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In Organello Transcription in Maize Mitochondria and Its Sensitivity to Inhibitors of RNA Synthesis[1]

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ABSTRACT

Purified mitochondrial preparations from etiolated maize shoots support the incorporation of radioactivity from labeled UTP into RNA. The incorporation is linear with time for up to 2 hours, shows Michaelis-Menton kinetics with respect to the concentration of the labeled substrate, UTP, and has salt and pH optima which are different than those previously reported for RNA synthesis by isolated chloroplasts. When a crude mitochondrial preparation is subjected to isopycnic sucrose gradient centrifugation, the bulk of the RNA synthetic activity co-sediments with mitochondrial marker enzymes and with the mitochondrial 26S and 18S rRNAs. Maize mitochondrial RNA synthesis is prevented by actinomycin D and ethidium bromide but unaffected by α-amanitin. It is strongly inhibited by rifampicin at concentrations which have no effect on nuclear and chloroplast RNA synthesis, but only moderately inhibited by rifampicin at concentrations which completely inhibit bacterial RNA synthesis. The optimization, cell fractionation, and inhibitor data all suggest that contaminating organelles and bacteria do not contribute appreciably to the RNA synthesis in purified mitochondrial preparations.

Mitochondrial genetic systems in higher plants differ from those of other organisms in a number of important respects. Plant mtDNAs are considerably more complex than those of animals and fungi (29) and are unique in their organization in that their sequences are distributed over sets of large recombining circular molecules, each of which may contain only a portion of the entire mitochondrial genetic complement (29). The synthesis of the α-subunit of the F_1 component of the ATPase (7, 20), the employment of a unique genetic code (13), and the presence of a 5S rRNA species (9, 24) constitute further differences between the mitochondrial systems of plants and other organisms.

Although our knowledge of the physical structure and information content of plant mtDNA has increased greatly in recent years, relatively little is known about the mechanisms through which this information is expressed. We have begun a study of plant mtDNA expression by investigating the characteristics of RNA synthesis by isolated maize mitochondria. Isolated organelles have been used successfully to study the transcription and processing of RNA in chloroplasts of higher plants (1, 21, 30), and in the mitochondria of yeast (3, 17, 27) and mammals (2, 14, 15, 22). We have already employed this approach to demonstrate the DNA independent mode of synthesis of a set of novel RNA species specific to the mitochondria of maize plants possessing the S-type male-sterile cytoplasm (11). We report here studies aimed at determining the optimal conditions and inhibitor sensitivities of DNA-dependent RNA synthesis in mitochondria isolated from maize plants with fertile (N) cytoplasm. We show that RNA synthesis by isolated maize mitochondria possesses several features that distinguish it from RNA synthesis by isolated nuclei and chloroplasts.

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MATERIALS AND METHODS

Materials. *Zea mays* L. inbred B73Ht seed was obtained from Mike Brayton Seeds, Ames, IA. Pyruvate kinase was from Boehringer Mannheim, Canada, and radioactive compounds were supplied by ICN Biomedicals, Canada.

Isolation of Mitochondria. Mitochondria were isolated from 5 to 7 d old etiolated maize shoots following established procedures (12, 23). All manipulations were done at 4°C. Finely chopped tissue was homogenized in 3 *v* of homogenization buffer (0.5 M mannitol, 10 mM Tes, pH 7.2, 1 mM EGTA, 0.2% [w/v] BSA, 0.05% [w/v] cysteine) using a mortar and pestle. The homogenate was passed through two layers of Miracloth (Calbiochem) and centrifuged at 1,000*g* for 10 min to remove cell debris. The mitochondria were collected from the supernatant by centrifugation at 12,000*g* for 10 min. The crude mitochondria were resuspended in 0.5 *v* homogenization buffer and the 1,000*g* centrifugation was repeated to remove residual cell debris. This supernatant was layered onto 2 *v* of sucrose cushion buffer (0.6 M sucrose, 10 mM Tes, pH 7.2, 20 mM EDTA) followed by centrifugation at 10,000*g* for 20 min. The resulting pellet was resuspended in a small volume of sucrose cushion buffer and layered onto sucrose gradients containing 0.1% (w/v) BSA, 100 mM Tricine (pH 7.2), 10 mM EGTA, and centrifuged at 100,000*g* for 30 min in a Beckman SW 41 Ti or SW 28 rotor. Typically, 20 to 60% (w/v) linear sucrose gradients were employed but for some experiments 30 to 60% (w/v) linear or 30/40/50/60% (w/v) step gradients were used. The mitochondria form a wide band at about 43% sucrose on a linear gradient or a sharp band at the 30/40% sucrose interface on a step gradient. The mitochondria were removed from the gradients either by fractionation through the bottom of the tube or by drawing into a Pasteur pipette through the top of the tube, slowly diluted with sucrose cushion buffer over a 15 min period, and collected by centrifugation at 12,000*g* for 10 min. Yields were typically 20 to 40 μg mitochondrial protein per gram starting material.

