Coordinate light-induction of two mRNAs, encoded in nuclei and chloroplasts, of ribulose 1,5-bisphosphate carboxylase/oxygenase

Kazuo Shinozaki, Yukiko Sasaki[+,*], Toshiko Sakihama[+] and Tadashi Kamikubo[+]

Department of Molecular Genetics, National Institute of Genetics, Mishima 411 and [+]*Department of Food Science and Technology, Kyoto University, Kyoto 606, Japan*

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1. INTRODUCTION

Several enzymes localized in chloroplasts are known to be synthesized by the cooperation of two organelles, nuclei and chloroplasts. Ribulose 1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) (RuBPCase) is typical of these enzymes. It is a multimeric protein composed of 8 large and small subunits. The large subunit is encoded in chloroplast DNA [1] and synthesized on chloroplast ribosomes [2]. The small subunit is encoded in nuclear DNA [3] and synthesized on cytoplasmic ribosomes in the form of a precursor, which is transported into chloroplasts [4–7]. The extra peptide of the precursor is removed during transport. Then the small and large subunits combine to form RuBPCase molecules.

This nucleo–chloroplast interaction is required for the biosynthesis of RuBPCase during light-induction. We have shown that small subunit mRNA increases in parallel with the induction of RuBPCase in pea plants [8]. This study shows that large subunit mRNA also increases with illumination time, and large and small subunit mRNA levels seem to be coordinately regulated during light-induction.

2. MATERIALS AND METHODS

2.1. *Materials*

Anti-small subunit IgG was prepared as in [8]. [^{35}S]Methionine (1000 Ci/nmol) and d[α-^{32}P]CTP (3000 Ci/mmol) were obtained from Radiochemical Centre (Amersham).

2.2. *Extraction of total RNA*

Pea seedlings (*Pisum sativum* cv. Alaska) grown for 7 days in darkness were exposed to light ($\sim$ 10 000 lux) for 0, 12, 24, 36 and 48 h, respectively. A 33% (w/v) apical bud homogenate was prepared in 100 mM Tris–HCl (pH 7.8) containing 200 mM NaCl, 10 mM magnesium acetate, 14 mM 2-mercaptoethanol, 1% SDS and 15 mM EDTA. Total RNA was extracted twice with a phenol–chloroform mixture, and precipitated twice with 2 M LiCl to remove DNA and twice with ethanol. This RNA contained $<$ 0.3% DNA as determined by the Burton method [9]. This preparation, containing both cytoplasmic and chloroplast RNAs, was used for the determination of large and small subunit mRNA levels.

2.3. *Determination of large subunit mRNA level*

RNA (10 μg total) extracted from apical buds was denatured in 8 μl a solution containing 1 M glyoxal, 50% dimethylsulfoxide, 10 mM sodium phosphate buffer, pH 7.0 at 50°C for 1 h [10,11]. The reaction mixture was cooled on ice and 2 μl of a 50% glycerol, 10 mM sodium acetate, 0.05% bromphenol blue mixture was added. The samples were electrophoresed on 1.2% agarose gel in 10 mM sodium phosphate (pH 7.0) at 80 V for 30 min. Glyoxalated RNA was transferred from the agarose gels to nitrocellulose paper as in [11]. The blots were dried and kept in a vacuum oven for 2 h at 80°C. The RNA blots were prehybridized and then hybridized

*To whom correspondence should be addressed

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with the ^{32}P-labeled spinach large subunit DNA probe as in [11]. The DNA fragment wich contained the spinach large subunit gene (900 000 M_r *Kpn*I fragment) was provided by Dr R.G. Herrmann, Düsserdorf, and labeled by nick-translation with d[α-^{32}P]CTP [12]. After washing in 2 × SSC, 0.05% SDS, the nitrocellulose paper was dried and allowed to expose Fuji X-ray film RX at −70°C. For quantitative estimation, the autoradiograms were traced with a microdensitometer (type 3CS, Joyce Loebl Co. Ltd).

2.4. *Determination of small subunit mRNA level*

RNA (10 μg total) was translated in 50 μl of a wheat germ cell-free system as in [8]. The translation products were immunoprecipitated by the addition of 25 μg control IgG, followed by adsorption by 12.5 μl of a 10% suspension of *Staphylococcal aureus* protein A (Pansorbin, Calbiochem) to remove non-specific immunoprecipitates as in [13]. Then 62 μg anti-small subunit IgG and 40 μl protein A suspension were added to the supernatant. The precipitates were washed 4 times with 1 ml solution containing 10 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.25% Nonidet P40 (Bethesda Research Lab.) and 10 mM methionine mixture, and dissolved in SDS-sample buffer. Electrophoresis was performed on 12.5% polyacrylamide gel essentially as in [14]. The gel was fixed, stained and destained. For fluorography, the gel was impregnated with En^{3}Hance (New England Nuclear), dried under vacuum, and allowed to expose Fuji X-ray film RX at −70°C for several days. For quantitative measurement, the gel was cut and the radioactivity counted as in [8].

