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The Role of Various Regions of the Bean Hypocotyl on Red Light-induced Hook Opening

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BERNARD RUBINSTEIN
Michigan State University-Atomic Energy Commission Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823

ABSTRACT

Measurement of various zones on the concave half of etiolated *Phaseolus vulgaris* L. (cv. Black Valentine) hypocotyls has shown that growth at the basal portion of the elbow and the contiguous upper portion of the shank was stimulated earliest by red light. Growth of these two zones was unaffected by the tissue of the convex half but was inhibited by tissue distal to them. The inhibition was alleviated by the continuous presence of shank tissue below the growing zones. Based on cuts made halfway through the hypocotyl at positions above, below, or between the two zones of growth, it is suggested that cells at the inner portion of the upper shank control in some way the light-induced growth of the elbow cells directly above.

During germination of bean seeds, the hypocotyl emerges as an inverted "U" and, if growth continues in the dark, this hook travels up the hypocotyl past the cotyledons into the epicotyl (7). Exposure to red light leads to asymmetric growth and unbending of the hook (3, 5). It was of interest, therefore, to locate the areas of growth along the hypocotyl which respond earliest to irradiation and attempt to analyze the relationships between those areas and other nonresponding ones. Regarding the location of the growth response, Darwin (1) observed that the opening of the hook was due to increased growth along its inner (concave) portion. Klein (5) showed that cortical cells of the inner part of the elbow region elongated during hook opening and eventually reached the length of the cells in the outer half whose size remained unchanged. This was confirmed in a more quantitative manner by Kang and Ray (3), but, as in earlier studies, their measurements were made only after considerable opening had occurred, thus making it difficult to determine which areas elongate first after irradiation.

Studies of the relationships between the various areas of the hook of bean have shown that tissue above the hook region exerts an inhibitory effect on opening (4, 6, 8), and the straight tissue below the elbow seems to stimulate opening (3, 4).

The purposes of this investigation are first to clearly define in both intact plants and isolated hypocotyls the zones of the hook which elongate soon after exposure to light; second, to determine what contributions the nongrowing areas might have to this elongation; third, to investigate the interrelationships between the growing zones themselves.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L. (cv. Black Valentine) were sown on water-moistened vermiculite and grown in complete darkness at 25 C. After 7 or 8 days, hypocotyls were excised to include the entire curved region with 5 mm of tissue above it and 3 cm of shank tissue below. Following isolation, the basal ends of the hypocotyls were embedded in 1% agar to a depth of 8 mm. For experiments utilizing intact plants, each 4-day-old seedling was transplanted into a 100-ml beaker filled with vermiculite. Plants 5 to 6 cm in height were then selected after 2 days of further growth in the dark.

The light source was a 40 w cool white fluorescent tube (Ken-Rad) wrapped in three layers of cellophane. The light was filtered further by a layer of Plexiglas (Rohm and Haas, No. 2444) which cuts off the wavelengths below 600 nm. Light energy as determined by a YSI-Kettering thermopile was approximately 500 erg/cm^2·sec. A green safelight, which conformed to the specifications of Withrow and Price (9), was used during handling of the material.

Hook opening and growth were determined by the methods of Kang and Ray (3). To measure hook opening, hypocotyls were first shadowgraphed. The angle made between a line perpendicular to the distal cut surface and a line parallel to the 5-mm portion of hypocotyl just below the concave portion was then determined with a goniometer. This angle is the same as the angle α of Powell and Morgan (8). Isolated hypocotyls which contained the entire curved region had a starting angle of 0 degrees; isolated hypocotyls with tissue distal to the basal portion of the elbow removed had a starting angle of 90 degrees. Growth was measured by delimiting zones 1.5 to 2.0 mm long on the hypocotyl with lanolin drops and shadowgraphing the tissue after various time periods. The increase in length of each section was measured under a dissection microscope at a magnification where 20 units of the ocular micrometer equalled 1 mm.

RESULTS

The sites of elongation of hypocotyl hooks after 12 hr *in situ* can be seen by comparing the data of Figure 1, A and B. In the dark, the most rapid growth occurs at sections 6 and 7 of the inside portion of the hook. Growth of section 6 leads directly to opening of the hook, but sections 4 to 7 at the outside of the hook are also increasing in length. This growth at the outer half results in reformation of the hook. When intact plants are exposed to red light, the growth of areas of the hook which is already occurring in the dark is stimulated further, so that the hook is simultaneously opening and reforming. This situation would not lead to an immediate opening of the hook, and it was, in fact, seen that with the light energies used here hooks on intact plants opened only after the curved portion had reached the cotyledonary node.

