Position and cell type-dependent microtubule reorientation characterizes the early response of the Arabidopsis root epidermis to ethylene

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The involvement of cortical microtubules in the control of plant cell expansion was studied in the Arabidopsis root epidermis. In the zone of fast elongation microtubules were transverse to the root axis in all epidermal cells. However when cells entered the differentiation zone cell type-specific microtubule reorientation took place. In the trichoblasts that were then approximately 130 μm long and formed the root hair bulge, the microtubules switched to a random distribution. In the adjoining atrichoblasts microtubules adopted a slightly oblique orientation. In more proximal parts of the differentiation zone atrichoblast microtubules were found in a more oblique and finally in a longitudinal orientation. Upon exposure to ethylene or 1-aminocyclopropane-1-carboxylic acid (ACC – the precursor of ethylene) at a saturating dose, cell elongation abruptly stopped. From then on trichoblast cells reached only a length of about 35 μm, and developed root hairs. Cortical microtubules changed orientation within 10 min. In trichoblasts they adopted the typical random orientation, in atrichoblasts however, they took up a longitudinal orientation. Microtubule reorientation was complete within 60 min. The possible role of microtubules in the control of cell elongation is discussed.

Introduction

One century after Neljubow's discovery of the effect of illuminating gas on pea seedlings (Neljubow 1901), the effect of ethylene on plant development is well documented. In natural conditions, environmental factors induce changes in the endogenous ethylene concentration (e.g. Jackson 1991, Abeles et al. 1992) and thus affect plant development. Additionally, in root growth ethylene plays a key-regulatory role. To understand the influence of ethylene on root morphogenesis, it is not only important to know the general effect, but also the timing and the cellular basis of the response (Whalen and Feldman 1986). An increase in ethylene concentration inhibits root elongation, induces ectopic root hair formation and stimulates radial expansion (Abeles et al. 1992, Dolan 1997, Smalle and Van Der Straeten 1997). These responses are however, not visible within the first 6 h of ethylene treatment.

Occasionally rapid responses have been reported (Eisinger 1983). For example, elongation in decapitated pea epicotyls responds to ethylene within 7 min (Warner and Leopold 1971) and in maize roots within 20 min (Whalen and Feldman 1986). In a previous study we have shown that in Arabidopsis primary root the early response to ethylene is established in the zone of fast elongation in a very specific way within tens of minutes (Le et al. 2001). The end of fast elongation is marked by onset of root hair formation in the trichoblast cell files. Cell elongation slows down and rapidly stops during further root hair outgrowth. Ethylene or its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) defines in

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Abbreviations – ACC, 1-aminocyclopropane-1-carboxylic acid; LEH, length of the first epidermal cell with visible root hair bulge, or the length of the hair-initiating trichoblast.

a dose-dependent way a new and shorter length for each epidermis cell, beyond which elongation is blocked. Thus, the cell length at which root hairs initiate (the length of the first epidermis cell with a visible root hair bulge or LEH) is reduced. The effect was saturated at $5\,\mu M$ leading to the maximal inhibition of cell elongation with a LEH of $35\,\mu m$. The elongation from $16\,\mu m$, the cell length at the beginning of the elongation zone, to $35\,\mu m$ was not affected by ACC. Control roots had a LEH of about $130\,\mu m$. On a cellular basis, the arrest imposed on elongation is irreversible.

The cell wall is generally considered to play a major role in the regulation of the extent and the direction of cell expansion (Cosgrove 1997, Darley et al. 2001). In the wall the orientation of cellulose microfibrils determines mechanical characteristics of the wall (Kerstens et al. 2001) and is thought to determine the direction of cell expansion (Cosgrove 1993, 1997). In view of the cellulose synthase constraint model (Giddings and Staehelin 1991) the most widely held belief is that microtubules control the directionality of expansion. Their orientation is typically perpendicular to the direction of maximal expansion (Taiz and Zeiger 1998).

Microtubules have been found to reorient during ethylene-induced growth changes (Steen and Chadwick 1981, Lang et al. 1982, Roberts et al. 1985, Shibaoka 1991, 1994). In the early ethylene response of the Arabidopsis root epidermis however, there is only a stop and no change in direction of cell expansion, and in addition this arrest in elongation coincides with root hair outgrowth (Le et al. 2001). In lettuce roots a link between microtubule randomization and root hair initiation has been reported recently (Takahashi et al. 2003). In Arabidopsis the fate of microtubules at the onset of differentiation is scarcely documented and the control mechanisms are as yet unclear (Andème-Onzighi et al. 2002). There is a need for an assessment of the role of cortical microtubules in cell expansion control, especially during the early ethylene response of Arabidopsis roots.

