Cell Division Activity during Apical Hook Development[1]

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Growth during plant development is predominantly governed by the combined activities of cell division and cell elongation. The relative contribution of both activities controls the growth of a tissue. A fast change in growth is exhibited at the apical hypocotyl of etiolated seedlings where cells grow at different rates to form a hook-like structure, which is traditionally assumed to result from differential cell elongation. Using new tools we show asymmetric distribution of cell division during early stages of hook development. Cell divisions in the apical hook were predominantly found in subepidermal layers during an early step of hook development, but were absent in mutants exhibiting a *hookless* phenotype. In addition, during exaggeration of hook curvature, which is mediated by ethylene, a rapid change in the combined activities of cell division and cell elongation was detected. Our results indicate a fast change in cell division activity during apical hook development. We suggest that cell division together with cell elongation contributes to apical hook growth. Our results emphasize the change in the relative contribution of cell division and cell elongation in a fast growing structure like the apical hook.

The growth rate of a specific organ or tissue in the plant can change during its development or in response to external stimuli. Growth is the net effect of cell production, expansion, arrest, and apoptosis. Although programmed cell death and growth arrest contribute to slow growth processes (Greenberg, 1996), in fast growing areas, it is mainly the combined activities of cell division and cell expansion that contribute to growth (Beemster and Baskin, 1998). The combined activities of cell division and cell elongation can affect cell differentiation in the leaf (Donnelly et al., 1999), and affect root growth (Beemster and Baskin, 1998). Along the root the relative contribution of both activities is changed in a zone-specific manner (Doerner et al., 1996; Beemster and Baskin, 1998). This illustrates that the relative contribution of cell division and cell elongation activities can change within a tissue, and this might also be the case in time-dependent growth.

A hook-like structure develops at the apical part of the hypocotyl in dark-grown seedlings. In Arabidopsis the apical hook is formed 24 h after germination and is maintained for about 4 d by a process of differential growth (Ecker, 1995; Raz and Ecker, 1999). During these 4 d the growth pattern of the apical hook undergoes through three phases: formation, maintenance, and opening (Raz and Ecker, 1999). Hook development is a result of asymmetric growth along the apical-basal axis (Silk and Erickson, 1978). Differential growth in the apical hook is traditionally assumed to result from differential cell elongation of epidermal cells (Silk and Erickson, 1978; Ecker, 1995). Here we examined the possibility that cell division may also contribute to differential growth in the apical hook, using a marker for dividing cells together with a quantitative analysis of cell numbers in the apical hook.

Apical hook development is regulated by plant hormones, including auxin and ethylene. Treatment of wild-type seedlings with auxin results in a reduced hook curvature, whereas ethylene enhances hook curvature (Ecker, 1995; Lehman et al., 1996). Ethylene sensitivity in the apical hook is restricted between d 2 and 3 of seedling growth. At that time ethylene response genes asymmetrically accumulated in the apical hook (Raz and Ecker, 1999). Although a molecular understanding of ethylene action in the apical hook is starting to be assembled, the mechanism by which ethylene mediates differential growth in the apical hook is still vague. We analyzed cell division activity in the ethylene-mediated exaggerated hook and in optical sections obtained from the confocal microscope. Based on these results we suggest a mechanism that describes enhanced differential growth, which results in exaggerated hook structure.

RESULTS AND DISCUSSION

Cell Division Activities during Apical Hook Development

Expression of the mitotic cyclin, Cyc1B, is restricted to cells undergoing mitosis. The transcription of Cyc1B is activated during G2, and by telophase the protein is degraded (Doonan and Fobert, 1997). Thus, the mitotic cyclin, Cyc1B, is an excellent expression marker for cells undergoing mitosis. The labile Cyc1B-β-glucuronidase (GUS) fusion protein exhibits a short expression in individual dividing cells and therefore mimicked the expression of the native protein (Colon-Carmona et al., 1999). Cyc1B-GUS exhibited a spatial expression in the apical hook during

early stages of hook development (Fig. 1A). High-magnification analyses showed that GUS staining is restricted for a single cell (Fig. 2C), which further indicates that each GUS spot represents a single cell division (Colon-Carmona et al., 1999). In addition, nuclear division in the apical hook was detected in optic sections obtained from confocal microscope analyses of 2-d-old seedlings (Fig. 2B). Neither dividing nuclei nor Cyc1B-GUS were found in the hypocotyl (not shown), which is in agreement with previous studies of hypocotyl growth (Gendreau et al., 1997). These observations indicate that although the apical hook and the hypocotyl are located in the same tissue along the apical-basal axis of the seedling, the cellular mechanisms regulating the growth of these two tissues are not completely identical.