In studies on the control of hook opening, the hypocotyl hook is generally isolated from the rest of the plant. In Figure 1, C and D, are shown the sites of growth of a hypocotyl hook

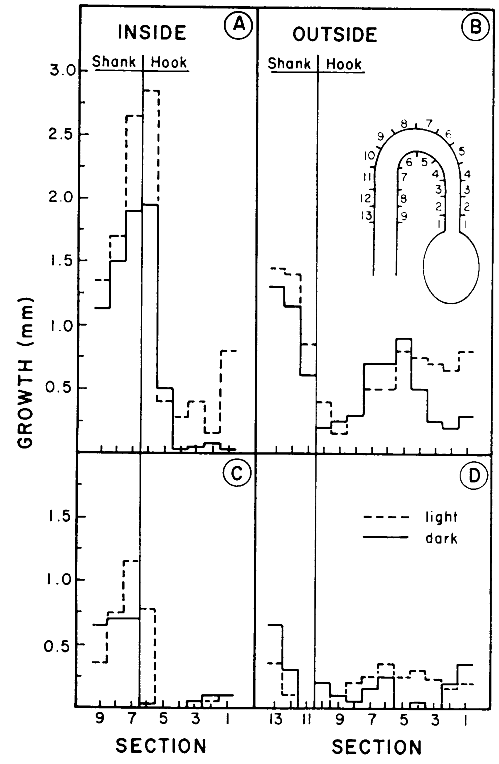


FIG. 1. Growth of various zones of the bean hypocotyl (numbered in inset) after 12 hr in the dark (solid lines) or in continuous red light (dotted lines). A and B: intact plants; C and D: isolated hypocotyl hooks; A and C: inside or concave surface; B and D: outside or convex surface. Vertical line indicates the transition between the curved (hook) and straight (shank) portions. Data are for individual plants. The experiment was repeated four times and, though the growth rates varied, the patterns of growth were identical.

removed from the intact plant by cutting just below the cotyledonary node and at 3 cm below the base of the elbow. Isolating the hypocotyl resulted in an almost complete cessation of growth for 12 hr on the inside portion of the hook (Fig. 1C). Growth in sections 6 and 7, however, could be stimulated by red light. Since little growth occurs at the outside of the hypocotyl in either dark or light (Fig. 1D), growth of section 6 at the concave half results in opening of the hook.

Before examining more carefully the effect of red light on the growing zones themselves, one must first determine what relationships, if any, exist between those areas which elongate in response to red light and the areas of the hook seemingly little affected by red light treatment. The effect of the outer (convex) portion of the hook which grows only slightly in light or dark on the elongation of the inner cells was tested using isolated hypocotyls. These were cut in half perpendicular to the plane of the hook (see inset to Fig. 2), and the inside and outside halves were incubated separately in red light or dark. The time courses (Fig. 2) show that the inside halves opened normally in red light. They seemed to close somewhat immediately after cutting, but the differences between the inside halves in light or dark at 12 and 24 hr, as well as the opening of dark controls after 24 hr, are all typical responses seen in intact hypocotyls (3, 6). The outside halves opened somewhat immediately after cutting and continued to open regardless of whether they were in light or dark. It thus appears that the elongation of the inner cells is not affected by cells on the outside portion and that growth of the cells of the outer half is not sensitive to red light.

The effects of tissue above and below the zone of light-induced elongation were tested by cutting away the tissue in question. The data in Figure 3 show that removal of tissue just distal to the basal elbow (column B) had no effect on opening in red light or in dark. If the distal area was left intact and all but 8 mm of the shank removed (column C), a marked retardation of opening in the light was apparent. Thus, the tip seems to be an inhibitory influence, but this effect is counteracted by the presence of the shank. If both tip and shank are removed, resulting in a piece of hypocotyl containing only the basal portion of the elbow and about 8 mm of shank (column D), the amount of opening is the same as in hooks with only the tips removed.

To determine if the presence of the shank acts as a trigger for the elongation leading to opening or whether the shank must be continuously present, shanks were removed 8 mm below the elbow either immediately after isolating the hypocotyls or at various times during hook opening. It can be seen in Figure 4 that removal of the shank (indicated by arrows) at any time, even after the hooks had opened 120°, resulted in a subsequent reduction in the rate of opening. Thus, the continuous presence of the shank is required to aid in relieving the inhibitor effect of the tip.

