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Higher-Plant Mitochondrial Ribosomes Contain a 5S Ribosomal Ribonucleic Acid Component

By CHRISTOPHER J. LEAVER and MATHEW A. HARMEY*
Department of Botany, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland, U.K.

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Ribosomes from higher-plant mitochondria contain 5S rRNA, in contrast with the mitochondrial ribosomes of animals and fungi, in which such a component has not been detected. In common with the ribosomes of prokaryotes and chloroplasts, higher-plant mitochondrial ribosomes do not appear to contain an RNA equivalent to the 5.8S rRNA that is found in eukaryotes hydrogen-bonded to the largest of the cytoplasmic rRNA species.

The cytoplasm of plants and animals contains ribosomes which sediment at approx. 80S and contain two high-molecular-weight ribosomal RNA species (25–28S, 1.3×10^6–1.75×10^6 mol.wt., and 18S, 0.7×10^6). The ribosomes of bacteria, blue-green algae and chloroplasts on the other hand sediment at approx. 70S and contain 23S (1.1×10^6) and 16S (0.56×10^6) rRNA species.

A point of similarity between the two types of ribosomes has been the demonstration of a low-molecular-weight RNA with a sedimentation coefficient of about 5S (38000 mol.wt.) as a constituent of the larger ribosomal subunit (Payne & Dyer, 1971). A major point of difference is that animal (Pene *et al.*, 1968), fungal and plant 80S ribosomes contain a 5.8S RNA (50000 mol.wt.) component which is hydrogen-bonded to the largest rRNA, but such a molecule has not been detected in the 70S ribosomes of bacteria (Pene *et al.*, 1968), chloroplasts or blue-green algae (Payne & Dyer, 1972).

It has become apparent that mitochondrial ribosomes cannot be characterized as being either of the 70S or the 80S type; their sedimentation coefficients range from 55 to 60S in animal mitochondria and from 70 to 80S in fungi and higher plants (Leaver, 1975). Mitochondrial ribosomes do, however, have several functional similarities to the prokaryotic 70S ribosome. For instance, they require a formylated methionine for initiation of protein synthesis, there is an interchangeability of protein factors required for protein synthesis and a selective sensitivity to certain antibiotics (Borst, 1972). However, a feature that suggested that mitochondrial ribosomes are unique has been the apparent absence of a low-molecular-weight RNA other than transfer RNA (4S, 25000 mol.wt.) from the mitochondria from several organisms (Borst & Grivell, 1971).

* Permanent address: Department of Botany, University College, Dublin, Republic of Ireland.

In this communication, we demonstrate that higher-plant mitochondrial ribosomes contain a 5S rRNA component which is present in approximately equimolar amounts with the large mitochondrial rRNA species. No 5.8S (50000 mol.wt.) RNA was disassociated from the largest mitochondrial rRNA by denaturing treatments which cause the release of such a molecule from the homologous high-molecular-weight rRNA from cytoplasmic ribosomes.

Materials and Methods

Mung-bean (*Phaseolus aureus*) seeds were surface-sterilized with 10% (w/v) sodium hypochlorite and allowed to imbibe overnight in sterile water and grown in sterile moist vermiculite (Dupre Vermiculite, Hertford, Herts., U.K.) in the dark at 25°C for 5 days. The etiolated hypocotyls were excised and surface-sterilized for 5 min in cold 0.5% sodium hypochlorite, and then washed with five changes of sterile water. Mitochondria were extracted and purified by gradient centrifugation as described by Leaver & Harmey (1973).

All media and glassware were sterilized before use, and all operations carried out between 0° and 2°C.

Purified mitochondria, suspended in 10mM-Tris/HCl (pH 8.5)/50mM-KCl/10mM-$MgCl_2$, were lysed by addition of Triton X-100 to a final concentration of 2% (v/v), and the lysate was clarified by centrifugation at 10000*g* for 10min. Nucleic acids were extracted from the supernatant and from total hypocotyl tissue by the phenol/detergent method of Parish & Kirby (1966). Nucleic acids were fractionated by polyacrylamide-gel electrophoresis below 5°C as described by Leaver (1973).

