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Variation in protein and RNA synthesis activity in isolated mitochondria of the developing rice (*Oryza sativa* L.) panicle

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Abstract We have studied variation in mitochondrial protein and RNA synthesis during the development of a specialized rice (*Oryza sativa* L.) reproductive organ in a bacteria-free environment. Mitochondria were prepared from the maturing panicle during microsporogenesis when meiosis occurred and from etiolated seedlings at two growth stages. We found (1) that there was no discernible qualitative difference among the polypeptides synthesized by these three mitochondrial samples; (2) that the quantity of proteins synthesized by panicle mitochondria was approximately 3 times that of the seedling mitochondria, while the two seedling samples exhibited only a minor quantitative difference; (3) that panicle and seedling mitochondria samples synthesized qualitatively the same RNA but at distinctly different rates and that more RNA products were synthesized by panicle than by seedling mitochondria. These results, taken together, suggest that either the regulation of mitochondrial transcription and translation or the copy number of mitochondrial DNA per mitochondrion change discretely in the developing panicle and consequently that the level of mitochondrial gene expression increases considerably during the development of the reproductive structure in rice.

Key words Mitochondria · Protein and RNA synthesis · *Oryza sativa* L. · Panicle

Introduction

The mitochondrial genomes of plants (ca. 200–2500 kb) are much larger and more complex than those of animals and fungi (ca. 15–78 kb) (Leaver and Gray 1982; Lonsdale 1984; Newton 1988; Levings and Brown 1989; Oda et al. 1992). The significance of this striking difference is obscured by the fact that the number of discrete polypeptides synthesized by isolated plant mitochondria does not differ appreciably from the number synthesized by isolated mitochondria of other organisms. In addition to previously identified proteins, 33 open reading frames, each with 60 or more amino acid residues of unknown function, have been identified from the complete sequence of a primitive plant mitochondrial genome, and 5 of these frames bear sequence similarity to counterparts whose functions are unknown in other plants (Oda et al. 1992). This result lends support to the suspicion that plant mitochondrial genomes, implicit from their large physical size, may encode more proteins than those which have thus far been identified. Recently, some 30 species of *in organello* translation products have been discerned in sugar beet mitochondria (Halldén et al. 1992).

Relative few studies have addressed the question of whether plant mitochondrial genomes encode, in addition to constitutively expressed genes, developmentally and/or environmentally regulated genes, or both. The synthesis of mitochondria-encoded polypeptides specific to a developmental stage or an organ has been documented only in the scutella of developing and germinating maize kernels (Newton and Walbot 1985) and in roots, leaves and flowers of sugar beet (Lind et al. 1991); differential expression of mitochondria-encoded proteins has been shown to occur in the same tissue during plant floral development (Conley and Hanson 1994). The relative proportion of several mitochondria-encoded polypeptides has been shown to alter during the aging of plant storage tissue (Forde et al. 1979), and tissue-specific regulation of a mitochondrial gene has been shown to be associated with the nuclear restoration of cytoplasmic male sterility in sunflower (Monéger et al. 1944). Isolated plant mitochondria have been shown not to synthesize a unique set of proteins in response to elevated temperatures (Nieto-Sotelo and Ho 1987; Dai et al. 1993b); bacteria were responsible for the synthesis of heat-shock proteins previously reported to oc-

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cur in plant mitochondrial samples (Cooper and Ho 1983; Nebiolo and White 1985; Sinibaldi and Turpen 1985).

Studies of protein synthesis by isolated rice mitochondria have underscored the critical importance of eliminating bacterial contamination; bacteria present even in numbers usually considered negligible often synthesize a significant amount of discrete [^{35}S]methionine-labeled polypeptides (Dai et al. 1991; 1993b). Having characterized a bacteria-free protein synthesis system with mitochondria isolated from aseptically-grown rice seedlings (Dai et al. 1993a), we investigated the possibility of an organ-specific alteration of protein synthesis by isolated rice mitochondria in a bacteria-free environment.

