Original research article

Effects of ethylene on the production, elongation, and differentiation of endodermal cells in maize primary root: An integrative analysis of the developmental process of a particular cell type

Ichirou Karahara, Kaori Matsuda, Yoshihiro Honma*

Department of Biology, Faculty of Science, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan
*Present address: Environmental Protection Laboratory, DOWA ECO-SYSTEM Co., Ltd., 65-1 Omoriyama-shita, Hanaoka, Odate, Akita, 017-0005, Japan
Corresponding author: I. Karahara, E-mail: karahara@sci.u-toyama.ac.jp, Phone/ Fax : +81-76-445-6630/ 6549

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Abstract: A unique integrative analysis is proposed to monitor changes in the developmental processes of a particular cell type in the root, i.e. the rates of cell differentiation, production, and elongation. As a model case, effects of exogenous ethylene on differentiation, division and elongation of endodermal cells were analyzed in maize primary roots. The distance from the lowest position of the Casparian strip, a morphological marker for endodermal cell differentiation, to the root tip decreased in response to ethylene in a dose-dependent manner. The endodermal cell flux in a single cell file, an indicator for cell division, decreased in response to ethylene, although the estimated time required for an individual cell to complete the formation of the Casparian strip, as an indicator for the cell differentiation rate, did not. As indicators for cell elongation, the mature cell length did not change in the presence of ethylene, although the period for cell elongation increased. The Casparian strip formed after the endodermal cell had ceased elongation, irrespective of the presence of ethylene.

Keywords: Casparian strip, endodermal cell, cell differentiation, cell elongation, ethylene, maize (*Zea mays* L.).

Introduction

A central question in the study of plant physiology is how plants modulate their body plan in response to environmental changes. Development of an individual plant body or organ is realized through a sequential addition of cells at the meristem. An individual cell in an organ undergoes its own life cycle processes: cell division, elongation, and differentiation. Each of these processes is modulated constantly during organ development in response to environmental changes, imparting morphological flexibility to a plant body. As a first step toward understanding how the responses of individual cells to environmental changes lead to morphological responses of the entire organ, it is necessary to develop a methodology to monitor morphological changes of each cell, i.e. changes in the rates of cell division, elongation, and differentiation in response to the environment. An individual root provides a good model system for that analysis because of its simple cell tissue organization and its one-dimensional growth.

Among the processes described above, the rates of cell division and cell elongation have been investigated quantitatively in organs because of their importance for organ growth (for a review, see Fiorani and Beemster 2006). Sophisticated methods, such as kinematic analysis (Silk 1984, Beemster and Baskin 1998) and cell-flow and the rate of cell production methods (Ivanov and Dubrovsky 1997), have been developed to monitor these processes in a whole organ. In addition to analysis of growth, analysis of cell differentiation is also important because cell differentiation is not only an essential process for an organ to exert its function: it affects cell morphology. Nevertheless, not many studies have monitored cell differentiation in an organ (Le et al. 2001) because monitoring this process in a dynamically growing organ is difficult. This difficulty is attributable to the

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fact that, in a plant organ, cell differentiation generally takes place after cell division and elongation during cellular development.

Endodermal cells develop a Casparian strip as an important morphological marker for cell differentiation. The literature demonstrates that changes in the Casparian strip formation occurring under different environmental conditions have long received attention (Wilcox 1962, Perumalla and Peterson 1986, Karahara and Shibaoka 1994, Reinhardt and Rost 1995, Enstone and Peterson 1998, Lux et al. 2004): the strip's structure plays a pivotal role in the radial transport of solutes as the apoplastic barrier and the gate of symplastic transport in roots (for review, see Clarkson et al. 1975, Peterson 1988, Schreiber et al. 1999). Understanding the developmental change of the endodermis in response to environmental factors is especially important. In those precedent studies, the distance from the root tip to the lowest position of the strip was used as an indicator to assess whether the development of the strip is delayed or advanced in response to the environment. Regulation of this distance is of physiological interest because the existence of this Casparian-strip free region might be advantageous for absorption of solutes and water, but might be disadvantageous under salt stress. However, this distance depends on the rate of cell division, the rate of cell elongation, and the time required for formation of the strip in each cell. Consequently, one cannot simply conclude that the strip development has been accelerated from the evidence that the strip is observed closer to the root tip. In this context, a method to monitor the rate of differentiation of endodermal cells has been proposed: examination of the cell production rate and analysis of the time required for an individual cell to complete formation of the Casparian strip (Karahara et al. 2004). It is possible to upgrade this earlier methodology to a more integrated one by adding analyses of cell elongation. Additional analyses of cell elongation will update and improve this earlier methodology to a more integrative one.

