*A RAPID PHOTOREVERSIBLE RESPONSE OF BARLEY ROOT TIPS IN THE PRESENCE OF 3-INDOLEACETIC ACID**

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For several years I have observed that root tips of some species of plants adhere to glass surfaces in water. Examination of this phenomenon shows that under suitable conditions the root tips of barley adhere to glass within 30 seconds when irradiated with red light and detach from the glass within 30 seconds when irradiated with far-red light. This rapid reversibility has been repeated for five or more cycles in numerous trials with different lots of seedlings. 3-Indoleacetic acid at very low concentrations and several other compounds are required for the reversible process.

Methods and Materials.—About 14 gm of barley seeds (*Hordeum vulgare* L., var *Compana*) are washed several times with deionized water. They are then spread over cheesecloth supported on a stainless steel wire net hung inside a porcelain crock. Six liters of deionized water from which chlorine has been removed by passing through charcoal are poured into the crock until the net just touches the water surface. The seeds are held in darkness for 2 days at 23°C with the water aerated continuously.

After 2 days, the seedlings are exposed to dim white light (0.3 ft-c) from a tungsten filament lamp for at least 1 hr before use in a constant-temperature room at 23°C. The dim light is used in subsequent handling of seedlings. Roots, after gentle blotting on filter paper, are placed on a ruled plastic block and 1-mm sections are quickly cut with a stainless steel razor blade from tips of the first two seminal roots of a seedling. Ten tips are washed into a Pyrex glass beaker (tall form) of 200-ml capacity containing about 25 ml of deionized water. The water is poured off after a few seconds and 10 ml of solution of the following composition is added immediately to the beaker: sodium adenosine-5′-triphosphate (ATP), 2.5×10^{-6} *M*; 3-indoleacetic acid (IAA), 10^{-8} *M*; L-ascorbic acid (AA), 10^{-6} *M*; $MnCl_2$, 2×10^{-6} *M*; $MgCl_2$, 2×10^{-4} *M*; KCl, 10^{-4} *M*; and HCl, 5×10^{-5} *M*. Solutions of the organic chemicals are freshly prepared each day. The pH of the final solution is 5.0.

The beaker is immediately placed in red light. After mixing with a gentle rotary motion for about 10 sec, the tips are allowed to settle and remain undisturbed in the central portion of the bottom for 20 sec. The beaker is then raised a few millimeters on one edge and very gently swirled 3 or 4 times. Sudden or jerky movements are avoided. The number of tips adhering, or showing signs of adhering, to the glass surface is then counted. Tips that do not adhere move smoothly away from the spot on which they have settled. After the gentle swirling motion, the tips are left undisturbed for about 5 sec. This process of dislodging, counting, and settling is repeated three times. A good count of the average number of tips adhering to the glass can be obtained by the end of the fourth dislodging. After 1 min in red light, the tips are exposed to far-red light for 1 min during which time the procedure done in red light is repeated. The red and far-red irradiation cycle is carried out for 5 cycles.

The source of red light is a 15-watt daylight fluorescent tube filtered with 2 layers of red cellophane. Both ends of the tube are covered with 15 cm of black plastic. The beaker is placed 55 cm from the tube where the radiant flux is 7.9 $\mu w/cm^2$ in the region of 600–700 nm.[1] The far-red light source is a 60-watt tungsten filament lamp covered with 6 sheets of red cellophane and 2 sheets of blue cellophane. The lamp is inverted so that the far-red light passes through 5.5 cm of water contained in a glass dish with a

watch-glass cover. The beaker is placed on the cover at a distance of 10 cm from the lamp where the radiant flux is 260 $\mu w/cm^2$ in the region of 700–750 nm. The tips can be observed best in the far-red light by suitable positioning of black paper. These types of light sources are in routine use for phytochrome studies at Beltsville.

