THE MITOCHONDRIAL RIBOSOME AND RIBOSOMAL RNA OF THE CHICK

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1. Introduction

Reports have appeared on large molecular weight RNAs associated with the mitochondria of higher organisms. These include HeLa cells [1], BHK-21 cells [2], mouse L-cells [3, 4], rat liver [4, 5] and *Xenopus* ovary [6]. This RNA consists of two predominant species found to be 17 S and 13 S by sedimentation [2, 3]. Values determined by electrophoresis for the larger species vary between 21 S–17 S and for the smaller species 13 S–12 S [1, 4–6]. Mitochondria of *Xenopus* ovary contain a 60 S RNP particle which was capable of using polyuridylic acid as an m-RNA [6] and from which was isolated 21 S and 13 S RNA. In BHK cells, the 17 S and 13 S RNAs were shown to be associated with separate RNP particles [7] (presumed to be ribosomal subunits) but in the other reports no function was assigned to the RNAs.

We report here the existence, in chick liver, of an RNP particle which sediments in sucrose gradients at 55 S. This particle is shown to be involved in mitochondrial protein synthesis and it can be regarded as the mitochondrial ribosome. Further, these ribosomes contain '18 S' and '12 S' RNA which are shown to be mitochondrial-gene products.

2. Materials and methods

New born chicks were given an intraperitoneal injection of 0.5 mCi of ^{3}H-uridine (Radiochemical Centre, Amersham, Bucks. 5 Ci/mmole) followed by a further injection of 0.5 mCi after 19 hr. Each chick was killed after a further 24 hr and liver mitochondria prepared by differential centrifugation in 0.3 M sucrose, 10 mM tris-HCl pH 7.6 containing either 1 mM EDTA (STE) or 10 mM $MgCl_2$ (STM) as specified. In some experiments a chick received an injection of ethidium bromide (Boots Pure Drug Co. Ltd., Nottingham) 4 hr before the first isotope injection (10 μg EB per g of body weight).

RNA was extracted at room temperature by the procedure of Brown and Littna [8] except that bentonite was used as a ribonuclease inhibitor. Polyacrylamide gel electrophoresis was carried out as described by Peacock and Dingman [9] at 4° with 28 S and 18 S cytoplasmic RNA as markers.

Abbreviations:
EB : ethidium bromide
Ma-RNA : total mitochondria-associated RNA
Ms-RNA : mitochondria-specific RNA
RNP : ribonucleoprotein

3. Results and discussion

Ma-RNA, prepared as described, is labelled efficiently with ^{3}H-uridine and has a specific activity between 2000–2500 cpm/μg RNA. Fig. 1 shows the acrylamide gel electrophoresis profile of such RNA: the electrophoresis was carried out for 3 hr, 4 S RNA being run off the end of the gel. For convenience the three species will be called 28 S, 18 S and 12 S RNAs, of which 28 S and 18 S have the same mobility as cytoplasmic ribosomal RNA markers.

To find whether any of these RNA species were associated with a mitochondrial ribosome we employed the technique described in a previous report [10] in which a 55 S mitochondrial ribosome was functionally characterized by pulse labelling of mitochondria in vitro with ^{14}C-amino acids. The ^{3}H-uridine labelled mitochondria prepared as described above, in STM medium, were allowed to synthesize protein

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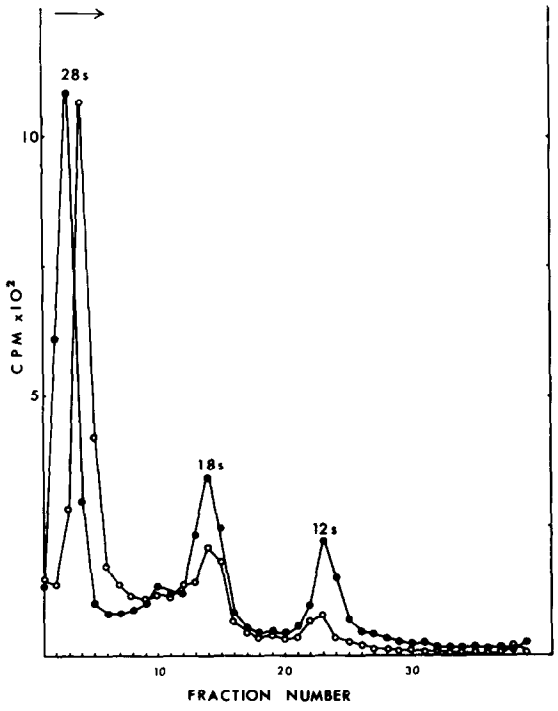

