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Phytochrome induces changes in the immunodetectable level of a wall peroxidase that precede growth changes in maize seedlings

(ELISA/monoclonal antibody/wall enzyme/wall extensibility/*Zea*)

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ABSTRACT **The regulatory pigment phytochrome induces rapid and opposite growth changes in different regions of etiolated maize seedlings: it stimulates the elongation rate of coleoptiles and inhibits that of mesocotyls. As measured by a quantitative immunoassay, phytochrome also promotes rapid and opposite changes in the extractable content of a M_r 98,000 anionic isoperoxidase in the cell walls of these same organs: it induces a decrease of this peroxidase in coleoptiles and an increase in mesocotyls. The peroxidase changes precede the growth changes. As measured by video stereomicroscopy or a position transducer, red light (R), which photoactivates phytochrome, stimulates coleoptile elongation with a lag of about 15–20 min and suppresses mesocotyl growth with a lag of 45–50 min. R also induces a 50% reduction in the extractable level of the anionic peroxidase in coleoptile walls in less than 10 min and a 40% increase in the level of this peroxidase in mesocotyl walls within 30 min. Ascorbic acid, an inhibitor of peroxidase activity, blocks the effects of R on mesocotyl section growth. These results are relevant to hypotheses that postulate that certain wall peroxidases can participate in light-induced changes in growth rate by their effects on wall extensibility.**

The regulatory pigment phytochrome can initiate rapid growth changes in plants. In etiolated seedlings of oats, maize, and other grasses, the photoactivation of phytochrome by red light (R) stimulates two distinct growth responses: it promotes coleoptile elongation and inhibits mesocotyl elongation (1).

Changes in the extensibility of cell walls can help to mediate certain environmentally stimulated growth changes in plants (2), including those induced by phytochrome (3). Cell wall extensibility is at least partially controlled in many plants by one or more wall-localized enzymes that catalyze the formation or breakage of cell wall bonds (4). Consistent with this finding, several authors have noted that there is an inverse correlation between wall peroxidase activity and the growth of cell walls (5); e.g., when cucumber hypocotyl elongation is inhibited by blue light, there is an increase in wall peroxidase activity (6). The inverse correlation is consistent with the function of wall peroxidases in cross-linking wall macromolecules (7). To the extent peroxidases can decrease wall extensibility, they may help to mediate the inhibitory effects Ca^{2+} can have on wall extensibility (8), for Ca^{2+} can stimulate both the activity and secretion of wall peroxidases (9).

There are numerous wall isoperoxidases, and there has been little or no previously published information correlating induced growth changes with changes in the content of any specific wall peroxidase isozyme. We recently characterized a monoclonal antibody, mWP3, which specifically cross-reacts with a M_r 98,000 anionic peroxidase, which represents 15% of the total peroxidases present in soluble extracts of wall proteins from maize seedlings (10). Analysis by immunogold localization methods revealed that more than 85% of this peroxidase is localized in the walls of cells in various maize tissues. Here we report the use of this antibody to follow light-induced changes in the anionic wall peroxidase of maize coleoptile and mesocotyl cells, and we correlate the timing of these changes with growth rate changes induced by light in the same organs.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seeds of maize (*Zea mays* L. cv. Merit) were surface-sterilized in 10% Clorox for 15 min, rinsed with distilled water for 8 hr, and sown on moist vermiculite. They were grown in the dark for 4.5–6 days at 23°C, preirradiated with white light (WL) for 10 min at 12 hr before harvesting, and then returned to darkness until harvesting. The pretreatment light source consisted of three 40-W daylight fluorescent tubes located 1.5 m distant (total fluence, 10 μmol/m^2). Seedlings for the mesocotyl experiments were not preirradiated with WL before harvesting. Harvesting and all subsequent steps before irradiations were carried out under a very dim green safelight (fluence rate was less than 0.01 μmol/m^2·sec at a distance of 2.5 cm with λ_{max} = 525 nm).

Light Treatments. Except for the data in Fig. 3, the R and far-red light (FR) sources (source A) used were those described by Chen and Roux (11). Fluence rates of R were 8–12 μmol/m^2·sec; fluence rates for FR were 15–20 μmol/m^2·sec. For the data in Fig. 3, R-irradiations were given in a 2-min pulse at 10–20 μmol/m^2·sec and measured by a quantum sensor (LI-185B; Li-cor, Lincoln, NE) by using a Schott KL1500 fiberoptic microscope illuminator (source B) filtered with a Schott RG610 filter.