The same beaker is used throughout an experiment. It is washed in a biodegradable detergent and rinsed several times with tap water followed by deionized water. The charge on the glass surface responsible for root tip adherence appears to be associated with phosphate. Beakers are treated once with a dilute solution of Na_2HPO_4 and washed several times with deionized water before use. An acid wash removes the adsorbed phosphate ion, but the detergent appears to have little or no effect on it.

Results.—Results from one experiment showing the essentiality of the various components of the solution for reversibility in red and far-red light are listed in Table 1. Reversibility of attachment is manifest in the complete solution. Without ATP the root tips do not detach in far-red light. The ATP cannot be replaced by ADP. IAA is necessary for both attachment in red light and detachment in far-red light. AA also seems to be necessary for both processes. Mn^{++} is essential only for adherence in red light. Both processes in red and far-red light require Mg^{++} which cannot be replaced by Ca^{++}. K^+ is required for detachment in far-red light, and Na^+ cannot substitute for it.

The effect of increasing ATP concentration on detachment of root tips in red and far-red light is shown by the results described in Table 2. Again, detachment does not occur in far-red light unless ATP is present. However, when the ATP concentration exceeds an optimum value (2.5×10^{-6} *M* in this experiment), the tips tend to adhere less and less even in red light.

The effect of increasing IAA concentration on adherence of root tips in red and far-red light can be seen in Table 3. When IAA is absent, attachment and detachment become progressively poorer as the irradiation cycles are run. A very low concentration of 2×10^{-10} *M* enables a large proportion of tips to adhere in red light, but detachment in far-red is not seen until a concentration of about $5 \times$

TABLE 1. *Essentiality of various compounds for the reversible adhesion of barley root tips to glass during one-minute red (R) or far-red (FR) irradiation.*

Media composition	Number of Root Tips Adhering to Glass				
	Cycle 1 R–FR	Cycle 2 R–FR	Cycle 3 R–FR	Cycle 4 R–FR	Cycle 5 R–FR
Complete	10–4	10–3	9–3	8–1	8–1
−ATP	9–10	10–10	10–10	10–10	10–10
−ATP, + ADP	9–9	10–9	9–8	8–7	8–8
−IAA	5–5	5–4	3–3	3–2	2–1
−AA	8–6	8–7	6–5	4–4	3–3
$-MnCl_2$	6–3	5–1	4–0	5–2	4–0
$-MgCl_2$	8–6	6–5	5–5	3–4	3–2
$-MgCl_2$, + $CaCl_2$	7–7	7–5	5–5	4–4	4–3
−KCl	10–10	10–9	9–9	9–8	9–9
−KCl, + NaCl	10–10	9–8	9–9	9–8	8–8

Length of root tip: 1 mm. Ten tips per run.

Composition of complete medium: ATP, adenosine-5′-triphosphate (2.5×10^{-6} *M*); IAA, 3-indoleacetic acid (10^{-8} *M*); AA, L-ascorbic acid (10^{-6} *M*); $MnCl_2$ (2×10^{-6} *M*); $MgCl_2$ (2×10^{-4} *M*), KCl (10^{-4} *M*), HCl (5×10^{-5} *M*).

Substitutions: ADP, adenosine-5′-diphosphate (2.5×10^{-6} *M*); $CaCl_2$ (2×10^{-4} *M*), NaCl (10^{-4} *M*).

R, red light; FR, far-red light.

The first number in each column indicates number of tips adhering in red light, second for number in far-red light.

TABLE 2. *Effect of increasing ATP concentration on the reversible adhesion of barley root tips to glass during one-minute red (R) or far-red (FR) irradiation.*

ATP concentration (*M*)	Number of Root Tips Adhering to Glass				
	Cycle 1 R–FR	Cycle 2 R–FR	Cycle 3 R–FR	Cycle 4 R–FR	Cycle 5 R–FR
0	10–9	10–9	9–10	9–9	9–9
1×10^{-6}	10–8	9–7	9–7	9–8	9–7
2×10^{-6}	10–7	10–8	10–7	9–6	10–6
2.5×10^{-6}	9–3	9–2	8–2	7–0	7–0
3×10^{-6}	9–3	8–1	8–0	7–0	6–1
4×10^{-6}	6–2	4–0	4–0	4–0	2–0

Except for the concentrations of ATP, tips were irradiated in the medium described as "complete" in Table 1.

