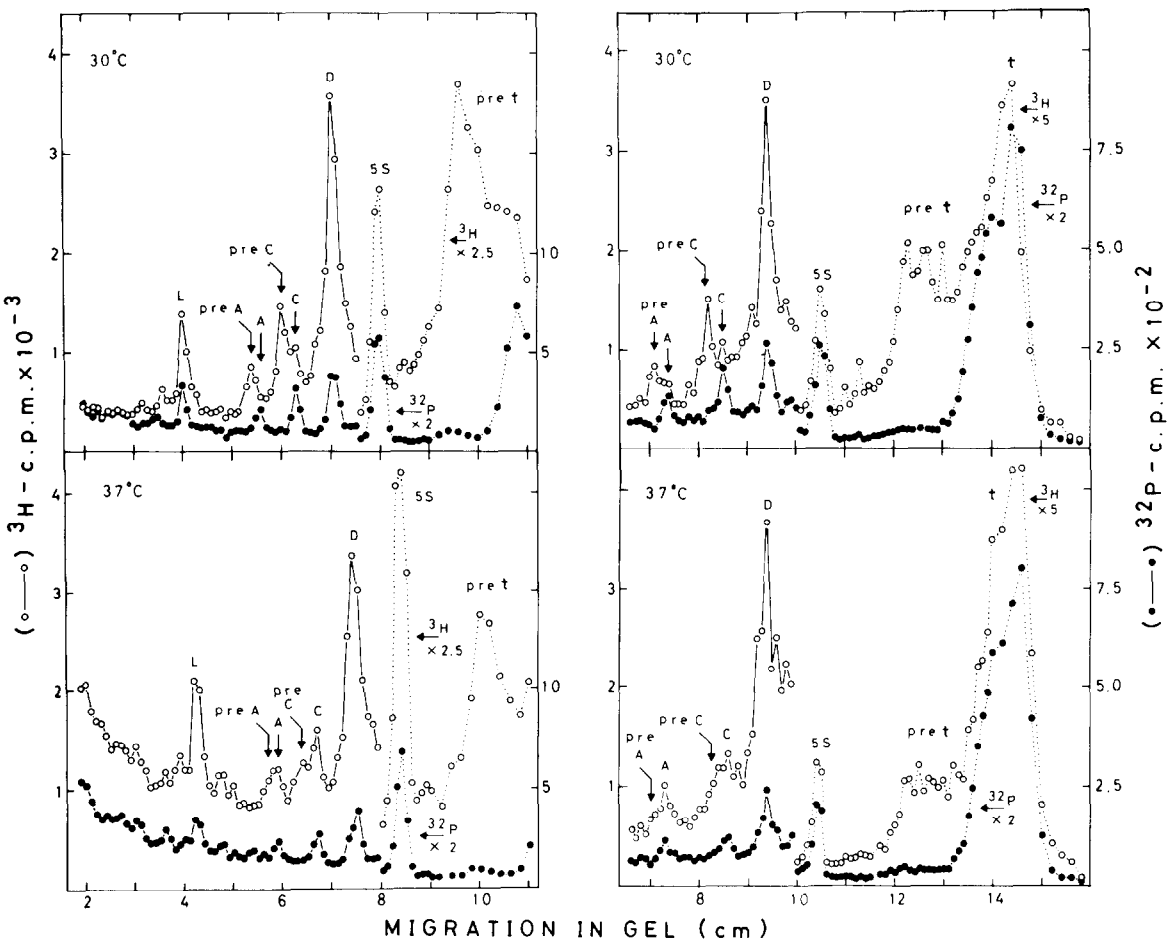

Fig.2. The effect of low growth temperature on the amounts of precursors for sm RNA and t-RNA. Cells were grown for 90 min as described in fig.1 except that one culture was grown at 30°C instead of 37°C.

Table 2
Relative amounts of precursors and products in cells grown at 30°C and 37°C

Component	Counts per minute		Ratio precursor/product	
	30°C	37°C	30°C	37°C
Pre A	965	540	1.6	0.45
A	600	1200		
Pre C	1950	1000	3.3	0.6
C	600	1750		
pre t-RNA	100 600	109 300	0.59	0.25
t-RNA	171 400	433 100		

Cells were grown for 90 min as described in fig.1 except that one culture was grown at 30°C instead of 37°C.

electrophoresed on 10% polyacrylamide gels, the pulse labelled peak of D (^{3}H) is 1 to 2 mm behind the ^{32}P peak in short pulses (50–60 min) but not in longer pulses (90 min). The specific activities (ratio ^{3}H cpm/^{32}P cpm) of pre A + A, pre C + C and D are very similar (table 3) so it is likely that either pre D is processed very rapidly or pre D is included in the D peak. With respect to component L the peak seen in pulse labelled RNA coincides with the one obtained from RNA labelled for long periods.

