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# Light-Enhanced Protein Synthesis in Gravitropically Stimulated Root Caps of Corn[1]

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## ABSTRACT

**Light stimulates gravitropic bending (downward growth) in roots of many cultivars of corn (*Zea mays*). In this work, using the cultivar Merit, we show that light stimulates protein synthesis in the root cap, with protein levels increasing 1.3 to 1.6 times that recorded for tissues maintained in continuous dark. Light enhances protein levels both in intact caps (attached to the root) and in caps in culture. Protein synthesis is optimal in cultured caps when 1 nanomolar indole-3-acetic acid is included in the culture medium. If cap tissue is illuminated and subsequently returned to the dark, in the 2-hour period following illumination protein levels decline to that observed in dark controls. It is proposed that light-stimulated protein synthesis mediates in part downward bending in roots of these cultivars of corn.**

In many cultivars of corn, if seeds are germinated in darkness or in dim green light, the seedling root fails to orient itself with respect to gravity, but rather grows in a direction determined by the original orientation of the seed (6). However, briefly illuminating seedling roots with white light initiates a set of events which culminate in downward growth (positive gravitropism). The region of the root which perceives the light is the root cap. Roots from which caps have been excised fail to bend downward when exposed to white light.

Light, therefore, acts to enhance or to initiate certain processes in the caps. One of these processes which appears to be required for downward root growth is protein synthesis (1). From earlier work with cultured root caps, we have shown that protein and RNA synthesis must occur in the cap if the events which culminate in root bending are to occur (1). Inhibiting protein synthesis in the caps retards or prevents completely the development of the gravitropic response in illuminated roots.

In this investigation, we explore further the effects of light on protein synthesis within the cap, and, in particular, address the question of whether light initiates downward bending by influencing aspects of protein synthesis and turnover.

## MATERIALS AND METHODS

Grains of *Zea mays* var Merit (Asgrow Seed Co., MI) were surface sterilized for 8 min in 3% NaOCl, rinsed three times in sterile distilled $H_2O$, and subsequently imbibed for 3 h in darkness at 23.5°C. Following imbibition, seeds were transferred to trays (39 × 30 × 5cm) and the trays covered with foil and returned to the dark at 23.5°C. Forty to 44 h later, seedlings with straight primary roots 10 to 20 mm in length were selected in dim green light (515–545 nm) and, with the aid of a dissecting microscope and fine dissecting tools, root caps were detached from the root. Separation occurs at the root cap junction (2). Following excision in green light, caps were transferred to media designated S2M (8), lacking sucrose, vitamins and 2,4-D but supplemented with either 1 nM IAA (auxin), or 1 μM cycloheximide, or 50 μCi $ml^{-1}$ L-[4,5-$^3$H]leucine (Amersham, 130 Ci $mmol^{-1}$) or to media supplemented with a combination of the above. Following transfer to media, tissues were returned to the dark, on a shaker (100 rpm), and allowed to equilibrate for 3 h with components of the medium. For this work, usually 50 to 100 caps were cultured in 100 to 150 μl of medium. Following equilibration, caps were either illuminated with white light ($1.3 \times 10^{-2}$ E $cm^{-2}$ $s^{-1}$) for 10 to 30 min and then returned to darkness or caps were maintained in continuous darkness.

For some experiments, roots were equilibrated in medium supplemented with [$^3$H]leucine only, illuminated, and then returned to the darkness on a shaker. After varying intervals of time, auxin was added (final concentration equals 1 nM) and the caps now in auxin plus [$^3$H]leucine returned to darkness on a shaker.

