MATURATION OF RIBOSOMAL RNA's IN SUSPENSIONS OF HIGHER PLANT CELLS A DNA–RNA HYBRIDIZATION STUDY

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1. Introduction

During recent years the existence of pre-ribosomal RNA's (pre-RNA's) has been established in higher plants [1–4]. In sterile suspensions of Sycamore (*Acer pseudoplatanus* L.) cells, four different pre-rRNA's have been characterized [5]. They have sedimentation constants (and molecular weights) of 42 S (2.9×10^6), 36 S (2.3×10^6), 27 S (1.4×10^6) and 20 S (0.9×10^6), respectively. We have already shown, using pulsechase experiments with Sycamore cells [5], that the 42 S molecule is the primary transcription product. It contains the sequences of both 26 S and 17 S rRNA's and the first step in rRNA processings is the cleavage of this large molecule into the 36 S pre-rRNA species; the mean half-life of this step is about 30 min.

However, the mechanism of the maturation process of ribosomal RNA's (rRNA's) is far from being completely understood in higher plants. In particular, it is not known whether the 36 S pre-rRNA is a common precursor of the two mature cytoplasmic rRNA's. Also, no work has been performed on the non-conserved sequences of pre-rRNA's and, for instance, it is not certain if they are methylated or not. Lastly, the possible existence of sequence homologies between the different polynucleotide sequences contained in the largest rRNA's especially the 26 S and 17 S molecules, has never been examined.

The aim of the present paper is to bring new insights into this matter by using the DNA–RNA molecular hybridization technique.

2. Methods

Sycamore cells were grown as previously described [5]. Ribosomal and pre-ribosomal RNA's were labelled with [methyl-^{3}H]methionine (11 Ci/mM), [^{32}P]phosphoric acid (without carrier) or [^{3}H]uridine (10 Ci/mM). Cells were exposed to these labelled products at the following concentrations and for the following times: 10 mCi/ℓ for 20 min ([methyl-^{3}H]-methionine), 50 mCi/ℓ for 4 hr ([^{32}P]phosphoric acid), and 6 mCi/ℓ for 4 days ([^{3}H]uridine). [^{32}P]phosphoric acid labelling was carried out in the absence of cold orthophosphate in the culture medium, and [^{3}H]uridine labelling was followed by a 24 hr chase in the presence of a 100-fold excess of cold uridine.

Cytoplasmic and nuclear RNA's were extracted as previously described, sedimented on sucrose gradients [5], and further purified from DNA and heterogeneous nuclear RNA's either by chromatography on methylated albumin columns [6] for the cytoplasmic rRNA's, or by polyacrylamide gel electrophoresis [5] for the nuclear pre-rRNA's. In this case, RNA's were eluted from the gels with phenol at 4°C. When a pancreatic ribonuclease treatment was carried out before sedimentation on sucrose gradients, all the absorbance and radioactivity peaks completely disappeared.

DNA was extracted from Sycamore cells using the technique of Smith and Halvorson [7] and further purified by hydroxylapatite chromatography [8]. DNA obtained in this way exhibited a hyperchromicity of 32% in 1 SSC (saline sodium citrate) buffer. Its modal sedimentation constant was 21 S, which

corresponds to an estimated mol. wt. of 7.8×10^6 [9]. The melting profile of DNA in a 1 SSC buffer gave a Tm of 84°C, which allows the calculation of a guanine plus cytosine content of 36% [10]. Because mature rRNA's or pre-rRNA's have been purified and behave as homogeneous, on polyacrylamide gels, molecular DNA–RNA hybridization was carried out on ultrafractionated DNA.

The hybridization technique used was that of Gillespie and Spiegelman [11], as modified by Teissere et al. [12].

3. Results

In fig. 1A are presented, using the linear representation of Bishop et al. [13], the saturation of Sycamore DNA by mature 17 S and 26 S rRNA's. The saturation values can be determined from the slope [13] of these plots and are 5.9×10^{-4} and 5.3×10^4 $\mu g/\mu g$/DNA, respectively. These values are quite compatible with other results obtained with higher plants [14, 15]. One can see that these saturation values are not in agreement with the molecular weight of the 17 S and 26 rRNA's (0.8×10^6 and 1.2×10^6, respectively). This is not due to a difference in the number of 17 S and 26 S rRNA's cistrons, (fig. 2, table 2) but rather to a considerable cross-hybridization between mature rRNA's, as shown in fig. 1. Results of fig. 1 clearly indicate that the base sequence of the cytoplasmic rRNA's are very similar.