In this paper we report on spatial and temporal changes in the microtubule cytoskeleton during the first 60 min of the ethylene response and relate them to cell elongation and cell type.

Materials and methods

Plant material and growth conditions

Seeds of *Arabidopsis thaliana* (Col-0) were obtained from the Arabidopsis Biological Resource Center (ABRC). Plants were grown under sterile conditions on a half strength Murashige and Skoog medium including vitamins (Duchefa, Haarlem, The Netherlands), supplemented with $10\,g\,l^{-1}$ sucrose, solidified with $4\,g\,l^{-1}$ Gelrite (Duchefa), pH 5.7. After an incubation overnight at $4°C$, the dishes were placed vertically in a growth chamber at $23 \pm 2°C$ in a $16/8\,h$ (light/dark) photoperiod under $40\,\mu mol\,m^{-2}\,s^{-1}$ PPFD illumination.

Measurement of elongation rate and cell length

To measure root elongation rates, the position of the root apex was marked on the culture Petri dishes at known time intervals.

Digital time-lapse images of growing roots were obtained using a Nikon DXM1200 digital camera (Nikon Europe, Badhoevedorp, The Netherlands) mounted on a Zeiss Axioskop microscope (Zeiss Oberkochen, Germany). Cell lengths on the images were measured with Scion Image (http://www.scioncorp.com). The spatial distribution of elongation rates was determined using a modified method of Sugimoto et al. (2000). Relative elemental growth rates (REGRs) were calculated using the following equation: REGR $(h^{-1}) = (\ln x_f - \ln x_i)/t$, where, x_i is the initial length of a cell, x_f is the final length of the same cell, and t is the time in hours between the two measurements. The averages of REGRs at specific distances from the root apex were plotted against the distance from the root apex at the final time point.

ACC treatment

The precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC, Sigma) was dissolved in water as stock solution. It was added to the melted Murashige and Skoog medium after filter sterilization, to result in final ACC concentrations of $5\,\mu M$. Seeds were sown and grown on control and on ACC-supplemented medium for 4 days under the same growth conditions as described above. For short-term treatments, 4-day-old seedlings were transferred to ACC-supplemented medium.

Ethylene treatment

Seedlings were grown on normal Murashige and Skoog medium for 4.5 days and then exposed to ethylene in a glass jar with inlet and outlet. In order to rapidly reach a steady-state ethylene concentration, jars of a volume of $4\,l$ were first flushed with air containing $100\,\mu l\,l^{-1}$ ethylene at a flow rate of $0.25\,l\,min^{-1}$. The desired concentration of $10\,\mu l\,l^{-1}$ was reached after 3 min 20 s. Subsequently, the plants were kept in an atmosphere with a constant ethylene concentration of $10\,\mu l\,l^{-1}$ and a constant flow rate of $0.25\,l\,min^{-1}$. Samples were then taken after 10, 20, 30 and 60 min.

Immunolocalization of microtubules and angle measurement

Whole seedlings were fixed for 3 h in 4% (w/v) paraformaldehyde and 0.5% glutaraldehyde (v/v) in PHEM [60 mM PIPES (1,4-piperazine-diethanesulphonic acid), 25 mM HEPES (N-2-hydroxoyethylpiperazine-N'-2-ethanesulphonic acid), 2 mM EGTA, 2 mM MgSO$_4$, pH 7.0], supplemented with $200\,\mu M$ phenylmethane sulphonyl fluoride (PMSF) and 0.05% Triton X-100 (Sera Laboratory, Crawley Down, UK). After three rinses with PHEM, the seedlings were further incubated in

0.1% NaBH$_4$ to reduce free aldehydes. To allow penetration of the antibodies through the cell wall, the specimens were digested for 25 min with a solution of 0.1% (w/v) pectinase (Serva, Heidelberg, Germany) and 0.05% (w/v) pectolyase (Seishin Pharmaceutical Co., Tokyo, Japan) in PHEM. A wash with phosphate-buffered saline (PBS) preceded cold methanol for 10 min at −20°C. After a 20-min treatment with 1% (w/v) bovine serum albumin, the seedlings were incubated overnight in the primary antibody (monoclonal rat anti yeast tubulin, MAS078c; Sera Laboratory). After four rinses with PBS, they were incubated overnight in the fluorescent secondary antibody (Fluorescein isothiocyanate-conjugated goat anti-rat IgG; Sigma). The roots were then examined with the confocal laser scanning microscope (Bio-Rad MRC-600 on a Zeiss Axioskop; Bio-Rad, Hemel Hempstead, UK) using the BHS filterset (excitation 488 nm, beamsplitter 510 nm and emission 515 nm).

