The Mitochondrial Ribosome of Baby-Hamster Kidney Cells

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Dubin & Montenecourt (1970) reported two 'actinomycin-resistant' high-molecular-weight RNA molecules associated with the mitochondrial fraction of hamster (BHK-21) cells. These RNA species sedimented in sucrose gradients at about 17S and 13S. By polyacrylamide-gel electrophoresis at room temperature molecular weights of 0.73×10^6 and 0.41×10^6 were obtained (equivalent to 18.5S and 14S). A later report (Montenecourt & Dubin, 1970) describes these RNA molecules as associated with ribonucleoprotein particles that sediment at 33S and 25S. These ribonucleoprotein particles were presumed to be mitochondrial ribosomal subunits, but no ribosomal monomer was identified. We now report the existence of a 55S ribosome monomer in mitochondria of BHK-21 cells. This monomer functions in mitochondrial protein synthesis *in vivo* and contains two high-molecular-weight RNA species (18S and 12S by electrophoresis at 4°C); it can therefore be identified with certainty as the mitochondrial ribosome.

Methods. BHK-21 cells were grown in suspension in Eagle's minimum essential medium (Eagle, 1959) plus 10% calf serum (Flow Laboratories, Irvine, Ayrshire, U.K.). The cells were incubated as indicated below at a concentration of 3×10^5 – 6×10^5 cells/ml, either (A) in medium containing one-tenth the normal quantity of leucine, plus 80 µg of emetine (E. Merck A.-G., Darmstadt, Germany)/ml with or without 50 µg of chloramphenicol (Parke, Davis and Co., Hounslow, Middx., U.K.)/ml for 5 min followed by the addition of 0.25 µCi of [³H]-leucine (The Radiochemical Centre, Amersham, Bucks., U.K.; sp. radioactivity 22 Ci/mmol)/ml and incubation for 1 min, or (B) for 30 min with 0.05 µg of actinomycin D (Sigma Chemical Co., St Louis, Mo., U.S.A.)/ml followed by a further 18 h with 0.25 µCi of [³H]uridine (The Radiochemical Centre; 5 Ci/mmol)/ml plus the actinomycin.

After incubation the cells were disrupted in 0.3 M-sucrose (Ca²⁺- and Mg²⁺-free medium) with an Ultra-Turrax homogenizer (Freeman, 1965). Immediately after disruption, 1 M-MgCl₂ was added to give a final concentration of 0.01 M. Mitochondria were prepared by differential centrifugation (the homogenate was centrifuged at 700g for 15 min, and mitochondria were prepared from the supernatant by two successive sedimentations at 8000g, each

for 10 min) and the final mitochondrial pellet was lysed with 0.01 M-tris-HCl (pH 7.6)–0.01 M-MgCl₂–0.01 M-KCl containing 1% Brij-58 (Honeywell-Atlas Ltd., Carshalton, Surrey, U.K.) and 0.1% sodium deoxycholate (Shell Chemicals, Sittingbourne, Kent, U.K.) plus either Krebs II ascites cell 80S ribosomes or *Escherichia coli* 50S ribosomal subunits as u.v.-absorption marker. The lysate (2 ml) was layered on a 15–40% (w/v) sucrose gradient (method A) or a 15–30% (w/v) gradient (method B) (28 ml) in 0.01 M-tris-HCl (pH 7.6)–0.01 M-MgCl₂–0.01 M-KCl. The gradients were centrifuged for 17 h at 17 500 rev./min in a Spinco SW25 rotor at 2°C and then fractionated into 1 ml fractions. The fractions were precipitated with 5% (w/v) trichloroacetic acid, the precipitates collected and their radioactivities counted with a Packard Tri-Carb scintillation counter.

In other experiments mitochondria were prepared from cells labelled as in method (B) with [³H]uridine. The mitochondria were lysed as described for method (B) but in the presence of 1 mg of yeast 'soluble' RNA (Hopkin and Williams Ltd., Chadwell Heath, Essex U.K.). The lysates were centrifuged in a 15–30% sucrose gradient and the 50S peak fractions (tubes 14–17) were precipitated with 2 vol. of ethanol. RNA was prepared from the precipitate as described elsewhere (Rabbitts & Work, 1971), and fractionated, alongside cytoplasmic rRNA as u.v.-absorption marker, on a polyacrylamide gel (2.5% acrylamide–0.5% agarose) for 4 h at 250 V at 4°C by the method of Peacock & Dingman (1968). The gel was sliced into 2 mm slices and its radioactivity counted on a Packard Tri-Carb scintillation counter.

