AN ABSTRACT OF THE THESIS OF

Kyoung-Hee Kim for the degree of Master in Science in Botany and Plant Pathology presented on April 25, 1997. Title: Gravity and Red Light Interaction in the *lazy-2* Tomato Mutant.

Redacted for Privacy

Abstract approved: _____

Terri L. Lomax

The mechanism of plant response to gravity is explained by the Cholodny-Went (C-W) theory which formalized observations on tropisms which had been made as early as Darwin. The C-W theory states that a growth-promoting substance moves to the bottom of gravitropically-stimulated stems and that the resulting asymmetric growth produces curvature. Although the C-W theory led to the discovery of the plant hormone auxin, considerable arguments have accumulated surrounding the theory. In these studies, the phytochrome-regulated reversed gravitropic response of the tomato mutant, *lazy-2* (*lz-2*) was used to test the C-W theory and to better understand the nature of the interaction between gravity and red light. The downward curvature of the *lz-2* mutant in red light was found to be the result of stimulated stem elongation and the apical region of *lz-2* hypocotyls was found to be necessary for the gravitropic response. Both of these results indicate that auxin plays a major role in the gravitropism of the *lz-2* mutant, more importantly in the red light-induced reversal of the gravitropism. In order to determine whether changes in auxin movement or sensitivity are involved, auxin redistribution in response to gravity was examined using a double-labeling technique. Intact wild-type and *lz-2* tomato seedlings were labeled with $^{3}$H-indole-acetic acid ($^{3}$H-IAA) and $^{14}$C-benzoic acid ($^{14}$C-BA) and used to demonstrate specific IAA transport which could be inhibited by 2, 3, 5-triiodobenzoic

acid (TIBA), an inhibitor of specific polar auxin transport. $^{3}$H-IAA redistribution was compared with $^{14}$C-BA distribution in the upper and lower halves of tomato hypocotyls during gravitropic curvature. In upward-bending hypocotyls, e.g., wild-type in the dark and red light and *lz-2* in the dark, higher levels of $^{3}$H-IAA were found in the lower half. In contrast, in downward-bending hypocotyls, i.e., *lz-2* in red light, $^{3}$H-IAA redistributed to the upper half. The kinetics of gravitropic curvature correlated with IAA redistribution in both wild-type plants and the *lz-2* mutant. These studies convincingly support the C-W theory by confirming that auxin mediates the gravitropic responses of plant stems. These studies are also the first report that gravity and red light interacts at the level of auxin transport regulation.

# Light and Gravity Interaction in the Tomato Mutant of *lazy-2*

by

Kyoung-Hee Kim

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master in Science

Completed 6 June, 1997
Commencement June 1998

Master in Science thesis of Kyoung-Hee Kim presented on April 25, 1997

APPROVED:

Redacted for Privacy

Major Professor, representing Botany and Plant Pathology

Redacted for Privacy

Chair of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Kyoung-Hee Kim, Author

Table of Contents

List of Figures

List of Tables

List of Diagrams

# Analysis of the Interactions Between Phytochrome and Auxin using THE GRAVITROPIC RESPONSE OF MUTANT TOMATO, *LAZY-2*

## I. INTRODUCTION

Tropisms are one strategy which plants have evolved in order to compensate for their lack of mobility. Unlike animals, which react to environmental changes by movement, plants depend largely on the growth of organs such as stems or roots. Tropisms have been an object of plant growth and development research ever since early studies by Darwin (1880). Darwin's observations on tropic movements in plants were later formalized as the Cholodny-Went theory (C-W theory) which provided a hypothesis for the more obvious forms of tropic behavior such as phototropism or gravitropism (Went and Thimann, 1937) . The C-W theory states that the differential growth which results in the tropic curvature of stems or roots is caused by movement of the growth-stimulating hormone, auxin, from one side of the responding organ to the other side.

The C-W theory has also triggered an immense volume of controversy (see Trewavas, 1992, for forum). Bruinsma and Hasegawa (1990) measured growth inhibitors moving in the opposite direction from auxin in the phototropism of radish seedlings. They suggested that the phototropic bending is caused by growth inhibition on the lighted side rather than growth promotion on the shaded side. Changes in tissue sensitivity to auxin instead of actual movement of the hormone have also been put forth as a possible

explanation for tropic curvatures induced by differential growth (Trewavas, 1981; Salisbury, 1993, and references therein). In these studies, the tissue responsiveness to auxin in the top and bottom of the gravitropically-responding stems was measured and higher sensitivity to auxin was found in the bottom.

During the past several decades, plant mutants have been shown to be a powerful tool for investigating complex processes in growth and development such as photomorphogenesis (Chory, 1993). Our lab has utilized mutants of tomato to provide insight into the mechanisms underlying plant responses to gravity. *lazy-2* (*lz-2*) is a single gene, recessive tomato mutant which exhibits a reversed shoot gravitropic response in the presence of light (Roberts, 1987; Gaiser and Lomax, 1992). In the dark, *lz-2* stems respond normally to gravity, i.e., they grow upward. In the presence of light, however, *lz-2* stems and petioles grow toward the gravity vector (Gaiser and Lomax, 1992). The gravitropic response of *lz-2* roots is unaffected by the mutation. Photobiological studies have shown that the downward gravitropic response in *lz-2* stems is mediated by the red light-absorbing photoreceptor, phytochrome (Gaiser and Lomax, 1993).

Although most plants can use the gravity signal to orient their stems or roots in the total absence of light, numerous studies have reported that light can modulate gravitropic responses (Blaauw, 1961; Wilkins and Goldsmith, 1964; Tepfer and Bonnett, 1972; McArthur and Briggs, 1979; Feldman and Briggs, 1987; Moitzik and Mohr, 1988; Kelly and Leopold, 1992; Liscum and Hangarter, 1993). In some species, the influence of light on gravitropism is extreme. The corn cultivar, Merit, exhibits a root gravitropic response only after exposure to light (Feldman and Briggs, 1987). In most cases, phytochrome is the photoreceptor and the direction of light is unimportant. This excludes the possibility that this is a phototropic response. Rather, it is considered to be a special version of photomorphogenesis, that is, light modulates many responses in plant growth and development which, in turn, can be modulated by gravity.

There have been several studies on cellular reactions which are regulated by both light and gravity signals. A rapid asymmetric accumulation of $Ca^{2+}$ is seen in the cell wall or vacuole during light-mediated gravitropism, suggesting that $Ca^{2+}$ may be a candidate for the point of intersection between the two signal transduction pathways (Roux and Serlin, 1987, and references therein). As the direction of asymmetric $Ca^{2+}$ accumulation is opposite to that of auxin, it was suggested that $Ca^{2+}$ inhibits the enzymes involved in cell wall loosening (Cleland and Rayle, 1977). Plant hormones may also link the two pathways which are involved in plant responses to light and gravity. Regulation of stem elongation, one of the major photomorphogenic processes mediated by phytochrome, occurs via modulation of plant hormones. The role of the hormones such as auxin and gibberellins in phytochrome-regulated stem elongation has been shown (López-Juez et al., 1995; Behringer et at., 1990; Behringer and Davies, 1992; Jones et al., 1991; Jones and Prasad, 1992). Although a recent study suggested that phytochrome and gibberellins employ independent pathways to regulate stem elongation (López-Juez et. al.., 1995), phytochrome has been shown to alter both auxin transport and binding (Beringer and Davies, 1992; Jones and Prasad, 1992). Other plant hormones such as brassinosteroids, cytokinins, and ethylene have also been reported to be involved in the phytochrome-mediated stem elongation response (Kronenberg and Kendrick, 1994).

The highly specific phenotype of the *lz-2* mutant provides a valuable tool for studying the mechanism of interaction between light and gravity. Unlike the other *lazy* mutant, *lazy-1*, which is altered in gravity perception, i.e., it is a starch mutation (Roberts, 1987), the *lz-2* mutation seems to affect either the transmission of or response to the gravity signal (Gaiser and Lomax, 1993). This provides an unique opportunity to examine the role of auxin in the plant gravitropic response. For example, if auxin movement across a gravitropically-stimulated stem is responsible for the asymmetric growth which produces curvature, then one would expect to find <u>increased</u> growth on the <u>upper</u> side of the *lz-2* stems which grow downward in red light. In contrast, if an inhibitory substance is the

primary factor regulating the gravitropic response then a decrease in growth should be observed on the lower side of red light-treated *lz-2* shoots.

In the research described here, initial studies were conducted in order to elucidate whether the downward curvature in the *lz-2* mutant is a result of stimulation or inhibition of stem growth. Intact *lz-2* seedlings were exposed to red light in order to induce downward curvature. This treatment increased elongation rates, which indicates the action of a growth stimulating substance rather than an inhibitor. Among the known plant hormones only auxin is thought to be synthesized exclusively at the shoot apex. I found that excision of the shoot apex completely eliminated gravitropic curvature in *lz-2* hypocotyls, but not in wild-type, which provides evidence that auxin plays a significant role in the gravitropic response of the *lz-2* mutant.

While the stimulation of growth and necessity of the shoot apex indicate a role for auxin in the reversed *lz-2* gravitropic response, these experiments still did not differentiate between alterations in auxin movement (the C-W theory) or changes in auxin sensitivity (Salisbury, 1993, for review) as the primary mechanism responsible for asymmetric curvature in response to gravity. In order to differentiate between these two possibilities, I developed a double-labeling technique to measure auxin redistribution in response to gravity in the *lz-2* mutant as well as in wild-type. This technique employs the major native auxin, indole-3-acetic acid (IAA), radioactively-labeled with tritium ($^{3}H$) and a $^{14}C$-labeled form of another weak acid, benzoic acid (BA), which has a size and pKa which is similar to IAA but which is not transported through the specific polar auxin transport stream. Using the double-labeling technique, I was able to detect specific polar auxin transport in the hypocotyls of intact tomato seedlings as well as lateral transport of the hormone in response to gravity. Higher levels of auxin were observed in the lower half of those hypocotyls which exhibited upward growth, i.e., in wild-type seedlings either in the dark or red light and in *lz-2* seedlings in the dark. In *lz-2* seedlings treated with red light which respond to gravity with downward curvature, higher auxin levels were detected in the

upper half of the hypocotyls. These results strongly confirm the C-W theory. More importantly, the studies described below demonstrate that red light can interact with gravity at the level of auxin transport regulation.

# II. PHYTOCHROME-MEDIATED DOWNWARD GRAVITROPIC CURVATURE IN THE *LAZY-2* MUTANT IS THE RESULT OF INCREASED STEM ELONGATION

## Abstract

Shoots of the *lazy-2* (*lz-2*) tomato mutant grow downward in response to gravity. Gaiser and Lomax (1993) have shown that the reversed gravitropic response of the *lz-2* mutant is mediated by phytochrome. In this study, we characterized stem elongation and the pattern of gravitropic bending in etiolated *lz-2* and wild-type hypocotyls. During red light-induced downward curvature, *lz-2* hypocotyls elongate faster than either wild-type or *lz-2* seedlings in the dark. The kinetics of gravitropic bending in *lz-2* hypocotyls also differ from wild-type. Upon gravistimulation, curvature is rapidly observed in the elongation zone of wild-type hypocotyls, whereas *lz-2* hypocotyls reorient more slowly. Excision of the shoot apex from intact tomato seedlings completely eliminates gravitropic curvature in *lz-2* hypocotyls, whereas decapitated wild-type hypocotyls respond to gravity in a relatively normal fashion. We suggest that the observed stimulation of stem elongation as well as the evidence that the shoot apex is required for the *lz-2* response indicates involvement of the plant growth regulator auxin in the phytochrome-mediated downward gravitropic response of *lz-2* hypocotyls.

## Introduction

Plants with altered gravitropic responses can provide a useful tool for elucidating the mechanisms underlying gravitropism (Bell and Maher, 1990; Roberts, 1987). *lz-2* is a single gene, recessive mutant of tomato which exhibits a unique gravitropic response in stems and petioles. Instead of growing away from the ground as normal plants do in response to gravity, the *lz-2* stems and petioles grow toward the ground. However, the gravitropic response in the *lz-2* root is normal, i.e., the roots exhibit downward growth. The reversed gravitropic response of *lz-2* stems occurs only in the presence of light. In the dark, *lz-2* stems grow upward like wild-type plants (Gaiser and Lomax, 1992; Roberts, 1987). Gravity perception in *lz-2* appears to involve normal amyloplast sedimentation (Roberts, 1984) and it has been demonstrated that *lz-2* shoots actively perceive the direction of the gravity signal (Gaiser and Lomax, 1992). The growth of *lz-2* stem segments in response to auxin as well as phototropism (which is also mediated by asymmetric stem growth) are also normal in the *lz-2* mutant (Gaiser and Lomax, 1992).

The greatest induction of downward growth of *lz-2* stems occurred in red light and the reversibility of that response by far-red light has been demonstrated (Gaiser and Lomax, 1993). This indicates a role for the red light receptor, phytochrome, in regulating the *lz-2* phenotype; however, other phytochrome-regulated responses such as chlorophyll and anthocyanin synthesis were shown to be normal in *lz-2* plants. Therefore, phytochrome seems to interact with gravity in a specific manner to alter the direction of stem growth.

Although the Cholodny-Went theory (C-W theory) is widely accepted as a hypothesis to explain the mechanisms by which plant gravitropic responses occur, some studies have been interpreted as conflicting with the C-W theory (see Trewavas, 1992, for forum). Cholodny and Went separately proposed the lateral movement of auxin toward the side of a stem which is nearest the gravity vector. This was postulated to induce increased

cell elongation on that side, leading to curvature away from gravity (Went, 1937). Opponents of the C-W hypothesis have proposed that curvature is actually the result of an inhibitory compound moving to the opposite side of the bending stems (Bruinsma and Hasegawa, 1990; Iino, 1982). Also in opposition to the C-W theory is the proposal that changes in auxin sensitivity rather than actual movement of the hormone provide the primary mechanism which results in tropic curvature (Salisbury, 1993; Trewavas, 1991).