Mitochondrial and cytoplasmic ribosomes were extracted and fractionated by centrifugation on sucrose gradients as described by Leaver & Harmey (1973).

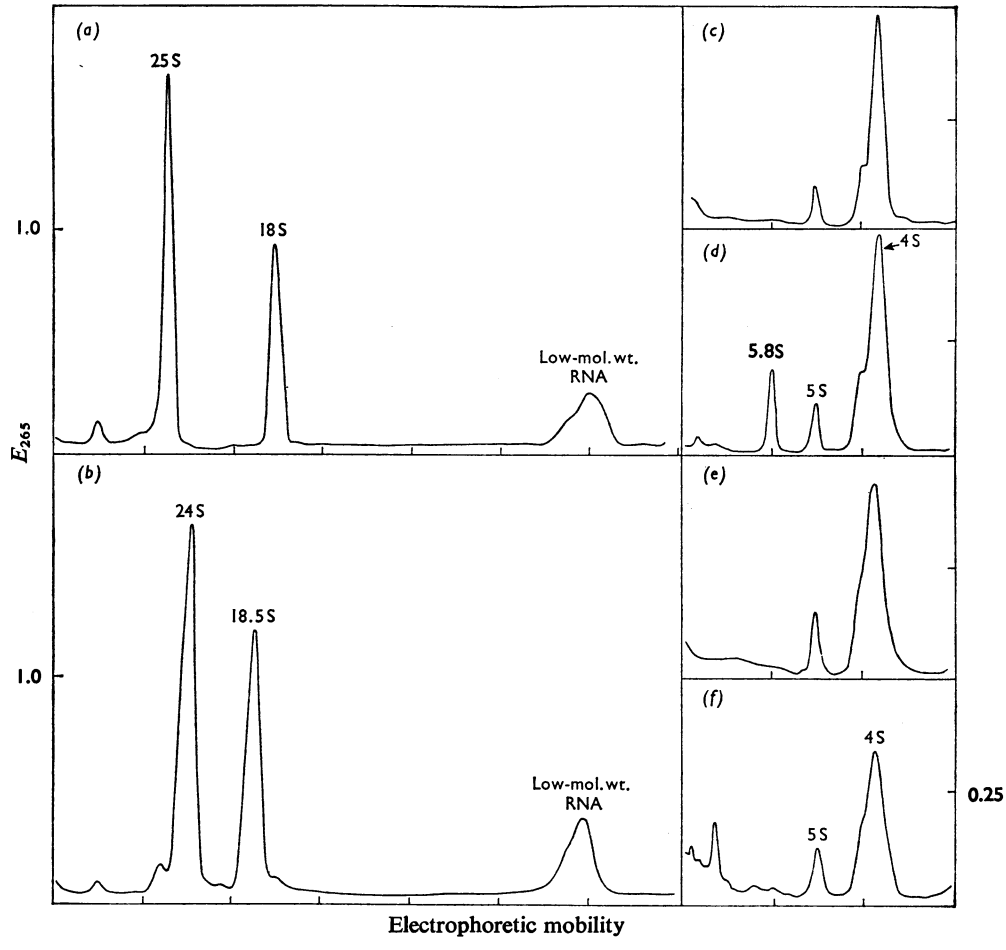

Fig. 1. *Polyacrylamide-gel electrophoresis of nucleic acids from mung-bean hypocotyls*

(*a*) Total cytoplasmic nucleic acids and (*b*) total mitochondrial nucleic acids were fractionated on 2.4% (w/v) polyacrylamide gels for 3.5h at 50V (3mA/9cm gel) and at 5°C. Portions of the same samples were fractionated on 10% (w/v) polyacrylamide gels for 5h at 50V to resolve further the low-molecular-weight nucleic acid components. (*c*) Total cytoplasmic nucleic acid; (*d*) as (*c*) but heated at 65°C for 10min and cooled by rapid immersion in liquid N_2 before fractionation; (*e*) total mitochondrial nucleic acid; (*f*) as (*e*) but heated at 65°C before fractionation.