Investigations were also conducted into the relationships among parts of the growing zones. This was done by making

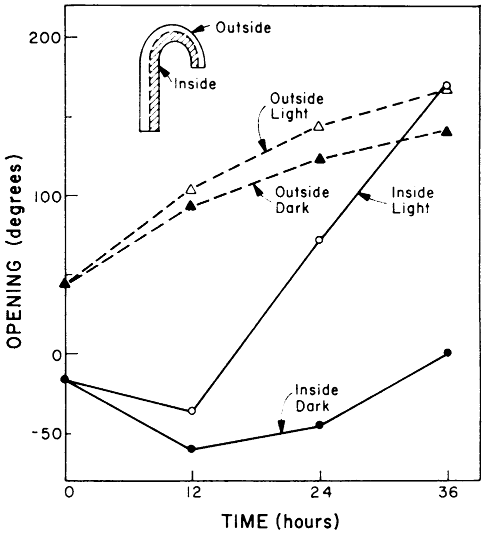
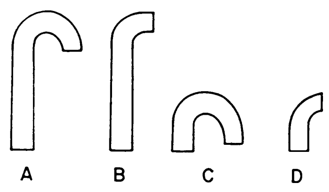


FIG. 2. Opening of inside and outside halves of bean hypocotyls. The tissue was cut lengthwise (dotted line on inset), and the resulting convex (triangles) or concave portions (circles) were incubated separately in the dark (filled symbols) or in continuous red light (open symbols). Each treatment consisted of 10 hypocotyls.



	A	B	C	D
DARK (degrees)	10 ± 6	14 ± 9	4 ± 2	8 ± 2
LIGHT (degrees)	55 ± 11	49 ± 5	18 ± 13	54 ± 7

FIG. 3. Effect of removal of tissue above or below the growing region of isolated hypocotyls. Data expressed as degrees opening with standard error after 18 hr of darkness or continuous red light. Each treatment consisted of 10 hypocotyls.

cuts approximately halfway through the hypocotyl at locations above, below, or between the regions of elongation so as to separate the basal portion of the elbow from the upper area of the shank. After cutting, growth of the tissue led to separation of the cut surfaces so that a notch rather than a thin slit resulted. Figure 5 shows that in the light the cut above the growing zone (C) had little effect on opening; a cut below the growing zone (A) markedly inhibited the elongation leading to hook opening. The latter result is in agreement with Klein (4). If a cut was made just at the base of the elbow (B), there was no noticeable inhibition of opening. A cut at this location did alleviate the inhibitory effect of a cut at A, however. When cuts were made at similar locations in the dark, no effects were observed except for a stimulation of opening of hooks cut at B and C. Cuts along the outer or convex portion of the hook are generally ineffective in agreement with Klein (4). An exception, however, is a 47 ± 20 degree reduction in hook opening when the cut is made on the outside of the hypocotyl opposite position B.

DISCUSSION

Measurements of growth rates at various zones of the hypocotyl hook of intact bean plants indicate that the hook continuously tends to open by growth at the inner, basal portion of the elbow. This is true even when the plants remain in the dark. The opening is counteracted, however, by a continuous reformation of the hook due to a greater growth at the outer, apical portion of the elbow. The net result is that in dark-grown seedlings the hook is always present, but appears to move up the hypocotyl.

Similar to hypocotyls of intact plants, growth of isolated hooks leading to hook opening begins at the inside, most basal portion of the elbow and proceeds acropetally with time. The data of Kang and Ray (3) also suggested this, but the authors recorded some light-induced growth even at the more distal end of the concave portion of the elbow. This discrepancy is probably due to the longer time period over which they measured growth (24 hr) compared to the 12 hr in this study. Upward movement of the zones of growth would have occurred during the extensive degree of opening seen after 24 hr.