To examine the orientation of individual microtubules, the angle of deviation from the direction transverse to the root axis was measured. Thus a transverse microtubule has an angle of 0°, whereas a longitudinal microtubule has an angle of 90°.

Results

Cell elongation rate and LEH

The distribution of cell elongation activity can be illustrated by plotting the relative elemental growth rates (REGR as defined by Erickson and Sax 1956) along the root. In the zone of fast elongation REGR increased in a basipetal way and was maximal between 750 and 900 μm away from the tip (Fig. 1). At about 900 μm from the tip, the first cells with a root hair bulge were found in the trichoblast cell files (indicated by the arrow in Fig. 1). These cells were then approximately 130 μm long. This stage of cell development is defined as trichoblasts reaching the LEH (length of the first epidermal cell with visible root hair bulge, or the length of the hair-initiating trichoblast), a kinetic parameter of cell elongation in
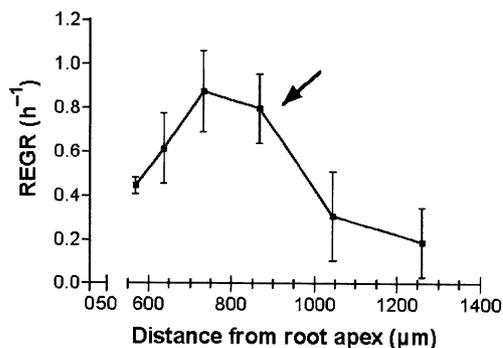

Fig. 1. The spatial distribution of elongation rates in 4-day-old wild-type Arabidopsis roots. Relative elemental growth rates (REGRs) are plotted against the distance from the root apex. Bars represent standard deviation. The arrow indicates the site of the first root hair bulges, the LEH.

root growth (Le et al. 2001). The REGR started to decrease just before the cells reached the LEH. It further decreased during root hair bulging, the first phase of root hair growth (Dolan et al. 1994). Further than 1500 μm away from the root tip, in the differentiation zone, the REGR was not statistically different from zero.

Microtubule patterns, cell position and cell type

In the zone of fast elongation, the microtubules in epidermal cells had a transverse orientation. This was the case at the beginning (Fig. 2A), in the middle (Fig. 2B), and also near the end (Fig. 2C) of the elongation zone. Furthermore, no difference in microtubule orientation was found between the trichoblast and atrichoblast cell lines (Fig. 2A–C). This transverse orientation was confirmed quantitatively by analysing the proportional distribution of microtubules deviating with a given angle from the transverse orientation (Fig. 3A). Short cells and long cells, from the beginning and the end of this elongation zone had the same distribution of microtubule orientations. Thus neither the increasing growth rate nor the cell length caused a change of microtubule patterning.

However, when cells arrived at the differentiation zone and trichoblasts reached the LEH, microtubule patterns changed (Fig. 2D). Microtubules had a random orientation in trichoblasts at the moment of root hair bulging, but they adopted a slightly oblique orientation in atrichoblasts. This difference between the two cell types was confirmed in quantitative terms (Fig. 3B). The distribution of angles of microtubule orientation for atrichoblasts was preferentially deviating about 30–50° from the transverse position, whereas in trichoblasts microtubules tended more to a deviation of 50–80°. In older atrichoblasts, further away from the root tip, microtubule orientation eventually became steeply oblique and finally longitudinal (Fig. 2E and F). The full reorientation from transverse to longitudinal took about 4–6 h.

