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Cell Elongation and Microtubule Behavior in the *Arabidopsis* Hypocotyl: Responses to Ethylene and Auxin

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Abstract

During elongation of the *Arabidopsis* hypocotyl, each cell reacts to light and hormones in a time- and position-dependent manner. Growth in darkness results in the maximal length a wild-type cell can reach. Elongation starts at the base and proceeds in the acropetal direction. Cells in the upper half of the hypocotyl can become the longest of the whole organ. Light strongly inhibits cell elongation all along the hypocotyl, but proportionally more in the upper half. The ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) is known to stimulate hypocotyl elongation in the light. Here we show that this stimulation only occurs in cells of the apical half of the hypocotyl. Moreover, ACC application can partially overcome light inhibition, whereas indole-3-acetic acid (IAA) cannot. On low-nutrient medium (LNM) in the light, elongation is severely reduced as compared to growth on rich medium, and both ACC and IAA can stimulate elongation to the levels reached on a nutrient-rich medium.
Furthermore, microtubule orientation was studied *in vivo*. During elongation in darkness, transverse and longitudinal patterns are clearly related with rates of elongation. In other conditions, except for the association of longitudinally orientated microtubules with growth arrest, microtubule orientation is merely an indicator of developmental age, not of elongation activity. A hypothesis on the relation between microtubules and elongation rate is discussed.

Key words: *Arabidopsis thaliana*; Auxin; Ethylene; Elongation; Hypocotyl; Microtubule; Tubulin

Introduction

Throughout the life of a plant the control of post-mitotic cell elongation is an important actor in the process of morphogenesis. The *Arabidopsis* hypocotyl is widely used as a model for physiological, cellular, and molecular studies of the mechanism of

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cell expansion. Indeed, the postembryonic growth of the *Arabidopsis* hypocotyl is mainly the result of cell elongation, because there is no significant cell division in the cortex or epidermis (Wei and others 1994; Gendreau and others 1997; Smalle and others 1997; Collett and others 2000; Saibo and others 2003). The hypocotyl epidermis has a simple structure with parallel cell files, each with approximately 20 cells. The effect of environmental signals on hypocotyl length is mediated by plant hormones regulating cell elongation (Reid and Howell 1995; vonArnim and Deng 1996; Smalle and others 1997; Gray and others 1998; Saibo and others 2003). Nearly all known plant hormones have been implicated in the control of hypocotyl elongation (reviewed in Vandenbussche and others 2005). Among these, auxin has the longest history as a cell elongation stimulator. Elevated endogenous auxin levels can stimulate hypocotyl elongation in *Arabidopsis* seedlings (Boerjan and others 1995; Gray and others 1998; Romano and others 1995). The effects of exogenous auxin on whole plants and its interaction with ethylene (Smalle and others 1997; Collett and others 2000; Wei and others 2000) have led to ambiguous results. Application of auxin did not promote hypocotyl elongation on normal Murashige and Skoog medium; in fact, it even inhibited elongation (Collett and others 2000), whereas it stimulated hypocotyl elongation on low nutrient medium (Smalle and others 1997; Saibo and others 2003). In pea, Barratt and Davies (1996) found that exogenous auxin stimulated growth, though only at a certain developmental stage. Elongation was inhibited by exogenous auxin at the early-expansion stage, but it was promoted at mid-expansion. It has been suggested that the effects of exogenous auxin depend on the variable endogenous auxin levels and the changes in auxin sensitivity over time. Ethylene can also both inhibit and promote cell elongation (Abeles 1992). In darkness, ethylene inhibits etiolated hypocotyl elongation as part of the well-known ''triple response'' (Knight and Crocker 1913). Conversely, in the light, ethylene promotes hypocotyl elongation of seedlings, particularly when grown on low-nutrient medium (Smalle and others 1997). Interactions of ethylene with gibberellins, auxins, and brassinosteroids can result in maximal elongation rates (Saibo and others 2003; Vandenbussche and others 2003; De Grauwe and others 2005). Gendreau and others (1997) studied *Arabidopsis* hypocotyl elongation at the cellular level. They found that cells within a given cell file had both a successive timing of initiation and a different extent of elongation from base to apex.

Apart from a clear implication of endoreduplication (Sugimoto-Shirasu and Roberts 2003), cell expansion results from a temporary imbalance between the extensibility of the cell wall and the turgor pressure inside the cell (Green 1980; Cosgrove 1993; Darley and others 2001). The orientation of cellulose microfibrils determines eventual anisotropy in the mechanical characteristics of the wall (Kerstens and others 2001), and is often related with anisotropic cell expansion or elongation (Green 1980; Niklas and others 1995; Verbelen and Kerstens 2000; Verbelen and others 2001). In view of the presumed role of microtubules in guiding cellulose synthase complexes (Giddings and Staehelin 1991), cortical microtubules have been considered to control the direction of expansion. Consequently, plant hormones were postulated to regulate cell elongation and plant morphology by changing the orientation of microtubules (Roberts and others 1985; Shibaoka 1994; Nick 1998, Foster and others 2003; Knowles and others 2005). The successful expression of the GFP-TUA6 construct (α-tubulin) under control of the 35S promoter in *Arabidopsis* hypocotyls (Ueda and others 1999) provides a tool to reveal *in vivo* the role of microtubules in events between a regulatory (hormone) signal and the resulting change in elongation rate. In this article, we present data on the position- and time-dependent cell elongation in *Arabidopsis* hypocotyls treated with ACC and auxin. In addition, we investigated the relation between elongation and microtubule orientation using a GFP-TUA line.

