PROCESSING OF PRECURSOR RIBOSOMAL RNA IN SUSPENSIONS OF HIGHER PLANT CELLS

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1. Introduction

The maturation of macromolecular ribosomal RNA's (rRNA's) is now well documented in animal cells [1]. However, in higher plants, this process is far from being completely understood. Up to 1967 there was no experimental evidence for the existence of large precursor-RNA molecules in these organisms. Since that time, various types of precursors were isolated and characterized in higher plants [2–5], and it was tentatively inferred [3, 6] that the mechanism of processing was analogous to the one known to occur in HeLa cells [7].

In fact, most of the work with higher plants was performed with large fragments of tissues, or even with entire organs. It is thus extremely difficult, with this type of material, to reach definitive conclusions from pulse–chase experiments, due to transport phenomena within the tissue. Moreover, RNA labelling was effected, in most cases, with [^{32}P]orthophosphate, [^{3}H]- or [^{14}C]uridine, and therefore the electrophoretic or sedimentation patterns, for short labelling periods, were obscured by labelled heterogeneous nuclear RNA's. For these reasons, it has been impossible to obtain information on the early events of precursor processing. Thus, the sequence of events leading to mature rRNA's in higher plants has not been experimentally established.

It is possible to avoid these difficulties by using both [methyl-^{3}H]methionine as a more specific tracer, and suspensions of higher plant cells as a biological system. The aim of the present paper is to present and to discuss the results obtained with Sycamore cells in sterile culture.

2. Methods

Sycamore (*Acer pseudoplatanus* L.) cells are grown in the presence of thiamine and 2,4-dichlorophenoxyacetic acid, using the technique of Lamport [8] modified by Lescure [9]. Sampling of the cells is effected during the exponential phase of cellular growth. [^{32}P]orthophosphate and [methyl-^{3}H]-methionine are sterilized by filtration through Millipore membranes with 0.22 μ porosity. RNA labelling with [methyl-^{3}H]methionine is done in the presence of adenosine and guanosine [10]. The cells are disrupted at 4° with a MSE sonifier in a phenol–cresol mixture and in a 0.02 M Tris-HCl buffer (pH 7.2) containing 0.01 M NaCl, 0.2% macaloid, 0.01 M EDTA and 50 μg/ml polyvinyl sulfate. "Nuclear" and "cytoplasmic" RNA's are extracted using a technique similar to that previously described [11]. However, the two following modifications were introduced: deproteinization was effected three times with the mixture chloroform–isoamylic alcohol; pectic compounds were precipitated with 10% ethanol in the presence of 0.15 M sodium acetate, and discarded.

Sedimentation of RNA precursors on sucrose gradients is effected as previously described [11]. Their sedimentation constant is estimated by reference to those already known for RNA's extracted from *E. coli* (strain K12).

Polyacrylamide gel electrophoresis of the RNA's is performed by the method of Peacock et al. [12]. Molecular weights of the precursors are obtained from their electrophoretic mobility.

3. Results

After labelling with either [^{32}P]orthophosphate or [methyl-^{3}H]methionine, "nuclear" RNA's from sycamore cells are sedimented in a linear sucrose gradient. The results obtained are presented in fig. 1. In both cases four peaks of radioactivity are seen. However, the sedimentation profile is much clearer using [methyl-^{3}H]methionine as a tracer. On assuming the values of 23 S and 16 S for the rRNA's of *E. coli*, the estimation of the sedimentation constants of the cytoplasmic rRNA's extracted from sycamore cells are 26 S and 17 S, respectively. Polyacrylamide gel electrophoresis gives the values of 1.2×10^6 daltons and 0.8×10^6 daltons for the same rRNA's. Both values are close to those previously given by Rogers et al. [13] for rRNA's extracted from roots. The modal sedimentation constants corresponding to the four radioactivity peaks of fig. 1 are, respectively, 42 S, 36 S, 27 S, and 20 S. Polyacrylamide gel electrophoresis of these RNA's gives the values of 2.9×10^6, 2.3×10^6, 1.4×10^6, and 0.9×10^6 daltons, respectively. Moreover, their strong labelling with [methyl-^{3}H]methionine clearly indicates they are ribosomal precursors. Whereas the 27 S and 20 S radioactivity peaks of fig. 1 correspond to mixtures of precursors and mature rRNA's, the 42 S and 36 S peaks are mostly composed of precursor molecules (fig. 2). In addition, the 42 S and 36 S precursors migrate as single symmetrical peaks on acrylamide gel electrophoresis.