The expression of Cyc1B-GUS in the apical hook showed a time-dependent pattern. Cyc1B-GUS expression was restricted to the early stages of hook development (d 1 and 2 of seedling growth; Fig. 1B). During a later phase of hook maintenance (after 60 h)

the expression of Cyc1B in the apical hook declined significantly and disappeared by the 3rd d of growth (Fig. 1B). These results indicate that the relative contribution of cell division and cell elongation activities is dynamic and changeable during hook development. Previous studies of differential growth in the apical hook have focused on epidermal cells (Ecker, 1995; Silk and Erickson; 1978). Using optical sections of the apical hook, dividing nuclei were localized in subepidermal layers (Fig. 2B), where Cyc1B-GUS was also detected by Nomarski optics (Fig. 2C). Moreover, subepidermal layers contained more cells compared with the epidermal layers (Fig. 2A). These observations indicate that the conditions for asymmetric growth can initiate from inner cell layers followed by outer cell layers. For that, using only epidermal cells to study growth of the apical hook can be misleading. Although we could not detect dividing nuclei or GUS-stained cells in epidermal layers, we cannot exclude the possibility that cell division events rarely occur.

Figure 1. Cyc1B expression in the developing apical hook. A, Cyc1B-GUS transgenic seeds were germinated in darkness for 30 (i), 36 (ii), and 48 h (iii) and then stained with GUS substrates. B, GUS-stained seedlings were analyzed for hook curvature (■) and the number of cells expressing GUS was counted (□). Average represents 20 seedlings. C, GUS expression in the apical hook region of *hookless* mutants. Cyc1B-GUS transgenic plants were crossed to *hls1-1* and *cop2* plants. Two-day-old seedlings were subjected to GUS staining. To avoid germination differences between batches of seeds, analyses were performed on wild-type and mutant seedlings segregating from F_2 population. i, Colombia wild type; ii, *hls1*; iii, *cop2*. Bar = 0.4 mm.