The effect of ACC on microtubule reorientation

The effect of ACC on microtubule orientation was studied in roots fixed after 1 h of treatment at the saturating concentration of 5 μM. The effect depended on the size of the cells, and thus on their position in the elongation zone. In cells shorter than 35 μm, which is the LEH specified by 5 μM ACC (Le et al. 2001), the transverse arrays of microtubules were not dramatically affected in both trichoblast and atrichoblast cell files as is illustrated by micrographs (Fig. 4A) and measured microtubule angles (Fig. 5A). However in cells equal to or longer than the ACC-specified LEH, ACC caused random microtubules in trichoblasts and longitudinal microtubules in atrichoblasts (Fig. 4B and C). The plot of microtubule angles deviating from the transverse position confirms this ACC-induced reorientation of microtubules in quantitative terms (Fig. 5B).
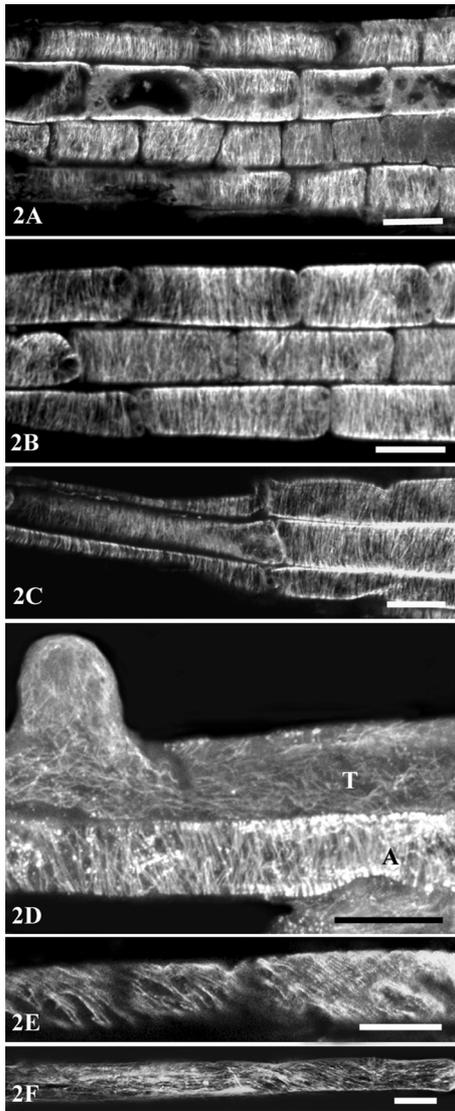
Fig. 2. Confocal microscopy images illustrate the orientation of microtubules in wild-type (Col-0) roots under normal conditions. The root tip points to the right. A–C, Transversely aligned microtubules occur as well in the beginning (A) as in the middle (B) and at the end (C) of the elongation zone. D, Microtubule orientation is random in trichoblasts and slightly oblique in atrichoblasts at the LEH, in the root hair initiation zone. E, In older atrichoblasts, microtubules are more oblique. F, In atrichoblasts far away from the root tip, microtubule orientation is longitudinal. Scale bar = 20 μm. T, trichoblast; A, atrichoblast.

Ethylene and the fast microtubule response

For defining the reaction time ethylene gas was used instead of ACC (see Le et al. 2001 or at http://www.uia.ac.be/bio/fymo/lejie/). Fig. 6A shows the highly aligned transverse microtubules in the elongation zone before ethylene treatment. Microtubule reorientation had started already after 10 min of ethylene treatment (Fig. 6B, arrowheads), but not yet in all cells. After 60 min, the microtubules had completed their rearrangement to purely longitudinal in atrichoblasts (A in
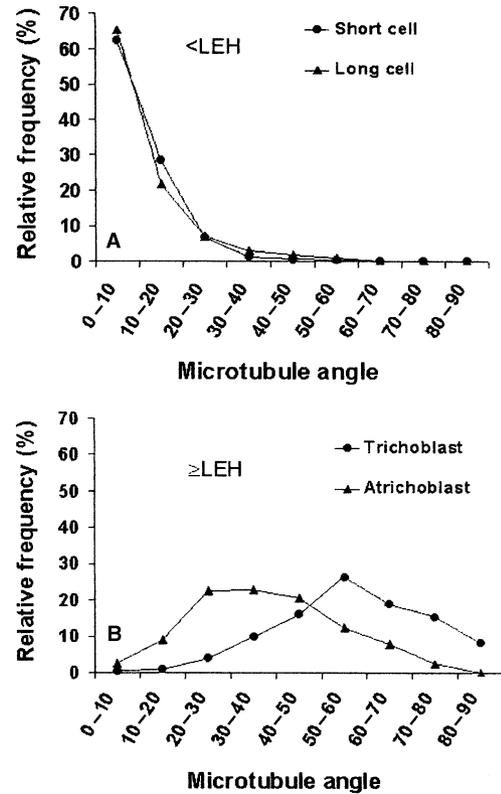