3. RESULTS AND DISCUSSION

Recent progress in gene technology enables us to analyze gene sequences in chloroplast DNA. The large subunit gene from maize [15], spinach [16] and tobacco [17] have already been cloned, and the DNA sequences were found to be conservative among these plants with 90% homology [17]. These results prompted us to determine the large subunit mRNA level in pea plants using cloned large sub-

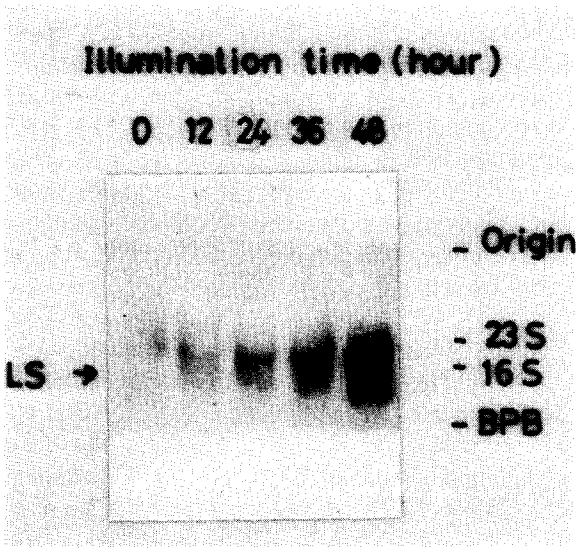

Fig.1. Effect of light on hybridizable mRNA for the large subunit. Each 10 μg total RNA isolated from pea seedlings which had been exposed to light for 0, 12, 24, 36, and 48 h, respectively, was denatured with glyoxal, electrophoresed and transferred to nitrocellulose paper. The blots were hybridized with the ^{32}P-labeled spinach large subunit DNA probe and autoradiographed. LS = Large subunit mRNA.

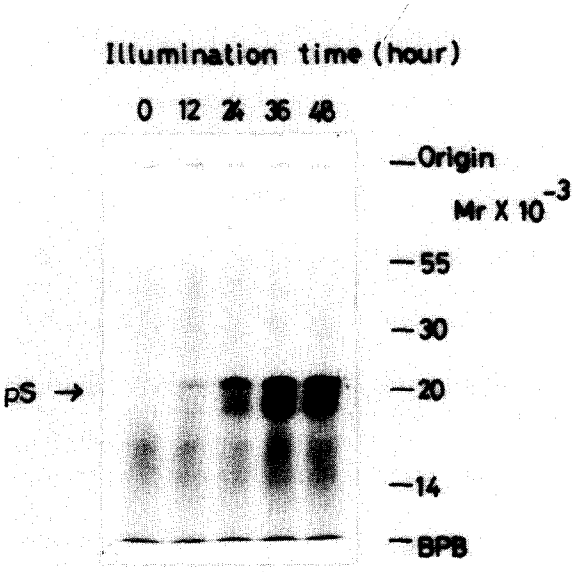

Fig.2. Effect of light on translatable mRNA for the small subunit. Each 10 μg total RNA isolated from pea seedlings exposed to light for 0, 12, 24, 36 and 48 h, respectively, was translated by a wheat germ cell-free system. The translation products were immunoprecipitated, electrophoresed and autoradiographed. pS = precursor of the small subunit.

unit DNA from another plant as a hybridization probe. We have used a cloned large subunit DNA fragment from spinach.

For quantitative analysis, we performed the Northern hybridization with the spinach DNA probe. After glyoxal treatment, 10 μg total RNA which had been separated on agarose gel was transferred to a nitrocellulose filter and hybridized with the DNA fragments labeled with ^{32}P by nick-translation. Fig.1 shows the effect of light on large subunit mRNA levels. The RNA from dark-grown seedlings (0 h) gives a weak band in the region corresponding to the M_r of large subunit mRNA and a more dense band appears with illumination time. These results indicate that large subunit mRNA level increases during light-induction.

To compare these results with the kinetics of small subunit mRNA induction the effect of light on the translatable mRNA level for the small subunit was examined using the same RNA preparation. The total mRNA activity of each RNA preparation did not differ very much, and all the translation products derived from 10 μg total RNA was used for immunoprecipitation. Fig.2 shows the fluorogram of translation products immunoprecipitated by anti-small subunit IgG. In the region corresponding to the M_r of the precursor of the small subunit ($\sim$ 20 000 M_r), a dark band appears for each lane except for 0 h. The dark band gradually becomes denser with increased illumination time as observed with the large subunit (fig.1). The amount of mRNA in dark-grown tissues is below the limit of detection by this method.

To obtain more subtle information about these increases, the relative amounts of mRNA were determined by densitometric tracing of the autoradiograms shown in fig.1 for the large subunit, and by counting the radioactivity of the precursor band for the small subunit. The results are shown in fig.3 in which the amounts of mRNA from seedlings illuminated for 48 h are arbitrarily set at unity. The profiles of the 2 mRNA levels indicate that the mRNA levels are coordinately regulated. The increase of the large subunit mRNA appears to be slightly later than that of the small subunit. These results are consistent with reports which show that the large and small subunit mRNAs synthesized under light are more abundant than those synthesized in darkness using the techniques of the Northern hybridization [18,19] and translation [20-22].

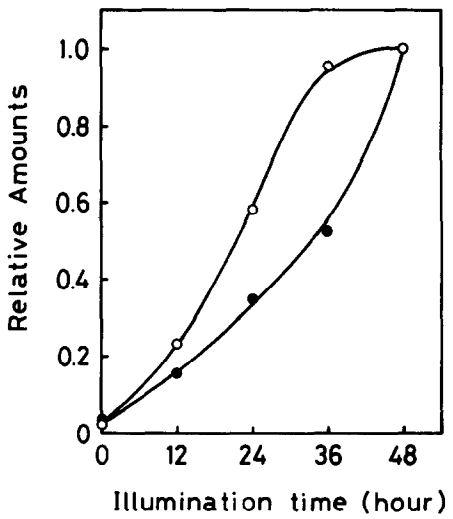

Fig.3. Relative amounts of the two mRNAs during illumination. Relative amounts of large subunit (•) and small subunit (○) mRNA were calculated on the basis of the results obtained in fig.1 and fig.2 with the value at 48 h taken as 1.

These present results, together with [8], indicate that the synthesis of RuBPCase is pre-translationally regulated during light-induction.

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