Fig. 1. Polyacrylamide gel electrophoresis of mitochondria-associated RNA. Mitochondria were prepared from a chick, injected with ^{3}H-uridine (with or without ethidium bromide), using the STE medium. The Ma-RNA from the mitochondria was dissolved in 25 mM tris-HCl pH 7.9 and was electrophoresed on 2.5% acrylamide, 0.5% agarose gels in 25 mM tris-citrate pH 7.9 at 4° for 3 hr. The gels were sliced in 2 mm fractions and counted in a Packard Tricarb scintillation counter. ●—● uridine only, ○—○ ethidium bromide + uridine. Direction of electrophoresis is indicated by the arrow.

in vitro for 5 min in the presence of ^{14}C-leucine, lysed and fractionated on a sucrose gradient. As shown in fig. 2 a peak of nascent ^{14}C-peptide cosediments with the 50 S optical absorbancy marker but the ^{3}H-RNA label of 80 S particles obscures the 50 S region. These 80 S particles are presumed to be contaminating cytoplasmic ribosomes since no significant ^{14}C-peptide labelling can be seen in association with them. The clarification of the 50 S region was facilitated by resedimentation of this region, as shown in fig. 3. It is now clear that coincident peaks of ^{3}H-uridine and

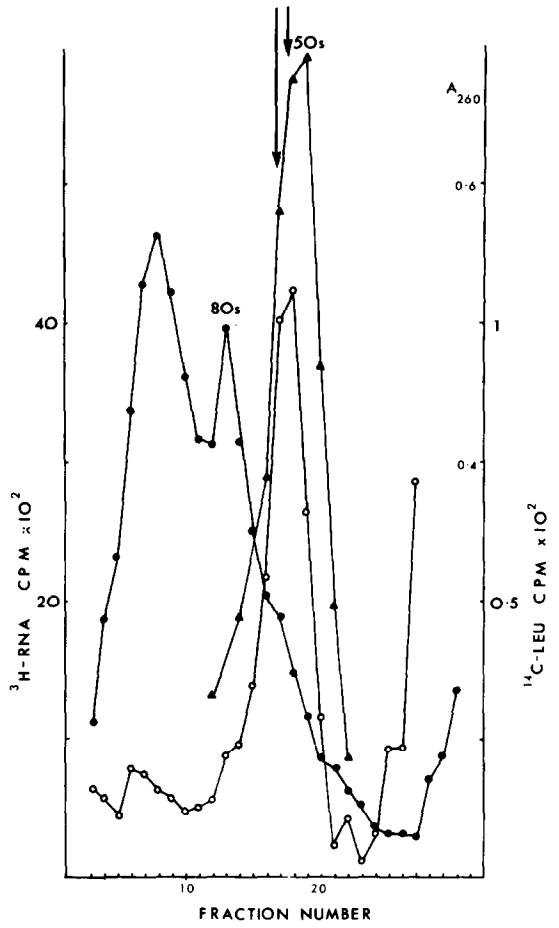

Fig. 2. Sucrose gradient profile of mitochondria lysates. ^{3}H-Uridine labelled mitochondria, prepared, using the STM medium, were incubated in vitro at 30° for 5 min in the presence of 0.7 μCi/ml of ^{14}C-leucine (311 mCi/mmole Amersham Radiochemical Centre) as described previously [10]. The mitochondria were recovered by centrifugation and lysed with 2 ml of 10 mM tris-HCl pH 7.6, 10 mM $MgCl_2$ and 10 mM KCl containing 1% Brij-58 (Honeywell-Atlas Ltd.), 0.1% sodium deoxycholate (Shell Chemicals) and 0.82 mg of *E. coli* 50 S ribosomal subunit as optical absorbancy marker. The lysate was layered onto a 15–30% sucrose gradient containing the buffer mixture above and spun of $3\frac{1}{4}$ hr at 2° in the Spinco 30 rotor at 27,000 rpm. 1 ml fractions were taken, TCA-precipitated and counted on a Packard Tricarb scintillation counter. ▲—▲ A_{260}; ●—● ^{3}H-uridine counts; ○—○ ^{14}C-leucine counts.