Extraction of Wall Proteins and Enzyme-Linked Immunosorbant Assay (ELISA). Two methods were used for extraction of the extracellular protein preparation including wall peroxidases.

Method A. This method was essentially the same as the centrifugation method of Kim *et al.* (10), except that after the 20-min distilled water rinse, the light-treated samples were irradiated with R for 10 min, then vacuum-infiltrated in cold buffer (10 mM NH_4HCO_3, pH 7.7/50 mM $CaCl_2$/1 mM $Na_2S_2O_5$/0.05% aprotinin), and centrifuged as described. The extracellular fluid extracted by this procedure was free of cytoplasmic marker proteins (10).

Method B. Either whole coleoptiles with enclosed young leaves or 1-cm sections of mesocotyls were frozen in liquid nitrogen at various time periods after being irradiated and then lyophilized to dryness. Subsequent steps before ELISA were at 4°C. The dried material was ground by mortar and pestle,

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Abbreviations: R, red light; WL, white light; FR, far-red light; IR, infrared light.

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extracted in the same buffer as described above for 20 min, and clarified by centrifugation for 10 min in an Eppendorf microcentrifuge (15,000 × g). In some experiments the pellet of this centrifugation was reextracted with the same buffer except 500 mM $CaCl_2$ was used instead of 50 mM $CaCl_2$. In another experiment the salt-extracted pellet was further extracted by treatment with highly purified cellulase and pectinase (Worthington) as described by Ros Barcelo et al. (12). The extracellular protein preparation in method A and the supernatants in method B were concentrated and desalted by passage through a Centricon-10 microconcentrator (Amicon) and then were stored until assayed by ELISA (less than 2 hr). Protein concentration was measured by the procedure of Lowry et al. (13). An indirect ELISA (10) was performed with mWP3 antibody partially purified by CM Affi-Gel Blue chromatography (Bio-Rad) as the primary antibody and phosphatase-labeled goat anti-mouse immunoglobulin (Kirkegaard & Perry Lab. Inc.) as the second antibody. The primary antibody was applied in 50 μl at the antibody concentration of 60 μg/ml, and the second antibody was applied in 100 μl at 20 μg/ml. After an incubation at room temperature and a wash, bound phosphatase activity was determined by adding 150 μl of substrate solution (*p*-nitrophenyl phosphate at 1 mg/ml in diethanolamine buffer) and reading the absorbance at 405 nm. Under the conditions of an ELISA, a dilution curve of partially purified peroxidase challenged with purified mWP3 was linear between 0.5 and 8 μg of total wall protein preparation. All ELISA results were measured within this linear range. No primary antibody and ascites controls gave equivalent background absorbances, and this background was subtracted from all ELISA readings.

Enzyme Assay. Peroxidase assays were carried out with 4-aminoantipyrine as a substrate as described (10).

Measurements of Kinetics of Growth Responses to R in Coleoptile Sections and Intact Mesocotyls. Coleoptile segments (about 1.4 cm) were cut from seedlings as described above and placed in 10 mM NH_4HCO_3 buffer (pH 7.7). A single segment was placed in a 2 mm wide × 1.5 mm deep trough of a Plexiglas rack. Both ends of the trough had shear vertical faces, and the basal end of the coleoptile segment was placed abutting one end of the trough. Thus, any elongation would result in the displacement of the unconfined end. The segment was then partially immersed in the same buffer as above to prevent drying, and the rack was placed on the stage of a Zeiss SV8 stereomicroscope. The above manipulations were carried out under a dim green flashlight (<0.001 μmol/m^2·sec at a distance of 15 cm with λ_{max} = 550 nm) from a 6-W white fluorescent bulb filtered by two layers of Roscolene 874 (green) and one layer of Roscolene 807 (yellow) acetate gel. The lens combination used gave a magnification of ×364 at the ocular lens. The segment was illuminated with a 3 × 8 array of gallium aluminum arsenide infrared light (IR)-emitting diodes (SEP 8703-001, Radio Shack; IR energy at 880 nm and radiant power output at 3.4 mW/cm^2) filtered by a 7-100 glass filter (Kopp Glass, Pittsburgh), which transmitted only 800 nm and longer wavelengths. The image was focused on a Panasonic WV-BL200 CCD camera. The magnification used led to a 0.6 mm × 0.5 mm field on screen. Hence, only the free edge of the segment was in the field. The image of the edge was recorded on video tape, and the tape was played back through an IBM PC running the Video Van Gogh video digitizer and software (Tekmatic Systems, Ben Lomond, CA; see ref. 14). A recognizable feature of the edge of a segment was located at 5-min intervals, and the changes of position were then used to calculate displacement. During the R irradiation and for 3 min thereafter, the IR source was turned off.