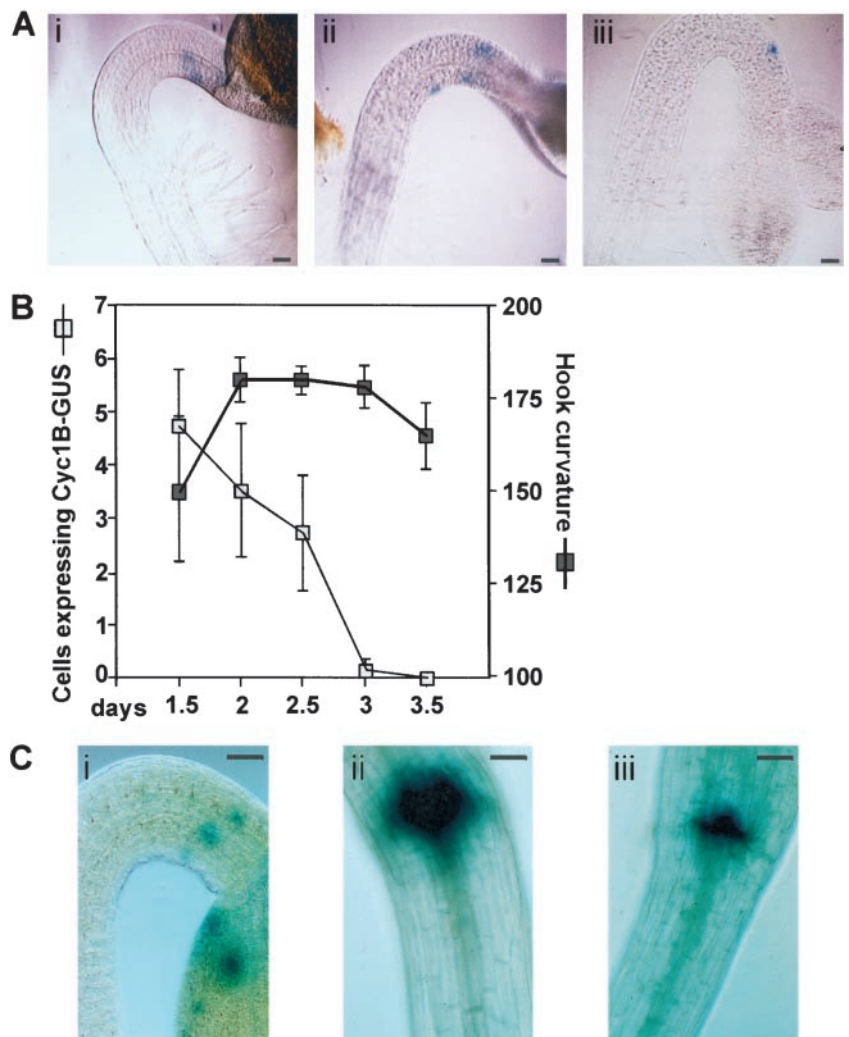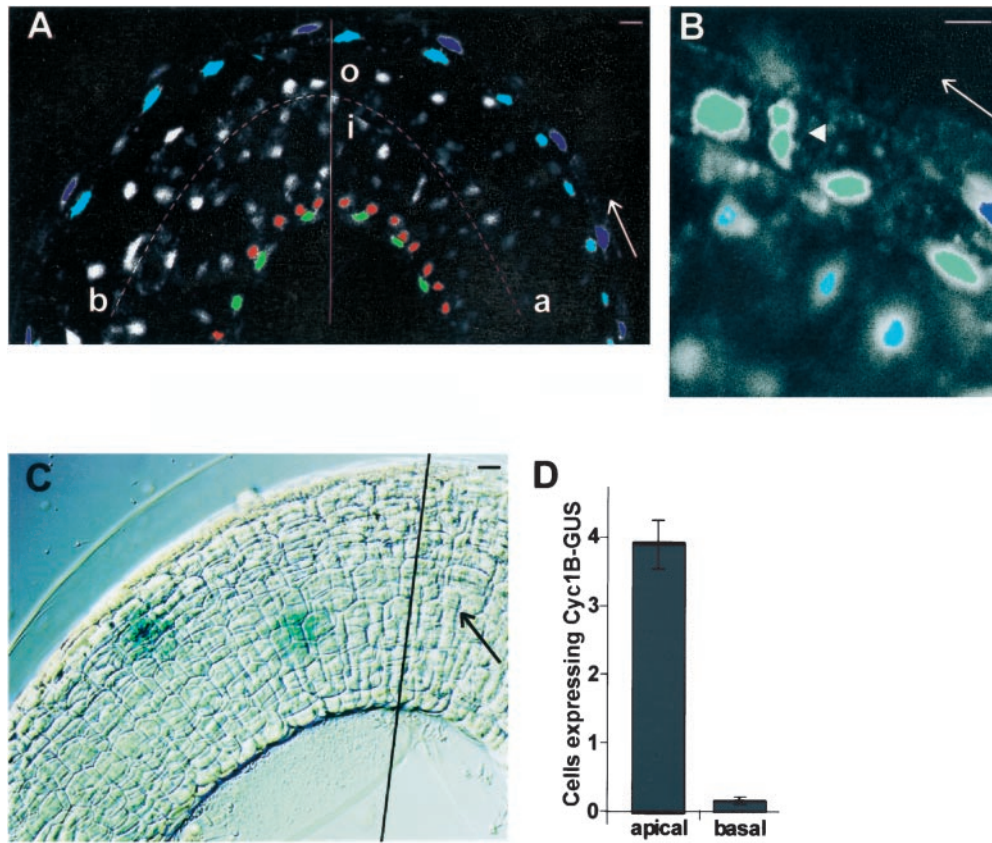
Figure 2. Asymmetric localization of cell division in the apical hook. A, Confocal section of the apical hook in wild-type 2-d-old etiolated seedlings. Stained dots are nuclei and each cell layer is represented by different color. Arrow shows the direction of cell flow over the hook. The vertical line shows the hook-mid-line, which separated the apical (a) and the basal (b) parts of the hook. A dashed curved line along the hook separates the inner (i) and outer (o) sides of the hook. Nuclei of epidermal cells at outer and inner edges are marked blue and green, respectively; subepidermal layers are marked with red and light-blue nuclei. The hook mid-line is indicated as a white vertical line. B, Confocal section of dividing nuclei in the apical hook. Nuclei in the epidermal layer are painted in dark blue and nuclei division (arrowhead) is observed in a subepidermal layer. Arrow shows the direction of cell flow over the hook. C, Cyc1B-GUS is expressed in subepidermal layers in the apical hook. Two stained cells are shown that are in subepidermal cells, but only one is in focus. The arrow indicates the direction of cell flow over the hook and the vertical line shows the hook mid-line. D, Asymmetric distribution of cells within the apical hook. Two-day-old seedlings were analyzed using a confocal microscope. Nuclei from subepidermal layers were counted from the inner side (dotted bars) or the outer side (black bars) of the hook. Using the hook mid-line, the vertical line in A, the number of nuclei was counted on the apical and basal parts of the hook. Averages represent eight to 10 seedlings. With increased light it was confirmed that each cell contains only one nucleus (not shown). Bars = 60 μm.