***In organello* Labeling of Mitochondrial RNA.** Purified mitochondria were resuspended at a concentration of 0.5 to 2 mg mitochondrial protein per milliliter in 0.05 to 0.5 ml standard incubation buffer (3 mg/ml BSA, 60 mM mannitol, 20 mM Tris phosphate [pH 7.3], 10 mM potassium phosphate, 150 mM KCl, 10 mM $MgCl_2$, 5 mM sodium succinate, 5 mM P-enolpyruvate, 20 μg/ml pyruvate kinase, 1 mM EGTA, 2.5 mM ATP, 0.3 mM CTP, 0.3 mM GTP, 0.1 mM UTP). Adjustments to the standard incubation buffer are indicated in the figures. After a 10 min preincubation, the labeling of mitochondrial RNA was initiated

by the addition of [5,6-^{3}H] or [α-^{32}P]UTP or [5,6^{-3}H]uridine to a final specific radioactivity of 1 to 3 Ci/mmol and incubated at 23°C for 60 min unless otherwise stated in the figures. The labeling reaction was terminated by adding 10 vol ice-cold 100 μg/ml ethidium bromide, 0.3 M sucrose, 5 mM Tes (pH 7.2), 5 mM EDTA, 2.5% (w/v) sodium pyrophosphate and collecting the mitochondria by centrifugation at 10,000*g* for 5 min. The pelleted material was lysed by resuspending it in 2% (w/v) SDS, 10 mM Tes (pH 7.2), 0.2% (v/v) diethyl pyrocarbonate, 10 mM EDTA, 2.5% (w/v) sodium pyrophosphate. The total radioactivity incorporated was determined by acid precipitating a portion of the lysate with cold 10% (w/v) TCA. The precipitated material was collected on a nitrocellulose filter (Millipore Type HA, 0.45 μ), washed five times with 5 ml cold 10% (w/v) TCA, 2.5% (w/v) sodium pyrophosphate, and washed twice with 95% ethanol. The filters were air dried several hours before counting in a scintillation counter in 5 ml Aquasol (New England Nuclear). A second portion of the lysate was assayed for protein content in order for the amount of incorporated radioactivity to be expressed per milligram of mitochondrial protein.

Purification and Electrophoretic Separation of Mitochondrial RNA. Products of the *in organello* labeling reactions were purified by making the mitochondrial lysates 1 mM in aurintricarboxylic acid followed by phenol extraction, ethanol precipitation, gel electrophoresis, gel staining, and autoradiography as previously described (11). The gels contained 1% (w/v) agarose, 5 M urea, 1 mM aurintricarboxylic acid in electrophoresis buffer (0.09 M Tris, 0.09 M boric acid, 2 mM EDTA). Because urea undergoes thermal degradation, it is necessary to keep the urea and agarose as separate solutions until the agarose has been melted. This was done by adding an equal volume of 10 M urea to a solution containing melted 2% (w/v) agarose in 2× concentrated electrophoresis buffer. Aurintricarboxylic acid was then added to 1 mM. Since the high urea content prevents gelation of the agarose at 23°C, the gels were allowed to solidify at 4°C for at least 12 h before use.