When mesocotyl elongation was measured in intact plants, the growth rate was determined by using a linear position transducer (Schaevitz Engineering, Pensauken, NJ) as described elsewhere (15). The growth of the mesocotyl was isolated from the coleoptile growth by modifying a technique used previously (16). In this case a 1-cm fine-gauge insect-mounting pin was passed through the stem at the node, and the thread used to attach the plant to the transducer was tied in a loop around the stem beneath the pin. An upward tension of 0.5 g held the thread taut between the seedling and the transducer core. Displacement was recorded by using a chart recorder, and growth rates were determined at 5-min intervals by measuring the slope of the trace over that period. Growth was recorded for at least 3 hr. Irradiation with R was given after a minimum of 1 hr of steady growth in the dark.

For mesocotyl section elongation, the tissue was prepared, incubated, and measured as reported (17) with the following modifications: (*i*) there was a 30-min preincubation period before irradiation; (*ii*) elongation was measured after 4 hr; (*iii*) sucrose was omitted from the medium; and (*iv*) 1 mM ascorbic acid (Sigma) was added as an experimental variable. Irradiations with R were with source A.

RESULTS

Within 10 min after R irradiation of coleoptile sections, there was a significant decrease in the level of an ELISA-detectable wall isoperoxidase extracted from them (Fig. 1*A*), but the overall activity of the wall peroxidases extracted increased during this same time (Fig. 1*B*). With the extraction method used (method A), the earliest time point that could be assayed was 10 min after irradiation. A control ELISA with monoclonal anti-cellulase antibody designated mWP18 (18) detected no significant cellulase change up to 1 hr after R-irradiation (Table 1). The results for the anionic peroxidase were confirmed by an independent method (method B): peroxidase in extracts from tissue rapidly frozen in liquid nitrogen after the irradiation showed a similar R-induced decline (Fig. 2). The R effect was fully reversible by 3 min of FR, while 5 min FR alone did not induce any significant change of the content relative to the dark control. Increasing the concentration of $CaCl_2$ in extraction buffer from 50 mM to 500 mM did not change the pattern of the light response (Table 1).

The R-induced change in peroxidase was reduced and FR reversibility of the effect of R was not complete for coleoptiles from seedlings that did not receive a WL pretreatment (data not shown). Such pretreatment irradiations can also alter growth responses to R (19). Since pretreated seedlings exhibited larger peroxidase responses, they were chosen for studies of elongation in coleoptile sections.

The effect of R on coleoptile elongation was superimposed on a general decline of elongation rate during the first 35 min of incubation of the sections in buffer (Fig. 3). Note Fig. 3 *Inset* shows the change in actual lengths of dark control sections over time. These sections were prepared and incubated in total darkness (i.e., without IR). The similarity of this result to the growth rates obtained for the dark control as measured by video microscopy validated the results of the latter technique.

Irradiation with R significantly retarded the decline in the growth rate of coleoptiles. The divergence of growth rates between irradiated and dark control sections was apparent from the 15-min time point onwards and was statistically significant (at $P < 0.05$ or better) from 25 min onwards. FR reversed the effect of R, as determined by direct measurement of sections (data not shown). The effects of R on growth and the overall decline in growth rate are both in the opposite direction from the pattern of peroxidase change detected by ELISA (see Fig. 1*A*).