Fig. 3. The orientation of microtubules in the elongation zone and the root hair initiation zone. Relative frequency is the percentage of microtubules deviating from the transverse orientation with a specific angle (n > 300). A, Short cell: cells in the beginning of the elongation zone; Long cell: cells in the middle and near the end of the elongation zone (but pre-LEH); B, Atrichoblast and trichoblast, atrichoblasts and trichoblasts in the root hair initiation zone (length equal or more than LEH).

Fig. 6C) and random in trichoblasts (T in Fig. 6C), even before root hair bulging was visible.

Discussion

In Arabidopsis, the developing root epidermis consists of trichoblast cell files interspersed with files of atrichoblasts (Dolan et al. 1994). Studies on mutants (Baskin et al. 1992, Benfey et al. 1993, Hauser et al. 1995) strongly indicated that root morphogenesis greatly relies on the direction and extent of cell expansion (Schiefelbein et al. 1997, Whittington et al. 2001). In most cases, ethylene affects cell elongation negatively (Bleecker et al. 1988, Kieber et al. 1993, Rodrigues-Pousada et al. 1993). The underlying mechanism of the control of cell elongation by ethylene remains largely unknown to date. Changes in microtubule orientation starting after 30 min of ethylene treatment have been reported in hypocotyls (Roberts et al. 1985). A role for microtubules in the inhibition of cell expansion has been suggested for many plant systems (e.g. Steen and Chadwick 1981, Lang et al. 1982, Roberts et al. 1985, Shibaoka 1991, 1994), but has also been questioned, especially in recent work on roots of
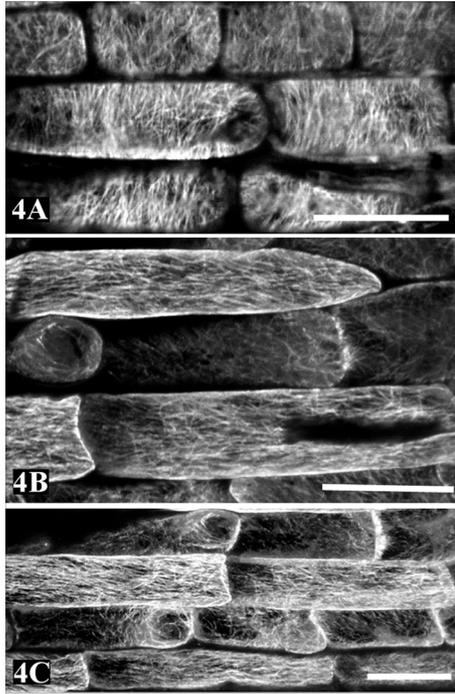
Fig. 4. ACC induces dramatic microtubule reorientation only in cells that are longer than the ACC-specified LEH. A, The transverse alignment of microtubules is nearly not affected in cells shorter than the ACC-specified LEH. B and C, In cells longer than the ACC-specified LEH microtubules realign from transverse to random (trichoblast) and to longitudinal (atrichoblasts) within 1 h. Scale bar = 35 μm, the specific LEH for 5 μm ACC.

Fig. 5. Quantification of the reorientation of microtubules responding to ACC. A, In short cells (shorter than 35 μm, the 5 μM ACC-specified LEH) microtubules keep predominantly a transverse orientation but are slightly random. B, In cells equal to or longer than 35 μm, the rearrangement of microtubules in atrichoblasts is dramatic towards longitudinal, whereas in trichoblasts, microtubule orientation is random. The microtubule distributions of the control are re-plotted from Fig. 3 as references.

Arabidopsis (Baskin et al. 1999, Sugimoto et al. 2000, 2001, 2003, Wiedemeier et al. 2002, Himmelspach et al. 2003). However detailed information on the fate of microtubules, specifically when trichoblasts and atrichoblasts enter the differentiation zone, was to our knowledge never published. We found that ethylene-induced microtubule reorientation in the root epidermis is position and cell type dependent.