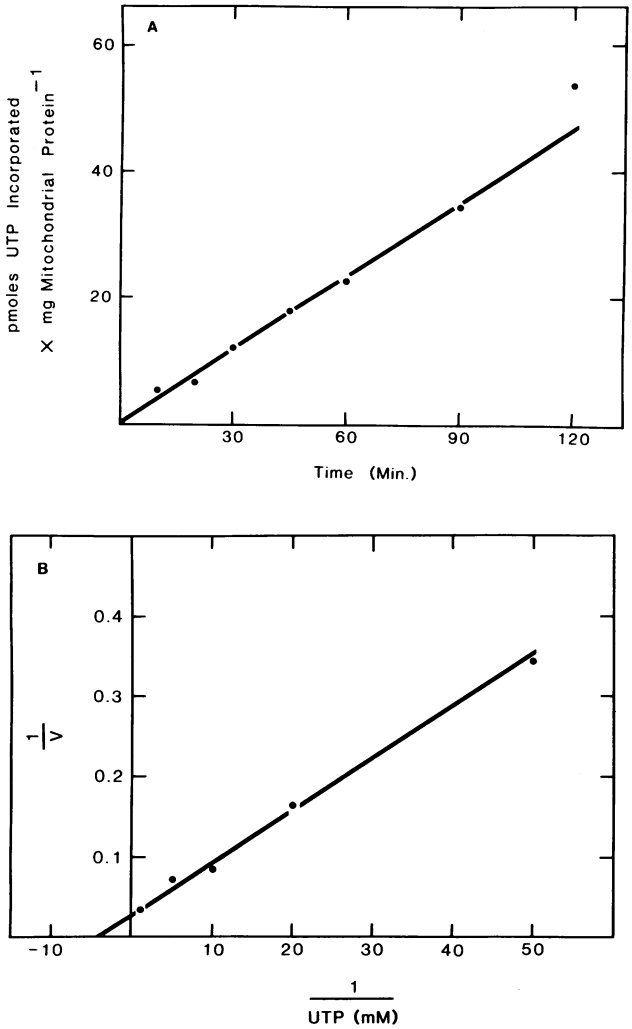

FIG. 1. Incorporation of [5,6-^{3}H]UTP by isolated maize mitochondria into acid insoluble material was determined with respect to time (A) and UTP concentration (B) as described in "Materials and Methods." In (B), the data is presented as a Lineweaver-Burk plot where V is expressed as pmol UTP incorporated per mg mitochondrial protein per 30 min.

Table I. *Effect of Reaction Mixture Components on RNA Synthesis by Isolated Maize Mitochondria*

The RNA synthesis activity of isolated maize mitochondria was determined as described in "Materials and Methods" using the standard reaction mixture with the indicated omissions or additions.

Incubation Conditions	[^{3}H]UTP Incorporation
	%
Standard	100
– [^{3}H]UTP, + [^{3}H]uridine	10
– Succinate	45
– GTP	62
– ATP	44
– Pyruvate kinase, p-enolpyruvate	63
– $MgCl_2$	19
– Succinate, + α-ketoglutarate, 5 mM	88
+ RNase, 1 μg/ml	111
– Energy[a]	16
– Energy[a], + acetate, 10 mM	13

[a] Succinate, ATP, pyruvate kinase, and p-enolpyruvate omitted.

Enzyme Assays. The mitochondrial marker enzymes succinate:Cyt *c* reductase and malate dehydrogenase were assayed at room temperature using slight modifications of published techniques (8, 10). The succinate induced reduction of Cyt *c* by succinate:Cyt *c* reductase was followed at 550 nm in an assay mixture containing 50 μM Cyt *c*, 9 mM sodium succinate, 24 mM sodium phosphate (pH 7.6), 5 mM NaN_3, 0.2 mM EDTA. For malate dehydrogenase the oxalacetate stimulated oxidation of NADH was followed at 340 nm in an assay mixture containing 0.1 mM NADH, 0.5 mM sodium oxalacetate, 17 mM sodium phosphate (pH 7.0), 5 mM NaN_3, 0.1% (w/v) EDTA.

RESULTS

RNA Synthesis in Isolated Mitochondrial Fractions from Maize. When mitochondria isolated from dark grown maize shoots are incubated in a mixture that includes the four nucleoside triphosphates, an oxidizable substrate and an ATP regenerating system, they support the incorporation of radioactivity from labeled UTP into acid insoluble material. The product of this reaction is RNA since the purified radiolabeled product is hydrolyzed completely by RNase A. The kinetics of this *in organello* RNA synthesis reaction are shown in Figure 1. The incorporation can remain linear with time for up to 120 min (Fig. 1A), and is linear with protein concentration over the range of 1 to 8 mg/ml (not shown). The rate of UTP incorporation is about 10 pmol UTP incorporated per mg mitochondrial protein in a 30 min incubation at 100 μM UTP (Fig. 1A).

A Lineweaver-Burk plot of the dependance of the incorporation on UTP concentration is shown in Figure 1B. The reaction shows typical Michaelis-Menton type kinetics with an apparent