Ethylene plays important roles in plant responses to biotic and abiotic stresses (for review, see Kende 1993, Dolan 1997, Wang et al. 2002). At the cellular level, ethylene affects not only cell division and elongation but also differentiation patterns of epidermal cells in roots after long incubation times (Tanimoto et al. 1995, Masucci and Schiefelbein 1996, Pitts et al. 1998). These facts indicate that ethylene is an important endogenous mediator regulating cellular life cycle processes, i.e., cell division, elongation, and differentiation; it therefore provides an ideal model case to explore and test this unique methodology for monitoring the modulation of cellular life cycle processes. Therefore, in this study, we examined the effects of ethylene on the endodermal cell elongation rate and elongation period (as indicators of cell elongation), cell flux (as an indicator for cell division), and the estimated time necessary for an individual cell to complete formation of the Casparian strip (as an indicator of the differentiation rate of endodermal cells), in maize primary roots.

Materials and Methods

Plant materials and growth conditions

Kernels of maize (*Zea mays* L. ssp. sacchorata; Sakata Seed Co., Yokohama, Japan) were soaked in distilled water (dH_2O) for 24 h in darkness at 25°C and were then sown on vermiculite (Asahi Kogyo Co. Ltd., Okayama, Japan), which was contained in a plastic container (S-1; Ritchell, Toyama, Japan) and moistened with dH_2O (control). One hundred mL of dH_2O was added per 250 mL of vermiculite. Using this brand of vermiculite, it has been confirmed that the root growth of the controls (grown in dH_2O) was typical of roots with a supply of 1 mM calcium sulfate (data not shown). Therefore, calcium was not added to the vermiculite. Seedlings were grown in darkness at 25°C for 4–8 days. The ages of seedlings are expressed in terms of the number of days from the start of imbibition.

Ethylene treatment

Kernels that had been sown on vermiculite were transferred to a 12.8 L gas-tight plastic chamber (K-Box S70; Asbel, Nara, Japan). Ethylene gas (Nippon Sanso, Tokyo, Japan) was first injected into a gastight intake gas bag (Tedlar bag 15 L; GL Sciences Inc., Tokyo, Japan) using a gastight microsyringe (1700 series; Hamilton Co., Reno, Nevada, USA). The intake gas bag was then connected to the chamber with silicon tubing (Tigers Polymer Corp., Osaka, Japan). The chamber was then connected to an exhaust gas bag, which is used for collection of exhaust gas from the opposite side of the chamber (Fig. 1).

Ethylene-containing air, which was retained in the intake gas bag, was then fully transferred to the chamber using a diaphragm suction pump (MV-6005VP; Enomoto Micro Pump Mfg. Co. Ltd., Tokyo, Japan) placed between the chamber and the exhaust gas bag. Ethylene-containing air inside the chamber was replaced with fresh ethylene/air mixture every 24 h. The replacement operation was carried out under dim green light. For the control treatment, the air without ethylene was used instead of ethylene-containing air; 10 g of activated charcoal was placed inside the control chamber to absorb ethylene.

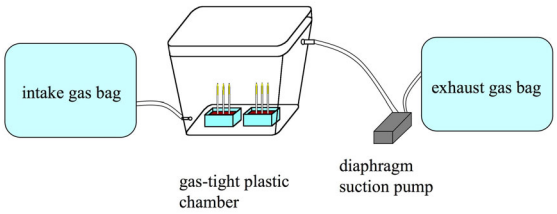

Fig. 1. A schematic illustration of the experimental system for the supply and replacement of ethylene-containing air.

To absorb CO_2, 20 mL of 20% (w/v) solution of KOH was placed in each chamber because CO_2 reportedly inhibits ethylene action (Burg and Burg 1965).

