The Effect of Red Irradiation on Plastid Ribosomal RNA Synthesis in Dark-grown Pea Seedlings

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ABSTRACT

Dark-grown pea seedlings (*Pisum sativum* L.) were irradiated for a short period each day with low intensity red light (662 nm), red light immediately followed by far red light (730 nm), or far red light alone. Other plants were transferred to a white light regime (14 hours light/10 hours dark). There was no change in the amount of RNA in the tissue on a fresh weight basis after the various treatments. However, compared with dark-grown seedlings, those plants irradiated with red light showed an increase in the net RNA content per stem apex. In addition there was a two- to three-fold increase in ribosomal RNA of the etioplasts relative to the total ribosomal RNA. These increases were comparable to those found in plants grown in the white light regime. The changes were much smaller if the dark-grown plants were irradiated either with red light followed by far red light, or with far red light alone. Thus continuous light is not essential for the production of ribosomal RNA in plastids, and the levels of ribosomal RNA found in chloroplasts can also be attained in etioplasts of pea leaves in the dark provided the leaf phytochrome is maintained in its active form.

Evidence from several laboratories has shown that phytochrome mediates in photoregulating the synthesis of chloroplast proteins (3, 5, 13–15, 19). Thus activation of the phytochrome system in dark-grown pea seedlings by a brief irradiation with red light results in a marked increase in the synthesis of ribulose-1,5-diphosphate carboxylase and other chloroplast enzymes in the dark (3, 19). Continuous light then is not required for the synthesis of Calvin cycle enzymes, nor is it required for synthesis of enzymes of the C_4-dicarboxylic acid pathway of photosynthesis (4).

At least some of these chloroplast proteins appear to be synthesized on chloroplast ribosomes (17, 19, 20), but it is not known whether the phytochrome-mediated increase in plastid protein synthesis involves an increase in the number of plastid ribosomes or whether these ribosomes are already present in the etioplasts of dark-grown plants. Continuous light is known to increase the amount of plastid rRNA (1, 8, 22) and also plastid RNA polymerase (2) in some plants; the extent of the increase in rRNA varies with the type of plant and the age of the leaves.

In the experiments reported here, we have measured the amount of plastid and cytoplasmic rRNA in the apices of dark-grown pea seedlings and in seedlings subjected to various light regimes. The leaves of dark-grown seedlings contained lower amounts of chloroplast-type rRNA (16 S and 23 S) compared with green leaves, but after irradiating the seedlings with light at 662 nm for short periods, the 16 S and 23 S RNA increased to the levels found in the leaves of green plants of the same chronological age.

MATERIALS AND METHODS

Pea seeds (*Pisum sativum* L., cv Greenfeast, obtained from Yates Seed Co., Sydney) which had not been treated with fungicide or preservatives were used. The seeds were washed in a dilute solution of detergent (1% Teepol, Shell Chemical Co.), soaked in 3% (v/v) Chlorize (Nightingale Chemical Co., Sydney) for 15 min and then washed in running water in the dark for 24 hr. The beginning of the washing period was taken as the start of germination. The seeds were planted in trays containing vermiculite and grown at 25 C in total darkness. Four days after the beginning of germination batches of seedlings were exposed to different light regimes. These were: (a) control, kept in total darkness; (b) red light for 3 min only each day; (c) red light for 3 min followed immediately by far-red light for 10 min; (d) far red light for 10 min; (e) white light (warm white fluorescent light) for 14 hr per day. The air temperature was kept at 25 C. Light from a projector outside of the dark-room and fitted with a 500 w tungsten lamp and a "red cut-off" filter (Corning C.S. 2-62; transmission less than 0.5% at wavelengths less than 579 nm) was passed into the darkroom through an interference filter (λ_{max} 662 nm, band half-width 16 nm, Type PIL, Schott and Gena, Mainz, Germany) to produce red light. A different interference filter (λ_{max} 730 nm, band half width 10 nm, Type PIL) was used for far-red light. The trays of seedlings were placed at 45° to the light path and rotated at regular intervals to ensure uniform irradiation. The intensity of the light at the seedling apex was: red light, 72 $\mu w/cm^2$; far red light, 20 $\mu w/cm^2$; white light, 1.4 mw/cm^2. The plant material was harvested under a green safelight (24). After the various red light treatments (b–d above) the seedlings showed morphological changes characteristic of induction of the phytochrome system (7) but remained etiolated, while the seedlings grown in white light were green and appeared normal. Two trays of seedlings selected at random were used for each treatment and samples of 1 to 2 g of pea apex material were harvested from just below the third node on each plant. Immediately after harvest, RNA was extracted from the tissue by phenol extraction (method "B" of Loening and Ingle (12)) and the RNA separated by gel electrophoresis (10) on 2.6% polyacrylamide gels, 8 cm long, at 5 ma/gel for 12 hr. After elec-