In the *lz-2* mutant, studying the alterations in stem elongation which result in the red light-induced downward gravitropic curvature, should allow us to differentiate whether growth-stimulating and/or inhibiting substances, or other mechanisms such as changes in tissue sensitivity lead to gravitropic curvature. In this study, I characterized stem elongation and the pattern of gravitropic bending. In particular, I examined the region of the hypocotyl where downward curvature is initiated by red light in the *lz-2* mutant. When closely examined using marker beads to aid growth measurement, downward curvature was first found to appear in the elongation zone of etiolated *lz-2* hypocotyls. Stem elongation was stimulated during downward curvature in red light as compared with upward-growing hypocotyls of wild-type plants in the dark or red light, or *lz-2* plants in the dark. I also excised the shoot apex of wild-type and *lz-2* seedlings and found that relatively normal, although reduced, gravitropic bending was still observed in wild-type hypocotyls, whereas the gravitropic curvature was completely eliminated in the *lz-2* hypocotyls in either the dark or red light. Upward curvature of *lz-2* hypocotyls in the dark is also slower than that of wild-type, which indicates that there is also a dark phenotype in the *lz-2* mutant. This and the elimination of gravitropic curvature upon excision of the shoot apex regardless of light condition indicate that the mechanism underlying the reversed gravitropic response by red light in *lz-2* hypocotyls is not simply a reversed version of upward curvature in wild-type hypocotyls.

The facts that the reversed gravitropic response of the *lz-2* mutant is a result of an increase in elongation rate and the necessity of the shoot apex for the gravitropic response

of the *lz-2* mutant strongly indicate that the plant growth hormone, auxin, is involved in the phytochrome-mediated downward growth in *lz-2* hypocotyls. This work supports the proposal that auxin is the primary factor regulating gravitropic curvature, as stated in the C-W theory.

## Materials and Methods

### *Plant Growth*

Seeds of the *lz-2* mutant of tomato (*Lycopersicon esculentum* Mill.) and the near-isogenic wild-type parent line, cv. Ailsa Craig, were originally supplied by Dr. Charles Rick, University of California, Davis, and subsequently propagated through numerous generations at the Botany and Plant Pathology farm at Oregon State University. *lz-2* is an ethylmethanesulfonate-induced, monogenic recessive mutation originally isolated in the tomato cultivar 'San Marzano' by Sorressi and Cravedi (1967) and extensively backcrossed into the cultivar Ailsa Craig (C. Rick, personal communication). Tomato seeds were soaked in 1% NaOCl for 15 min and rinsed well with tap water. Tomato seeds were sown in individual plastic caps from 7ml scintillation vials after each was filled with vermiculite and moistened with distilled water. This was followed by incubation in the dark at 28 °C for 3.5 days. Seedlings 1 to 1.5 cm in hypocotyl length were selected using a caliper under a dim green safe light for use in experiments.

### *Measurement of Stem Elongation and Gravitropic Curvature*

Resin beads (Amberlite, 400-500 μm, Bio Rad) were used as markers for stem elongation measurements. Resin beads were first coated with lanolin (Sigma) and carefully mounted on the surface of hypocotyls along the hook and in the elongation zone. In etiolated tomato seedlings, the elongation zone encompasses 6 to 8 mm of the hypocotyl

immediately below the hook. A styrofoam rack was used to hold the seedlings in the scintillation vial caps. To orient the seedlings with respect to gravity, the styrofoam rack was placed either rightside-up (rsu), upside-down (usd), or horizontally (GS: gravistimulated). After incubation in either the dark or red light, the seedlings were photocopied. This allowed subsequent measurement of both stem elongation and gravitropic bending. For stem elongation, the distance between resin beads or the apex of the hook was measured by a ruler and a caliper. For gravitropic bending, the angle of hypocotyl curvature was measured using a ruler and a protractor. To measure gravitropic curvature in decapitated hypocotyls, the apical region of the hypocotyls was excised at the basal end of the hook with a razor blade.

*Light Sources*

Red light was provided by two overhead fluorescent tubes (40 W Shoplight, General Electric) filtered through red acrylic (Shinkolite 102, Argo Plastics Co., Los Angeles, CA). Fluence rates used for all experiments were in the range of 20 to 40 μmol $m^{-2}s^{-1}$. The fluence rate of the dim safe light used for handling seedlings in the dark was 0.3 μmol $m^{-2}s^{-1}$.

## Results

*Localization of the region of downward curvature*

The behavior of wild-type and *lz-2* hypocotyls in response to red light is shown in Figure II.1. Beads placed around the hook and the upper portion of the hypocotyl at zero time migrated along the hook and down to the hypocotyl as the seedlings grew. The three beads nearest the apex, which were located in the hook region at time zero, were displaced to the upper region of the hypocotyl in 6 hours. Downward curvature was first observed

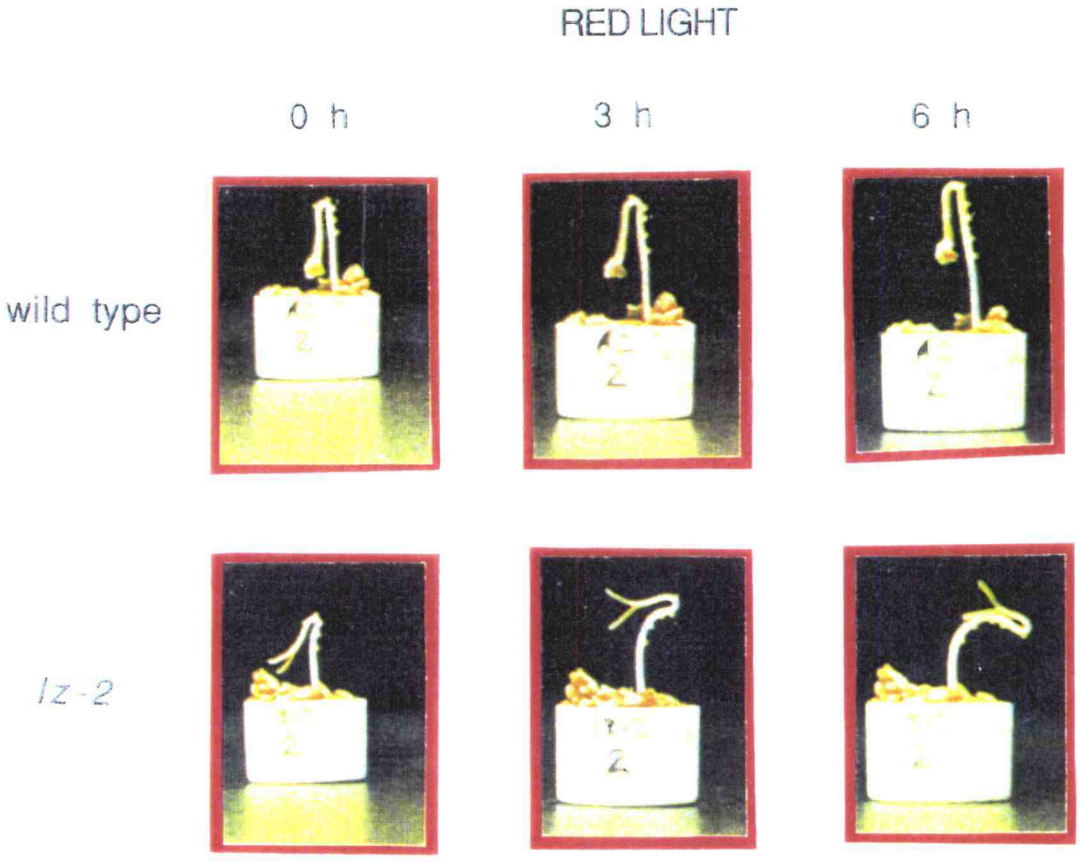


**Figure II.1.** Red light-induced downward growth in *lz-2* hypocotyls. 3.5d-old dark-grown wild-type (upper panel) and *lz-2* (lower panel) seedlings approximately 15mm in length were irradiated with red light (20 - 40 $\mu molm^{-2}s^{-1}$) from above. Before irradiation, resin beads coated with lanolin were applied to the surface of hypocotyls to aid in documenting stem elongation and downward growth. Photographs were taken at time zero (left panel), 3 hours (middle panel), and 6 hours (right panel) after red light illumination.

directly below the hook (see, e.g., the 3 h time point) and appeared to be further down the stem as the upper part of the hypocotyl grew (see 6 h). The hypocotyl region where the downward curvature initiated in *lz-2* hypocotyls in red light corresponds to the elongation zone of dicot plants (Mohr and Schopfer, 1995).

In order to determine whether the reversed curvature in *lz-2* was a result of increased growth on what would become the "convex" side, or decreased growth on the "concave" side, I placed a marker bead 3 mm below the hook in the elongation zone and incubated plants for 6 hours either in the dark or red light. The increase in hypocotyl length was then determined by measuring the distance from the bead to the hook (Figure II.2A). In the dark, wild-type and *lz-2* plants showed no difference in the elongation of this stem region (Figure II.2B). Wild-type hypocotyls in red light also elongated the same amount as wild-type and *lz-2* stems in the dark. In red light, however, while the concave side grew the same amount as any of the straight-growing hypocotyls, i.e., wild type and *lz-2* seedlings in the dark and wild-type seedlings in red light, the convex side of *lz-2* hypocotyls elongated significantly more, resulting in downward curvature.

*Reorientation toward the gravity vector specifically stimulates elongation in lz-2 plans*

We hypothesized that *lz-2* hypocotyls would exhibit a wild-type response to the gravity signal in red light if placed in the reversed position, i.e., upside-down. Indeed, when placed upside-down in red light, *lz-2* hypocotyls grew straight toward the gravity vector (data not shown). We also speculated that the straight upside-down *lz-2* hypocotyls would elongate less than rightside-up *lz-2* hypocotyls where curvature is induced.

In Table II.1, stem elongation is compared between *lz-2* and wild-type seedlings positioned rightside-up in either the dark (a) or red light (b), and between red light-treated *lz-2* seedlings positioned either rightside-up or upside-down (c). In the dark, rightside-up *lz-2* and wild-type plants, both of which grow straight upward, increased the same amount (ratio 1.0). In red light, rightside-up *lz-2* seedlings exhibited a 1.3 fold increase in stem

**Figure II.2.** Stem elongation in the elongation zone of wild-type and *lz-2* hypocotyls. 3.5d-old dark-grown wild-type and *lz-2* seedlings were incubated in the dark or red light for 6 hours. A resin bead coated with lanolin was applied to the surface of hypocotyl 3 mm below the hook at time zero. Seedlings were photocopied at time zero and at the end of incubation. The distance from the bead to the basal end of the hook was measured from the photocopies (A). Red light-treated *lz-2* hypocotyls were measured on both sides of the downward-curving stem (e.g., the concave and convex side). The percentage increase in stem length over 6 hours is presented (B). Each bar represents average of at least 3 experiments ± S.E (n=3). 10-15 seedlings were used in each experiment.

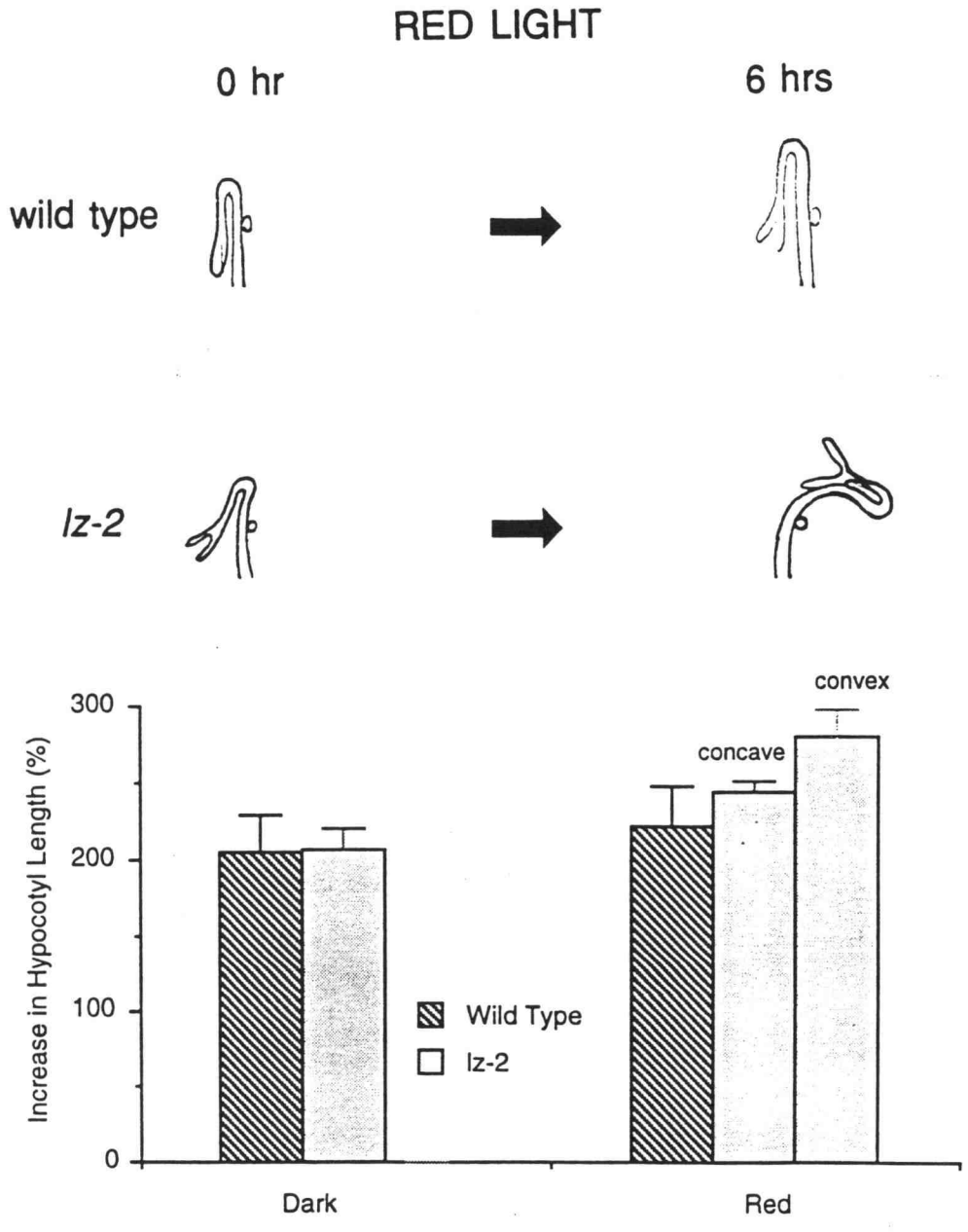
RED LIGHT
0 hr
6 hrs
wild type
lz-2
Increase in Hypocotyl Length (%)
300
200
100
0
convex
concave
Wild Type
lz-2
Dark
Red