The lengths of primary roots that had grown for 8 days in the presence or the absence of ethylene were measured and a frequency distribution was constructed. When the class width of the frequency distribution was set at 25 mm, the most frequent and second most frequent length classes were as follows: 150–199 mm in the control, 100–149 mm in 0.5 µL L^{-1}, and 25–74 mm in 2.5 µL L^{-1} (n=122–123). These frequent length classes were used for observation of the Casparian strip and quantification of the cell length.

2,5-Norbornadiene treatment

An inhibitor of ethylene action, 2,5-Norbornadiene (NBD) (bicyclo[2.2.1]hepta-2,5-diene) (Tokyo Kasei Kogyo Ltd., Tokyo, Japan), initially in the liquid phase, was poured on a glass petri dish, which was then placed in the chamber to yield 1.25 mL L^{-1} in the gas phase after its evaporation. Then ethylene was injected directly into the chamber through a plug attached to the chamber using a gastight microsyringe. The air containing NBD and ethylene inside the chamber was replaced with fresh NBD/ethylene/air mixture every 24 h.

The lengths of primary roots that had grown for 8 days were measured; a frequency distribution was produced. When the class width of the frequency distribution was set at 25 mm, the most and the second most frequent length classes were 125–175 mm in 1.25 mL L^{-1} NBD, 100–149 mm in 1.25 mL L^{-1} NBD and 0.5 µL L^{-1} ethylene. The primary roots in the most and the second most frequent length class for each treatment were used for observation of the Casparian strip.

Observations of the Casparian strip using fluorescence microscopy

Hand-cut cross sections were prepared from the roots at 1 mm intervals and stained with 0.5% (w/v) berberine and 0.1% (w/v) aniline blue for observations of the Casparian strip, as described previously (Fig. 2, Karahara et al. 2004, Brundrett et al. 1988). The stained sections were mounted on a glass slide; they were then observed under a fluorescence microscope (BX-50 FLA; Olympus Corp., Tokyo, Japan) equipped with a filter assembly for excitation by ultraviolet light (U-MWU: excitation filter, BP330-385; absorption filter, BA420; dichroic mirror, DM-400; Olympus Corp.). For fluorescence photographs, 50-µm-thick frozen cross sections were prepared with a sliding microtome, as described previously (Karahara and Shibaoka 1998). Fluorescence micrographs were taken using a digital camera (Cool Snap cf; Nippon Roper KK, Tokyo, Japan) fitted to the microscope. Because the observations of the Casparian strip through a root usually took several hours and because only one or two roots were observed for each condition (control or treatments) in one experiment, too few samples were able to be handled in one experiment to produce meaningful results for quantitative analyses. For that reason, the experiment was repeated and the data were combined. Consequently, one biological repetition was used. The sample size, or the number of plants, is the sum of the numbers of plants examined in separate experiments.

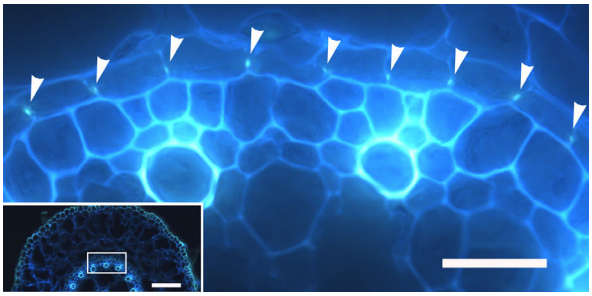

Fig. 2. Fluorescence micrographs showing the Casparian strip in a control 8-d old maize primary root. Magnified view of a region around the endodermis of a frozen cross section cut at 8 mm from the root tip. Location of this region is shown in the white rectangular in inset micrograph although the sections are not the same. Arrowheads: the Casparian strip. Bars = 20 µm and 200 µm (inset micrograph).