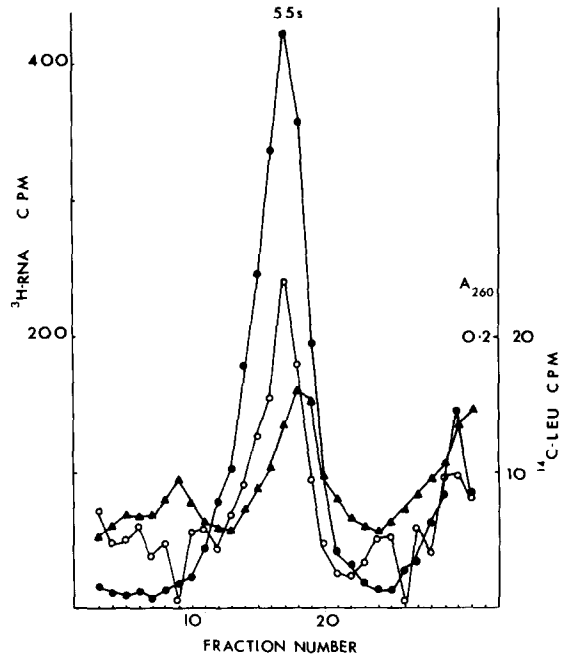

Fig. 3. Sucrose gradient profile of pooled material from fig. 2. The two peak fractions from a gradient similar to fig. 2 (arrows indicate these fractions) were pooled and diluted to 15% sucrose with 10 mM tris-HCl pH 7.6, 10 mM $MgCl_2$, 10 mM KCl. This was then layered onto a further 15–30% sucrose gradient and treated as in fig. 2. ▲—▲ A_{260}; ●—● ^{3}H-uridine counts; ○—○ ^{14}C-leucine counts.

^{14}C-leucine occur in a peak which sediments slightly faster than the optical density marker. The nature of the ^{3}H-RNA in this 55 S mitochondrial ribosome is shown in fig. 4. This shows the electrophoresis of RNA extracted from the 55 S region of the gradient. In order to obtain undegraded RNA from the sucrose gradient it was found necessary, as described in the legend to fig. 4, to add soluble RNA to the medium before lysing the mitochondria. This RNA probably provides a substrate for nucleases present in the mitochondrial fraction since if this RNA is omitted the mitochondrial RNA is degraded. The extracted RNA travels as 18 S and 12 S RNA and there is no 28 S in this profile. Thus, the RNP particles, active in protein synthesis, sediment at 55 S in sucrose and contain only 18 S and 12 S RNA (excluding the soluble RNA).

The problem of whether these two types of RNA are products of mitochondrial or nuclear genes was answered by use of ethidium bromide. This drug preferentially inhibits synthesis of RNA on the circular mitochondrial DNA: Zylber et al. [11] have shown, in HeLa cell mitochondria, that the synthesis of 21 S and 12 S species of Ma-RNA was inhibited in vivo by EB. The effect of EB on Ma-RNA is shown in fig. 1B where it can be seen that the labelling of 28 S RNA is unaffected but that of 18 S RNA is inhibited by about 40% and 12 S by about 80%. It therefore appears that the 12 S and, at least, part of the 18 S RNA are true mitochondria-gene products (i.e. the mitochondrial ribosomal 18 S and 12 S RNA): the 40% inhibition of the 18 S RNA would indicate that it is a mixture of two or more species, of which one is mitochondrial. The most likely explanation is that extramitochondrial 28 S and 18 S RNA are contaminants of cytoplasmic ribosomes. The unusually high ratio of 28 S:18 S may be due to the EDTA washing of the mitochondria. Sabatini et al. [12] have shown that EDTA washing of microsomes results in the preferen-

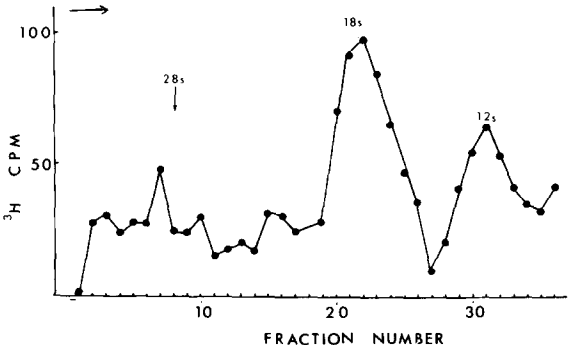

Fig. 4. Polyacrylamide gel electrophoresis of RNA from the 55 S peak of sucrose gradients. Mitochondria were prepared, lysed and centrifuged on sucrose gradients as in figs. 2 and 3 except that the incubation in vitro with ^{14}C-leucine was omitted prior to lysis of mitochondria and the lysis medium contained 1 mg of yeast soluble RNA (Hopkin and Williams Ltd.). Fractions 13–16 from the second sucrose gradient were pooled and the RNA material precipitated, overnight at −20°, with 2 volumes of ethanol. RNP particles were pelleted by centrifugation on an MSE 18 centrifuge at 4° for 20 min at 10,000 rpm. The 'pellet' was resuspended at room temperature in 0.4 ml 10 mM sodium acetate pH 5.2 containing 50 mM sodium chloride and 2% sodium dodecyl sulphate; this was then cooled on ice to precipitate the SDS (which was removed by centrifugation). The RNA was precipitated, overnight at −20°, with 0.05 volumes of 2 M sodium acetate plus 2 volumes of ethanol, then dissolved and electrophoresed as in fig. 1, but for $3\frac{3}{4}$ hr.

tial loss of the small ribosomal subunit. The same is probably true for microsomes contaminating the mitochondrial fraction.