In addition to being regulated by the nuclear genome, the expression of the mitochondrial genome also appears to depend on its interactions with the chloroplast genome. The abundance of mitochondrial genes and their transcripts per cell were found to decrease co-linearly with a gradient of increasing photosynthetic competency in cells starting at the basal meristem and ending at the distal tip of wheat leaf (Topping and Leaver 1990). To minimize the chance of a similar influence by chloroplasts on the expression of mitochondrial genes in rice, we compared protein synthesis by mitochondria isolated from a developing rice panicle, a naturally occurring non-green tissue, with protein synthesis by mitochondria from the hypocotyl of etiolated seedlings at two growth stages.

We found no discernible qualitative difference among the proteins synthesized by these three mitochondrial samples, but the quantity of the polypeptides synthesized per mitochondrion-protein equivalent was approximately 3 times higher for panicle mitochondria than for seedling mitochondria. This discrete increase in protein-synthesis activity was accompanied by a comparable increase in RNA synthesis activity in isolated panicle mitochondria. These results suggest (1) that a sizable quantitative increase of mitochondrial gene expression occurred during rice panicle development; and (2) that such an increase was accomplished by a discrete change in either the regulation of mitochondrial transcription and/or translation or in DNA copy number per mitochondrion in panicle mitochondria.

Materials and methods

Preparation of rice seedlings

We used *Oryza sativa* cv 'Tainung 67', a *japonica* variety widely cultivated in Taiwan. The plants and seeds used in this study were cultivated and harvested at the Institute of Botany, Academia Sinica, Taipei. The details of the treatment of seeds, germination and growth of aseptic rice seedlings have been described previously (Dai et al. 1991).

Isolation of rice panicles

Developing panicles were isolated at a developmental stage when the meiotic divisions in microsporogenesis occurred. Rice plants visually determined to be at approximately this stage were cut in the field, immediately immersed in water and brought back to the laboratory. To obtain bacteria-free panicle mitochondria, we sterilized the entire surface of the whole plant by repeated and careful rubbing with 70% ethanol before removal of the sheaths and leaves. The developmental stage of each panicle was ascertained by measuring the distance between the pulvinus of the boot leaf and the pulvinus of leaf sheath (Wu 1967); those panicles for which this distance measured about 1 cm were collected for mitochondria isolation under aseptic conditions.

Isolation of bacteria-free rice mitochondria

Mitochondria were isolated from the collected panicles described above and from 8- and 14-day-old seedlings grown aseptically (Dai et al. 1991). The composition and sterilization of solutions/buffers, the pre-treatment of glass-/plasticware and the procedure of seedling mitochondria isolation and sucrose-gradient purification have been described in detail by Dai et al. (1991).

Protein synthesis by isolated mitochondria

Purified mitochondria, equivalent to 80 µg of mitochondrial protein, were incubated in a 250 µl reaction mixture at 30 °C for 60 min with constant rotatory shaking. The filter-sterilized reaction mixture contained 250 m*M* mannitol, 90 m*M* KCl, 10 m*M* Tricine-KOH (pH 7.2), 5 m*M* potassium phosphate (pH 7.2), 10 m*M* $MgCl_2$; 1 m*M* EGTA, 2 m*M* dithiothreitol, 1 m*M* GTP, 25 µ*M* each of 19 amino acids (excluding methionine), 225 p*M* [^{35}S]methionine (ca. 37000 GBg/m*M*); and neutralized energy-generating supplements consisting of either (1) 2 m*M* ADP, and 10 m*M* sodium succinate or (2) 6 m*M* ATP, 8 m*M* phosphocreatine and 4 units of creatine phosphokinase (Forde et al. 1978). Aliquots of 5 µl of this reaction mixture were diluted serially and plated on Luria's broth plates to determine the number of contaminating bacteria, if any. At the termination of the incubation period, 2.5 µl aliquots of the reaction mixture were used to determine the extent of [^{35}S]methionine incorporation into protein according to a previously described procedure (Dai et al. 1991, 1993a).

