Journal of Plant Biology, August 2007, 50(4) : 484-489

Cell Type Identity in *Arabidopsis* Roots Is Altered by Both Ascorbic Acid-Induced Changes in the Redox Environment and the Resultant Endogenous Auxin Response

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Redox plays a critical role in controlling many cellular processes of plant growth and development. To understand the effect of changes in redox on cell-type determination in the root meristem, we examined the influence of a strong reducing agent -- ascorbic acid (AA) – on both the expression patterns of several cell type-specific promoters and the endogenous auxin sensitivity of auxin-responsive DR5::GUS transgenic plants. AA treatment altered the regular expression of columella-specific markers. Moreover, when the same treatment was applied to the DR5::GUS lines, normal expression of the GUS reporter was completely abolished in the auxin maximum, while exogenous auxin restored AA-driven depletion of that maximum. Interestingly, the level of DHA (dehydroascorbate, an oxidized form of AA) in the AA-treated roots was greatly increased. This indicates that changes in cell-type specificity and the sensitivity to endogenous auxin may result from an increase in the cellular DHA that is metabolized from exogenously supplied AA. Therefore, we propose that redox changes in the root meristem alter auxin homeostasis, perhaps causing a change in cell types within the root meristem.

Keywords: ascorbic acid, auxin, quiescent center, redox, root cell type identity

A wide range of biological processes is regulated by cellular redox (Foyer and Noctor, 2005). For instance, reactive oxygen species (ROS) are important signaling messengers in plants and vertebrates (Neil et al., 2002). Plant thioredoxins are key regulators in the oxidative stress responses because they play a fundamental role in conferring tolerance against oxidative stresses (Park et al., 1998; Santos and Rey, 2006). Glutaredoxin, for example, works as an oxidoreductase that is involved in deglutathionylation (Rouhier et al., 2004).

Redox homeostasis, which controls and maintains the levels and ratio of the reduced and oxidized forms of redox couples (i.e., GSH/GSSG, AA/DHA), determines the overall redox status in cells and tissues (Foyer and Noctor, 2005). Ascorbic acid (AA), reduced glutathione (GSH), and tocopherol act as antioxidants that ameliorate this oxidative stress (Foyer and Noctor, 2005). AA is a major antioxidant, protecting plant cells by scavenging free radicals and other ROS (Kang et al., 1998; Foyer and Noctor, 2005). Root nodules produce AA in the infected zone, guarding the host cells from peroxide damage (Matamoros et al., 2006). Ascorbate also serves as a cofactor of several enzymes (Smirnoff and Wheeler, 2000), and it regulates several aspects of plant growth and development by controlling the cell cycle, cell elongation, and programmed cell death (Foyer et al., 2006). In the tobacco cultivar Bright Yellow 2 (BY-2) system, AA stimulates cell division while DHA decreases the mitotic index (de Pinto et al., 1999).

The quiescent center (QC), located near the root tip, is composed of mitotically inactive cells. These are one of the most important cell types in root development because they can integrate many cellular processes leading to root meristem establishment and maintenance (Jiang and Feldman, 2003). The QC is surrounded by meristematic initial cells that are mitotically active. These initial cells are defined by their location within the root apical meristem, and their identity is determined and maintained by positional information that originates from the surrounding cells, including the QC (van den Berg et al., 1997). To build proper organization within the root, a balance must be achieved between self-renewing activity and differentiation of the QC. However, the control of this phenomenon in plants is not well understood. In animal systems, this balance between self-renewal and differentiation in dividing glial precursor cells can be modulated by redox signals (Smith et al., 2000).

