A Kinetic Analysis of Phytochrome Controlled Mesocotyl Growth in *Zea mays* Seedlings

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ABSTRACT

Mesocotyl elongation in 4 day old etiolated seedlings immediately following 3 hours of white light (3 h W) is reversibly controlled by phytochrome. Time-lapse video measurements were made of the 5 millimeter zone just below the coleoptile which is the main growth region of the mesocotyl. The growth kinetics were determined for five contiguous 1 millimeter zones subtending the coleoptile node for nonirradiated seedlings, for seedlings given 3 h W, and 3 h W followed by terminal far-red (FR) or red subsequent to the far-red (FR/R) irradiation. Each zone in nonirradiated seedlings exhibits exponential elongation kinetics during the early stages of elongation. This finding suggests that during elongation, a growth limiting factor is also exponentially increasing. Following 3 h W differences in the kinetic responses were found for each zone. In all zones, the inhibitory effect following the 3 h W is totally FR reversible. The effect of FR is reversed by R. The upper zone exhibits the fastest response and is the most plastic in its growth response. The three upper zones all exhibit spontaneous and sharp recoveries with time. It is suggested that the control by phytochrome is not inductive but rather continuous, the controlling factor being either the level of the far red-absorbing form of phytochrome (Pfr) or the ratio Pfr to total phytochrome.

Mesocotyl elongation in etiolated cereal seedlings is inhibited by light, especially R[1] (2, 6). Whether this inhibition is mediated by pytochrome is unclear as FR reversibility often is partially (19) or totally absent (2, 10). The mesocotyl growth region which is located just below the coleoptile node (8, 14), is not an homogeneous tissue. In a region contiguous to the node, no more than 1 mm in length, cells are meristematic (14). Below this, is a small transition zone where cell division ceases and elongation commences. This is followed by a rather large zone where cells rapidly elongate. Merging with this zone is a zone where elongation slows and cells undergo maturation (8). Since previous kinetic studies analyzed the effect of light on whole organ or whole plant growth (3, 6, 12, 17, 19), or on the growth of large sections which spanned the various zones (8), or employed measurement techniques with low resolution (6, 8) they would not have detected differential effects in the different regions.

Here, we present a detailed kinetic analysis of the effect of light on mesocotyl growth, for the 5 mm region just below the coleoptile node which is the main region of growth and for each mm within this region. These measurements are made with corn

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[1] Abbreviations: R, red light; D, dark; W, whitelight; FR, far-red light; RGR, relative growth rate; Ptot, total phytochrome.

seedlings given a prior light treatment of 3 h W which increases the responsiveness of the mesocotyl growth to phytochrome control.

MATERIALS AND METHODS

Plant Material. *Zea mays* seeds (var. Jubilee F1, Rogers Brothers Seed Co., Idaho Falls, Idaho) were soaked for 4 h in running tap water and sown either in plastic trays (end point measurements done at Tel Aviv University) or in cuvettes ¾ filled with 1.0% water agar (kinetic time-lapse video measurements performed at the Macaulay Institute for Soil Research, Aberdeen, Scotland). The seedlings were grown in the dark at 25°C for 4 d.

Light Treatments. Etiolated seedlings were exposed to 3 h W from 4 fluorescent 40 watt tubes (1 cool white + 3 warm white) placed 60 cm above the seedlings. The mesocotyls were marked just prior to the end of the 3 h W either with India ink, at the indicated distance below the coleoptile node for end point measurements, or with beads spaced at about 1 mm intervals just below the node as previously described (9) for video measurements. Seedlings then either received no further treatment, or were irradiated with 10 min FR or 10 min FR followed by 5 min R from a light source consisting of a slide projector and a 650 (R) or 730 nm (FR) interference filter (Ditric Optics, Inc., Hudson, Mass.; half band widths less than 15 nm). The fluence of the FR and the R at Aberdeen were 0.2 and 0.40 nmol cm^{-2}s^{-1} and at Tel Aviv, 0.38 and 0.57 nmol cm^{-2}s^{-1}, respectively. Dark control seedlings were marked under green safelight.

