PROTEIN COMPLEMENTS OF 80 S RIBOSOMAL PRECURSOR PARTICLES FROM *TETRAHYMENA PYRIFORMIS*

Claudina RODRIGUES-POUSADA, Paula CONTENÇAS and D. H. HAYES[+]

Laboratório de Genética Molecular, Instituto Gulbenkian de Ciência, Apartado 14, 2781 Oeiras Codex, Portugal and *[+]Laboratoire de Chimie Cellulaire, Institut de Biologie physico chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France*

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1. Introduction

In all organisms the 2 large rRNAS are transcribed from a common transcriptional unit. In the protozoa *Tetrahymena pyriformis*, the genes for these rRNAS are organized in an extrachromosomal palindromic rDNA [1,2], and their primary transcript is a common precursor molecule with a sedimentation coefficient of 36 S [3,4]. This precursor rRNA, as in other eukaryotic cells becomes associated with proteins forming ribonucleoprotein complexes. In kinetic studies [5] using precursors to both RNA and proteins, we have established a scheme for ribosome biogenesis which is close to that observed in higher eukaryotic cells [6]. Some small differences exist however, for instance, ribosomal precursor RNAs as well as the mature rRNAS in the ribosomal subunits are smaller than the corresponding species in higher eukaryotic cells.

Detailed characterization of the complete series of nuclear ribosome precursors is an essential prerequisite for the study of the regulation of ribosome biogenesis, of the enzymology of the maturation process, and of the mechanism by which nascent ribosomal subunits are transported to the cytoplasm. Only fragmentary data of this kind has so far been obtained in work with eukaryotic cells [7,8].

We have now established the protein composition of the 80 S ribosome precursor particles of *Tetrahymena pyriformis* using 2-dimensional electrophoretic analysis. We find that 80 S precursor particles contain 26 proteins from the large and 13 from the small ribosomal subunits. In addition we detect 11 proteins in these precursor particles which are not present in the mature cytoplasmic ribosomes.

2. Materials and methods

2.1. *Cultures and labelling*

Tetrahymena pyriformis CGL was grown aerobically at 28°C as in [9] and collected in the log phase at 10^5 cells/ml. When labelling was used, cells were grown in a medium as in [3] in the presence of a mixture of [^{3}H]amino acids (100–130 μCi/ml; TRK 440), purchased from Radiochemical Centre, Amersham.

2.2. *Cell fractionation*

Unless otherwise stated, all operations were done at 0–4°C and all solutions and glassware were sterilised before use. Cells were harvested by centrifugation at 2000 × *g* for 15 min and washed in 10 mM Tris–HCl (pH 7.5) buffer containing 0.25 M sucrose, 5 mM $MgCl_2$, 10 mM KCl and 20 μg potassium poly-(vinyl) sulphate/ml. After washing, cells were resuspended in the same buffer and lysed at 0°C by addition of Nonidet P_{40} (final conc. 0.23%). After 10 min in ice, the lysate was centrifuged at 3000 × *g* for 5 min. The 3000 × *g* pellet was then resuspended in the same buffer and centrifuged as in [5] yielding a pellet of purified nuclei.

2.3. *Preparation of nuclear ribosomal precursor particles*

Purified nuclei were resuspended in the same medium containing 0.1 mM $MgCl_2$ and sonicated using an MSE ultrasonic generator (24 Kc/s, 2 microns of magnitude, 4 times 15 s), Mg^{2+} was made up to 2 mM and heparine added to 0.5%. The resulting viscous nuclear lysate was treated for 5 min with RNase-free DNase (100 μg/ml) followed by mixing in a Vortex for 10 min, and then centrifuged at 15 000 × *g* for 20 min. The supernatant fraction contained the ribonucleoprotein particles.

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2.4. *Analysis of ribonucleoprotein particles*

Analysis of ribonucleoprotein particles was performed by centrifugation through linear 10–30% sucrose gradients prepared in 10 mM Tris–HCl (pH 7.4) containing 2 mM $MgCl_2$ and 20 mM KCl. Gradients loaded with 80–100 A_{260} of nuclear extract were centrifuged in the SW 27-1 rotor of a Beckman L5-50B at 17 500 rev./min for 16.5 h. Centrifuged gradients were fractionated and their A_{260} profiles recorded.

Fractions containing the 80 S ribonucleoprotein precursor particles were pooled, the Mg^{2+} made up to 8 mM and the particles were precipitated (30 min, −20°C) by addition of 2 vol. ethanol. After centrifugation at 10 000 × *g* for 15 min the pellet was resuspended in the following buffer: 20 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 250 mM KCl and 6 mM β-mercaptoethanol. In some experiments the 80 S precursor particles were concentrated by centrifugation as described for the mature ribosomal subunits. No differences were detected in the electrophoretic patterns of precursor particle proteins prepared by the 2 methods.