Analysis of products of protein synthesis *in organello*

After 60 min of incubation, 2.5 µl of protein-synthesis chasing solution (10 m*M* methionine and 1.8 m*M* each of the 19 other amino acids) were added; the incubation was continued at 30 °C for 15 min. The reaction was terminated by adding 1 ml of protein-synthesis stopping solution [0.4 *M* mannitol, 10 m*M* Tricine-KOH (pH 7.2), 10 m*M* methionine, 1 m*M* EGTA, and 1 m*M* each of six protease inhibitors: PMSF, TLCK, TPCK, *t*-epoxysuccinyl-L-leucylamido-(4-guanidino)-butane, pepstatin A and bestatin] to the reaction mixture. Mitochondria were pelleted at 12000 g for 10 min and then were either solubilized in 100 µl of sample loading buffer by heating at 95 °C for 2 min or were quick frozen in liquid nitrogen and stored at –80 °C for later analysis. The total mitochondrial lysate was analyzed by 15% (w/v) SDS-polyacrylamide (acrylamide: bisacrylamide = 150:1) gel electrophoresis. Gels were stained with Coomassie blue, destained till the protein bands were visible, immersed in 1 M sodium salicylate for 1 h and then dried on Whatman 3 MM filter paper. Positions of the protein size markers in the dried gels were inscribed on the filter paper, and the gels were then exposed to Kodak XAR-5 X-ray film with an intensifying screen at –80 °C.

RNA synthesis by isolated mitochondria

A previously described procedure for studying RNA synthesis by maize mitochondria (Finnegan and Brown 1986) was adopted with minor modifications. Purified rice mitochondria, equivalent to 200 µg mitochondrial protein, were preincubated at 25 °C for 5 min with shaking in a reaction mixture containing 150 m*M* KCl, 0.3% (w/v) bovine serum albumin, 60 m*M* mannitol, 20 m*M* TRIS-phosphate

(pH7.3), 10 m*M* potassium phosphate (pH7.3), 10 m*M* $MgCl_2$, 1 m*M* EGTA, 5 m*M* sodium succinate, 5 m*M* phosphoenolpyruvate, 10 units of pyruvate kinase, 2.5 m*M* ATP, 0.3 m*M* CTP, 0.1 m*M* GTP and 0.1 m*M* UTP. Three and seven-tenth MBg α-[^{32}P] GTP (14800 GBg/m*M*) and 1.1 MBg α-[^{32}P] UTP (14800 GBg/m*M*) were then added to start the RNA synthesis reaction. Actinomycin D, rifampicin, ampicillin or streptomycin was added as indicated in the legend of Fig. 3.

Analysis of products and rate of RNA synthesis *in organello*

The incubation of the RNA synthesis reaction at 25°C was clocked to begin after the addition of [^{32}P]UTP/GTP to the reaction mix. At 30-min intervals during incubation, mitochondria were pelleted and immediately lysed in 50 m*M* TES (pH 7.2), 10 m*M* EDTA, 0.2% (v/v) diethyl-pyrocarbonate and 2% (w/v) SDS. Mitochondrial RNA was extracted three times with phenol and then with chloroform. The RNA was precipitated overnight at –70°C with ethanol and was then analyzed on a 15% formaldehyde agarose gel (Maniatis et al. 1982).

To assay for [^{32}P]UTP/GTP incorporation, 1/20 volume of each RNA synthesis reaction sample was taken from the mitochondria lysate prior to phenol/chloroform extraction. Following dilution to a total volume of 100 µl, two 5-µl aliquots were spotted directly on a pair of filters (Whatman GF/C) to measure the total radioactivity in each reaction mix. [^{32}P]UTP/GTP-labeled RNA in the remaining 90-µl aliquot was precipitated onto a filter with 10% trichloroacetic acid in the presence of carrier tRNA (100 µg/ml). The filters were dried and counted in a liquid scintillation counter without scintillating fluid.

Results

The protein-synthesis characteristics of panicle mitochondria

Choices in selecting a non-green rice organ at a discrete developmental stage for the study of mitochondrial protein synthesis *in organello* was limited by the high silicon content and the porous, uneven surface of the rice plant (Kaufman 1955; Dayanandan et al. 1983), which together made it virtually impossible to isolate bacteria-free mitochondria from a tissue other than the developing panicle. This organ is tightly wrapped by tubular leaves before its eventual emergence from meristem. We timed the isolation of rice panicles to coincide with a discrete developmental stage in which meiosis of microsporogenesis occurred (Wu 1967).