In mesocotyl tissue both WL-pretreated and nonpretreated seedlings displayed peroxidase changes in response to R. Pretreated seedlings showed much more variability than nonpretreated seedlings both in their peroxidase response

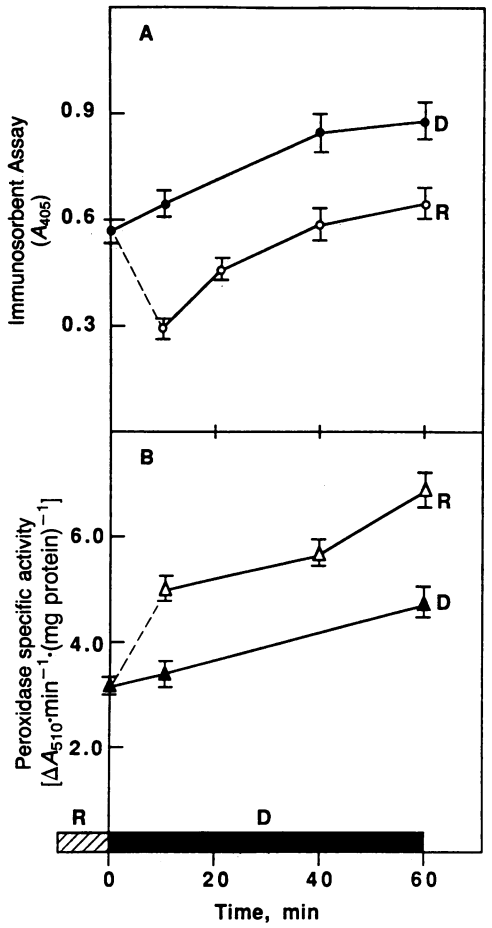

FIG. 1. Time course for change in the immunodetectable content of M_r 98,000 peroxidase in maize coleoptile sections (*A*) and in the total specific peroxidase activity (*B*) induced by a 10-min irradiation with R. Subapical coleoptile sections were cut from 4- to 5-day-old seedlings and loaded into plastic barrels. Irradiation took place immediately after loading and distilled water rinse. Sections were then left in buffer in the dark for the lag periods shown, and then vacuum-infiltrated. Cell wall proteins were extracted by centrifugation (method A in *Materials and Methods*). Dark controls (D) were extracted at the same times after loading as were irradiated sections (R). ELISAs were run on 1.5 μg of total wall protein for each datum point shown. Each point is the mean for five (*A*) or three (*B*) replicate experiments. Error bars indicate SEM.

and in their mesocotyl elongation response to R (data not shown). Only the responses of nonpretreated seedlings are presented here.

In mesocotyl tissue, the response to R is slower than in coleoptiles, for both the peroxidase change and the change in elongation rate. Both of these changes are in the opposite direction from the changes seen in the coleoptile. Fig. 4

Table 1. ELISA of cell wall enzymes extracted from coleoptiles under different conditions: R effects

Wall antigen assayed	Extraction condition*	Ratio of enzyme level from R-treated/untreated coleoptile
Peroxidase	Method B	0.45
Peroxidase	B + 500 mM $CaCl_2$	0.56
Cellulase	Method A	0.98

Peroxidase and cellulase were assayed by ELISA by using mWP3 and mWP18, respectively.
*Methods A and B and the extraction with 500 mM $CaCl_2$ are described in *Materials and Methods*.

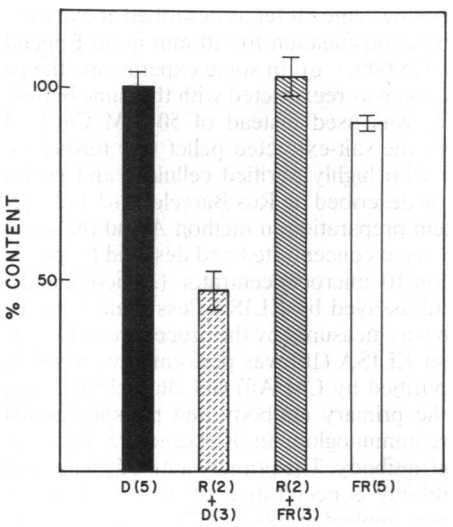

FIG. 2. FR reversibility of the R effect on immunodetectable content of M_r 98,000 peroxidase extracted from maize coleoptile sections. Cell wall proteins were extracted from a set of 10 coleoptiles cut at 2 cm below the node (i.e., with tip intact and 2 cm of mesocotyl). This tissue was treated with no light (D) or with R and/or FR for 5 min (numbers in parentheses indicate the minutes of exposure to each treatment). The attached mesocotyl tissue was then cut off, and the coleoptiles, with enclosed leaves, were frozen in liquid nitrogen. Cell wall proteins were extracted from freeze-dried tissue by method B. For each ELISA measurement a 4-μg total protein sample was used. Results shown are the means of three replicate experiments. Error bars represent SEM.