**Figure II.2**

**Table II.1.** Ratios of elongation between downward-bending vs straight-growing hypocotyls. 3.5-d-old dark-grown wildtype (wt) and *lz-2* seedlings were incubated in the dark or red light for 10 hours. In red light, *lz-2* plants were held either rightside-up (rsu) or upside-down (usd) for comparison of elongation rates between downward bending and straight growth of hypocotyls, respectively. In red light, wild-type seedlings were kept rightside-up for comparison to the downward bending in rightside-up *lz-2* hypocotyls. In the dark, both *lz-2* and wild type seedlings were kept rightside-up so that their straight growth could be as a control. One bead was placed directly below the hook at the beginning of incubation and after 10 hours, the plants were photocopied to measure the distance from the bead to the hook. Ratios between treatment were determined for individual experiments and then averaged.

| Light Regime | Compared genotypes | Position[a] | Growth Pattern | Elongation Ratio[b] |
|---|---|---|---|---|
| dark | *lz-2*/ wt | rsu/ rsu | straight/ straight | 1.0 ± 0.07 |
| red | *lz-2*/ wt | rsu/ rsu | downward/ straight | 1.3 ± 0.13 |
| red | *lz-2*/*lz-2* | rsu/usd | downward/ straight | 1.3 ± 0.16 |

[a]rsu: rightside-up, usd: upside-down

[b]means of three experiments ± standard error (more than 20 seedlings were used in each experiment)

elongation as compared with wild-type hypocotyls during the transition from the straight growth to downward bending. In red light, when curving rightside-up *lz-2* seedlings were compared with upside-down *lz-2* plants which grow straight toward the ground, a ratio of 1.3 was also observed.

*The shoot apex is necessary for gravicurvature in lz-2 plants*

Analysis of migration of the marker bead distribution revealed that the zone of curvature of *lz-2* hypocotyls in red light begins directly below the hook and remains there as cells which were previously part of the hook become part of the elongation zone (Figure II.1). This indicated a role of the apical region of etiolated *lz-2* seedlings in the red light-induced downward growth. In order to establish which tissues are necessary for gravitropic curvature, wild-type and *lz-2* hypocotyls were excised at the basal end of the hook (above the elongation zone) and placed horizontally for gravi-stimulation as in Figure II.3A. After 10 hours in red light, intact wild-type hypocotyls reoriented upward with respect to the gravity vector and intact *lz-2* hypocotyls curved downward. Decapitation of wild-type hypocotyls significantly reduced gravitropic curvature but upward bending clearly still occurred. In comparison, decapitation completely eliminated gravitropic bending in *lz-2* hypocotyls.

*lz-2 hypocotyls respond more slowly to gravity in both darkness and red light*

Analysis of the kinetics of gravitropic bending revealed (Figure II.3B) that wild-type hypocotyls responded rapidly to gravity both in the dark and in red light. Within one hour the wild-type seedlings had reached approximately half of the full upward curvature in either the dark or red light. Decapitated wild-type hypocotyls exhibited similar kinetics, although they never reached as great a final curvature as observed for intact hypocotyls. The gravitropic response in *lz-2* plants was more complicated. Like wild-type seedlings, intact *lz-2* hypocotyls bent upward in the dark, but the response was much slower than for

**Figure II.3.** The gravitropic response of intact or decapitated hypocotyls of wild-type and *lz-2* seedlings. 3.5d-old dark-grown wild-type and *lz-2* seedlings with hypocotyl length of 10 to 15 mm were selected under dim green light. Half of the seedlings were decapitated directly below the hook. Both intact (IT) and decapitated (DC) seedlings were placed horizontally to provide gravistimulation and incubated for 10 h in the dark or red light. Photocopies were taken at each time point for subsequent measurement of gravitropic curvature. Typical gravitropic responses in red light (A) and kinetics of gravitropic curvature in either darkness (B, left panel) or red light (B, right panel) are shown. In B, each data point represents the mean of 3 experiment ± S.E.(10 to 15 seedlings/experiment).

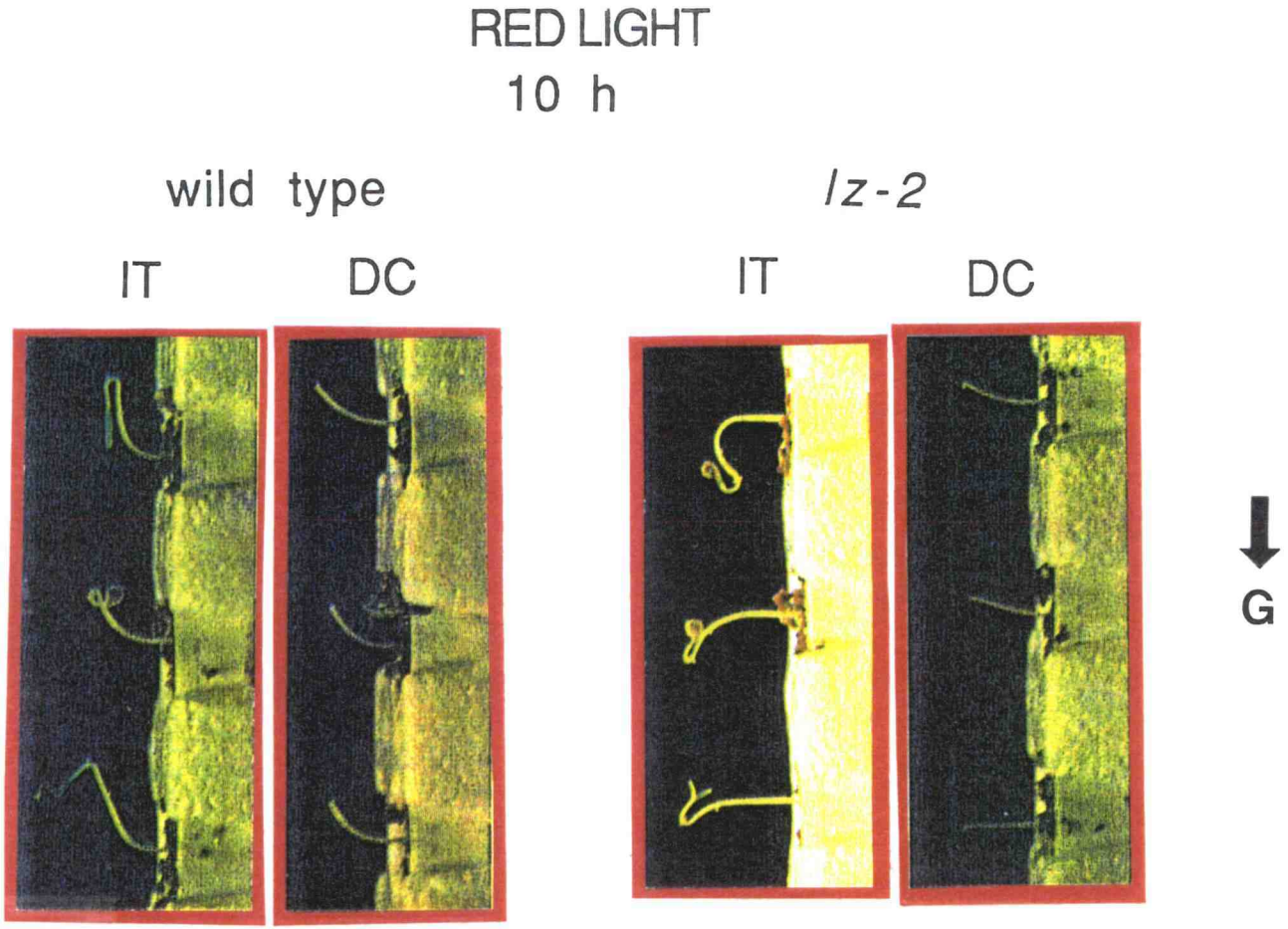


**Figure II.3A.**

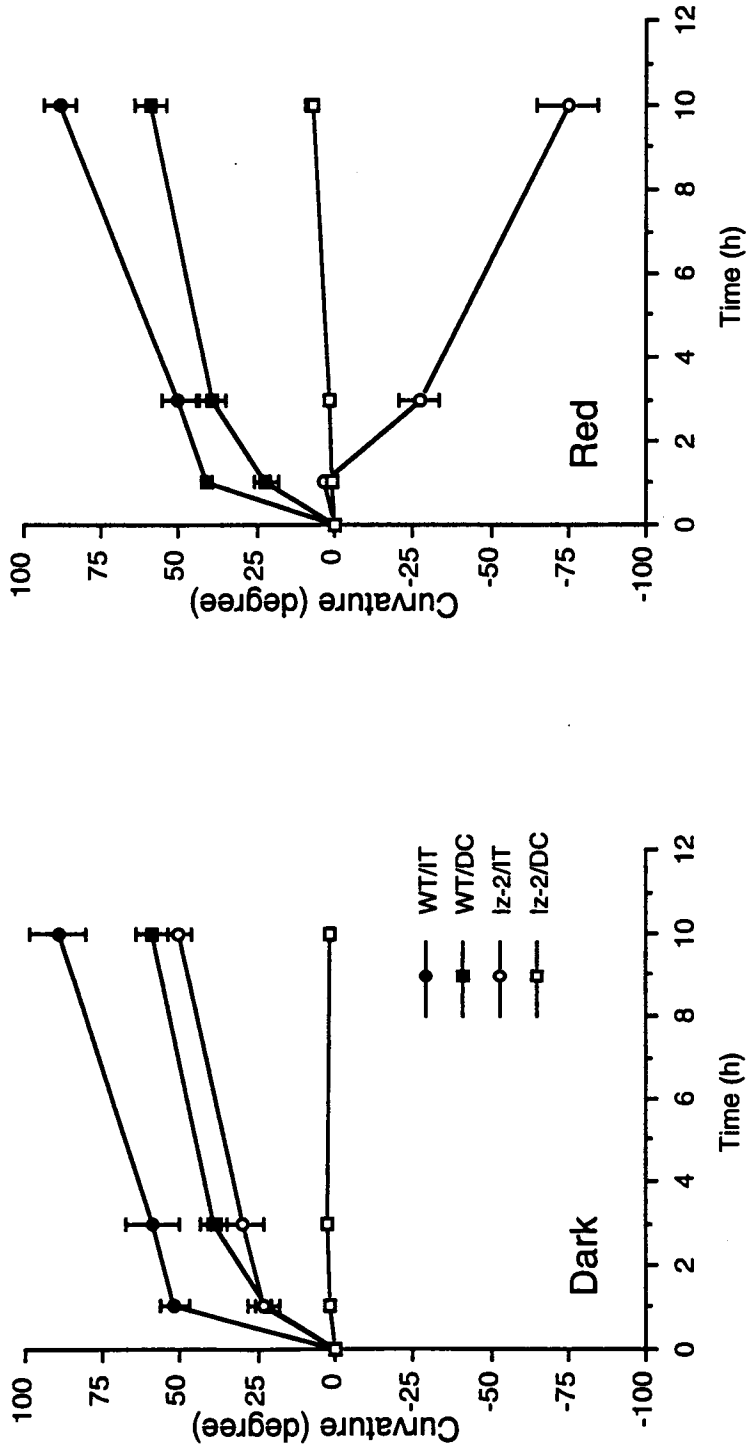
Curvature (degree)
100
75
50
25
0
-25
-50
-75
-100
0
2
4
6
8
10
12
Time (h)
Red
Dark
WT/IT
WT/DC
lz-2/IT
lz-2/DC

**Figure II.3B.**

wild-type, and the curvature achieved by 10 h was significantly less. In fact, the kinetics of upward bending for intact *lz-2* hypocotyls resembled that of decapitated wild-type hypocotyls. Downward curvature in red light by intact *lz-2* hypocotyls was even slower. Distinct downward curvature was not detected until 3 hours of gravistimulation. Again, decapitated *lz-2* hypocotyls failed to curve in either the dark or red light (Figure II.3B).

## Discussion

*Downward bending occurs in the elongation zone*

Gaiser and Lomax (1993) found no difference in stem elongation between wild-type and *lz-2* hypocotyls. However, they used plants which had been illuminated for 2 days with red light and they measured the entire length of hypocotyls to compare stem elongation. By this stage, *lz-2* plants have completed reorientation, with the downward curvature apparent throughout the length of the hypocotyl. It thus appeared more critical to characterize the initial phase of downward curvature as to when and where curvature is first induced by red light. Using marker beads, we were able to observe that the downward curvature begins directly below the hook about 3 hours after red light- illumination and it progresses further away from the hook as the hypocotyl elongates (Figure II.1). Cells which were situated within the hook at the time of red light illumination were in the elongation zone and participated in curvature of *lz-2* hypocotyls by the time that response occurred (Figure II.1, 6 hours). Silk and Erickson (1978) showed that cells move along the hook, elongating more on the outer side and less on the inner side to maintain hook shape, and as they arrive below the hook and reach the elongation zone, the hypocotyl grows taller (see also Salisbury and Ross, 1992). In *lz-2* , it appears that the elongation zone is the site of downward curvature. Therefore, I examined stem elongation in this particular region more closely during the early phases of downward curvature. The convex side of downward-curving *lz-2* hypocotyls exhibited a marked increase in length as

compared with the concave side (Figure II.2A), which increased at a level similar to that of straight-growing hypocotyls, e.g., wild-type hypocotyls in the dark and red light, and *lz-2* seedlings in the dark (Figure II.2B). Therefore, stimulation of stem elongation, not inhibition, is the mechanism behind downward curvature in *lz-2* hypocotyls exposed to red light. Our results, thus, do not agree with the suggestion that tropisms are the result of asymmetrical redistribution of a growth inhibitor (Bruinsma and Hasegawa, 1990).