Quantification of the longitudinal length and ordinal numeral of cells in longitudinal sections

Segments (2 mm long) of roots were sampled from the region between the root tip and the mean location of the lowest position of the Casparian strip in controls and in roots grown in the presence of 0.5 µL L^{-1} ethylene for 8 days (see Table 1). Embedding of samples and quantification of the cells located between the lowest position of the Casparian strip and the endodermal initial cells in longitudinal sections

were carried out as described previously (Karahara et al. 2004). Light photomicrographs were taken using the digital camera fitted to a microscope, as described above. Quantification of the longitudinal length of cells in the sections was performed using software (Openlab Darkroom; Improvision, Coventry, UK) as follows. Two endodermal cell files were observed in one longitudinal section. The lengths of two cells at the same distance from the initial cells in each corresponding cell file were averaged and plotted against the ordinal numeral (cell number) from the initial cell for each root. Binomial smoothing was carried out using software (Igor Pro version 5; WaveMetrics, Inc., Lake Oswego, Oregon, USA). The ordinal numeral and the length of a cell that had just started elongation and that had ceased elongation were determined from the smoothed curve (Fig. 5). Mature cell length was analyzed by measuring lengths of cells located in the region from where the cells had ceased elongation to the lowest position of the Casparian strip. Because cell length data were obtained from individual roots nested within treatments, nested ANOVA was performed, using software (JMP version 6; SAS Institute Inc., North Carolina, USA), to test whether the effect of ethylene on cell length was significant.

Endodermal cell flux in one cell file

Seedlings were grown for six days in the presence or absence of 0.5 μL L^{-1} ethylene. The lengths of primary roots that had grown for six days were measured and a frequency distribution was constructed. When the class width of the frequency distribution was set at 25 mm, the most and the second most frequent length classes were 100–129 mm in the control and 60–89 mm in 0.5 μL L^{-1} (n=189–197). The primary roots in the most and the second most frequent length class for each treatment were used in the following experiment.

Determination of endodermal cell flux in one cell file was carried out as described previously (Karahara et al. 2004, cell flux was referred to as the apparent rate of production of cells in this citation) with a slight modification. Roots of the length in range within mean ± 5 mm of controls and of treatment with 0.5 μL L^{-1} ethylene were used (see *Results*). Roots were picked clean of vermiculite and marked with indelible black ink (Magic Ink, Tokyo, Japan) under dim green light at 50 mm in the case of controls, and at 30 mm in the case of treatment with 0.5 μL L^{-1} ethylene, from the root tip where cell elongation had already ceased. The roots were returned to the vermiculite for 7.5 h. Then roots were again picked out of the vermiculite and were marked at the same distance from the root tip for each treatment. The distance between the two marks represented the elongation of roots during the 7.5-h period.

Segments (2 mm long) were cut from this region, fixed, and embedded as described above. The endodermal cells in longitudinal sections were quantified as described above. The numbers of endodermal cells in two files observed in each section were added together, divided by two files and by 7.5 h. The result corresponded to the number of endodermal cells produced in 1 h in one cell file, namely, the cell flux in an endodermal cell file. The numbers of cells produced in 7.5 h in an endodermal cell file should be equal to the number of cells produced per cell file at the root meristem in 7.5 h if it is assumed that the rate of cell division remained constant during the time when the cells in this region were produced.

Results

Effects of ethylene on primary root growth and on the distance from the root tip to the lowest position of the endodermal Casparian strip

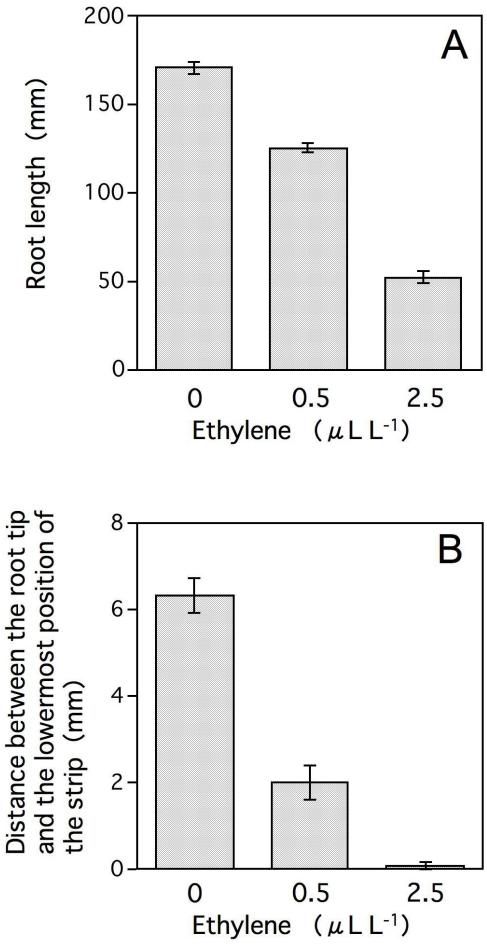

Fig. 3. Effect of ethylene on root growth and on the distance from the maize root tip to the lowest position of the Casparian strip. (A) Length of 8-d old root. (n = 122–123). (B) The distance from the maize root tip to the lowest position of the Casparian strip (n = 12–14).

