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RELATIONSHIP BETWEEN PHOTOMORPHOGENESIS AND RNA SYNTHESIS IN OAT AND PEA SEEDLINGS*

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Abstract—Brief red light treatment causes a temporary rise in the rate of cytoplasmic *r*RNA synthesis in etiolated pea seedling buds and a temporary fall in excised epicotyl sections. In each case a lag of 1–3 hr occurred before the changes were detectable. No changes could be detected in *r*RNA synthesis in excised apical sections of dark-grown oat coleoptiles. By depressing *r*RNA synthesis selectively with 5-fluorouracil it was possible to show that red light treatment did not cause any changes in the overall rate of polydisperse RNA synthesis. Neither 5-fluorouracil nor actinomycin D selectively inhibited the red light induced changes in growth in pea and oat sections. The results suggest that phytochrome does not operate through the regulation of transcription.

INTRODUCTION

THERE are two basic hypotheses of the primary action of phytochrome. The first of these proposed that phytochrome acts directly on gene transcription, selectively regulating the formation of enzymes.[1] The later hypothesis proposed that phytochrome acts by regulating the permeability, and perhaps other properties, of certain critical membranes in plant cells, thus bringing about unknown but profound metabolic changes within the various cellular compartments.[2] Attempts to decide between these two models have centred on investigations of light-induced changes in RNA synthesis and in the levels of certain enzymes. It has been shown, for example, that phenylalanine ammonia-lyase levels in several plants are sensitive to light treatment and this has in certain cases been taken as evidence that light-induced enzyme synthesis is involved.[3–6] Similarly, inhibition of the light-induced increases in phenylalanine ammonia-lyase by inhibitors of RNA and protein synthesis has also been taken as supporting evidence for the gene-transcription hypothesis.[7] Recently, the density-labelling method has been used to provide direct evidence for the phytochrome mediated synthesis of phenylalanine ammonia-lyase in mustard seedlings.[8]

On the other hand, conflicting reports have appeared recently of the effects of red light

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[6] T. H. ATTRIDGE and H. SMITH, *Biochem. Biophys. Acta* **148**, 805 (1967).
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treatment on the rate of RNA synthesis in etiolated seedlings. Jaffe,[9] for example, has reported that red light treatment of pea seedlings leads to an increase in *r*RNA* synthesis in the terminal buds 24 hr after irradiation, and has concluded that the increased synthesis of RNA is involved in the phytochrome-mediated stimulation of bud growth. Consistent with this was the report of Bottomley[10] that red light treatment results in an increased specific activity of RNA-polymerase in pea seedling terminal buds. On the other hand, Dittes and Mohr[11] were unable to detect any differences in the rate of formation of rapidly-labelled RNA after light treatment of etiolated mustard seedlings within the lag phase of the known growth and biochemical responses in that organism. They thus concluded that rapid changes in RNA synthesis were not involved in the developmental responses to light treatment. This paper reports investigations into the effects of red light treatment on RNA synthesis in peas and oats and on the effects of 5-FU and of Act D on these responses.

The phytochrome-mediated growth responses studied were as follows: (a) the stimulation of the growth of pea seedling terminal buds; (b) the inhibition of extension growth in isolated pea epicotyl sections; and (c) the stimulation of extension growth in isolated apical sections of oat coleoptiles.

RESULTS

The Effect of Light on Growth and RNA Synthesis

Table 1 shows the overall changes in growth, RNA and protein content which occurred in each test object during a period of 24 hr in darkness following treatment with red and/or far-red light. It is clear that although in all cases the growth responses are evident within 24 hr, there are no significant differences in the levels of RNA and protein. In the intact seedlings, both RNA and protein levels increase, whereas in both of the isolated sections, RNA and protein levels decrease during the experimental period.

To determine whether or not an early phytochrome-mediated effect on RNA synthesis occurred, the tissues were exposed to ^{32}P for various periods of time followed by extraction of the RNA and separation on acrylamide gels. In this way it is possible to assess the rates of synthesis of *r*RNA (i.e. the rate of ^{23}P incorporation into the *r*RNA fractions). In Fig. 1, the specific radioactivity (i.e. dpm/mg RNA) of the heavy ribosomal component (i.e. the RNA fraction having a molecular weight of $1{\cdot}3 \times 10^6$ daltons, or the 1·3 M RNA) in the red light treatments is plotted as a percentage of the corresponding dark control against time after treatment. (Typical gel scans can be seen in Fig. 2, upper curves.) It is not reasonable to place much reliance on differences of less than 20% using this method, but even so it is clear from Fig. 1 that red light causes a transient stimulation of *r*RNA synthesis in the pea buds and a transient inhibition of *r*RNA synthesis in the pea stems, both processes becoming detectable about 2–3 hr after red light treatment. In each case the curves extrapolate to the 100% level at approximately 1 hr after red light treatment. Light had no detectable effect on RNA synthesis in oat coleoptile sections at any stage of treatment.