In a previous study (Le et al. 2001) we introduced the LEH as a marker for the end of cell elongation and the onset of differentiation in ethylene treated roots. The LEH reflects the length a trichoblast can reach in the elongation zone just before root hair outgrowth starts. In wild-type plants it can vary between 35 μm in saturating ethylene conditions and 180 μm in ethylene-free conditions. These values are mirrored in the *ctr1–1* and *etr1–3* mutants. In normal growth conditions there is seemingly always a response to the endogenous level of ethylene, resulting in a LEH of about 100–130 μm.

In wild-type plants grown in standard conditions microtubules are strictly transverse to the root axis throughout the elongation zone. However when cells reach the LEH the microtubules drastically change to a nearly random orientation in trichoblasts whereas in the neighbouring atrichoblasts a slightly oblique orientation is found. The pattern found in trichoblasts at the LEH stage, is similar to the pattern found in all root cells of
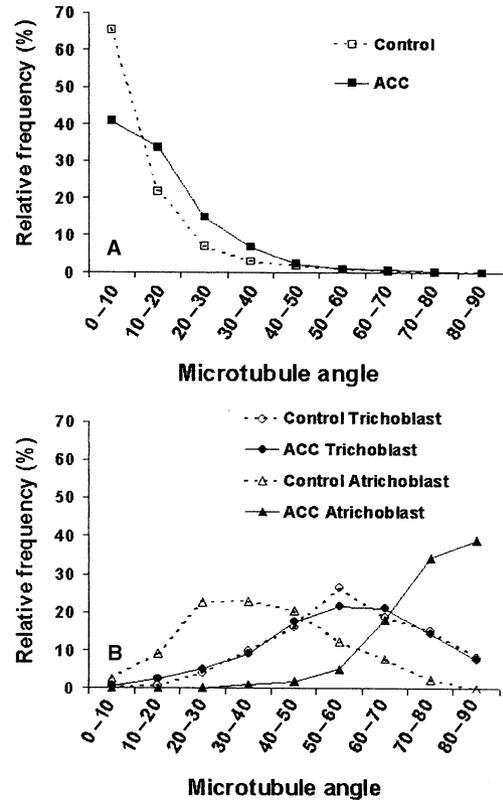
Medicago truncatula, a species that does not have atrichoblasts (Sieberer et al. 2002) and in lettuce where it coincides with root hair initiation (Takahashi et al. 2003). A longitudinal orientation of microtubules is found in atrichoblasts much further away from the root tip that have already stopped all elongation activity, a finding consistent with the observations of Sugimoto et al. (2000). This striking difference in microtubule pattern between trichoblasts and atrichoblasts may be typical for the fate of the cells (Andème-Onzighi et al. 2002). It appears that bulging of entire cells (Andème-Onzighi et al. 2002) or of part of the cell for forming root hairs (this study) is correlated with random microtubules.

Addition of ACC or ethylene defines a new, lower value of LEH and elongation stops irreversibly in cells exceeding or reaching this size (Le et al. 2001). Microtubules are not affected in cells shorter than the new LEH, but in trichoblasts equal to or longer than the new LEH, microtubules switch from a transverse to a random orientation. In the adjacent atrichoblasts they adopt a longitudinal orientation. These patterns of the microtubules are reminiscent of cells in control plants that have fully stopped elongating and that are at least
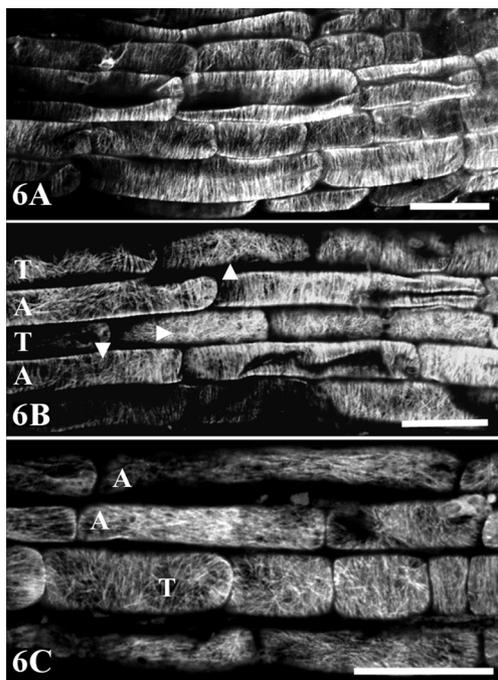
Fig. 6. Kinetics of the effect of $10 \mu l^{-1}$ ethylene on microtubule orientation in the elongation zone. A, Before ethylene treatment microtubules are transversely aligned in all cells. B, After 10 min of ethylene treatment, microtubules start reorientation as well in trichoblast as atrichoblast cell lines. Arrowheads indicate re-orientating microtubules. C, After 60 min of ethylene treatment, the reorientation from transverse to longitudinal in atrichoblast and to random in trichoblasts is completed. Scale bar = $40 \mu m$; A, atrichoblast; T, trichoblast.

could influence the deposition or formation of wall polysaccharides other than cellulose or influence the secretion or activity of wall proteins (Sugimoto et al. 2003).