shows that R irradiation causes a gradual increase in the immunodetectable content of the peroxidase as measured by method B. The increase is apparent at 30 min after R-irradiation and reaches 70% at 1 hr after irradiation. To

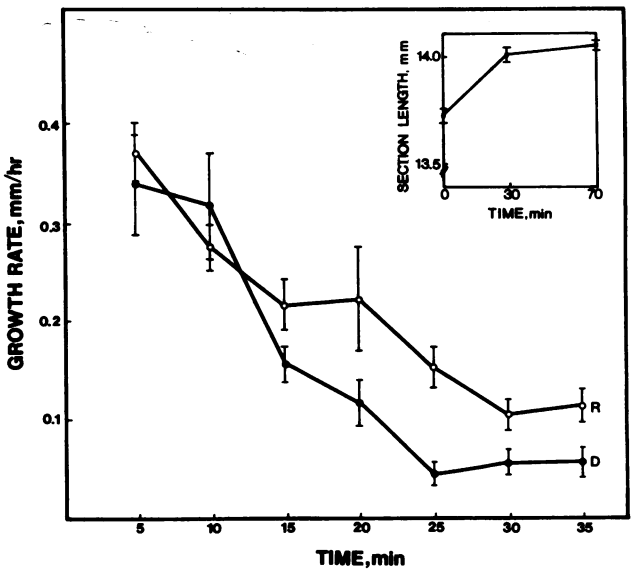

FIG. 3. Effect of R on elongation rates of maize coleoptile sections cut from 4- to 5-day-old dark-grown seedlings. Rates were determined at 5-min intervals from the observation of a video image of the section taken through a high magnification stereo microscope. Illumination was with IR only. R-treated sections (○) were cut, placed in buffer, and given a 2-min pulse of R (fluence rate 10–20 μmol/m^2·sec) at graph time zero (within 25 min after cutting). Dark controls (●) were observed for the same time period as irradiated sections. Each curve shown is the average for six replicate sections. Error bars indicate SEM. (*Inset*) Total length of dark-control sections measured directly with an ocular lens from sections incubated for the indicated time period in complete darkness (no IR).

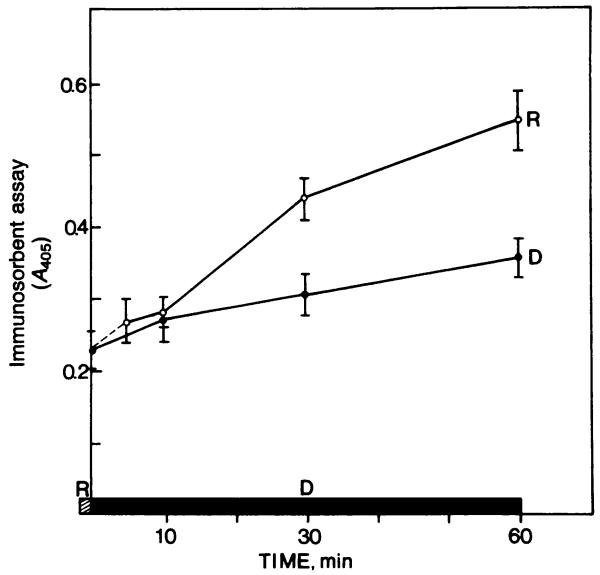

FIG. 4. Time course of change in immunodetectable content of M_r 98,000 peroxidase upon R irradiation in maize mesocotyls. Each datum point was generated from the extract of eight mesocotyl sections. Sections including both mesocotyl and coleoptile were cut 2 cm below the node and immediately irradiated with R for 2 min then returned to darkness (R). After an indicated period in the dark, 1-cm mesocotyl sections were cut from just below the node, rapidly frozen in liquid nitrogen, and then freeze dried. Dark controls (D) were taken for the same time period as irradiated sections. Proteins were extracted from the freeze-dried tissue by method B as in Fig. 2, and assayed by ELISA. For each ELISA measurement, 5 μg of total protein were assayed. Error bars indicate SEM.

parallel the coleoptile experiments, most tests of R-induced peroxidase changes in mesocotyls used tissue that was cut just before the R treatments. However, a test utilizing samples cut after the R treatment yielded kinetic and quantitative results similar to those shown in Fig. 4 (data not shown).