TABLE 3. *Effect of increasing 3-indoleacetic acid concentration on the adhesion of barley root tips to glass during one-minute red (R) or far-red (FR) irradiation.*

IAA concentration (*M*)	Number of Root Tips Adhering to Glass				
	Cycle 1 R–FR	Cycle 2 R–FR	Cycle 3 R–FR	Cycle 4 R–FR	Cycle 5 R–FR
0	8–7	5–3	5–3	3–2	2–1
2×10^{-10}	8–7	6–6	6–6	7–6	7–5
5×10^{-10}	9–9	9–10	10–8	10–8	8–6
1×10^{-9}	10–8	10–9	9–7	9–7	8–6
2×10^{-9}	10–9	10–8	9–7	9–5	8–3
5×10^{-9}	10–6	9–5	9–2	8–1	8–0
1×10^{-8}	9–3	10–3	10–1	8–1	8–0

Except for the concentrations of IAA, tips were irradiated in the medium described as "complete" in Table 1.

10^{-9} *M* is reached. Good reversibility is achieved at about 10^{-8} *M* concentration. Results from some unreported experiments indicate that at higher concentrations of IAA the tips adhere to glass even in far-red light.

After the experiments reported in Tables 1–3 were completed, it was found that when the deionized water used to make up the solution is placed in sunlight for about one week (in a greenhouse), the tips respond to a much lower concentration of IAA. Some results are reported in Table 4. The tips without IAA do not show a pronounced lack of it until after several cycles. These results suggest that something (probably chlorine) in the water destroyed some of the endogenous and exogenous IAA in the previous experiments. The response of the tips

TABLE 4. *Effect of water quality on the influence of indoleacetic acid on attachment of barley root tips to glass during one-minute red (R) or far-red (FR) irradiation.*

IAA concentration (*M*)	Number of Tips Adhering to Glass						
	Cycle 1 R–FR	Cycle 2 R–FR	Cycle 3 R–FR	Cycle 4 R–FR	Cycle 5 R–FR	Cycle 6 R–FR	Cycle 7 R–FR
0	10–8	10–6	8–5	7–5	7–4	7–5	5–4
10^{-10}	10–6	10–3	10–3	10–3	9–4	8–6	8–6
2×10^{-10}	10–4	10–3	10–2	9–2	9–3	—	—
3×10^{-10}	10–4	10–2	10–2	10–2	10–1	9–1	8–1
5×10^{-10}	10–4	10–4	10–3	10–4	10–4	9–4	9–3
10^{-9}	10–7	10–7	10–5	10–6	9–5	10–4	—
5×10^{-9}	10–10	10–9	10–9	10–8	10–8	—	—

Deionized water, exposed to sunlight for 1 week, was used to make up solution. ATP concentration, 4×10^{-6} *M*; concentrations of other compounds as shown in Table 1.

in far-red light to IAA can now be seen when the concentration is as low as $2 \times 10^{-10} M$. A higher concentration than $10^{-9} M$ causes the tips to adhere in both red and far-red light.

Discussion.—The photoreversible attachment and detachment of barley root tips on a glass surface in light corresponding to the spectral regions of phytochrome action indicate that the phenomenon is mediated by phytochrome. The specificities of the several compounds, which correspond with known physiological actions, and their requirements in physiologically effective concentrations make it unlikely that the system is an artificial one. In addition, it looks as if phytochrome expression is controlled not only by light, but is modified by endogenous levels of IAA, ATP, and AA.