Results and discussion. BHK cells were pulse-labelled, as described in the Methods section, with [³H]leucine in the presence of emetine, a potent inhibitor of cytoplasmic ribosome activity (Perlman & Penman, 1970), and the mitochondria were prepared from these cells. These mitochondria were lysed with detergent and the lysate was sedimented on a sucrose gradient. Fig. 1(a) shows the radioactivity profile of such a sucrose gradient, in which two peaks can be seen, corresponding to particles sedimenting at 55S and at 75S. If chloramphenicol is also present during the incubation of the cells, the labelling of the particles sedi-

Fig. 1. Sucrose-density-gradient sedimentation of mitochondrial lysates and polyacrylamide-gel electrophoresis of mitochondrial ribosomal RNA from BHK-21 cells. (a) BHK cells were incubated, in the presence of 80 μg of emetine/ml, with or without 50 μg of chloramphenicol/ml for 5 min, followed by the addition of 0.25 μCi of [^3H]leucine/ml and incubation for 1 min. Mitochondria were prepared from these cells, lysed and layered on 15–40% sucrose gradients, which were centrifuged, fractionated and the radioactivities counted as described in the Methods section. (b) Cells were incubated for 30 min with 0.05 μg of actinomycin D/ml, followed by a further 18 h in the presence of 0.25 μCi of [^3H]uridine/ml plus the actinomycin. The mitochondria were prepared and lysed and 15–30% sucrose-gradient-centrifugation analysis was carried out as described in the Methods section. (c) Cells were incubated, mitochondria prepared and gradient centrifugation was carried out as described in (b). The RNA was extracted from the 50S peak (fractions 14–17) and subjected to electrophoresis on 2.5% acrylamide–0.5% agarose gels for 4 h as previously described (Rabbitts & Work, 1971). O, [^3H]uridine; $\blacktriangle$, [^3H]leucine+emetine; $\bullet$, [^3H]leucine+emetine+chloramphenicol. In (b) the [^3H]uridine radioactivity (c.p.m.) for fractions 1–20 is shown on a different scale (left-hand scale) from that of fractions 21–30 (right-hand scale).

menting at 55S and 75S on sucrose gradients is inhibited. Since chloramphenicol is known to inhibit mitochondrial, but not cytoplasmic, protein synthesis (Kroon, 1963; Ashwell & Work, 1968;

Firkin & Linnane, 1968) both the 55S and 75S peaks must be due to mitochondrial particles active in protein synthesis. Therefore either the 75S peak represents the mitochondrial ribosomal monomer and the 55S its large subunit, or the 55S particle is the monomer and the 75S represents a polyribosome or aggregate of the 55S particle. The results obtained by labelling of the RNA in these particles indicates that the latter is the true situation.

Cells were grown in the presence of [^3H]uridine and actinomycin D [to minimize labelling of cytoplasmic rRNA (Dubin, 1967)], mitochondria were prepared and a sucrose gradient of the mitochondrial lysate was analysed (Fig. 1b). In this gradient the 55S peak was labelled substantially, and no clear peak appeared in the 75S region. The RNA present in this 55S peak was extracted from the 55S region of a gradient similar to that used in Fig. 1(b) and subjected to polyacrylamide-gel electrophoresis. Fig. 1(c) shows the gel-electrophoresis pattern: only two peaks are present, which correspond to RNA species of 18S_E* and 12S_E (excepting 4S RNA which, under these conditions of electrophoresis, moves off the gel). These values are very near to those obtained by Dubin & Monteneourt (1970) for the actinomycin-resistant mitochondria-associated RNA of BHK-21 cells. Since the two species of RNA, 18S_E and 12S_E, are both associated with the 55S ribonucleoprotein particle and this particle is involved in the chloramphenicol-sensitive mitochondrial protein synthesis *in vivo*, the 55S particle must be the mitochondrial ribosomal monomer of BHK-21 cells. As the 55S particle is the ribosomal monomer, the nature of the 75S particle is not clear. Attardi & Ojala (1971) have reported a similar particle of 78S, as well as a 60S particle, in HeLa-cell mitochondria. Whether these 75S or 78S particles represent polyribosomes or merely aggregates of the 55S particle is not clear from either our own results or those of Attardi & Ojala (1971).

Swanson & Dawid (1970) have described a 60S ribonucleoprotein particle, from the mitochondria of *Xenopus* oocytes, which contains 21S and 13S RNA (measured by electrophoresis). This 60S particle was shown to be ribosomal in nature, since it was active in the incorporation of phenylalanine in cell-free systems with polyuridylic acid as the mRNA. Further, we have found a similar 55S particle in chick liver mitochondria (Rabbitts & Work, 1971) which possesses two RNA species, 18S and 12S as measured by electrophoresis. Reports by Attardi & Ojala (1971) and Brega & Vesco (1971) show that HeLa cells contain 60S or 55S mitochondrial ribosomes possessing two types of RNA (16S

* Abbreviation: S_E is the electrophoretic mobility obtained by comparison with the mobility of 28S and 18S cytoplasmic rRNA as references.

and 12S by sedimentation in the former, 21S and 12S by electrophoresis in the latter). In these two reports, as well as the report of Swanson & Dawid (1970), presumptive ribosomal subunits were isolated, each containing one of the RNA species.

The results now available indicate the universality of the 55S mitochondrial ribosome, containing two ribosomal RNA species, in higher eukaryotes. This 55S monomer is capable of dissociation into two subunits, each carrying one of the RNA species, in a manner similar to eukaryotic and bacterial ribosomes.

T.H.R. is a Medical Research Council Research Scholar.

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