Growth Measurements. (a) End point measurements of mesocotyl elongation following various irradiation treatment were performed by measuring growth to the nearest mm with metric paper, after 20 h in the dark. Each datum point is the average of at least two replicates of 20 plants each. (b) Kinetic measurements were performed in Aberdeen using time-lapse video techniques as previously described (7). Two pairs of selected uniform seedlings, each pair having received a different light treatment, were placed before the camera and photographed with nonactive infrared radiation at given intervals. The resolution of the system was about 100 μm. Each datum point is the average of 7 to 10 experiments.

RESULTS

The mesocotyls of etiolated 4 d old seedlings were marked at 5 mm intervals down from the mesocotyl node and net elongation after 20 h measured (Table I). The main elongation zone is in the 5 mm marked interval just below the coleoptile node; 78% of the elongation is contributed by cells in this zone. Cells in the zones below 15 mm are apparently no longer elongating.

If seedlings are irradiated with W, marked, and returned to D

Table I. *Distribution of Growth Along the Mesocotyl of 4 Day Old Etiolated Maize Seedlings*

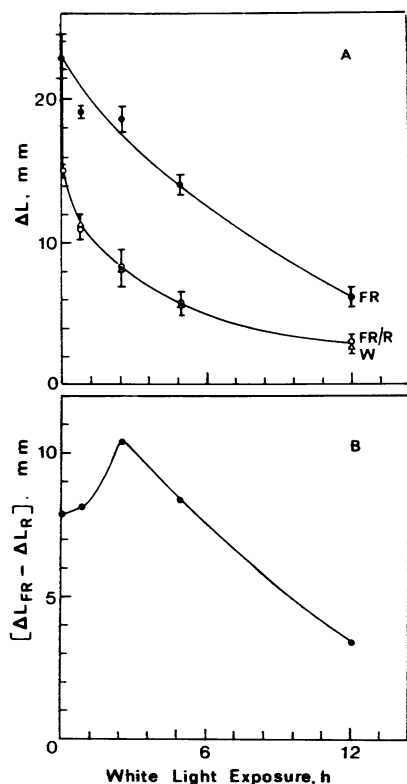| Distance from Node	Net Elongation	Increase
mm	*mm/20 h*	%
0–5	17.6 ± 5.0	352
5–10	4.1 ± 2.0	82
10–15	1.0 ± 0.5	20
15–20	0	0

FIG. 1. End-point measurement of elongation of 5 mm apical zone of mesocotyl of 4 d dark grown corn seedlings irradiated with W (Δ) for indicated duration and given either no terminal irradiation, a terminal irradiation of 10 min FR (●) or 10 min FR followed by 5 min R (O). (A) Total elongation of marked zone and (B) net FR stimulatable—FR/R reversible elongation after 20 h subsequent growth in the dark.

for 20 h, mesocotyl elongation becomes progressively inhibited with increasing periods of irradiation (Fig. 1A, W). A terminal FR partially reverses (Fig. 1A, FR), while R following FR completely reverses the FR effect, and the inhibition is as with W alone (Fig. 1A, FR/R). Since the maximal stimulation of net elongation by terminal FR is after about 2.5 h W (Fig. 1B), we chose a period of 3 h W as our standard prior light treatment. The complete FR/R reversibility for a number of cycles confirms the sole involvement of phytochrome in the FR stimulation of growth following 3 h W (Table II).

Video time lapse photography was used to measure the growth

of marked (beaded) portions of mesocotyls after various irradiation regimes. The growth curves for the marked zone following various irradiation programs appear nonlinear (Fig. 2A). When plotted on a semilog scale as $L(t)/L(O)$, biphasic linear curves are obtained (Fig. 2B), indicative of exponential growth. Growth can then be expressed as $L(t) = L(o)e^{r\Delta t}$ where $L(o)$ is the initial length, Δt is the time interval of growth and r is the slope in the semilog plot and is termed the 'relative growth rate' (RGR) (5). The RGR in nonirradiated controls is linear for about 10 h and then declines. Following 3 h W, the initial RGR of the marked zone upon transference to D is inhibited by about 55%. A terminal FR almost completely reversed the inhibition after a lag of about 1 h. R terminal to the previous FR treatment cancels the FR effect and the RGR is essentially that measured with seedlings which received only 3 h W.

