Red Light-Regulated Growth

I. Changes in the Abundance of Indoleacetic Acid and a 22-Kilodalton Auxin-Binding Protein in the Maize Mesocotyl

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ABSTRACT

We examined the changes in the levels of indoleacetic acid (IAA), IAA esters, and a 22-kilodalton subunit auxin-binding protein (ABP1) in apical mesocotyl tissue of maize (Zea mays L.) during continuous red light (R) irradiation. These changes were compared with the kinetics of R-induced growth inhibition in the same tissue. Upon the onset of continuous irradiation, growth decreased in a continuous manner following a brief lag period. The decrease in growth continued for 5 hours, then remained constant at 25% of the dark rate. The abundance of ABP1 and the level of free IAA both decreased in the mesocotyl. Only the kinetics of the decrease in IAA within the apical mesocotyl correlated with the initial change in growth, although growth continued to decrease even after IAA content reached its final level, 50% of the dark control. This decrease in IAA within the mesocotyl probably occurs primarily by a change in its transport within the shoot since auxin applied as a pulse moved basipetally in R-irradiated tissue at the same rate but with half the area as dark control tissue. In situ localization of auxin in etiolated maize shoots revealed that R-irradiated shoots contained less auxin in the epidermis than the dark controls. Irradiated mesocotyl grew 50% less than the dark controls even when incubated in an optimal level of auxin. However, irradiated and dark tissue contained essentially the same amount of radioactivity after incubation in [14C]IAA indicating that the light treatment does not affect the uptake into the tissue through the cut end, although it is possible that a small subset of cells within the mesocotyl is affected. These observations support the hypothesis that R causes a decrease in the level of auxin in epidermal cells of the mesocotyl, consequently constraining the growth of the entire mesocotyl.

R2 causes the elongation rate of the cells in the apical 1 cm of the etiolated maize mesocotyl (e.g., 16, 29) and cell division just below the node (25) to decrease in a fluence-dependent manner. Type I and probably type II phytochromes (27) mediate these light responses. How phytochrome mediates the decrease in growth is not known, although there are several hypotheses. One hypothesis (12, 30) is that the level of the growth-promoting hormone, IAA, decreases in the mesocotyl and there is evidence for such a decrease (6, 11). However, it has also been noted that a decrease in hormone alone can not account entirely for the observed change in growth rate (11, 26). It is also unclear how a decrease in the auxin pool might occur since either decrease in its synthesis or transport (6) or increase in its degradation or conjugation (2) could serve to decrease the free auxin pool. Other hypotheses on the mechanism of light-regulated growth include a role for inhibitors (19), calcium (9), cell wall enzymes (34), cell wall properties (35), and the auxin receptor (16, 33). Although it is likely that each of these molecular changes is involved, it is unclear which of these changes is directly mediated by phytochrome.

We have reexamined this problem by looking at the kinetics of the R-induced changes in IAA level, ABP1 level (18, 23), and growth kinetics. Because there is some indirect evidence that ABP1 may mediate auxin effects on cell walls (e.g., 3), it was of interest to determine if changes in its abundance could be a primary step in light-regulated growth.

MATERIALS AND METHODS

Plant Tissue, Chemicals and Light Treatments

Maize (Zea mays L.) caryopses (B73 × Mo17) (Jacque Seed Co., Lincoln, IL) were soaked in tap water for 2 h, then sown in wet vermiculite and grown in darkness at 26°C for 3.5 days. [3H]-N,IAA was prepared by the method of Melhado et al. (22). [14C]IAA (26 μCi·mmol) was purchased from Amer- sham (Arlington, IL). NAA was purchased from Sigma Chemical Co. (St. Louis). Continuous R was produced with a 60 W incandescent lamp covered with one layer of red acetate (600 nm cutoff, 7.5 μmol·m−2·s−1). Pulsed light (60 s, 15 μmol·m−2·s−1) was provided as described in Jones et al. (16). All manipulations of etiolated tissue were performed in less than 60 min under dim green light (511 nm peak, 40 nm half-band width, 100 nmol·m−2·s−1 at bench level) except the