trophoresis the gels were soaked in water for 4 hr and their absorbance at 260 nm scanned in a chomatogram scanner fitted to a Shimadzu MPS-50L spectrophotometer. The amount of RNA in the peaks was determined from triplicate gels by planimetry or by cutting out and weighing the peaks from the chart paper. The absence of a significant peak at 13 S indicated that there was little breakdown of the relatively unstable 23 S plastid rRNA. Experiments with rRNA from *Escherichia coli* showed that the area under a peak on the gel scan was proportional to the amount of RNA in the peak on the gel, up to at least 60 μg of RNA per peak. The S values of the RNA species found were established by coelectrophoresis with *E. coli* rRNA (16). RNA in the material harvested was also measured chemically (18).

RESULTS

Effects of Irradiation with Red Light on RNA Content of Pea Stem Apices.

Pea plants were germinated and grown in the dark and on the 4th day after the beginning of germination batches of seedlings were irradiated daily for a brief period with one of the following regimes: red (662 nm) light, red light followed by far red (730 nm) light, or far red light alone. Other batches were either kept in darkness or allowed to green in white light (see "Materials and Methods"). After 4 consecutive days of the various light treatments, the plants were harvested on the 5th day and the RNA content measured chemically. There was no change of RNA concentration per gram fresh weight in the stem apices after the various irradiations (Table I). This contrasts with the results of Jaffe (9) who found an increase in RNA per gram fresh weight in the buds of dark-grown peas (var. Alaska) after treatment with red light. The ratio of RNA to DNA in the pea stem apex also did not change as the result of the irradiation with red light (unpublished data). Figure 1a shows that throughout the period of irradiation in the experiment shown in Table I, the RNA concentration (per gram fresh weight) in dark-grown and red-irradiated pea stem apices remained about the same. However, the RNA content of each stem apex increased about 4-fold as a result of irradiation with red light (Table I, Fig. 1) and was similar to the changes in RNA content found in the green plant. The increase in RNA content was less marked if the irradiation with red light was followed immediately by far red light, or if the plants were irradiated only with far red light (Table I).

rRNA in Pea Stem Apices.

It has been pointed out that ribosomes may be classified on the basis of their size and the sizes or molecular weights of their constituent subunits and RNA (11, 19, 23). In plants the 80 S cytoplasmic ribosome has

Table I. *Effect of Red Irradiation on the RNA Content of Pea Stem Apices*

Pea seeds were germinated, grown in the dark, and then subjected to various light treatments on the 4th and subsequent days after germination, as described in the text. The pea stem apices were harvested on the 8th day after the beginning of germination, and the RNA was measured (18).