Light-induced changes in mesocotyl elongation rate (Fig. 5) lagged behind light-induced changes in peroxidase level. R caused a detectable reduction in elongation rate within 45 min after irradiation, and this decline was statistically significant (at $P < 0.05$) from 55 min onward. Individual plants showed apparent declines within 30 min, and they also showed an

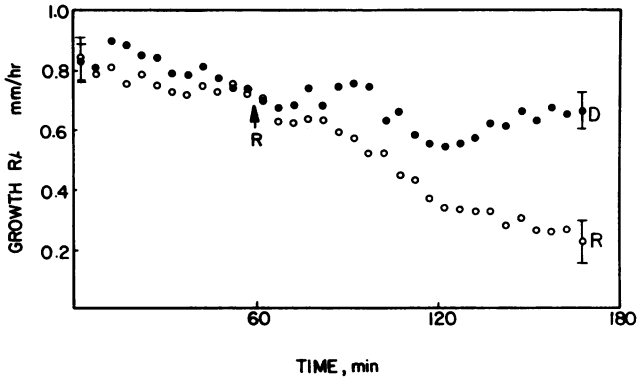

FIG. 5. Effect on R on elongation rates of mesocotyl regions of intact 4- to 5-day-old dark-grown maize seedlings. Seedlings were attached to position transducers as described, and seedling growth was recorded for at least 1 hr in the dark. At 1-hr graph time, R-treated seedlings (○) were given a 2-min pulse of R (arrow, total fluence of 1,200 μmol/m^2), while dark controls (●) remained unirradiated. Each curve shown is the average of five replicate seedlings. Error bars at the beginning and end of each curve represent the average of the SEMs for the data points shown.

Table 2. Elimination of R-induced inhibition of mesocotyl section elongation by 1 mM ascorbic acid

Treatment	Ascorbic acid (1 mM)	
	Without	With
D	10.8 ± .06	10.8 ± .09
R	10.5 ± .09	10.8 ± .08

Sections were cut to an initial length of 10.3 mm and placed in 5 mM potassium phosphate buffer (pH 5.9) with or without 1 mM ascorbic acid. All sections were preincubated for 30 min in darkness at 25°C, after which irradiated sections received a 2-min pulse of R (total fluence of 1,200 μmol/m^2). Results shown are the mean final length (±SEM) after 4 hr from three replicate experiments.

oscillation in growth rate, which is the basis of the slight oscillation seen in the averaged growth data. Similar kinetics for R-induced inhibition of mesocotyl elongation have been reported in nonpretreated maize plants (16). The elongation of mesocotyl sections was also inhibited by R. Ascorbic acid, an inhibitor of peroxidase activity (15), abolished this light effect without altering control elongation (Table 2). The addition of 1 mM ascorbic acid did not change the pH of the incubation buffer.

DISCUSSION

The results presented here provide several lines of evidence that a specific cell wall peroxidase helps to mediate light-induced growth changes in maize seedlings. The effects of R on the peroxidase and on elongation in two separate organs of maize seedlings are consistently opposite: when R stimulates a growth rate increase, extractable peroxidase levels decline, and vice versa. These observations and the fact that the wall peroxidase changes precede the growth rate changes are consistent with peroxidase acting to inhibit cell elongation. Further implicating peroxidases in the control of R-induced growth changes is the finding that ascorbic acid, an inhibitor of peroxidase activity (15), blocks the inhibition of mesocotyl elongation by R (Table 2).

The opposition in the direction of change between extractable peroxidase and growth extends beyond the effect of R. The declining growth rate of coleoptile sections (a typical response ascribed to the loss of auxin by the tissue, see ref. 20) is paralleled by an increase in peroxidase levels with time after cutting. Taken together, the results form an argument for a cause-and-effect relationship between the wall peroxidase changes and growth rate changes.

Several earlier reports support this argument (5, 6, 21), but in these publications authors generally assay only changes in the soluble peroxidases without giving parallel information about the kinetics of the changes in growth rate. None of these reports distinguishes whether the peroxidase changes observed are attributable to a specific isoperoxidase or to one or more of a mixed population of peroxidases. In our study, shifts in the overall wall peroxidase activity induced by light are not in the same direction as the shifts we observed in the immunodetectable anionic peroxidase (Fig. 1*B*), so all wall peroxidases may not be equally modulated by light.