An examination of the growth kinetics of each 1 mm zone within the 5 mm apical growth zone revealed differences in response (Figs. 3 and 4). For nonirradiated seedlings the most apical zone (I) exhibits the lowest initial RGR with the RGR of zones II, III, IV, and V being, respectively, 1.5, 1.65, 1.5, and 1.3 times greater. After about 12 h, the RGR of zone I accelerates. In contrast, that of zone II remains constant while that for zones III, IV, and V exhibit declines respectively after 13, 9, and 6 h.

The RGR of zone I following 3 h W is the most strongly inhibited, followed by zones II and III while the RGR for zones IV and V exhibit lower initial levels of inhibition (Fig. 4). FR terminal to 3 h W completely or nearly completely reverses the effect of 3 h W, with a lag of less than 1 h for zone I and a lag of 1 to 2 h for zones II to V. R terminal to the FR reversed the effect of FR for all zones.

It should be noted that the RGRs for zones I, II, and III exhibited sharp and spontaneous recoveries 4 to 6 h after being transferred to D following 3 h W. In the case of zone I there is a second sharp increase after about 14 h. In contrast, in zones IV and V, the growth response following transfer to darkness is markedly different. For these two zones the RGRs continuously decrease with time. This behavior is more marked for zone V than for zone IV.

DISCUSSION

Our results that the exposure of etiolated maize seedlings to W increases the responsiveness of mesocotyl extensive growth to phytochrome control, is in agreement with the results of Duke *et al.* (4). Maximum responsiveness is after about 2.5 to 3 h of W. With completely etiolated seedlings, the inhibitory effects of R are not photoreversible by low intensities of FR (10, 19), but requires FR fluences, about three orders of magnitude more than that reported here. It is known, in many cases, that the responsivity toward photomodulation is increased by a prior light treatment (1, 13, 15, 16). This increase in responsivity to phytochrome is mediated by phytochrome itself (1, 13), the photoreceptive site being in the apex of the mesocotyl (11). It should be noted that the D-control seedlings used in the present study were exposed to green safelight during marking. Thus, the RGR of the D-control seedlings is probably less than would have been found for seedlings grown and handled in total darkness (8, 10). Thus, for both D-control seedlings, and for seedlings given a

Table II. *Effect of Terminal Irradiations Given after 3 h W on the Net Elongation of the 5 mm Mesocotyl Apex*

	Post White Irradiation[a]				
	None	FR	FR/R	FR/R/FR	FR/R/FR/R
Net elongations (mm)[b]	5.4 ±0.6	13.7 ± 0.4	5.4 ± 0.4	13.7 ± 0.8	5.2 ± 0.6