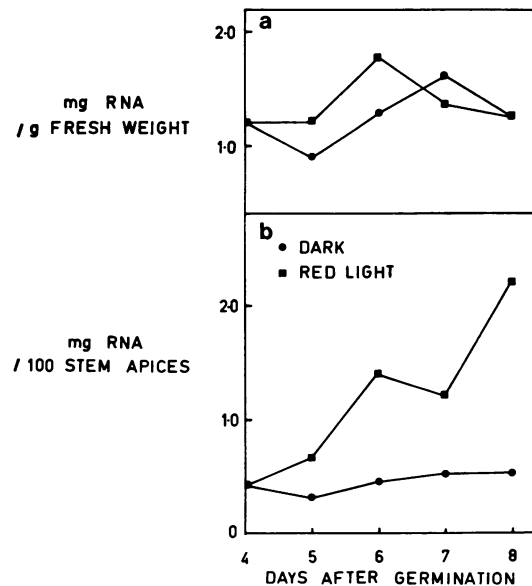| Treatment	RNA Content	
	Per gram fresh weight	Per 100 stem apices
	mg	
Dark	1.21 ± 0.05	0.53 ± 0.02
Red light	1.21 ± 0.04	2.20 ± 0.07
Red light, then far red light	1.30 ± 0.10	1.31 ± 0.10
Far red light	1.18 ± 0.09	0.68 ± 0.05
White light	1.22 ± 0.07	2.14 ± 0.12

FIG. 1. Effect of red light treatment on RNA content of pea stem apices. Dark-grown peas were irradiated with red light for 3 min daily from the 4th day after germination. Each day before the irradiation treatment, stem apices were harvested, and the RNA was measured chemically. a: mg RNA/g fresh weight; b: mg RNA/ 100 stem apices. ●: Plants kept in the dark; ■: plants irradiated with red light.

subunits of 60 S and 40 S containing respectively 25 S and 18 S rRNA. The chloroplast or etioplast ribosome, like the ribosome of procaryotic cells, is about 70 S and has 50 S and 30 S subunits containing 23 S and 16 S rRNA, respectively. These values can vary slightly among species and also depend on the techniques used to extract and measure the RNA.

Electrophoresis on polyacrylamide gels has been used to separate cytoplasmic rRNA from plastid rRNA after extraction of the RNA from pea leaves. On the basis of several experiments using *E. coli* rRNA as a standard, it was found that the S values for cytoplasmic rRNA are 26 S and 19 S, and for plastid rRNA they are 23 S and 17 S. However, in order to avoid confusion the commonly cited values of 25 S and 18 S for cytoplasmic rRNA and 23 S and 16 S for plastid rRNA are used throughout the text.

Effect of Red Light on the rRNA Content of Pea Stem Apices.

By determining the area under the peaks after gel electrophoresis of RNA extracted from the leaves of plants under various light conditions, the total amount of plastid rRNA relative to total rRNA could be measured. The results of three such experiments are presented in Table II. In experiment 1 in Table II, there was three times as much etioplast rRNA in etioplasts of plants irradiated with red light as in plants grown in the dark. Although there is some variation from experiment to experiment, the over-all effect of red light is to double or triple the amount of plastid rRNA in the apices to levels about the same as that found in plants grown under a white light regime.

Also shown in Table II is the amount of RNA extracted from the pea stem apices by phenol treatment compared with the amount of RNA in the apices as estimated chemically. In the three experiments shown, different recoveries were found and so it is unlikely that differential extraction of plastid and cytoplasmic rRNA could account for any of the differences in the ratio of plastid to cytoplasmic RNA shown in Table II.

Figure 2 shows the time course of the increase of rRNA in etioplasts relative to cytoplasmic rRNA in plants irradiated

with red light. Four days after germination of the seedlings the etioplasts accounted for only 6.5% of the total rRNA and, over the next 4 days in the dark, this amount rose slowly to about 10% of the total. However, in plants irradiated with red light the amount of etioplast ribosomal RNA relative to cytoplasmic rRNA increased over the 4 days of the irradiations to a value which corresponded to the normal ratio of chloroplast to cytoplasmic rRNA found in green leaves.

DISCUSSION

There was no significant change in the concentration of RNA per unit fresh weight or in the ratio of RNA/DNA in the pea stem apices in these experiments. Thus at first sight, the increase in RNA content/100 stem apices after irradiation with red light simply reflects the additional growth of the plant that is induced by the irradiation.