Table 1. Effects of ethylene on the distance from the lowest position of the endodermal Casparian strip to the root tip, the number of endodermal cells located in this region, endodermal cell flux, and the estimated time necessary to form the Casparian strip in each cell

Parameter	Control		Ethylene 0.5 μL L^{-1}			
	mean (± SE)	*n*	mean (± SE)	*n*	*P*	Statistics (*z*)
Distance from the lowest position of the Casparian strip to the root tip (mm)	9.7 ± 0.3	13	4.9 ± 0.3	12	<0.0001	-4.288
Number of cells located between the lowest position of the Casparian strip and the root tip in one cell file	142 ± 8	5	95 ± 8	5	0.016	-2.402
Endodermal cell flux in one cell file (cells / h)	4.3 ± 0.6	6	2.5 ± 0.2	6	0.010	-2.562
Estimated time required for formation of the endodermal Casparian strip in each cell (h)	33.0		38.1			

Analyzed roots were 5–13. The Mann-Whitney *U*-test (two-tailed) was used to determine whether the differences were statistically significant.

Root growth was inhibited by ethylene treatment in a dose-dependent manner (Fig. 3A). As a first step to examine effects of ethylene on the differentiation of the endodermal cells, the distance from the root tip to the lowest position of the endodermal Casparian strip in primary roots of 8-d-old seedlings that had been grown in the presence or the absence of ethylene was examined. The distance from the root tip to the lowest position of the strip decreased with increased concentration of ethylene (Fig. 3B).

In fact, NBD binds to the ethylene receptor, thereby inhibiting ethylene activity (Bleecker et al., 1987). The effects of NBD on these parameters were examined to elucidate whether the decrease in the root growth and the decrease in the distance from the root tip to the lowest position of the strip are specific effects of ethylene. Because NBD did not recover inhibition of root growth by ethylene treatment at the concentration of 0.25 mL L^{-1} and did inhibit root growth at concentrations greater than 2.5 mL L^{-1}, NBD was applied at 1.25 mL L^{-1}. Ethylene was supplied at the concentration of 0.5 μL L^{-1}.

Root growth was inhibited to 75.9 ± 4.5% (mean ± SE, *n*=130) of the control in the presence of ethylene (0.5 μL L^{-1}); it recovered to 92.4 ± 5.7% (mean ± SE, *n*=128) in the presence of both ethylene (0.5 μL L^{-1}) and NBD (1.25 mL L^{-1}). The experiment was repeated three times to confirm the reproducibility of the results. The decrease in the root growth by ethylene was partially but significantly recovered by NBD (unpaired *t*-test, *t*=-2.294, *p*=0.0226, two-tailed).

The distance from the root tip to the lowest position of the strip decreased to 64.1 ± 5.0% (mean ± SE, *n*=17) in the presence of ethylene (0.5 μL L^{-1}), and was recovered to 75.2 ± 5.7% (mean ± SE, *n*=17) in the presence of both ethylene (0.5 μL L^{-1}) and NBD (1.25 mL L^{-1}). The decrease in the distance from the root tip to the lowest position of the strip by ethylene was partially recovered by NBD, although it was not statistically significant (Mann-Whitney *U*-test, *z*=-1.349, *p*=0.1775, two-tailed).

Effects of ethylene on endodermal cell flux and the estimated time required to form the Casparian strip in each cell

The time required for a cell to complete the formation of the strip was estimated by dividing the number of cells in an endodermal cell file in the region between the root tip and the lowest position of the strip by the number of cells produced in an hour, namely, cell flux, in an endodermal cell file (Karahara et al. 2004).

