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Light-Independent Regulation of Chloroplast Translation Elongation Factor Tu Gene Expression in Three Types of Grass: Rice, Maize, and Barley

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We have analyzed the effect of light on the steady-state level of the chloroplast elongation factor EF-Tu (*tuf*A) mRNAs during chloroplast biogenesis in rice. The steady-state level of the *tuf*A mRNAs in continuously light-grown seedlings was almost equal to that in the continuously dark-grown seedlings. Furthermore, the *tuf*A transcript level was hardly changed in the transition of dark-grown seedlings to the continuous light and during all chloroplast developmental stages examined. We have previously shown that light had a similar effect on the *tuf*A mRNA level during chloroplast biogenesis in maize. Therefore, we also examined the possibility of the light-regulation of barley *tuf*A mRNA. It turned out that light had no effect on the barley *tuf*A mRNA level either. Although only three types of grass were examined, these results may show a difference of *tuf*A gene expression between grasses and legumina.

Keywords: chloroplast EF-Tu, light, mRNA, rice (*Oryza sativa* L.), *tuf*A

INTRODUCTION

Elongation factor Tu (EF-Tu) plays an essential role in protein synthesis: it binds aminoacyl-tRNAs and GTP, associates with the A-site of ribosomes, and consequently directs the elongation of polypeptides on ribosomes. These functions are performed by EF-Tu in prokaryotes and eukaryotic organelles and by EF-1α in eukaryotes (Miller and Weissbach, 1977). In lower photosynthetic eukaryotes, including *Chlamydomonas reinhartii* (Baldauf and Palmer, 1990), *Euglena gracilis* (Montadon and Stutz, 1983), and probably most green algae (Baldauf *et al.*, 1990), EF-Tu proteins are encoded by the chloroplast genome. However, higher plant chloroplast EF-Tu proteins are encoded by nuclear DNA (Baldauf and Palmer, 1990; Baldauf *et al.*, 1990), synthesized as precursors in the cytoplasm, and then imported into chloroplasts (Schmid and Mishkind, 1986; Keegstra and Olsen, 1989). The EF-Tu protein shows some interesting characteristics. First, EF-Tu is a multi-functional protein in *Escherichia coli* (Blumenthal *et al.*, 1980; Vijgenboom *et al.*, 1988). It was reported that the EF-1α is also involved in other cellular processes other than translation elongation in higher plants (Roth *et al*, 1987; Rao and Slobin, 1988; Shepherd *et al*, 1989). Second, the EF-Tu gene is considered to be one of the genes which have been evolutionally transferred from the chloroplast genome to the nuclear genome (Baldauf and Palmer, 1990).

In *E. gracilis*, Spremulli and coworkers showed that the activities of several chloroplast protein synthesis factors, such as EF-G (Breitenberger *et al.*, 1979), EF-Ts (Fox *et al.*, 1980), EF-Tu (Sreedharan *et al.*, 1985), IF-2 (Gold and Spremulli, 1985), and IF-3 (Kraus and Spremulli, 1986), were light-induced. The activity of a pea chloroplast elongation factor G (EF-G) is also regulated in response to light (Akaya and Breitenberger, 1992). EF-G activity was almost undetectable in the extracts from dark-grown pea seedlings. When 13 day dark-grown pea seedlings were exposed to light, EF-G specific activity reached a higher value 2 to 3 days later than observed in seedlings grown under continuous light (Lamppa and Bendich, 1979). In *C. reinhartii*, the expression of the *tuf*A gene was regulated at the level of posttranscription by light (Silk and Wu, 1993). In the soybean, the steady-state level of *tuf*A mRNA was sharply changed when the dark-grown seedlings were illuminated (Bonny and Stutz, 1993). The nuclear genome of the soybean contains a family of four *tuf* genes coding for the chloroplast EF-Tu protein; the four *tuf* genes belong to two subfamilies

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(type A and type B) (Maurer *et al.*, 1996), and these two subfamilies were expressed in a tissue-specific manner. The tobacco *tuf*A and *tuf*B mRNAs were also differentially expressed according to tissue and plastid types (Sugita *et al.*, 1994).