Materials and Methods

Plant Materials and Growth Conditions

Arabidopsis ecotype Columbia (Col-0) and ethylene mutants, *etr1-3* were obtained from the Arabidopsis Biological Resource Center (ABRC). GFP-TUA6 was constructed for constitutive expression of a fusion of the smGFP and *Arabidopsis thaliana* α-tubulin 6 proteins (Ueda and others 1999). Seeds were surface sterilized in 6% (v/v) commercial bleach for 15 min and rinsed five times with sterile distilled water. For experiments on rich medium, seeds were germinated on a half-strength Murashige and Skoog medium including vitamins (Duchefa, The Netherlands), supplemented with 10 g L^{-1} sucrose and solidified with 4 g^{-1} Gelrite (Duchefa, The Netherlands) at pH 5.7. Low-nutrient medium (LNM) was prepared by using mineral water (SPA, Belgium) and 8 g L^{-1} agar at pH 5.8. After incubation overnight at 4°C, the dishes were placed vertically in a

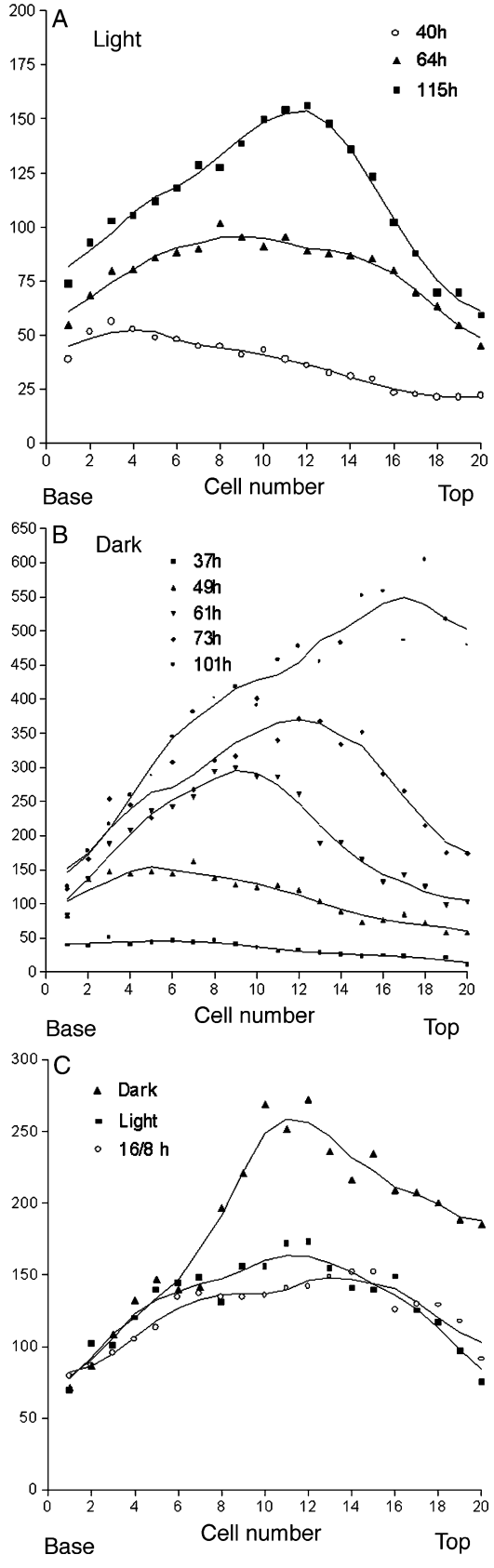

Figure 1. **A.** Cell elongation profiles in hypocotyls of light-grown GFP-TUA6 plants on ½ Murashige and Skoog (MS) medium. Cells from base to top are numbered from 1 to 20. Time (h) indicates time after transfer into the growth chamber. **B.** Cell elongation profiles of dark-grown GFP-TUA6 plants on ½ MS medium. Cells exhibit a much more pronounced elongation in darkness as compared to light-grown seedlings. The strongest elongation occurs at the top of the hypocotyl. **C.** Cell elongation in hypocotyls of GFP-TUA6 seedlings under different light conditions (½ MS). After three cycles of 16 h/8 h of light/dark, seedlings are given continuous light or darkness for another 3 days. The cell length profile is not changed by continuous light treatment. However, 3 days of continuous darkness results in promotion of elongation in the upper half of the hypocotyl.

growth chamber at 23° ± 2°C in a 16 h/8 h (light/dark) photoperiod under 40 μmol m^{-2} s^{-1} Photosynthetic Photon Flux Density (PPFD) illumination. For growth in continuous darkness, Petri dishes were wrapped in three layers of aluminum foil.

ACC, Auxin, and Anti-microtubule Treatments

Stock solutions of 1-aminocyclopropane-1-carboxylic acid (ACC, Sigma) were made in water; those of indole-3-acetic acid (IAA, Sigma) and propyzamide (Alltech Associates, Inc., Belgium), in dimethylsulfoxide (DMSO). The compounds were added to the autoclaved medium after filter sterilization, to yield final concentrations of 10 or 50 μM ACC, 5, 10, or 50 μM IAA, and 20 μM propyzamide.

Cell and Hypocotyl Length Measurement

Measurements of cell and hypocotyl lengths were performed with a Nikon DXM1200 digital camera or Bio-Rad MRC-600 confocal laser scanning microscope mounted on a Zeiss Axioskop. Cell lengths were measured in 3 to 5 cell files per hypocotyl, in a total of at least 20 hypocotyls. Each experiment was independently repeated at least once. Cell and hypocotyl lengths were measured on the images using the ScionImage program, which is freely available on the World Wide Web at http://www.scioncorp.com. The results were analyzed and smoothed using the software of GraphPad Prism (http://www.graphpad.com). Data represent averages of all measurements.