If, after a short pulse of [methyl-^{3}H]methionine, a large excess of cold methionine is introduced into the incubation medium, a loss of radioactivity is clearly detected in the nuclear fractions within 15 min or less (fig. 3A), while the label is incorporated into the cytoplasmic rRNA's. This situation is thus the most favorable so far described with plant material since, for comparison, in artichoke tuber tissue and in pea embryo [13, 5], the label incorporation continues for 45 min and 70 min, respectively, during the chase incubation.

Since the 42 S (2.9×10^6 daltons) and 36 S (2.3×10^6 daltons) peaks of the sedimentation and the electrophoretic profiles appear to be homogeneous and since the pulse–chase experiments give significant results with the material used, there is no difficulty in the investigation of high molecular weight precursor processing in sycamore cells. The results obtained are presented in fig. 3B and 3C. The kinetics of label incorporation into the 2.9×10^6 and the 2.3×10^6 precursors are very different during a chase incubation. While the incorporation of label into the 2.9×10^6 component decreases within 15 min or less, the amount of radioactivity incorporated into the 2.3×10^6 pre-rRNA increases for

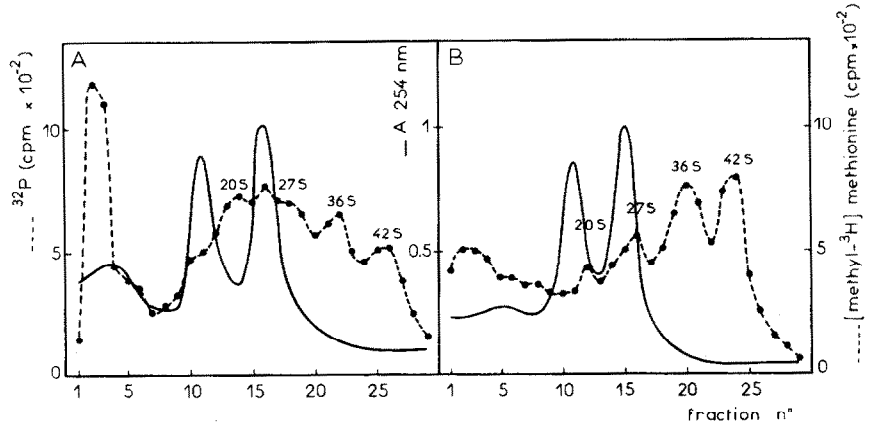

Fig. 1. Sedimentation analysis of "nuclear" RNA's from sycamore cells. Cells are labelled for 20 min with either 40 mCi/l [^{32}P]orthophosphate or with 25 mCi/l [methyl-^{3}H]methionine (600 mCi/mM). RNA's are sedimented in sucrose gradients (10 to 30%). Centrifugations are carried out in a Spinco SW 25-1 rotor for 13 hr 30 min at 21 000 rpm [11]. The determination of sedimentation constants is effected by using linear gradients of 5–20% sucrose [18], and by reference to the known values of rRNA's extracted from *E. coli* (strain K12, RNase$^-$ and Met$^-$).