The validity of these hybridization experiments can be checked by the fact that no competition occurs between the rRNA's extracted from Sycamore and from *E. coli* cells (table 1). Moreover, data of table 1 show that the same results are obtained, whethet the hybridization is effected in one or in two steps.

When competition hybridization are performed between one of the labelled 42 S or 36 S pre-rRNA's, and unlabelled 17 S rRNA, or 26 S rRNA, or an equimolar mixture of these two rRNA's, the results of fig. 2 are obtained. The proportion of sequences of ^{32}P-labelled 42 S and 36 S pre-rRNA's competed against by an equimolar mixture (at infinite concentration) of unlabelled 17 S and 26 S rRNA's are 66% and 86% respectively (fig. 2A). These values are equal

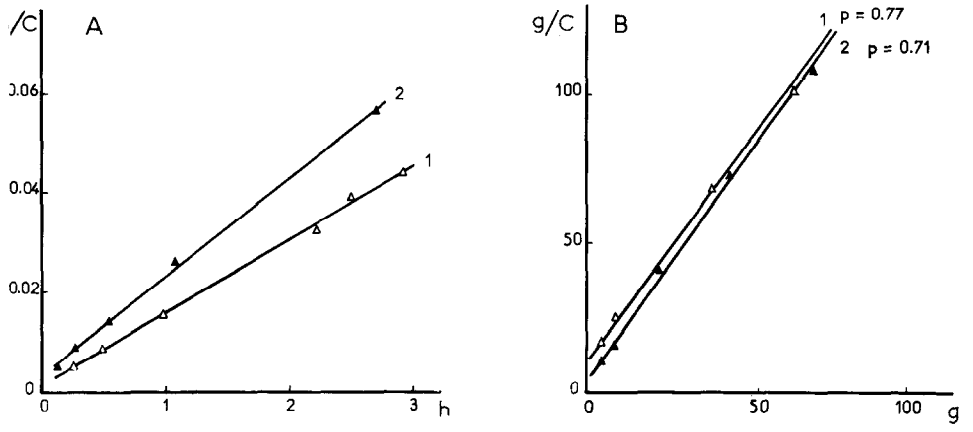

Fig. 1. Hybridization of Sycamore DNA with cytoplasmic ribosomal RNA's: A) Hybridization of DNA with either [^{3}H]uridine labelled 17 S rRNA (curve 1) or [^{3}H]uridine labelled 26 S rRNA (curve 2). Hybridization was carried out for 21 hr, at 41°C, in 6× SSC, 0.1% SDS buffer containing 40% formamide (v/v). h is the concentration of the labelled RNA (μg/ml) and C the number of hybridized cpm per μg of DNA. The reciprocal of the slope corresponds to the maximum number of cpm which can hybridize with DNA at an infinite concentration of labelled RNA; B) Hybridization competition between [^{3}H]uridine labelled 26 S rRNA and unlabelled 17 S rRNA (curve 1) or [^{3}H]uridine labelled 17 S rRNA and unlabelled 26 rRNA (curve 2). If co and c are the numbers of cpm hybridized/μg of DNA, either in the absence (co) or in the presence (c) of unlabelled RNA, the competition is defined as C = (co-c)/co. g is the concentration of the unlabeleld RNA. The reciprocal of the slope corresponds to the proportion of sequences p, in labelled RNA, that would be competed against by an infinite concentration of the unlabelled RNA. 2.5 μg/ml of labelled RNA were used. The specific activities of the 17 S and the 26 S rRNA were 113 300 cpm/μg and 93 300 cpm/μg, respectively. 25 μg of DNA were immobilized on the filters.

Table 1
Hybridization competition between [^{3}H]uridine labelled Sycamore (S.) cell rRNA's and the same unlabelled rRNA's or *E. coli* rRNA's.

Labelled rRNA	Unlabelled rRNA	Hybridization competition values obtained	
		With the one-step procedure	With the two-step procedure
26 S rRNA (S. cells)	26 S rRNA (S. cells)	97%	97%
26 S rRNA (S. cells)	17 S rRNA (S. cells)	77%	77%
17 S rRNA (S. cells)	26 S rRNA (S. cells)	71%	
26 S rRNA (S. cells)	16 S + 23 S rRNA's (*E. coli* cells)	0%	

In the two-step procedure the filters were pre-incubated, for 18 hr, with increasing amounts of the unlabelled RNA. They were then incubated for 21 hr with saturating amounts of the labelled RNA (4 μg/ml).

to the theoretical percentages (table 2) computed by assuming that the sequences of the two mature rRNA's are obtained once only in the 36 S and in the 42 S pre-rRNA's. If hybridization competitions are carried out, using pre-ribosomal RNA's labelled with [methyl-^{3}H]methionine, one can observe (fig. 2B, C, D) that competitio n values obtained with the mixture of the two mature rRNA's (at an infinite concentration) are higher than with any of these molecular species alone. Further, they are very close (95% and 94%) to those obtained for an homologous competition (97%) (table 1).