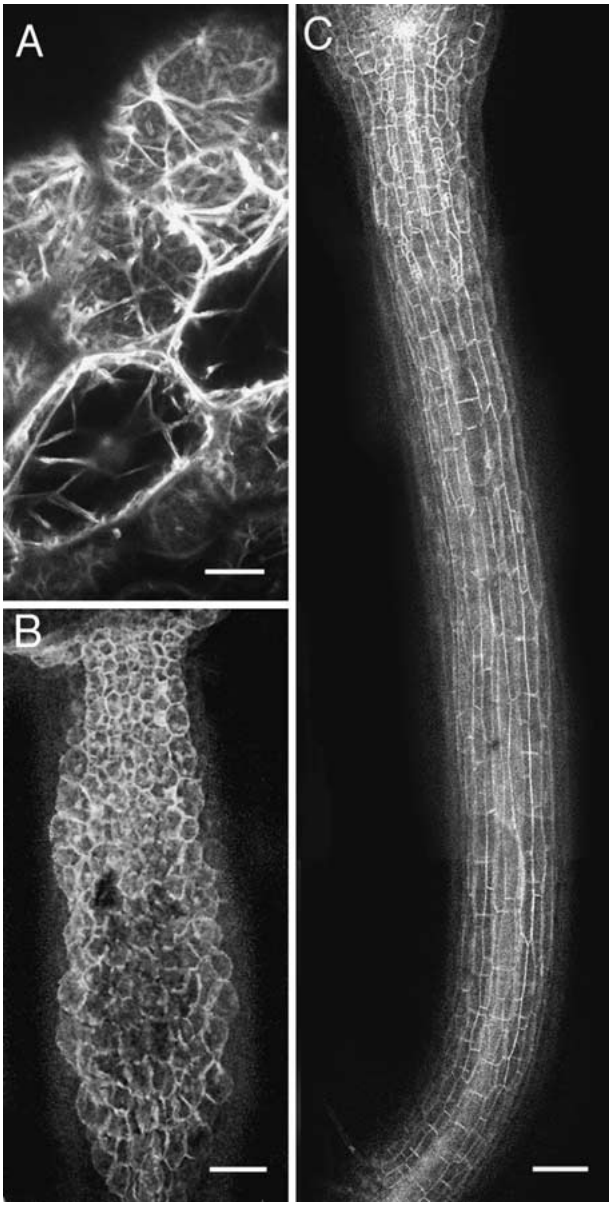

Figure 2. Intact microtubules play a crucial role in hypocotyl elongation. Seedlings were grown on ½ MS. **A.** Cortical microtubules are disrupted by propyzamide, and fluorescence is diffuse all over the cytoplasm. **B.** Propyzamide-induced isotropic expansion of cells results in a stunted hypocotyl (3 days). **C.** Control plant. Scale bar = 20 μm in **A**; 100 μm, in **B** and **C**.

Observation of Cortical Microtubules in Living Cells

Microtubules were observed with a Bio-Rad MRC-600 confocal laser scanning microscope using the BHS filterset (excitation 488 nm, beamsplitter 510 nm and emission 515 nm). All the images were collected within 20 min after removal of the plants from the growth chamber.

Results

Both light- and dark-grown GFP-TUA6 seedlings have the same characteristics of hypocotyl cell growth as the wild-type (Figure 1A and Figure 10; Ueda and others 1999). Using GFP-TUA6 therefore not only allows microtubule orientation to be examined *in vivo*, but also facilitates cell size measurement as cell boundaries are easily discerned. The earliest measurements were made after 40 h. At that time, the hypocotyls had a length of 0.85 ± 0.10 mm, and all epidermis cells had a length of between 25 and 50 μm. At 115 h, cell length varied between 75 and 150 μm, with the longest cells occurring in the middle of the hypocotyl (cells 10–12, Figure 1A; Gendreau and others 1997).

At the base of the hypocotyl, cells attained a length of 150 μm in darkness, compared to 80 μm in the light. However, the apical cells (cells 15–20) showed the strongest elongation during skotomorphogenesis (Figure 1B). They reached a length of 500 μm, whereas they grew only to 70 μm in the light.

This stimulation of elongation by darkness can be induced at any moment during hypocotyl growth in the light until maximal elongation has been reached. After 3 days of cycles of 16 h light and 8 h darkness, seedlings were given continuous darkness or light. Three days later, cell lengths were measured (Figure 1C). Darkness strongly promoted cell elongation in the top two thirds of the hypocotyl, from cell 8 upwards. The elongation of cells in the basal part of the hypocotyl was not affected. It is also noteworthy that switching to continuous light had no impact on cell elongation as compared to seedlings grown in light/dark cycles.

Microtubule Orientations in Hypocotyl Cells Correlate with Different Elongation Activities in Light and in Darkness

The crucial role of cortical microtubules in both photomorphogenesis and skotomorphogenesis of the hypocotyl was confirmed by germinating and growing seedlings on propyzamide. Cortical microtubules were disrupted and dissociated tubulin subunits resulted in diffused fluorescence all over the cytoplasm (Figure 2A). Epidermis cells expanded isotropically, yielding a stunted, radially expanded hypocotyl in 3-day-old seedlings (Figure 2B), in comparison with normal hypocotyls (Figure 2C). The same impact was noted in dark-grown hypocotyls (data not shown).

