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Ribonucleic Acid Synthesis in Chloroplasts

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Chloroplasts isolated from young spinach leaves incorporate [^{3}H]uridine into RNA. This incorporation shows an absolute requirement for light and does not occur in lysed chloroplasts. Fractionation by polyacrylamide-gel electrophoresis of the RNA synthesized *in vitro* reveals a major discrete product of molecular weight 2.7×10^6 and two minor products of molecular weight 1.2×10^6 and 0.47×10^6. These discrete products are superimposed on a background of polydisperse RNA. The incorporation of $^{32}P_i$ into chloroplast rRNA species (mol.wt. 1.05×10^6 and 0.56×10^6) in excised spinach leaves proceeds after a distinct lag period compared with the incorporation into cytoplasmic rRNA species (mol.wt. 1.34×10^6 and 0.7×10^6). Incorporation of $^{32}P_i$ into chloroplast RNA species of molecular weight 2.7×10^6, 1.2×10^6, 0.65×10^6 and 0.47×10^6 proceeds without such a time-lag. The kinetics of labelling of the individual RNA components is consistent with the rapidly labelled RNA species of molecular weight 1.2×10^6 and 0.65×10^6 being precursors to the more slowly labelled rRNA species of molecular weight 1.05×10^6 and 0.56×10^6 respectively.

It has been established that chloroplasts contain DNA which may code for a limited number of chloroplast components. Recent studies on the kinetics of renaturation of denatured chloroplast DNA suggest that both algal and higher-plant chloroplasts contain multiple copies of a DNA molecule of kinetic complexity 1×10^8–2×10^8 daltons (Wells & Birnstiel, 1969; Stutz, 1970; Tewari & Wildman, 1970; Wells & Sager, 1971). This value is close to that of 1.3×10^8 daltons for bacteriophage T_4 DNA (Wetmur & Davidson, 1968). which is known to contain at least 100 different genes (Wood & Edgar, 1967). It has also been established that chloroplasts contain ribosomes which differ from those of the cytoplasm in several major respects (for a recent review, see Boulter *et al.*, 1972). One specific difference is that the high-molecular-weight RNA components of chloroplast ribosomes (mol.wt. 1.1×10^6 and 0.56×10^6) are smaller than their respective cytoplasmic counterparts of molecular weight 1.3×10^6 and 0.7×10^6 (Stutz & Noll, 1967; Loening & Ingle, 1967; Dyer *et al.*, 1971; Leaver & Ingle, 1971).

Although it is known that nuclear genes control the synthesis of several chloroplast components, there has been no direct identification of a gene product of chloroplast DNA (recent reviews by Kirk & Tilney-Bassett, 1967; Smillie & Steele-Scott, 1970; Boulter *et al.*, 1972). One likely candidate is chloroplast rRNA, since molecular hybridization experiments have shown that 0.5–1.5% of chloroplast DNA hybridizes with chloroplast rRNA (Steele-Scott & Smillie, 1967; Tewari & Wildman, 1968; Ingle *et al.*, 1970). However, chloroplast rRNA has also been shown to hybridize with nuclear DNA (Tewari & Wildman, 1968; Ingle *et al.*, 1970). Tewari & Wildman (1968) calculated that, on a per cell basis, there is three times more potential coding information for chloroplast rRNA in a tobacco leaf mesophyll cell nucleus than contained in chloroplast DNA. This raises the question as to the location of the functional genes for chloroplast rRNA. There is some indirect evidence which indicates that it is the chloroplast genes, and not those in the nucleus which function in the synthesis of chloroplast rRNA. This idea is based on the finding that *Chlamydomonas reinhardi* cells grown in the presence of rifampicin, an inhibitor of *C. reinhardi* chloroplast but not nuclear RNA polymerase, lose their chloroplast ribosomes, presumably by dilution (Surzycki *et al.*, 1970).

The most direct way to establish that chloroplast DNA codes for chloroplast rRNA is to demostrate the synthesis of rRNA or its possible macromolecular precursor(s) in isolated chloroplasts. Berger (1967) demonstrated that chloroplasts isolated from enucleated *Acetabularia mediterranea* cells incorporate [^{14}C]uracil or a [^{14}C]nucleoside triphosphate into RNA species which co-sediment on sucrose density gradients with *Escherichia coli* rRNA species and tRNA with an additional product sedimenting at 9S. In view of the controversy about the precise sizes of *Acetabularia* chloroplast rRNA species (Woodcock & Bogorad, 1970) and the recent finding that isolated 'chloroplasts' may in fact consist of chloroplasts associated with variable amounts of cytoplasm surrounded by a membrane (Bidwell, 1972), caution should be exercised in the interpretation of these results. In the case of chloroplasts isolated from higher plants, the RNA synthesized *in vitro* has

proved to be heterogeneous in size. No discrete or recognizable RNA species have been observed (Spencer & Whitfield, 1967; Tewari & Wildman, 1969; Spencer *et al.*, 1971). In these experiments, nucleoside triphosphates were used as RNA precursors and the chloroplasts were deliberately lysed before incubation. Presumably lysed chloroplasts were used to achieve satisfactory rates of incorporation, since it has been shown by Heldt (1969) and Walker (1969) that the intact chloroplast envelope is relatively impermeable to nucleoside triphosphates. However, it is possible that lysis leads to the loss, by dilution, of necessary transcriptional control factors. An additional problem is the ease of degradation of chloroplast rRNA (Ingle, 1968; Leaver & Ingle, 1971).

As yet there is little information on the mechanism of synthesis of chloroplast rRNA. The synthesis of cytoplasmic rRNA in animals, higher plants and green algae is thought to proceed via the specific cleavage of a large rRNA precursor which contains one of each of the rRNA sequences and some non-conserved 'excess' RNA (Maden, 1971; Grierson *et al.*, 1970; Leaver & Key, 1970; Brown & Haselkorn, 1971). The genes coding for cytoplasmic rRNA are present in multiple copies and are clustered in the nucleolus (Birnstiel *et al.*, 1971). In bacteria and blue-green algae, the high-molecular-weight RNA species of the large and small ribosomal subunits are derived from slightly larger precursor molecules (Hecht & Woese, 1968; Adesnik & Levinthal, 1969; Dahlberg & Peacock, 1971; Szalay *et al.*, 1972). Whether or not these two precursor molecules are derived from a 'unit of transcription' containing both their sequences is not unambiguously known, although there are indications that this may be the case (Kossman *et al.*, 1971; Pettijohn *et al.*, 1970). Ingle (1968) showed that the chloroplast 1.1×10^6- and 0.56×10^6-molecular-weight rRNA species of radish cotyledons are synthesized in a 1:1 molar ratio. The accumulation of these rRNA species during light-induced development is restricted to a short interval and the accumulated chloroplast rRNA turns over at a slower rate than the cytoplasmic rRNA. Similar observations were made for chloroplast rRNA synthesis in *Phaseolus vulgaris* leaves by Treharne *et al.* (1970). Chloroplast rRNA in *Euglena gracilis* was shown by Brown *et al.* (1970) to be synthesized by a rifampicin-sensitive RNA polymerase. These authors were unable to detect precursors to the chloroplast rRNA species and label entered the latter without a time-lag. However, it is unlikely that the resolution afforded by sucrose-density-gradient centrifugation, used by these authors to fractionate RNA, would be sufficient to distinguish between mature chloroplast rRNA species and possible precursors of 5–10%-higher molecular weight.

In the present paper, we describe experiments in which intact isolated spinach chloroplasts show a light-dependent incorporation of [^{3}H]uridine into discrete high-molecular-weight RNA species. Similar species of RNA are synthesized *in vivo* by spinach leaves, and kinetic experiments suggest that some of them may be precursors to chloroplast rRNA species.