It is not possible using these methods to obtain any information on possible effects of light on the formation of *m*RNA, since *m*RNA is thought to run as a broad polydisperse

* Abbreviations: *r*RNA, ribosomal-RNA; *m*RNA, messenger-RNA; FU, 5-fluorouracil; act D, actinomycin D; SLS, sodium lauryl sulphate; 1·3 M RNA, the fraction of *r*RNA with a molecular weight of $1{\cdot}3 \times 10^6$ daltons.

[9] M. J. JAFFE, *Physiol. Plant.* **22,** 1033 (1969).
[10] W. BOTTOMLEY, *Plant Physiol.* **45,** 608 (1970).
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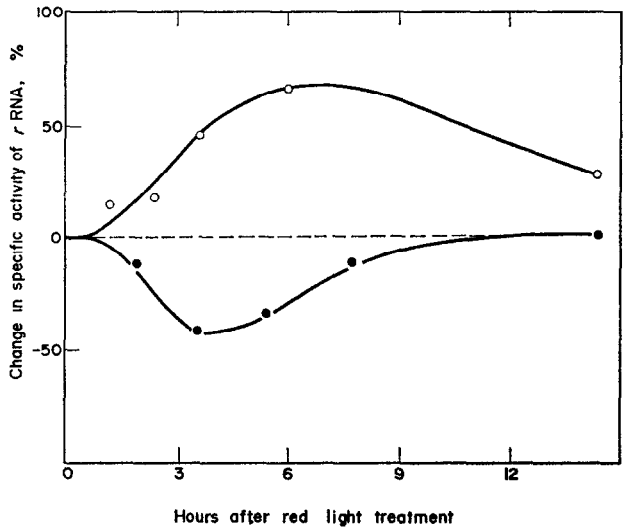

FIG. 1. THE EFFECT OF BRIEF RED LIGHT TREATMENT ON THE RATE OF SYNTHESIS OF RIBOSOMAL-RNA IN THE TERMINAL BUDS OF INTACT PEA SEEDLINGS (○) AND IN EXCISED PEA EPICOTYL SECTIONS (●). The percentage difference between the specific activity of the 1·3 M RNA in the treated materials and that in the corresponding dark controls is taken as a relative measure of the rate of cytoplasmic *r*RNA synthesis.

band on the gels. The purine analogue FU is known to inhibit preferentially the synthesis of *r*RNA and to have very little effect on *m*RNA (i.e. polydisperse-RNA) synthesis.[12] Thus, it was considered that the use of FU may prevent *r*RNA synthesis and enable possible

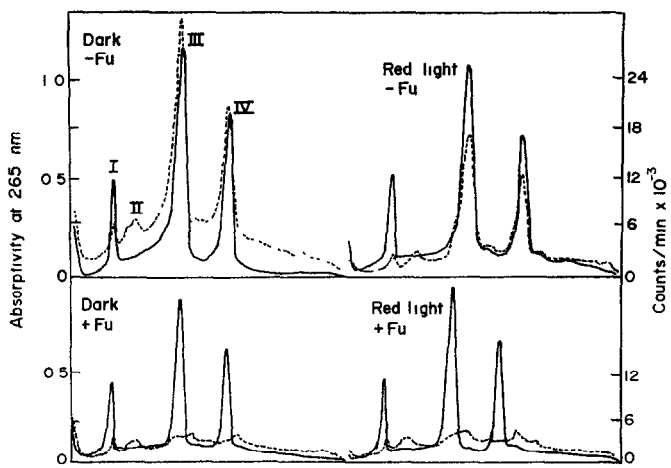

FIG. 2. GEL ELECTROPHORETOGRAMS OF TOTAL NUCLEIC ACID EXTRACTS PREPARED FROM DARK-GROWN AND RED LIGHT TREATED PEA EPICOTYL SECTIONS INCUBATED IN ^{32}P AND IN THE PRESENCE OR ABSENCE OF 600 μg/ml FU.
————, absorptivity; - - - -, radioactivity; Peak I, DNA; Peak II, *r*RNA precursors; Peak III, 1·3 M RNA; Peak IV, 0·7 M RNA.