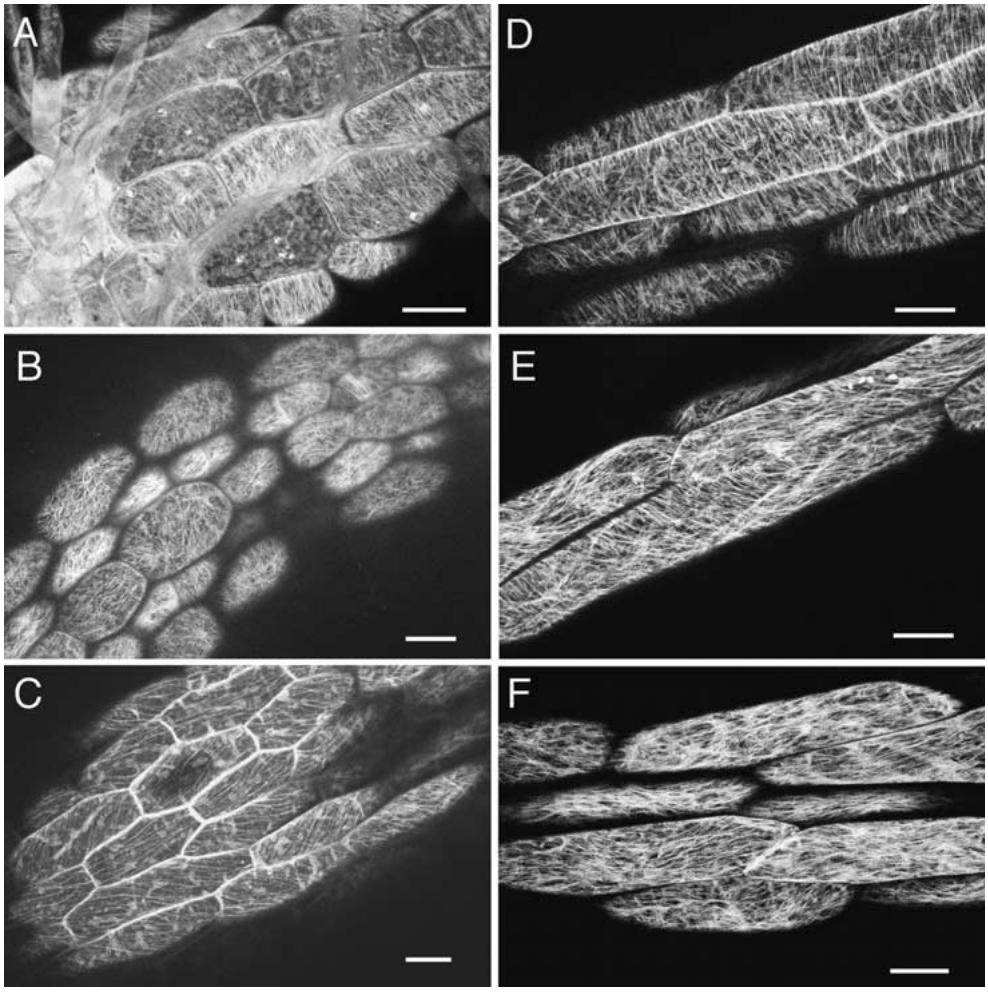

Figure 3. **A** and **B.** Microtubule orientation in light-grown GFP-TUA6 seedlings on ½ MS at day 3 (48–72 h after transfer into growth chamber) **A.** Transverse microtubules can be found in elongating cells at the base. **B.** Various microtubule orientations in the middle and at the top of the hypocotyl. **C–F**. Microtubule orientation in a hypocotyl of a 5-day-old light-grown GFP-TUA6 seedling on ½ MS. **C.** Longitudinal to oblique microtubules in basal cells. **D–F**. In the upper part of the hypocotyl, various types of microtubule patterns (transverse, oblique, and longitudinal) can be observed. Scale bar = 20 μm.

Because GFP-TUA6 was poorly expressed in the hypocotyls during the first 2 days, the orientation of microtubules was investigated from day 3 onward (48–72 h after transfer into the growth chamber). When seedlings were grown for 3 days in the light on rich medium, ½ Murashige and Skooge (MS/2), microtubules were transverse in the elongating cells at the base (Figure 3A) and had a randomized orientation in the upper part of the hypocotyl (Figure 3B). At day 5, microtubules in the basal part had a longitudinal or steeply oblique orientation (Figure 3C), whereas in the upper part they were organized in different patterns. Transverse, oblique, and longitudinal orientations (Figure 3D, Figure 3E and Figure 3F) occurred simultaneously in a population of seedlings, indicating the highly variable character of the microtubule population in these cells in the light.

In 3-day-old plants grown in darkness, the cortical microtubules in the cells at the base of the hypocotyl (cells 1 to 7) were longitudinal, parallel to the long axis of the cell (Figure 4A). In the middle (cells 8–14) and at the top (cells 15–20) of the hypocotyl, the microtubules were predominantly transverse to the long axis of the cell (Figure 4B and Figure 4C). During the following days, basal cells showed fewer and random microtubules, including longitudinally oriented microtubules, whereas cells in the middle switched from a transverse to a longitudinal orienta-

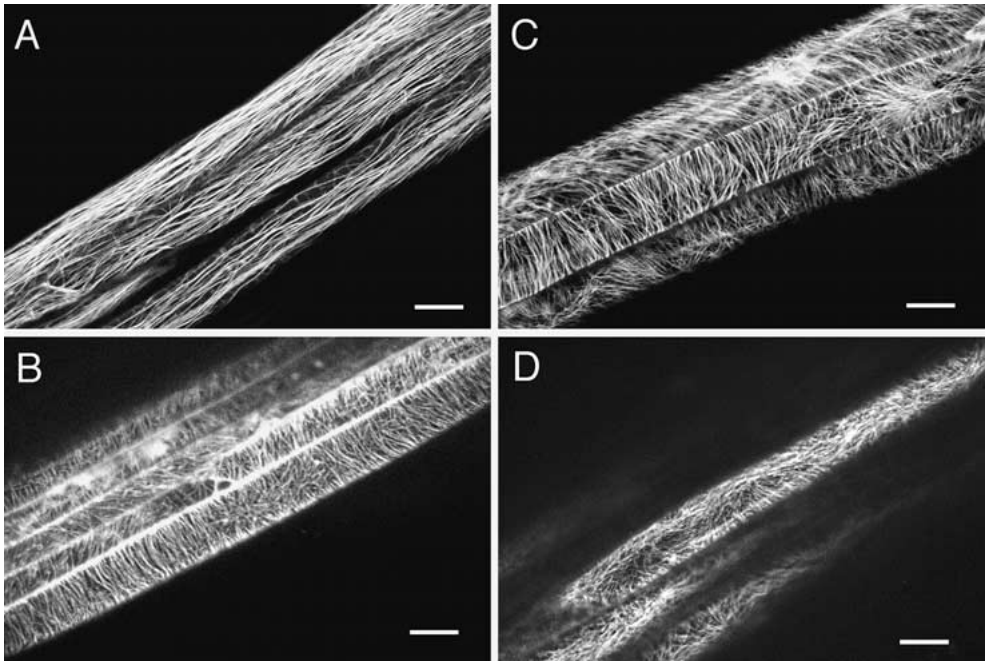

Figure 4. Microtubule orientation in hypocotyls of dark-grown GFP-TUA6 seedlings on ½ MS medium. **A.** Longitudinal microtubules in the cells at the base. **B** and **C**. Transverse aligned microtubules in cells in the middle and at the top. **D**. Microtubule reorientation from transverse to longitudinal during exposure to white light. Scale bar = 20 μm.

tion, and transverse microtubules were maintained in the cells at the top (data not shown). Hence, the reorientation developed in the acropetal direction. The number of cells with transverse microtubules decreased continuously. When etiolated plants were exposed to white light, an inhibitor of extension growth, the microtubule orientation at the base of the hypocotyl was maintained (data not shown). However, in the upper part, light induced a microtubule rearrangement from transverse into oblique and longitudinal (Figure 4D) within 50–70 min of exposure, as reported by Ueda and Matsuyama (2000).

Another well-known inhibitor of elongation growth in skotomorphogenic seedlings is ethylene. Four and 5-day old seedlings grown in the presence of the ethylene precursor ACC, had a relatively smaller zone at the hypocotyl top (below the apical hook), showing a transverse microtubule orientation. Further, when compared with untreated seedlings (Figure 5A and Figure 5B), the ACC-treated plants had a higher percentage of longitudinally oriented microtubules in the middle and near the top (below the apical hook) (Figure 5C and Figure 5D). We therefore conclude that there is an association between longitudinal orientation of microtubules and diminished growth. On day 7, when maximal elongation was reached (Gendreau and others 1997) both untreated and ACC-treated seedlings showed predominantly randomized orientation of microtubules along the hypocotyl, with longitudinal orientated microtubules at the top (data not shown).