The cells in endodermal cell files located in the region between the root tip and the mean lowest position of Casparian strips were counted directly in sections. The number of endodermal cells in this region decreased significantly in the presence of 0.5 μL L^{-1} ethylene (Table 1). Endodermal cell flux was examined using 6-d-old roots grown in the presence or absence of ethylene. The roots of 6-d-old seedlings were 78.1 ± 2.7 mm long in the controls and 75.0 ± 2.7 mm long in the presence of 0.5 μL L^{-1} ethylene (mean ± SE, *n*=189–197). The endodermal cell flux in one cell file was reduced significantly in response to ethylene (Table 1). The estimated time required for an individual cell to complete the formation of the strip in the presence of ethylene appears to be not very different from the time required in the control if it is

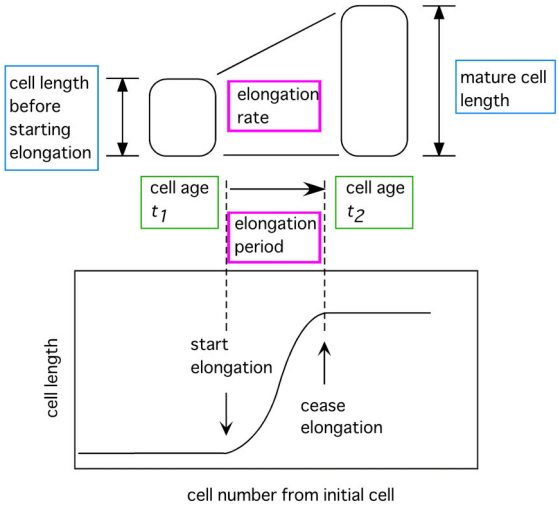

Fig. 4. A schematic illustration of the basic concept for analysis of cell elongation process.

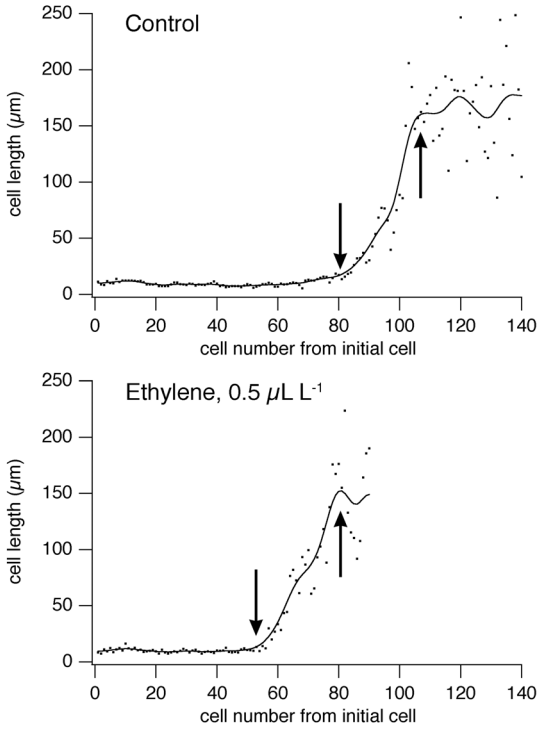

Fig. 5. Effect of ethylene on the length of the endodermal cells of a maize primary root. Cell lengths were plotted against the ordinal numerals of the cells from root tip to the mean lowest position of Casparian strips. Profiles of typical roots. Each dataset was smoothed using binominal smoothing (20 passes). Downward arrow: location of a cell starting elongation. Upward arrow: location of a cell that has ceased elongation.

assumed that the endodermal cell flux remained constant from 6 days after the start of imbibition (Table 1).

Effects of ethylene on elongation of the endodermal cells

Cell elongation processes were assessed as follows. For simplicity, the cell elongation process is described by the elongation period and elongation rate (Fig. 4). The elongation period is calculated as the difference between the age of a cell that has started elongation, and of the age of a cell that has ceased elongation. The cell elongation rate is calculated in each cell by dividing the difference between the length of a cell which has just started elongation and that of a cell which has ceased elongation by the elongation period. The age of a cell is estimated by dividing the ordinal numeral of the cell by the endodermal cell flux.