The panicle mitochondria failed to synthesis polypeptides if acetate was used as the energy source; consistent with this result, no bacterial colony was obtained by plating the diluted protein synthesis reaction mix (Fig. 1, lane 3). In the presence of ADP, oxidizable substrate, succinate was efficiently utilized by panicle mitochondria to generate energy required for protein synthesis (Fig. 1, lane 1). An external ATP-regenerating system independent of the mitochondrial membrane was 32% less efficient in supporting protein synthesis by the isolated panicle mitochondria (Fig. 1, lane 2; Table 1). These results indicate (1) that the panicle mitochondria prepared under our experimental conditions were functionally active with apparently intact membranes; and (2) that developing panicle mitochondria could be isolated free of bacteria and therefore were suitable for *in organello* protein synthesis.

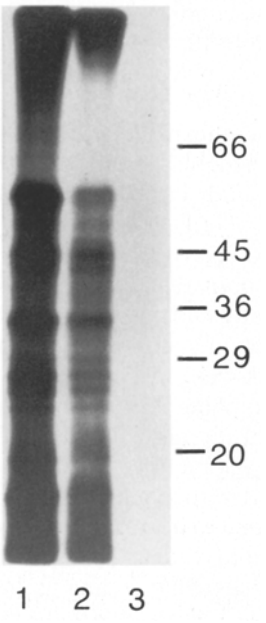

Fig. 1 Autoradiograph of [^{35}S]methionine-labeled polypeptides synthesized by rice mitochondria isolated from developing panicles during microsporogenesis when meiosis occurred. Experimental conditions for protein synthesis and product analysis are described in the Materials and methods. Sucrose-gradient-purified panicle mitochondria, equivalent to 80 µg mitochondrial protein, were used for each protein synthesis reaction. After 60 min of incubation, the mitochondria were recovered by centrifugation and solubilized. The lysate was then loaded in its entirety for analysis. The X-ray film was exposed for 24 h at –80°C with an intensifying screen. The energy source used for the three protein synthesis reactions was as follows: *lane 1*, 2 m*M* ADP and 10 m*M* sodium succinate; *lane 2*, 6 m*M* ATP, 8 m*M* phosphocreatine and 4 units of creatine phosphokinase, *lane 3*, 20 m*M* sodium acetate. Plating data indicated that all three of the 250 µl protein-synthesis reaction mixes contained no bacteria

Comparison of panicle and seedling mitochondria protein-synthesis patterns

The electrophoretic profile of the polypeptides synthesized by panicle mitochondria (Fig. 1, lanes 1 and 2) was the same as that which we had characterized previously for bacteria-free seedling mitochondria (Dai et al. 1993a). In the present study, two control mitochondria samples were isolated from 8- and 14-day old etiolated seedlings grown under aseptic conditions. To facilitate a direct comparison, the same amount of mitochondria were used to run all protein synthesis reactions and to subsequently load all gel wells for the analysis of the products. As shown in Fig. 2, the polypeptides synthesized by the panicle mitochondria contained the most [^{35}S]methionine label and the 14-day-old seedling mitochondria the least. In all three mitochondria samples, more [^{35}S]methionine-labeled polypeptides were synthesized when the membrane-dependent ATP-generating system (succinate/ADP) was used as the energy source (Fig. 2, lanes 1, 3, and 5) instead of the external ATP-regenerating system (Fig. 2, lanes 2, 4, and 6). By direct counting, 3.6 times more [^{35}S]methionine was shown to have been incorporated with the membrane-dependent

Table 1 Comparison of protein and RNA synthesis in isolated mitochondria of rice seedlings and panicles

Protein synthesis energy source or RNA synthesis	Incubation time (min)	Percent of incorporation				Ratio of incorporation
		Seedling mitochondria[a]		Panicle mitochondria[b]		Panicle mitochondria
		[^{32}P]-UTP/GTP into RNA	[^{35}S]-met into protein	[^{32}P]-UTP/GTP into RNA	[^{35}S]-met into protein	Seedling mitochondria
ADP/succinate	60	–	6.3	–	22.6	3.6
ATP/kinase	60	–	4.3	–	12.0	2.8
ADP/succinate ATP/kinase	60	–	1.5	–	1.9	81.3
RNA synthesis	0[c]	0.02[c]	–	0.2	–	10.5
RNA synthesis	10	0.2	–	0.9	–	4.8
RNA synthesis	30	–	–	2.0	–	–
RNA synthesis	60	0.9	–	2.3	–	2.5

