Phytochrome-mediated accumulation of chloroplast DNA in pea leaves

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Pea seedlings grown for 5 days in the dark were treated with red light for 5 min and grown for 2 more days in the dark. Effects of the red light on chloroplast DNA levels in the pea leaves were examined using probe DNA of the chloroplast-coded large subunit and nuclear-coded small subunit of ribulosebisphosphate carboxylase/oxygenase. The gene dosage of the large subunit, but not of the small subunit, was increased by red light. The increase was inhibited by subsequent far-red light treatment. These results indicate that accumulation of chloroplast DNA in the cell is mediated by phytochrome. Probably the replication of chloroplast DNA is mediated by phytochrome.

chloroplast DNA *Gene dosage* *Light induction* *(Pea seedling)* *Phytochrome* *Ribulose-bisphosphate carboxylase*

1. INTRODUCTION

Chloroplast development is a light-induced process accompanied by chloroplast DNA synthesis. The light induction of chloroplast DNA levels is not well characterized because the replication of DNA, both plastid and nuclear, and the division of both plastids and cells are superimposed.

Recent progress in gene engineering has made available several plant clones from nuclear and chloroplast DNA [1–4]. Using the cloned DNA fragments as probes for the nuclear and chloroplast DNA, we can estimate changes in chloroplast DNA levels compared with those of nuclear DNA irrespective of the division of chloroplasts and nuclei. We have reported for greening pea leaves that the amounts of the large subunit gene, encoded in the chloroplast genome, of ribulosebisphosphate carboxylase/oxygenase (RuBisCO) change with illumination time due to changes of the chloroplast DNA level, while those of the small subunit, encoded in nuclei, do not [5]. So we can follow changes in chloroplast DNA levels relative to nuclear DNA using the large and the small subunit gene as probes for chloroplast and nuclear DNA, respectively.

Here, we examine the effects of red and far-red light treatment on the gene dosage of the large and the small subunit to see if the light-induced increase in the chloroplast DNA level is mediated by phytochrome in pea leaves.

2. EXPERIMENTAL

2.1. *Growth of plants and light treatment*

Pea seedlings (*Pisum sativum* var. Alaska) grown for 5 days in the dark at 20°C were treated once with various kinds of light: no light (C), far-red light (λ_{max} 760 nm) for 5 min (FR), red light (λ_{max} 660 nm) for 5 min (R), or red light for 5 min followed by far-red light for 5 min (R + FR). The red and far-red light were the same as those described [6]. All of the treated plants were grown for 2 days more in the dark and divided into 2 groups. One group was harvested and called the dark sample. Another group was illuminated with white fluorescent lamps of about 13000 lux for 1

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day and called the light sample. From the apical buds of these 2 sets, DNA was extracted.

2.2. *Extraction of DNA*

DNA from the apical buds was extracted as described in [5]. From 1.5 g apical buds, 400–500 μg of total DNA was obtained. The deoxyribose contents were measured by the diphenylamine method [7] to check that the DNA was RNA-free.

2.3. *Dot-blotting and hybridization*

A sample of each DNA preparation was sheared, denatured and dot-blotted on a nylon membrane filter, Biodyne A (Pall), as described elsewhere [5]. The membrane was baked for 2 h at 80°C in a vacuum. The filter-bound DNA was prehybridized and hybridized with a nick-translated ^{32}P-labeled DNA probe, then the filter was washed and autoradiographed as described in [5].

The probe used for the detection of chloroplast DNA was a tobacco large subunit DNA (1.25 kbp *Bam*HI fragment), which codes for two-thirds of the amino acids from the C-terminal of the large subunit [8]; it was provided by Dr K. Shinozaki. The probe for the detection of nuclear DNA was a pea small subunit cDNA (0.7 kbp *Eco*RI-*Bam*HI fragment of pGR 407) provided by Dr S.M. Smith. It codes for all the mature sequence and the C-terminal 13 amino acids of the transit peptide [1].

3. RESULTS AND DISCUSSION

To see if the light-induced change of chloroplast DNA is mediated by phytochrome, we surveyed the effects of brief light treatment (5 min) with red and far-red light on the gene dosage of the large and small subunits of RuBisCO. Pea seedlings grown in the dark for 5 days were exposed to red or far-red light for 5 min and placed back in darkness for 2 days. We did 2 sets of experiments; one was an experiment without further white-light illumination after this treatment (dark sample), the other was one with 1 day of white-light illumination to reinforce the treatment in the dark (light sample). A sample of total DNA extracted from these apical buds was dot-blotted and hybridized with nick-translated ^{32}P-labeled probes as described in section 2.