In plants, redox homeostasis also may play an important role in maintaining and activating the QC, which is more oxidized than its actively dividing neighbor cells (Sanchez-Fernandez et al., 1997; Jiang et al., 2003). For example, the redox state affects post-embryonic root cell division and organization in an *Arabidopsis* mutant, *rml*1 (Vernoux et al., 2000), where a mutation is found in the γ-glutamylcysteine synthetase gene that is the committal enzyme in glutathione synthesis. Although AA treatment increases the rate of cell division in plants (Liso et al., 1988; Kerk and Feldman, 1995), treatment with DHA has the opposite effect (Potters et al., 2004). Progression of the cell cycle is sensitive to oxidative stress, and QC cells are arrested in the G1 stage by such stress (Juliano, 2003).

Auxin is a major phytohormone controlling the development and maintenance of normal patterning of the root meristem. It determines cell fate, planes of cell division, mitotic activity of the QC, zones of elongation/differentiation, and polarity of the epidermal root hair cells (Sabatini et al., 1999). Auxin is synthesized in the apical cells of the stem, then polarly transported through conductive tissues to

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Abbreviations: AA, ascorbic acid; AAO, ascorbic acid oxidase; DHA, dehydroascorbate; GFP, green fluorescent protein; GUS, glucouronidase; QC, quiescent center

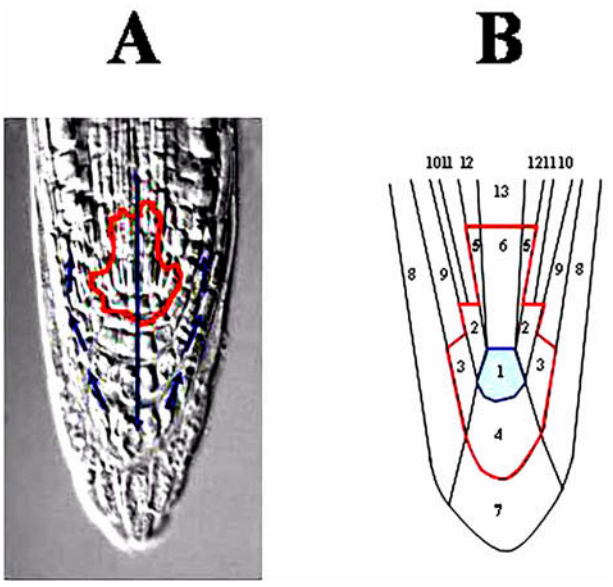

Figure 1. Morphology of *Arabidopsis* root meristem. (**A**) Region of initials, including QC (red closed area), and direction of auxin transport (blue arrows). (**B**) Quiescent center cells are located proximal to columella stem cells (initials) and columella cells of root cap. 1, QC; 2, cortex/endodermis initials; 3, epidermis/lateral root cap initials; 4, columella initials; 5, pericycle initials; 6, vasculature initials; 7, columella; 8, lateral root cap; 9, epidermis; 10, cortex; 11, endodermis; 12, pericycle; 13, vasculature.

the root meristem, where it regulates growth and development (Fig. 1A). As a result of this transport, the auxin maximum sits at the end of the vascular boundary, establishing a distal organizer in the root (Sabatini et al., 1999). The sinks for this auxin maximum are the QC cells, which influence the differentiation of surrounding cells, e.g., distally located columella initial cells (Fig. 1B for the cellular architecture of roots.). The QC and columella initial cells serve as a positional signal for the auxin response by the entire root apex (Jiang and Feldman, 2005). Intensive genetic studies using auxin mutants that are defective in their auxin response and transport have revealed a strong correlation between cellular localization of the auxin maximum and patterned cell division within the root meristem (Sabatini et al., 1999).

Recent progress has been made toward molecularly dissecting and understanding how changes in redox potential affect the establishment of root apical meristems (Jiang and Feldman, 2005). Nonetheless, the molecular mechanisms by which the QC forms and is maintained are not well defined, and it is still unclear what regulates the determination of cell fate and its elaboration. In this study, we investigated the effect of redox on the QC and its neighboring cells to further understand the influence of redox on cell-type specification in *Arabidopsis*.