Experimental

Materials

Spinach seeds (*Spinacea oleracea* var. Monstrous Virofly) were purchased from Thompson and Morgan, Ltd., Ipswich, Suffolk, U.K. Pancreatic ribonuclease (type 1-A; aqueous solutions containing 1 mg of protein/ml were incubated at 80°C for 10 min to inactivate any deoxyribonuclease contaminant) and UTP (type 1, trisodium salt) were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Ribonuclease-free deoxyribonuclease 1 was from Worthington Biochemical Corp., Freehold, N.J., U.S.A. [^{3}H]Uridine (TRK·178, 30 Ci/mmol, 1 mCi/ml) and $^{32}P_i$ in dilute HCl solution (PBS 1, 40–70 Ci/mg of phosphorus) were from The Radiochemical Centre, Amersham, Bucks., U.K. Tri-isopropylnaphthalenesulphonic acid (sodium salt) was from Eastman Organic Chemicals, Rochester, N.Y., U.S.A. Acrylamide (Fluka A.G., Buchs, Switzerland) and *NN'*-methylenebisacrylamide (Eastman Organic Chemicals) were recrystallized from chloroform and acetone respectively as described by Loening (1967). Actinomycin D was kindly supplied gratis by Merck, Sharpe and Dohme, Rahway, N.J., U.S.A.

Methods and procedure

Plant tissue. Spinach seeds were germinated in Arthur Bowers potting compost. After 7 days seedlings were removed, their roots washed free of compost, and transferred into aerated Huntner's medium (Huntner, 1953), adjusted to pH 6.4. Seedlings were grown under a 12 h photoperiod of 10000 lx white light at 22±2°C. The first-formed leaf pair of 14–16-day-old plants was used for chloroplast isolation and for labelling studies *in vivo*. At this age, each leaf is about 1 cm in length.

Chloroplast isolation. Chloroplasts were isolated by a modification of the method of Kalberer *et al.* (1967). Laminae (2–5 g) were homogenized for 4 s in 100 ml of a semi-frozen slurry of sterile grinding medium {0.35 M-sucrose–25 mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid]–NaOH buffer (pH 7.6)–2 mM-EDTA (disodium salt)–2 mM-sodium isoascorbate} with a Willems Polytron P.T.35 (Northern Media Supply, Ltd., Hull, Yorks., U.K.). The homogenate was squeezed through four layers of absorbant muslin, filtered through a further eight layers of muslin and centrifuged at 0°C at 2500*g* for 1 min. The supernatant was decanted and

the pellet gently resuspended with the aid of a glass rod and small piece of absorbent cotton-wool in 2–5ml of sterile ice-cold medium containing 66 mM-Tricine [*N*-tris(hydroxymethyl)methylglycine]–KOH buffer (pH8.3)–6.6mM-$MgCl_2$–0.2M-KCl (hereafter referred to as Tricine–$MgCl_2$–KCl medium). Visual investigation of chloroplast suspensions by phase-contrast microscopy showed that 60–80% of the chloroplasts were intact, i.e. possessed a complete limiting envelope as characterized by their highly refractive appearance (Kahn & von Wettstein, 1961). When lysed chloroplasts were required, the chloroplast pellet was resuspended in 66mM-Tricine–KOH buffer (pH8.3)–6.6mM-$MgCl_2$, the suspension incubated at 0°C for 3min, then an equal volume of 66mM-Tricine–KOH buffer (pH 8.3)–6.6mM-$MgCl_2$–0.4M-KCl added to restore the composition of the resuspending medium to that of Tricine–$MgCl_2$–KCl medium. Visual investigation of such a chloroplast suspension by phase-contrast microscopy revealed that all the chloroplasts had ruptured limiting envelopes as characterized by their granular appearance (Kahn & von Wettstein, 1961).

The chlorophyll concentration of the chloroplast suspension was determined by the method of Arnon (1949).

Assay of [^3H]uridine incorporation into a trichloroacetic acid-insoluble product by isolated chloroplasts. Reaction mixtures contained 200μmol of KCl, 6.6μmol of $MgCl_2$, 66μmol of Tricine–KOH buffer (pH8.3), 10μCi of [^{3}H]uridine and chloroplast suspension containing 50–100μg of chlorophyll in a total volume of 1.0ml. Reaction mixtures were incubated at 20°C for 20min in an illuminated water bath similar in design to that described by Hill & Walker (1959). Filtered red light ($\lambda > 600$nm) provided an illumination intensity of 15000 lx at the bottom of the reaction tubes. Reactions were stopped by the addition of 1ml of 20% (w/v) trichloroacetic acid containing 10mM-sodium pyrophosphate. The suspensions were left to stand in ice for 30min then transferred quantitatively on to 2.5cm Whatman GF/C glass-fibre discs. The precipitates were washed three times with 10ml of 5% (w/v) trichloroacetic acid containing 10mM-sodium pyrophosphate, twice with 10ml of Hokins reagent [quoted by Bollum (1968); prepared by mixing 935ml of ethanol, 60ml of acetic acid and 4.5ml of 2M-NaOH], once with 10ml of Hokins reagent–diethyl ether (1:1, v/v) and finally with 5ml of ether. Filters were air-dried and placed in scintillation vials to which 5ml of toluene-based scintillation fluid [0.5% (w/v) 2,5-diphenyloxazole, 0.03% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene] was added. The radioactivity was determined in Packard Tri-Carb model 3320 liquid-scintillation spectrometer at a counting efficiency of 24%. The value (c.p.m.) of a zero-time control, which was in all cases less than 10% of the maximum value for incubated reaction mixtures, was subtracted from all values given in c.p.m.

Labelling of nucleic acid in excised leaves. Leaves were excised from stems at the bases of their petioles, and the cut ends placed in sterile water contained in 1cm-diameter wells drilled in a block of Perspex. Each well contained 0.5ml of water and four excised leaves. The leaves were illuminated for 1h under 10000-lx white light, then 50μCi (100μl) of $^{32}P_i$ was added to each well. Leaves were removed from the wells after the times indicated, and washed in sterile water.

Preparation of nucleic acid. (a) Total leaf nucleic acid: the phenol–detergent method of Parish & Kirby (1966) as modified by Loening (1969) was used.

(b) Chloroplast nucleic acid: nucleic acid was extracted from chloroplast-containing reaction mixtures by the method of Leaver & Ingle (1971) except that the Triton X-100-fractionation step was omitted. The Mg^{2+} concentration was maintained at 10mM throughout the extraction procedure. The ethanol-precipitated nucleic acid fraction was washed twice with 80% (v/v) ethanol containing 10mM-$MgCl_2$.

The washed nucleic acid pellets were drained to remove excess of ethanol and dissolved in electrophoresis buffer containing 7% (w/v) sucrose. The nucleic acid concentration was estimated from the E_{260}. It was assumed that 1mg of nucleic acid/ml has an E_{260}^{1cm} of 20.

Fractionation of nucleic acid. The nucleic acid preparations were fractionated by polyacrylamide-gel electrophoresis as described by Loening (1967), but with the EDTA-containing buffer [36mM-Tris–30mM-NaH_2PO_4–1mM-EDTA with 0.2% (w/v) sodium dodecyl sulphate added in the buffer compartments] described by Loening (1969). The nucleic acid sample (30μg) at a concentration of 1mg/ml was applied to a 2.2% or 2.4% polyacrylamide gel and fractionated by electrophoresis at 50V (approx. 6mA/9cm gel) for 3–4h. The gels were scanned at 265nm, either in a Joyce–Loebl Chromoscan fitted with a 265nm interference filter, or in a Gilford 2000 spectrophotometer fitted with a 2410S Linear Transport scanning attachment.

For radioactivity determinations, scanned gels were transferred into aluminium foil troughs, frozen to a length of 90mm in powdered solid CO_2 and sectioned transversely into 0.6 or 0.8mm slices with a Mickle gel slicer. Gel slices were dissolved in 0.2ml of 100vol. H_2O_2 at 60°C for 5h in capped scintillation vials and dispersed in 10ml of Triton X-100–toluene scintillation fluid [0.4% (w/v) 2,5-diphenyloxazole, 0.05% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene–Triton X-100 (2:1, v/v)]. Samples were counted for radioactivity in a Packard Tri-Carb model 3320 liquid-scintillation spectrometer at an efficiency of 25% for ^{3}H and 93% for ^{32}P. In samples in which ^{3}H and ^{32}P were counted simultaneously,

correction was made for a 9% spill-over of ^{32}P counts into the ^{3}H channel. The molecular weights of the RNA components were calculated with reference to *E. coli* rRNA species of assumed molecular weight 1.1×10^6 and 0.56×10^6.