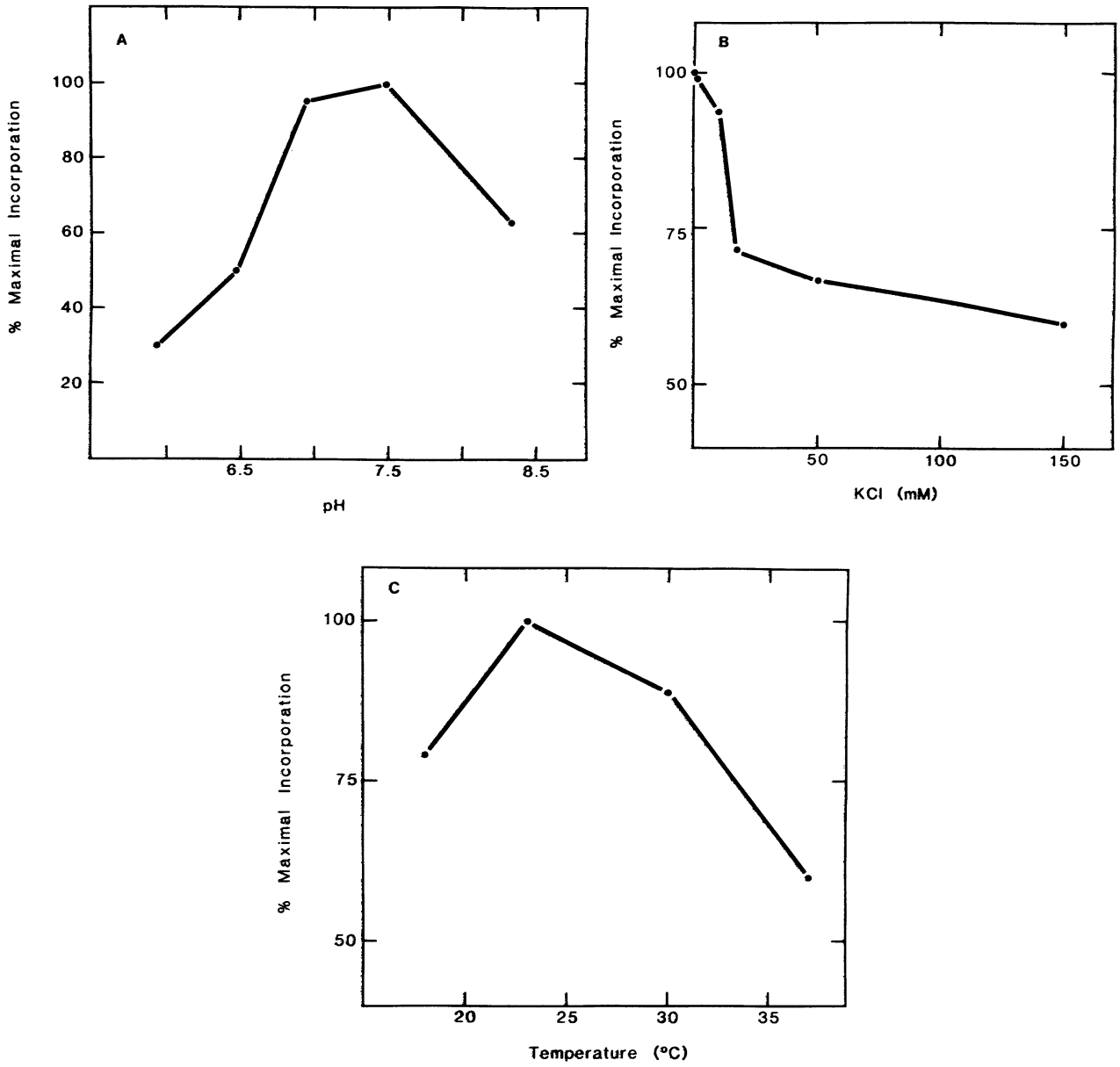

FIG. 2. Effect of varying pH (A), KCl concentration (B), and temperature (C) on the incorporation of radiolabeled UTP into acid insoluble material by isolated maize mitochondria was determined. In (B), the potassium phosphate buffering system of the standard RNA synthesis reaction was omitted. In addition, sucrose was added to those reactions containing less than 150 mM KCl to maintain a constant osmolarity.

K_m of 200 μM UTP (Fig. 1B). When [^{3}H]uridine is used as the radioactive precursor, the incorporation expressed as pmol substrate/mg mitochondrial protein, is one-tenth that obtained when UTP is the labeled substrate (Table I). Thus, UTP is utilized considerably more efficiently than uridine for RNA synthesis by isolated maize mitochondrial fractions.

The dependence of the rate of RNA synthesis on various components of the reaction mixture is also shown in Table I. Elimination of the oxidizable substrate, one of the triphosphates or the ATP regenerating system results in an approximately 50% decrease in incorporation. As in other isolated organelle systems (15, 17, 30) Mg^{2+} is essential. Elimination of this cofactor results in an over 80% drop in incorporation. The system shows at least a partial specificity for succinate as the oxidizable substrate, since α-ketoglutarate is not as effective in supporting the reaction. The inclusion of RNase A has no effect, indicating that the product of the reaction remains enclosed in an RNase impermeable compartment, presumably the mitochondrion. As with maize mitochondrial protein synthesis (12), acetate, in the absence of an additional energy source, does not stimulate the reaction, suggesting that contaminating bacteria do not contribute appreciably to the incorporation.

pH, K^+, and Temperature Optima for Mitochondrial RNA Synthesis. Several experiments were performed to determine the optimal conditions for RNA synthesis by isolated mitochondria and to compare these to those of other *in organello* systems. The effect of varying the pH of the incubation medium is shown in Figure 2A. The amount of incorporation varies approximately 4 fold over the pH range 6.0 to 8.5, and attains a maximum value between pH 7.0 and 7.5. This pH optimum is comparable to that found for RNA synthesis by isolated yeast (17) and human (15) mitochondria, but different from that found for isolated spinach chloroplasts and nuclei (30). Potassium ions inhibit incorporation (Fig. 2B), an effect again similar to that observed

