Deoxyribonucleic Acid-dependent Ribonucleic Acid Polymerase Activity of Nuclei and Plastids from Etiolated Peas and Their Response to Red and Far Red Light in Vivo

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ABSTRACT

DNA-dependent RNA polymerase activity has been found in both the nuclei and etioplasts of dark-grown pea seedlings (Pisum sativum). Although these enzymes had similar overall characteristics with respect to substrate, pH, and inhibitor responses, they could be distinguished by their different sensitivities to sonicication.

Brief irradiation of the seedlings with red light resulted in an increase in the activity of both the nuclear and plastid enzymes, but the time course of this response showed it to be too slow to be considered an early result of phytochrome control of metabolism. Far red light following red treatment largely prevented the increase in the activity of the nuclear enzyme. The plastid enzyme responded to far red light in a manner similar to its response to red light so that no reversal was observed when far red treatment followed red.

Although the physiological consequences of the stimulation of the nonphotosynthetic pigment phytochrome have been extensively documented, relatively few studies of the effect of this photosystem on specific biochemical reactions have been made. Phenylalanine deaminase has been shown to be sensitive to phytochrome control in mustard seedlings (5, 19) and etiolated pea buds (2). Surry (21) demonstrated a phytochrome response of lipoxidase activity in squash seedlings, and Graham et al. (7) reported large increases in several Calvin cycle enzymes in pea seedlings following repeated daily doses of red light. Marcus (14) showed increases in TPN triose phosphate dehydrogenase, and Margulies (15) found increases in glyceraldehyde 3-phosphate dehydrogenase following red light irradiation. Both these effects were also reversed by subsequent far red treatments.

The work described here demonstrated the presence of DNA-dependent RNA polymerase (nucleoside triphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) in both the nuclei and etioplasts of the apical buds of dark-grown pea seedlings. Some of the characteristics of the enzymes from the two organelles are compared, and the response of the two enzyme activities following red and far red light treatment of the seedlings is examined. This enzyme has previously been reported in the organelles of a number of plants including the nuclei of green peas (11).

MATERIALS AND METHODS

Plant Material. Seeds of Pisum sativum cv. Greenfeast were soaked in water for 5 hr and planted in vermiculite. They were grown in complete darkness before light treatment and were usually harvested when 7 days old.

Light Sources. Red light was provided by a bank of three Phillips 20 W red fluorescent tubes covered with four layers of red cellophane suspended approximately 20 cm from the apical buds of the plants. Far red light was obtained from three Mazda 150 W reflector incandescent lamps suspended in a water bath over a sheet of Rohm and Haas Black Plexiglass FR700.

The emission intensities of the two sources are shown in Figure 1.

Isolation of Nuclei and Plastids. During isolation and assay, all solutions contained 10 mM mercaptoethanol and 25 pg ml chloramphenicol. The chloramphenicol had no detectable effect on the enzyme activity. During isolation all solutions were kept at 0 to 4°C. Approximately 10 g of terminal buds of the etiolated peas were harvested, cooled in ice, and chopped with a razor blade in 20 ml of 0.05 M tris (pH 8.3 at 4°C) containing 10 mM MgCl₂ and 0.4 M sucrose, by the method of Spencer and Wildman (20). After filtration through two layers of Miracloth (Chicopee Mills, Inc.), the suspension was centrifuged at 700 g for 10 min. The pellet was resuspended in 5 ml of the 0.4 M sucrose-tris-MgCl₂/chopping buffer and layered on a discontinuous sucrose gradient consisting of 5 ml each of 1.4 M, 1.1 M, 1.0 M, 0.8 M, and 0.6 M sucrose in 0.05 M tris (pH 8.3 at 4°C) containing 10 mM MgCl₂. The gradient was centrifuged at 1000 g for 15 min, after which time the intact etioplasts were recovered from the 1.0 M sucrose layer and the nuclei from the pellet at the bottom of the tube.

Carotenoid Estimation. A 0.2 ml aliquot of the plastid suspension was diluted with 0.8 ml of acetone, and the solution was shaken, warmed, and centrifuged to clarify it. The absorbance of the supernatant at 473 nm was determined, and the carotenoid content was calculated assuming E₄₇₃ to be 2500 (6).

The total carotenoid in the buds was determined by macerating 0.5 g of tissue with 10 ml of acetone in an Omnimixer. The macerate was filtered, washed, and the combined filtrates were made up to a standard volume with 80% acetone. Carotenoid was measured as described above.