Cell Division in the Apical Hook Is Absent in Mutants Exhibiting a *hookless* Phenotype

The first phase of hook development is regulated by *HLS1* and *COP2* genes, which confer a hookless phenotype when mutated (Lehman et al., 1996; Raz and Ecker, 1999). Because Cyc1B-GUS expression in the apical hook occurs at the time that the hookless morphology is exhibited in the *hookless* mutants, we examined cell division in the apical end of *hls1* and *cop2* mutants (Fig. 1C, ii and iii, respectively). It was observed that the expression of Cyc1B-GUS was not expressed in the apical hypocotyl region of the *hookless* seedlings and nuclear division was absent as determined by confocal microscope analyzes (not shown). The absence of hook curvature and cell di-vision activity in the *hls* mutants indicates the functional contribution of cell division during early growth of the apical hook. Cyc1B-GUS was not abolished in other parts in the *hls* seedlings, as in the root and the shoot meristems. *hls1* and *cop2* mutants exhibit a larger shoot apical meristem (SAM) during seedling growth (Lehman et al., 1997). Larger SAM can be the result of unregulated cell division activity in the meristem (Jacobsen et al., 1999), or due to under-regulated differentiation rate (Schoof et al., 2000). Although the molecular basis for the enlarged SAM in the *hookless* mutants is not known, the enhanced expression of Cyc1B-GUS in *cop2* and *hls1* SAM (Fig. 1C) may suggest unregulated cell division activity in the meristem. The enhanced activity of

Cyc1B-GUS in *hls* SAM alternatively can indicate a faster seedling growth, which results in an earlier transfer into the adult phase in the mutants.

To confirm the functional role of cell division on apical hook curvature, 1-d-old seedlings were treated with cell cycle inhibitors, aphidicolin, and hydroxyurea block cell cycle at the G_1-to-S transition (Tamura et al. 1999). Hook curvature in Col WT was reduced when etiolated seedlings grew in the presence of the cell cycle inhibitors, aphidicolin, or hydroxyurea (Fig. 3A); hypocotyl morphology, however, was not affected (not shown). The observation that application of cell cycle inhibitors resulted in a reduced but not a complete absence of the hook curvature further indicates that the combined activity of cell elongation and cell division are required for curving of the apical hook.

The Distribution of Cell Division in the Apical Hook Is Asymmetric

Kinematic studies of hook curvature showed that the curvature is maintained by differential growth between cells at the inner and outer sides, whereas cells flow over the hook mid-line as defined in Raz and Ecker (1999). On the apical part of the hook the relative growth rate of the outer side exceeds that of the inner side. Once cells pass the hook mid-line, the difference in growth rate between outer and inner sides reverses and the structure is straightened. To understand how cell division contributes to differential growth, four different parts of the hook were defined (apical [a], basal [b], inner [i], and outer [o]; Figure 2A), and the number of subepidermal cells in each part was counted. The major difference in cell number was found between apical and basal parts (Fig. 3B). The apical part contained 1.7 times more cells compared with the basal part. In addition, Cyc1B-GUS was predominantly expressed at the apical part of the hook, as determined by the number of cells expressing GUS (Fig. 2D).

These results indicate that the differential growth along the apical-basal axis of the hook is, in part, due to asymmetric distribution of cell division between the apical and basal part of the hook. The difference in cell number between inner and outer sides was much smaller compared with the difference between apical and basal parts (Fig. 3B). However, small, but significant differences were found in the ratio between the number of cells on the outer and inner sides (Fig. 3B). When seedlings were treated with aphidicolin the number of cells at the basal part was comparable with air-grown seedlings, but at the apical-inner part the number of cells was reduced (Fig. 3B). Cells at the outer side, however, are 3.5 to 2.3 times longer compared with cells at the inner side at the same apical-basal position. Basal to the apical hook cells on opposite sides of the hypocotyl exhibit similar length (not shown). In the *hls1* and *cop2* mu-
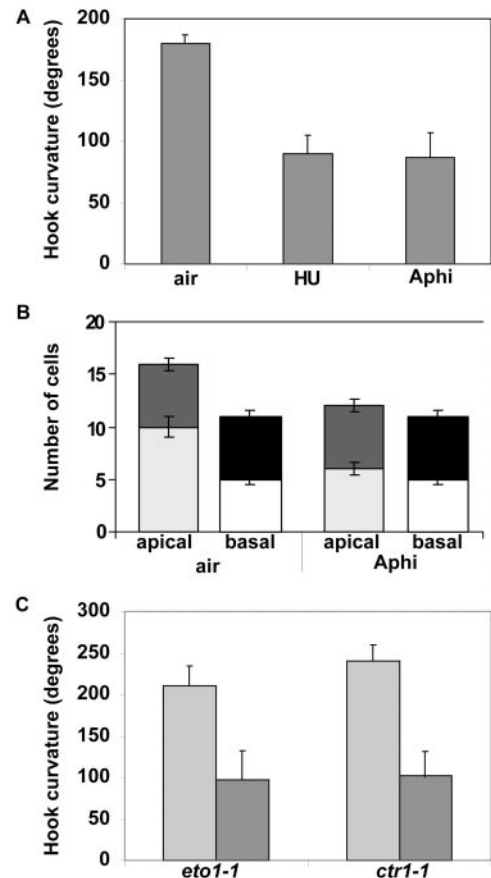