[a] FR 10 min each; R 5 min each. [b] Net elongation determined after 20 h dark growth subsequent to final irradiation.
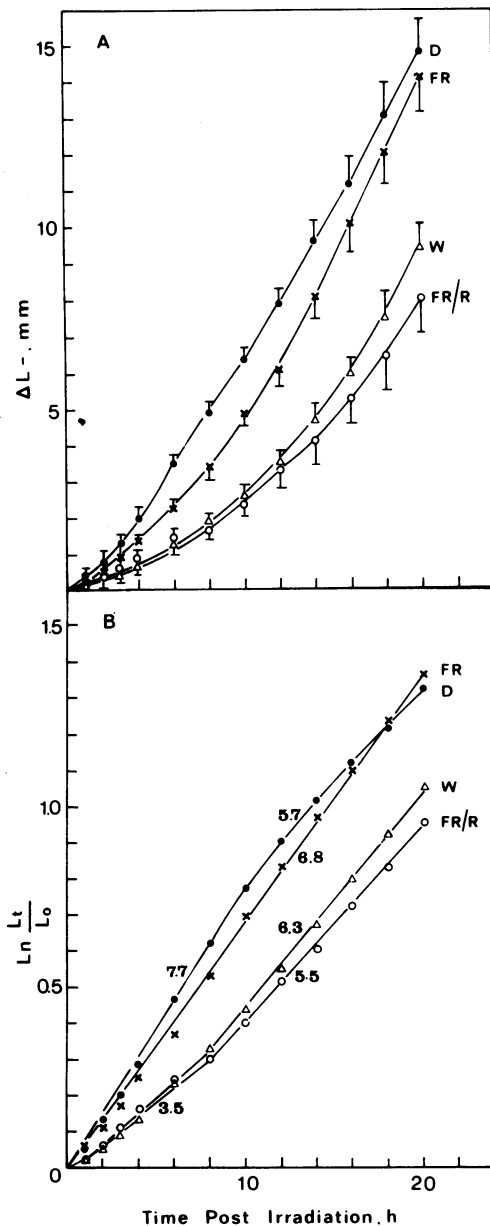
FIG. 2. Time-lapse video measurements of elongation of 5 mm apical zone of mesocotyls of D controls (●) and of mesocotyls given 3 h W (△), 3 h W terminated with 10 min FR (×), or 3 h W terminated with 10 min FR followed with 5 min R (O). Each point is the average of 4 to 7 experiments of 2 repeats each. (A) Net elongation, $L(t) - L(o)$, (B) data from A, replotted as $\ln[L(t)/L(o)]$. Numbers on curves are RGR × 10^2.
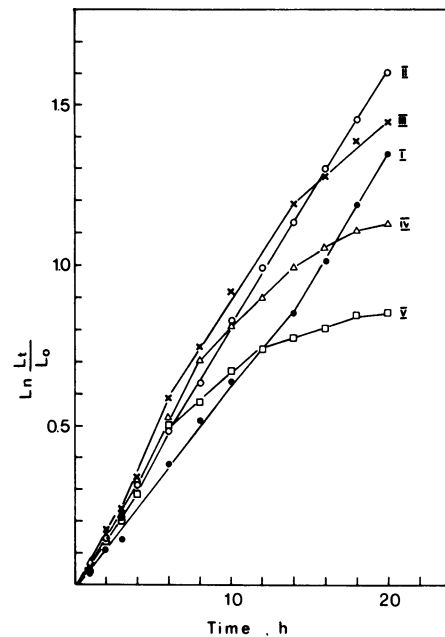

FIG. 3. Time-lapse video measurements of relative growth, plotted as $\ln[L(t)/L(o)]$, of 5 sequential 1 mm zones of mesocotyl apex of dark control seedlings. Zone I is uppermost and zone V is lower most region.

prior W-irradiation the very low irradiance reaction is no longer operating.

An analysis of mesocotyl growth kinetics of the D control revealed exponential growth (Figs. 2 and 3) especially during the early phases of growth. It is important to note that when growth is exponential, end-point measurements may give misleading results. The percent of growth or of inhibition will depend on time of end point measurement. In exponential growth $L(t) = L(o)e^{kt}$. Thus, $L(m)/L(c) = [L(o)e^{k(m)t} - L(o)]/[L(o)e^{k(c)t} - L(o)]$ where $L(m)$ is the net elongation after time t of treatment m, and $L(c)$ is the net elongation after time t of the control. For kt large, *i.e.* where growth is significant, $L(m)/L(c) = e^{(k(m) - k(c))t}$, *i.e.* the result is exponentially dependent on time. For kt small, *i.e.* where growth is small, one gets pseudo-linear kinetics and

the equation reduced to $L(m)/L(c) = k(m)/k(c)$, and which is not dependent on time.

Cell division is not prerequisite for exponential growth. Cells undergoing extensive growth alone can exhibit exponential character; this can be seen on reploting on a semilog scale the data published by various workers (3, 8, 14, 17). The implication of exponential growth is that a growth limiting factor is increasing in an exponential fashion in concert with the volume (length). In contrast, general protein content shows very little increase during cell elongation. Thus, we suggest that mesocotyl elongation is not a function of a fixed number of preexisting growth centers, but that these centers are increasing in an exponential manner concomitant with growth. We find that following 3 h W, all marked regions continued to elongate in an exponential manner, but with a decreased RGR. This could be due either to a reduction in the number of 'growth sites' or to a reduction in the rate constant of the growth site. The rapid reversal by FR suggests the latter as more likely.