DNA Estimation. The DNA was isolated following the method of Spencer and Whitfield (19), and the estimation was carried out by the diphenylamine method of Burton (4).

RNA Polymerase Assay. These assays were carried out in duplicate following the method of Spencer and Whitfield (18). The incubation mixture consisted of 0.2 ml of 0.05 M Tes, pH 8.3, containing 10 mM MgCl₂; 30 μmole each of the triphosphates of cytidine, guanosine, and uridine; 1 μmole of phosphoenol pyruvic acid cyclohexy lammonium salt; 1 μl of pyruvate kinase sus-
pension (type II, Sigma Chemical Co.); and 2 μl of a solution of
3H-ATP (1 mc/ml; 7.1 c c/mole, Schwarz Bioresearch Inc.). To
this was added 0.2 ml of a suspension of organelles, equivalent
to 1 g of fresh weight of tissue, in 0.05 M Tes, pH 8.3, containing
10 mM MgCl₂. Incubation was carried out for 10 min at 25°C
after which the reaction was stopped with 0.5 ml of 0.1 M sodium
pyrophosphate (pH 3.0) and 1 ml of 10⁻⁶ M trichloroacetic acid.
The precipitates were collected and assayed as described by
Spencer and Whitfield (18).

**Sonication.** Sonication was carried out at 0 to 4°C in the Tes-
MgCl₂ buffer with a Raytheon 50W, 10-kc sonic oscillator (Ray-
theon Manufacturing Co. Waltham, Mass.). Following sonication
the suspensions were centrifuged at 1000 g for 5 min, and the
pellets were resuspended in Tes-MgCl₂ for assay.

**Isolation of the RNA Product.** For isolation of the RNA a sus-
pension of organelles isolated from 8 to 10 g fresh weight of pea
buds was incubated with tritiated 3H-ATP and cofactors. After
20 min the mixture was frozen, phenol was added, and the RNA
was extracted by the method of Spencer and Whitfield (19). The
product was dissolved in 0.2 ml of water and layered on a 5 to 20°C
linear sucrose gradient and centrifuged at 37,000 g for 4 to 5 hr.
The bottom of the tube was pierced, fractions were collected, the
RNA was estimated spectrophotometrically, and aliquots were
assayed for radioactivity.

**RESULTS**

The cells of etiolated pea apical buds are much smaller than
those of green tissue, and the difficulties associated with breaking
the cells open without rupturing the organelles are greater. The
best results were obtained by chopping with a razor blade by the
method of Spencer and Wildman (20) with a mechanical chopper.
Even this method yielded only about 2% of the total etioplasts
in the purified preparation as determined by carotenoid content.
This low yield made it impossible to use tissue weight as a basis
for comparison of different treatments, and so the RNA poly-
merase activities of the plastids were compared on the basis of
carotenoid content and those of nuclei on DNA content. This
specific activity was found to be reasonably constant from prep-
aration to preparation, as well as remaining constant during
normal manipulations even when part of the activity was lost.

In studies that have been reported previously, the RNA poly-
merase activity in spinach (19), broad bean (12), and tobacco (17)
chloroplasts was either greater than or about equal to that of the
nuclei on a per cell basis. In etiolated peas the nuclei isolated
from preparations contributed three to five times as much ac-
tivity as the plastids in the preparation. This is a species difference
rather than a characteristic of etiolated tissue since the chloro-
plasts and nuclei of light-grown peas had a similar ratio of RNA
polymerase activity.

Because it was extremely difficult to prepare the plastids com-
pletely free of nuclei, it was important to know to what extent
the contaminating nuclei were contributing to the activity of the
plastid fraction. It was found that the RNA polymerases from
the two sources differed markedly in their stability to sonication.
While the nuclear polymerase activity was virtually unaffected
after 15 min of sonication, that from the etioplasts was almost
completely destroyed within 5 min (Fig. 2). Since the polymerase
in the etioplast preparations was reduced to 5 to 8% of its original
activity under conditions where the nuclear polymerase was unaf-
fected, it follows that the nuclear contribution to the measured
activity of this purified plastid fraction must be very low and
could be safely ignored.