4–6 h older. Under ethylene treatment this rearrangement of microtubules however, starts within 10 min and is completed within 1 h. The kinetics correlate well with older data on ethylene effects in pea and mung bean stems (Roberts et al. 1985).

The data reported here confirm the role of ethylene as a main regulator of rapid cell elongation control in Arabidopsis roots. It is however, doubtful that the microtubule reorientation causes the arrest of cell elongation. Indeed in-non-treated control plants longitudinal microtubules only occur in cells that have already stopped elongation, while various angles of orientation occur during the last phases of cell growth. In ethylene treated plants cell elongation stops within 10 min in cells longer than or equal to the ethylene-specified LEH, while it still takes 1 h to establish the longitudinal microtubule orientation.

It therefore remains a challenge to unravel the precise mechanism of the ethylene-mediated control of cell elongation. Ethylene may have a fast impact on cell wall metabolism. In that case transverse microtubules would not directly mediate but allow cell elongation. Even so reorientation into longitudinal microtubules would not control the extent of elongation but would be instrumental to fix the final cell size, once elongation has been stopped. Such a role for microtubules is in accordance with a recent hypothesis that microtubule orientation

References

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in Plant Biology. Academic Press, Inc, New York

Andème-Onzighi C, Sivaguru M, Judy-March J, Baskin TI, Driouich A (2002) The reb1–1 mutation of Arabidopsis alters the morphology of trichoblasts, the expression of arabinogalactan – proteins and the organization of cortical microtubules. Planta 215: 949–958

Baskin TI, Betzner AS, Hoggart R, Cork A, Williamson RE (1992) Root morphology mutants in *Arabidopsis thaliana*. Aust J Plant Physiol 19: 427–437

Baskin TI, Meekes HTHM, Liang BM, Sharp RE (1999) Regulation of growth anisotropy in well-water and water-stressed maize roots. II. Role of cortical microtubules and cellulose microfibrils. Plant Physiol 119: 681–692

Benfey PN, Linstead PJ, Roberts K, Schiefelbein JW, Hauser M-T, Aeschbacher RA (1993) Root development in Arabidopsis: four mutants with dramatically altered root morphogenesis. Development 119: 57–70

Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science 241: 1086–1089

Cosgrove DJ (1993) How do plant cell walls extend? Plant Physiol 102: 1–6

Cosgrove DJ (1997) Assembly and enlargement of the primary cell wall in plants. Annu Rev Cell Dev Biol 13: 171–201

Darley CP, Forrester AM, McQueen-Mason SJ (2001) The molecular basis of plant cell wall extension. Plant Mol Biol 47: 179–195

Dolan L (1997) The role of ethylene in the development of plant form. J Exp Bot 48: 201–210

Dolan L, Duckett CM, Grierson C, Linstead P, Schneider K, Lawson E, Dean C, Poethig S, Roberts K (1994) Clonal relationships and cell patterning in the root epidermis of Arabidopsis. Development 120: 2465–2474

Eisinger W (1983) Regulation of pea internode expansion by ethylene. Annu Rev Plant Phys 34: 225–240

Erickson RO, Sax KB (1956) Rates of cell division and cell elongation in the growth of the primary root of *Zea mays*. Proc Am Philos Soc 100: 499–514

Giddings TH, Staehelin LA (1991) Microtubule-mediated control of microfibril deposition: a re-examination of the hypothesis. In: Lloyd CW (ed) The Cytoskeletal Basis of Plant Growth and Form. Academic Press, New York, pp 85–100

Hauser M-T, Morikami A, Benfey PN (1995) Conditional root expansion mutants of Arabidopsis. Development 121: 1237–1252

Himmelspach R, Williamson RE, Wasteneys GO (2003) Cellulose microfibril alignment recovers from DCB-induced disruption despite microtubule disorganization. Plant J 36: 565–575