Figure 3. The effect of cell cycle inhibitors on hook curvature. A, Columbia wild-type 1-d-old seedlings were grown in air or treated with 10 μg mL^{-1} aphidicolin (Aphi) or 100 μM hydroxyurea (HU) for 24 hours and hook curvature was measured. Average represents 50 seedlings. B, Asymmetric distribution of cells within the apical hook in air-grown and aphidocolin-treated seedlings. Two-day-old seedlings were analyzed using a confocal microscope. Nuclei from sub-epidermal layers were counted from the inner side (dark bars) or the outer side (light bars) of the hook. Using the hook mid-line, the vertical line in Fig. 2A, the number of nuclei was counted on both apical (natural colors) and basal (gray) sides of the hook. Averages represent eight to 10 seedlings. With increased light it was confirmed that each cell contains only one nucleus (data not shown). C, *eto1-1* and *ctr1-1* 2-d-old seedling mutants were grown in air (light gray boxes) or treated with 100 μM hydroxyurea (dark gray boxes) for 24 hours, and hook curvature was measured. Average represents 50 seedlings.

tants, however, an equal number of cells was counted on both sides of the apical part of the hypocotyl. These results together indicate that differences in the number of cells on both sides of the apical hook result in curvature of the structure. The difference in cell numbers is mainly between apical and basal parts of the hook, and corellated with Cyc-1B-GUS expression (Fig. 2D).

The results presented here indicate that different rates of cell elongation on both sides of the hook contribute to differential growth between the inner and the outer sides. Differential growth at the apical-basal axis, however, is determined by the combined
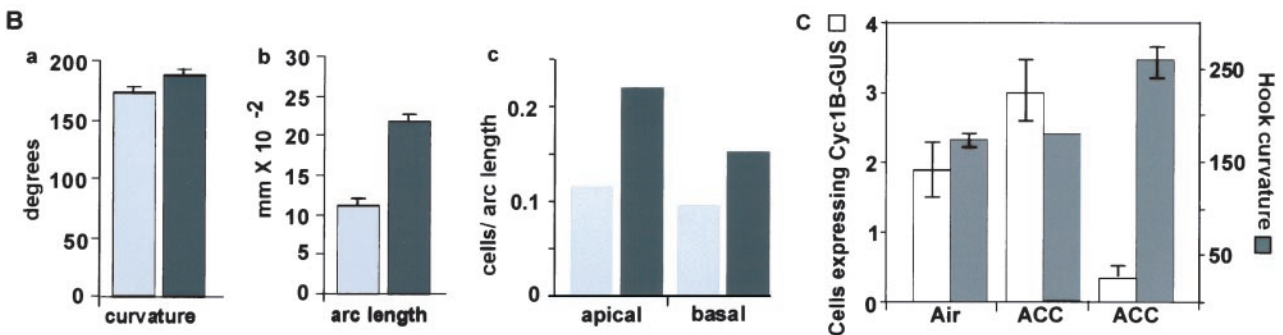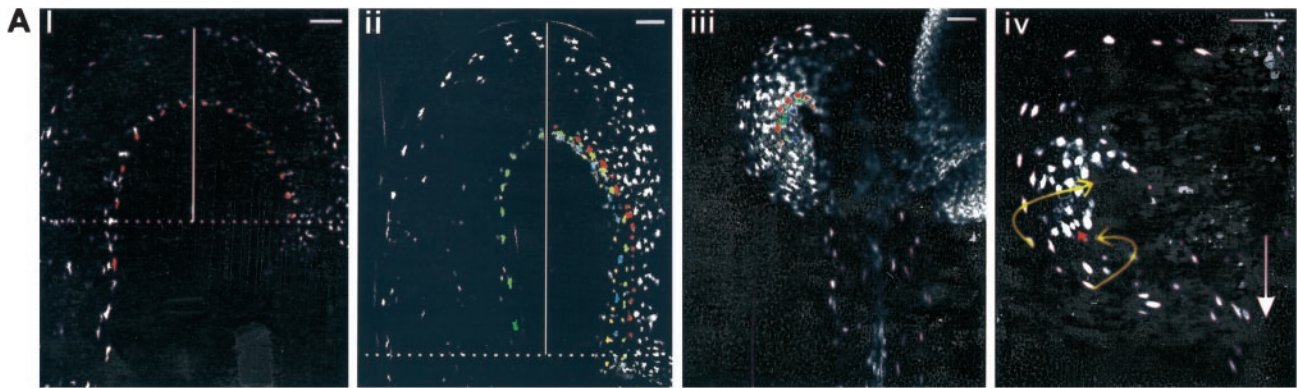
Figure 4. Steps in ACC-mediated exaggerated apical hook. A, Confocal sections of the apical hook during ACC application. Seedlings were grown in air (i) or treated with ACC for 20 h (see Materials and Methods; ii, iii, iv). Two steps in exaggerated hook development were observed: ii, arc elongation; iii and iv, arc twisting. Stained dots are nuclei and each cell layer is represented by a different color. The horizontal dotted line that starts from the SAM shows the basis of the apical hook, whereas the vertical continuous line shows the hook mid-line. The red arrowhead in iv shows a dividing nucleus; the green arrows show the direction of twisting and white arrow shows the basal direction of the apical-basal axis. a- and b-type seedlings were subjected for further analyses. B, Quantitative analyses of hook development. A, ACC increased arc length before exaggeration of the hook. Hook curvature was measured in 10 seedlings grown in air (light gray bars) or ACC-treated (dark gray bars). The same seedlings were further used for analyses in b and c. b, For arc length calculations the hook basis (dotted line in Fig. 4A) was used as the radius and the hook-mid-line as height (continuous line in Fig. 4A). c, ACC increased the number of cells per arc length in the apical part of the hook. Subepidermal cells were counted at the apical and basal parts of the hook from air or ACC treatments. To compare between the two treatments the number of cells per arc was calculated for the same arc length on both sides of the hook mid-line. C, Cyc1B-GUS expression in the apical hook differs between the two steps of ACC-mediated exaggerated apical hook. Cyc1B-GUS seeds were treated with ACC as described in Materials and Methods, and were stained for GUS. Seedlings were grown in air or treated with ACC were preselected according to hook curvature (gray bars). Left bars show air-grown seedlings. Middle bars show ACC-treated seedlings with air-like curvature, and right bars show ACC-treated seedlings with exaggerated curvature. Blue cells were counted from preselected seedlings (white bars). Averages represent 20 seedlings.