2.5. *Isolation of ribosomal subunits*

Ribosomal subunits were isolated as in [9]. After sucrose gradient centrifugation, 40 S and 60 S ribosomal subunits were pooled from the appropriate sucrose gradient fractions, collected by centrifugation, and dissolved in 20 mM Tris–HCl, 5 mM $MgCl_2$, 100 mM KCl and 6 mM β-mercaptoethanol.

2.6. *Ribosomal protein analysis*

Ribosomal proteins were extracted from ribosomal subunits, and from nuclear ribosomal precursor particles by the acetic acid method [10] and concentrated

a b

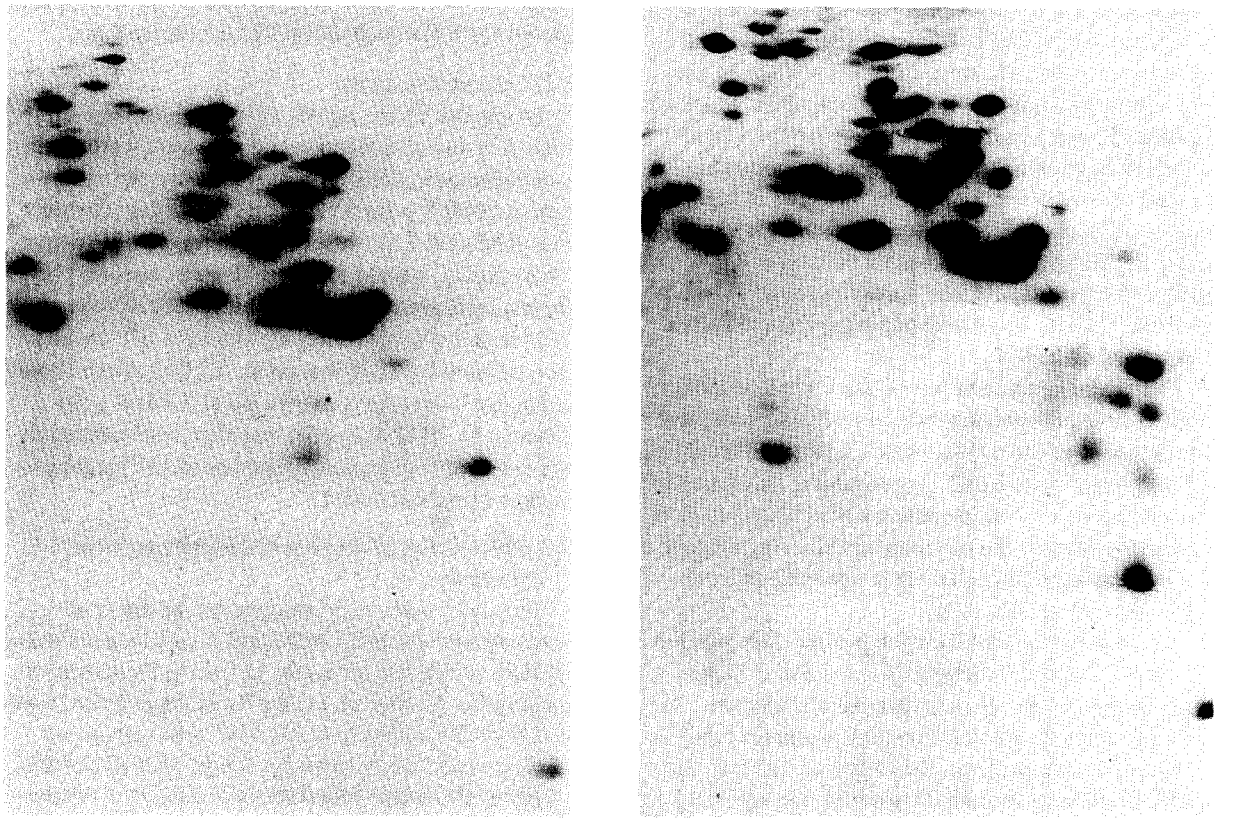

Fig.1. 2-Dimensional gel electrophoretic migration pattern of 80 S preribosomal particle proteins (a) and its schematic representation (b). Standard conditions were used for electrophoresis. The anode is to the left in the first dimension and at the top in the second. Protein samples contained ~800 μg.

by acetone precipitation [11]; they were analysed by 2-dimensional polyacrylamide gel electrophoresis as in [9]. When ^{3}H-labelled proteins comigrated with cold proteins, the slabs were destained and individual spots were cut out. These gel pieces were then completely digested in sealed glass scintillation vials, using 0.3 ml of 30% (v/v) hydrogen peroxide, at 60°C overnight and radioactivities were counted.