Both nuclear and plastid polymerases had a broad pH optimum

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**Table 1. Effect of Cofactors and Inhibitors on the Activity of RNA Polymerase in Preparations of Nuclei and Plastids**

<table>
<thead>
<tr>
<th>Relative Activity</th>
<th>Nuclei</th>
<th>Etioplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>UTP</td>
<td>36</td>
<td>45</td>
</tr>
<tr>
<td>CTP</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>GTP</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>UTP, CTP, and GTP</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>34</td>
<td>23</td>
</tr>
<tr>
<td>MgCl₂ + MnCl₂ (1 mm)</td>
<td>50</td>
<td>67</td>
</tr>
<tr>
<td>+Actinomycin D (10 μg/ml)</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>+DNase² (10 μg/ml)</td>
<td>41</td>
<td>8</td>
</tr>
<tr>
<td>+RNase² (10 μg/ml)</td>
<td>27</td>
<td>18</td>
</tr>
</tbody>
</table>

1 With the complete medium under the conditions used the nuclei incorporated 500 μg of the added 3H-ATP per mg of DNA. The plastids incorporated 15 μg of added 3H-ATP per μg of carotenoid.

² The DNase and RNase treatments were incubated for 5 min prior to addition of the 3H-ATP.
centered at pH 8.3 and were almost completely dependent on the supply of all four nucleoside triphosphates (Table I). They had a requirement for magnesium, with an optimum at 10 mM, which could not be replaced completely by manganese (Table I). Low concentrations of manganese (0.5 to 2.0 mM) also had a stimulatory effect, but the increase was only about 60 to 70% of that obtained with magnesium. In the presence of optimal concentrations of magnesium even low concentrations of manganese inhibited the activity in both nuclei and etioplasts. The incorporation of ATP was strongly inhibited by DNase and RNase and also by actinomycin D (Table I), indicating that the activity was due to a DNA-dependent RNA polymerase. The sensitivity to DNase together with the almost complete dependence on nucleoside triphosphates eliminated the possibility of significant contribution by contaminating microorganisms. This conclusion was confirmed by the lack of any increase in activity when a reaction mixture was incubated for 2 hr at 25°C prior to addition of the 

The polymerase activity was relatively insensitive to added DNA. High levels of added calf thymus DNA (250 μg/ml) resulted in increases of about 40%.

**Nature of the Product of the Reaction.** When the RNA was isolated from incubation mixtures of either plastids or nuclei and subjected to sucrose density gradient centrifugation in the presence of unlabeled pea root RNA, the labeled RNA formed a polydisperse band which did not correspond with either the ribosomal RNA or the transfer RNA but, in fact, exhibited a sedimentation pattern that is usually associated with messenger RNA (7). No significant differences were observed between the density gradient patterns from the RNA of red light-treated plants and that from dark controls, suggesting that there was no gross change in the type of RNA being synthesized.

**Effect of Red and Far Red Light.** When the dark-grown peas were given a short irradiance (5 sec–5 min) with red light 16 hr prior to harvest, the activity of the RNA polymerase in the nuclei increased by about 50% over that of the dark control (Table II). Treatment with far red light immediately following the red treatment reduced this increase to the level of that obtained from plants which had received only far red light (Table II). The polymerase from the plastids showed a similar increase in activity under the influence of red light, however, since far red light treatment alone also resulted in a comparable increase, the sequence red-far red exhibited no reversal of the stimulation.

**Table II. Effects of Red and Far Red Light**

<table>
<thead>
<tr>
<th></th>
<th>Nuclear RNA</th>
<th>Etioplast RNA</th>
<th>Fresh Weight</th>
<th>Carotenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Far red</td>
<td>31</td>
<td>48</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Red</td>
<td>51</td>
<td>54</td>
<td>42</td>
<td>29</td>
</tr>
<tr>
<td>Red-far red</td>
<td>32</td>
<td>52</td>
<td>23</td>
<td>27</td>
</tr>
</tbody>
</table>

1 Differences between the treatments were not significant at the 5% level. All other differences were significant at the 0.1% level. All light treatments were significantly different to the dark controls at the 0.1% level.

Henshall and Goodwin (10) have shown that the amount of carotenoid in etiolated pea buds is increased following red light treatment, that this effect is most marked in 4-day-old seedlings, and that it decreases with seedling age. If the carotenoid content and polymerase activity were similarly affected by light, it would be expected that no change in specific activity would be observed even if the total activity changed. With the 7- to 9-day-old plants used in the present experiments there was some effect of light on carotenoid content, but these were small (Table II) and could not significantly affect the pattern of RNA polymerase activity.