Results and Discussion

We have previously shown that a range of higher-plant mitochondria contain mitochondrial ribosomes which sediment at 77–78S and which can be dissociated into large and small subunits (Leaver & Harmey, 1973). These subunits contain discrete rRNA species, which have molecular weights estimated to be 1.12×10^6–1.24×10^6 (24S) and 0.69×10^6–0.78×10^6 (18.5S) with slight variation between the plant species (Leaver & Harmey, 1973).

These observations have been independently confirmed for maize (*Zea mays* L.) (Pring, 1974), where its mitochondrial ribosomes sediment at 78S and contain high-molecular-weight rRNA species with molecular weights of 1.25×10^6 and 0.76×10^6 (as determined under denaturing conditions) (Pring & Thornbury, 1975).

Polyacrylamide-gel electrophoresis on 2.4% (w/v) gels of total cytoplasmic nucleic acid (Fig. 1*a*) and total mitochondrial nucleic acid (Fig. 1*b*) from etiolated mung-bean hypocotyls allows a comparison of the high-molecular-weight rRNA components. In addition to rRNA the mitochondria contain low-molecular-weight RNA with molecular weights between 25000 and 40000. A greater resolution of these low-molecular-weight RNA species was obtained by electrophoresis in 10% (w/v) polyacrylamide

gels. When preparations of either total cytoplasmic nucleic acid (Fig. 1*c*) or total mitochondrial nucleic acids (Fig. 1*e*) were fractionated, two low-molecular-weight fractions were distinguishable. These were indistinguishable from the 4S and 5S RNA species of *Escherichia coli* on co-electrophoresis and had calculated molecular weights of 25000 and 37500 respectively. When the nucleic acid preparations were heated at 65°C for 10min, quickly cooled and electrophoresed on 10% (w/v) polyacrylamide gels, an additional low-molecular-weight RNA component was detected in the total cytoplasmic nucleic acid preparation (Fig. 1*d*), but not in the mitochondrial nucleic acid preparation (Fig. 1*f*). This additional component had a calculated *s* value of 5.7S and a molecular weight of 50000. It is the ribosomal component, found hydrogen-bonded to the high-molecular-weight rRNA in the 80S ribosome of animals (Pene *et al.*, 1968; King & Gould, 1970) and plants (Payne & Dyer, 1972). Our failure to detect an equivalent low-molecular-weight RNA component in the mitochondrial nucleic acid preparation suggests that this molecule is absent from plant mitochondrial ribosomes, a finding in accord with reports on mitochondrial ribosomes from animal and ascomycete sources (Borst, 1972).

Essentially similar results to those described above were obtained if mitochondrial 78S and cytoplasmic 80S ribosomes were first of all purified by sucrose-gradient centrifugation. In addition, we have confirmed these observations with mitochondrial RNA from both artichoke (*Helianthus tuberosus*) tuber mitochondria and turnip (*Brassica napus*) root mitochondria.

Calculations, based on the ratio of E_{265} of the 5S and high-molecular-weight rRNA species from nucleic acid preparations from both purified mitochondria and mitochondrial ribosomes, indicate that the mitochondrial 5S rRNA is present in approximately equimolar proportions to the large mitochondrial rRNA species.

These results demonstrate that plant mitochondrial ribosomes do contain a 5S rRNA, in contrast with the mitochondrial ribosomes of *Neurospora* (Lizardi & Luck, 1971), yeast and animal mitochondrial ribosomes, which do not (see Borst, 1972).

As has been suggested by Borst (1972), the failure to detect an RNA species with the electrophoretic mobility of 5S in ascomycete or animal mitochondria does not exclude the possibility that these mitochondria may contain a functionally equivalent molecule which migrates with tRNA at 3–4S or alternatively that the 5S RNA function may be carried out by part of the larger mitochondrial rRNA molecule. Support for the former suggestion comes from work of Dubin *et al.* (1974), who described an unmethylated 3S RNA, which might be the mitochondrial equivalent of 5S RNA, in hamster mitochondria.

The presence of a specific mitochondrial 5S rRNA in and the absence of a 5.8S rRNA from the ribosomes of higher-plant mitochondria demonstrate a further similarity between mitochondrial, chloroplast and prokaryotic ribosome structure.

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