activities of cell elongation and cell division. Yet it is not clear how the combined activities of cell elongation and cell division regulate differential growth. Optical sections of the hook (Fig. 2A) show that the number of cells at the apical-inner curve is 3 to 2.5 times higher than that at the apical-outer curve, but cells at the outer side are 3.5 to 2.3 times more elongated than these at the inner side. Aphidicolin-treated seedlings exhibited a reduced cell density at the apical-inner curve, 0.66 compared with air-grown seedlings. These observations suggest that cell density can be the cause for differential growth during curving. At the outer curve cell density along the file is lower, cells can elongate more, and the file stretches. At the inner curve cell density is higher,

due to cell division events, and the rate of cell elongation is reduced. At the basal part cell division is reduced, cell density along the file reduces, and cells elongate more. As a result the structure straightens as previously suggested based on kinematic studies (Silk and Erickson, 1978).

The definition of four parts in the apical hook separates two axes of asymmetric growth: the apical-basal axis, which is a line along the seedling apical-basal line; and the inner-outer axis, which makes a transverse line connecting inner and outer sides of the hook (Fig. 2A). We provide data showing that each part of the hook exhibit a different growth profile. The differences in growth between the parts direct curving or opening of the structure.
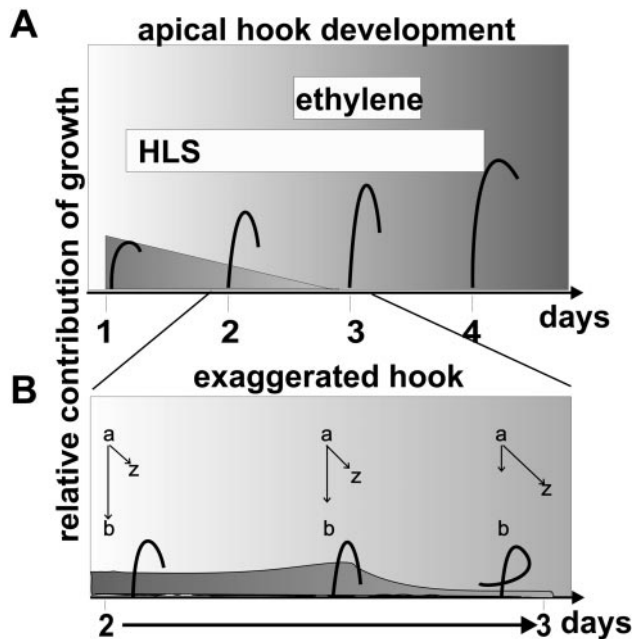
Figure 5. Schematic representation of the relative contribution of cell division and cell elongation during apical hook development. A, The relative contribution of cell division (dark gray area) and cell elongation (light gray area) mechanisms to differential growth is changing during time. During early steps of hook development, at the time that *HLS* genes regulate hook curvature, the relative contribution of cell division is essential. By the 3rd d, when the apical hook shows ethylene sensitivity, cell division in the hook is negligible and cell elongation is the main contributor to differential growth. B, Exaggeration of the apical hook. In air-grown seedlings differential growth is predominantly contributed by growth in the apical-basal axis (a and b). In addition, the structure of the hook also twisted (*z*-axis). During exaggeration of the apical hook, first the arc length is increased, keeping its growth in apical-basal axis. Later, growth in this axis is predominantly arrested, whereas growth in the third dimension increases resulting in enhanced twisting of the hook.