Pulse-chase experiments were conducted in the following manner. Tissues were equilibrated for 3 h in [$^3$H]leucine-containing medium with or without 1 nM auxin. Following equilibration, caps were illuminated (as before) for 10 min and then rinsed and transferred to a chase solution identical to the incubation medium except now the [$^3$H]leucine was replaced by 0.5 mM unlabeled leucine plus 0.1 mM penicillin G. Tissues were returned to the dark on a shaker and at intervals (0–25 h) lots harvested. Measurement of [$^3$H]leucine uptake and incorporation was assessed by first rinsing the caps quickly in the chase solution followed by homogenization on ice in 2 ml of 50 mM Tris-HCl (pH 7.4). The resulting homogenate was then spun at 10,000 rpm for 30 min and, following centrifugation, an aliquot of the supernatant was removed, placed in a scintillation vial, and the liquid evaporated. To the remaining portion of the supernatant, 50% (v/v) TCA was added so that the final TCA concentration was 10%. Precipitation of proteins was allowed to proceed for 1 h and the resultant precipitate collected on a Whatman GF/A glass fiber filter paper. Following filtration, the filter was air dried and then immersed sequentially into four washes of 5% TCA plus 0.1 M leucine (25

Table I. *Average Curvature of Roots to Which Have Been Affixed Caps Cultured in the Presence or Absence of IAA*

| | Amount of IAA | |
|---|---|---|
| | 0 | 1 nM |
| | *degrees ± SE* | |
| Plus light | 5 ± 3 | 21 ± 5 |
| Continuous darkness | 6 ± 4 | 7 ± 2 |

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Table II. *Average Curvature of Roots to Which Cycloheximide-Treated Caps Have Been Affixed*

| | Amount of Cycloheximide | | |
|---|---|---|---|
| | 0 | 0.1 μM | 1 μM |
| | *degrees ± SE* | | |
| Plus light | 30.5 ± 7 | 17 ± 3 | 11 ± 3 |
| Continuous darkness | 5.5 ± 4 | 6 ± 5 | 6.5 ± 4 |

Table III. *Protein, as Measured by the Method of Lowry et al., from Root Caps Illuminated 10 Minutes and then Returned to the Dark for Varying Periods of Time After Which Caps Are Assayed for Protein*

| Time in Dark after 10 Min Light | Protein Content | Increase above Time 0 |
|---|---|---|
| *min* | *μg/cap*[a] | *-fold* |
| 0 (dark control) | 1.3 | |
| 30 | 1.7 | 1.3 |
| 60 | 1.5 | 1.1 |
| 120 | 1.3 | 1.0 |

[a] Average value from 150 root caps.

Table IV. *Radioactivity ([$^3$H]Leucine) in Protein (TCA Precipitable Fraction) from Root Caps Illuminated for 10 Minutes and then Returned to the Dark for Varying Periods of Time after Which the Caps Are Homogenized and Assayed for Protein*

| Time in Dark after 10 Min Light | Radioactivity | Increase over Time 0 |
|---|---|---|
| *min* | *dpm × $10^{-3}$/-50 caps*[a] | *-fold* |
| 0 (dark (dark control) | 35.6 | |
| 30 | 57.6 | 1.62 ± 0.03 |
| 60 | 47.7 | 1.34 ± 0.04 |
| 120 | 34.8 | 0.98 ± 0.02 |

[a] From 50 caps labeled continuously.

ml/wash) and agitated gently for 20 min in each wash. Following these washes, the filters were rinsed five times with 95% ethanol, air dried, and then placed in scintillation vials. Samples were stored in the dark at 0 to 5°C for 25 h prior to counting in a Beckman LS7000 liquid scintillation counter.

For other experiments, caps were attached (intact) to the root at the time of the illumination. Two sorts of experiments were done with intact caps. In the first, dark-grown roots were illuminated with white light (as before) for 10 min and then the roots returned to the dark for varying periods of time. Dark controls (tissue never exposed to white light) were also carried out. Subsequently, caps were excised in green light and collected in cold Tris buffer (pH 8.0), and protein assayed by the method of Lowry *et al.* For the second series of experiments with intact caps, seedlings (in green light) were oriented vertically (root end down) by inserting a pin through the seed kernel into a styrofoam support covered with moistened filter paper. Vertically oriented seeds were then returned to the dark in a sealed chamber (humidity 90–95%). Subsequently, the seeds were illuminated for 10 min with white light (as above) and then the chambers, with the roots still in the vertical position, returned to complete darkness. At varying intervals after the illumination (0–5 h), in green light, the chambers were reoriented so that the roots were now positioned horizontally. Five h after reorientation, bending was assessed by shadow-graphs made from the roots.