The inverse correlation between ELISA-detectable peroxidase and elongation is consistent with models for peroxidase-mediated regulation of cell wall extensibility (7). In forming bridges between phenolic residues on neighboring cell wall proteins or polysaccharides, peroxidases should act to reduce cell wall extensibility (7, 22). We did not measure wall extensibility, but Warner and Ross have reported that R induces an increased extensibility of maize coleoptiles within 20 min after irradiation (3)—i.e., in the expected direction and within the time frame of our observed changes in elongation and extractable peroxidase levels. Also, Yahalom *et al.* (23) have reported that phytochrome decreases wall

extensibility and inhibits elongation of maize mesocotyls within 3 hr after they are irradiated.

The FR reversibility of the R-induced responses in coleoptiles clearly indicates phytochrome regulation. Several rapid growth responses have been attributed to control by phytochrome, and phytochrome-mediated changes in peroxidase activity in maize have been reported (24), but these two phenomena had been measured only separately in previous publications. Because our goal in the mesocotyl studies was only to test whether R effects in this tissue contrasted with those in coleoptiles, we did not test the FR reversibility of the changes observed. However Vanderhoef *et al.* (16) have reported that R effects on growth in maize mesocotyls are FR reversible, and hence, under phytochrome control. It is probable that the R response observed here in mesocotyls is also under phytochrome control.

Our results indicate that phytochrome can regulate the immunodetectable content of a specific anionic isoperoxidase extractable from maize cell walls within minutes of R irradiation. Whether phytochrome does this by altering the immunoreactivity of the peroxidase, its rate of turnover in the wall, its rate of delivery to the wall, or its binding state in the wall is unknown. Because two independent methods of peroxidase extraction yielded similar results, the R-induced changes were unlikely to be due to changes that occurred during the extraction process. It seems unlikely that phytochrome affects ionic binding of the M_r 98,000 peroxidase after irradiation, since the R effects are observed whether the wall is extracted with 50 or 500 mM $CaCl_2$ (Table 1). It does not appear that R is changing the covalent association of the anionic peroxidase with pectins or with cellulose, for an initial test indicates that digesting these wall components with highly purified cellulase and pectinase does not significantly alter the relative levels of peroxidase released from the walls of R-treated and untreated coleoptiles (data not shown). The failure to observe R-induced changes in extractable wall cellulase indicates that the R effects are not global effects on all soluble wall enzymes.

Phytochrome has been reported to induce a number of transport changes across the plasma membrane that could be expected to affect wall peroxidase biochemistry. Phytochrome-induced Ca^{2+} fluxes could influence the rate of peroxidase secretion into the wall (9). Phytochrome-induced changes in the pH (25) or pCa^{2+} (26) of the wall could result in enzyme activity changes that could in turn affect the immunoreactivity or extractability of the anionic peroxidase. For example, if phytochrome-induced ionic changes in the wall altered wall peroxidase activity, a peroxidase-mediated formation of isodityrosine bonds in the anionic peroxidase itself could result in both epitope modification and insolubilization of the protein (27).

The most obvious way the ELISA-detected peroxidase changes would have relevance to cell growth would be if they are linked to and reflect changes in the activity of the M_r 98,000 anionic peroxidase. Such a connection is clearly possible no matter what is the biochemical basis of the peroxidase changes observed. A change in the binding state or solubility of the peroxidase could modify its activity in the wall by altering its accessibility to its substrate. Even if the light-induced peroxidase change is only an epitope change, this could readily result in activity changes, because the particular epitope recognized by mWP3 is important for activity of the peroxidase (10).

In the coleoptile the R-induced peroxidase changes were so rapid that a high-resolution measurement of the growth response in that organ was essential to evaluate whether the biochemical changes preceded or followed the growth changes. However, obtaining short-term kinetics for the growth response of coleoptile sections proved to be a challenging problem. Our solution was a unique integration of previously used techniques. An IR source similar to that used by Iino (28) was combined with a video camera sensitive to IR so that growth changes could be recorded without the interference of actinic wavelengths of light. This was then combined with the use of microscopy to detect small changes in section length (as in ref. 29), and reproducible quantitation was obtained by processing video images through a computer-driven image digitizer (as in ref. 14). The IR video microscopy technique successfully detects rapid effects of R on coleoptile elongation. The results also indicate that the IR illumination is a true "safe light" under the conditions used. The IR video data acquisition system described here would be valuable also for the study of a wide range of other rapid morphological changes induced by phytochrome.