Estimation of cytoplasmic and chloroplast high-molecular-weight rRNA species. Spinach leaves (10–100, depending on size) were homogenized in a VirTis '45' (VirTis Co. Inc., Gardiner, N.Y., U.S.A.) at 45krev./min for 10s with 10ml of the phenol–detergent solution described by Loening (1969). The homogenate was transferred quantitatively into glass centrifuge tubes and centrifuged at 1000*g* for 10min at 7–8°C. The aqueous layer was removed and the phenol layer plus interface re-extracted three times by shaking on each occasion with an equal volume of detergent solution, and centrifuging. Nucleic acid in the combined aqueous layers was precipitated by the addition of 2vol. of ethanol and storage for 16h at 0°C. The precipitate was washed with 70% (v/v) ethanol containing 50mM-NaCl. The precipitate was dissolved in electrophoresis buffer containing 7% (w/v) sucrose, and the total nucleic acid content determined from the E_{260}. A portion (30μg) was fractionated by electrophoresis in a 2.4% polyacrylamide gel and the amounts of cytoplasmic (mol.wt. 1.34×10^6 and 0.7×10^6) and chloroplast (mol.wt. 1.05×10^6 and 0.56×10^6) rRNA species estimated from the E_{265} scan by measurement of the areas of the respective peaks. These areas were expressed as a % of the total area and related to the μg of nucleic acid applied to the gel. Values were corrected for the partial degradation of the chloroplast 1.05×10^6-molecular-weight rRNA as described by Ingle (1968).

Alkaline hydrolysis of chloroplast RNA synthesized in vitro and determination of ^{3}H in the nucleotides. Nucleic acid samples were dissolved in 0.5ml of sterile solution containing 50mM-Tris–HCl buffer, pH7.6, and 10mM-$MgCl_2$. The sample was applied to a column (12cm×1cm) of Sephadex G-25 and eluted with 50mM-Tris–HCl buffer (pH7.6)–10mM-$MgCl_2$. The E_{260} of eluate fractions (0.5ml) was determined and nucleic acid in the peak fraction was precipitated with 2vol. of ethanol and stored at −20°C for 4h. The precipitate was collected by centrifugation, dried *in vacuo*, dissolved in 100μl of 0.3M-KOH and incubated for 15h at 37°C in a sealed tube. The solution was adjusted to pH6.0 by the addition of 3M-$HClO_4$ and the suspension stored at 0°C for 30min. The suspension was centrifuged at 0°C, portions (20μl) of the supernatant applied to a strip (57cm×13cm) of Whatman 3MM paper and subjected to electrophoresis in buffer containing 10% (v/v) acetic acid, 1% (v/v) pyridine and 1mM-EDTA for 2h at 2.5kV. The paper was dried overnight at 60°C and nucleotides located under a u.v. lamp. Squares (2cm×2cm) of the electrophoretogram containing the nucleotides were cut out and placed directly into scintillation vials containing 15ml of toluene-based scintillation fluid [0.5% (w/v) 2,5-diphenyloxazole, 0.3% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene]. Radioactivity was determined in a Packard Tri-Carb model 3320 liquid-scintillation spectrometer at 24% efficiency for ^{3}H. The radioactivity in each nucleotide was expressed as a percentage of the total radioactivity in all four nucleotides.

Results and Discussion

Accumulation of cytoplasmic and chloroplast rRNA species during spinach-leaf development

The accumulation of cytoplasmic (mol.wt. 1.34×10^6 and 0.7×10^6) and chloroplast (mol.wt. 1.05×10^6 and 0.56×10^6) rRNA species during development of the first-formed leaf pair of spinach seedlings is shown in Fig. 1. There is a rapid accumulation of both chloroplast and cytoplasmic rRNA species from day 10 to day 20, after which the rate of accumulation declines until a maximum content of each is attained at about day 25. Leaves of 14–16-day-old plants, which show a maximum rate of accumulation of chloroplast rRNA, were used in all subsequent experiments on chloroplast RNA synthesis.

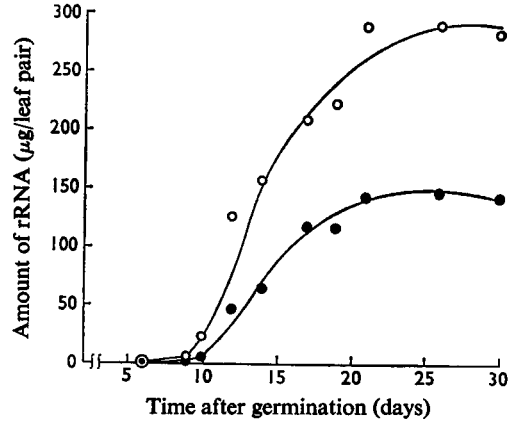

Fig. 1. *Accumulation of cytoplasmic and chloroplast rRNA species during spinach-leaf development*

Total nucleic acid was quantitatively extracted from the first-formed leaf pair of spinach seedlings and samples were fractionated by electrophoresis in 2.4% polyacrylamide gels, as described under 'Methods and procedure'. The amounts of cytoplasmic rRNA species of molecular weight 1.34×10^6 and 0.7×10^6 (○) and chloroplast rRNA species of molecular weight 1.05×10^6 and 0.56×10^6 (●) were estimated as described under 'Methods and procedure'.

Characteristics of [^{3}H]uridine incorporation by isolated chloroplasts

Time-courses for the incorporation of [^{3}H]uridine into a trichloroacetic acid-insoluble product by intact chloroplasts in the light and dark, and lysed chloroplasts in the light and dark, are shown in Fig. 2. Incorporation by intact, illuminated chloroplasts proceeds at an approximately linear rate with time for about 20min and stops after about 30min. The rates of incorporation by intact chloroplasts in the dark and lysed chloroplasts in the light and dark are all less than 10% of the rate shown by intact illuminated chloroplasts. This result shows that incorporation is light-dependent and occurs only in intact chloroplasts; both these characteristics suggest that photophosphorylation is required to convert [^{3}H]uridine into [^{3}H]UTP. The fact that incorporation is light-dependent, and is abolished after exposure of the chloroplasts to hypo-osmotic conditions suggests that any bacterial contaminant derived from the leaves is not significantly contributing to the total activity. In contrast with this, the incorporation of [^{14}C]ATP in the presence of CTP, GTP and UTP is not light-dependent and proceeds at a faster rate in lysed chloroplasts than intact chloroplasts (Hartley, 1971).

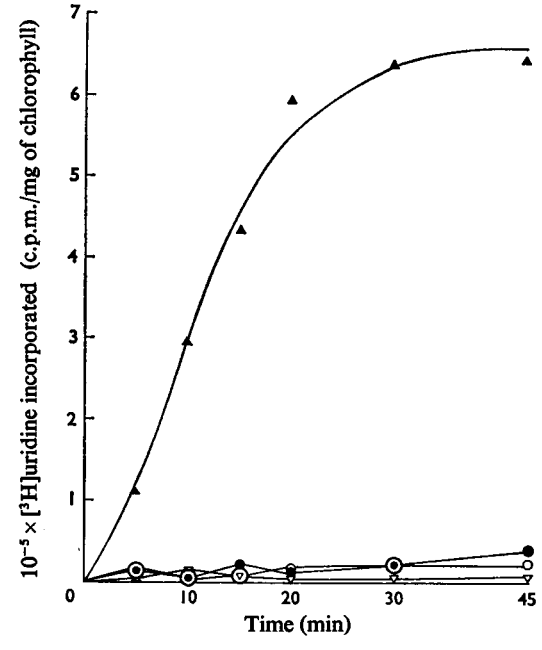

Fig. 2. *Effect of illumination and lysis on the incorporation of [^{3}H]uridine into an acid-insoluble product by chloroplasts*

Intact and lysed chloroplasts were prepared from the leaves of 15-day-old spinach plants and assayed for [^{3}H]uridine-incorporating activity with illumination, or in darkness, as described under 'Methods and procedure'. Reaction mixtures contained 63μg (intact chloroplasts) or 72μg (lysed chloroplasts) of chlorophyll. ▲, Intact, illuminated; ●, intact, in darkness; ○, lysed, illuminated; ▽, lysed, in darkness.