Growth during Ethylene-Induced Hook Exaggeration

During a later phase of hook maintenance (2.5–3.5 d of seedling growth) the cells become ethylene sensitive (Raz and Ecker, 1999) and seedlings treated with ethylene form a hook with an enhanced curvature (Ecker, 1995). Although many mutants in which the hook structure is modified, including mutants in the ethylene biosynthesis and signal transduction pathways have been isolated, the mechanism for ethylene-induced differential growth in the apical hook is unclear. To address this question, 2-d-old air-grown seedlings were treated with the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), for an additional 20 h and were grown at a lower temperature to reduce growth rate. Seedlings that were treated with ACC fell into two major groups based on the degree of curvature: those with an intermediate stage exhibiting an air-like curvature (approximately 180°) and an enhanced and exaggerated curvature (240°–

270°; Fig. 4, A and C). In ACC-treated seedlings with an air-like curvature (Fig. 4Bi) the arc length of the curvature was about twice as long than in air-grown seedlings with the same curvature (Fig. 4Bii) and these contained 2 to 1.5 times more cells per equivalent arc length (Fig. 4Biii). The increase in cell number was related to an increase of Cyc1B-GUS expression (Fig. 4C). The expression of Cyc1B-GUS in air-grown and ACC-treated seedlings was predominantly localized in the apical part of the hook (not shown). Both the *ethylene overproduction1* (*eto1*) and *constitutive triple response1* (*ctr1*) mutants exhibit exaggerated hook when grown in air (Ecker, 1995). The curvature of the exaggerated hook in *eto1-1* and *ctr1-1* was significantly reduced when the seedling was treated with hydroxyurea (Fig. 3C). During the second step the hook twisted while the cell flow over the hook significantly slowed down (Raz and Ecker, 1999), as well as cell division activity (Fig. 4C). Thus, the hook exaggeration is developed in two steps, which differ in their growth mechanisms: First, asymmetric cell elongation and cell division at the apical part take place; later, both growth activities are arrested while twisting continues, resulting in an enhanced curving.

In addition to the asymmetric growth along the apical-basal axis and between the inner and the outer sides, epidermal cells rotate around the apical-basal axis at the apical part of the hypocotyl (Raz and Ecker, 1999). Optical sections revealed that ACC treatment enhanced the rotation rate (Fig. 4A). In air-grown seedlings only 2 cell layers could be observed in a single optical section of the epidermal layer (Fig. 2Ai). Using similar optical sectioning parameters, more layers could be observed in ACC-treated seedlings (Fig. 4A, ii and iii). The rotation was enhanced at the apical part compared with the basal part as exemplified by the position of the red and green layers (Fig. 4Aii). During hook exaggeration, arc twisting was furthermore enhanced (Fig. 4Aiv), as a layer at a mid-position in the radial axis reached an inner position after a shorter distance (the red layers in Fig. 4A, ii and iii) and soon after disappeared from the optical plane (Fig. 4Aiv). Together these results indicate that the exaggeration of the hook can be divided into two sequential steps: increase in arc length, followed by twisting of the hook (Fig. 5B). During the first step, cell elongation and cell division in the apical-basal axis contribute to the increase in arc length. Growth in the apical-basal axis gradually is reduced while the structure continues twisting, which results in enhanced curvature of the hook (Fig. 5B). The regulation of growth during curving of the apical hook differs from hypocotyl elongation. Although we show that both cell elongation and cell division are required for differential growth in the apical hook, hypocotyl growth in etiolated seedlings is governed by cell elongation activity (Gendreau et al., 1997). Our studies presented here indicate that the number of cell division events can be

changed within a tissue, as suggested for root or leaf growth (Tsuge et al., 1996; Beemster and Baskin, 1998). Furthermore, our studies in the apical hook indicate that the relative contribution of both mechanisms can be transiently changed during time. Growth into the third dimension might be directed by a change in cell polarity elongation, as suggested for leaf growth (Tsuge et al., 1996).