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2 Abbreviations: R, red light; ABP1, maize 22-kD subunit auxin-binding protein; FR, far-red light; NAA, naphthalene-1-acetic acid; [3H]-N,I AA, tritiated 5-azidoindole-3-acetic acid; SIM, selected ion monitoring.
experiment shown in Figure 1, which was performed in absolute darkness with the aid of an infrared viewer (Find-R-Scope, FJW Industries, Elgin, IL). For this experiment, fluence was provided by changing the irradiance and time in the following manner: (a) between 10 pmol·m⁻² and 100 nmol·m⁻², plants were irradiated with 1/1000 to 1 s R pulses (500 nmol·m⁻²·s⁻¹), (b) between 100 nmol·m⁻² and 10 mmol·m⁻², plants were irradiated with 1/100 to 10 m pulses of R (15 μmol·m⁻²·s⁻¹), and (c) between 10 mmol·m⁻² and 1 mmol·m⁻², plants were irradiated for 10 min to 15 h. Reciprocity was not tested. In all other experiments, pulsed R means 60 s of R at an irradiance of 15 μmol·m⁻²·s⁻¹ to give a fluence of approximately 1 mmol·m⁻².

**ABP Purification, Antibody and Fusion Protein Production**

Partially purified ABP was obtained by the procedures of Napier et al. (23). Approximately 100 μg of this lyophilized protein was resuspended in water and emulsified with two parts of Freund's complete adjuvant. A female New Zealand white rabbit (designated IB) was immunized at multiple intradermal sites plus a single intraperitoneal injection. A boost was given similarly, but with 10 μg in Freund's incomplete adjuvant, on d 180. Acceptable titer antiserum was obtained by d 60, and bleeds at 10-d intervals continued through month 9. Different routes were followed in a second rabbit (designated R) for injection of an SDS-PAGE purified β-galactosidase-ABP fusion protein, designated AJ3. In this case, the acrylamide pieces containing AJ3 were crushed without adjuvant and injected at three sites on the back by intradermal, subcutaneous, and intramuscular routes and also injected into the peritoneum. AJ3 was expressed in *Escherichia coli* containing plasmid pAJ3 by standard procedures (1). The plasmid pAJ3 was prepared by cloning the *EcoRI-HindIII* fragment from pKlambt (28) into the *Ri-HindIII* sites of pWR590 (10). Monospecific antibodies (IBa22, IBaAJ3, and RoAJ3) were prepared as described by Ausubel et al. (1) where the antigens used to select antibodies were ABP1 for IBa22 and AJ3 for IBaAJ3 and RoAJ3.

**Growth Measurements**

Instantaneous growth rates were measured at Ohio State University using an angular transducer described by Evans (8). The seedlings were excised in vermiculite and were connected to the transducer by means of a pin inserted at the coleoptilar node. The average rate of growth determined over the 2 h prior to the beginning of irradiation is the initial dark control rate. The dose response curve for NAA-induced growth in dark and irradiated mesocotyl was demonstrated as described by Melhado et al. (21).

**IAA Content Analysis and Auxin-Binding Activity**

The apical 1-cm section of etiolated or R-irradiated mesocotyls was harvested for IAA analysis, frozen immediately in liquid nitrogen and stored at −80°C until ready for extraction. The extractions (at Chapel Hill) and GC-SIM-MS analyses (at Beltsville) were performed as described in Chen et al. (7). The NAA binding assay was performed as described in Jones et al. (17).

**Auxin Transport and in Situ Auxin Localization**

Etiolated shoots were excised 1 cm above and 1 cm below the coleoptilar node and for 10 min placed apical end down into a vial containing 200 μL of [7-3H]N-IAA (1 μM) then rinsed and held vertical by being inserted into a 1% agar slab (30 mm sucrose, 5 mM potassium phosphate, pH 6) kept in a humid chamber. Agar receiver blocks were placed on the basipetal ends of these shoots to collect transported radioactivity over 20-min intervals. The radioactivity was measured by liquid scintillation counting. The relative level of transported auxin in individual cells of the shoot tissue was determined by microautoradiography as previously described by Jones (15). Briefly, shoots excised from dark or irradiated seedlings are allowed to transport [3H]5-N-IAA for 4 h then irradiated with intense UV. Tissue is fixed, sectioned, and subjected to *in situ* autoradiography.