Light is essential for normal chloroplast development in certain strains of algae and in higher plants (Thompson, 1991). In some species, light also induces changes in the expression of a number of nucleus- and plastid-encoded genes, which are named 'photogenes', at the levels of transcription (Klein *et al.*, 1988) and posttranscription (Klein and Mullet, 1987; Gruissem *et al.*, 1988; Klein and Mullet, 1988; Gruissem, 1989). In higher plants, chloroplasts contain their own protein biosynthetic apparatus (Steinmetz and Weil, 1989). However, while only one-third of the chloroplast ribosomal proteins are encoded in chloroplast DNA, the remaining two-thirds are nuclear-encoded, synthesized as precursors in the cytoplasm, and then imported into chloroplasts (Sugiura, 1992). Therefore, the biosynthesis of many plastid protein complexes requires the coordinated expression of genes located in the nucleus and the plastid. For example, gene expression of the two subunits (*rbc*L and *rbc*S) of ribulose 1,5-*bis*phosphate carboxylase/oxygenase is co-regulated by environmental signals such as light (Zhu *et al.*, 1985; Klein and Mullet, 1987). In most higher plants, *rbc*L and *rbc*S gene expression is regulated by light at the level of steady-state mRNA accumulation, but the effect of light on the gene expression has also been shown to vary among different plant species. *rbc*L and *rbc*S mRNA levels have been shown to be strongly light-regulated in pea (Smith and Ellis, 1981) and soybean (Berry-Lowe *et al*, 1982) plants, moderately in cucumber (Walden and Leaver, 1981), maize (Nelson *et al.*, 1984), rice (Kapoor *et al.*, 1994), and amaranth (Berry *et al.*, 1985) plants, but rarely in barley (Klein and Mullet, 1987).

We had previously shown that the *tuf*A mRNA level was barely regulated by light in maize (Lee *et al*, 1997). In this study, we have examined if this is also true in rice and barley-maize's close relatives.

MATERIALS AND METHODS

Plant Growth Conditions

To study the light-regulated patterns of the EF-Tu gene, seedlings of *Oryza sativa* L. (subsp. *japonica* cv. Il pum-byeo) were grown in continuous light and dark conditions in plant growth chambers under controlled environmental conditions (temperature 28°C, humidity 70%, light intensity 12,000 Lux). For the light-induction studies, seedlings were grown for 7 days in continuous darkness, and then exposed to light for a specified duration. Seedlings of maize (*Zea mays* L. cv. Golden × Bantam 70), barley, and tobacco (*Nicotiana tabacom* NC-82) were grown under the same conditions.

Preparation of Total RNA and Northern Blot Analysis

Total cellular RNAs were isolated from leaf materials using the method of De Vries *et al.* (1988). Twenty micrograms of denatured total RNA samples were resolved on 1% agarose-formaldehyde gels.

RNA was transferred to a Hybond-N membrane (Amersham International Inc. UK.) according to the capillary method, and then the blots were fixed by U.V irradiation. Prehybridization was carried out in a hybridization oven containing 20 mL prehybridization solution comprising of 50% formamide, 5 × SSC, 5 × Denhardt's solution, 0.1% SDS, and 100 μg/mL of denatured salmon sperm DNA at 37°C (*tuf*A) or 40°C (*rbc*L and 25S rDNA) for 2 h. For hybridization, a specific DNA probe to *tuf*A, *rbc*L (ribulose 1,5-*bis*-phosphate carboxylase/oxygenase large subunit), or 25S rDNA was labelled with [α-^{32}P]dCTP (Amersham) using the random DNA labelling system (Boehringer Mannheim). The source of the hybridization probes was previously described in Lee *et al.*, (1997). Hybridization was performed at the same temperatures with prehybridization for 15 to 17 h.

After hybridization, each membrane for total RNA was washed according to the gene-specific conditions. For the *tuf*A probe, the membrane was washed in 2 × SSC with 0.1% SDS once at 37°C for 10 min, in 1 × SSC with 0.1% SDS twice at 40°C for 10 min, and in 0.1 × SSC with 0.1% SDS once at 40°C for 10 min. For the *rbc*L probe, the membrane was washed in 1 × SSC with 0.1% SDS once at 40°C for 10 min and in 0.1 × SSC with 0.1% SDS once at 42°C for 10 min. For the 25S probe, the membrane was washed in 1 × SSC with 0.1% SDS once at 42°C for 20 min and in 0.1 × SSC with 0.1% SDS once at 42°C for 20 min. For the detection of transcripts, the membranes were scanned and signals were quantified by Phosphoimager (Fuji-BAS).

RESULTS AND DISCUSSION

In order to examine if the steady-state *tuf*A mRNA

level of rice is light-regulated, we performed Northern analysis on the steady-state levels of the rice *tuf*A transcripts under four different conditions (Fig. 1). The rice *tuf*A transcript was detected as a single band, and its size was similar to that of the tobacco *tuf*A transcript. As expected, the steady-state levels of the 25S rRNA did not change in all tested conditions, and the *rbc*L transcript levels were significantly affected by light. Under the same conditions, the transcript level of the *tuf*A gene was hardly influenced by light. Because *tuf*A mRNA accumulation was an early event during the greening process in *Chlamydomonas* (Silk and Wu, 1993) and soybean (Bonny and Stutz, 1993), we have also examined the effect of light on the level of rice *tuf*A transcript during shorter greening periods (Fig. 2). After dark-to-light transition, the transcript levels did not change with increasing duration of light exposure up to 24 hours. Under the same conditions *rbc*L transcript levels showed a similar pattern that was known previously (Klein *et al.*, 1988). The steady-state levels of the 25S rRNA did not change as expected. These results suggest that rice *tuf*A transcripts barely accumulated in early greening periods.