For some experiments, caps were excised in green light and explanted with the basal end down to media previously described, but now supplemented with 1% agar. As before, tissues were allowed to equilibrate with components of the medium and then either illuminated or maintained in complete darkness. One-half to 2 h after illumination, these caps were substituted for caps on roots maintained previously in total darkness (for a more complete description of this procedure, see Ref. 1). Following this substitution, a pin was inserted through the seed kernel into a styrofoam support and the seedling root to which was affixed the cultured cap positioned horizontally in a moistened chamber (90–95% humidity) in darkness at 23.5°C. Five h later, shadow-graphs were made and the angle of root bending recorded.

All experiments assessing root curvature were done at least twice, and 15 to 20 roots were used in computing average curvature at the specified time points.

Table V. *Effect of IAA on [$^3$H]Leucine Uptake into Root Tissue Maintained Continuously in Darkness or Illuminated for 10 Min*

| Treatment | Radioactivity in | |
|---|---|---|
| | Protein | Soluble pool |
| | *dpm × $10^{-3}$/50 caps* | |
| + Light | | |
| + IAA | 32 | 308 |
| − IAA | 16 | 298 |
| In dark | | |
| + IAA | 24 | 275 |
| − IAA | 17 | 350 |

Table VI. *Radioactivity ([$^3$H]Leucine) in Protein (TCA Precipitable Fraction) from Root Caps Cultured in the Presence of 1 μM Cycloheximide, in the Light or the Dark*

| Treatment | Radioactivity |
|---|---|
| | *dpm × $10^{-3}$/50 caps* |
| Light + cycloheximide | 3.7 |
| Dark + cycloheximide | 4.2 |
| Light, no cycloheximide | 55.1 |
| Dark, no cycloheximide | 42.1 |

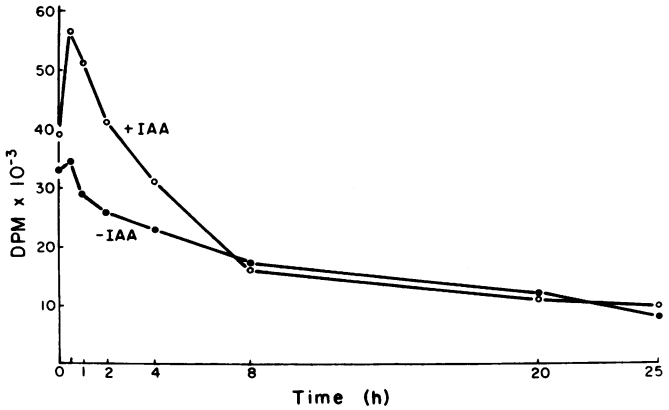


FIG. 1. Loss of radioactivity from [$^3$H]leucine-labeled *Zea* root cap proteins during a 25-h chase. Lots of 50 caps were incubated in the label for 3 h in the dark in media ± IAA, then illuminated for 10 min and subsequently placed in the dark in identical media now containing an excess of unlabeled leucine. At intervals, groups of caps were removed from the chase solution and assayed for $^3$H in proteins by precipitation with TCA.