**RESULTS**

**The Kinetics of Red-Light-Induced Inhibition of Mesocotyl Growth Is Monophasic**

Figure 1 shows the three fluence-dependent phases of mesocotyl growth inhibition (shown here for the first time in a single figure). All three phases occur with this maize variety and they occur at the fluences and level expected from previously reported values for other maize varieties (11, 29). The kinetics of R inhibition of mesocotyl growth were monitored continuously using linear transducers as described by Evans (8). Growth rate declined soon after continuous irradiation began and continued to decline for 4 h when a rate of 25% of the dark control was established (Fig. 2A). The biphasic

![Figure 1](https://example.com/figure1.png)

Figure 1. Fluence response of red light inhibition of maize mesocotyl growth. The apical 1 cm of etiolated maize mesocotyls was marked with ink in absolute darkness with the aid of an infrared viewer then irradiated with R of the indicated fluence by varying irradiance and time as described in "Materials and Methods." Seedlings were grown in the dark for 15 h and the distance between marks recorded. Growth (final length minus 1 cm) is expressed as a percent of the dark controls. Each point is the mean of at least 10 seedlings. The symbols distinguish data points from 15 experiments. Data from an individual experiment share the same symbol.
pattern of inhibition, as was observed by Vanderhoef et al. (29), was not seen with this maize variety. Instead, the kinetics of growth inhibition resembled those reported by Bleis and Smith (4) for wheat seedlings.

Validation of the AntiABP Antibodies as Reagents to Quantitate the Abundance of ABP1

Two different antisera against ABP1 are shown to recognize a band at 22 kD as well as several other bands (Fig. 3A). These other bands are probably recognized by contaminant antibodies, because monospecific antibodies recognize a single band at 22 kD (Fig. 3A). For this reason, monospecific antibodies were chosen to quantitate the abundance of the ABP1 in subsequent experiments. The antibodies also recognize a β-galactosidase-ABP1 fusion protein (Fig. 3B), indicating that the antibodies recognize the previously reported ABP. The level of the ABP1 exactly correlates with the auxin-binding activity in every type of chromatography (e.g. anion exchange, size exclusion, hydrophobic interaction) to which we have subjected the ABP1-containing samples, indicating that these antibodies are specific to an auxin-binding protein (e.g. Fig. 3C). The polyclonal antibody against ABP1, but not the preimmune antiserum, immunoprecipitates a 22-kD protein photolabeled with [3H]5-N-IAA. The recognition of the 22-kD band by these antibodies comigrated with recognition by antibodies previously described by Napier et al. (23). Furthermore, antiseria used by Napier et al. (23) also recognized the AJ3 fusion protein (data not shown) indicating that the auxin-binding protein previously reported by four groups (13, 18, 23, 28) is the same.

Red Light Causes a Decrease in the Levels of IAA and the Abundance of ABP1 in Mesocotyls

The level of IAA and IAA esters in the apical mesocotyl was determined by GC-SIM-MS using 14C-IAA as an internal standard as described by Chen et al. (7), and it was found that 2 h after continuous irradiation the free IAA level was approximately 50% of the dark control tissue (Fig. 2B). This level remained more or less constant up to 8 h. The level of IAA esters measured 8 h after continuous R was 89% of the dark control (0 h). ABP1, quantitated by immunoblot analysis, did not significantly decrease in abundance until 7 h after continuous irradiation (Fig. 2C). By 12 h after irradiation, the ABP1 level was approximately 60% of the dark control. This value agrees with the steady state level of ABP1 previously observed after 15 h of R (16). Site I auxin-binding activity, postulated to be specific to this protein (16), decreases with kinetics parallel to those of ABP1 abundance and as previously shown by Walton and Ray (33).