[a] Seedling mitochondria were isolated from 8-day-old etiolated rice seedlings grown aseptically
[b] Panicle mitochondria were isolated from rice panicles at a developmental stage in which the meiotic divisions of microsporogenesis occurred
[c] The reaction mix was centrifuged immediately following the addition of [^{32}P]-labeled UTP and GTP for this 0-min incubation time point; the recovered mitochondrial pellet was then processed according to the same experimental conditions as used in Figs. 2 and 4 to determine the incorporation of labeled nucleotides. These incorporation data are also presented graphically in Fig. 4

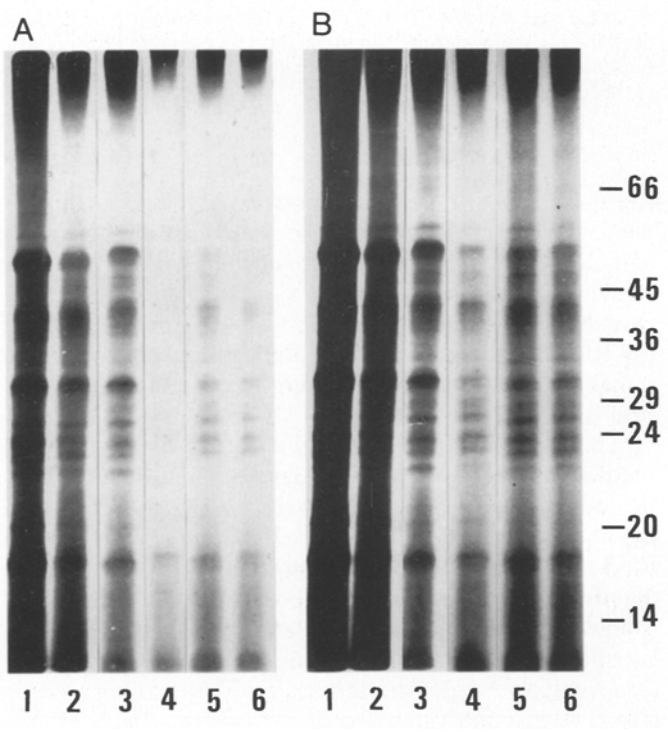

Fig. 2A, B Comparison of [^{35}S]methionine-labeled polypeptides synthesized by rice panicle and seedling mitochondria. The result was obtained from experiments independent of that shown in Fig. 1, but the experimental conditions were the same. The autoradiographs shown in *Panel A* and *Panel B* were produced from the same gel with an exposure length of 16 and 48 h, respectively. The mitochondrial sample used in the six protein-synthesis reactions was as follows: *lanes 1 and 2*, panicle mitochondria; *lanes 3 and 4*, 8-day-old seedling mitochondria, *lanes 5 and 6*, 14-day-old seedling mitochondria. The energy source used for the six protein synthesis reactions was as follows: *lanes 1, 3 and 5*, 2 m*M* ADP and 10 m*M* sodium succinate, *lanes 2, 4 and 6*, 6 m*M* ATP, 8 m*M* phosphocreatine and 4 units of creatine phosphokinase

energy source into panicle mitochondrial proteins than into seedling mitochondrial proteins; the ATP- regenerating system yielded a 2.8-fold increase (Table 1).

There were no obvious differences in the relative proportions or in apparent molecular mass of the newly synthesized polypeptides among the three mitochondria samples (Fig. 2). Discernible constituents were 65.8, 63, 54.2, 53, 50.3, 47, 44, 40.2, 38, 36.5, 33, 31.4, 28.5, 26.5, 23, 22.4, 21 19.2 and 17 kDa in size (calculated from Fig. 1).

Characterization of RNA synthesis by isolated rice mitochondria

Although the panicle and two seedling mitochondria samples synthesized the same set of discernible polypeptides (Fig. 2), [^{35}S]methionine incorporation was approximately 3 times higher in panicle mitochondria than in 8-day-old seedling mitochondria (Table 1). This difference suggested that more proteins were actually synthesized by panicle mitochondrion than by seedling mitochondrion. Next, we examined RNA synthesis by isolated panicle and seedling mitochondria to ascertain whether a coordinated increase in panicle mitochondrial transcription and translation was in effect. To this end, the RNA synthesis by isolated rice mitochondria was first characterized.