The longitudinal lengths that were measured on sections were plotted against the ordinal numerals of cells in the region from the root tip to the mean lowest

Table 2. Effects of ethylene on the parameters that represent the elongation process of the endodermal cells

Parameter	Control		Ethylene 0.5 μL L^{-1}			
	mean ± SE	*n*	mean ± SE	*n*	*P*	Statistics (*z*)
Cell that has just started elongation						
Ordinal numeral	67 ± 10	5	56 ± 8	5	0.249	-1.152
Cell age (h) [a]	15.5 ± 2.3	5	15.5 ± 3.7	5	0.600	-0.524
Cell that has just ceased elongation						
Ordinal numeral	95 ± 10	5	86 ± 10	5	0.530	-0.629
Cell age (h) [a]	22.1 ± 2.4	5	34.6 ± 4.1	5	0.047	-1.984
Cell elongation period (h)	6.4 ± 0.5	5	12.0 ± 1.2	5	0.008	-2.643
Cell elongation rate (μm / h)	19.7 ± 2.6	5	12.2 ± 1.3	5	0.016	-2.402

Five roots were analyzed. The Mann-Whitney *U*-test (two-tailed) was used to determine whether the difference were statistically significant. [a]: the parameter was calculated using the values of endodermal cell flux in one cell file (Table 1).

Table 3. Effects of ethylene on the mature longitudinal length of the endodermal and epidermal cells and results of nested ANOVA

Source of variation	Control		Ethylene 0.5 μ L L^{-1}					
	mean ± SE	*n*	mean ± SE	*n*	df	SS	*P*	*F*
Endodermal cells								
Length of cells that have not started elongation (μm)	10.7 ± 0.3	150	9.6 ± 0.2	146	1	92.4	0.268	1.419
Mature cell length (μm)	142.0 ± 9.0	191	152.0 ± 7.3	34	1	416.4	0.773	0.088
Epidermal cells								
Mature cell length (μm)	106.7 ± 4.0	257	103.5 ± 4.2	67	1	78280.1	0.275	1.370

Five roots were analyzed. Values for mean, SE, and *n* were calculated across all roots. SS: sum of squares.

position of the Casparian strip for each root (Fig. 5). Cell length remains constant until a certain time point (Fig. 5, downward arrows), then increases rapidly, and subsequently becomes constant after a certain time point (Fig. 5, upward arrows). The ordinal numeral of a cell that has just started elongation and that of a cell that has just ceased elongation were determined from the curve. Then, the age of a cell that has just started elongation and that of a cell that has just ceased elongation were calculated. The cell elongation period, calculated as the difference between the age of the cell that has just started and that of a cell that has just ceased elongation increased significantly in response to ethylene (Table 2). The cell elongation rate decreased significantly in response to ethylene (Table 2). However, ethylene did not significantly affect the age of the cell that had just started elongation as well as that which had just ceased elongation (Table 2). In addition, for the control treatment plants and those treated with 0.5 μL L^{-1} ethylene (Table 3), no significant differences were found between the lengths of cells when they had not yet started elongation and when they had ceased elongation.

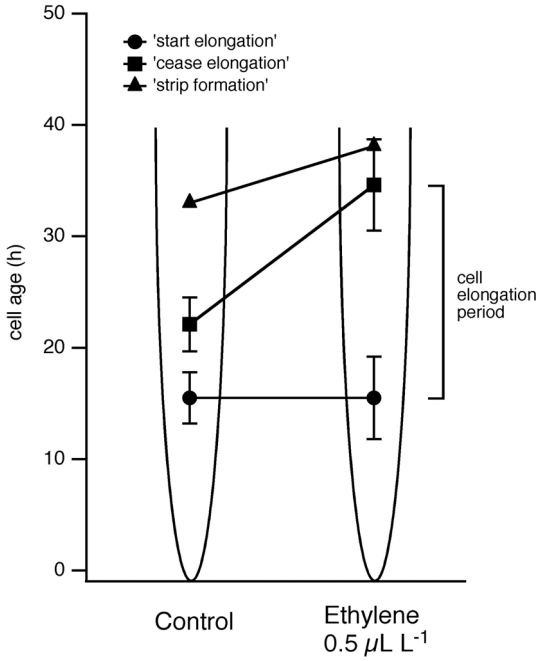

Fig. 6. A comprehensive diagram showing the effects of ethylene on the ages of endodermal cells when they have just started elongation, when they have ceased elongation or when they have formed the Casparian strip. Data values (mean or mean ± SE) are the same as presented in Table 1 or 2.