Growth Recovery in Dark. The RGR of the mesocotyl of corn seedlings given 3 h W spontaneously recovers after 8 h D growth (Fig. 2). A similar recovery was reported by Iino (8) for totally etiolated corn seedlings. However, Duke *et al.* (4) in studies made with maize seedlings given a prior W irradiation, and Vanderhoef *et al.* (19) working with maize seedlings which had received only 5 min R, found no such recovery. In studies with etiolated oat seedlings given a brief R, no recovery was found (14, 15). In the work of Duke *et al.* (4), the corn seedlings received 12 h W and thus they may behave differently. The conclusion of Vanderhoef *et al.* (19) that the rate of elongation of the light inhibited corn mesocotyl does not recover, is not clear cut. This conclusion was based on short term, very high precision transducer measurements, which were terminated prior to the time where we observe recovery (8 h) and on low-precision long-term end-point measurements which could be redrawn to show a recovery (see Figs. 3 and 4 in Vanderhoef *et al.* [19]). *Avena* may indeed behave differently.

Regional Differences in Growth Rate. Zone I which exhibited the highest level of inhibition as well as the fastest recovery following FR is the main region of cell division. Previously it has
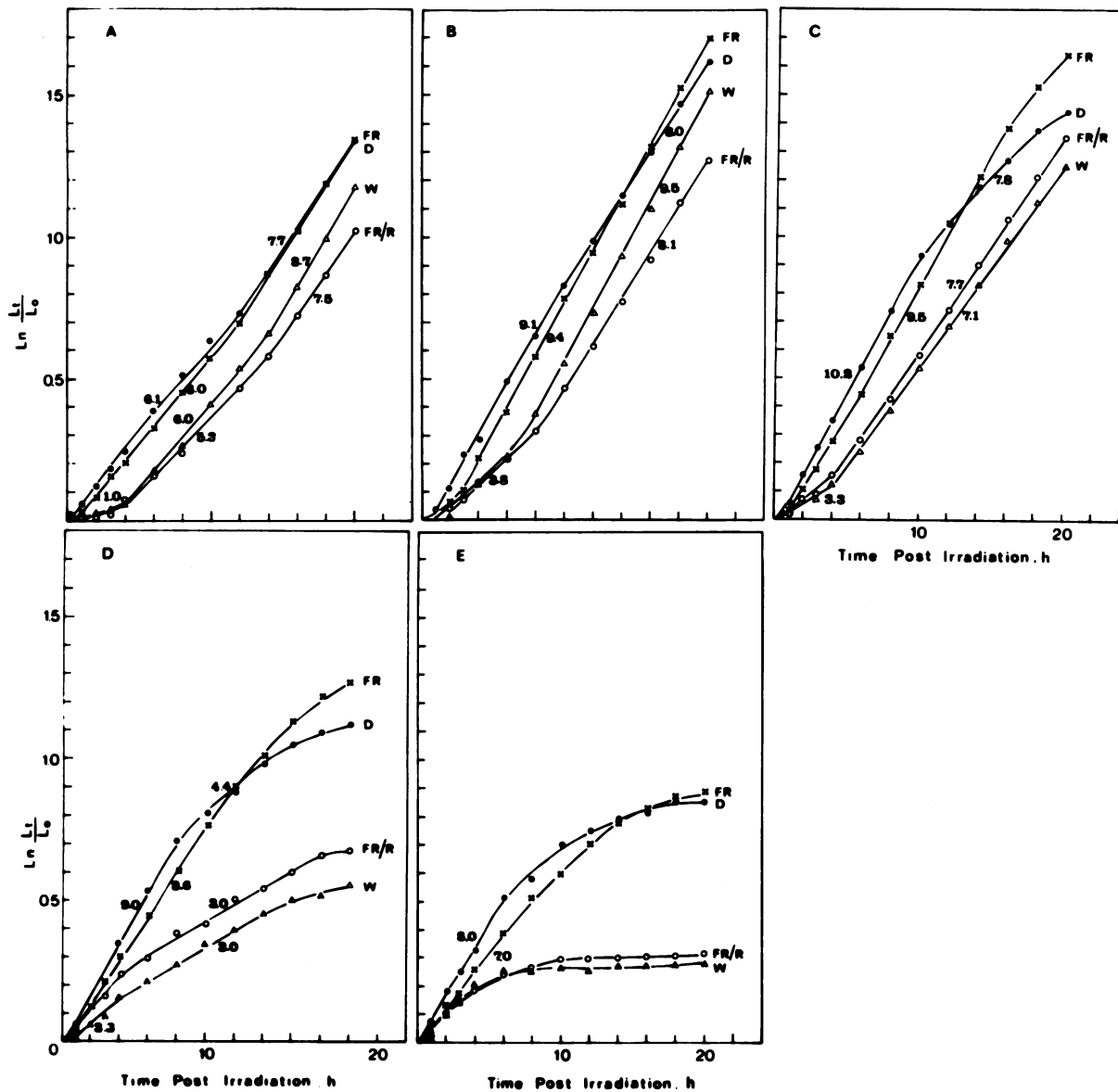
FIG. 4. Time-lapse video measurements of relative growth, $\ln[L(t)/L(o)]$, of mesocotyl zone I (A), II (B), III (C), IV (D), V (E), following no irradiation (●) or following 3 h W (△), 3 h W terminated with 10 min FR (×), 3 h W + 10 min FR terminated with 5 min R (O). Zones demarked as in Figure 3. Numbers on curves are RGR × 10^2.