*The direction of stem growth is actively changed by stimulated elongation*

Shoots of *lz-2* seedlings can recognize the gravity signal (Roberts, 1987; Gaiser and Lomax, 1992) and *lz-2* seedlings actively reorient to gravity by changing the direction of stem growth (Gaiser and Lomax, 1992). This was confirmed when we placed *lz-2* plants upside-down in red light as their hypocotyls grew straight toward the ground (data not shown). If the altered direction of growth in red light in upright *lz-2* plants is due to increased stem elongation, the upside-down *lz-2* hypocotyls should elongate less than the rightside-up *lz-2*, i.e. with an elongation rate similar to wild-type plants in the upright position. Indeed, upside-down *lz-2* hypocotyls grew at the same rate as wild-type plants in the upright position, while upright *lz-2* plants showed a 1.3-fold higher elongation rate (Table II.1). Thus, when oriented toward the gravity vector, *lz-2* hypocotyls behave like wild-type plants, i.e., growth is straight with a similar magnitude of stem elongation.

*The downward gravitropic response in lz-2 is not a simple reversal of the normal wild-type response*

The apical meristem of dicot plants is not only the region of cell division, but also the site of synthesis of the plant hormone, auxin. The necessity of the shoot apex for tropic responses prompted Darwin (1880) to propose that when a stimulus (e.g. light or gravity) is perceived at the apex, a message is transmitted asymmetrically toward the base of the stem. However, tropic curvatures have been demonstrated not only in the absence of the shoot apex (Iwami, 1974; Firn et al, 1981), but with isolated stem segments (Hart and

MacDonald, 1984). Hart and MacDonald (1984) pointed out, however, that while tropic responses can occur without the shoot apex, a certain amount of apical tissue is necessary for tropic curvature to be completed.

MacDonald and Hart (1987) modified the Cholodny-Went theory to explain the gravitropic response mechanism in the hypocotyls of dicot plants. They proposed that differential growth resulting in gravitropic curvature occurs as two distinct phases which are separable in time and location. The primary response occurs within minutes of gravity stimulus and both growth inhibition and stimulation on the upper and lower surface of horizontally-oriented hypocotyls, respectively, contribute to the formation of gravitropic curvature. The secondary response takes place one or two hours later and involves the basipetal resumption of stem growth which is hormone-mediated.

During the course of stem elongation measurements, we noticed that the apical part of *lz-2* hypocotyls is more important for the gravitropic response than is the wild-type apex. Downward curvature did not occur at once over the entire body of the *lz-2* hypocotyl (Figure II.1). The bending initiated immediately below the hook in the elongation zone and developed further as the hypocotyl increased in length; that is, rather than migration of the curvature from the elongation zone to the basal part of the hypocotyl, further bending occurred as more cells of the *lz-2* migrated from the hook to the elongation zone. Therefore, the *lz-2* downward gravitropism in red light requires growth of the hypocotyl to continue to bend.

The experiments in which the shoot apices were excised provided further support for the observation that the shoot apex is important for gravitropic bending in *lz-2*. Decapitation completely eliminated gravitropic curvature in *lz-2* hypocotyls regardless of the light condition (Figure II.3B), whereas relatively normal gravitropic bending was detected in decapitated wild-type hypocotyls. This result provided for the first time an indication that the hypocotyl gravitropic response is altered by the *lz-2* mutation not only in red light but also in the dark. The kinetics of gravitropic curvature in the dark further

indicate that the gravitropic response of *lz-2* hypocotyls is different from that of wild type. This implies that the downward gravitropic curvature in red light is not a simple reversal of the upward curvature of wild type (Figure II.3B).

MacDonald and Hart's two-phase model can be applied to explain the lack of gravitropic curvature in decapitated hypocotyls as well as the altered gravitropic response in intact *lz-2* hypocotyls. First of all, *lz-2* plants could have completely lost the first phase of the response which occurs in the absence of the shoot apex. This would explain the fact that *lz-2* hypocotyls are unable to respond to gravity in the absence of shoot apex both in the dark and red light. The second phase of the gravitropic response which requires the shoot apex could be under red light control. In the dark, normal gravitropic curvature would appear in the intact *lz-2* hypocotyls due to the second phase which requires stem elongation, but it is slow (or reduced) because the first phase did not occur. However, in red light, the second phase could somehow be modified such that the direction of stem growth is reversed by the *lz-2* mutation. Without the first response, downward growth in red light would appear as slow as the upward growth in the dark. Kinetic studies showed that intact *lz-2* hypocotyls either remained horizontal or sometimes curved slightly upward during the early response to gravity (Figure II.3B).

*Auxin as a candidate to link red light and gravity*

Stem elongation can be regulated by phytochrome. When dark-grown plants are exposed to light, reduction of stem elongation occurs as a part of the photomorphogenesis which is mediated by phytochrome (Kronenberg and Kendrick, 1994). During phytochrome inhibition of stem elongation, decreased levels of both auxin and gibberellins are found (Behringer et al., 1990; Behringer and Davies, 1992; Jones et al, 1991). Recent evidence indicates that phytochrome and gibberellins utilize separate mechanisms to inhibit stem elongation response, although they possibly interact at the response level (López-Juez et al., 1995). However, phytochrome does seem to act via changing auxin levels to alter

stem elongation (Behringer and Davis, 1992;Jones et al, 1991). Jones and Prasad (1992) have suggested that phytochrome modifies auxin transport in order to regulate auxin levels in those plant tissues where cell elongation takes place.

The experiments in this study were originally designed to provide evidence as to which, if any, plant growth hormone is involved in the phytochrome-mediated reversal of the gravitropic response in *lz-2* the mutant. Our findings that elongation is stimulated and that the shoot apex plays a critical role strongly suggests the involvement of auxin and excludes the possibility of an inhibitory compound. If auxin is the point of interaction between phytochrome and gravity, it is possible that the reversed gravitropic curvature is induced by red light altering the lateral redistribution of auxin. Therefore, studying auxin movement in response to gravistimulation is important to our understanding of the mechanism of the interaction between red light and gravity. Furthermore, analysis of the mode of auxin transport in *lz-2* hypocotyls should provide significant evidence as to the ability of the C-W theory to explain the gravitropic response mechanism in dicot stems. These studies are described in the following chapter.

# III. THE REVERSED GRAVITROPIC RESPONSE OF THE TOMATO MUTANT, *LAZY-2*, CORRELATES WITH REVERSED AUXIN REDISTRIBUTION

## Abstract

Shoots of the gravitropic mutant of tomato, *lazy-2* (*lz-2*), exhibit a reversed growth response to gravity in the presence of light, in a phytochrome-mediated manner (Gaiser and Lomax, 1993). A role for the plant hormone auxin in the induction of downward growth in *lz-2* shoots is indicated by evidence that; a) stem elongation is stimulated and b) excision of the shoot apex eliminates the *lz-2* gravitropic response. To investigate auxin transport in the *lz-2* mutant, we developed a double-labeling technique which utilizes tracer amounts of $^{3}$H-indole-acetic acid ($^{3}$H-IAA) and $^{14}$C-benzoic acid ($^{14}$C-BA). Benzoic acid is a weak acid similar in size and pKa to IAA, but inactive as an auxin and is used as a control for non-auxin-specific transport. Using this technique, we have demonstrated specific polar transport of $^{3}$H-IAA in the hypocotyls of intact, upright wild-type and *lz-2* plants. In response to gravity, $^{3}$H-IAA is redistributed laterally to the lower half of wild-type hypocotyls in the dark or red light. The *lz-2* hypocotyls in the dark exhibited the same pattern of IAA redistribution as seen for wild-type. However, in red light the IAA asymmetry in the *lz-2* hypocotyls was reversed, as was gravitropic bending, with similar kinetics. The correlation between auxin redistribution and gravitropic bending strongly confirms the Cholodny-Went theory and indicates that modulation of the gravitropic response by red light occurs at the level of auxin transport regulation.

## Introduction

The Cholodny-Went theory (C-W theory) is the classic and most widely accepted hypothesis proposed to explain tropic responses such as phototropism and gravitropism. It states that differential growth between two sides of an organ responding to light or gravity is caused by the movement of the growth-regulating hormone, auxin, from one side of the organ to the other (Went and Thimann, 1937). Although it was studies of plant tropic responses which led to the discovery of auxin, controversies and arguments have accumulated which challenge the C-W theory (see Trewavas, 1992, for forum). The C-W theory is probably not sufficient to explain all aspects of all tropic responses. For example, Moore and Evans (1986) have suggested that $Ca^{2+}$ is an important factor in root gravitropism, while an asymmetry in auxin sensitivity rather than distribution has been proposed to be responsible for differential elongation during shoot gravitropism (Trewavas, 1991; Salisbury, 1993). Additionally, asymmetric redistribution of an undefined growth inhibitor has been proposed as the mechanism for phototropism (Bruinsma and Hasegawa, 1990).

We have found that the phytochrome-mediated downward growth of shoots of the *lz-2* mutant of tomato is a result of increased stem elongation on the outer or convex side. In the dark, *lz-2* hypocotyls respond more sluggishly to gravity than wild-type plants, even though curvature is in the same direction. In red light, *lz-2* hypocotyls display a slight upward bending at the beginning of the gravitropic response and the subsequent downward curvature is much slower. More striking results were revealed from experiments in which the shoot apex was excised. While decapitated wild-type hypocotyls were able to respond to gravity in a relatively normal (although reduced) fashion, the decapitated *lz-2* hypocotyls did not bend in response to gravity. Complete elimination of gravitropic responses in decapitated hypocotyls of *lz-2* occurred both in the dark and red light. This indicates that

there is a dark phenotype for *lz-2* plants and that the shoot apex plays an important role in the altered gravitropic response of the mutant.

Auxin is synthesized at the shoot apex and transported to other parts of a plant via a specific polar transport stream which occurs primarily in the cells surrounding the vascular bundles, the starch sheath cells (see Lomax, et al. 1995 for review). It is known that the gravity signal is perceived by the sedimentation of amyloplasts located in the starch sheath cells and that this somehow leads to the lateral movement of auxin (Pickard, 1985 for review). The stimulation of stem elongation during downward curvature in red light and the complete elimination of curvature upon excision of the shoot apex, strongly indicate auxin involvement in the altered *lz-2* gravitropic response. If the C-W theory holds true, auxin redistribution should be reversed with kinetics that correspond to the red light-induced downward gravitropic curvature in *lz-2* hypocotyls.

In order to measure auxin redistribution in wild-type and *lz-2* hypocotyls, we developed a double-labeling technique which utilizes tracer amount of $^{14}$C-benzoic acid ($^{14}$C-BA) as well as $^{3}$H-indole-3-acetic acid ($^{3}$H-IAA; IAA is a natural form of auxin). Benzoic acid is a weak acid similar in size and pKa to IAA, but inactive as an auxin, so that it can be used as a control for non-auxin-specific diffusion through tissues (Lomax, et al. 1995). Using this technique, we were able to show specific polar IAA transport in the intact hypocotyls of upright wild-type and *lz-2* plants. In response to gravity, wild-type hypocotyls curved upward in either dark or red light and $^{3}$H-IAA was redistributed laterally to the lower half. The same was true for the *lz-2* seedlings which were incubated in the dark. In red light, however, the IAA asymmetry in the *lz-2* hypocotyls was found to be reversed in a manner similar to the reversed gravitropic bending. That is, more $^{3}$H-IAA was found in the upper half of downward-curving hypocotyls.

This correlation between the pattern of auxin redistribution and the pattern of gravitropic bending in both *lz-2* and wild-type hypocotyls provides strong confirmation of the Cholodny-Went theory. More importantly, this study reports the first evidence that, at

least in dicot hypocotyls, red light regulation of the gravitropic response occurs by modifying the direction of auxin transport.

## Materials and Methods

*Plant Material*

Seedlings of the *lz-2* mutant of tomato (*Lycopersicon esculentum* Mill.) and the near-isogenic wild type parent line (cv. Ailsa Craig) were used in all experiments. Seeds were soaked in 1% NaOCl (a 20% solution of commercial bleach) for 15-20 min and rinsed well with tap water for 30 min. 30 caps from 7 ml mini scintillation vials, each 10 mm in diameter were packed in a Petri-dish and then gently filled with vermiculite and moistened with distilled water. Wild-type and *lz-2* seeds were sown individually in each scintillation cap and incubated for 3.5 - 4 days in the dark at 28 °C. Etiolated seedlings were selected under a dim green safe light when their hypocotyls reached 10 to 15 mm long and any seed coats which remained were carefully removed before application of the radioactive compounds.

*Measurement of Auxin Transport*

$^{3}$H-IAA and $^{14}$C-BA were purchased from Amersham and American Radiolabeled Chemicals Inc., respectively. 10 µl of each $^{3}$H-IAA (23 Ci/mmol) and $^{14}$C-BA (53 mCi/mmol) were diluted in 130 µl ethanol and 1 µl of this mixture was used for labeling intact tomato seedlings. The final concentration of $^{3}$H-IAA and $^{14}$C-BA delivered in the 1 µl drop were 3.1 µM and 7.2 mM respectively.