**Time Course of the Response of RNA Polymerase Activity to Red Light.** The kinetics of the increase in RNA polymerase activity in response to red light was determined by harvesting apical buds at various times after irradiation. It was found that virtually no changes in activity could be detected in less than 4 hr in either nuclei or etioplasts.

**DISCUSSION**

There are two current ideas concerning the mechanics of phytochrome control. The first envisages phytochrome as selectively controlling the permeability of membranes and exerting its influence by the regulation of the levels of substrates and other essential substances (9). The evidence for this mechanism rests largely on the rapidity of some phytochrome responses and on observed changes in electrolyte flux following phytochrome activation. The second invokes control of selective activation of genes leading to the synthesis of new enzymes (16).

This second theory would involve, as an early step, the transcription of new messenger RNA from the newly activated genetic material. It is possible, therefore, that phytochrome activation would be reflected in an early rise in the activity of the enzyme system that is responsible for the synthesis of mRNA, namely DNA-dependent RNA polymerase.

This enzyme is known to be present in three types of organelles—nuclei, plastids, and mitochondria. The nuclei and etioplasts isolated from dark-grown pea buds by sucrose density centrifugation both had RNA polymerase activity although, in contrast to other plants already reported, the nuclear polymerase was considerably more active than the plastid polymerase. This ratio of activity also held true for chloroplasts and nuclei isolated from light-grown peas.

The polymerases had the usual characteristics of RNA polymerase from other sources and had a requirement for magnesium which could not be replaced entirely by manganese. Kirk (13) found that light-grown broad bean chloroplasts responded to magnesium and manganese in a similar manner but that, in contrast to peas, the nuclear polymerase was unaffected by 2 mM MnCl2 in the presence of 10 mM MgCl2. The polymerase activity from the two organelles differed in their sensitivity to sonication. No attempt was made to find out whether this difference was due to difference in sensitivity of the enzymes themselves or of the DNA from the two sources. If the enzyme exists in association with the DNA template, then it can be imagined that sonication may shear the DNA, leaving only a very short template to be transcribed. This would lead to decreased activity provided that the polymerase was unable to dissociate from the template or to reassociate with another DNA strand. The former explanation is supported by the finding of Anthony et al. (1), who showed that _Escherichia coli_ RNA polymerase was bound more strongly to sonicated DNA than to native DNA. That this difference on sonication reflects differences in sensitivity of the DNA is also supported by the observation that DNA-dependent DNA polymerase activity from nuclei and etioplasts of peas show similar differences (W. Bottomley, unpublished results). None of the activity lost by sonication of etioplasts could be restored by the subsequent addition of calf-thymus DNA to the preparation.
The increases in fresh weight of the pea buds 16 hr after irradiation with red and far-red light indicated that the plants were responsive to the phytochrome system and these changes were paralleled by changes in the activity of the RNA polymerase of the nuclei. The RNA polymerase in the etioplasts also was stimulated by red light, but, unlike the nuclear polymerase, there was no far-red reversal and, in fact, far-red light appeared as effective as red itself in increasing the activity of this enzyme. The total increase in activity of RNA polymerase was not very great, and, in addition, no increase in activity could be detected in either nuclei or etioplasts in less than 4 hr. Since by this time it is also possible to detect gross changes in metabolism, such as fresh weight increases (3), it followed that increasing activity of RNA polymerase is not one of the early manifestations of phytochrome regulation. This does not discount the "genetic expression" thesis of phytochrome action, since there could be qualitative changes in mRNA synthesis without any net change in the total RNA polymerase activity. This would lead to the synthesis of a range of new mRNA molecules which may then effect the later changes in metabolism.

The possibility that the RNA polymerase from the etioplasts escaped from phytochrome control before the far-red irradiation was applied was minimized by using times as short as 10 sec between the beginning of red treatment and the beginning of far-red treatment when similar results to those reported were obtained.

The differences in reaction of the two polymerase systems, particularly to far-red light, may be due either to differences in sensitivity to control by phytochrome or to the control being exercised by two different pigment systems. Turner and Vince (22) have recently suggested that in lettuce seedlings two different photoreceptors exert control on anthocyanin synthesis through different photosensitive pigments.

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LITERATURE CITED