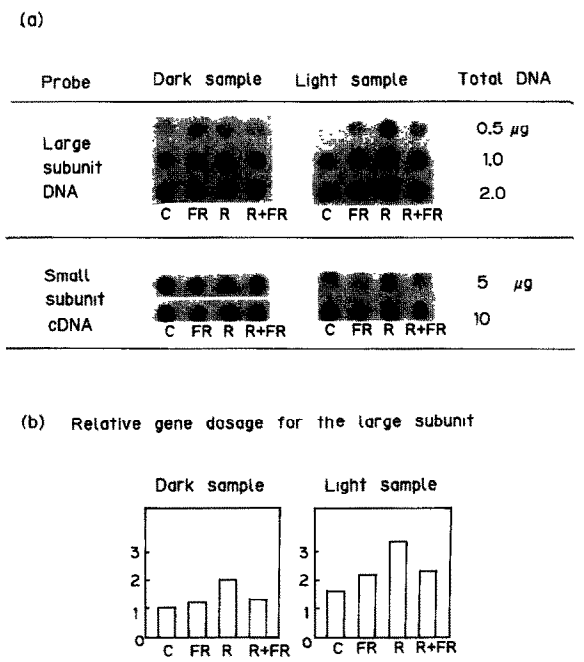

Fig.1. Effects of red and far-red light treatment on gene dosage for the large and the small subunits. The light treatments of pea seedlings and hybridization were done as described in section 2. 2–0.5 μg DNA was dot-blotted for measurement of the large subunit gene and 10 and 5 μg DNA for the small subunit gene. The specific activity of both probes was about 0.3–0.5 × 10^8/μg and the probe concentration in the hybridization buffer was about 0.1 μg/ml. The exposure time was 2–3 h for the large subunit gene and 7 days for the small subunit gene. The mean value of 3 analyses for the large subunit gene was calculated and expressed relative to that of the control (C) in the dark sample. Dark sample means the seedlings without white-light illumination after treatment, light means with white-light treatment as described in section 2.

Fig.1a is a typical autoradiogram showing the relative change of the 2 gene dosages. The black dot of the large subunit gene changes with light treatment, while the dots of the small subunit gene are thin and appear to be invariable, although the latter dots contain 5-fold the total DNA and are exposed for longer time. These are the results we expected. The small subunit genes are found as a small multigene family of 6 copies [9]; about 12 copies are present in diploid cell. The large subunit gene is present in one copy in the chloroplast DNA [10]. Some 5000–10000 copies of chloroplast DNA

are present in a cell [11], so the amount of the large subunit gene in diploid cell is about 500-fold that of the small subunit. For this reason, the dots of the small subunit gene are so pale that it is difficult to compare the density compared to that of the large subunit. We used the results of the small subunit gene as an indicator simply to guarantee the constant presence of nuclear DNA.

The autoradiogram for the large subunit gene was measured by a densitometer scan. The mean value of 3 analyses in the concentration range in which the measured area was proportional to the amounts of dotted DNA was calculated. The value was expressed relative to that of the control in dark sample, i.e. 7-day-old seedlings without illumination (fig.1b). Red light (R) enhanced the gene dosage of the large subunit, but far-red light treatment (R + FR) immediately following decreased them to the level of the far-red light control (FR) in 7-day-old seedling. These effects of a red light pulse, given in darkness, are reinforced when white light irradiates to the plant (light sample). This implies that light-induced increase of the large subunit gene is mediated by phytochrome. These changes of the gene dosage are attributable to those of chloroplast DNA levels because the change in gene dosage of the large subunit is due to that in chloroplast DNA levels rather than to a specific gene amplification of the large subunit [5]. Recently a sequence homologous to the large subunit gene was found in maize mitochondrial DNA [12]. However, the homologous sequence is not present in pea mitochondrial DNA [13]. Mitochondrial DNA does not increase with illumination [14], so the observed increase of the large subunit is ascribable to an increase of the chloroplast DNA.

These are the changes of the chloroplast DNA level in the total DNA. Total DNA from pea leaves contains both nuclear and extra-nuclear DNA. The amount of extra-nuclear DNA is at most 13% of the total DNA in mature pea leaves, because mitochondrial DNA is about 1% [14] and chloroplast DNA about 12% [15]. So, the bulk of total DNA is nuclear and we can assume that the gene dosage in total DNA is nearly equal to that per nuclear DNA level. Thus the increase of the gene dosage, being inducible by red light and reversible by far-red light, indicates that the increase of the chloroplast DNA in cell is mediated by pigment-protein phytochrome. This result agrees with the preliminary observations [16]. Light-dependent changes in quality and quantity of phytochrome have been reported with etiolated pea seedlings [17,18]. The increase of the chloroplast DNA is probably due to phytochrome-mediated stimulation of chloroplast DNA replication. However, DNA replication involves so many steps that at present we do not know which steps are mediated by phytochrome. Changes of several mRNA levels are mediated by phytochrome [6,16,19–22]. The mechanism involved in the process from the initial red-light signal to the effect of transcription of these mRNA is not known.

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