IDENTIFICATION OF MITOCHONDRIAL rRNA FROM PLANT CELLS

Francis QUETIER and Fernand VEDEL

Laboratoire de Biologie Moléculaire Végétale associé au CNRS (L. A. 40), Faculté des sciences, 9140 5 Orsay, France

Received 18 March 1974

1. Introduction

Mitochondria of ascomycetes and animals have been shown to contain unique species of ribosomes and rRNA. The mitochondria from ascomycetes are characterized by 73 S ribosomes and by 23 S and 16 S rRNA whereas animals, ranging from man to locust, contain 55–60 S mitochondria (mt) ribosomes and 16 S and 12 S mt rRNA [1]. The mitochondrial ribosomes and rRNA are thus generally smaller in size than their cytoplasmic counterpart. *Tetrahymena pyriformis* is an exception, with mitochondrial and cytoplasmic ribosomes similar in size (80 S), although mt rRNA are 21 S and 14 S compared to 26 S and 17 S for cytoplasmic rRNA [2].

The difference in size between mt ribosomes and mt rRNA of lower and higher organisms also occurs at the mt DNA level. Ascomycetes and protozoa contain long mt DNA molecules (25 μm for yeast, 17 μm for *Tetrahymena pyriformis*) whereas animals exhibit shorter mt DNA molecules (5 μm whatever the species so far studied) [3].

The position of higher plants has been recently reported; they display long mtDNA molecules: 19.5 μm for red bean [4], 45 μm for pea, [5], 30 μm for potato tubers [6]. The plant mt rRNA have not been evidenced with certainty [7,8]. Indeed, this material brings on several difficulties, like the cellular heterogeneity, the non uniform labelling by radioactive precursors and the bacterial contamination encountered with naturally growing plants. Moreover, the presence in higher plants of several kinds of organelles adds to the above difficulties; particularly chloroplasts have been shown to contain specific 23 S and 16 S rRNA molecules [9,10].

This paper reports for the first time, as far as we know, the identification and characterization of mt rRNA extracted from plant cells, asceptically cultured in liquid nutrient medium.

2. Materials and methods

Parthenocissus tricuspidata L. cells are grown asceptically, in liquid nutrient medium using the 'roller bottle' technique [11]. The detailed culture conditions will appear elsewhere. The cells are harvested during the exponential phase of cellular growth, washed with distilled water and cooled at 4°C. The cellular disruption is performed with a French Press, at 2000 psi, at 4°C in a 5×10^{-2} M Tris–HCl buffer (pH 7.2) containing 0.3 M sucrose, 3×10^{-3} M EDTA, 10 g/l polyethylene glycol 4000, 1g/l bovine serum albumin fraction V and 5×10^{-3} M β–mercaptoethanol [12].

The homogenate is centrifuged at 1100 *g* for 7 min; the supernatant is collected and centrifuged at 15 000 *g* for 15 min. The pellet is resuspended in the homogenization buffer and the suspension centrifuged again successively at low and high speed as above. The crude mitochondrial pellet obtained is then differently purified before either DNA or RNA extraction:

– mt DNA extraction: the crude mitochondrial pellet has to be submitted to a DNase treatment at 4°C and then to a centrifugation on a 30–60% linear sucrose gradient as described previously [6]. The mitochondrial band observed after centrifugation in the middle of the sucrose gradient is recovered and centrifuged. The mtDNA is extracted from the purified mitochondrial pellet as already described [6].

– mtRNA extraction: the crude mitochondrial pellet is washed with the homogenization buffer several times, as stated in the results. The last pellet is fractionated by centrifugation on a 30–60% linear sucrose gradient. The organelle band is removed and the mitochondria are pelleted and washed several times. The mtRNA is prepared in the cold by a 5 min homogenization of the organelle pellet in one volume of 0.1 M acetate buffer (pH 5.0) containing 0.1. M NaCl, 0.02 M magnesium acetate and 2% (w/v) tri-isopropylnaphthalenesulfonate. The lysed mitochondria are gently extracted with two volumes of phenol–chloroform (1:1, v/v) for 15 min. The mt RNA is precipited from the aqueous phase by two volumes of cold ethanol and stored at −20°C.