As diagrammed in Figure III.1, 1 µl of the isotope mixture was applied between the two cotyledon leaves of etiolated intact tomato seedlings. To determine the amount of radioactivity which reached the elongation zone of hypocotyls, a 1 cm segment was excised directly below the hook. For the zero time point, hypocotyl segments were excised

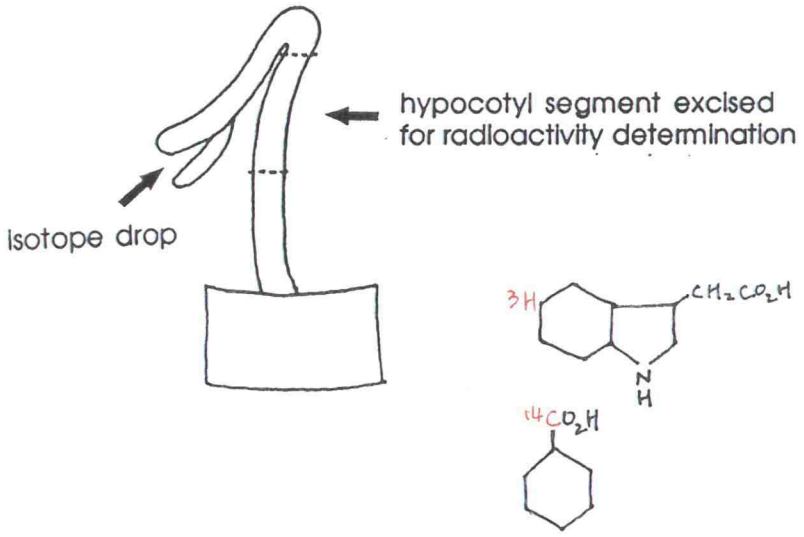


**Figure III.1.** Auxin transport and redistribution in intact tomato seedlings. 3.5d-old dark-grown seedlings 15 mm in length were selected and any remaining seed coats were carefully removed from the cotyledons. A 1µl drop of the $^{3}$H-IAA and $^{14}$C-BA mixture in 95% ethanol was applied between the cotyledons under a dim green safe light. The labeled seedlings were incubated either upright (for auxin transport) or horizontally (for auxin redistribution) before the 10 mm apical hypocotyl segments were excised directly below the hook and used for measurements of both $^{3}$H and $^{14}$C levels.

immediately after the isotopes were applied. The labeled plants were incubated in the dark at 28°C and the hypocotyl segments were excised at the given time intervals. To measure radioactivity, each hypocotyl segment was individually extracted in 4 ml liquid scintillation cocktail overnight and the entire extract was counted using a Beckman LS 6500.

*IAA Redistribution and Gravitropic Bending*

Seedlings were labeled with 1 µl of the $^{3}$H-IAA and $^{14}$C-BA mixture using the same method as described for the uptake experiments and incubated for 1 hour in the dark to allow the isotopes to be transported to the elongation zone. The labeled seedlings were anchored in a 10 hole-styrofoam rack which was then horizontally reoriented for gravistimulation. At the given time intervals following incubation in the dark or red light (Figure III.2), plants were photocopied as in (A) to measure gravitropic bending and then excised and dissected as in (B) for determination of isotope ratios between the upper and the lower half of the gravitropically-bending hypocotyl segments

*Calculation of IAA redistribution*

Radioactivity in the upper and the lower half segment of the gravitropically-bending hypocotyls were measured for both $^{3}$H-IAA and $^{14}$C-BA using separate channels of the scintillation counter. Table III.1 gives two examples of the calculations used to determine the distribution of auxin between the upper and lower halves. The % radioactivity which was detected in the lower half of the hypocotyl segment was first calculated for $^{3}$H-IAA and $^{14}$C-BA separately. The % of $^{3}$H-IAA in the lower half was then compared to that of $^{14}$C-BA. The IAA asymmetry was determined by subtracting 1 from the relative content of $^{3}$H-IAA to $^{14}$C-BA in the lower half, and then multiplied by 100 to give % redistribution. For example, +15% indicates that 15% more IAA was found in the lower half of upward bending hypocotyls and -14% means 14% more IAA distributed to the upper half of downward bending *lz-2* hypocotyls in red light.

**Figure III.2.** Measurement of gravitropic curvature and auxin redistribution. Wild-type and *lz-2* seeds were sown individually in single scintillation vial caps and grown for 3.5d in the dark. Seedlings with 15mm-long hypocotyls were labeled with the $^{3}$H-IAA and $^{14}$C-BA mixture as described for Figure III.1 and incubated upright for 1 hour in the dark. The scintillation vial caps were reoriented horizontally in a styrofoam rack and incubated in the dark or red light. At the given time intervals, the seedlings were photocopied for later determination of gravitropic curvature (A) and the elongation zone below the hook was dissected as diagrammed in the figure to determine auxin redistribution (B).

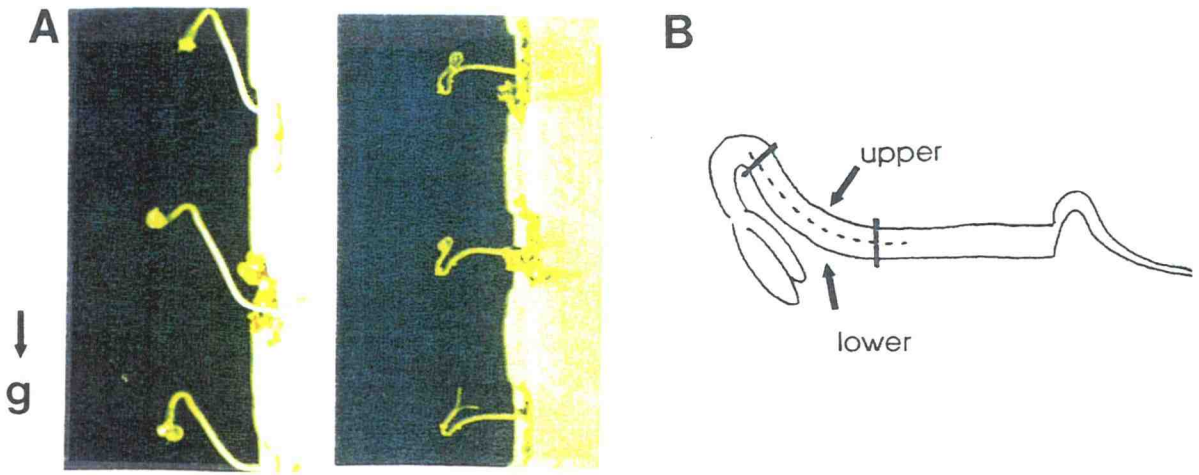


**Figure III.2**

**Table III.1.** Calculation of auxin redistribution. Two examples are given to demonstrate how auxin redistribution was determined for Figure II.6.

| Curvature | | Upward | | Downward | |
|---|---|---|---|---|---|
| Isotopes | | $^{3}$H-IAA | $^{14}$C-BA | $^{3}$H-IAA | $^{14}$C-BA |
| Radioactivity (CPM) | upper half | 518 | 380 | 534 | 368 |
| | lower half | 692 | 357 | 412 | 382 |
| lower/total (%) | | 55 | 48 | 44 | 51 |
| IAA/BA | | 55 / 48 = 1.15 | | 44 / 51 = 0.86 | |
| IAA asymmetry (%) | | (1.15 - 1) x 100 = 15 | | (0.86 - 1) x 100 = -14 | |

*Light Conditions*

Red light was provided by two overhead fluorescent tubes (40 W Shoplight, General Electric) filtered through red acrylic (Shinkolite 102, Argo Plastics Co., Los Angeles, CA). Fluence rates used for all experiments were in the range of 20 to 40 μmol $m^{-2}s^{-1}$. The dim safe light used for handling seedlings in the dark had a fluence rate of 0.3 μmol $m^{-2}s^{-1}$.

## Results and Discussion

*Uptake of radioactive compounds into intact tomato hypocotyls*

In etiolated tomato seedlings, there is significant growth of the hypocotyl above the hook. In order to examine auxin transport under conditions as natural as possible, whole intact seedlings were used for isotope labeling. One μl of the $^{3}$H-IAA and $^{14}$C-BA mixture in ethanol was applied directly between the two cotyledon leaves of upright seedlings (Figure III.1). The isotope drop remained between the cotyledons for about one half minute before it was absorbed. If the radioactive compounds were taken up into the plant via the apical meristem between the cotyledons and transported to the hypocotyl, we would expect radioactivity to move into the ca. 1 cm-long hypocotyl section directly below the hook where cells elongate most actively (the elongation zone). This is the region where the IAA redistribution leading to gravitropic bending is known to occur (Pickard, 1985, for review). After labeling, the 1 cm hypocotyl segment below the hook was excised and the level of isotopes was measured at time intervals up to 6 hours (Figure III.3). At time zero (i.e. hypocotyl segments were excised immediately after the isotope was applied to the cotyledons), no radioactivity was detected for either $^{3}$H-IAA or $^{14}$C-BA. At 30 min after the labeling, approximately 0.4% of the applied $^{3}$H-IAA and 0.3% of the total $^{14}$C-BA applied were detected in the elongation zone of both wild type and *lz-2* hypocotyls. At 1 hour $^{3}$H-IAA uptake increased approximately two-fold as compared with the levels at 30

**Figure III.3.** Transport of $^{3}$H-IAA and $^{14}$C-BA into wild-type (A) and *lz-2* hypocotyls (B). 3.5d-old dark-grown wild-type and *lz-2* seedlings were labeled with 1µl of $^{3}$H-IAA and $^{14}$C-BA mixture as described for Figure III.1 and incubated in the dark at 28°C. At given time intervals, 10 mm hypocotyl segments were excised directly below the hook and extracted overnight in 4 ml LSC. The radioactivity presented in the figure is the % of the total radioactivity applied to the cotyledons which was subsequently retrieved from the hypocotyl segment. Each point represents the mean of 3 experiments (10-15 seedlings/experiment) with ±S.E..

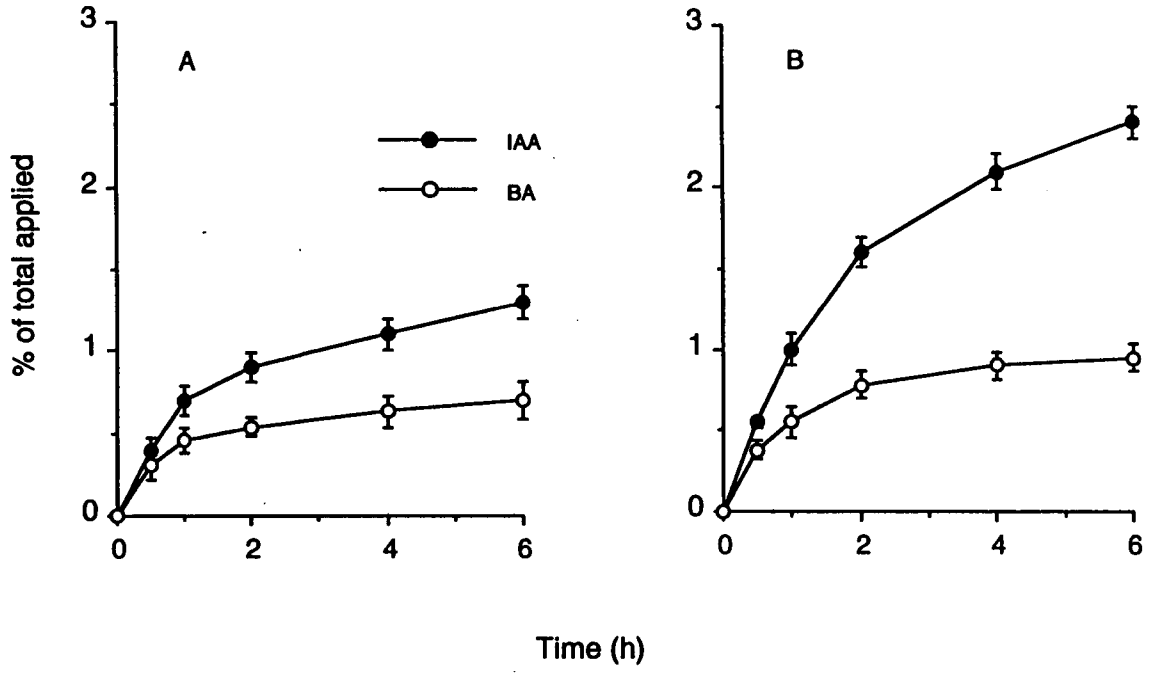


**Figure III.3**

min. $^{14}$C-BA uptake was also increased but at approximately half the level seen for $^{3}$H-IAA. The amount of $^{3}$H-IAA uptake began to fall after 2 hours , then remained constant thereafter (4 and 6 hours for wildtype in Figure III.3A). In *lz-2* seedlings (Figure III.3B), the uptake pattern for both isotopes was similar to that observed in wild-type plants. However, the magnitude of $^{3}$H-IAA uptake was almost twice high in *lz-2* throughout the uptake time course. The level of $^{14}$C-BA uptake found in *lz-2* hypocotyls was also higher than in wild-type, although the magnitude of the difference was not so great. The maximal levels of the two compounds in these tissues was below 7.8 nM (2.5% of 3.1 μM) for $^{3}$H-IAA and 72 μM (1% of 7.2 mM) for $^{14}$C-BA. The levels reaching the region of gravicurvature were, therefore, well below physiological levels of IAA or where weak acids should have any effect and thus served only a tracer function.

*Inhibition of IAA uptake by TIBA*

The fact that $^{14}$C-BA reached half-maximal accumulation in the 1 cm hypocotyl sections at about the same time as seen for half maximal $^{3}$H-IAA and while only half as much $^{14}$C-BA as $^{3}$H-IAA was recovered from the hypocotyl segments confirmed previous observations that there is more than one transport pathway in dicot hypocotyls; one which is non-specific one, i.e., both BA and IAA move together, and one which is specific for IAA, i.e., the polar auxin transport system in the starch parenchyma cells which actively transports IAA at a constant rate of 1-2 cm/hr. This led us to hypothesize that the difference in uptake levels between $^{3}$H-IAA and $^{14}$C-BA observed here might reflect the amount of $^{3}$H-IAA transported via the specific polar transport stream.

TIBA (2, 3, 5 - triiodobenzoic acid) is a specific inhibitor of polar auxin transport (Jacob and Rubery, 1988). To test whether the tracer amounts of $^{3}$H-IAA or $^{14}$C-BA applied to the cotyledons are at least partially transported to the hypocotyls via the polar auxin transport system, 1 μl TIBA ($10^{-3}$M) was applied between the cotyledons exactly as for the isotope application. The TIBA-treated plants were incubated for 1 hour in the dark

before application of the drop of $^{3}$H-IAA and $^{14}$C-BA mixture. The effect of TIBA on the transport of $^{3}$H-IAA and $^{14}$C-BA in wild-type and *lz-2* hypocotyls is shown in Figure III.4. Interestingly both in wild-type and *lz-2* plants, TIBA affected only $^{3}$H-IAA transport, not $^{14}$C-BA transport. The level of $^{3}$H-IAA uptake decreased to the same level as $^{14}$C-BA upon application of TIBA prior to the isotope application. It thus appears that the amount of $^{3}$H-IAA uptake inhibited by TIBA (approximately 40%) represents specific polar auxin transport and the uptake of $^{3}$H-IAA and $^{14}$C-BA which is unaffected by TIBA occurs via non-specific transport. This validates the use of this method to analyze the native auxin transport system. In addition, the levels of both $^{14}$C-BA and $^{3}$H-IAA detected in each hypocotyl segment were sufficient for both radioactively-labeled compounds to be used to analyze specific IAA redistribution. This technique was thus used to monitor the specific auxin transport involved in IAA redistribution during the gravitropic response mechanism.