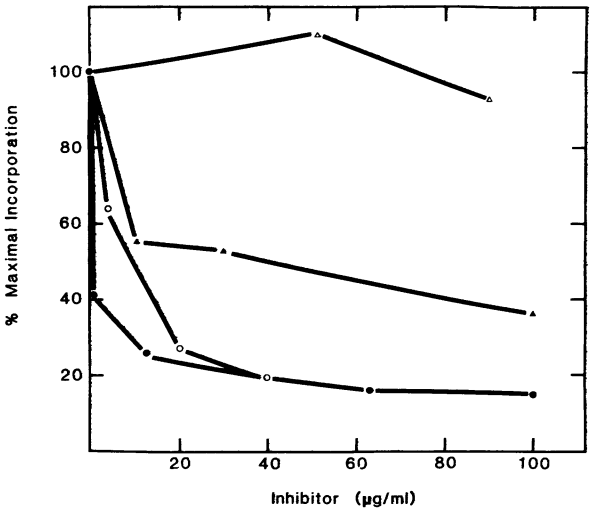

FIG. 3. Isolated maize mitochondria were incubated as described in "Materials and Methods" in reaction mixtures containing increasing concentrations of actinomycin D (●), ethidium bromide (○), rifampicin (▲), and α-amanitin (△).

in other mitochondrial systems (15) but different from that observed in isolated chloroplasts (25). Temperature has a moderate effect on incorporation (Fig. 2C), with maximal activity occurring at about 25°C.

Effects of Inhibitors of RNA Synthesis on Mitochondrial Incorporation. The effects on the reaction of increasing concentrations of various inhibitors of DNA-dependent RNA synthesis are shown in Figure 3. Incorporation is strongly inhibited by low concentrations of both ethidium bromide (20 μg/ml) and actinomycin D (12 μg/ml), indicating that the observed RNA synthesis is DNA-dependent. Rifampicin is also partially inhibitory, although inhibition is only moderate at concentrations (10–30 μg/ml) which completely inhibit RNA synthesis in bacteria (0.01 μg/ml; 16). Since RNA synthesis by isolated maize chloroplasts and etioplasts is unaffected by rifampicin concentrations which strongly inhibit this system (100 μg/ml; 6), the concentration dependence of the rifampicin inhibition distinguishes the maize mitochondrial RNA synthesis from both plastid and bacterial RNA synthesis. α-Amanitin, at concentrations up to 90 μg/ml, has no effect on the *in organello* RNA synthesis reaction. This indicates that the nuclear RNA polymerases II and III do not make detectable contributions to the incorporation, since, in plants, these enzymes are inhibited 50% by 0.01 to 0.05 μg/ml and 10 to 100 μg/ml α-amanitin, respectively (19). The observations that purified nuclei are very inefficient at incorporating UTP into RNA under mitochondrial incubation conditions (11) and that addition of RNase to the incubation mixture does not affect incorporation (Table I) provide further evidence that a significant nuclear contribution does not occur. Data from both the inhibitor and optimization experiments, therefore, indicate that mitochondria are primarily responsible for the observed RNA synthesis.

Co-Purification of RNA Synthesis Activity with Mitochondria.