The RNA pellet is dissolved in 10 mM Tris–HCl buffer (pH 7.2) containing 0.1 M NaCl and 3×10^{-2} M $MgCl_2$, DNase–treated (10 μg/ml) for 1 hr at 4°C and precipitated by ethanol. The high molecular weight rRNA is electrophoretically separated on polyacrylamide gels [13]; after electrophoresis the gels are scanned at 260 nm with a Polyfrac (Joyce–Loebl, England).

3. Results and discussion

The RNA extracted from a total cellular ribosomal fraction (see legend to fig. 1) presents only the 25 S and 18 S cytoplasmic components after electrophoresis on polyacrylamide gels (fig. 1a). The electrophoretic profile of the RNA extracted from a crude chloroplast pellet (prepared from cells of *Partheno-*

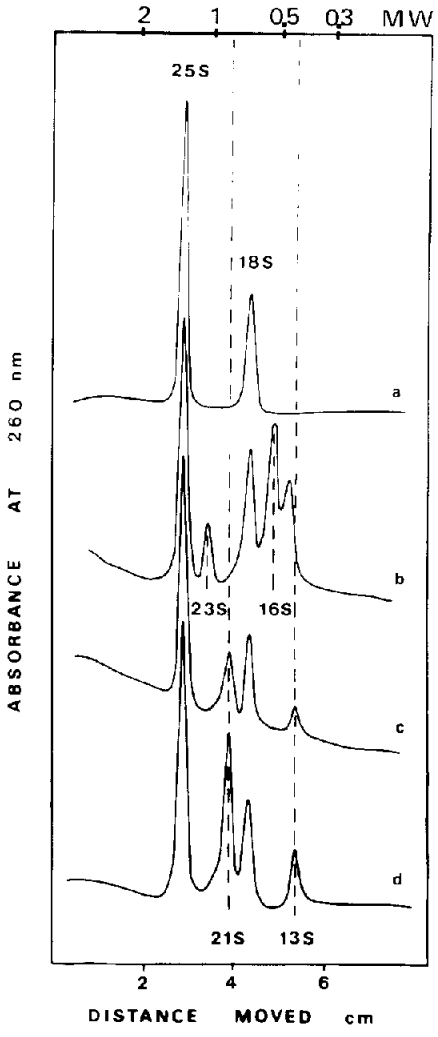

Fig. 1. Electrophoretic separation of rRNA from different cellular fractions of *Parthenocissus tricuspidata* L. cells. a–total rRNA from a ribosomal pellet isolated from cells grown in darkness. The cells are broken at 4°C with a French Press in 0.02 M Tris–HCl buffer pH 7.5, containing 0.25 M sucrose, 0.01 M $MgCl_2$, 0.015 M KCl 5 mM β-mercaptoethanol and 2% Triton X 100. The homogenate is stirred 5 mn and centrifuged at 30 000 *g* for 20 min. The supernatant is centrifuged at 120 000 *g* for 3 hr through a layer of 1 M sucrose; the ribosomal pellet is extracted for RNA. b– Chloroplastic rRNA from light-grown cells. The cellular disruption is performed at 4°C with a French Press in Honda medium. The homogenate is filtered through a nylon screen (40 μ) and the filtrate fractionated by centrifugation through a discontinuous sucrose gradient, for 1 hr at 20 000 *g*, 4°C, using SW 25-II-rotor. Five layers containing successively 80, 66, 54, 40 and 25% sucrose in Tris–HCl buffer, pH 7.8, with 2 mM $MgCl_2$ and 5 mM β-mercaptoethanol, constitute the gradient. The chloroplastic fraction is removed from the 66–54% sucrose interface and pelleted at 1500 *g* for 10 min before the RNA extraction. c–mt rRNA from a crude mitochondrial pellet. d–mt rRNA from a purified mitochondrial pellet. In c and d, the cells are grown in darkness and mitochondria prepared as described in Materials and methods. Electrophoresis is carried out for 4.5 hr with 2.6% polyacrylamide gels, at 5 m A/gel, at 20°C using a buffer containing magnesium (40 mM Tris, 20 mM sodium acetate, 0.3m M EDTA, 6mM magnesium acetate, pH 7.8 with acetic acid [12], 0.1% SDS).