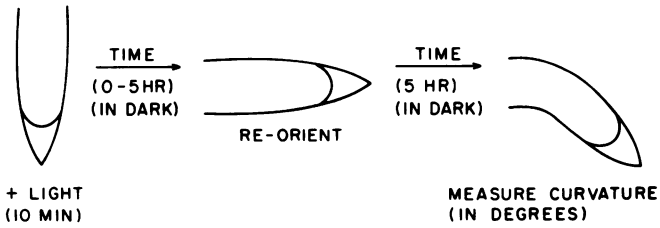


FIG. 2. Diagram indicating the timing and the sequence of steps in root reorientation experiments. See Table VII.

Table VII. *Root Reorientation*

| Time Interval between Illumination (10 min) and Reorientation | Curvature | |
|---|---|---|
| | + Light | Dark control |
| *h* | *degrees* ± SE | |
| 0 | 45 ± 5 | 4 ± 3 |
| 1 | 40 ± 3 | 6 ± 4 |
| 2 | 34 ± 3 | 5 ± 4 |
| 3 | 30 ± 3 | 5 ± 3 |
| 4 | 14 ± 4 | 6 ± 3 |
| 5 | 4 ± 2 | 5 ± 4 |

Table VIII. *[$^3$H]Leucine Incorporation into Protein in Auxin-Treated Tissues*

Auxin is added at varying times after a 10-min light treatment. Tissues are then left an additional 1 h in the dark and assayed for [$^3$H]leucine incorporation into protein

| Treatment | Radioactivity |
|---|---|
| | *dpm/100 caps* |
| Auxin present continuously | 110,400 |
| Auxin added 30 min after light | 92,437 |
| Auxin absent | 61,593 |
| | |
| Auxin present continuously | 96,408 |
| Auxin added 60 min after light | 46,136 |
| Auxin absent | 43,978 |

## RESULTS

When cultured root caps were substituted for caps of dark-grown roots, downward bending was observed only in those roots to which had been affixed caps cultured in the presence of 1 nM auxin (Table I). Illuminated caps cultured in the absence of IAA were unable to induce bending. Similarly, no bending was recorded for roots with caps maintained on auxin-supplemented media, but in darkness. When caps cultured in the presence of cycloheximide plus light and auxin were substituted for caps from dark-grown roots, downward bending of these roots is reduced compared to roots with non-cycloheximide treated caps (Table II).

Total root cap protein, as measured by the method of Lowry (5), reached maximum levels approximately 30 min after the illuminated roots (a 10-min illumination) were returned to the dark. With increased intervals of time between the light treatment and protein assays, we observed a decline in protein levels so that 2 h after illumination protein levels declined to levels recorded for caps maintained continuously in the dark (Table III). Protein synthesis, as measured by [$^3$H]leucine incorporation, is maximal in root caps illuminated for 10 min and subsequently returned to the dark for 30 min. With increased intervals of time in the dark following the light treatment, the amount of radioactivity associated with proteins declines to levels recorded for caps maintained continuously in darkness (Table IV).

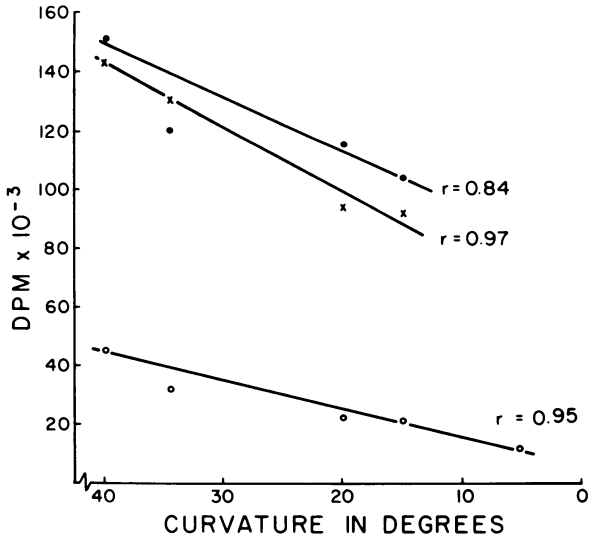


FIG. 3. Graph showing the correlation between loss of radioactivity from protein (data obtained from pulse-chase experiments) and the loss of the ability with time of roots to bend when reoriented from the vertical to the horizontal with respect to gravity. Reorientation experiments were conducted as shown in Figure 2.