Overall, these results clearly support the fact that microtubule orientation is correlated with cell-elongation activity, in light or in darkness, as well as upon ACC treatment in darkness.

Both IAA and ACC Can Stimulate Elongation of Hypocotyl Cells, Although with Clear Positional Differences

Contrary to the situation in dark-grown seedlings, Smalle and others (1997) reported that both exogenous ethylene (or its precursor ACC) and auxin were capable of promoting hypocotyl elongation of seedlings grown on LNM (see *Materials and Methods*) in the light. As shown in Figure 6A, the growth of the hypocotyl on LNM was not as vigorous as on ½ MS medium. Except for the cells at the base of the hypocotyl, elongation was greatly reduced (Figure 6A, compared with Figure 1A). Low-nutrient medium was supplemented with 50 μM ACC and 5 μM IAA, saturating concentrations for the hypocotyl elongation response (Smalle and others 1997;

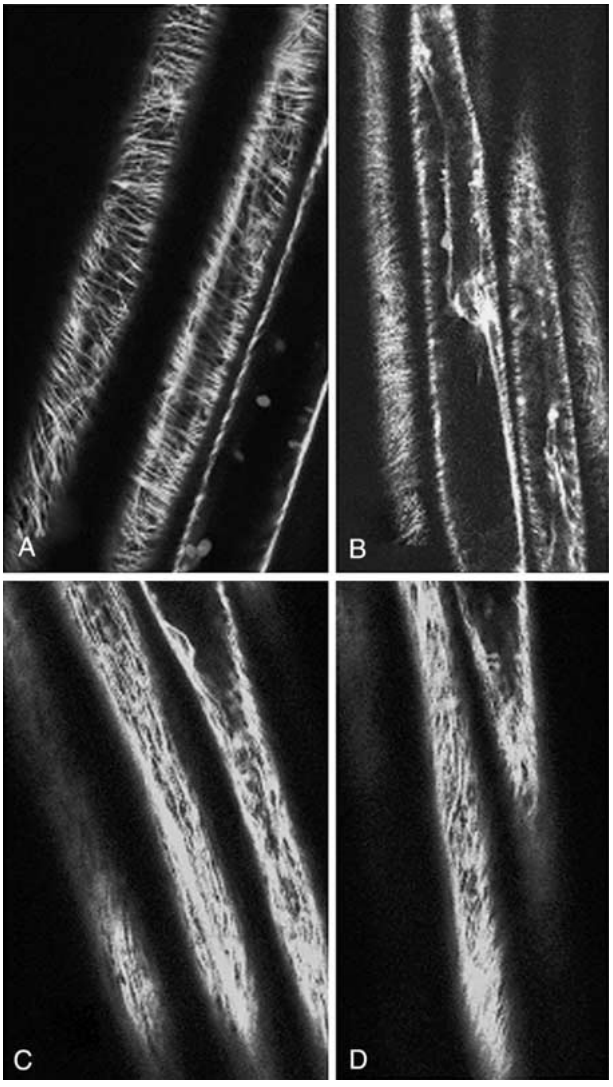

Figure 5. Microtubule orientation in hypocotyls of dark-grown GFP-TUA6 seedlings grown on ½ MS medium without (**A** and **B**) or with exogenously added 50 μM ACC (**C** and **D**). **A** and **C**. Microtubules in the cells in the middle part of the hypocotyl. C and D. Microtubules in the cells in the upper part of the hypocotyl.

Vandenbussche and others 2003). Both auxin and ACC could restore cell elongation to a level similar to that on medium containing MS salts, although with a clear positional difference. On day 5, seedlings treated with ACC were clearly more stimulated, in the basal and central regions, as compared to IAA-grown seedlings where the ''elongation wave'' moved faster from base to top (Figure 6B and Figure 6C compared with Figure 1A). Hence, the cell elongation profiles of ACC-treated hypocotyls exhibited a delay of 2 days compared to the IAA-treated plants, although ultimately the same hypocotyl length was attained. This implies that the initial rate of elongation is faster in IAA-treated hypocotyls than in ACC-treated ones. Smalle and others (1997) demonstrated that the stimulatory effect of ACC or ethylene on hypocotyl growth was also visible on rich (MS/2) medium, whereas this was not the case for auxin (Collett and others 2000). This additional stimulation upon ACC treatment on rich medium (Figure 7) results from cell elongation responses in the upper half of the hypocotyl, which is similar to the effect on LNM. The lower half of the hypocotyl is clearly not involved in the response to exogenous ACC.

Upon Hormone Treatment of Light-grown Seedlings, Changes in Microtubule Patterns Are Not Unambiguously Correlated with Growth

On LNM in the light, hypocotyl growth is strongly reduced as compared to rich medium. Nevertheless, microtubules again adopt specific temporal and positional patterns. For instance, at day 5, microtubules were transverse to slightly oblique at the top and in the central zone (Figure 8A and Figure 8B), yet they were longitudinal to steeply oblique at the base of the hypocotyl (Figure 8C). In IAA-treated seedlings of the same age, similar orientations were observed (for example, the middle part in Figure 8D). However, transverse microtubules dominated the cells in the middle part of the hypocotyls in ACC-treated seedlings (Figure 8E). At day 7 microtubules were longitudinal to very oblique in the three batches of plants that were investigated (data not shown). This again supports an association of the presence of longitudinally oriented microtubules and growth arrest, and of non-longitudinal orientation and growth potential.

On rich (MS/2) medium, microtubule orientation showed similarities to that of LNM grown seedlings. As demonstrated in Figure 3, cells at the top (Figure 9A) and in the middle (Figure 9B) of the hypocotyl contained numerous microtubules showing various orientations from transverse to slightly oblique. In contrast, cells at the base of the hypocotyl had relatively few microtubules, with a steeply oblique to longitudinal orientation (Figure 9C). Treatment of the seedlings with auxin did not affect microtubule orientations (data not shown). However, when seedlings were treated with ACC, microtubules were predominantly longitudinal in the central zone (Figure 9E) and oblique at the top (Figure 9D), indicating an association of a non- transverse orientation with elongating tissue in the upper half of the hypocotyl.