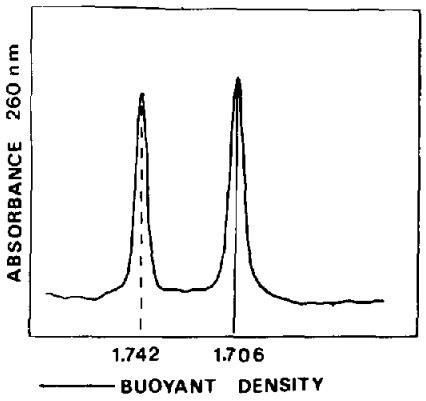

Fig. 2. DNA extracted from purified mitochondria of *Parthenocissus tricuspidata* L. cells: microdensitometer tracing of UV photograph after analytical density gradient centrifugation in CsCl. ϕE DNA is used as a density marker at 1.742 g/cm^3.

cissus grown under light) is presented on fig. 1b. The 23 S and 16 S RNA originate from chloroplastic ribosomes as previously stated [9,10]. The light band represents a degradation of the 23 S molecule [15,16] and the 25 S and 18 S RNA are cytoplasmic contaminants [9,10,12].

The procedure used to extract plant mitochondria has been shown to give highly purified organelles from potato tubers, as seen by electron microscopy and DNA analysis [6]. In fig. 2, the buoyant density profile in neutral CsCl of the DNA extracted from the purified mitochondria of *Parthenocissus* is displayed. The profile shows only one sharp and symmetrical band at 1.706 g/ml, a value that seems unique for all the mt DNA from higher plants [6,12,14].

The RNA isolated from a crude mitochondrial pellet washed three times with the homogenization medium shows two minor bands in addition to the 25 S and 18 S rRNA (fig. 1c). The relative proportion of these minor bands is markedly enhanced (fig. 1d) when the RNA extraction is performed on a purified mitochondrial pellet, obtained after three washings, one centrifugation on sucrose gradient and two subsequent washings. The electrophoretic patterns on fig. 1b and fig. 1d indicate that the two mt rRNA migrate more rapidly than 23 S and 16 S chloroplastic rRNA respectively.

Electrophoresis of the mt rRNA fraction have been achieved at 20°C and 4°C in either Mg (see legend to fig. 1) or EDTA-containing buffers, with different concentrations of acrylamide and at 60°C under denaturing conditions [17]; in every case, the mt rRNA migrate as stable species. The molecular weights are respectively 0.84×10^6 and 0.42×10^6, corresponding to about 21 S and 13 S, whatever the electrophoretic conditions.

A ribosomal fraction has been isolated from a purified mitochondrial pellet by treatment with 2% (w/v) Triton X 100 (in 5×10^{-2} M Tris–HCl buffer, pH 7.2, containing 5×10^{-2} M $MgCl_2$, 5×10^{-2} M KCl and 5×10^{-3} M β-mercaptoethanol). The rRNA extracted gives identical electrophoretic profiles as those on fig. 1d.

In spite of up to six washings (including a sucrose gradient), the mt rRNA is still contaminated by cytoplasmic rRNA; this difficulty has been described also for animals [1,18]. The mt rRNA of higher plants appears intermediary in size between that of ascomycetes and animals. Higher plants show a unique situation, since the cytoplasm,the chloroplasts and the mitochondria contain specific rRNA.

The detailed structural and metabolic properties of the higher plant mt rRNA will appear elsewhere.

Acknowledgements

The liquid culture technique for plant cells was set up with the help of J. M. Grienenberger; we are greatly indebted to Drs. C. Peaud-Lenoel, A. M. Lescure and J. P. Jouanneau for their help in this field.

This work has been partly supported by the CNRS (Laboratoire associé N° 40), the INSERM (grant n° 73 1030.2) and the 'Fondation pour la Recherche Médicale'.

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