*IAA redistribution and Gravitropic bending*

To measure the lateral redistribution of auxin, $^{3}$H-IAA and $^{14}$C-BA were loaded and allowed to transport as in the uptake experiments. Plants were reoriented horizontally 1 hour after isotope application and incubated in the dark or red light. At the given time intervals, the plants were photocopied to document gravitropic curvature and dissected to determine radioactivity levels in the upper and lower halves of the gravitropically-bending hypocotyls as diagrammed in Figure III.2.

The kinetics of gravitropic bending and IAA redistribution are compared in Figure III.5. Positive curvature represents upward bending and negative values represent downward bending. In the course of a 6 hour-gravitropic response, wild-type plants grew upward slowly in red light than in the dark. *lz-2* hypocotyls in the dark responded to gravity more slowly than wild-type did. The kinetics of upward gravitropic bending in the *lz-2* hypocotyls in the dark were similar to those of wild-type hypocotyls in red light. In

**Figure III.4.** Inhibition of $^{3}$H-IAA and $^{14}$C-BA transport by TIBA. 3.5d-old dark-grown wild-type (A) and *lz-2* (B) seedlings were fed with 1 µl of $10^{-3}$M TIBA applied between the cotyledons and incubated in the dark for 1 hour. 1 µl of $^{3}$H-IAA and $^{14}$C-BA mixture was then applied to the cotyledons and the 10 mm hypocotyl segment was excised after incubating the labeled seedlings for various time points as in Figure III.3. Triplicate experiments with 10 to 15 seedlings were used for each time point. Standard error is indicated for each time point (n=3).

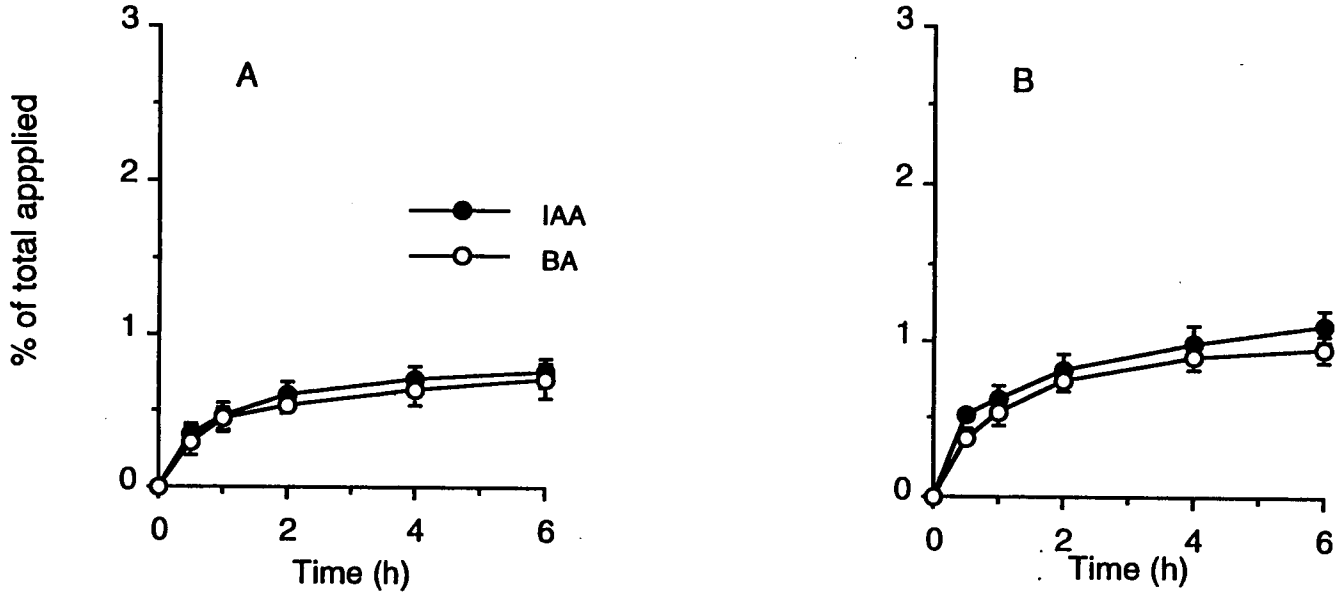


**Figure III.4**

**Figure III.5.** Kinetics of gravitropic curvature and auxin distribution in wild-type and *lz-2* hypocotyls. 3.5-d-old dark-grown wildtype and *lz-2* seedlings were labeled with 1 µl of the $^{3}$H-IAA and $^{14}$C-BA mixture and incubated upright for 1 hour in the dark. The labeled seedlings were horizontally placed in the dark (left panels, A and C) or red light (right panels, B and D). At given time intervals, seedlings were photocopied to measure curvature (upper panels, A and B) and the hypocotyl segments was bisected longitudinally to measure auxin redistribution (lower panels, C and D). The upper and the lower halves of the hypocotyl segment was extracted separately in LSC overnight to determine radioactivity. IAA redistribution was calculated as explained in *Material and Methods*. Each time point represents the mean of three experiments (> 10 seedlings/experiment) ±S.E.

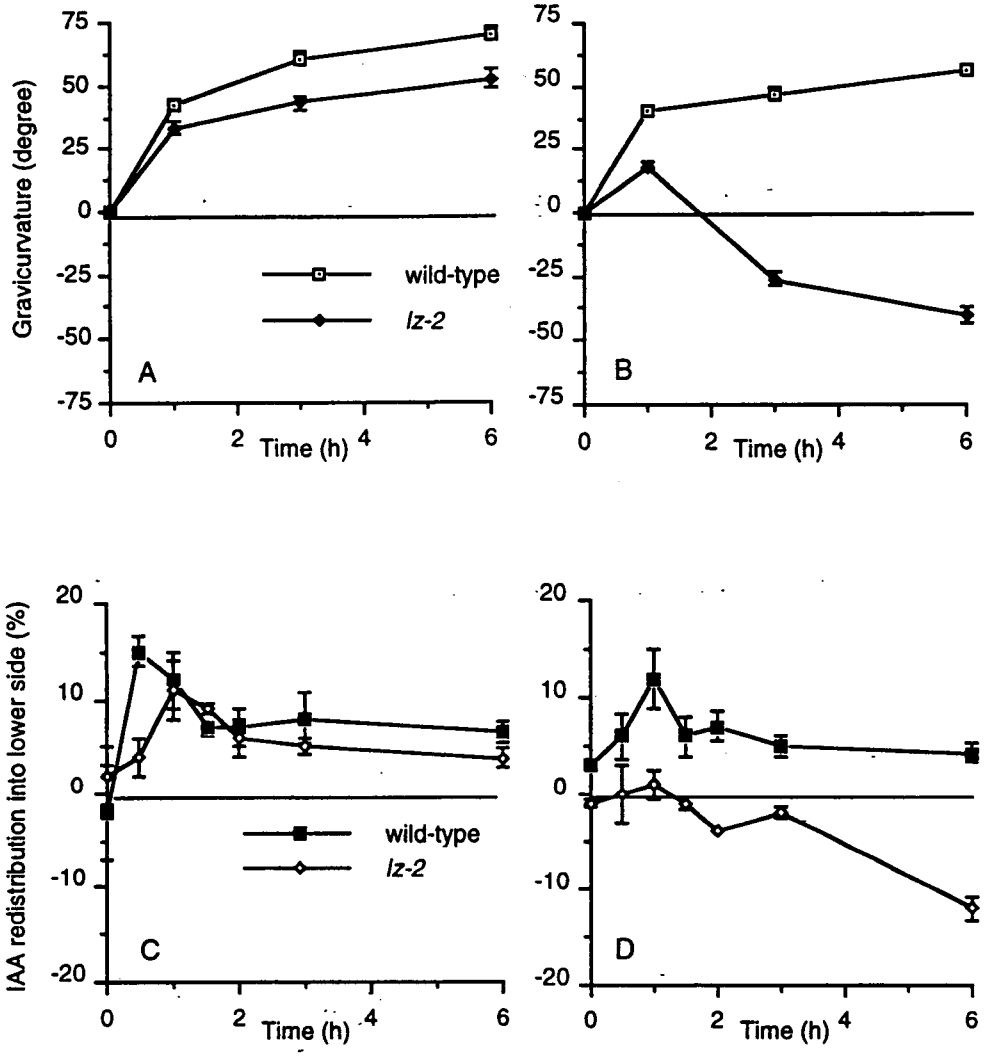
Gravicurvature (degree)
75
50
25
0
-25
-50
-75
wild-type
lz-2
A
B
0
2
4
6
Time (h)
IAA redistribution into lower side (%)
20
10
0
-10
-20
C
D

**Figure III.5**

red light-incubated *lz-2* seedlings, a slight upward bending was observed in the first hour, while downward bending first appeared one hour or more later which confirms the observation in Chapter II.

The IAA redistribution values presented in Figure III.5 are calculated by normalizing $^{3}$H-IAA distribution due to any inaccurate longitudinal dissection (examples given in Table III.1) by comparison with the distribution of $^{14}$C-BA. Distribution of $^{14}$C-BA between the upper and the lower half of the hypocotyls was found to consistently be symmetrical during all gravitropic responses ($50 \pm 0.9\%$ distribution into the lower half, N=200). To better facilitate comparison of IAA redistribution with gravitropic bending, the percentage of $^{3}$H-IAA distributed to the lower half of hypocotyls is used; positive numbers indicate that IAA redistribution is greater in the lower half while negative numbers indicate net IAA redistribution to the upper half of the gravitropically-bending hypocotyls.

IAA redistributes to the lower side of hypocotyls which grow upward in response to gravity, i.e., wild-type hypocotyls in the dark or red light, and *lz-2* hypocotyls in the dark, while IAA redistributes to the upper half of *lz-2* hypocotyls in red light. Thus, the direction of IAA redistribution correlates with the direction of gravitropic bending in both downward growth and upward growth, as predicted by the Cholodny-Went theory. Maximal redistribution of IAA occurred within 1 hour of gravistimulation for all upward-bending hypocotyls (i.e., when IAA is redistributed to the lower side). The peak redistribution of IAA, ca. 15% to the lower side of wild-type hypocotyls, was observed after 30 min of gravistimulation in the dark, whereas a peak redistribution of similar magnitude appeared after 1 hour of gravistimulation of wild-type hypocotyls in red light. In *lz-2* seedlings in the dark, maximal IAA redistribution was also observed after 1 hour gravistimulation at levels similar to those observed for wild-type seedlings in red light. This correlates with the similar patterns of gravitropic bending observed for wild-type seedlings in red light and *lz-2* seedlings in the dark.

A significant level of IAA redistribution could not be reliably detected in red light-treated *lz-2* seedlings until 2 hours after gravistimulation. By 6 hour, however, IAA redistribution was -12 %, a level which is similar to those measured in upward-bending hypocotyls. As was observed in the case of upward bending, IAA redistribution returned to an equilibrium level (data not shown). At 1 hour, although it is not statistically significant, higher IAA was detected in the lower half of the red light-treated *lz-2* hypocotyls, which correlates with slight upward bending observed in the gravitropic curvature.

The time course used in this study was not sufficient to determine whether IAA redistribution occurs early enough to induce gravitropic bending (Harrison and Pickard, 1989). It will be necessary to investigate patterns of both IAA redistribution and gravitropic curvature at earlier times and with shorter intervals in future studies. Nevertheless, the overall pattern of IAA redistribution including the strikingly similar kinetics between auxin redistribution and curvature provides evidence that a) the movement of auxin is involved in gravitropic bending and b) the direction of auxin movement determines the direction of gravitropic bending in wild-type and *lz-2* hypocotyls. These results strongly support the C-W theory. The fact that both IAA redistribution and the direction of gravitropic bending are reversed by red light treatment in the *lz-2* mutant is an even more striking result. This not only supports the C-W theory but also provides new evidence that red light participates in the gravitropic response mechanism at the level of auxin transport regulation.

# VI. CONCLUSION

## Phytochrome regulation of gravitropic response in plant stems

Although plant seeds are able to germinate in absolute darkness, the natural condition for germination and growth of the first root and shoot is often not completely dark. Some light is transmitted through soil and reaches plant seeds underground (Kronenberg and Kendrick, 1994). Plants have therefore evolved systems for the detection of light conditions necessary for germination of seeds and early growth of seedlings in soil. In relative darkness, i.e., in soil, seeds of dicot plants develop into a seedling of unique morphology (skotomorphogenesis). Growth of the hypocotyl is modulated such that the apical region is hook-shaped. When seedlings grow through the soil, the apical meristem is protected by the hook which protrudes from the soil first. Once exposed to a sufficient level of light, the hook opens. Hook opening along with other events of photomorphogenesis is regulated by the photoreceptor, phytochrome (Smith and Whitelam, 1980; MacDonald et al, 1982).

It has been suggested that formation of the hook is a response to gravity (Meyer et al., 1993; MacDonald et al , 1983). During germination, the radical (the first root), appears first from the dicot seed followed by emergence of the hypocotyl. Establishment of asymmetric elongation in the hypocotyl determines the direction of hook formation which ought to be toward the surface, i.e., away from the gravity vector. Therefore, a germinating dicot seed must be able to detect gravity and establish asymmetric hypocotyl growth with respect to the direction of gravity.