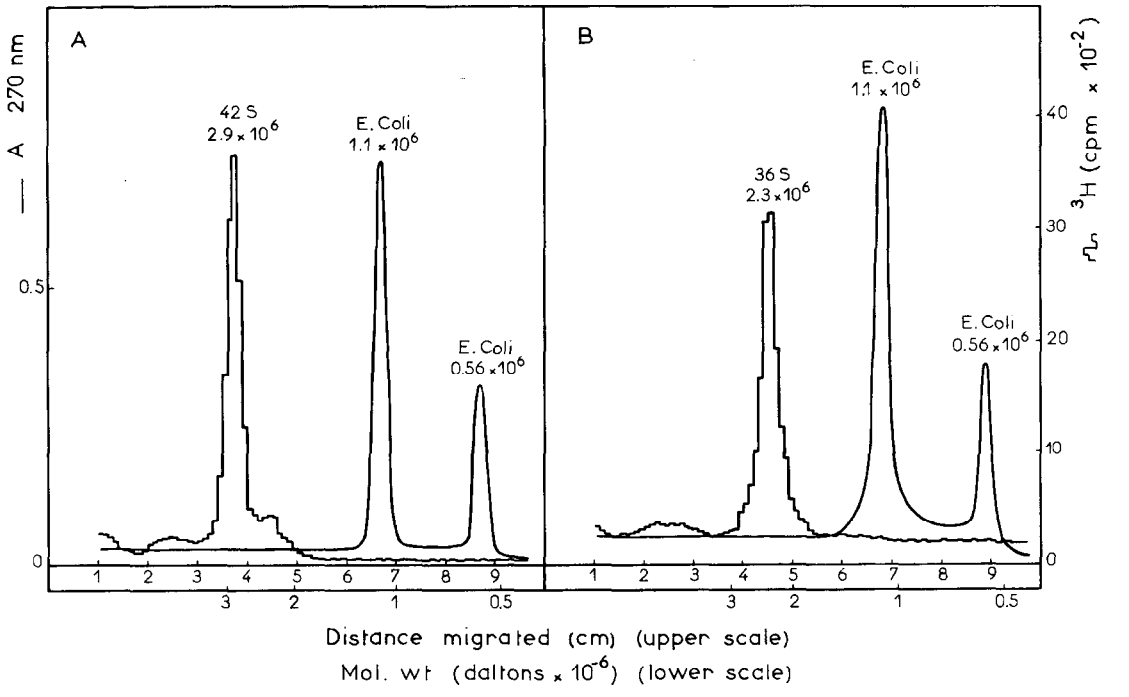

Fig. 2. Electrophoresis profiles of 42 S (A) and 36 S (B) precursors. Fractions corresponding to the 42 S and 36 S pre-RNA's are collected separately after a sedimentation analysis. They are mixed with rRNA's extracted from *E. coli* (strain K12, RNase$^-$ and Met$^-$), precipitated with ethanol and analyzed on 2% polyacrylamide gels according to Peacock and Dingman [12] (9.7 × 0.7 cm gels, 110 V, 2.5 mA/gel, 3.5 hr migration). Gels are scanned at 270 nm with a Gilford spectrophotometer equipped with the 2410 device, and cut into 1 mm sections using a Gilson fractionator. Polyacrylamide is digested overnight at 80° in 30% H_2O_2. 10 ml of Instagel (Packard Instruments) are added before counting in a Beckman LS 150 spectrometer.

25 min. The experimental results of fig. 3B can be interpreted only as the transformation of the 42 S (2.9×10^6 daltons) component into the 36 S (2.3×10^6 daltons) pre-rRNA. This early step in the processing of the high molecular weight precursor would imply the loss of either one excess piece (molecular weight 0.6×10^6 daltons) or of various pieces. The data of fig. 3D allow the calculation of the half-life of the 42 S precursor. The value obtained is 23 min. While the label is being lost from these nuclear precursors it appears in the cytoplasmic mature rRNA's (fig. 3A and 3C). The 0.8×10^6 species is more rapidly labelled than the 1.2×10^6 component (fig. 3C).

4. Discussion

Although rRNA precursors with molecular weights of about 2.9×10^6 and 2.3×10^6 daltons have been isolated from higher plants [3, 4], no experimental proof for the transformation of the first component into the second one has been presented so far. Thus the possibility that the precursors of weight 2.8 and 2.2×10^6 daltons arise by transcription of different genes has sometimes been entertained [4]. The present results rule out this possibility. For the first time the mean half-life of the high molecular weight ribosomal precursor has been calculated in higher plants. The value is close to that previous-