The size of pre A and pre C was calculated from the migration ratios in 10% polyacrylamide gels and recent data on the Size of D, C and A [4] and on the corresponding components from Novikoff hepatoma cells. The primary structure of these components

Table 3
Specific activity of different RNA components

Experiment No	Time of labelling	Spec. act. of component				
		L	pre A + A	pre C + C	D	5S
1	50 min	2.7	7.4	5.6	6.3	3.9
	75 min	5.5	9.8	7.2	8.8	5.8
	90 min	5.2	9.5	9.7	9.9	5.9
2	55 min	3.3	9.8	7.6	8.2	5.0
	80 min	7.4	11.3	9.8	11.9	7.7
	55 min + actinomycin D 55 → 80 min	4.8	8.7	9.4	9.6	7.0
3	55 min	6.5	5.4	5.9	7.8	4.2
	90 min	7.9	9.1	7.9	9.9	8.9
	30°C – 90 min	5.9	5.1	6.8	9.9	5.0

Experimental conditions as described in fig.1.
The specific activity of the components are calculated from the ratio ^{3}H cpm/^{32}P cpm in the different peaks.

(U_1, U_2 and U_3) have been studied in detail [10–12] and the total sequence of U_1 and U_2 is known. Assuming no major differences in conformational state between precursor and product, pre A and pre C are about 10 nucleotides longer than the product and the possible pre D is less than 5 nucleotides longer than component D.

Pre-t-RNA is transported into the cytoplasm and here further processed to mature t-RNA. The sm RNA components have been considered nuclear components [13–15]. Recently we found, however, that components A, C and D are present in the cytoplasm in amounts corresponding to 40–60% of the total cellular RNA and that 90% or more of component L is in the cytoplasm [16,17]. These results have been obtained employing several techniques for cell fractionation including the conventional aqueous techniques as well as the non-aqueous technique described by Siebert [18]. The demonstration of relatively large quantities of sm RNA in the cytoplasm is in agreement with results obtained by fractionation of Chinese Hamster Ovary cells [19]. It was therefore of interest to see of the precursors for sm RNA were also found in the cytoplasm. BHK cells were homogenized with RSB in a Dounce homogenizer. The homogenate was centrifuged 5 min at 1000 *g* which gave the cytoplasmic fraction and a crude nuclear pellet. The nuclei were treated with 0.5% nonidet and the suspension centrifuged 5 min at 1000 *g*. RNA was extracted from the three fractions (cytoplasm, nonidet wash and nuclei) with phenol. The nonidet extract contain 10% of component L, 3% of 5S RNA and 1% of t-RNA (profile not shown). The RNA profiles of nuclear and cytoplasmic RNA are shown in fig.3. In this experiment 40–45% of A, C and D is present in the cytoplasm and 90% of L. It is also seen that pre A and pre C are present in both the nuclear and the cytoplasmic fraction. The significance of this finding requires additional studies on the amounts and nature of the precursors in the two fractions. The components described in the present paper may correspond to the unstable RNA components demonstrated in the cytoplasm of Hela cells [20,21].

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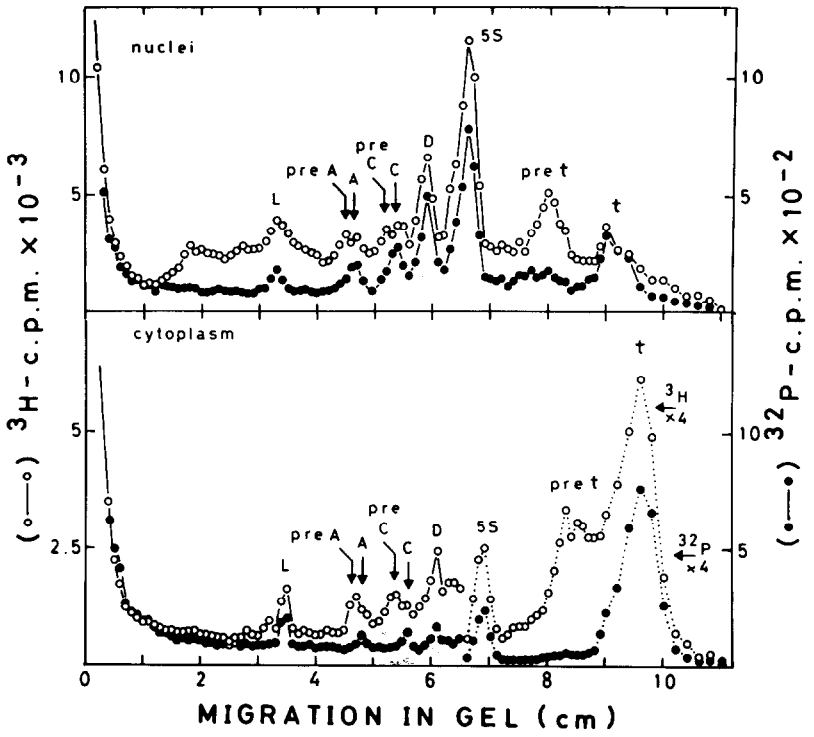

Fig.3. The presence of sm RNA precursors in nuclei and cytoplasm. Experimental conditions as in fig.1 except that the cells were labelled for 60 min. The harvested cells were allowed to swell in RSB and homogenized in a Dounce homogenizer [3,22]. The homogenate was centrifuged 5 min at 1000 *g* which gave the cytoplasmic fraction and a crude nuclear pellet. The nuclear pellet was suspended in 0.5% nonidet in RSB and treated with 3 strokes in a Dounce homogenizer. The suspension was centrifuged for 5 min at 1000 *g*. The cytoplasm, nonidet extract and nonidet treated nuclei were extracted with RSB and phenol at 0°C and RNA analyzed on 10% polyacrylamide gels [5] (nonidet fraction not shown).

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