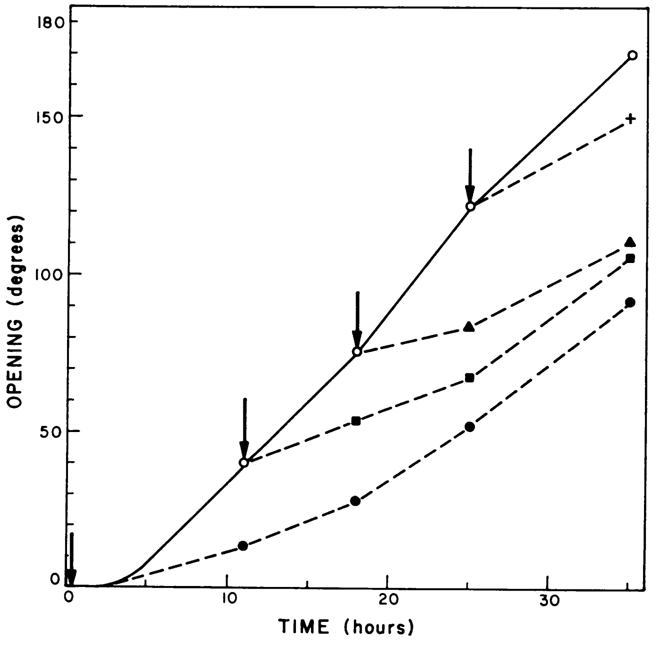
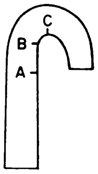


FIG. 4. Effect of shank removal on hook opening in continuous red light. Shanks were cut off 8 mm below the base of the elbow at times indicated by vertical arrows.



Location of Cut	OPENING (degrees)	
	Dark	Light
NONE	7±5	67±19
C	28±7	86±11
B	33±5	69±17
A	3±7	12±10
A and B	29±6	54±16

FIG. 5. Hook opening of 10 isolated hypocotyls cut at the position indicated. Data expressed as degrees opened ± standard error after 24 hr in darkness or in continuous red light.

From an inspection of Figure 1, it appears that red light effects on hook opening are only quantitative in nature. This is suggested by the fact that hooks of completely etiolated plants are opening continuously as well as reforming and that light stimulated only those areas on the intact hypocotyls which are already growing (Fig. 1, A and B). Evidence for a quantitative effect of light can also be deduced from the data of Klein *et al.* (6) and Kang and Ray (3) who showed that isolated hooks kept in complete darkness eventually opened although the rate was slower than in light-treated hooks. Thus, the effect of red light on hypocotyl hooks is to accelerate the rate of elongation where it is already occurring on intact plants, or where growth would have occurred if the hypocotyl had not been isolated from the plant.

In order to characterize more clearly the stimulatory effect of red light on elongation, the relationship of the nonelongating zones to the areas of growth was investigated. The convex half of the hypocotyl has little or no effect on light-induced growth of the inner half (Fig. 2). Tissue located just distal to the growing zone, however, can inhibit the elongation leading to opening. This inhibitory influence can be seen when the shank is removed. The failure of Powell and Morgan (8) to observe an inhibition after shank removal was probably because they removed only the lower portion of the hypocotyl. As shown by Klein (4) and confirmed here (unpublished data), the shank must be removed close to the base of the hook in order to demonstrate a retardation of opening.

Klein (4) proposed that a stimulatory substance is produced in the shank which travels acropetally to the elbow region. If such a substance were important, one would expect that removal of the shank would lead to a slower rate of opening. The data in Figure 3 show, however, that once the tip is removed, the presence of the shank has no stimulatory effect on hook opening. It is concluded, therefore, that the shank serves merely to alleviate the inhibitory effect exerted by tissue distal to the zone of growth.

By making incisions similar to those of Klein (4) halfway through the hypocotyl, it was possible to determine the relationship existing between the growing zones of the elbow (Fig. 1C, section 6) and the upper portion of the shank (Fig. 1C, section 7). A cut just below the shank growing zone (Fig. 5, position A) creates a physiological state resembling that of dark controls, and the elongation of the elbow cells as measured by hook opening is decreased. A cut made between the growing zones of the shank and elbow (Fig. 5, position B) which results in inhibited growth of the area below (unpublished data) is only slightly inhibitory in the light but stimulates opening in the dark. One possible explanation for these phenomena is that the elbow cells would elongate under any conditions, but the upper portion of the shank contains a light sensor for the system and growth here leads directly to growth in the elbow cells above. Thus, when a cut is made between the elbow and the shank in darkness or is made in the light along with another cut below the shank zone of growth, the physiological coupling between the two areas is broken and opening occurs. The importance of the shank as a light receptor in hook unbending has already been pointed out by Klein (4). He showed that exposure of the shank alone to red light resulted in almost as much hook opening as when the entire hypocotyl was irradiated. Light had a very small effect when only a portion of the hook area was exposed.

A polar mechanism may also be involved in the control of hook opening, since a cut below the areas of elongation inhibits unbending while a cut above these areas (Fig. 5, position C) has no marked effect in the light.

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