Basipectal Auxin Transport Is Affected by Low Fluence R Irradiation

The velocity of movement of an auxin pulse in the etiolated maize shoot is unaffected by low fluence R (1 mmol·m⁻²·min⁻¹, Fig. 1). In both R-irradiated and control shoots, the auxin pulse moved at a velocity of 0.9 mm·h⁻¹. However, the amount of label transported in R-irradiated shoots was ap-
approximately half of the dark control (Fig. 4). The same effect of light on auxin transport capacity but not on velocity was observed by Naqvi and Gordon (24) using the coleoptile.

In situ localization of transported auxin ([3H]5-N1IAA) within low fluence R-irradiated (1 mmol·m⁻²·s⁻¹ by 60-s pulse, Fig. 1) and dark shoots was performed by microautoradiography as previously described (15). Figure 5 shows that R-irradiated shoot tissue contained less radioactive auxin in the shoot epidermis than the dark control. This is evident in both the coleoptile and mesocotyl epidermis. Higher magnification revealed that the cortical tissue of R-irradiated shoots also contained less radioactivity but the difference was not as great as in the epidermis (data not shown). Occasionally, the in situ autoradiography of tissue sections showed less difference in the amount of photolabel in the epidermis between R-irradiated and dark shoots, but rarely more.

**Low Fluence R Causes a Decrease in the Maximum Elongation Induced by Auxin**

If the growth rate of the mesocotyl within the first 2 h after continuous R irradiation is directly controlled by the amount of auxin this tissue receives from the coleoptile, then one would predict that the growth of shoots irradiated with a minimal amount of R would be identical to the dark controls if provided with the optimum auxin concentration during this time. This was shown not to be the case in the following experiment. Shoots were irradiated with low fluence R (1 mmol·m⁻²·s⁻¹, Fig. 1), followed by 30 min of darkness. The apical 1 cm of the mesocotyl was then harvested and placed in the indicated concentrations of auxin for 8 h. The irradiated mesocotyls grew 50% less than the dark control at optimal auxin concentrations (Fig. 6). When the shoots were irradiated with FR instead of R, the apical cm of the mesocotyl grew 35% less than the dark control. When R was followed by FR the effect was reversed to the FR treatment level. The concentration to induce 50% maximal growth (C₅₀) was the same for all treatments. This light effect on growth capacity does not occur if the apical portion of the mesocotyl is excised from the seedling immediately after irradiation (versus 30 min dark period), and growth measurements are made on the excised tissue (data not shown). In two experiments, the basal level of growth (no auxin) was higher in the dark control. However, in a third experiment the amount of growth without auxin was the same for all treatments. The low fluence R effect on growth capacity (Fig. 6) is similar in extent to that previously observed using high fluence R (26, 33).

**Figure 3.** Characterization of polyclonal antibodies directed against ABP1. A, Immunoblot strips from SDS-PAGE of maize mesocotyl extract were probed with polyclonal antiserum directed against purified ABP1 (IB) or against a β-galactosidase-ABP1 fusion protein designated AJ3 (R). The R and IB sera were antigen-purified as described elsewhere (1). IBα22 was purified using ABP1, and IBαAJ3 and RαAJ3 are IB and R antisera, respectively, purified using AJ3 fusion protein. B, Immunoblot strips from SDS-PAGE of E. coli expressing AJ3 were probed with the indicated antibodies or IB preimmune serum. C, Crude maize extract was subjected to DEAE chromatography as previously described (23) and eluted using the indicated NaCl concentrations (units are mm). W is the no salt wash. NAA binding and immunoblot analysis using IBαAJ3 was performed on each eluted fraction as described in "Materials and Methods."

**Figure 4.** Red light-induced changes in auxin transport in maize shoots. The rate of movement and size of a 10-min radioactive auxin pulse ([3H]5-N1IAA) in dark (right panel) or R-irradiated (left panel, 1 mmol·m⁻²·s⁻¹, 60 s) maize shoots was determined as described in "Materials and Methods." The radioactivity in receiver blocks for three dark and three R-irradiated shoots at the indicated time is shown. The data from each shoot are shown with a separate symbol. This experiment was repeated twice with the same results.
The amount of auxin entering the sections through the cut ends of the incubated mesocotyl tissue was shown to be the same for both R and dark-treated tissue by incubating mesocotyls in \( [{^{14}C}]\text{IAA} \) over time then sectioning the rinsed tissue and counting the radioactivity by liquid scintillation (Fig. 7). Therefore, the R effect on growth capacity shown in Figure 6 is not due to differences in the amount of auxin presented to the cells of the mesocotyl.