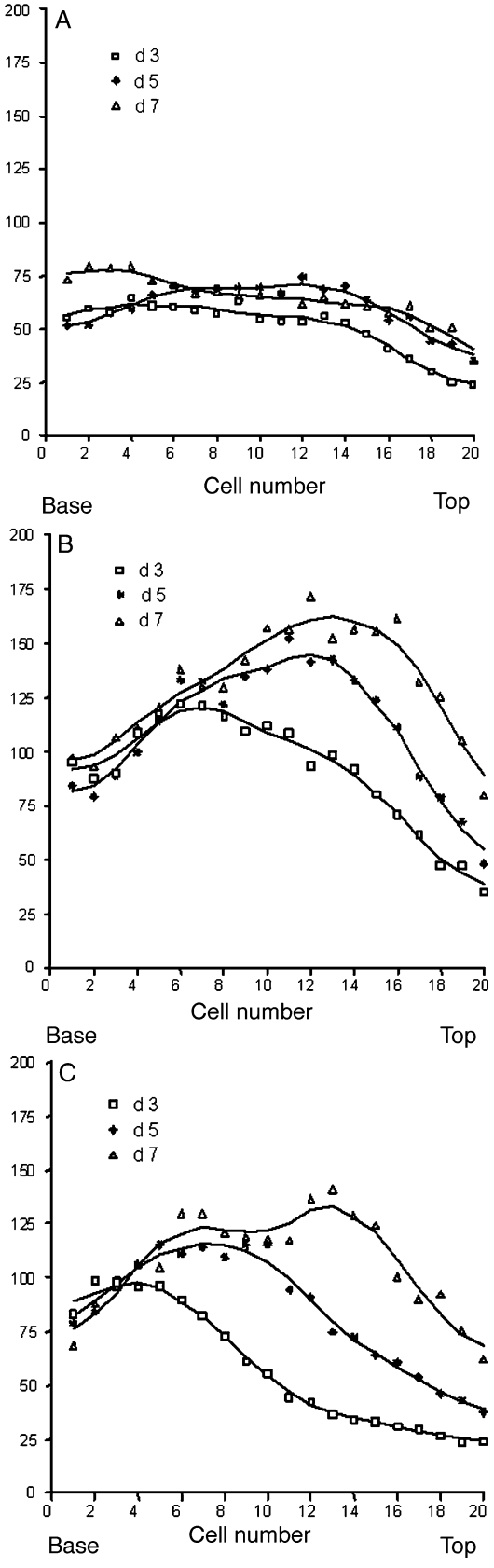

Figure 6. Cell elongation patterns of light-grown seedlings on low-nutrient medium (LNM) and LNM supplemented with hormones. **A.** On LNM, cell elongation is blocked, especially in the middle and top region of the hypocotyl. **B.** On IAA-supplemented LNM, cell elongation is fully restored to the cell length profile obtained on ½ MS medium. **C.** On ACC-supplemented LNM, cell elongation was also restored, but this restoration was delayed by 1 or 2 days.

Discussion

Regulation of Hypocotyl Cell Elongation Potential by Light and Hormones

In the light, initial elongation is found at the hypocotyl base. However, the cells in the middle (cells 10–13) elongate maximally and can reach a length of about 150 μm. The cells of the upper part of the hypocotyl (cells 14–20) elongate substantially, but never exceed this length. Remarkably, the cells in the upper half of the hypocotyl are most responsive to exogenous hormones. On MS/2 medium, ACC can considerably promote elongation in these cells whereas under IAA they only reach the size of untreated controls. In contrast, on LNM in the light, both ACC and IAA can rescue cell elongation, and the cells reach the size obtained on normal medium.

Hypocotyl elongation in darkness is however fundamentally different. Darkness promotes elongation in all cells, but the acropetal gradient in cell elongation is extremely well expressed as the cells at the top reach the longest final size. In the lower half of the hypocotyl however cells can grow to about 2.5 times the size they reach in the light, whereas in the upper half this is even more pronounced and cells become longer the closer they are to the top. Cells 16–20 are 6–7 times longer in darkness than they are in the light. The promotive effect of darkness can be induced also after a period of development in the light. Only the cells that are still capable of responding at the onset of darkness will further elongate. These cells and hence the entire hypocotyl will reach a size between that found in dark grown and in light grown plants, respectively.

The study of elongation of individual cells allows us to draw a detailed picture of elongation control on the cellular level (Gendreau and others 1997). In the light, the longest cells occur in the middle of the hypocotyl. Light exerts the strongest inhibition on cell elongation at the top of the hypocotyl. Ethylene can partly restore this light mediated inhibition. It is remarkable that in darkness, the same top cells form

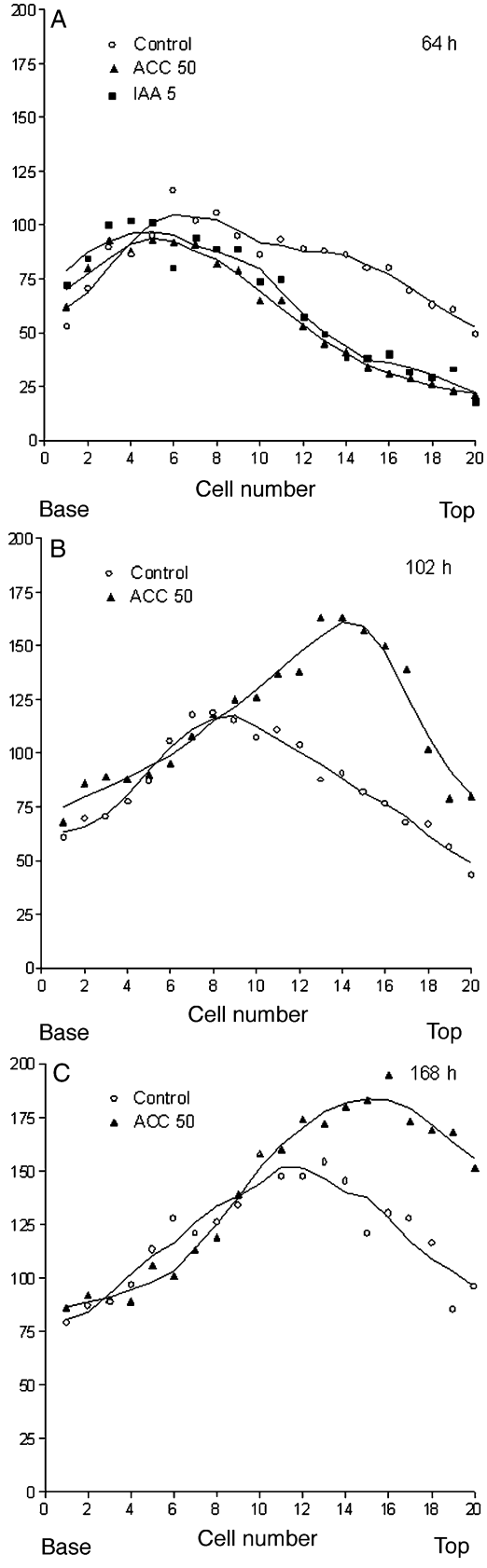

Figure 7. Cell elongation profiles as affected by exogenous ACC in wild-type seedlings grown on ½ MS in the light. **A.** At 64 h. **B.** At 102 h. **C.** At 168 h.