Characteristics of the product synthesized in vitro

Gel-electrophoretic fractionations of total nucleic acid extracted from intact chloroplasts incubated with [^{3}H]uridine for 45min are shown in Fig. 3. The major u.v.-absorbing components are DNA and the chloroplast rRNA species of molecular weight 1.05×10^6 and 0.56×10^6. The identity of DNA was confirmed by running duplicate gels of two nucleic acid samples, one of which had been treated with deoxyribonuclease; this treatment resulted in a specific loss of the component so marked. The major, discrete radioactive product synthesized by illuminated chloroplasts has a mobility corresponding to a molecular weight of 2.7×10^6. We reproducibly observe small peaks of radioactivity with mobilities corresponding to molecular weights of 1.2×10^6 and 0.47×10^6. In addition, there is a radioactive product which penetrates the gel to a distance of less than 5mm corresponding to a single-strand molecular weight of greater than 4×10^6. All of these discrete products are superimposed on a background of heterogeneous radioactive product. Qualitatively similar radioactivity profiles to that described were observed in gel fractionations of samples extracted from reaction mixtures incubated for 10min to 90min. None of these radioactive products is synthesized in chloroplasts incubated in the dark (Fig. 3*b*). The light-dependent synthesis of the radioactive products is inhibited by actinomycin D (Fig. 3*c*), and all the products are susceptible to ribonuclease digestion on incubation of the extracted nucleic acid with ribonuclease (Fig. 3*d*). This indicates that the radioactive components are the products of DNA-dependent RNA polymerase activity.

Localization of [^{3}H]uridine-incorporating activity within the chloroplast preparation

The chloroplast preparations used in the experiments described are likely to contain contaminating nuclei, nuclear fragments and mitochondria. The finding that the incorporation of [^{3}H]uridine into RNA is light-dependent is not sufficient evidence alone to exclude the possibility that a significant proportion of this incorporation is taking place in nuclei or mitochondria. The [^{3}H]uridine may be phosphorylated to [^{3}H]UTP in the chloroplast,

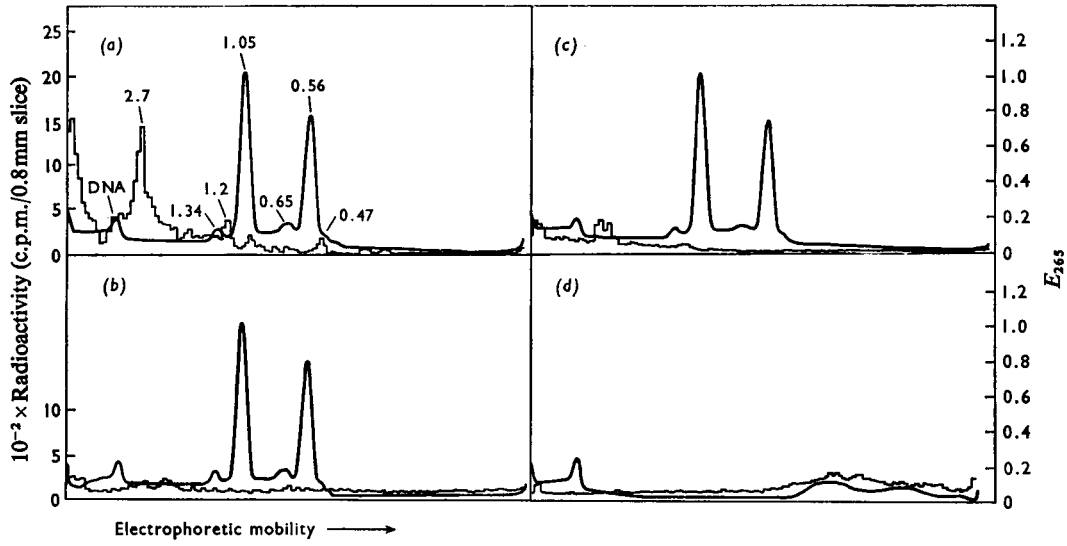

Fig. 3. *Gel-electrophoretic fractionation of the RNA synthesized in vitro*

Chloroplasts were isolated from leaves of 16-day-old plants and suspensions containing 69μg of chlorophyll incubated in [^{3}H]uridine-incorporation reaction mixtures (see under 'Methods and procedure') with illumination, or in darkness. Further experimental details are given in the text. Nucleic acid extraction and gel electrophoresis were performed as described under 'Methods and procedure'. (*a*) Illuminated; (*b*) in darkness; (*c*) illuminated, plus actinomycin D (10μg); (*d*) illuminated, extracted nucleic acid dissolved in electrophoresis buffer (sodium dodecyl sulphate omitted) and incubated for 10min at 4°C with ribonuclease (10μl of a 100μg/ml solution) before gel electrophoresis. ——, E_{265}; ——, (histogram) radioactivity. RNA components are referred to as 10^{-6} × molecular weight.

diffuse out and serve as co-substrate for the nuclear or mitochondrial polymerases. Providing that incorporation of [^{3}H]uridine into RNA is occurring via [^{3}H]UTP, this possibility may be tested by taking advantage of the relative impermeability of the chloroplast envelope to exogenous UTP, compared with [^{3}H]uridine.

The distribution of radioactivity was determined among the four 2′(3′)-nucleotides, obtained by alkaline hydrolysis of total RNA extracted from illuminated chloroplasts incubated with [^{3}H]uridine. Since 80% of the total radioactivity in the four nucleotides was recovered in UMP, the majority of [^{3}H]uridine incorporation into RNA must have occurred via [^{3}H]UTP. Chloroplast suspensions were assayed for [^{3}H]uridine incorporation in reaction mixtures containing [^{3}H]uridine/UTP molar ratios of between 1:1 and 1:1000. Over this concentration range, UTP caused no decrease in [^{3}H]uridine incorporation, which suggests that this incorporation is occurring exclusively within chloroplasts.

It has been reported that protein synthesis by intact isolated chloroplasts is not inhibited by exogenous ribonuclease (Margulies *et al.*, 1968; Blair & Ellis, 1972). This relates to the finding that RNA in intact chloroplasts is protected from degradation by ribonuclease (Ellis *et al.*, 1973). This finding may be used to support the contention that the RNA synthesized by chloroplast preparations is located in intact chloroplasts. Four reaction mixtures (*a*, *b*, *c* and *d*) containing chloroplast suspension and [^{3}H]uridine were incubated with illumination, for 40min, then placed in the dark to prevent further RNA synthesis. The following additions were then made to the reaction mixtures: (*a*) water (75μl); (*b*) ribonuclease (50μl of a 1mg/ml solution) plus water (25μl); (*c*) ribonuclease (50μl of a 1mg/ml solution) plus Triton X-100 [25μl of a 20% (v/v) solution] to lyse the chloroplasts; (*d*) Triton X-100 [25μl of a 20% (v/v) solution] plus water (50μl). Incubation was continued in the dark for 15min at 20°C, then nucleic acid was extracted by the phenol–detergent method and the samples were fractionated by gel electrophoresis. The E_{265} scans and radioactivity profiles of the gels are shown in Fig. 4. The E_{265} scans and radioactivity profiles of samples (*a*) (Fig. 4*a*) and (*b*) (Fig. 4*b*) are qualitatively similar to each other, and to those previously described (Fig. 3*a*). In

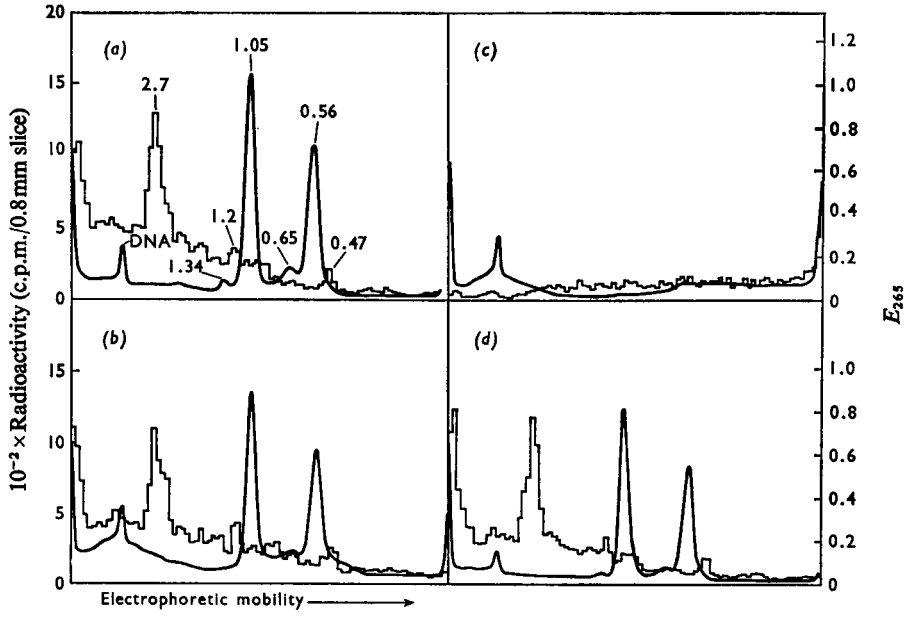

Fig. 4. *Effect of exogenous ribonuclease on the RNA synthesized in vitro in intact and lysed chloroplasts*