After the seedling mitochondria were incubated for 60 min in an RNA synthesis reaction mix, the newly synthesized RNA products consisted of high-molecular-weight precursor and low-molecular-weight partially degraded RNA species together with a population of not well-resolved RNA species with intermediate molecular weights (Fig. 3, lane 1). RNA synthesis was completely blocked by actinomycin D (Fig. 3, lane 2) and, partially inhibited by rifampicin (Fig. 3, lane 3). As expected, the

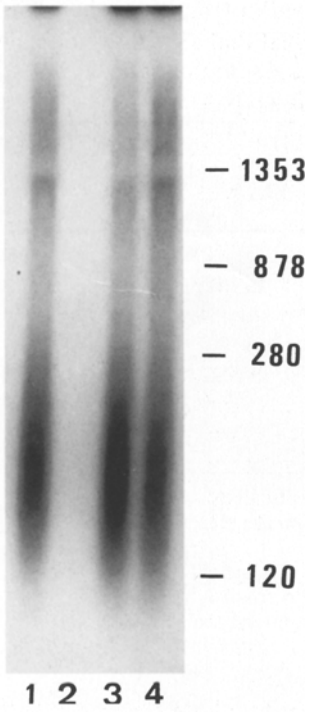

Fig. 3 Autoradiograph of [^{32}P] UTP/GTP-labeled RNA synthesized by isolated rice seedling mitochondria. Experimental conditions for *in organello* RNA synthesis and product analysis are described in the Materials and methods. Mitochondria were isolated from 8-day-old seedlings. Sucrose-gradient-purified mitochondria equivalent to 200 µg mitochondrial protein were used in each reaction. Antibiotic was supplemented in the four RNA synthesis reactions as follows, *lane 1*, control, no supplement, *lane 2*, 100 µg/ml actinomycin D, *lane 3*, 175 µg/ml rifampicin, *lane 4*, 100 µg/ml ampicillin and 100 µg/ml streptomycin. After 60 min of incubation at 25°C, RNA was extracted from the recovered mitochondria of each reaction and loaded in its entirety in a formaldehyde agarose gel. The X-ray film was exposed for 48 h at –80°C with an intensifying screen

RNA synthesis was not affected by ampicillin and streptomycin, which were included as a control for any possible growth of contaminants and their release of RNase during the incubation period (Fig. 3, lane 4). These results indicate that RNA synthesis by isolated rice seedling mitochondria was double-stranded DNA dependent and derived mostly from the mitochondrial DNA template. The characteristics of rice mitochondrial RNA synthesis and of the product profile (Fig. 3) were similar to those of maize (Finnegan and Brown 1986, 1987).

Comparison of panicle and seedling mitochondrial RNA synthesis rates

To measure the rate of RNA synthesis by isolated panicle and seedling mitochondria, mitochondrial samples were preequilibrated in the RNA synthesis reaction mix, which contained an excess of unlabeled UTP and GTP; the incubation was clocked to commence the sampling process after the addition of [^{32}P]-labeled UTP and GTP. As shown in Fig. 4, the rate of incorporation of labeled UTP/GTP into

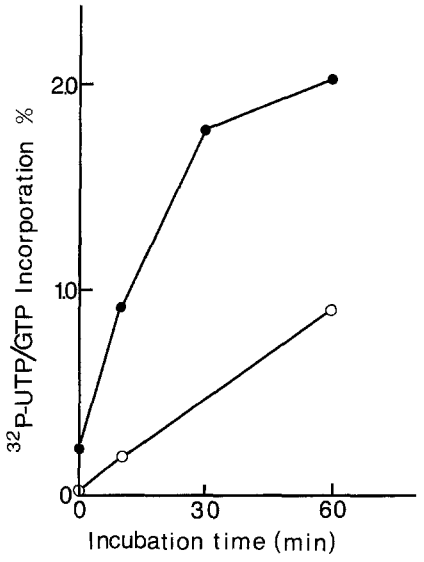

Fig. 4 The rate of RNA synthesis by isolated rice mitochondria. Experimental conditions for *in organello* RNA synthesis were the same as in Fig. 3; the results were obtained from the experiment shown in Fig. 5. At 30-min intervals after the addition of [^{32}P]UTP/GTP into the reaction mix, mitochondria were recovered by centrifugation and immediately lysed. A 1/20 aliquot of this lysate was used to determine the percentage of [^{32}P]UTP/GTP incorporation into RNA as described in the Materials and methods. The counting data are also presented in Table 1. *Closed circle* panicle mitochondria, *open circle* seedling mitochondria

RNA was initially linear for both mitochondria samples, while the rate of RNA synthesis was much higher in the panicle mitochondria than in seedling mitochondria. Thirty minutes later, the rate of RNA synthesis in panicle mitochondria decreased to a level almost comparable to that of the RNA synthesis rate of seedling mitochondria, which remained unchanged over the entire 60-min labeling period (Fig. 4).