the apical hook, which is exaggerated in the presence of ethylene (Gendreau and others 1997; Bleecker and others 1988; Raz and Ecker 1999). Differential growth in the apical hook results from a differential auxin signal (Friml and others 2002). This could be a permissive signal. Ethylene could be a modulator of the severity of the phenotype, in concert with gibberellins and brassinolide (Vriezen and others 2004; De Grauwe and others 2005). Hence, both in light and in darkness, ethylene stimulates the growth of the apical cells.

Involvement of Microtubules in Hypocotyl Growth

It is widely accepted that cortical microtubules are intimately related with cell elongation through their presumed role in the orientation of the cellulose fibrils and cellulose fibril arrays, and hence in the mechanics of the cell wall (Baskin 2000; Baskin and others 2004). Auxin-promoted elongation was found to be preceded by a reorientation of microtubules into transverse arrays (Bergfeld and others 1988; Nick and others 1990; Sakoda and others 1992) and may be linked with orientation dependent turnover (Wiesler and others 2002). However, the relation between microtubules and wall extension has been frequently questioned (Zandomeni and Schopfer 1993; Emons and Mulder 2000; Verbelen and others 2001), especially in elongation zones of the *Arabidopsis* root (Wiedermeier and others 2002; Sugimoto and others 2003; Baskin and others 2004).

Given the seemingly contradictory data on the hormonal regulation of cell elongation in the hypocotyl and the existing criticism on the role of microtubules in that process, we reassessed this topic. For this purpose we used *Arabidopsis* plants expressing the GFP-TUA6 fusion construct, resulting in green fluorescent microtubules (Ueda and others 1999).

Etiolated GFP-TUA plants exhibit patterns of microtubules generally considered as ''normal'' (Wasteneys 2004): they are transverse in elongating cells and longitudinal in cells that have stopped elongation. This also holds true for hypocotyls of ACC treated dark grown seedlings. In the light, not only transverse but also other orientations were found in untreated as well as in ACC-stimulated elongating cells on rich medium, and even in non elongating cells on LNM. Therefore, in plants grown in the light, apart from the presence of longitudinally oriented microtubules as a sign for elongation-arrest, microtubule orientations as seen *in vivo*

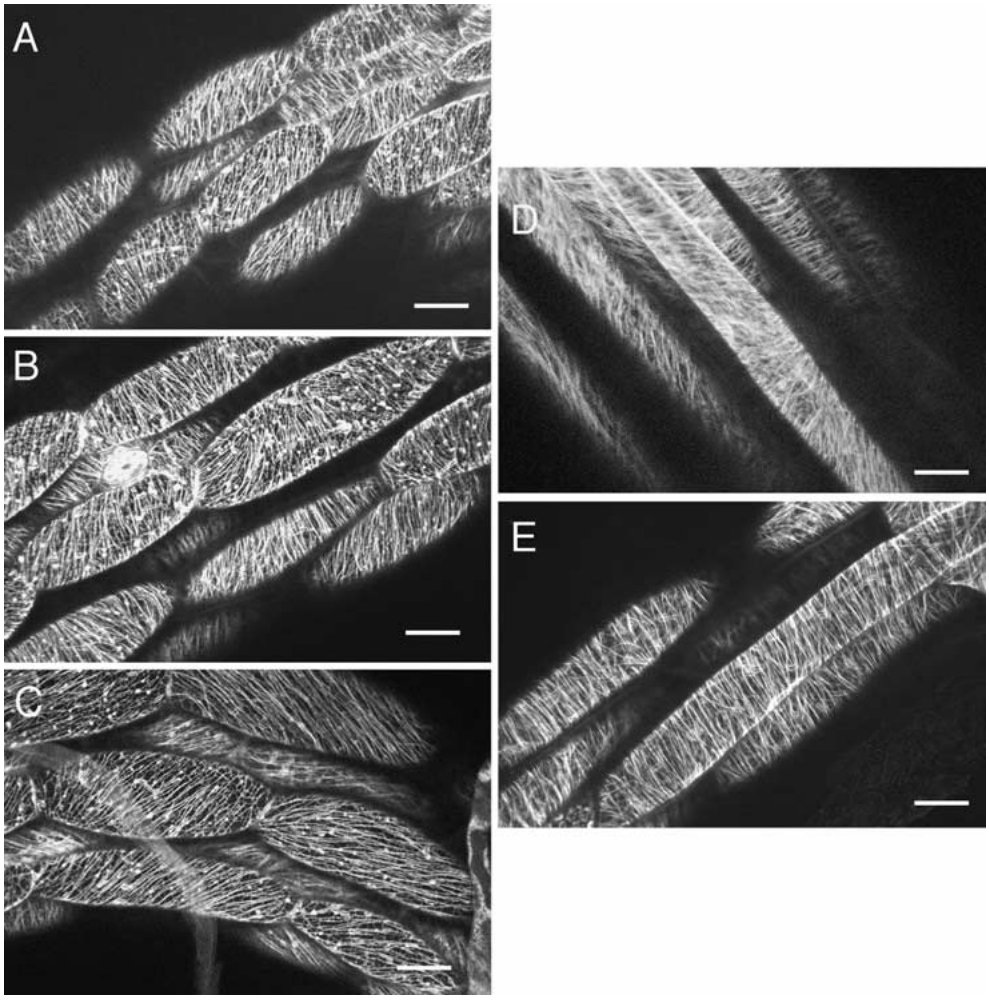

Figure 8. Microtubule pattern at day 5 in hypocotyls of GFP-TUA6 seedlings grown on LNM. **A–C**, from top to base. Transversely organized microtubules often appear in the middle and at the top. **D** and **E.** Transverse microtubule orientation in the middle part of GFP-TUA6 hypocotyls on IAA-supplemented (**A**) or ACC-supplemented (**B**) LNM at day 5. Scale bar = 20 μm.

cannot be unambiguously related with cell elongation rate. Along the same line, transcript levels of many tubulin and tubulin like genes are significantly upregulated in dark grown plants, whereas only a few are regulated by hormone treatments in the light (Vandenbussche and Van Der Straeten unpublished results; Leu and others 1995). In view of the growth rate of hypocotyls in darkness, this increase in tubulin production, together with the optimal spreading of cortical microtubules perpendicular to the cell axis, may be the only way to keep up with the extreme stretching of cells.