Chloroplasts were isolated from leaves of 15-day-old plants and suspensions containing 74 μg of chlorophyll incubated in [^{3}H]uridine-incorporation reaction mixtures (see under 'Methods and procedure') with illumination. Further experimental details are given in the text. Nucleic acid extraction and gel electrophoresis were performed as described in the text. (*a*), Intact chloroplasts incubated without exogenous ribonuclease; (*b*), intact chloroplasts incubated with exogenous ribonuclease; (*c*), lysed chloroplasts incubated with exogenous ribonuclease; (*d*), lysed chloroplasts incubated without exogenous ribonuclease. ▬, E_{265}; —— (histogram), radioactivity. RNA components are referred to as 10^{-6} × molecular weight.

sample (*c*) (Fig. 4*c*) there has been a complete degradation of both u.v.-absorbing RNA species and radioactive components. The E_{265} scan and radioactivity profile of sample (*d*) (Fig. 4*d*) is similar to that of (*a*) (Fig. 4*a*) showing that chloroplast lysis alone does not result in RNA degradation, and that Triton X-100 does not activate endogenous ribonuclease activity. These results, together with those described previously, show that the RNA synthesized *in vitro* is located in ribonuclease-impermeable Triton X-100-labile particles, and that these particles are intact chloroplasts.

RNA species made in vivo

Excised spinach leaves from 15-day-old seedlings were labelled via their petioles with $^{32}P_i$ for periods of up to 6h. Total leaf nucleic acid was extracted from these samples and fractionated by gel electrophoresis (Fig. 5). The major u.v.-absorbing components are DNA, the cytoplasmic rRNA species (mol.wt. 1.34×10^6 and 0.7×10^6) and the chloroplast rRNA species (mol.wt. 1.05×10^6 and 0.56×10^6). After 2h labelling, three distinct species of radioactive RNA are detectable (Fig. 5*a*). Two of these correspond in mobility to the cytoplasmic rRNA species (mol.wt. 1.34×10^6 and 0.7×10^6) the third, with a molecular weight of 1.45×10^6, does not correspond in mobility to a u.v.-absorbing component. This 1.45×10^6-molecular-weight component is known to be the immediate precursor to the cytoplasmic 1.34×10^6-molecular-weight rRNA (Leaver & Key, 1970; Rogers *et al.*, 1970; Grierson & Loening, 1972). In addition, a heterogeneous labelled RNA is detectable which appears to have two components: a major component of molecular weight 2.45×10^6 and a minor component of molecular weight 2.7×10^6. All of these labelled components are superimposed on a background of polydisperse labelled RNA. In contrast with this result, after 6h labelling the chloroplast and cytoplasmic rRNA species have attained an approximately equal specific radioactivity (Fig. 5*b*). Thus there is a time-lag in the incorporation of $^{32}P_i$ into chloroplast rRNA. There are two likely explanations

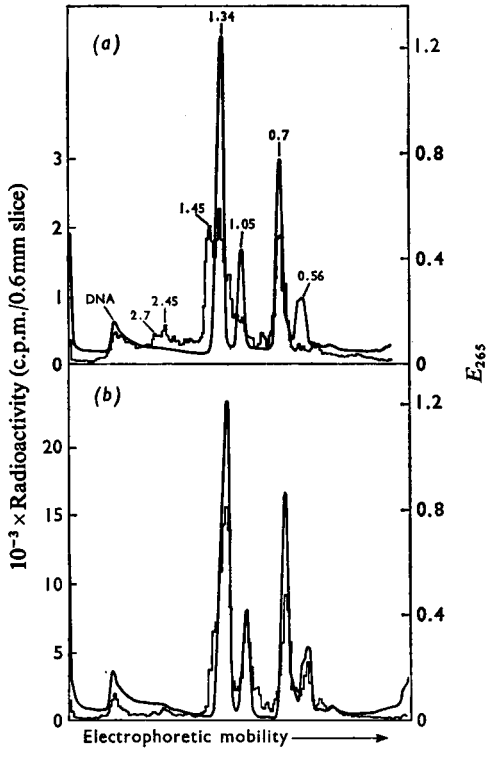

Fig. 5. *Gel-electrophoretic fractionation of nucleic acid extracted from leaves labelled for 2h (a) and 6h (b) with $^{32}P_i$*

Leaves were excised from 15-day-old plants and labelled via their petioles with $^{32}P_i$. Nucleic acid extraction and gel electrophoresis were performed as described under 'Methods and procedure'. ——, E_{265}; ——(histogram), radioactivity. RNA components are referred to as 10^{-6} × molecular weight.

for this result: (i) the chloroplast nucleotide RNA precursor pool equilibrates with $^{32}P_i$ more slowly than that of the cytoplasm; or (ii) the chloroplast rRNA species are processed from macromolecular precursors of similar sizes to the cytoplasmic rRNA species and are thus obscured by the latter on gel fractionation.

To investigate these possibilities, and to determine which of the labelled RNA species observed in total leaf nucleic acid preparations are located in the chloroplast, nucleic acid samples extracted from chloroplasts, isolated from leaves after various times of labelling with $^{32}P_i$, were analysed on gels (Fig. 6). The E_{265} scans show DNA, the chloroplast rRNA species (mol.wt. 1.05×10^6 and 0.56×10^6) and two minor components of molecular weight 1.34×10^6 and 0.65×10^6, a proportion of which probably represents cytoplasmic rRNA contaminant (to be discussed). After 40min labelling, four major radioactive peaks (omitting incorporation into DNA) with mobilities corresponding to molecular weights of 2.7×10^6, 1.2×10^6, 0.65×10^6 and 0.47×10^6 are discernible above the background of polydisperse labelled RNA (Fig. 6*a*). These radioactive RNA species become more distinct after 1h labelling (Fig. 6*b*). With further times of labelling (2h and 4h) radioactivity appears in the chloroplast rRNA species, the 1.05×10^6-molecular-weight rRNA becoming labelled more rapidly than the 0.56×10^6-molecular-weight rRNA (Fig. 6*c* and 6*d*). During this transition the radioactivity in the rapidly labelled RNA species of molecular weights 2.7×10^6, 1.2×10^6, 0.65×10^6 and 0.47×10^6 becomes a progressively smaller proportion of total radioactivity, until after 6h labelling the E_{265} scan and radioactivity profile approximate to each other (Fig. 6*e*).

Cellular location of labelled RNA species

Before discussing the possible significance of these results, it must be ascertained whether the labelled RNA species described above are chloroplast in location, or represent cytoplasmic or nuclear contamination. Contamination by nuclear RNA species may be discounted, since the labelled RNA of molecular weight 1.45×10^6, which is observed in total leaf nucleic acid (Fig. 5*a*) and assigned exclusively to the nucleus by Leaver & Key (1970) is not present in the chloroplast fraction. As stated previously, a proportion of the minor u.v.-absorbing components of mobilities corresponding to molecular weights of 1.34×10^6 and 0.65×10^6 probably represent cytoplasmic rRNA contaminants. An expanded E_{265} scan of a gel in which nucleic acid, extracted from chloroplasts isolated from leaves labelled for 75min with $^{32}P_i$, had been fractionated is shown in Fig. 7. The radioactive component of molecular weight 1.2×10^6 is clearly separated from the u.v.-absorbing component of molecular weight 1.34×10^6 and co-electrophoreses with a reproducibly detectable, partially resolved, minor u.v.-absorbing component. The radioactive component of molecular weight 0.65×10^6 co-electrophoreses with a u.v.-absorbing component, although both are somewhat heterogeneous and may represent more than one RNA species. In this respect, it has been shown by Adesnik & Levinthal (1969) and Dahlberg & Peacock (1971) that the macromolecular precursor to the 0.56×10^6-molecular-weight rRNA in *E. coli* occurs as at least two species, differing in electrophoretic mobility. A similar observation has been made for the precursor to the 0.56×10^6-molecular-weight rRNA of the blue–green alga *Tolypothrix distorta* (D. Grierson, personal communication).