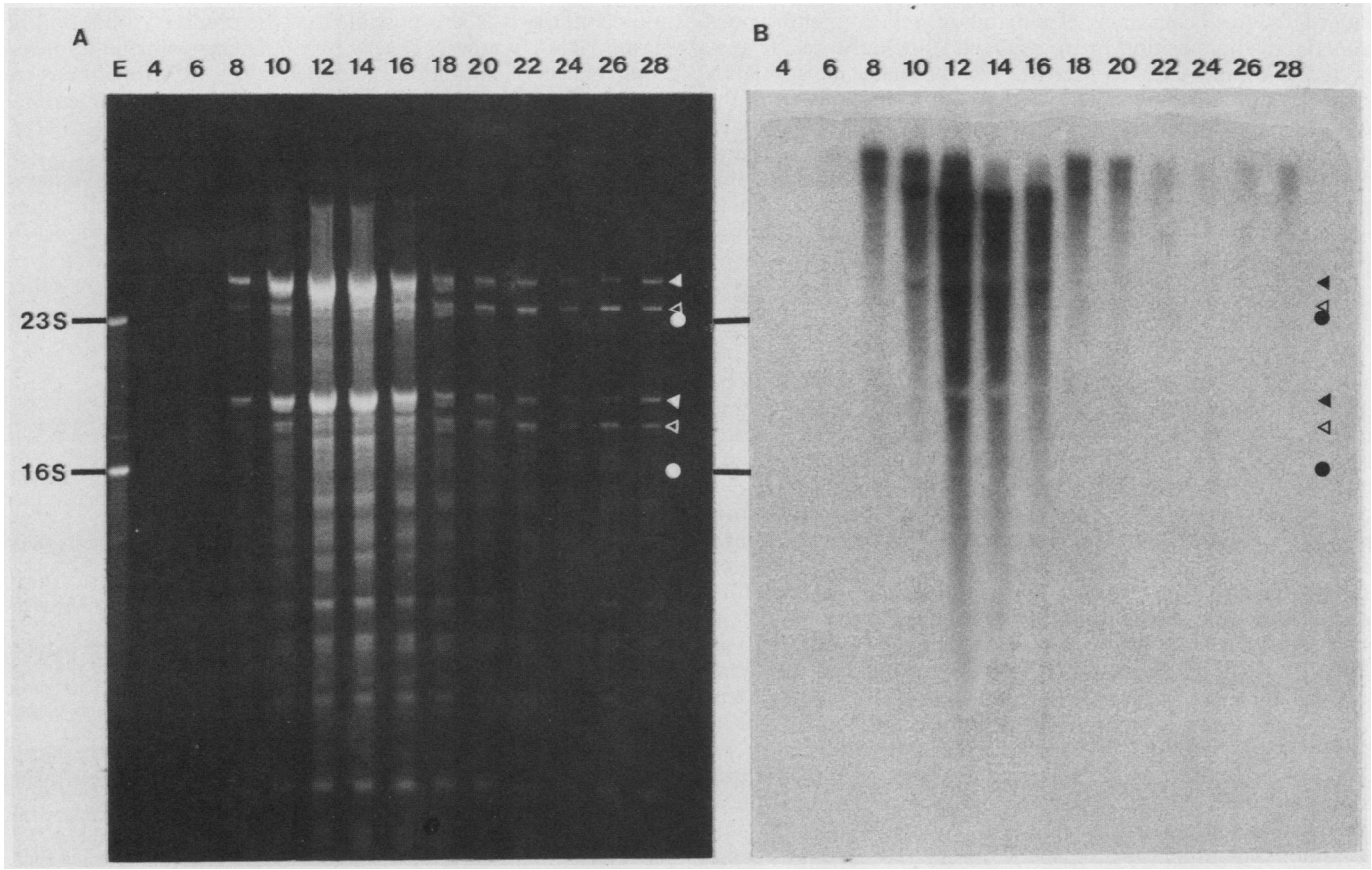

FIG. 4. Ethidium bromide staining (A) and autoradiographic (B) patterns of RNA isolated from some even numbered fractions (lanes 4–28) of a linear 30% (w/v) to 60% (w/v) sucrose gradient. The RNA was labeled *in organello* with [α-^{32}P]UTP, purified and subjected to electrophoresis as described in "Materials and Methods," using *Escherichia coli* rRNA as mobility marker (lane E). The mitochondrial (◀), cytoplasmic (◁) and plastid (●) rRNAs are indicated. Gradient fractions 1 and 28 contained 60 and 30% sucrose, respectively.

More direct evidence that the RNA synthesis is due to mitochondria and not to contaminating organelles was obtained by first subjecting mitochondria, purified from a tissue homogenate by differential centrifugation, to isopycnic centrifugation on a 30 to 60% (w/v) linear sucrose gradient. Individual gradient fractions were then assayed for *in organello* RNA synthesis activity. When the purified labeled products were separated on a 1% (w/v) agarose gel containing 5 M urea, the bulk of the ethidium bromide staining material was found in fractions 12 to 14 (Fig. 4A). The predominant RNA species in these fractions are the mitochondrial 26S and 18S rRNAs (solid arrows); these fractions contain relatively little contaminating cytoplasmic (open arrows) or plastid (circles) rRNAs. As the density of the fractions decreases (fractions 20–28), the total amount of RNA present also decreases and the relative amounts of mitochondrial and cytoplasmic rRNAs become more equal. An autoradiograph of this gel (Fig. 4B) indicates that fractions 12 and 14 contain the peak *in organello* RNA synthesis activity, which thus co-purifies tightly with the mitochondrial rRNAs. Two of the major products of RNA synthesis by these fractions have mobilities similar to those of the 26S and 18S mitochondrial species seen in the stained gel.

The mitochondrial nature of the peak fractions was confirmed by assaying for the mitochondrial inner membrane and matrix marker enzymes succinate:Cyt *c* reductase and malate dehydrogenase, respectively (data not shown). Both enzymes sedimented as a broad peak that extended from fractions 11 to 17 and corresponded to the 26S and 18S RNA peak. Maximal activities of the enzymes occurred in fraction 14 at a sucrose concentration (43%) consistent with previous reported values for the bouyant density of maize mitochondria (12).

Although fractions 22 to 24 appeared to contain etioplasts, as judged by the occurrence of a band of yellow membranous material at this position in the gradient, they only weakly supported RNA synthesis (Fig. 4B). The products of this RNA synthesis were diffuse in size and different in mobility from the mitochondrial products. These observations suggest that even prior to the sucrose gradient purification step, plastid contaminants make only a small contribution to the transcriptional activity of mitochondrial preparations. Their contribution to RNA synthesis by gradient-purified mitochondrial preparations would therefore appear to be negligible.