3. Results and discussion

80 S precursor particles were isolated from *Tetrahymena pyriformis* as in [5], with some minor modifications (section 2). The nuclear extract was prepared from large cultures to obtain sufficient amounts of these particles to permit protein analysis by two-dimensional polyacrylamide gel electrophoresis. Sucrose gradient centrifugation profiles of nuclear extracts were very similar to those obtained in previous work (fig.1, in [5]). 80 S precursor particles were present in a large mono-disperse peak; 44 S and 60 S precursor particles were also present but in much smaller amounts probably due to the high rate of ribosome formation in *Tetrahymena* [12], and rapid conversion of these species to nascent 40 S and 60 S ribosomal subunits which are transported to the cytoplasm.

Proteins extracted from 80 S particles were separated by 2-dimensional gel electrophoresis analysis. Since protein samples were loaded on top of first dimension gels, the proteins which are negatively charged at pH 8.7 (the pH of the separation gel), are lost. Photographs of basic protein patterns are shown in fig.1(a) and the corresponding schematic drawings in fig.1(b). Electrophoretic analysis carried out to search for acidic proteins in 80 S precursor particles gave uniformly negative results (not shown) even when gels were heavily overloaded (2 mg protein). Comparison of the electrophoretic pattern of proteins isolated from 80 S precursor particles and from 40 S + 60 S subunits shows that some proteins are common to the 2 patterns but that others are present in only 1 of the 2 patterns. In order to correlate the species shown in fig.1(a) with the basic proteins of the mature 40 S and 60 S ribosomal subunits it was necessary to

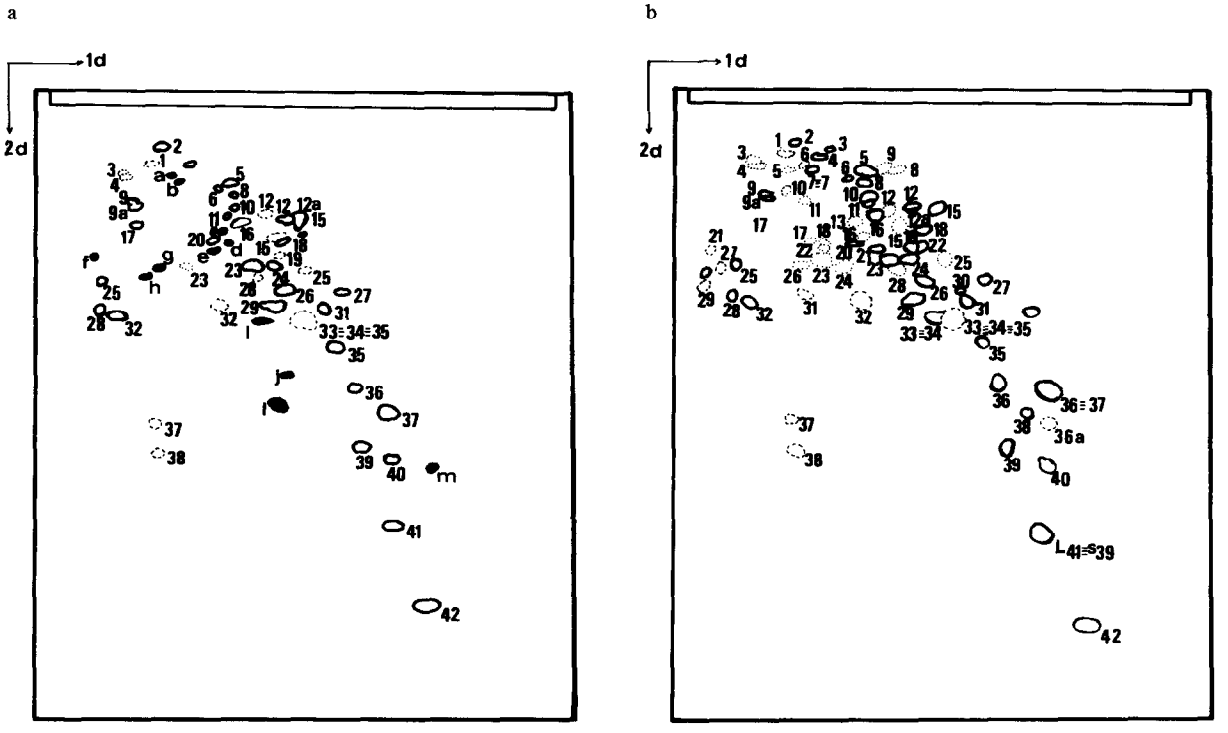

Fig.2. 2-Dimensional gel electrophoretic migration pattern of mixed 40 S + 60 S mature proteins (a) and its schematic representation (b). The conditions and the amount of material used are the same as in fig.1.