Finally, we examined if the transcript abundance of the rice *tuf*A gene was influenced by the age of the seedlings (Fig. 3). The *tuf*A transcript levels were examined during a period of 7 to 10 days in continuous dark, light, or dark-to-light transition. The steady-state levels of the 25S rDNA transcript did not change under any of the examined conditions. However, the *rbc*L transcripts were significantly affected by light, as expected. Under the same conditions, the steady-state levels of the rice *tuf*A transcripts did not change to a significant extent.

Based on the above results, we concluded that the expression of the rice *tuf*A gene was not light-regulated significantly at the mRNA level. Interestingly,

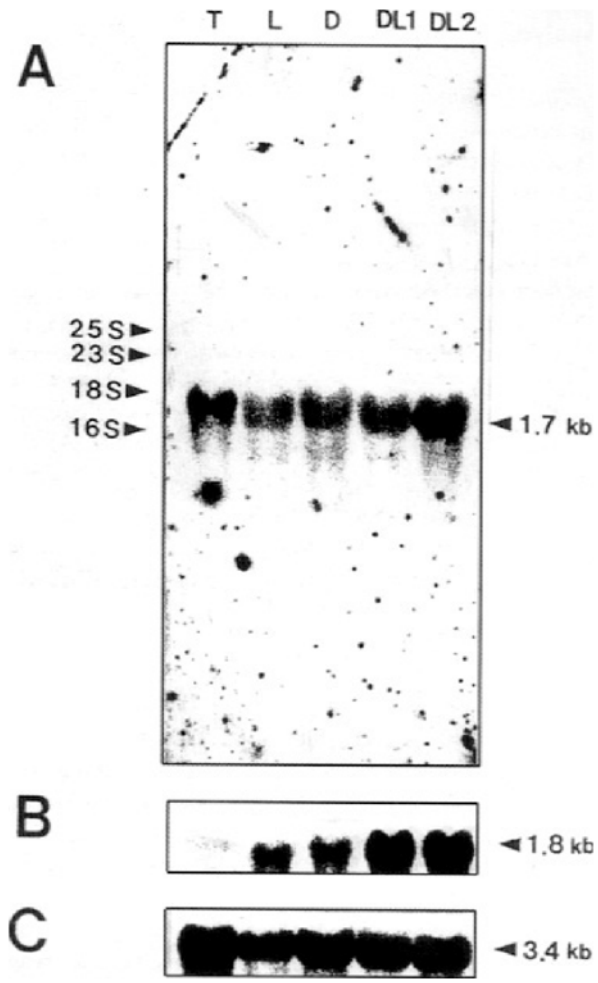

Fig. 1. Northern hybridization analysis of total RNAs isolated from tobacco or rice leaves grown in continuous light, continuous dark, and dark-to-light transitions. Total RNAs were isolated from leaves grown in continuous light (9 days, L), in continuous darkness (9 days, D), and in transition of continuous darkness (7 days) to light for 24 hours (DL1) or 48 hours (DL2). 20 μg of total RNA were loaded on each lane and hybridized with labelled probes from *tuf*A (A), *rbc*L (B), or 25S rDNA (C). Tobacco total RNA (20 μg) was also loaded as a control (T). Size markers are rice cytoplasmic rRNAs (25S and 18S) and chloroplast rRNAs (23S and 16S). The sizes of the *tuf*A, *rbc*L, and 25S rDNA transcripts are indicated in kilobases.

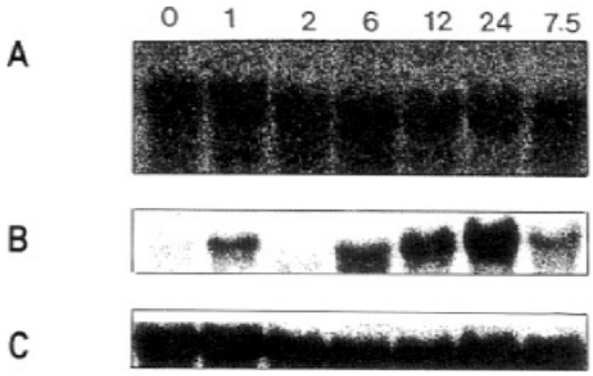

Fig. 2. The changes of rice leaf RNA during shorter greening periods. Dark-grown seedlings (7 days) were illuminated for the indicated hours (0, 1, 2, 6, 12, 24 hours), and total RNA was isolated right after illumination. As a control, total RNA was also isolated from 7.5 days-dark-grown seedlings. 20 μg of total RNA were loaded on each lane and hybridized with labelled probes from *tuf*A, *rbc*L, or 25S rDNA. Northern blots with the *tuf*A (A), *rbc*L (B), or 25S rDNA (C) probe were performed under the same conditions described in Fig. 1.