Formation of the hook in an etiolated dicot seedling requires a remarkable asymmetry in the cell elongation response between the cells in the outer- and inner-most layers of the hook tissue. Examination of 4 day-old etiolated tomato seedlings (Figure IV.1) shows a substantial difference in cell size between the cells in the outer- and the inner-most epidermal layers of the hook (Diagram IV.1, the purple zone). Cells in the

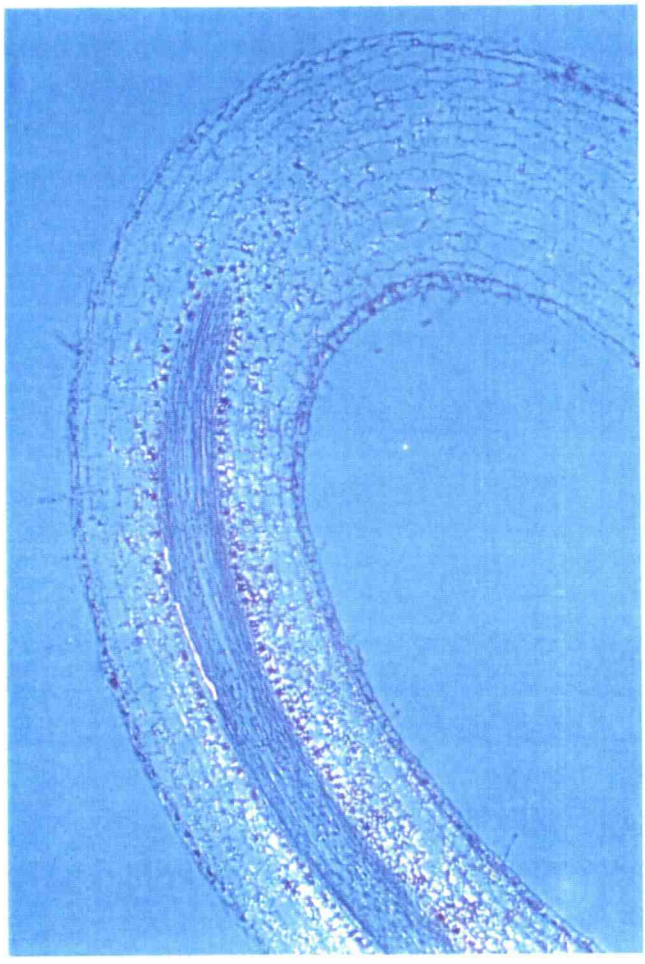

**Figure IV.1.** A median section of an etiolated tomato hypocotyl. The region of the hook was photographed to observed the morphological detail of the cells in the tissue.

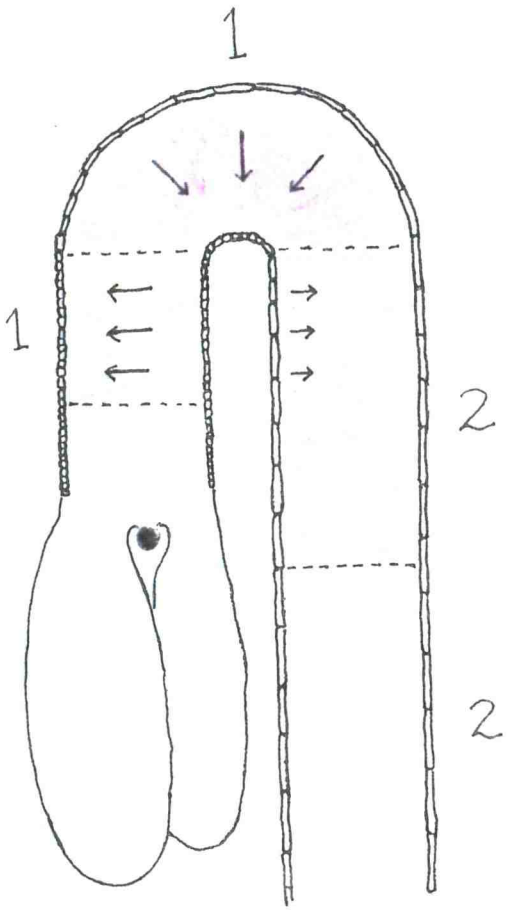


**Diagram VI.1**. Lateral movement of auxin as a mechanism of hook maintenance and hypocotyl elongation. Green zone 1and 2 is hypocotyl tissue above the hook where outward auxin movement occurs. Purple zone 1 is hypocotyl tissue in the hook where inward auxin movement occurs.

outer layer are longitudinally elongated and their size is rather similar to the cells lying below the hook, that is, in the elongation zone. Cells in the inner side of the hook are very compact and their morphology appears to be similar to cells in the apical region above the hook. In dicot seedlings, cell divisions occur in the meristem tissue at the tip of hypocotyl which is buried between the cotyledons (Diagram IV.1). Therefore, cells in the apical region which are closer to the meristem are smaller and less differentiated.

In longitudinal sections of tomato seedlings, the apical tissue between the meristem and the hook appears to be uniformly undifferentiated cells (Figure IV.1). Moving from the apex to the hook (from green zone1 to purple zone 1), cells in the outer epidermal layer become greatly elongated whereas the cell size in the innermost layer remain similar to those cells proximal to the apex. However, in the region between the hook and the elongation zone (from purple zone 1 to green zone 2), the compact and rather primitive cells in the inner side of the hook become increasingly elongated as they move into the elongation zone, where the size of the outer and the inner epidermal cells becomes similar. This implies that epidermal cells undergo two major cell enlargement events which are temporally and spatially separated. The first burst of cell enlargement occurs in the outer epidermal cells in the area between the apex and the hook (Diagram IV.1, between green zone 1 and purple zone1). The second phase of cell enlargement occurs in the inner epidermal cells between the hook and the elongation zone of the hypocotyl (Diagram IV.1, between purple zone 1 and green zone 2).

Exaggerated hook formation is a well-established phenomenon which has been successfully used to select plants which are mutant in ethylene responses (Guzman and Ecker, 1990). It has been proposed that ethylene arrests cell elongation activity in the inner side of the hook, which creates the growth asymmetry resulting in hook formation (Kang and Burg, 1972). Auxin is the plant hormone which has most often been linked with the promotion of cell elongation in young seedlings (Cleland, 1995, for review). Therefore, auxin is likely to be involved in the regulation of cell elongation which controls formation

of the hook in etiolated tomato seedlings and ethylene most likely acts by inhibiting the auxin-induced elongation.

Silk and Erickson (1978) demonstrated migration of cell markers through the hook as an etiolated lettuce seedling elongated (see also Salisbury and Ross, 1990). They suggested that the hook is not only formed by cell elongation in the elongation zone, but is also maintained by the flow of cells migrating through the hook and arriving in the elongation zone. Our anatomical examinations show that the inner epidermal cells between the hook and the elongation zone undergo a remarkable longitudinal expansion while cells in the outside remained rather unchanged (Diagram IV.1- purple zone1 and green zone 2). It seems that stimulation of cell elongation in the inner epidermal layer of the hook results in straightening of the hook. Tissues previously constituting the hook region now reside in the elongation zone (Diagram IV.2 - purple zone 2 in I and II). At the same time, however, stimulated elongation again occurs in the outer epidermal cells above the hook in the apical region ( green zone 3 in I) leading to continuous formation of the hook (green 3 in II). Cell division activity in the meristem supplies new generations of compact young cells which replace the apical region (purple II-4). When this pattern of developmental program is continuously exerted in the dark, etiolated dicot seedlings maintain their hook while the hypocotyl elongates (Diagram IV.2, I-II-III).

If auxin is involved in hook formation and straightening, auxin asymmetry has to be established in the apical region and the hook. Whatever the mechanism is, e.g., actual movement of the hormone, control of conjugation (Szerszen et al., 1994), or changes in tissue sensitivity (Trewavas, 1981), auxin activity must be altered <u>laterally</u> from one side to the other of the apical tissues. However, this lateral signal transmission mode must be precisely controlled such that it occurs in an <u>outward</u> direction above the hook (Diagram 1, the arrows in green 1) and then reverses <u>inward</u> in the hook region (the arrows in purple 1 in Diagram IV.1). At present, we have no data to speculate whether this mode of auxin asymmetry uses the same machinery as utilized in phototropism or gravitropism.

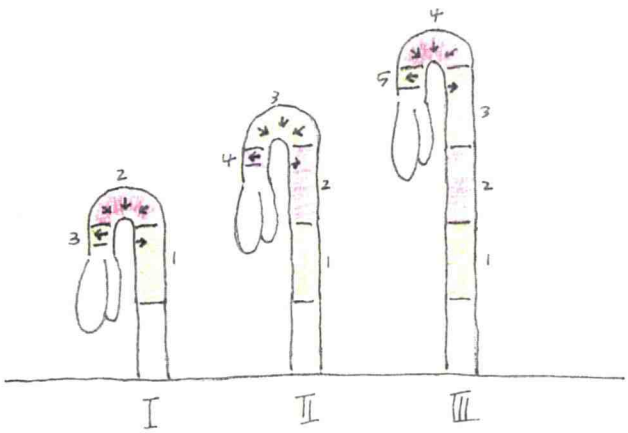


**Diagram VI.2.** Maintenance of the hook and hypocotyl elongation in wild-type tomato seedlings.

However, as indicated earlier, etiolated seedlings grow away from the gravity vector and the direction of hook formation is determined by the gravity vector. At least in the dark, a communication system must exist for the proper integration of the different occurrences of auxin asymmetry necessary for hook formation and straightening and the gravitropic response, in order to produce the proper dark-grown morphology in young seedlings.

Once exposed to adequate levels of light, the hook of etiolated dicot seedlings opens and stem elongation is reduced (Kronenberg and Kendrick, 1994). Hook opening can be explained by changes in auxin activity which are under phytochrome regulation. One scenario for hook opening is that the outward signal transmission for auxin activity above the hook no longer occurs so that there is no more stimulation of elongation in the outer epidermal cells (Diagram IV.3). Alternatively, inhibition of elongation by ethylene on the inner side of the hook could cease. Whichever is the case, the inward auxin activity in the hook (Diagram IV.1, purple zone 1) will not stop until this existing hook is opened. Phytochrome inhibition of polar auxin transport would result in less auxin available for cell elongation in the inside of the hook and thus straightening of the hook in response to phytochrome could be accompanied by a reduction in stem elongation (Diagram IV.3, purple 2 and green 3).

In fact, our studies with the *lz-2* mutant showed that the induction of downward hypocotyl growth by red light is the result of a growth asymmetry produced by stimulation of elongation and often accompanied by opening of the hook later. Results from the double labeling study indicate that the phytochrome-mediated downward gravitropism in *lz-2* hypocotyls is caused by reversed auxin redistribution. Therefore, at least in the case of gravitropism, the redistribution of auxin activity described earlier occurs as a result of actual movement of the hormone across the hypocotyl tissue (although the possibility that auxin sensitivity is altered cannot be eliminated). If phytochrome also regulates the auxin movement responsible for hook opening, this may occur by inhibiting the outward

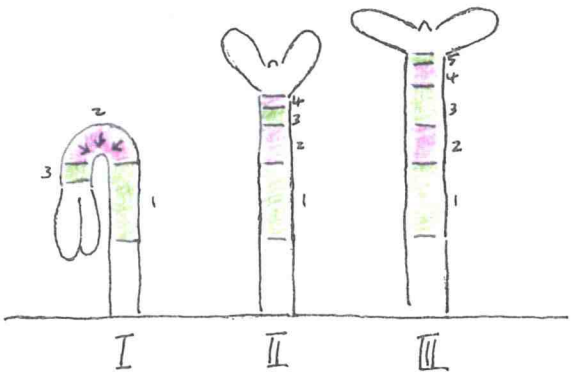


**Diagram IV.3.** Opening of the hook upon exposure to light.

movement of auxin above the hook and promoting the inward movement of auxin in the hook region itself until the cell elongation in the outer side is compensated for by the elongation in the inner side. In the *lz-2* mutant, the phytochrome-regulated auxin flow might be altered in such way that movement of auxin to the inner side continues to occur (Diagram IV.4). This "over-flow" auxin could establish the 'reversed' elongation asymmetry in the elongation zone (II, purple 2) which results in downward curvature (III).

Overall, this creates a complex picture with the lateral movement of auxin responsible not only for gravitropic responses but also for phytochrome-mediated early development in young seedlings. Phytochrome regulation of auxin movement seems to occur not only at the level of polar transport but also in the lateral transport involved in gravitropism. It will be absolutely fascinating to unravel each of these pathways and elucidate the mechanisms by which they communicate, i. e., how gravity and red light signals are integrated by the lateral auxin transport system and how phytochrome activation can trigger this different mode of auxin transport.

In this study, the gravitropic responses of decapitated hypocotyls have contributed to understanding the mechanisms underlying the gravitropic response and the interaction between light and gravity. In decapitated hypocotyls, gravitropic curvature was observed in wild type hypocotyls but not in *lz-2* hypocotyls. In addition, elimination of gravitropic curvature in *lz-2* hypocotyls occurred both in the dark and red light. As suggested by McDonald and Hart (1987), two phases of gravitropic responses accomplish the full induction of stem curvature in etiolated dicot seedlings. The first phase is conducted by rapid auxin movement across hypocotyl tissues, mostly in the elongation zone. This might be considered a 'priming action' to guide the stem as to which way to bend. Certain characteristics of etiolated dicot seedlings may create different morphological tensions in the hypocotyls tissues, especially in the elongation zone. Although it was not noticeable in wild type, *lz-2* hypocotyls respond differently to gravity depending on the orientation of the hook. In general, etiolated tomato seedlings bend more easily toward the hypocotyl side of

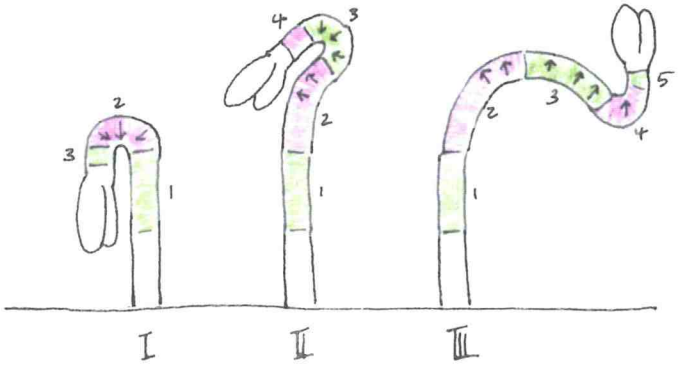


**Diagram IV.4**. Downward growth of *lz-2* hypocotyls upon exposure to red light.

the hook than in the direction of the cotyledon side. This tendency is obvious in red light-treated *lz-2* hypocotyls. When the hook is oriented such that the cotyledons face toward the gravity vector, *lz-2* hypocotyls first start bending upward like wild type before reversing toward the gravity after sufficient illumination of red light (Gaiser, 1994). Upright *lz-2* seedlings always bend toward the hypocotyl rather than the cotyledons (see, e.g., Figure II.1).