The results of fig. 2 demonstrate that the sequences of the mature cytoplasmic rRNA's are present together, once only, in the sequences of both 42 S and 36 S molecules. Moreover, the difference in the degree

Table 2
A comparison of experimental and calculated hybridization competition values between labelled pre-rRNA's and unlabelled mature rRNA's.

Labelled RNA	Unlabelled RNA	Experimental hybridization competition values*	Calculated hybridization competition values**
[^{32}p] 42 S pre-rRNA	Both 17 S and 26 rRNA's	66%	66%
[^{32}p] 36 S pre-rRNA	Both 17 S and 26 S rRNA's	86%	86%
[methyl-^{3}H] 42 S pre-rRNA	Both 17 S and 26 S rRNA's	95%	100%
	26 S rRNA	80%	60%***
	17 S rRNA	70%	40%***
[methyl-^{3}H] 36 S pre-rRNA	Both 17 S and 26 S rRNA's	94%	100%
	26 S rRNA	72%	60%***
	26 S rRNA	77%	40%***
[^{3}H]uridine 26 S rRNA	26 S rRNA	97%	100%

* Experimental values are derived from plots of fig. 2.

** These values are calculated, from molecular weight, by assuming, first, that the sequences of the two mature rRNA's are together present, once only, in the sequences of the precursor RNA's, and second, that the non-conserved sequences of the latter molecules are not methylated and have no sequence homologies with the mature rRNA's.

*** These are theoretical values that would be obtained if there were no sequence homology between the two mature rRNA's (the degree of methylation of the two rRNA's are the same).