In summary, we have described here two rapid growth responses mediated by phytochrome, which lag slightly behind changes in a wall-localized anionic peroxidase that are also under phytochrome control. The correlative data argue that the peroxidase could be a direct participant in the phytochrome regulated growth responses, a hypothesis consistent with the evidence that peroxidases help regulate cell wall extensibility.

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1. Gaba, V. & Black, M. (1983) in *Encyclopedia of Plant Physiology, New Series: Photomorphogenesis*, eds. Shropshire, W., Jr. & Mohr, H. (Springer, Berlin), Vol. 16A, pp. 358–400.
2. Taiz, L. (1984) *Annu. Rev. Plant Physiol.* **35**, 585–657.
3. Warner, T. J. & Ross, J. D. (1981) *Plant Physiol.* **68**, 1024–1026.
4. Rayle, D. L. & Cleland, R. E. (1972) *Planta* **104**, 282–296.
5. Goldberg, R., Imberty, A. & Chu-Ba, J. (1986) *Phytochemistry* **25**, 1271–1274.
6. Shinkle, J. R. & Jones, R. L. (1986) in *Cell Walls '86*, eds. Vian, B., Reis, D. & Goldberg, R. (Université Marie et Pierre Curie, École Normale Supérieure, Paris), pp. 190–193.
7. Fry, S. C. (1986) *Annu. Rev. Plant Physiol.* **37**, 165–186.
8. Cleland, R. E. & Rayle, D. L. (1977) *Plant Physiol.* **60**, 709–712.
9. Penel, C. (1986) in *Molecular and Physiological Aspects of Plant Peroxidases*, eds. Greppin, H., Penel, C. & Gaspar, T. (Univ. of Geneva Press, Geneva), pp. 155–164.
10. Kim, S.-H., Terry, M. E., Hoops, P., Dauwalder, M. & Roux, S. J. (1988) *Plant Physiol.* **88**, 1446–1453.
11. Chen, Y.-R. & Roux, S. J. (1986) *Plant Physiol.* **81**, 609–613.
12. Ros Barcelo, A., Munoz, R. & Sabater, F. (1987) *Physiol. Plant.* **71**, 448–454.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
14. Nelson, A. J. & Evans, M. L. (1986) *J. Plant Growth Regul.* **5**, 73–83.
15. Shinkle, J. R. & Jones, R. L. (1988) *Plant Physiol.* **86**, 960–966.
16. Vanderhoef, L. H., Quail, P. H. & Briggs, W. R. (1979) *Plant Physiol.* **63**, 1062–1067.
17. Shinkle, J. R. (1986) *Plant Physiol.* **81**, 533–537.
18. Kim, S.-H., Terry, M. E. & Roux, S. J. (1987) in *Physiology of Cell Expansion During Plant Growth*, eds. Cosgrove, D. J. & Knievel, D. P. (American Society of Plant Physiologists, Rockville, MD), pp. 289–291.
19. Blaauw-Jansen, G. & Post, Th. H. (1985) *Physiol. Plant.* **65**, 529–532.
20. Vesper, M. J. & Evans, M. L. (1978) *Plant Physiol.* **61**, 204–208.
21. Gardiner, M. G. & Cleland, R. (1974) *Phytochemistry* **13**, 1095–1098.
22. Cosgrove, D. J. (1989) *Planta* **177**, 121–130.
23. Yahalom, A., Epel, B. L. & Glinka, Z. (1988) *Physiol. Plant.* **72**, 428–433.
24. Sharma, R., Sopory, S. K. & Guha-Mukherjee, S. (1976) *Plant Sci. Lett.* **6**, 69–75.
25. Pike, C. S. & Richardson, A. E. (1977) *Plant Physiol.* **59**, 615–617.
26. Hale, C. C. & Roux, S. J. (1980) *Plant Physiol.* **65**, 658–662.
27. Everdeen, D. S., Kiefer, S., Willard, J. J., Muldoon, P., Dey, P. M., Li, X.-B. & Lamport, D. T. A. (1988) *Plant Physiol.* **87**, 616–621.
28. Iino, M. & Carr, D. J. (1981) *Plant Sci. Lett.* **23**, 263–268.
29. Jacobs, M. & Ray, P. M. (1976) *Plant Physiol.* **58**, 203–209.