The cellular location of these RNA species has

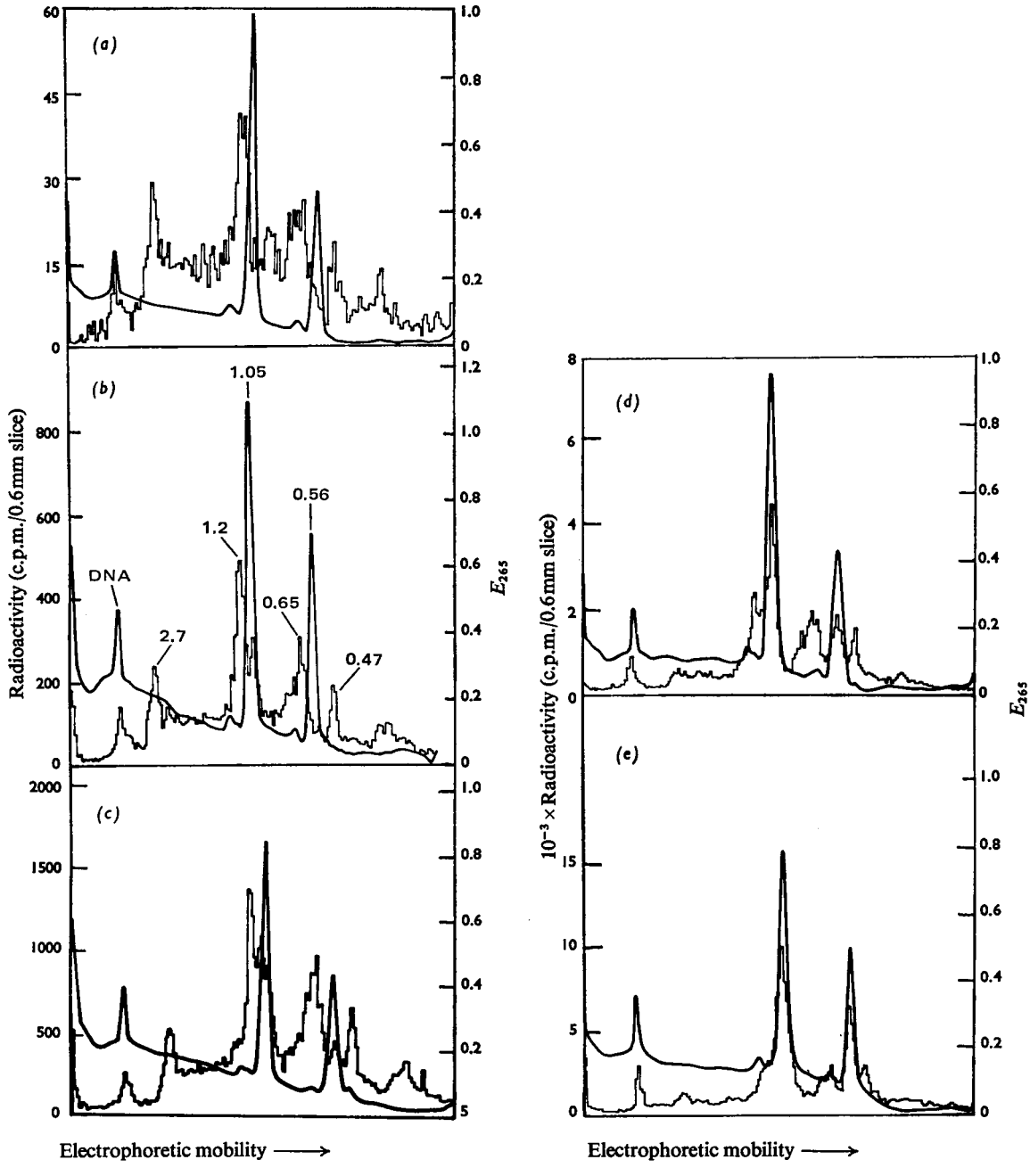

Fig. 6. *Gel-electrophoretic fractionation of nucleic acid extracted from chloroplasts prepared from leaves labelled for 40 min (a), 1 h (b), 2 h (c), 4 h (d) and 6 h (e) with* $^{32}P_i$

Leaves were excised from 14-day-old plants and labelled via their petioles with $^{32}P_i$. Chloroplast extraction, nucleic acid preparation and gel electrophoresis were performed as described under 'Methods and procedure'. ▬, E_{265}; —— (histogram), radioactivity. RNA components are referred to as 10^{-6} × molecular weight.

been resolved by using the finding that the RNA in intact chloroplasts, i.e. those possessing a complete limiting envelope, is not susceptible to ribonuclease digestion, whereas RNA in lysed chloroplasts, from which the limiting envelope has been lost, is susceptible to ribonuclease digestion. The gel-electrophoretic fractionations shown in Fig. 8 are of nucleic acid samples extracted from chloroplasts isolated from leaves labelled for 1h with $^{32}P_i$. In sample (*a*) (Fig. 8*a*) chloroplasts were resuspended in 1ml of Tricine–$MgCl_2$–KCl medium and incubated for 20min at 15°C before nucleic acid extraction. In sample (*b*) (Fig. 8*b*) chloroplasts were similarly resuspended and incubated, but with the addition of 50μg of pancreatic ribonuclease. In sample (*c*) (Fig. 8*c*) chloroplasts were resuspended in 0.5ml of Tricine–$MgCl_2$ medium to lyse them. After incubation of the suspension for 3min at 0°C, 0.5ml of Tricine–$MgCl_2$ medium containing 0.4M-KCl was added [to restore the composition of the resuspending medium to that of samples (*a*) and (*b*)] together with 50μg of pancreatic ribonuclease, and the suspension incubated for a further 20min at 15°C before nucleic acid extraction. The E_{265} scan and radioactivity profile of sample (*a*) (Fig. 8*a*) are similar to those shown in Fig. 6(*b*). In sample (*b*) (Fig. 8*b*) the radioactivity profile is qualitatively similar to that of sample (*a*). The E_{265} scan of sample (*b*) is also similar to that of sample (*a*) except that the RNA component of molecular weight 1.34×10^6 is not present in sample (*b*). Thus exogenous ribonuclease specifically degrades the 1.34×10^6-molecular-weight component. In sample (*c*) (Fig. 8*c*) there has been a complete degradation of all the high-molecular-weight u.v.-

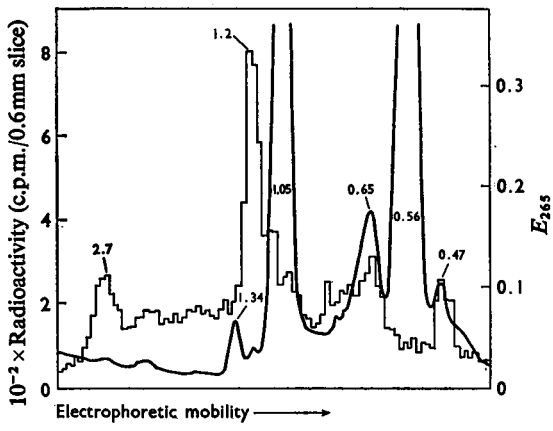

Fig. 7. *Detection of minor RNA species in nucleic acid extracted from the chloroplast fraction*

Leaves were excised from 14-day-old plants and labelled via their petioles for 75min with $^{32}P_i$. Chloroplast preparation, nucleic acid extraction and gel electrophoresis were performed as described under 'Methods and procedure'. ▬ E_{265}; —— (histogram), radioactivity. RNA components are referred to as $10^{-6}\times$molecular weight.