Several reports of fast phytochrome-mediated responses in plants have been recently reviewed by Hillman.[2] Up to now the earliest measurable result from phytochrome activity is that reported by Fondeville *et al.*[3] who have shown that the effect of red light on the closing movement of *Mimosa* leaflet can be seen five minutes after irradiation. Hendricks and Borthwick[4] in a detailed treatment of rapid responses suggest that phytochrome controls permeability of plant cell membranes. Because the phenomenon reported here can be detected within 15 seconds from the beginning of exposure time and is largely completed within 30 seconds, it is quite likely an initial expression of phytochrome action.

A molecular explanation of the reversible attachment is possible in terms of known biochemical and biophysical principles. Because phosphate causes the glass surface to be negatively charged, adherence of the tips to the surface suggests that they are positively charged. Detachment takes place when the tips become either neutral or negatively charged. The changes in charge could arise from molecular conformation or configuration changes in the protein moiety of phytochrome, particularly about its isoelectric point. Jones[5] and Jones[6] suggest a structural change in a contractile protein to explain the action of ATP on detachment of animal cells from glass and other cells. They postulate that this protein is at the cell surface, and that in the relaxed state it has positive charges which cause the cells to adhere to glass and other cells. Upon the addition of ATP, the protein contracts and the positive charges are decreased, resulting in less adhesion to negatively charged surfaces. ADP and other relaxing factors reestablish adhesiveness.

An IAA requirement in this phenomenon was unexpected. An external source of IAA was found to be necessary only after the seedlings were exposed for some time to dim white light. A review of the literature gives only a slight inkling of a possible connection between phytochrome and IAA. Liverman and Bonner[7] reported an interaction between IAA and red light on enhancing growth of *Avena* coleoptile. They suggest that several processes effected by red light and IAA may share in a common mechanism. Recently, Furuya and Torrey[8] have reported that IAA-induced lateral root initiation in pea root segments is inhibited by red light. The red-light inhibition is reversed by far-red light. They suggest a possible relationship between IAA action and phytochrome action. The results presented in this report clearly implicate IAA as an essential component of the phytochrome system.

A requirement for AA also became apparent only after the seedlings were exposed for some time to dim white light. While AA is a reducing agent, its low effective concentrations (about 10^{-6} M) suggest that it probably serves in some other capacity. Fawcett[9] has reviewed several papers showing a relationship between the actions of IAA and AA.

A promotion of a charge by weak light seems implicit in some investigations of Haupt.[10] He found that weak light induced the attachment of chloroplast to the cell wall of an alga, *Vaucheria*. In addition, Haupt's observations suggest that phytochrome is located in the periphery of the cell. The system reported in this paper also indicates that phytochrome is located near or in the cell membrane. Its reaction to external compounds and its quick response (within 30 sec) upon their addition point to such a location. If phytochrome is in the membrane, it could very well control permeability as suggested by Hendricks and Borthwick.[4]

When repeating these experiments, some practice will possibly be required in rotating the beaker with the right force. A concentration study of ATP is helpful to acquire the necessary skill. Also, because of differences in experimental setup, the quantities of the necessary compounds might vary considerably from those reported here. This is likely to be true especially for IAA and possibly for AA and ATP.

Preliminary results show that a similar system is present in mung bean root tips (*Phaseolus aureus*) with one notable difference. In addition to the seven factors listed in Table 1, mung bean root tips require Ca^{++}.

Summary.—A very rapid photoreversible attachment of barley root tips to wet glass surfaces has been found to be mediated by phytochrome. Adhesion of the tips to glass can be seen within 30 seconds in red light. They are released within 30 seconds in far-red light. Both processes are reversible for several light cycles. 3-Indoleacetic acid in very low concentrations is necessary for reversibility. Adenosine-5′-triphosphate, ascorbic acid, Mn^{++}, Mg^{++}, and K^{+} are also required in the system. This phenomenon is probably a result of the first physiological response to the change in form of phytochrome induced by radiation. 3-Indoleacetic acid is essential for this phytochrome action.

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[1] Measurement of radiant energy was made by K. H. Norris (MQRD; ARS, USDA, Beltsville, Maryland).

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