been reported that cell division is also inhibited by light (6, 8, 14). Cells in this region are largely undifferentiated (3) and are thus probably highly plastic in their ability to respond to environmental changes. Even in the absence of a terminal FR treatment, this zone undergoes a spontaneous recovery. The fact that the growth rate after 20 h is equal to, or greater than, that of the D control, suggests that cell division in addition to elongation, may have recovered. Possibly with prolonged W there is progressive loss in the ability of this zone to recover (Fig. 2) (Fig. 3 in Duke et al. [4]). Vanderhoef et al. (19) found that continuous W caused two distinct inhibition phases in mesocotyl elongation, one detectable after 20 min but needing 2 h for full development, and a second inhibition developing after 6 h continuous W. This second phase is apparently irreversible.

The elongation rates of zones II and III were not nearly as sensitive to 3 h W, the very sensitive initial phase observed in zone I being either absent, or exhibiting a very fast spontaneous recovery. Furthermore, in contrast to zone I, the growth rate in

zones II and III did not immediately recover following a terminal FR. Zone II exhibited a 3 h lag while zone III exhibited a 2 h lag followed by a full recovery.

In zones IV and V the cells are undergoing differentiation and even in the absence of W there is a spontaneous decrease in the RGR after about 8 and 6 h, respectively. Surprisingly the initial growth rate following 3 h W was only slightly inhibited relative to D control. However, 3 to 4 h after the termination of the 3 h W prior light treatment there was, relative to the D control a major (zone 4) or an almost total inhibition (zone 5) in RGR. In these two zones, light is apparently accelerating maturation as has been previously suggested by Thomson (18).

Correlation with Phytochrome. Duke et al. (4) suggested that mesocotyl growth is correlated with Ptot levels. Our data suggest that Pfr or Pfr/Ptot levels may be the more likely controlling factors, at least under the conditions we employed. Following 3 h W the level of Ptot is reduced due to irradiation induced destruction (4). Nonetheless, the growth rate of nonirradiated

control mesocotyls and those given a terminal FR after 3 h W are nearly identical. The recovery phenomenon also suggests that it is the Pfr level or the Pfr/Ptot ratio which is controlling, since with time Pfr and Pfr/Ptot should be decreasing due to continuous dark destruction of Pfr.