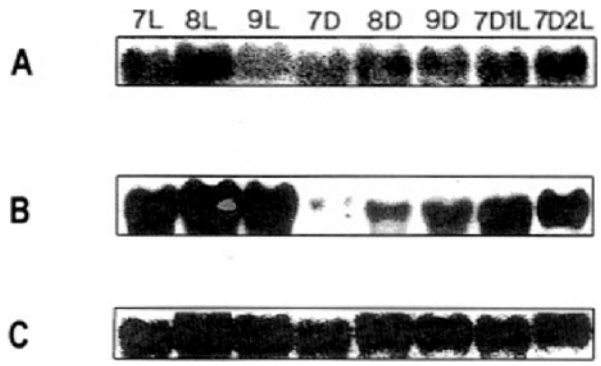

Fig. 3. Accumulation of rice leaf transcripts during light growth, dark growth, and dark-to-light transition. Total RNA was prepared from leaves grown in continuous light (from 7 days to 9 days: 7L, 8L, and 9L. respectively), or continuous darkness (from 7 days to 9 days: 7D, 8D, and 9D. respectively) for the indicated days. At 7 days, dark-grown plants were illuminated for 1 day or 2 days (7D1L or 7D2L), and total RNA was isolated immediately after illumination. Twenty μg of total RNA was loaded on each lane and hybridized with labelled probes from *tuf*A (A), *rbc*L (B), and 25S rDNA (C).

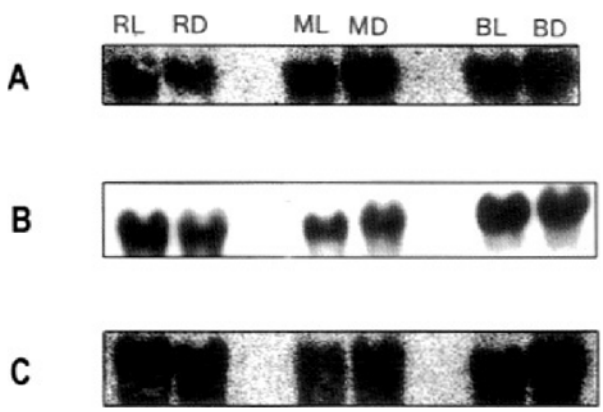

Fig. 4. Comparitive northern analysis in three grasses:rice, maize, and barley. Total RNAs were isolated from rice (R), maize (M) and barley (B) leaves. They were grown for 9 days in continuous light (L) and continuous darkness (D). Twenty μg of total RNA was loaded on each lane and hybridized with labelled probes from *tuf*A (A), *rbc*L (B), and 25S rDNA (C). The maize's data are consistent with those previously shown (Lee *et al.*, 1997).

it was reported that in the soybean (Bonny and Stutz, 1993) and pea, dicotyledonous plants, the *tuf*A mRNA level was light-regulated. Up to the present, it has not been reported that the steady-state *tuf*A mRNA levels of monocotyledonous plants are influenced by light. Therefore, we have been interested in the cases of other monocotyledonous plants. The steady-state levels of the *tuf*A mRNAs were examined from maize and barley seedlings which were grown in continuous light or dark for 9 days (Fig. 4). We could not detect any changes at the *tuf*A mRNA levels in either maize or barley.

It was proposed that chloroplast translation machinery played a role in coordinating nuclear events with chloroplast gene expression during light-induced chloroplast biogenesis (Talyor, W.C., 1989). In addition, the chloroplast translation elongation factor EF-Tu (*tuf*A) has been known to be light-regulated at its activity or RNA level in some algae and leguminous plants (Sreedharan *et al.*, 1985; Bonny and Stutz, 1993; Silk and Wu, 1993).

Therefore, *tuf*A gene regulation may affect coordination between nuclear gene expression and chloroplast gene expression during light-induced chloroplast development. We previously showed that unlike the leguminous plants, maize *tuf*A mRNA was barely regulated by light during chloroplast biogenesis (Lee *et al.*, 1997).

This led us to examine how the *tuf*A mRNA level would be regulated by light in rice and barley, which are the closest relatives of maize beside C_3 plants. It turned out that the rice and barely *tuf*A mRNA levels were barely regulated by light as well. In contrast, the accumulation of *rbc*L transcripts has been known to be regulated to different extents by light among these three plants (Nelson *et al.*, 1984; Klein and Mullet, 1987; Kapoor *et al.*, 1994).

Although the *tuf*A mRNA levels of these three plants were not regulated by light, their proteins or activity levels might be regulated by light. Here we propose with caution that our results may show the difference of *tuf*A gene expression between grass and leguminous plants.

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