[12] J. L. KEY, *Pl. Physiol.* **57**, 1257 (1966).

gross differences in the synthesis of the polydisperse-RNA to be detected, and at the same time provide a test as to whether continuous *r*RNA synthesis is necessary for the growth responses.

TABLE 1. THE EFFECTS OF VARIOUS LIGHT TREATMENTS ON THE GROWTH, RNA CONTENT AND PROTEIN CONTENT OF THE THREE EXPERIMENTAL MATERIALS USED

Experimental material	Parameter	Initial I	Dark D	Red R	Red/Far Red R/FR
Pea (terminal bud)	Fresh weight (mg)	5·2	9·8	12·8	8·6
	RNA/bud (μg)	37·0	56·0	64·0	45·0
	Protein/bud (μg)	92·0	188·0	254·0	200·0
Pea (epicotyl section)	Length	6·0	8·95	8·01	8·45
	Fresh weight	16·9	23·7	21·2	23·3
	RNA section	17·5	12·0	11·1	11·5
	Protein/section	125·0	129·0	131·0	136·0
Oat (coleoptile section)	Length	6·0	8·9	11·0	9·5
	Fresh weight	4·8	11·2	14·0	11·9
	RNA/section	7·5	5·1	5·0	5·2
	Protein-section	120·0	107·0	90·0	107·0

Due to the problems of penetration and transport, FU was only tested on isolated sections. Figure 2 shows the effects of FU at 600 μg/ml on nucleic acid synthesis in pea sections as affected by red light. FU prevented the incorporation of ^{32}P into the *r*RNA, although incorporation into the high molecular weight precursors to *r*RNA was not prevented. It is also clear that in the presence of FU there is no inhibitory effect of red light on the incorporation of ^{32}P into the polydisperse-RNA. This could be taken as evidence that light does not influence the overall rate of *m*RNA production. On the other hand, treatment of sections with red light in the absence of FU appears to show an inhibition of polydisperse-RNA synthesis (Fig. 2, upper curves). It is likely however, that the high level of radioactivity in the polydisperse-RNA in the dark controls reflects the tailing of the *r*RNA peaks on the gel and is not only due to *m*RNA. Similar experiments were carried out with oat sections with substantially similar results except that red light had no effect on the synthesis of any fraction of nucleic acid.

TABLE 2. THE EFFECTS OF 5-FLUOROURACIL ON THE GROWTH AND RNA CONTENT OF PEA AND OAT SECTIONS IN CONTINUOUS DARKNESS OR TREATED WITH RED LIGHT

Plants	Treatment*	Elongation (mm) Dark	Red	R/D%	Fresh weight (mg) Dark	Red	R/D%	RNA (μg) Dark	Red	R/D%
Peas	Control	2·3	1·5	65·2	6·8	4·4	64·8	12·0	11·4	95·0
	FU	2·0	1·6	80·0	6·8	4·8	70·6	10·8	11·0	101·8
Oats	Control	3·0	5·1	170·0	5·2	7·0	134·6	5·8	5·6	96·6
	FU	3·1	5·1	164·5	5·2	7·0	134·6	6·2	6·4	103·2

* 5-Fluorouracil applied at 600 μg/ml.

FU also has no selective effect on the photoinduced growth responses in either peas or oats, as is shown in Table 2. Elongation growth and fresh weight increase of the pea sections are inhibited to the same extent by red light in the presence or absence of FU, and there is no change in total RNA content. In the oat coleoptile sections the red light mediated increases in length and fresh weight are found both in the presence and absence of FU. Again total RNA content is unchanged. These results suggest that the synthesis of *r*RNA is not an essential step in the developmental responses to red light.

In order to determine whether or not the synthesis of other fractions of RNA was essential for the light response, the effects of Act D were investigated. Table 3 shows the effect of Act D on the growth changes in both pea and oat coleoptile sections. In neither case does Act D selectively inhibit the light responses, although higher concentrations increasingly inhibit growth both with and without light treatment.