The profiles of the RNA synthesized by panicle and seedling mitochondria at different time points (Fig. 5) confirmed the results derived from the [^{32}P]UTP/GTP incorporation data shown in Fig. 4. These data indicate that RNA synthesis activity was substantially higher and the amount of RNA synthesized discretely higher in panicle mitochondria than in seedling mitochondria.

Discussion

The present study revealed no qualitative difference between the polypeptides synthesized by isolated panicle mitochondria and the polypeptides synthesized by seedling mitochondria. Hence, it is most likely that the rice mitochondrial genome does not encode any panicle-specific protein. Alternative interpretations are conceivable. For instance, the rice mitochondria genome may encode a panicle-specific polypeptide(s), which, under our experimental conditions, was either not synthesized by the isolated

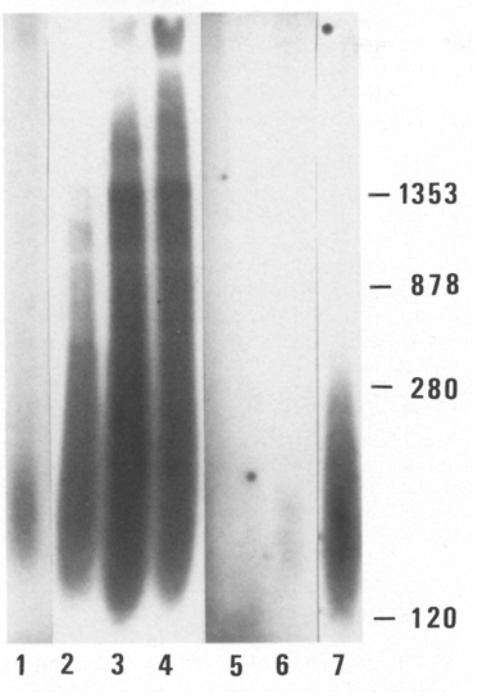

Fig. 5 Comparison of the rate of RNA synthesis *in organello* by rice seedling and panicle mitochondria. Experimental conditions for *in organello* RNA synthesis and product analysis were the same as in Fig. 3; the results were obtained from the experiment shown in Fig. 4. At 30-min intervals after the addition of [^{32}P]UTP/GTP into the reaction mix, mitochondria were recovered by centrifugation and immediately lysed. A 19/20 aliquot of this lysate was extracted successively with phenol/SDS and chloroform to isolate RNA. Total RNA isolated from mitochondria equivalent to 190 μg mitochondrial protein was loaded in each lane. Lanes *1, 2, 3 and 4* RNA synthesized by panicle mitochondria following an incubation period of 0, 10, 30 and 60 min, respectively. *Lanes 5, 6 and 7* RNA synthesized by seedling mitochondria following an incubation period of 0, 10 and 60 min, respectively. The X-ray film was exposed for 24 h at −80°C with an intensifying screen

panicle mitochondria or synthesized but not recognized as a discrete polypeptide due to its low relative proportion.

Despite the fact that no panicle-specific protein synthesis product was detected in rice, all polypeptides synthesized by panicle mitochondria uniformly incorporated about 3 times as much [^{35}S]methionine than did the polypeptides synthesized by seedling mitochondria (Fig. 2; Table 1). If we assume that the quantity of translational machinery is not rate-limiting for protein synthesis in isolated mitochondria, higher [^{35}S]methionine incorporation could have resulted from (1) an increase in [^{35}S]methionine uptake, (2) a decrease in the free methionine pool or (3) an increase in mitochondrial RNA transcript copy number. These alternatives have to be evaluated before we can properly interpret the higher [^{35}S]methionine incorporation into panicle mitochondrial proteins.