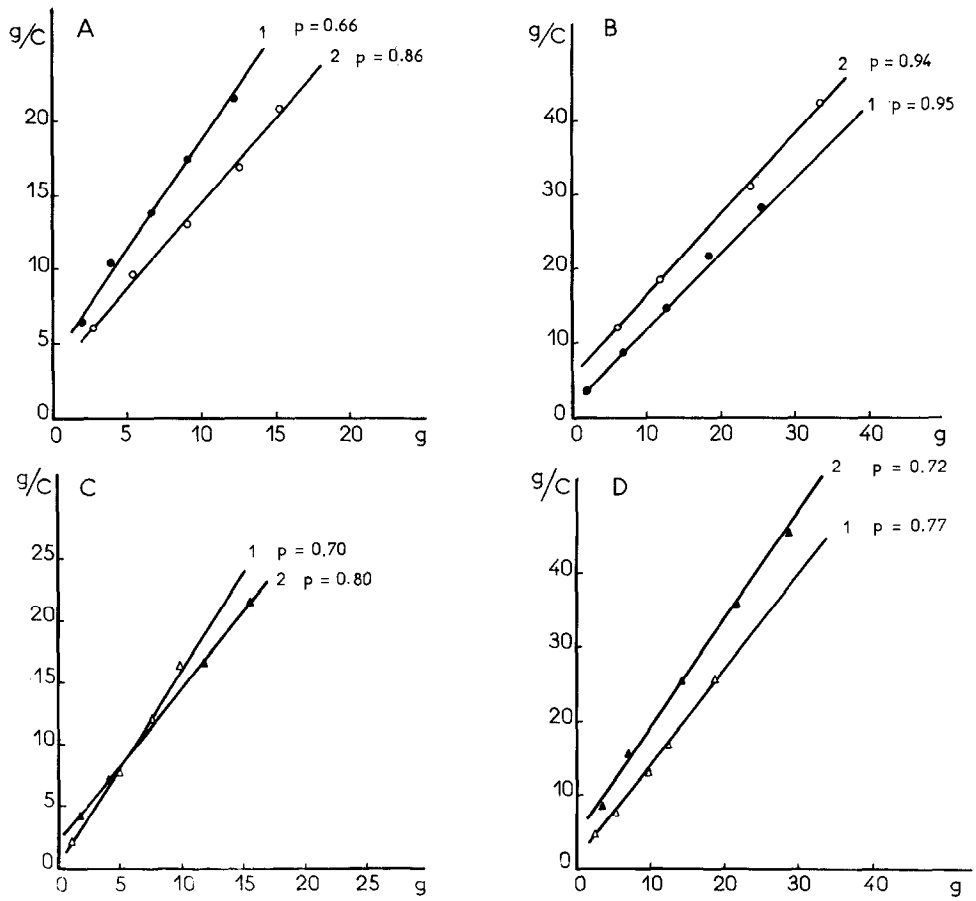

Fig. 2. Hybridization competition between labelled pre-ribosomal RNA's and unlabelled cytoplasmic ribosomal RNA's; A) ^{32}P-labelled 42 S (curve 1) or 36 S (curve 2) pre-rRNA's and an equimolar mixture of unlabelled 17 S and 26 S rRNA's. The specific activities of the 36 S and the 42 S molecules were 45 000 cpm/μg and 60 000 cpm/μg, respectively. Each filter (50 μg of DNA) was incubated with either 1.8 μg/ml of 36 S pre-rRNA or 1.3 μg/ml of 42 S pre-rRNA; B) methyl-^{3}H-labelled 42 S (curve 1) or 36 S (curve 2) pre-rRNA's and an equimolar mixture of unlabelled 17 S and 26 S rRNA's; C) methyl-^{3}H-labelled 42 S pre-rRNA and unlabelled 17 S rRNA (curve 1) or 26 S rRNA (curve 2); D) methyl-^{3}H-labelled 36 S pre-rRNA and unlabelled 17 S rRNA (curve 1) or 26 S rRNA (curve 2). In B, C, and D, 100 μg of DNA were immobilized on the filters. The specific activities of the 36 S and the 42 S pre-rRNA were 4200 cpm/μg and 9800 cpm/μg, respectively. The filters were incubated with either 6 μg/ml of 36 S pre-rRNA or 2.5 μg/ml of 42 S pre-rRNA.

of competition when labelling is effected with either [^{32}p] or [methyl-^{3}H]methionine (see fig. 2 and table 2) proves that the non-conserved sequences of pre-rRNA's exhibit no similarity to mature rRNA's, and that they are not methylated. Also, the fact that the competition values of the 36 S and the 42 S pre-rRNA's either by the 17 S rRNA or the 26 S rRNA are superior to those expected from molecular weight values (table 2) again argues in favor of a strong sequence similarity between the two cytoplasmic rRNA's.

4. Discussion

Previous results [5] have shown that the 42 S pre-rRNA (2.9×10^6 daltons) is a common precursor of the mature cytoplasmic rRNA's and is cleaved into the 36 S pre-rRNA (2.3×10^6 daltons), a sequence (or a sum of sequences) of 0.6×10^6 daltons being lost. The present hybridization studies show that the non-conserved sequences exhibit no sequence similarity with the mature ribosomal RNA's, and that they are not methylated. Moreover, similar conclusions can be

drawn for the 36 S species, namely that it is a precursor of the two mature rRNA's, and that the non-conserved sequences (0.3×10^6 daltons) have the same characteristics as those of the 42 S species. Similar results are obtained with animal cells [16], but not with lower plants [17], where the lost sequences appear to be methylated. It is thus tempting to suppose that, in higher plants, and in animal cells as well, the degree of methylation of the different stretches of the precursor rRNA's plays an important role in their processing. Recently Jakob et al. [18] have claimed that it is extremely difficult to label higher plant rRNA's with methyl-^{3}H because of a rapid incorporation of methyl groups into pectins of the cell wall. Moreover, they suggested that our previous results [5], could be, at least in part, artefacts due to pectin contaminations of RNA's. Since in the present work, as well as in the previous paper [5], all the methyl-^{3}H radioactivity can be removed by RNAase, this claim and this reservation are invalid.

The numerical values obtained from the cross-hybridization experiments cannot be taken as an exact measurement of the base sequence homology between the two mature rRNA's. In fact, it seems likely that the uridine or methyl groups are not equally distributed among the nucleotide sequences of rRNA's [15]. Mature ribosomal RNA's are extracted from Sycamore cells in equimolar amounts. Moreover, they migrate in polyacrylamide gels as homogeneous species, and their nucleotide compositions are very different. It thus appears impossible that the considerable cross-hybridization observed can be due to contamination of the 17 S component by fragments of the 26 S component, and the results obtained strongly suggest that the 26 S species consists of two parts exhibiting considerable similarities with the 17 S rRNA. From this point of view, the ribosomal RNA's of Sycamore cells are very different from their counterparts extracted from animal cells [19, 20], where no sequence homology has been found. On the other hand, they are very much like rRNA's of lower organisms, such as yeast [17] or bacteria [15].

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