Although ACC-treated seedlings exhibited an increase in cell division (Fig. 4C), ethylene sensitivity and cell division show only a partial overlap during apical hook development in air-grown seedlings (Fig. 5B). In *ein4-1* and *ein2-5* mutants, Cyc1B-GUS expression is abolished in the 3-d-old seedlings (not shown), and the mutant seedlings exhibit an open hook. These observations suggest that ethylene directs cell division in the apical hook. However, cell division in the apical hook during ethylene treatment may reflect the combined effect of additional hormones, which are involved in exaggerated hook formation. Auxin is involved in the regulation of differential growth in the apical hook (Doonan and Fobert, 1997) and its effect on cell division is well established (Hirt, 1996). The interaction between the two hormones during differential growth in the apical hook is unclear. However, the *ctr1* mutant, which exhibits an exaggerated hook when grown in air, exhibited a *hls* phenotype when grown in the presence of the auxin transport inhibitor naphthylphthalamic acid (Lehman et al., 1996). In addition, auxin treatment of *ein2* seedlings resulted in increase curvature of the apical hook (V. Raz, unpublished data). These experiments suggest that the apical hook of wild-type seedlings show sensitivity to auxin and ethylene at the same stage of development. Thus the increase in cell division during the first phase of ACC treatment may be the result of a combined action of hormones.

Final Remarks

The relative contribution of both mechanisms is changed at the apical part of the hook during hook development (Fig. 5A) and in response to ethylene (Fig. 5B). Because differential growth is mediated by differential cell elongation between opposite sides of the hook few but functionally significant cell division events localized in the apical inner side of the hook, we suggest that cell elongation is reduced and the arc length is shorter. Lower density of cells in the files at the outer side permits cell elongation and the arc length increases. This difference in rate of cell elongation results in curving. At the basal side, cell density between inner and outer sides is similar because there is no cell division; thus, the rate of cell elongation is equal on both sides and the structure straightens. The concept of growth as a dynamic process in which the relative contribution of cellular mechanisms is dynamic and changeable can be analyzed in additional growth processes, which are time dependent.

MATERIALS AND METHODS

Plants

All mutants and transgenics used are in the Colombia ecotype. For hook development studies, seeds were plated on 8% (w/v) water-agar plates and incubated at 4°C for 3 d and then incubated at 22°C to 24°C in darkness for the indicated times. For ACC treatments, seedlings were first grown for 40 h in air and then ACC was added onto the plates at a final concentration of 1 μM ACC and continued to grow further at 19° to 20°C for 20 h. For the inhibitor treatments, seeds were germinated in the dark for 24 h and the inhibitors aphidicolin (Sigma, St. Louis) and hydroxyurea (Sigma) were added onto plates to a final concentration of 10 μg mL^{-1} aphidicolin and 100 μM hydroxyurea. Hook curvature was measured after 2 d on Columbia wild-type seedlings or after 60 h on *eto1-1 and ctr1-1 mutants.*

GUS Staining and Imaging

Seeds from transgenic line FA4C homozygous for the Cyc1B-GUS construct (Colon-Carmona et al., 1999; received from P. Doerner) were germinated and seedlings were vacuum-infiltrated for 3 min with GUS buffer containing 100 mM NaP, pH 7, 10 mM EDTA, 0.1% (w/v) Tween 20, 1 mM potassium ferricyanide, 1 mM potassium ferrocyanide, and 1 mg mL^{-1} of 5-bromo-4-chloro-3-indolyl β-D-GlcUA. The staining reaction was incubated at 37°C in darkness for 30 h. Seedlings were washed three times with water and analyzed with Nomarski optics using a microscope (Optiphot, Nikon, Tokyo).

Confocal Microscope Analyses

Etiolated seedlings were stained with 1 μg mL^{-1} of propidium iodine and immediately after washing they were analyzed with a laser confocal microscope (CoMOS 7 operating software, Bio-Rad, Hercules, CA). Optic sections of subepidermal layers were chosen for quantitative analyses. The images were further processed in Adobe Photoshop 5.0 (Adobe Systems, Mountain View, CA); nuclei from the same layer were painted with the same color. In optic sections with an increased laser beam it was shown that each cell in the hook contains one nucleus, unless cell division occurred.

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