TABLE 3. THE EFFECTS OF ACTINOMYCIN D ON THE RED-LIGHT INDUCED CHANGES IN THE GROWTH OF PEA AND OAT SECTIONS

Plants	Treatment	Elongation (mm)			Fresh weight (mg)		
		Dark	Red	R/D%	Dark	Red	R/D%
Peas	Control	2·9	2·1	72·4	6·8	4·7	69·1
	Act D 2 μg/ml	2·4	2·0	83·4	5·8	4·7	81·0
	Act D 10 μg/ml	1·9	1·5	78·9	5·0	2·0	40·0
Oats	Control	3·6	5·7	158·3	4·5	7·9	175·6
	Act D 2 μg/ml	3·6	4·9	136·1	1·5	3·3	220·0
	Act D 10 μg/ml	2·5	3·5	140·0	2·2	2·7	122·0

DISCUSSION

Red light treatment of pea seedlings temporarily increased the rate of *r*RNA synthesis in the terminal buds, whereas treatment of isolated pea sections temporarily reduced the rate of *r*RNA synthesis. These changes reflect the changes in growth rate and the time courses indicate that the synthesis of *r*RNA is part of the growth process, but not necessarily directly under phytochrome control. In both of these tissues cell division plays an important part in the growth process, and it would seem likely that the normal formation of new cells would involve *r*RNA synthesis. It is clear from the inhibitor experiments, that cessation of *r*RNA synthesis due to FU treatment, does not fully prevent the light-induced changes in growth.

With the oat coleoptile sections, extension growth occurs in the absence of cell division, and it may be that such growth occurs without a concomitant increase in ribosome formation. The lack of effect of red light on *r*RNA synthesis can therefore be attributed to the non-essentiality of *r*RNA synthesis for growth in this system. Again, if *r*RNA synthesis is prevented by FU application, the phytochrome mediated growth responses are still evident. It seems reasonable to conclude, therefore that phytochrome does not act through a mechanism which regulates *r*RNA synthesis.

The more important question as to whether or not *m*RNA synthesis is implicated in the light responses is more difficult to resolve. Methods are not yet available for the separation and estimation of total or specific *m*RNA in plants and thus it is only possible at the present time to make deductions from indirect observations. When *r*RNA synthesis was prevented by FU treatment, no obvious differences were observable in the label incorporated into the

polydisperse fractions in the dark-grown and light-treated sections. This may be taken to indicate that marked overall changes in total *m*RNA synthesis are not involved in the light responses. On the other hand, it would seem more likely, if phytochrome were acting directly on gene transcription as suggested by Mohr,[1] that any changes in *m*RNA formation would be small and specific. It would be expected in this case, that the light responses would be particularly sensitive to Act D application, as was observed by Mohr and Lange[13] for the photoinduction of anthocyanin synthesis in mustard. In the photomorphogenic responses studied here, Act D did not selectively suppress the growth effects; in fact the only generalisation that can be made is that Act D is rather more inhibitory to rapidly growing sections, whether they are light-treated or dark-grown, than it is to more slowly growing sections. In the case of anthocyanin synthesis in *Sinapis*,[13] it was necessary to preincubate in Act D to get significant inhibition, which suggests that the availability of unstable *m*RNA species is necessary for the phytochrome-induced effects, but does not suggest that *m*RNA synthesis *per se* is required.

It does not seem likely from these results that phytochrome operates through the regulation of transcription, although it is not possible at the present time to conclude that regulation of enzyme synthesis occurs at the level of translation since observations with phenylalanine ammonia-lyase[14] suggest that enzyme synthesis occurs in both light- and dark-grown plants, but that inactivation of the enzyme occurs most rapidly in plants that have not been treated with light. The most logical conclusion at this point in time appears to be that the observed effects on RNA and enzyme synthesis are relatively remote consequences of the primary photoact, and although they may be implicated in the ultimate developmental processes, they cannot be regarded as being intimately involved in the primary action of phytochrome.

EXPERIMENTAL

Incubation of tissues. Epicotyl sections (6 mm) of the 3rd internode just below the apical hook, of seven day old etiolated *Pisum sativum*, cv. Alaska seedlings, were cut and incubated in 2·5 mM succinate buffer pH 6,2, containing 2% (w/v) sucrose, 20 μg/ml streptomycin, 20 μg/ml penicillin, and 60 μg/ml gramicidin. This medium is called SS-medium. 20 sections were shaken in 3 ml medium in the dark at 26°.

Apical coleoptile sections (6 mm) were cut from 4-day-old dark-grown seedlings of *Avena sativa*, var. Blenda. The leaves were pulled out and the sections incubated as the pea sections. All seeds were obtained from Carters Seeds Ltd., Wimbledon, Surrey.