Discussion

Using this method, it is possible to compare the timings, or cell ages, when different developmental events occur in a particular cell type. Because the Casparian strips, which are composed of rigid secondary cell wall components such as lignin (Zeier et al. 1999, Schreiber et al. 1999), surround the endodermal cells in radial walls, the cells might therefore no longer be able to continue elongation once the strip has been formed. The ages of a cell forming the strip and that of a cell that had ceased elongation were compared (Fig. 6). Results showed that the formation of the Casparian strip, which was at the cell age of 33.0 h, followed the cessation of elongation at the age of 22.1 h in the case of the control (Tables 1 and 2). It was also the case even though the elongation period was delayed by ethylene (formation of the Casparian strip, 38.1 h; cessation of elongation, 34.6 h; Tables 1 and 2). These results suggest that the formation of the strip occurs after the cessation of cell elongation in maize roots, which in turn suggests involvement of some signal transduction mechanism. The cessation of cell elongation leads to initiation of strip formation. It will be interesting to examine what kind of signal it is.

Regarding the ethylene action on cell differentiation, Le et al. (2001) showed that the timing of root

hair bulging, i.e. the differentiation rate of the root hair cell, was unaffected by short-term exposure to ethylene. Our observation in this study is consistent with their study. On the other hand, ethylene is known to affect differentiation by inducing ectopic root hair formation in atrichoblast cell files by treatment for a longer time and at higher concentration (Tanimoto et al. 1995, Masucci and Schiefelbein 1996, Pitts et al. 1998).

Regarding the ethylene action on cell production, ethylene has been shown to inhibit cell division in pea and maize root meristem (Apelbaum and Burg 1972, Barlow 1976). This was also confirmed for the endodermis in this study.

Data of cell elongation kinetics were analyzed in the case of endodermal cells in this study. For analyses of growth, the method developed in this study deals with fixed and embedded roots and is therefore not as precise as sophisticated methods such as kinematic analysis. However, our method can be applied to any type of tissue or root and be used as a first step for diagnostic purposes as well. Judging from the elongation kinetics of the endodermal and epidermal cells, the major cause of the inhibition of maize root growth by exogenous ethylene under the conditions used for this study is suggested to be the reduction of cell production because mature cell length did not change. However, results of a previous study have shown that both ctr1 mutation and treatment with ACC cause a significant reduction in the mature length of epidermal cells in Arabidopsis (Masucci and Schiefelbein 1996). Furthermore, treatment of Arabidopsis root with AVG, an ethylene inhibitor, increases the mature length of epidermal cells (Rahman et al. 2000, Le et al. 2001). It is possible that the mature cell length was not affected by exogenous ethylene in this study because the response was saturated as a result of the endogenous ethylene level.

Ethylene is involved in the response of roots to soil environmental stresses such as water stress (Sharp and LeNoble 2002) and flooding stress (Jackson and Colmer 2005). Arabidopsis roots respond to phosphorus stress by changing ethylene signal transduction pathways (Ma et al. 2003). Under such soil stresses, the function of the Casparian strip as an apoplast barrier would be important to protect the plant body. The distance from the root tip to the lowest position of the endodermal Casparian strip was decreased by ethylene treatment (Fig. 3). The ethylene-induced decrease in the region from the root tip to the lowest position of the strip, where the apoplast barrier is absent because the Casparian strip is immature, would contribute to reduction of the loss of water/oxygen to soil in the case of water or waterlogging stress. The decrease in the distance between the root tip and the lowest position of the strip in the presence of ethylene is considered to be attributable to the decrease of both the cell flux and the cell elongation rate because neither the time required for strip formation nor the mature cell length changed.

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Dr. Ichirou Karahara is a plant physiologist with primary research interest in regulation of root tissue development in response to environmental changes.

Ms. Kaori Matsuda studied endodermal development in maize roots and received her Master of Science degree in Biology from University of Toyama in 2003. She is currently working for Yamazawa Co., Ltd., based in Yamagata.

Mr. Yoshihiro Honma mainly studied endodermal development in Arabidopsis hypocotyls and received his Master of Science degree in Biology from University of Toyama in 2004. His current research interests include environmental management.