DISCUSSION

We chose to develop an *in organello* RNA synthesis system as a first step in the characterization of the transcriptional process in plant mitochondria. The observation that RNA synthesis under the conditions employed here co-fractionates with mitochondrial enzymatic activity and produces RNAs similar in size to the major mitochondrial rRNA species provides strong evidence that isolated maize mitochondria support the incorporation of radiolabel from UTP into RNA. In the course of studies aimed at determining optimal conditions for this incorporation and the sensitivity of the reaction to transcriptional inhibitors, we have found several features which distinguish RNA synthesis in maize mitochondria from transcription in bacteria and in isolated nuclei and plastids. These distinctive characteristics collectively rule out an appreciable contribution from these potential contaminants to RNA synthesis by the mitochondrial fraction. These features can be summarized as follows:

Substrate Preference. UTP is utilized approximately 10 times more efficiently than uridine as a precursor for RNA synthesis by isolated maize mitochondria. Although under appropriate conditions, chloroplasts are capable of supporting the incorporation of UTP into RNA (1), isolated intact chloroplasts (21) and bacteria preferentially utilize uridine over UTP.

Ribonuclease Insensitivity. Inclusion of RNAse in incorporation mixtures strongly inhibits RNA synthesis by isolated spinach nuclei (30). The complete insensitivity of RNA synthesis by the maize mitochondrial fraction to exogenous ribonuclease is therefore an indication that nuclei or nuclear fragments do not make an appreciable contribution to the transcriptional activity. Similarly, it is unlikely that broken chloroplasts or mitochondria contribute significantly.

Size of Products. The major RNA products formed in isolated chloroplasts (21) and in bacteria, when uridine is used as precursor, have electrophoretic mobilities expected of 23S and 16S rRNA species. No abundant 23S or 16S sized RNAs are observed in the products of RNA synthesis by the mitochondrial fraction. Moreover, a heterogenous distribution of low mol wt RNAs such as those synthesized by isolated chloroplasts when UTP is used as precursor (1) is not observed in the mitochondrial products.

Rifampicin Sensitivity. Rifampicin partially inhibits the mitochondrial reaction at concentrations which have no effect on RNA synthesis in isolated maize nuclei or plastids (6), but which totally inhibit RNA synthesis in bacteria (16).

It has been observed that *in vivo* chloroplast rRNA synthesis in maize leaves and in crude plastid preparations is inhibited significantly by rifamycins while the activity of a more purified plastid preparation is not (4). The latter observation has been confirmed by others using partially purified plastid extracts (5, 18, 26, 28). From these results, it has been suggested (4) that during purification of the chloroplast DNA-dependent RNA polymerase activity, an initiation factor or a rifamycin-sensitive RNA polymerase of plastid origin was removed. The finding that RNA synthesis by isolated maize mitochondria is partially sensitive to rifampicin suggests to us that the rifamycin-sensitive polymerase activity associated with crude plastid preparations may not have been of plastid origin but was due to contaminating mitochondria. It is also possible that the observed inhibition of plastid rRNA synthesis *in vivo* by rifamycins was due to pleiotropic effects of inhibition of mitochondrial RNA synthesis by these antibiotics. Clearly, it would be desirable to determine whether a purified maize mitochondrial DNA-dependent RNA polymerase is sensitive to the antibiotic.

The system described here has already proven useful in demonstrating that the mitochondria of S-type cytoplasmic male sterile maize plants contain an autonomously replicating RNA plasmid system (11). This *in organello* mitochondrial RNA synthesis system might be employed in future studies on other aspects of plant mitochondrial gene expression, such as transcript maturation and transcriptional control.

LITERATURE CITED

1. ALTMAN A, BN COHEN, H WEISSBACH, N BROT 1984 Transcriptional activity of isolated maize chloroplasts. Arch Biochem Biophys 235: 26–33
2. BHAT KS, GR KANTHARAJ, NG AVADHANI 1984 Nature of steady-state and newly synthesized mitochondrial messenger ribonucleic acids in mouse liver and ehrlich ascites tumor cells. Biochemistry 23: 1695–1701
3. BOERNER P, TL MASON, TD FOX 1981 Synthesis and processing of ribosomal RNA in isolated yeast mitochondria. Nucleic Acids Res 9: 6379–6390
4. BOGORAD L, CLF WOODCOCK 1971 Rifamycins: the inhibition of plastid RNA synthesis *in vivo* and *in vitro* and variable effects on chlorophyll formation in maize leaves. *In* NK Boardman, AW Linnane, RM Smillie, eds, Autonomy and Biogenesis of Mitochondria and Chloroplasts. North-Holland, Amsterdam, pp 92–97
5. BOTTOMLEY W, HJ SMITH, L BOGORAD 1971 RNA polymerases of maize: partial purification and properties of the chloroplast enzyme. Proc Natl Acad Sci USA 68: 2412–2416
6. BOTTOMLEY W, D SPENCER, AM WHEELER, PR WHITFELD 1971 The effect of a range of RNA polymerase inhibitors on RNA synthesis in higher plant chloroplasts and nuclei. Arch Biochem Biophys 143: 269–275
7. BOUTRY M, M BRIQUET, A GOFFEAU 1983 The α subunit of a plant mitochondrial F_1-ATPase is translated in mitochondria. J Biol Chem 258: 8524–8526
8. BROWN GG, DS BEATTIE 1978 Formation of the yeast mitochondrial membrane. V. Differences in the assembly process of cytochrome oxidase and coenzyme QH_2: cytochrome *c* reductase during respiratory adaptation. Biochim Biophys Acta 538: 173–187
9. CUNNINGHAM RS, L BONEN, WF DOOLITTLE, MW GRAY 1976 Unique species of 5S, 18S, and 26S ribosomal RNA in wheat mitochondria. FEBS Lett 69:

116–122
10. DOUCE R, CA MANNELLA, WD BONNER JR 1973 The external NADH dehydrogenases of intact plant mitochondria. Biochim Biophys Acta 292: 105–116
11. FINNEGAN PM, GG BROWN 1986 Autonomously replicating RNA in mitochondria of maize plants with S-type cytoplasm. Proc Natl Acad Sci USA 83: 5175–5179
12. FORDE BG, RJC OLIVER, CJ LEAVER, RE GUNN, RJ KEMBLE 1980 Classification of normal and male-sterile cytoplasms in maize. I. Electrophoretic analysis of variation in mitochondrially synthesized proteins. Genetics 95: 443–450
13. FOX TD, CJ LEAVER 1981 The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell 26: 315–323
14. GAINES G, G ATTARDI 1984 Intercalating drugs and low temperatures inhibit synthesis and processing of ribosomal RNA in isolated human mitochondria. J Mol Biol 172: 451–466
15. GAINES G, G ATTARDI 1984 Highly efficient RNA-synthesizing system that uses isolated human mitochondria: new initiation events and *in vivo*-like processing patterns. Mol Cell Biol 4: 1605–1617
16. GALE EF, E CUNDLIFFE, PE REYNOLDS, MH RICHMOND, MJ WARING 1981 The Molecular Basis of Antibiotic Action, Ed 2. Wiley, London
17. GROOT GSP, N VAN HARTEN-LOOSBROEK, GJB VAN OMMEN, HLA PIJST 1981 RNA synthesis in isolated yeast mitochondria. Nucleic Acids Res 9: 6369–6377
18. GRUISSEM W, BM GREENBERG, G ZURAWSKI, DM PRESCOTT, RB HALLICK 1983 Biosynthesis of chloroplast transfer RNA in a spinach chloroplast transcription system. Cell 35: 815–828
19. GUILFOYLE TJ 1981 DNA and RNA polymerases. *In* PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 6. Academic Press, New York, pp 207–247
20. HACK E, CJ LEAVER 1983 The α-subunit of the maize F_1-ATPase is synthesized in the mitochondrion. EMBO J 2: 1783–1789
21. HARTLEY MR, RJ ELLIS 1973 Ribonucleic acid synthesis in chloroplasts. Biochem J 134: 249–262
22. KANTHARAJ GR, KS BHAT, NG AVADHANI 1983 Mode of transcription and maturation of ribosomal ribonucleic acid *in vitro* in mitochondria from Ehrlich ascites cells. Biochemistry 22: 3151–3156
23. KEMBLE RJ, RE GUNN, RB FLAVELL 1980 Classification of normal and male-sterile cytoplasms in maize. II. Electrophoretic analysis of DNA species in mitochondria. Genetics 95: 451–458
24. LEAVER CJ, MA HARMEY 1976 Higher plant mitochondrial ribosomes contain a 5S ribosomal ribonucleic acid component. Biochem J 157: 275–277
25. MIKULOVICH TP, IM KUKINA 1982 RNA synthesis in intact chloroplasts from excised pumpkin cotyledons: some characteristics of transcription and the effect of phytohormones. Biochem Physiol Pflanzen 177: 419–429
26. NARITA JO, KE RUSHLOW, RB HALLICK 1985 Characterization of a *Euglena gracilis* chloroplast RNA polymerase specific for ribosomal RNA genes. J Biol Chem 260: 11194–11199
27. NEWMAN D, N MARTIN 1982 Synthesis of RNA in isolated mitochondria from *Saccharomyces cerevisiae*. Plasmid 7: 66–76
28. POLYA GM, AT JAGENDORF 1971 Wheat Leaf RNA polymerases I. Partial purification and characterization of nuclear, chloroplast and soluble DNA-dependent enzymes. Arch Biochem Biophys 146: 635–648
29. PRING DR, DM LONSDALE 1985 Molecular biology of higher plant mitochondrial DNA. Int Rev Cytol 97: 1–46
30. SPENCER D, PR WHITFELD 1967 Ribonucleic acid synthesizing activity of spinach chloroplasts and nuclei. Arch Biochem Biophys 121: 336–345