Addition of IAA to the culture medium is required for light-induced protein synthesis (Table V). Auxin alone in the dark can stimulate some protein synthesis but the levels observed were only about two-thirds that recorded for illuminated caps cultured in an auxin-containing medium. Neither light nor auxin appear to affect the uptake of the [$^3$H]leucine.

Addition of cycloheximide reduced by 90% the amount of [$^3$H] leucine incorporated into protein irrespective of whether caps were illuminated or maintained in continuous darkness (Table VI).

Pulse label and chase experiments were carried out to assess protein turnover in caps cultured on media ± IAA. Dring the first 8-h period, overall loss of the label occurred at a greater rate for caps cultured in the presence of IAA compared to caps on media lacking IAA. In the 8- to 25-h interval of the chase, the loss of radioactivity was almost identical for the two treatments (Fig. 1). Four to 5 h after beginning the chase, the radioactivity recorded for caps on IAA-supplemented media reached the initial levels observed at the start of the experiment for caps cultured on auxin-free media (approximately $33 \times 10^{-3}$ dpm). A peak in radioactivity is observed for tissues from both treatments (± IAA) approximately 0.5 h after commencing the chase (Fig. 1). Note that the peak for caps on IAA-free medium is only slightly greater than at the beginning of the experiment ($34.5 \times 10^{-3}$ *versus* $33 \times 10^{-3}$ dpm) compared to the increased radioactivity in protein in caps on IAA-supplemented media ($56 \times 10^{-3}$ *versus* $41 \times 10^{-3}$ dpm).

In certain experiments, roots were reoriented from the vertical to the horizontal at varying intervals after the light treatment (Fig. 2). Roots reoriented 4 to 5 h after the light treatment show bending, differing little from that observed in roots maintained in darkness (dark control) (Table VII).

In cultured caps, light was most effective in stimulating protein synthesis when caps were cultured continuously in the presence of auxin. Alternatively, if caps were illuminated while in an auxin-free medium, returned to the dark and then after varying periods of time in the dark transferred to auxin-supplemented media, a stimulation of protein synthesis was observed, but only if the transfer occurred within 30 min of the illumination (Table VIII). Addition of auxin 60 min after the light treatment was without effect on protein synthesis compared to tissues cultured continuously on auxin-free media.

## DISCUSSION

In some cultivars of corn, light has a role in stimulating the gravitropic response mechanism of roots. Light is believed to mediate downward bending initially through the photochemical transformation of a photoreceptor (7) and subsequently through the initiation of a series of intermediate events leading to the downward bending of the root. Some of these suggested light-stimulated intermediate events include: changes in the NADPH/NADP levels (7); changes in membrane permeability (3); an increase in the root cap of inhibitory growth substances (4).

The work reported in this investigation focuses on light-stimulated protein synthesis in the root cap and examines the role of these proteins in gravitropic root bending. The initial observation suggesting a role for protein synthesis in root bending derives from work showing that inhibition of protein synthesis (with cycloheximide) in the cap alone was sufficient to prevent or retard downward bending when the root tissue was exposed to light (1). However, because cycloheximide is not a specific inhibitor for protein synthesis, interpretation of its effect on root cap metabolic processes must be made with a degree of caution. In this work, we have extended these observations to include a more precise quantification of the effects of light on protein synthesis.

From both radiolabel data and Lowry data, it is seen that light increases measurable protein 1.3 to 1.6 times that observed in dark control cap tissue (Tables III and IV). The peak in protein is recorded approximately 0.5 h after illuminating the cap tissue and is observed in both cultured and in intact root caps. With increased intervals of time in the dark following light treatment, protein levels decline so that 2 h after illumination protein levels are the same as in caps maintained continuously in darkness. We therefore conclude that the ability of light to stimulate protein synthesis is of a short-term nature, and that in the intact cap the effect of light is lost approximately 2 h after illuminating cap tissues.