It is possible that early developmental programs for young etiolated seedlings are 'primed' to construct a morphological passage for lateral auxin movement for the first phase of the gravitropic response; lateral auxin movement might occur preferentially toward the inner side of the elongation zone. When the cotyledons face toward the gravity vector, the etiolated seedling is situated in such a way that the primed auxin transport takes place immediately. On the other hand, when the cotyledons face up, the lateral auxin transport involved in the first phase of gravitropism has to occur 'against' the morphological design. In this case, an 'adjusting program' might operate to allow the first phase to take place. In wild-type seedlings, the adjustment of lateral auxin transport with respect to the hook orientation seems to occur rapidly. Hook orientation in wild type does not seem to affect the gravitropic responses in hypocotyls.

According to the McDonald and Hart's two-phase model (McDonald and Hart, 1987), gravitropic curvature continues until completed by the second phase. This second phase involves stem elongation which requires auxin supplied by the shoot apex (Hart and MacDonald, 1984). Intact *lz-2* hypocotyls exhibited a slow gravitropic response which is eliminated by excision of the shoot apex. This indicates that the *lz-2* gravitropic response is a result of only the second phase, i.e., the component which requires the apical region. That second phase of the gravitropic response is almost certainly influenced by developmental events in the apical region such as hook formation. If lateral movement of auxin mediates cell elongation for both hook morphology and gravitropic curvature in wild-type plants, two auxin movement systems must be coordinated to ensure the proper

development of the young dicot seedlings with respect to both light and gravity. In the *lz-2* mutant, the second phase of the gravitropic response results in normal upward growth in the dark whereas it is reversed resulting in downward growth in red light. This provides evidence that regulation of auxin movement during the second phase of gravitropic response is tightly connected with the phytochrome-regulated auxin transport involved in hook morphology.

It is a novel observation that in the absence of the shoot apex, *lz-2* hypocotyls cannot respond to gravity regardless of the light condition. The gravitropic curvature observed in decapitated wild-type hypocotyls might be the result of the first phase of the gravitropic response which appears to be lacking in plants with the *lz-2* mutation. The surgical elimination of the gravitropic response might indicate that the *lz-2* lesion alters the morphological construction of the auxin passage in the hypocotyl tissue where the first phase takes place, i.e., the elongation zone. Defective auxin transport machinery in the *lz-2* elongation zone might abolish the auxin asymmetry which is responsible for the first phase gravitropic curvature.

It is also possible that the reversed auxin movement responsible for downward growth in *lz-2* hypocotyls is the result of altered cell differentiation in the apical region. If phytochrome is involved in a developmental program which is required for morphological construction for auxin transport in the apical region, e.g, for hook opening, the *lz-2* lesion could affect this phytochrome-regulated process in the apical region and later in the elongation zone, auxin transport could be reversed in response to gravity. Excision of shoot apex in the *lz-2* mutant may be useful in defining the function of the *lz-2* gene product and its relationship with a phytochrome-regulated auxin transport. Different portions of the apical tissue can be excised to examine the gravitropic response in *lz-2* hypocotyls either in the dark or red light. The region of the apex which is important for upward gravitropism in the dark may or may not be different from the region responsible for downward curvature in red light. In our study, lateral auxin distribution was examined

only in the elongation zone. The pattern of auxin redistribution in the apical region can be important in understanding not only the mechanism of auxin transport in response to gravity, but also the role of phytochrome. Dissection of the apical tissue, however, can be laborious and inaccurate. Microsectioned hypocotyl tissues probed with radioactive auxin should yield better information (Jones et al, 1991). Auxin binding proteins or auxin-regulated genes may also provide valuable tools for elucidation of the mechanism of auxin action at the cellular and molecular levels (Jones and Prasad, 1992; Hagen, 1995, for review). Use of these probes to indicate active auxin concentration in wild type and *lz-2* hypocotyl tissues and examination of the effect of phytochrome on the distribution of auxin-regulated genes should be productive areas of study in the future.

## LITERATURE CITED

**Behringer, F., P. Davies, and J. Reid.** (1990). Genetic analysis of the role of Gibberellin in the red light inhibition of stem elongation in etiolated seedlings. Plant Physiol. **94**: 432-439.

**Behringer, F., and P. Davies.** (1992). Indole-3-acetic acid levels after phytochrome-mediated changes in the stem elongation rate of dark- and light-grown *Pisum* seedlings. Planta. **188**: 85-92.

**Bell, C. J. and E. P. Maher.** (1990). Mutants of *Arabidopsis thaliana* with abnormal gravitropic responses. Mol. Gen. Genet. **220**: 289-293.

**Blaauw, O. H.** (1961). The influence of blue, red and far-red light on geotropism and growth of the *Avena* seedlings. Acta Bot. Neerl. **10**: 397.

**Bruinsma, J., and K. Hasegawa.** (1990). A new theory of phototropism - its regulation by a light-induced gradient of auxin-inhibiting substances. Plant Physiol. **79**: 700-704.

**Chory, J.** (1993). Out of darkness: mutants reveal pathways controlling light-regulated development in plants. Trends. in Genetics. **9**: 167-172.

**Cleland, R. E.** (1995). Auxin and cell elongation. In: *Plant Hormones. Physiology, biochemistry, and molecular biology* (P. J. Davies ed.) Kluwer Academic Publishers, Dordrecht, pp 214-227

**Cleland, R. E., and D. L. Rayle.** (1977). Reevaluation of the effect of calcium ions on auxin-induced elongation. Plant Physiol. **60**: 709.

**Darwin, C.** (1880). The power of movements in plants. (London, John Murray Press.)

**Feldman, L., and W. Briggs.** (1987). Light-regulated gravitropism in seedling roots of maize. Plant Physiol. **83**: 241-243.

**Firn, R. D., J. Digby, and A. Hall.** (1981). The role of the shoot apex in geotropism. Plant Cell Env. **4**: 125-129.

**Gaiser, J.C.**(1994). Ph.D. dissertation. Oregon State University, Corvallis, USA.

**Gaiser, J.C., and T. L. Lomax.** (1992). The gravitropic mutant *lazy-2* is altered in signal transduction. In: *Progress in Plant Growth Regulation.* (C. M. Karssen, L. C. Loon, and D. Vreugdenhil. eds). Kluwer Academic Publishers, Dordrecht, pp 928-937.

**Gaiser, J. C., and T. L. Lomax.** (1993). The altered gravitropic response of the *lazy-2* mutant of tomato is phytochrome regulated. Plant Physiol. **102**: 339-344.

**Guzman, P., and J. R. Ecker.** (1992) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell. **2:** 513-524.

**Hagen, G.** (1995). The control of gene expression by auxin. In: *Plant Hormones. Physiology, biochemistry, and molecular biology* (P. J. Davies ed.) Kluwer Academic Publishers, Dordrecht, pp 228-245.

**Harrison, M.A., and B.D. Pickard** (1989). Auxin asymmetry during gravitropism by tomato hypocotyls. Plant Physiol. **89**: 652-657

**Hart, J. W., and I. R. MacDonald**. (1984). Is there a role for the apex in shoot geotropism. Plant Physiol. **74**: 272-277.

**Iino, M.** (1982). Inhibitory action of red light on the growth of the maize mesocotyl: evaluation of the auxin hypothesis. Planta. **156**: 388-395.

**Iwami, S., and Y. Masudad**. (1974). Geotropic response of cucumber hypocotyls. Plant Cell Physiol. **15**: 121-129.

**Jacob, M. and P.H. Rubery**. (1988). Naturally occurring auxin transport regulators. Science. **241:** 346-349

**Jones, A., D. Cochran, P. Lamerson, M. Evans, and J. Cohen**. (1991). Red light-regulated growth I. Changes in the abundance of Indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. Plant Physiol. **97**: 352-358.

**Jones, A. M., and P. V. Prasad**. (1992). Auxin-binding proteins and their possible roles in auxin-mediated plant cell growth. BioEssays. **14**: 43-48.

**Kang, B. G. and S. P. Burg**. (1972). Ethylene as a natural agent including plumular hook formation in pea seedlings. Plants. **104**: 275-281.

**Kelly, M., and A. Leopold**. (1992). Light regulation of the growth response in corn root gravitropism. Plant Physiol. **98**: 835-839.

**Kronenberg, G. H. M., and R. E. Kendrick.** (1994). Photomorphogenesis in plants. Kluwer Academic Publishers, Dordrecht, The Netherlands.

**Liscum, E., and R. P. Hangarter**. (1993). Genetic evidence that the red-absorbing form of phytochrome B modulated gravitropism in *Arabidopsis thaliana*. Plant Physiol. **103**: 15-19.

**Lomax, T. L., G. K. Muday, and P. H. Rubery.** (1995). Auxin Transport. In: *Plant Hormones. Physiology, biochemistry, and molecular biology* (P. J. Davies ed.) Kluwer Academic Publishers, Dordrecht, pp 531-546.

**López-Juez, E., M. Kobayashi, A. Sakurai, Y. Kamiya, and R. Kendrick.** (1995). Phytochrome, gibberellins, and hypocotyl Growth; a study using the Cucumber (*Cucumis sativus* L.) *long hypocotyl* mutant. Plant Physiol. **107**: 131-140.

**MacDonald, I. R., D. C. Gordon, and J. W. Hart**. (1982). Analysis of growth during light-induced hook opening in cress. Plant Cell Env. **5**: 355-360.

**MacDonald, I. R., D. C. Gordon, and J. W. Hart, E. P. Maher.** (1983) The positive hook: the role of gravity in the formation and opening of the apical hook. Planta. **158**: 76-81.

**MacDonald, I. R., and J. W. Hart.** (1987). Tropisms as indicators of hormone-mediated growth phenomena. In: *Hormone action in plant development.* (G. V. Haod J. R. Lenton, M. B. Jackson, and R. K. Atkin. ed). London, UK, Butterworths.

**McArthur, J. A., and W. R. Briggs.** (1979). Effect of red light on geotropism in pea epicotyls. Plant Physiol. **63**: 218-220.

**Meyer, A. B., R. D. Firn, and J. Digby.** (1993). Gravitropic sign reversal-A fundamental feature of the gravitropic perception or response mechanism in plant organs. J. of Exp. Bot. **101**: 567-572.

**Mohr, H. and P. Schopfer.** (1995). *Plant Physiology.* Springer Verlag, Berlin.

**Moitzik, F., and H. Mohr.** (1988). Control of hypocotyl gravitropism by phytochrome in a dicotyledonous seedlings (*Sesamum indicum* L.). Plant Cell Env. **11**: 663-668.

**Naqvi, S., and S. Gordon.** (1966). Auxin transport in *Zea* maize coleoptiles II. Influence of light on the transport of $^{14}$C-IAA. Plant Physiol. **42**: 138-143.

**Pickard, B.G.** (1985). Early events in geotropism of seedling shoots. Ann.Rev.Plant.Physiol. **36**: 55-75.

**Roberts, J. A.** (1984). Tropic responses of hypocotyls from normal tomato plants and the gravitropic mutant *lazy-1*. Plant.Cell.Env. **7**: 515-520.

**Roberts, J. A.** (1987). Mutants and gravitropism. In: *Developmental Mutants in Higher Plants. SEB Seminar Series 32.* (H. Thomas and D. Grierson. ed). Cambridge, Cambridge University Press.

**Rorabaugh, P., and F. Salisbury.** (1989). Gravitropism in higher plants. VI. Changing sensitivity to auxin in gravistimulated soybean hypocotyls. Plant Physiol. **91**: 1329-1338

**Roux, S., and B. Serlin.** (1989). Cellular mechanisms controlling light-stimulated gravitropism: role of calcium. Crit. Rev. Plant Science **5**: 205-236

**Salisbury, F. B.** (1993). Gravitropism: Changing ideas. Horticultural Reviews. **15:** 233-278.

**Salisbury, F. B., and C. W. Ross.** (1992). *Plant Physiology.* (Belmont, CA, Wadsworth Publishing Company.) pp344-345.

**Silk, W. K. and R. O. Erickson.** (1978). Kinematics of hypocotyl curvature. Am. J. Bot. **65**(3): 310-319.

**Smith, H. and G. C. Whitelam.** (1990). Phytochrome, a family of photoreceptors with multiple physiological roles. Plant Cell Env. **13**: 695-707.

**Sorressi, G. P. and P. Cravedi.** (1967). Tomato mutants obtained by means of X-ray and ethylmethanesulfonate (EMS) treatments. Rep.Tom.Genet.Coop. **7**: 51.

**Szerszen, J. B., K. Szczyglowski, and R. S. Bandurski.** (1994). *iaglu*, a gene from *Zea mays* involved in conjugation of growth hormone indole-3-acetic-acid. Science. **265**: 1699-1701.

**Tepfer, D. A. and H. T. Bonnett.** (1972). Role of phytochrome in the geotropic behavior of *Convolvulus arvensis*. Planta. **106**: 311-314.

**Trewavas, A. J.** (1991). How do plant growth substance work? II. Plant Cell Env. **14:** 1-12.

**Trewavas, A. J.** (1992). What remains of the Cholodny-Went Theory: A Forum. Plant Cell Env. **15**: 759-794.

**Went, F. W. and K. V. Thimann.** (1937). *Phytohormones*. MacMillan, New York, NY

**Wilkins, M. B. and M. H. Goldsmith.** (1964). The effect of red light, far-red and blue light on geotropic response of *Zea mays*. J. Exp. Bot. **15**: 600.