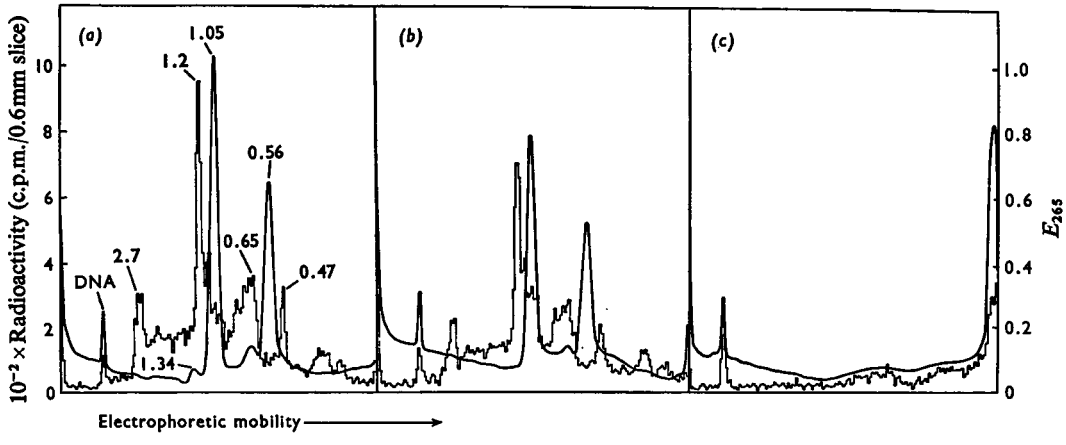

Fig. 8. *Effect of exogenous ribonuclease on RNA species in intact and lysed chloroplasts*

Leaves were excised from 15-day-old plants and labelled via their petioles with $^{32}P_i$ for 1h. Chloroplasts were isolated as described under 'Methods and procedure' and suspensions of lysed or intact chloroplasts incubated with or without exogenous ribonuclease, as described in the text. (*a*) Intact chloroplasts incubated without exogenous ribonuclease; (*b*) intact chloroplasts incubated with exogenous ribonuclease; (*c*) lysed chloroplasts incubated with exogenous ribonuclease. Nucleic acid extraction and gel electrophoresis were performed as described under 'Methods and procedure'. ▬, E_{256}; —— (histogram), radioactivity. RNA components are referred to as $10^{-6}\times$molecular weight.

absorbing and radioactive components into low-molecular-weight products. We interpret these results to indicate that the radioactive and/or u.v.-absorbing components of molecular weight 2.7×10^6, 1.2×10^6, 1.05×10^6, 0.65×10^6, 0.56×10^6 and 0.47×10^6, present in the chloroplast fraction after incubation with ribonuclease, are chloroplast in location, whereas the component of molecular weight 1.34×10^6, present in the chloroplast fraction incubated in the absence of exogenous ribonuclease but lost after incubation with ribonuclease, is an extra-chloroplast contaminant. We have not been able to clearly resolve the 0.65×10^6-molecular-weight chloroplast RNA from the 0.7×10^6-molecular-weight cytoplasmic rRNA. In gel fractionations of total leaf nucleic acid, the former is probably represented by the small shoulder on the trailing edge of the 0.7×10^6-molecular-weight cytoplasmic rRNA peak (Fig. 5). The radioactive RNA of molecular weight 2.45×10^6, present in total leaf nucleic acid from $^{32}P_i$-labelled leaves (Fig. 5) is extra-chloroplast in location, since it is not detectable in chloroplast preparations. By inference from other studies on plant cytoplasmic rRNA synthesis (Leaver & Key, 1970; Rogers *et al.*, 1970; Grierson *et al.*, 1970; Brown & Haselkorn, 1971; Kochert, 1971; Grierson & Loening, 1972), this component probably represents a large cytoplasmic rRNA precursor molecule.

Kinetics of labelling in vivo

To gain some insight into the interrelationships of the chloroplast RNA components labelled *in vivo*, the kinetics of labelling of the individual RNA species were calculated from the results shown in Fig. 6. From these kinetic data, shown in Fig. 9, we conclude the following: (i) there is a time-lag in the appearance of $^{32}P_i$ into the chloroplast rRNA species of molecular weight 1.05×10^6 and 0.56×10^6 compared with the RNA species of molecular weight 2.7×10^6, 1.2×10^6 and 0.65×10^6. This time-lag is especially marked in the case of the 0.56×10^6-molecular-weight rRNA. Assuming that all these RNA species are synthesized from the same nucleotide precursor pool, this result suggests that macromolecular precursors are involved in the pathway of biosynthesis of the chloroplast rRNA species. (ii) There is an approximately exponential decline with time in the percentage of the total radioactivity in the 2.7×10^6-molecular-weight RNA. (iii) The percent of the total radioactivity in the RNA species of molecular weight 1.2×10^6 and 0.56×10^6 remains approximately constant between 40min and 4h labelling and thereafter declines. These RNA species cannot be degradation or aggregation products of the chloroplast rRNA species since they are labelled before the latter.

There are difficulties in interpreting the results obtained in this type of experiment which stem from

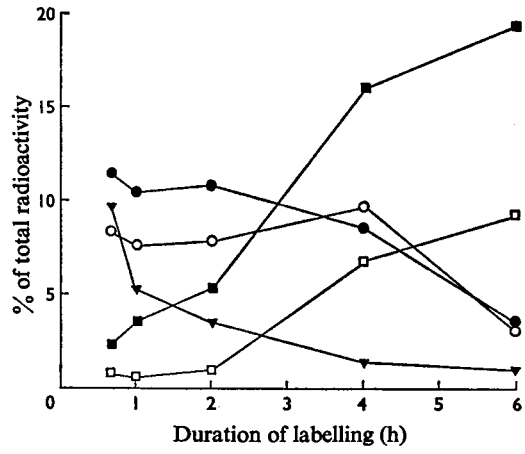

Fig. 9. *Kinetics of labelling of chloroplast RNA species during continuous labelling of leaves with* $^{32}P_i$

Values were calculated from the gel-electrophoretic fractionations shown in Fig. 6. Each point represents the radioactivity in a specific RNA species expressed as a percentage of the total radioactivity present in the gel (omitting incorporation into DNA). RNA components shown have molecular weight: ▼, 2.7×10^6; ●, 1.2×10^6; ■, 1.05×10^6; ○, 0.65×10^6; and □, 0.56×10^6. The difference between the summation of the radioactivity at each time-point on the graph and the total radioactivity is accounted for by the polydisperse RNA and the 0.47×10^6-molecular-weight component.

the nature of the tissue under investigation. We estimate that it takes more than 6h for the supplied $^{32}P_i$ to equilibrate with the chloroplast nucleotide RNA precursor pool, since the rate of incorporation of $^{32}P_i$ into chloroplast total nucleic acid increases up to at least this time. If, as seems likely from their kinetics of labelling, the chloroplast rRNA species are derived from macromolecular precursors, and the time required for the processing of these precursors is appreciable compared with the duration of labelling with $^{32}P_i$, then the precursors will attain a higher specific radioactivity than the newly synthesized products to which they give rise. Hence in this situation steady-state precursor–product kinetics will not be found. Bearing this in mind and assuming that chloroplast rRNA species are derived from precursors of higher molecular weight than the mature rRNA species, the data shown in Fig. 9 are consistent with the 1.2×10^6- and 0.65×10^6-molecular-weight RNA species being precursors to the 1.05×10^6- and 0.56×10^6-molecular-weight rRNA species respectively.

The high-molecular-weight rRNA species of the large and small ribosomal subunits of both mammals

and bacteria are synthesized in a 1:1 molar ratio (Maden, 1971; Mangiarotti *et al.*, 1968; Avery & Midgley, 1971). If this is the case in chloroplasts, as suggested by Ingle (1968), and if synthesis of the rRNA species proceeds via the 1.2×10^6- and 0.65×10^6-molecular-weight precursors, then the value of the ratio:

$$\frac{\frac{A}{1.2}+\frac{B}{1.05}}{\frac{C}{0.65}+\frac{D}{0.56}}$$

in which A, B, C and D represent the radioactivity in the RNA components of molecular weight 1.2×10^6, 1.05×10^6, 0.65×10^6 and 0.56×10^6 respectively, should theoretically be 1. The value of this ratio is independent of both the absolute and relative rates of processing of the precursors, and of the absolute amount of $^{32}P_i$ incorporation in any one sample. The experimentally determined values of this ratio, shown in Table 1, were calculated from the results presented in Fig. 6. The values are close to 1 for all of the RNA samples extracted between 40min and 6h labelling. This fact supports, but does not prove, that the chloroplast rRNA species of molecular weight 1.05×10^6 and 0.56×10^6 are synthesized in a 1:1 molar ratio via precursors of molecular weight 1.2×10^6 and 0.65×10^6 respectively.