**DISCUSSION**

We investigated the role of IAA and a putative auxin receptor in R-regulated growth of the maize mesocotyl. Our quantitation of IAA by GC-SIM-MS confirms the R-induced change of IAA levels reported by Lino (11), who used a fluorescence method. The kinetics of the IAA decrease in the apical mesocotyl only match the early kinetics of growth change suggesting either that a subset of the cells in the mesocotyl that control the growth of the mesocotyl experience a R-induced loss of auxin or that some other primary event is involved. Jones et al. (16) recently investigated a light-
induced change in the abundance of an ABP that may mediate auxin action at the cell wall. In that study, it was found that the abundance of this putative receptor measured 15 h after continuous R irradiation decreased by 50% of the dark control. In the present study, we demonstrate that the early kinetics in the ABP decrease do not correlate with the kinetics in growth rate and therefore conclude that a change in the level of ABP1 is not important in the early kinetics of R-regulated growth. Our data, however, neither support nor refute the hypothesis that ABP1 mediates auxin-induced elongation (3).

In contrast to a report by Bandurski et al. (2) who used white light-irradiated seedlings, we did not observe an increase in the IAA-esters pool following R treatment, probably because we focused on auxins in the apical portion of the mesocotyl where growth changes are localized or possibly because of the difference in light treatments. We conclude that changes in free IAA level do not occur by a net change in the level of conjugation within the mesocotyl. More likely, the R-induced change in IAA is occurring by a change in auxin transport. While the mechanism of this change in transport is unknown, one possibility would be a R-induced increase in negative regulators of auxin transport for which there is circumstantial evidence (5, 14). Bottomley et al. (5) have shown that the level of quercitin glucosides increases after R irradiation, and recently Jacobs and Rubery (14) found that quercitin inhibits auxin transport. What is needed to assess the possible role of these phytochrome-regulated flavonoids in R-regulated auxin transport is the kinetics of the R induction of free quercitin.

It is possible that the R-induced change in auxin transport establishes a growth-limiting level of auxin primarily within epidermal cells. The effect of light is to decrease the net level of auxin in the cell. This hypothesis is consistent with our observation that R-irradiated tissues grew 50% less than the dark controls even though the tissues were bathed with an optimal level of exogenous auxin. This would imply that a component of auxin perception is internal to the cell as has been suggested (32), yet does not exclude the possibility of an external site of perception as well (e.g. 31). Since the phytochrome effect on growth capacity does not occur if mesocotyl tissue is excised from the shoot immediately after irradiation, the data also indicate that this R effect requires a lag period and is not tissue autonomous. This result is predicted if R decreases in the growth capacity occur by inducing the synthesis of auxin-transport inhibitors in another tissue as speculated above.

It has been proposed that R inhibits the transport of auxin in the coleoptile which then affects the growth of the subtending mesocotyl (6). Our data, however, indicate that auxin transport is affected in the mesocotyl as well, especially the epidermal cells of the mesocotyl. Targeting a decrease in IAA almost exclusively to the epidermis, which represents only a small volume of the whole shoot, resolves the discrepancy between auxin tissue level and growth rate after 2 h of R (cf. Fig. 2, A and B). This specific effect is consistent with the fact that the epidermis controls the growth rate of the shoot (20). While the light effect on growth of the mesocotyl appears mechanistically simple, i.e., explained by changes in the auxin pool within the epidermis, it may not be exclusive of other primary light effects. Changes in other variables are expected and, as mentioned before, are observed. Therefore, our efforts should now focus on the relationships between these variables, the degree to which any of these changes are primary, and the feedback controls that probably exist.

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