Jackson MB (1991) Ethylene in root growth and development. In: Matto AK, Suttle JC (eds) The Plant Hormone Ethylene. CRC Press, Boca Raton, pp 159–181

Kerstens S, Decraemer WF, Verbelen J-P (2001) Cell walls at the plant surface behave mechanically like fiber-reinforced composite materials. Plant Physiol 127: 381–385

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell 72: 427–441

Lang JM, Eisinger WR, Green PB (1982) Effects of ethylene on the orientation of microtubules and cellulose microfibrils of pea epicotyl cells with polylamellate cell walls. Protoplasma 110: 5–14

Le J, Vandenbussche F, Van Der Straeten D, Verbelen J-P (2001) In the early response of Arabidopsis roots to ethylene, cell elongation is up- and down regulated and uncoupled from differentiation. Plant Physiol 125: 519–522

Neljubow DN (1901) Uber die horizontale nutation der stengel von *Pisum sativum* und einiger anderen Pflanzen. Beih Bot Zentralbl 10: 128–139

Roberts IN, Lloyd CW, Roberts K (1985) Ethylene-induced microtubule reorientation: mediation by helical arrays. Planta 164: 439–447

Rodrigues-Pousada RA, De Rycke R, Dedonder A, Van Caeneghem W, Engler G, Van Montagu M, Van Der Straeten D (1993) The Arabidopsis 1-aminocyclopropane-1-carboxylate synthase gene is expressed during early development. Plant Cell 5: 897–911

Schiefelbein JW, Masucci JD, Wang H (1997) Building a root: the control of patterning and morphogenesis during root development. Plant Cell 9: 1089–1098

Shibaoka H (1991) Microtubules and the regulation of cell morphogenesis by plant hormones. In: Lloyd CW (ed) The Cytoskeletal Basis of Plant Form. Academic Press, London, pp 159–168

Shibaoka H (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. Annu Rev Plant Physiol Plant Mol Biol 45: 527–544

Sieberer BJ, Timmers ACJ, Lhuissier FGP, Emons AMC (2002) Endoplasmic microtubules configure the subapical cytoplasm and are required for fast growth of *Medicago truncatula* root hairs. Plant Physiol 130: 977–988

Smalle J, Van Der Straeten D (1997) Ethylene and vegetative development. Physiol Plant 100: 593–605

Steen DA, Chadwick AV (1981) Ethylene effects in pea stem tissue, evidence of microtubule mediation. Plant Physiol 67: 460–466

Sugimoto K, Himmelspach R, Williamson RE, Wasteneys GO (2003) Mutation or drug-dependent microtubule disruption causes radial swelling without altering parallel cellulose microfibril deposition in Arabidopsis root cells. Plant Cell 15: 1414–1429

Sugimoto K, Williamson RE, Wasteneys GO (2000) New techniques enable comparative analysis of microtubule orientation, wall texture, and growth rate in intact roots of Arabidopsis. Plant Physiol 124: 1493–1506

Sugimoto K, Williamson RE, Wasteneys GO (2001) Wall architecture in the cellulose-deficient rsw1 mutant of *Arabidopsis thaliana*: microfibrils but not microtubules lose their transverse alignment before microfibrils become unrecognizable in the mitotic and elongation zones of roots. Protoplasma 215: 172–183

Taiz L, Zeiger E (1998) Plant Physiology. Sinauer Associates Inc, Sunderland, MA, pp 660

Takahashi H, Hirota K, Kawahara A, Hayakawa E, Inoue Y (2003) Randomization of cortical microtubules in root epidermal cells induces root hair initiation in lettuce (*Lactuca sativa* L.) seedlings. Plant Cell Physiol 44: 350–359

Warner HL, Leopold AC (1971) Timing of growth regulator responses in pea. Biochem Biophys Res Commun 44: 989–994

Whalen MC, Feldman LJ (1986) The effect of ethylene on root growth of *Zea mays* seedlings. Can J Bot 66: 719–723

Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO (2001) MOR1 is essential for organizing cortical microtubules in plants. Nature 411: 610–613

Wiedemeier AMD, Judy-March JE, Hocart CH, Wasteneys GO, Williamson RE, Baskin TI (2002) Mutant alleles of Arabidopsis RADIALLY SWOLLEN 4 and 7 reduce growth anisotropy without altering the transverse orientation of cortical microtubules or cellulose microfibrils. Development 129: 4821–4830