These results show that in chick liver mitochondria there exists a ribonucleoprotein particle with sedimentation of approximately 55 S. It is ribosomal in character since it is involved in mitochondrial protein synthesis in vitro. Also, this particle contains two RNA species, 18 S and 12 S (by electrophoresis) which seem to be gene products of the mitochondrial DNA as shown by their EB-sensitivity.

Similar results have been obtained with BHK-21 cells, in which a 55 S mitochondrial ribosome containing 18 S and 12 S RNA has been identified [13]. Also, rat brain mitochondria have been shown to possess 55 S ribosomes [14], and the pattern of Ma-RNA in chick brain is similar to that described here for chick liver (unpublished results).

Molecular weight determination was carried out on the 18 S and 12 S RNA by electrophoresis [15, 16]. The apparent molecular weights of the 18 S and 12 S RNA were 0.70 and 0.33×10^6 daltons (assuming 1.7 and 0.7×10^6 daltons for chick liver cytoplasmic RNA [17] and 1.1 and 0.56×10^6 for *E. coli* ribosomal RNA [18]). The relationship of the chick Ms-RNA species to RNA found by other investigators is explained by a recent report of Groot et al. [19] in which different molecular weights values for rat mitochondrial RNA were obtained at different temperatures of electrophoresis. Hence it seems that the apparent molecular weight differences from different laboratories are due to variation in the secondary structure of the RNA at different temperatures. The mitochondrial RNA isolated by various workers to which reference has been made in this report are probably all mitochondrial ribosomal RNA and the true molecular weights are essentially the same. They are all structural RNAs of a 55 S mitochondrial ribosome of these higher eukaryotes.

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Additional note

Since this manuscript was completed two reports have appeared on mitochondrial ribosomes of HeLa cells. In one [20] a 60 S ribosome apparently contains RNA sedimenting at 16 S and 12 S, and in the other [21] a 55 S ribosome gave RNA electrophoresing at 21 S and 12 S. In both reports these RNAs were associated with presumptive ribosomal subunits. Our present data show clearly that a functional 55 S ribosomal monomer exists in chick liver mitochondria and that this monomer contains two types of RNA of 18 S and 12 S (by electrophoresis). Together with the recent report of Montenecourt and Dubin [7] that, in the mitochondria of BHK-21 cells, two RNP particles sedimenting at 33 S and 25 S contain RNA molecules sedimenting at 17 S and 13 S, and our own finding of a 55 S RNP particle in BHK cells [13], the present results settle beyond doubt that the 55 S particle identified as the functional unit in mitochondrial protein synthesis is a ribosome-like particle.

References

[1] C.Vesco and S.Penman, Proc. Natl. Acad. Sci. U.S. 62 (1969) 218.
[2] D.T.Dubin and B.S.Montenecourt, J. Mol. Biol. 48 (1970) 279.
[3] B.S.Montenecourt, M.E.Langsam and D.T.Dubin, J. Cell Biol. 46 (1970) 245.
[4] B.Bartoov, R.S.Mitra and K.B.Freeman, Biochem. J. 120 (1970) 455.
[5] C.Aaij and P.Borst, Biochim. Biophys. Acta 217 (1970) 560.
[6] R.F.Swanson and I.B.Dawid, Natl. Acad. Sci. U.S. 66 (1970) 117.
[7] B.S.Montenecourt and D.T.Dubin, Biochem. Biophys. Res. Commun. 41 (1970) 458.
[8] D.D.Brown and E.Littna, J. Mol. 8 (1964) 669.
[9] A.C.Peacock and C.W.Dingman, Biochemistry 7 (1968) 668.
[10] M.A.Ashwell and T.S.Work, Biochem. Biophys. Res. Commun. 39 (1970) 204.
[11] E.Zylber, C.Vesco and S.Penman, J. Mol. Biol. 44 (1969) 195.
[12] D.D.Sabatini, Y.Tashiro and G.E.Palade, J. Mol. Biol. 19 (1966) 503.
[13] T.H.Rabbitts and T.S.Work, manuscript in preparation.
[14] A.Hernandez and T.S.Work, manuscript in preparation.

[15] D.H.L.Bishop, J.R.Claybrook and S.Spiegelman, J. Mol. Biol. 26 (1967) 373.
[16] U.E.Loening, Biochem. J. 113 (1968) 131.
[17] A.S.Spirin and L.P.Gavrilova, in: The Ribosome, eds. A.Kleinzeller, G.F.Springer and H.G.Wittman (Springer, 1969).
[18] C.G.Kurland, J. Mol. Biol. 2 (1960) 83.
[19] P.H.E.Groot, C.Aaij and P.Borst, Biochem. Biophys. Res. Commun. 41 (1970) 1321.
[20] G.Attardi and D.Ojala, Nature New Biology 229 (1971) 133.
[21] A.Brega and C.Vesco, Nature New Biology 229 (1971) 136.