However, the polyacrylamide gel analyses show that there has been a change in the type of RNA being produced. After irradiation with red light there is a relatively higher amount of plastid rRNA in the irradiated apices, similar to the amount found in the green plant. This response to red irradiation appears to be mediated by the phytochrome system. The rise in plastid rRNA following irradiation with red light suggests that there is a corresponding increase in the number of plastid ribosomes and possibly an increased capability in the etiolated apex to make plastid proteins.

In related experiments (3), increases ranging from 10- to 91-fold on a per apex basis were found in the amounts of fraction I protein and the activities of ribulose-1,5-diphosphate carboxylase and NADP-glyceraldehyde-3-phosphate dehydrogenase. Most of these increases occur subsequent to the increase in plastid rRNA. Thus the increase in plastid rRNA shown in our experiments would explain, in part, the increased synthesis of plastid proteins observed by Graham et al. (3). However, the supply of ribosomes in the etioplasts is probably not the only factor limiting the synthesis of plastid proteins and irradiation with red light may also accelerate synthesis of plastid-specific mRNA, tRNA and aminoacyl tRNA synthetase. Total synthetase activity does increase in etiolated pea seedlings following irradiation with red light, but, as in the case of total RNA, the increase is proportional to the increase in tissue weight (6).

Table II. *Changes in Chloroplast Ribosomal RNA in Pea Stem Apices*

rRNA was extracted from pea stem apices subjected to various light treatments as described in the text. The amount of plastid rRNA as a percentage of the total rRNA was estimated by polyacrylamide gel electrophoresis. The yields of RNA obtained by phenol extraction in each experiment is also shown and is expressed as a percentage of the amount of RNA measured chemically.

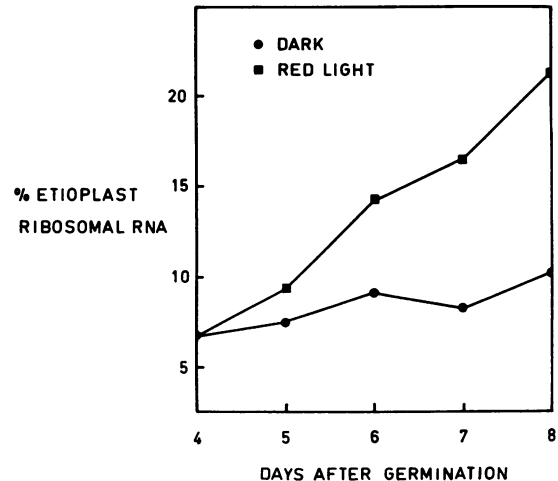| Treatment	Plastid RNA as Percentage of Total RNA		
	Experiment 1	Experiment 2	Experiment 3
Dark	7.3	12.9	7.8
Red light	22.0	18.1	14.0
Far red light	7.1	8.5	9.8
Red, then far red light	12.8	10.7	10.9
White light	18.5	21.2	18.9
Percentage of RNA extracted	35–50	30–38	79–81

FIG. 2. The relative amount of plastid rRNA in pea stem apices irradiated with red light. Irradiations were begun four days after germination. The amount of plastid rRNA relative to total rRNA was measured by polyacrylamide gel electrophoresis as described in the text. ■: Irradiated with red light; ●: plants kept in darkness.

It is not clear whether the effect of irradiation with red light is to change the course, as well as to increase the pace of the development of the dark-grown pea. As Figure 2 shows, there is a slow increase in the plastid rRNA content of dark-grown peas with time, and it may be that dark-grown peas over a long period could reach a similar stage of development as peas irradiated with red light were their seed reserves adequate. In barley seedlings where the rate of elongation of the leaves in the dark approximates that in the light, the ratio of plastid rRNA to cytoplasmic rRNA continues to increase as the etiolated leaves elongate (21).

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