Comparison of RNA synthesis in vitro and in vivo

To compare the rRNA species synthesized *in vivo* with those synthesized *in vitro* chloroplasts were isolated from leaves labelled with $^{32}P_i$ for 1h, then incubated *in vitro* with [^{3}H]uridine for 45min. The nucleic acid was extracted, fractionated in gels and the gels were analysed for ^{32}P (the label *in vivo*) and ^{3}H (the label *in vitro*). The result (Fig. 10) shows that the major discrete RNA synthesized *in vitro* co-electrophoreses exactly with the RNA of molecular weight 2.7×10^6 synthesized *in vivo*. Similarly, the minor RNA species of molecular weight 1.2×10^6 and 0.47×10^6 synthesized *in vitro* co-electrophoreses with their counterparts synthesized *in vivo*. Our interpretation of this result is that isolated chloroplasts have similar RNA biosynthetic capabilities as chloroplasts in excised leaves, but in the former system post-transcriptional modification is prematurely arrested. For reasons which are not clear, we have on no occasion observed the synthesis of an RNA species *in vitro* with a mobility corresponding to a molecular weight of 0.65×10^6, that of the proposed 0.56×10^6-molecular-weight rRNA precursor. In this respect it is interesting to note that Pettijohn *et al.* (1970) were able to demonstrate the synthesis of the precursor to 23S rRNA in extracts of *E. coli*, but not that of the precursor to 16S rRNA, whereas both of these precursor molecules are synthesized *in vivo* (Adesnik & Levinthal, 1969; Dahlberg & Peacock, 1971).

The chloroplast RNA of molecular weight 2.7×10^6 synthesized both *in vivo* and *in vitro* shows kinetics of labelling consistent with it being a precursor molecule containing sequences of both the 1.05×10^6- and 0.56×10^6-molecular-weight rRNA species (Fig. 9*a*). However, since any RNA species of unspecified function with a high rate of turnover would be expected to show similar kinetics of labelling, it remains an open question as to the nature and function of this 2.7×10^6-molecular-weight RNA. It is noteworthy that a rapidly labelled RNA species of similar molecular weight has recently been demonstrated in the blue–green alga *Anacystis nidulans* (Szalay *et al.*, 1972).

We have no evidence of an interrelationship between the chloroplast rapidly labelled RNA of molecular weight 0.47×10^6 and the proposed rRNA macromolecular precursors. Leaver & Ingle (1971) showed that spinach chloroplast rRNA of molecular weight 1.05×10^6 is unstable at room temperature in the presence of EDTA and/or in the absence of

Table 1. *Ratios of radioactivity in chloroplast rRNA species and their proposed precursors during continuous labelling of leaves with $^{32}P_i$*

Values were calculated from the gel-electrophoretic fractionations shown in Fig. 6. The numbers in parentheses refer to $10^{-6}\times$molecular weight of the respective RNA species, i.e. (c.p.m. 1.2) represents the radioactivity in the 1.2×10^6-molecular-weight RNA. For further explanation see the text.

Duration of labelling	Value of ratio $\frac{\frac{\text{(c.p.m. 1.2)}}{1.2}+\frac{\text{(c.p.m. 1.05)}}{1.05}}{\frac{\text{(c.p.m. 0.65)}}{0.65}+\frac{\text{(c.p.m. 0.56)}}{0.56}}$
40min	0.87
1h	0.85
2h	1.03
4h	0.82
6h	1.01

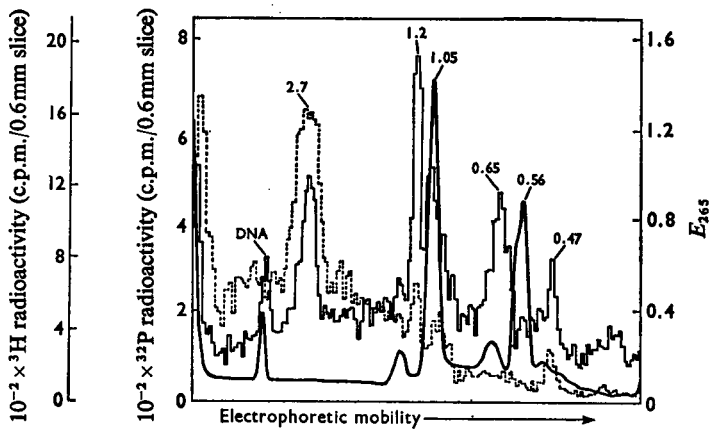

Fig. 10. *Gel-electrophoretic fractionation of chloroplast RNA species synthesized in vivo and in vitro*

Leaves were excised from 14-day-old plants and labelled via their petioles for 1 h with $^{32}P_i$. Chloroplasts were isolated as described under 'Methods and procedure' and resuspended in Tricine–$MgCl_2$–KCl medium. Chloroplast suspension (1 ml, containing 98 μg of chlorophyll) was incubated with illumination with 10 μCi of [^{3}H]uridine for 45 min at 20°C. Nucleic acid extraction, gel-electrophoretic fractionation and radioactivity determination were performed as described under 'Methods and procedure'. ▬ E_{265}; —— (histogram), ^{32}P radioactivity; ----, ^{3}H radioactivity. RNA components are referred to as 10^{-6} × molecular weight.

bivalent metal ions, and degrades into specific fragments, one of which has a molecular weight of approximately 0.47×10^6. It is unlikely that the labelled 0.47×10^6-molecular-weight RNA described in the present paper is derived from the 1.05×10^6-molecular-weight rRNA, since it acquires a high specific radioactivity before the 1.05×10^6-molecular-weight rRNA is labelled (Fig. 6). Grierson & Loening (1972) demonstrated that the properties of rapidly labelled RNA from mung-bean leaves differed in two respects from that of the roots: (i) the leaves contained a component of molecular weight 0.45×10^6 that specifically hybridized to the ribosomal genes of total mung-bean DNA and which was not demonstrable in roots; (ii) leaves, but not roots, contained a 2.9×10^6-molecular-weight component which was partially resolved on gels from a 2.7×10^6-molecular-weight component of roots. Grierson & Loening (1972) suggest that the 0.45×10^6-molecular-weight component is a piece of 'excess' RNA cleaved from the 2.9×10^6-molecular-weight component which they assume to be the initial cytoplasmic rRNA precursor in leaves. In view of the results presented here, an alternative interpretation of the results of Grierson & Loening (1972) is that the 0.45×10^6-molecular-weight RNA is a chloroplast component, analogous with the 0.47×10^6-molecular-weight RNA. Were this component derived from the ribosomal genes in chloroplast DNA it might be expected to hybridize to total DNA of buoyant density in CsCl characteristic of cytoplasmic rRNA genes in nuclear DNA, since Ingle *et al.* (1970) have shown that ribosomal genes in nuclear DNA hybridize to both cytoplasmic and chloroplast rRNA species. Similarly, the 2.9×10^6-molecular-weight component of Grierson & Loening (1972) may be analogous to the 2.7×10^6-molecular-weight RNA species of chloroplast origin described here.

There is a strong similarity between the proposed mechanism of chloroplast rRNA synthesis described here and that described for blue–green algae and bacteria. This similarity extends to the kinetics of labelling of the two rRNA species, for it has been shown both for *Anacystis nidulans* and *E. coli* that the rate of processing of the precursor to the 0.56×10^6-molecular-weight rRNA is slower than that of the precursor to the 1.1×10^6-molecular-weight rRNA (Szalay *et al.*, 1972; Adesnik & Levinthal, 1969; Dahlberg & Peacock, 1971). These findings support the contention that prokaryotic organisms and chloroplasts are closely related in evolution.

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