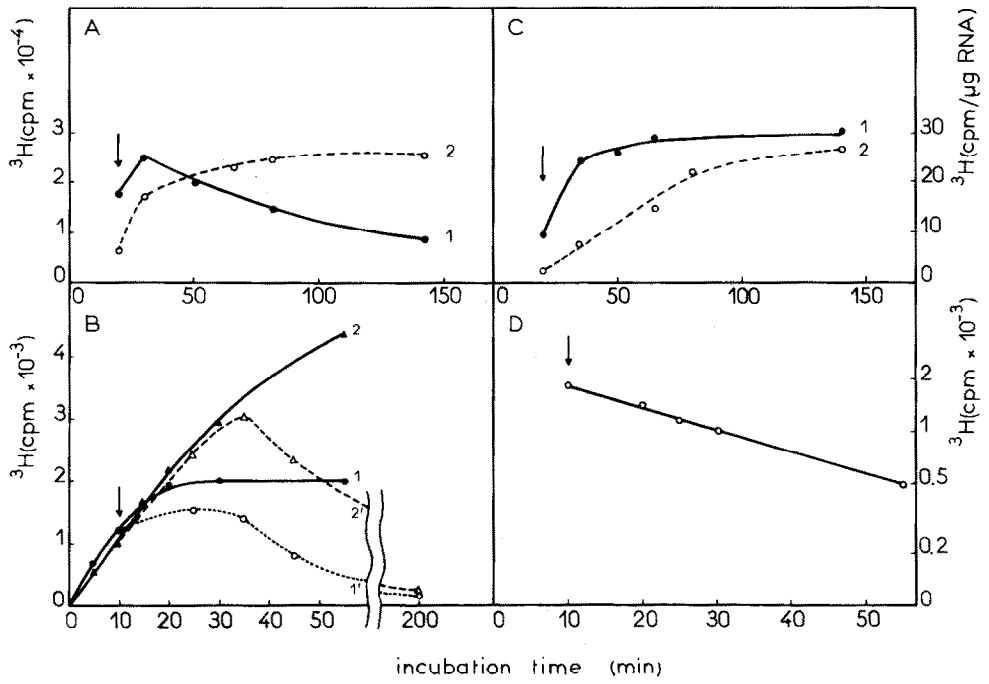

Fig. 3. Evolution of label incorporated in RNA's during pulse-chase experiments. Cells are incubated for either 20 min (A,C) or 10 min (B,D) with [methyl-^{3}H]methionine as for experiment described in fig. 1. One thousand-fold excess of cold methionine is added (arrows in the figure) and samples are collected after various intervals of time. Radioactivity incorporated in the RNA's is estimated from sedimentation patterns. A) Evolution of radioactivity incorporated in "nuclear" (curve 1) and "cytoplasmic" (curve 2) RNA's. B) Incorporation of radioactivity in 42 S (curve 1) and 36 S (curve 2) RNA's during a time-course labeling. Evolution of label incorporation in the same RNA's (curve 1' for 42 S precursor and curve 2' for 36 S precursor) during a chase incubation. C) Evolution of specific radioactivity of "cytoplasmic" mature rRNA's (curve 1 for 17 S rRNA and curve 2 for 26 S rRNA). D) Half-life estimation of 42 S precursor (semi-logarithmic coordinates).

ly given for the 45 S precursor of HeLa cell rRNA [14].

The question of heterogeneity of this precursor (2.9×10^6 daltons) has been discussed at length by Loening and his group [6]. Although some heterogeneity seems to occur in the present material on labelling RNA's with [^{32}P]orthophosphate or [^{14}C]uridine, the precursor appears to be nearly homogeneous when [methyl-^{3}H]methionine is used as a tracer. It is thus clear that, in the present material at least, part of the apparent heterogeneity is in fact due to the labelling of heterogeneous nuclear RNA, and is thus an artefact. On the other hand, the apparent homogeneity of the 42 S precursor is by no means a proof for the existence of only one molecular species with a molecular weight of 2.9×10^6 daltons. A similar point was recently discussed [15].

The present work does not give definitive information on the transformation of the 36 S precursor. However, one can notice that the sum of the molecular weights of the 27 S and 20 S components is equal to 2.3×10^6 daltons, which is exactly the molecular weight of the 36 S pre-RNA. Thus, it can be tentatively assumed that the 36 S precursor is cleaved without any loss of sequences, to give the 27 S and the 20 S components which, in turn, are converted into the 26 S and 17 S mature rRNA's, respectively. The steps of precursor processing would thus be (with different molecular weights), identical to those established by Weinberg et al. [7] for HeLa cells. This scheme is also consistent with the one postulated by Loening [6] for mung bean.

Kinetics of label incorporation is different for the two mature cytoplasmic rRNA's. If the above scheme

is valid, the results of fig. 3C would suggest the step $0.9 \times 10^6 \rightarrow 0.8 \times 10^6$ to be more rapid than the step $1.4 \times 10^6 \rightarrow 1.2 \times 10^6$. These results are similar to those described with HeLa cells [7, 14].

In conclusion, it cannot be overemphasized that the power of cell culture technique is far and away superior to other techniques for rRNA maturation studies in animal [7, 16, 17] and in higher plants as well.

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