In cultured tissues, it is clear that auxin is required for light-stimulated protein synthesis (Table V). Caps cultured in media lacking auxin, but illuminated, typically show reduced incorporation of [$^3$H]leucine into protein compared to caps illuminated while maintained on an auxin-supplemented medium. From our present results, we are unable to clarify more precisely how auxin affects protein synthesis. We cannot now decide whether auxin acts simply to maintain or to enhance general protein synthesis or whether the synthesis of a limited number of polypeptides is modified, or alternatively, whether the turnover of specific proteins is regulated by auxin. Auxin in dark-cultured tissues can stimulate some protein synthesis, but this synthesis is enhanced considerably with the addition of light (Fig. 1; Table V). This observation suggests that there may be differences in the manner in which auxin affects protein synthesis, depending on whether the tissues are illuminated or maintained in continuous darkness.

To examine further the interactions between auxin and light in regulating both protein synthesis and gravitropic root bending, experiments were carried out with tissues which were illuminated while on auxin-free media and then returned to the dark. After varying periods of time in the dark (still on auxin-free media), auxin was added to these tissues and after an interval of time the tissues assayed for label in protein (Table VIII). The data in Table VIII indicate that cultured root cap tissue in the absence of auxin can 'remember' the light treatment for periods up to 0.5 h following the light treatment. Addition of auxin during this 0.5-h interval significantly enhances [$^3$H]leucine incorporation into protein, whereas addition of auxin 1 h after illumination yields little if any stimulation of [$^3$H]leucine incorporation into protein. From this experiment, we had hoped to decide whether light and auxin affect protein synthesis via the same or different mechanisms. At present, we are unable to distinguish between these two alternatives and can now only say that the root cap tissue has a 'memory' for the light treatment and that in caps in culture the effect of the light treatment can be enhanced considerably if auxin is added within a certain interval of time following illumination.

From pulse-chase experiments (Fig. 1), we have shown in IAA-cultured caps that 4 to 5 h after beginning the chase the amount of label remaining in protein approximately equals the amount of label at the start of the chase in protein from caps cultured in media lacking IAA. We interpret these data to mean that, in the 4- to 5-h interval following illumination, much of the IAA-dependent/light-stimulated incorporated radioactivity is turned over.

Ultimately, one would hope to link light-induced processes occurring in the root cap with the gravitropic bending response of roots. From this work, we wish to propose that there is a direct correlation between light-induced protein synthesis in root caps and root bending (Fig. 3). Figure 3 shows this correlation. This figure has been prepared by graphing the dpm data recorded at various times (*e.g.* 1, 2, 3, 4, 5 h) from several pulse-chase experiments (graphed on the '*y*' axis), and for the '*x*' axis, using the data (in degrees) from root reorientation experiments in which roots were reoriented from the vertical to the horizontal at various times after illumination (*e.g.* 1, 2, 3, 4, 5 h). Plotting the data from the corresponding time points shows a high correlation between the loss in radioactivity (turnover in protein) and the loss of the ability of reoriented roots to bend. After 5 h in a vertical position, roots reoriented to the horizontal no longer bend downward. We interpret these results to mean that, during the 4- to 5-h interval following illumination, changes have occurred in the cap so that the gravity stimuli are without effect in initiating downward bending.

Exactly what change occurs in the cap in the 4- to 5-h interval is not known; however, the high correlation between light-stimulated protein synthesis and light-stimulated gravitropic bending strongly implies a role for proteins in translating gravity stimuli into downward root growth. The manner in which proteins mediate this response is not known. Currently, we are investigating what changes may occur in specific proteins in the 5-h interval following illumination of root cap tissue.

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