Table 1
Ribosomal affiliation of proteins of the 80 S precursor particle

Proteins		Radio-act.	Proteins		Radio-act.	Specific proteins
60S subunit	Precursor particles	(cpm)	40 S subunit	Precursor particles	(cpm)	in 80 S rRNP (cpm)
L2	L2	487	S1	S1	159	a – 37
L3			S3	S3	161	b – 27
L4			S4	S4	245	c – 35
L5	L5	842	S5			d – 44
L6	L6	183	S6			e – 33
L7			S7			f – 25
L8	L8	159	S8			g – 45
L9	L9		S9			h – 37
L9a	L9a	604	S9a			i – 30
L10	L10	793	S10			j – 35
L11	L11	298	S11			l – 30
L12	L12		S12	S12	338	
L12a	L12a	333	S13			
L15	L15	1261	S15	S15	372	
L16	L16	640	S16			
L17	L17	1610	S17			
L18	L18	371	S18			
L20	L20	368	S19	S19	245	
L21			S21			
L22			S22			
L23	L23	519	S23	S23	234	
L24	L24	979	S24			
L25	L25	276	S25	S25	546	
L26	L26	407	S26			
L27			S27			
L28	L28	1380	S28	S28	354	
L29	L29	1085	S29			
L30			S30			
L31	L31	1178	S31			
L32	L32	255	S32	S32	442	
L33			S33	S33	520	
L34			S34	S34	1846	
L35	L35	229	S35	S35	332	
L36			S36			
L37			S36a			
L38	L38	400	S37			
L39	L39	1990	S38			
L40	L40	1860	S39			
L41						
L42	L42	439				

2-Dimensional electrophoretic analyses were carried out on samples containing unlabelled 80 S precursor proteins (800 μg) and ^{3}H-labelled 40 S ribosomal subunit proteins (10 μg containing 17 100 cpm) or ^{3}H-labelled 60 S ribosomal subunit (20 μg containing 30 700 cpm). All stained spots in the resulting gels were cut out and their radioactivity measured. Results are tabulated. The gel was cut around the spots to determine background (mean values ~ 30 cpm)

establish the positions of the latter proteins in the 2-dimensional gels in which a mixture of 40 S and 60 S proteins had been separated. This was done by comparing 2-dimensional gel separation patterns of a series of protein samples (40 S, 60 S, and 40 S + 60 S proteins mixed in various ratios). The results of these experiments summarized in fig.2(a) (photograph) and 2(b) (corresponding schematic drawing) agree with those in [13].

We have verified that the electrophoretic pattern of proteins of the 80 S precursor particle is not altered by isolation of this species in the presence of the protease inhibitor PMSF suggesting that protease activity is not responsible for the presence of proteins (a–l, fig.1(b)) which do not migrate with ribosomal proteins.

To confirm the identification of proteins in fig.1(b) we have subjected mixtures containing unlabelled proteins of 80 S precursor particles and ^{3}H-labelled proteins of mature 40 S and 60 S ribosomal subunits to electrophoretic analysis. Stained spots in the resulting gel slabs were cut out and their radioactivity measured. The results (table 1) show that the majority of 80 S precursor proteins comigrate with 40 S or 60 S subunit proteins but that spots designated a–l in fig.1(b) do not do so since they do not contain radioactivity. The large differences observed in the degree of labelling could be explained by the different size of individual ribosomal proteins as well as their different amino-acid content and also by different labelling kinetics of the proteins. This has been observed by other authors [14] in mammalian cells. Our results were reproducible in several experiments.

As reported in other eukaryotic cells [7,8] it seems that in 80 S precursor particles isolated from *T. pyriformis* there are 2 sets of proteins; one which remains in the nucleus (11 species in the case of this protozoa) and another set which is transferred to the cytoplasm with the nascent cytoplasmic ribosomal subunits (41 species in this eukaryotic cell). Finally, we would like to draw attention to the fact that we have not detected any acidic proteins in the 80 S precursor particles. This suggests that these proteins are added at a later stage of ribosome maturation, for instance in 44 S or 66 S precursor ribosomal particles.

Acknowledgements

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