Our observations lead us to propose the following hypothesis. In cells with potential to elongate, there is always a rather constant pool of cortical microtubules present. When the elongation rate is high as for instance in darkness, the wall synthesis activity at the cell membrane recruits most available cortical microtubules in transverse arrays. When the rate of cell elongation is much lower as is the case in the light, with or without auxin or ethylene, or practically zero, as on LNM, a smaller fraction of the microtubule pool is recruited. The remainder of the microtubules can freely change orientation. This would explain the variety of orientations seen in the light on rich medium. When cells are about to stop elongation, their microtubules switch to a longitudinal position. Finally, when cells no longer have the possibility to elongate (for example, an aged cell at the base) the microtubules have a longitudinal orientation but are often reduced in number. In addition, other processes than microtubule orientation could be more determinant in stimulating cell elongation. Our future research will focus on the

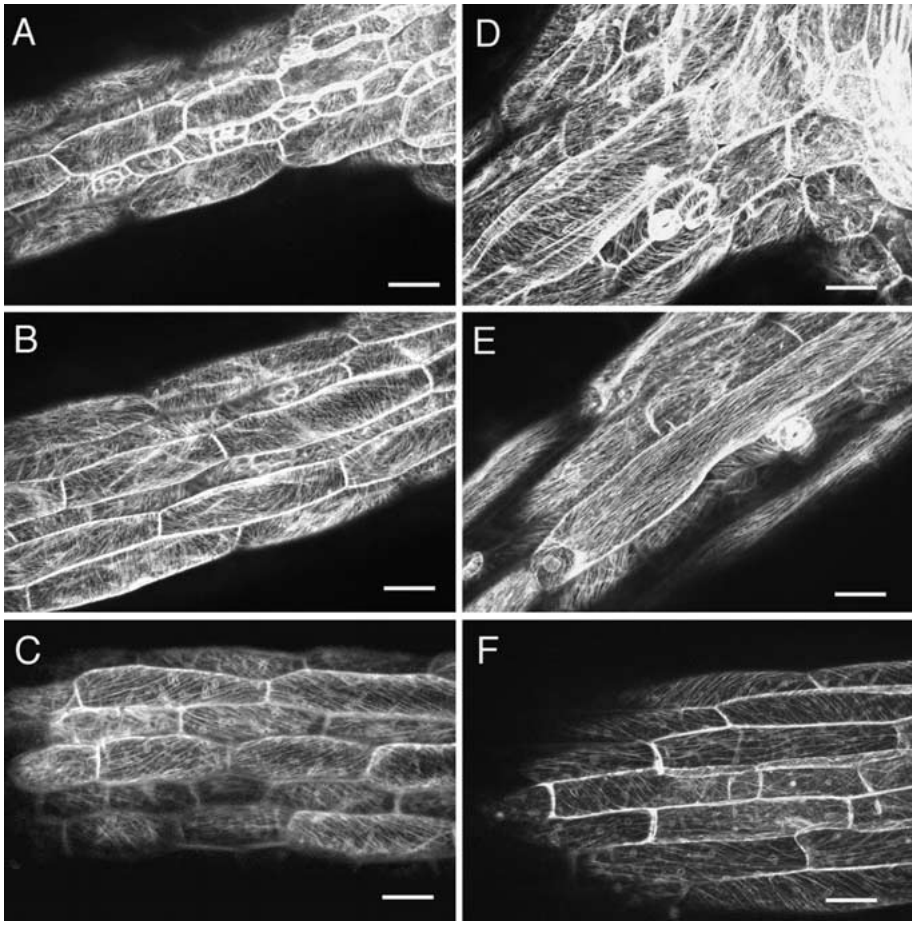

Figure 9. Effect of hormones on microtubule orientation in hypocotyls of light-grown GFP-TUA6 seedlings (4-day-old, ½ MS). **A–C.** Microtubule orientation in a control plant, from top to base. **D–F.** Oblique and longitudinal microtubules in most cells of ACC-treated hypocotyls, from top to base. Scale bar = 20 μm.

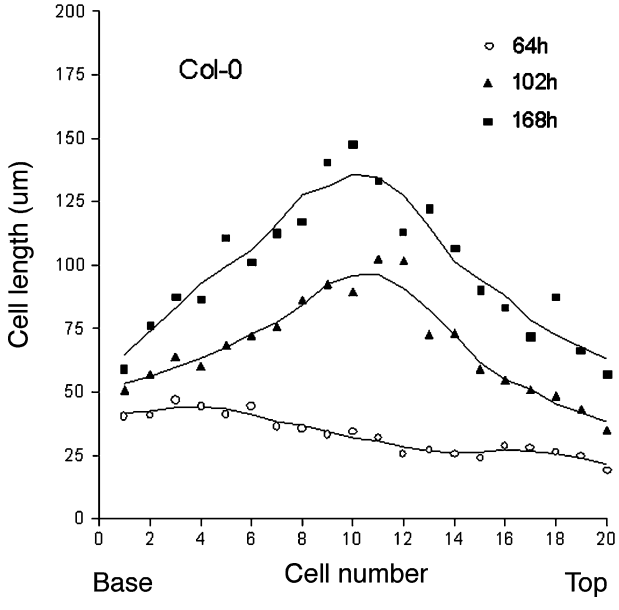

Figure 10. Cell elongation profiles in hypocotyls of light-grown Col-0 wild-type plants on ½ MS medium. Cells from base to top are numbered from 1 to 20. Time (h) indicates time after transfer into the growth chamber.

regulation of turgor pressure and of other cell wall restructuring enzymes, such as xyloglucan endo transglycosylases/hydrolases.

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