Incubation with ^{32}P and inhibitors of RNA synthesis. Carrier-free ^{32}P–Na_3PO_4 (PBS-1, Radiochemical Centre, Amersham, England) at a final concentration of 50–100 μCi/ml was added to the SS-medium just before irradiation. 5-FU (Koch-Light Laboratories Ltd., Liverpool) at a final concentration of 600 μg/ml was added to the incubation medium 2 hr before irradiation. Act D (Calbiochem, California) was added at a final concentration of 2 or 10 μg/ml at the beginning of irradiation. ^{32}P was applied to intact pea seedlings by placing the seedlings with their roots in ^{32}P solution under constant temperature conditions. The seedlings were left for 24 hr prior to light treatment.

Light treatments. The red light source consisted of three red fluorescent tubes (47 cm, 15 W, Atlas Double Life red) filtered through one layer of Cinemoid No. 1 Yellow, and one layer Cinemoid No. 14 Ruby (Strand Electrics, Kingsway, London). The wavelength range was 600–690 nm with a peak transmission at 660 nm and an intensity of 700 erg cm^{-2} sec^{-1} at plant height. The far red light source consisted of 5 Osram tungsten bulbs (150 W, 69 VL) filtered through 5 cm H_2O and one layer No. 5A Deep Orange and one layer No. 20 Deep Blue Cinemoid. No light was emitted below 735 nm although transmission extended beyond 1100 nm. The intensity was 10 K erg cm^{-2} sec^{-1}, much of this at wavelengths longer than those absorbed by P_{FR}.

The dark-grown tissue was cut under a dim green safe light consisting of one Osram tungsten bulb (60 W) filtered through 3 layers of Cinemoid No. 39 Primary Green, which cut off at 600 nm. Exposure of either of the plant tissues to this source did not result in a measurable growth response. The sections were cut under the safe light, then irradiated with red light for 5 min, placed on a shaker and further incubated in the dark, or the red light treatment was immediately followed by 10 min irradiation with far-red light.

[13] H. LANGE and H. MOHR, *Planta* **65**, 107 (1965).
[14] M. ZUCKER, *Pl. Physiol.* **47**, 442 (1971).

Phenol extraction of RNA. A phenol-method as described by Kirby[15] was followed with modifications. The tissue was homogenized in 1 ml 0·01 M Tris-HCl buffer pH 7·4 containing 50 mM NaCl and 1% (w/v) *p*-amino-salicylic acid in a mortar and transferred into centrifuge tubes. The mortar was rinsed with two 1·5-ml portions of the same solution and combined with the homogenate. An equal volume of H_2O-saturated phenol containing 0·1% (w/v) 8-hydroxyquinoline and 14% (v/v) m-cresol was added, mixed thoroughly and centrifuged. The phenol layer was removed and 1 ml 1·3 M NaCl and 4 ml phenol-cresol were added, mixed and centrifuged again. The tris layer was then removed and extracted once more with 5 ml phenol-cresol. The final tris layer was made with 0·02 M with respect to NaOAc and the RNA was precipitated with 3 vol. of EtOH overnight at 4°. After centrifugation the RNA precipitate was washed with 80% (v/v) aq. EtOH, centrifuged and dissolved in 2 ml 0·15 M NaOAc and 0·25% (w/v) SLS. The RNA was precipitated by addition of 6 ml EtOH at −20° for 1 hr.

RNA separation. The final precipitate was dissolved in electrophoresis buffer containing 6% (w/v) sucrose. Total nucleic acid values were determined by measuring the absorptivity at 260 nm of this solution using an extinction coefficient of $E^{260}_{1\%} = 240$. The RNA was separated by polyacrylamide gel electrophoresis following the method of Loening[16] except that 2·6% gels were used and the electrophoresis buffer contained 36 mM Trisma base, 3 mM NaH_2PO_4, pH 6·8 and 0·02% (w/v) SLS. Electrophoresis was for 3·5 hr at 60V and 5mA/gels. The gels were scanned at 260 nm with a modified Unicam SP500 monochromator equipped with a Hilger-Gilford photometric unit and a Rikadenki recorder, frozen in dry ice and cut into 1 mm sections. The sections were placed onto adhesive labels, dried and placed into toluene-based scintillation fluid and counted in a Nuclear Enterprise (Edinburgh, Scotland) scintillation counter.

Protein estimation. Estimation of soluble protein was carried out by the Lowry method.[17]

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[16] U. E. Loening, *Biochem. J.* **102**, 251 (1967).
[17] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

Key Word Index—Pisum sativum; Leguminosae; pea; *Hordeum sativum*; Gramineae; oat; photomorphogenesis; RNA-synthesis; phytochrome.