From our data it is clear that phytochrome control of growth following 3 h W is not of the inductive type, but rather that Pfr is continuously exerting its regulation on growth, as long as the Pfr/Ptot is above a certain threshold. This can be deduced from the observation that even after 3 h W, where the growth rate is already strongly inhibited (Fig. 2B), FR reverses the inhibition. The spontaneous recovery from inhibition which is observed, leads us to suggest a threshold type of control. The recovery is not a gradual and continuous process but a rather abrupt one (Figs. 2 and 4), while no recovery is observed if the plants are given continuous irradiation (not shown). It should be noted that after very prolonged W, such as 12 h, FR does not completely reverse the inhibition. This is probably a twofold effect: first, the irradiation is not only inhibiting cell elongation, but probably cell division, and with prolonged irradiation the potential pool of cells entering the elongation zone has been decreased; second, as a consequence of the prolonged irradiation, more and more cells are entering zones IV and V which are irreversibly inhibited and no new elongating cells are being produced to replenish zones II and III.

LITERATURE CITED

1. BEGGS CJ, W GEILE, MG HOLMES, M JABBEN, AM ROSE, E SCHÄFER 1981 High irradiance response to promotion of a subsequent light induction response in *Sinapis alba* L. Planta 151: 135–140
2. BLAAUW OH, JG BLAAUW-JANSEN, WT VAN LEEVWEN 1968 An irreversible red light-induced growth response in Avena. Planta 82: 87–104
3. CAUSTON DR, CL MER 1966 Analytical studies of the growth of the etiolated seedling on *Avena sativa*. I. Meristematic activity in the mesocotyl with special reference to the effect of carbon dioxide. New Phytol 65: 87–99
4. DUKE SO, AW NAYLOR, JL WICKLIFF 1977 Phytochrome control of longitudinal growth and phytochrome synthesis in maize seedlings. Physiol Plant 40: 59–68
5. ERICKSON RO 1976 Modeling of plant growth. Annu Rev Plant Physiol 27: 407–434
6. GOODWIN RM 1941 On the inhibition of the first internode of Avena by light. Am J Bot 28: 325–332
7. GORDON DC, IR MACDONALD, JW HART 1982 Regional growth patterns in the hypocotyls of etiolated and green cress seedlings in light and darkness. Plant Cell Environ 5: 347–353
8. IINO M 1982 Inhibitory action of red light on the growth of the maize mesocotyl: evaluation of the auxin hypothesis. Planta 156: 388–395
9. MACDONALD IR, JW HART, DC GORDON 1982 Analysis of growth during light induced hook opening in cress. Plant Cell Environ 5: 355–360
10. MANDOLI DF, WR BRIGGS 1981 Phytochrome control of two low irradiance responses in etiolated oat seedlings. Plant Physiol 67: 733–793
11. MANDOLI DF, WR BRIGGS 1982 The photoreceptive sites and the function of tissue light-piping in photomorphogenesis of etiolated oat seedlings. Plant Cell Environ 5: 137–145
12. MER CL, CAUSTON DR 1967 Analytical study of the growth of the etiolated seedling of *Avena sativa*. II. The effect of growth modifying factors on cell divisions and extension. New Phytol 66: 665–681
13. MOHR H, H DRUMM, R SCHMIDT, B STEINITZ 1979 The effect of light pretreatments on phytochrome-mediated induction of anthocyanin and phenyl alanine ammonialyase. Planta 146: 369–376
14. SCHAER JA, DF MANDOLI, WR BRIGGS 1983 Phytochrome-mediated cellular photomorphogenesis. Plant Physiol 72: 706–712
15. SCHÄFER E, M EBERT, M SCHWEITZER 1984 Control of hypocotyl growth in mustard seedlings after light-dark transitions. Photochem Photobiol 39: 95–100
16. SCHMIDT R, H MOHR 1981 Time dependent changes in the responsiveness to light phytochrome-mediated anthocyanin synthesis. Plant Cell Environ 4: 433–437
17. SCHOPFER R, KH FIDELAK, E SCHÄFER 1982 Phytochrome controlled extension growth of *Avena sativa* L. seedlings. I. Kinetic characterization of mesocotyl coleoptile and leaf response. Planta 154: 224–230
18. THOMSON BF 1951 The relation between age at time of exposure and response of part of the Avena seedling to light. Am J Bot 38: 635–638
19. VANDERHOEF LN, PH QUAIL, WR BRIGGS 1979 Red light inhibited mesocotyl elongation in maize seedlings. II. Kinetic and spectral studies. Plant Physiol 63: 1062–1067