Panicle mitochondria utilized succinate, an endogenous oxidizable substrate 1.9 times more efficient than an exogenous ATP-regenerating system as an energy source for protein synthesis *in organello*; this ratio was only 1.5 in the case of seedling mitochondria (Table 1). Although these data suggest that all energy-dependent protein synthesis processes, including amino acid uptake, were more efficient in isolated panicle mitochondria than in seedling mitochondria, the differential ratio of these two energy-efficiency coefficients, 1.3, appeared too low to account for the 2.8- to 3.6-fold higher [^{35}S]methionine incorporation in panicle mitochondrial proteins than in seedling mitochondrial proteins (Table 1). Hence, the efficiency of amino acid uptake per se by isolated mitochondria was unlikely to have changed significantly between these two mitochondrial samples.

The difficulty in obtaining a sufficient quantity of panicle mitochondria to measure directly their free amino acid or nucleotide pool prompted us to compare RNA synthesis by panicle mitochondria with RNA synthesis by seedling mitochondria. The data indicate that these two mitochondria samples synthesized qualitatively the same RNA products at discretely different rates and that more RNA was synthesized by panicle than by seedling mitochondria (Figs. 3–5).

The results of this study, taken together, suggest that either the mitochondrial transcription/translation regulation or the DNA copy number per mitochondrion changed substantially in the developing panicle mitochondria to promote the synthesis of more protein molecules in panicle mitochondria. This qualitative alteration of mitochondrial protein synthesis products complements results obtained in maize kernel (Newton and Walbot 1985), in different organs in sugar beet (Lind et al. 1991) and changes in the quantity of selected mitochondrial proteins in Jerusalem artichoke tuber (Forde et al. 1979). This indiscriminate increase of all mitochondrial proteins in the developing rice panicle is yet another example of mitochondrial gene expression variation that accompanies the development of specialized structures in plants.

The developing rice panicle consists of several types of cells (Wu 1967). It is not known whether the increase in mitochondrial transcription/translation activity reflects a disproportionately large increase of such activity in a small fraction of cells of a specific type(s) or whether it reflects an increase in all types of cells. During pollen formation in maize, the number of mitochondria in tapetal cells has been found to increase by 40 fold (Lee and Warmke 1979).

During cereal leaf development, the level of mitochondrial gene expression appears to be inversely related to chloroplast development and gene expression (Dean and Leech 1982; Mayfield and Taylor 1984; Martineau and Taylor 1985; Topping and Leaver 1990). Our study suggests that in the development of an etiolated, specialized organ, mitochondrial gene expression may be regulated independently of the proplastid to chloroplast transition. It seems likely that proplastids may act similarly to chloroplasts in their interaction with mitochondria and the nucleus in a tripartite regulatory interaction/coordination that ultimately controls cellular growth and differentiation in plants.

The large, complex genome of plant mitochondria and their high ribonuclease content are likely responsible for

the disproportionate abundance of low-molecular-weight, RNA synthesis products consisting mainly of truncated and degraded RNA (Figs. 3 and 5). The profiles of newly synthesized RNA by rice mitochondria and by maize mitochondria (Finnegan and Brown 1986; 1987) are very different from those of HeLa cell (Gaines and Attardi 1984) and yeast (Boerner et al. 1981; Groot et al. 1981; Newman and Martin 1982). This difference may also reflect the fact that in contrast to mammalian and yeast cells, rice seedling and panicle tissues contain multiple cell types. Unlabeled RNA extracted by a variety of procedures from rice mitochondria that were not subjected to a prior RNA synthesis reaction exhibited consistently sharp, discrete electrophoretic banding patterns (unpublished data), indicating that the profile of newly synthesized rice mitochondrial RNA was not an artifact generated by our experimental conditions.

Even though the preparation of newly synthesized rice mitochondrial RNA contained almost no discrete mRNA species in the expected size range, a considerable amount of mitochondrial mRNA molecules must have remained functionally intact inside the organelle, otherwise no discrete polypeptides would be synthesized by isolated rice mitochondria. Thus, degradation of the newly synthesized mitochondrial RNA may have occurred after the mitochondria were lysed for RNA extraction. It is not known whether all discrete polypeptides synthesized *in organello* arise only from those mRNA species with a high degree of inherent stability or whether a fraction of all mRNA species is degraded and such degradation is unrelated to the *in organello* protein synthesis pattern.

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