MATERIALS AND METHODS

Materials and Growth Conditions

Arabidopsis seeds were surface-sterilized by washing them first with isopropanol, then with 20% Clorox. After they were rinsed several times with sterile deionized water, they were sowed either on sterile filter paper mounted on a solid MS medium (0.6% agar and 0.5X MS; pH 5.7) or directly on an MS medium (0.8% agar and 0.5 MS; pH 5.7), before being stratified in the dark at 4°C for 2 d. For microscopic observations, two-week-old seedlings grown under continuous light were put in a 1-mL solution for 24 h, with or without additional chemicals. To quantify AA and DHA, seedlings grown for two weeks under the same conditions were treated with a sterile 5 mM AA agar/MS solution (0.4% agar and 0.5X MS; pH 5.7) and incubated for 24 h in a continuous-light chamber. Roots were excised surgically and either placed directly into 5% metaphosphoric acid (10:1 ratio of volume / fresh weight) for AA measurements or put into a fixing solution for GUS-staining.

Measurement of AA Levels

Tissues were homogenized in 5% metaphosphoric acid on ice, then centrifuged at 14000 rpm in a microfuge for 15 min at 4°C. To chromatically measure total AA the reduced AA plus the oxidized AA (DHA) - and the amount of reduced AA alone, we mixed 100 µL of the supernatant with 250 µL of 0.15 M K_2HPO_4 buffer (containing 5 mM EDTA; pH 7.4) in the presence of either 50 µL of 10 mM DTT (for total AA) or the same volume of water (for the reduced AA). Samples were incubated for at least 10 min at room temperature before 50 µL of 0.5% N-ethylmaleimide was added. A color-developing solution containing 200 µL of 10% TCA, 200 µL of 44% *o*-phosphoric acid, 200 µL of 4% α,α′-dipyridyl in 70% ethanol, and 11 µL of 30% $FeCl_3$ was added to each of the above mixtures (450 µL). These were then vigorously mixed and incubated at 40°C for 40 min before absorbance at A_{525} was measured.

GUS-Staining of Roots

GUS-staining was performed according to the method of Jefferson (1987), with some modifications. Tissues were fixed in 90% acetone for 1 h at -20°C, then washed twice for 5 min in washing buffer (0.1 M phosphate [pH 7.0], 10 mM EDTA, and 2 mM $K_3Fe(CN)_6$) under vacuum. Afterward, a staining buffer (0.1 M phosphate [pH 7.0], 10 mM EDTA, 2 mM $K_4Fe(CN)_6 \cdot 3H_20$, and 1 mg mL^{-1} X-GLUC) was infiltrated into the washed tissues by a brief vacuum treatment, and the specimens were incubated overnight at 37°C. The GUS-stained tissues were fixed in a 3:1 mixture of ethanol:acetic acid, then cleared and mounted in a drop of clearing solution. Digital images were compiled via Adobe Photoshop 7.

Starch-Staining of Root Caps

Seedlings were stained for 10 min in an I-KI solution (0.2% iodine in 2% of a potassium iodide solution), then mounted in 20% glycerol for our microscopy analysis.

Microscopy

We used a Nikon Eclipse TE2000-U inverted microscope to observe the GUS- and starch-stainings. GFP expression was examined via confocal laser imaging analysis. A Zeiss LSM 510 Meta NLO-UV confocal laser scanning microscope was equipped with an argon 2 ion laser (488 nm) and a He/

Ne 1 ion laser (543 nm). An HFT488 chromatic beam splitter and a BP500-550 IR bandpass filter were used to generate the GFP signal, and an HFT 543 beam splitter and a BP 565-615 bandpass filter aided in our observations of the propidium-iodide (PI) staining. Images were compiled with LSM 5 image browser software. For PI-staining, the seedlings were incubated for 30 min in 5 μg mL^{-1} propidium iodide (Sigma-Aldrich, USA).

RESULTS

AA Treatment Alters Cell Type-Specific Gene Expression in *Arabidopsis* Root Meristems

Previous research has shown that the QC has a relatively higher level of auxin and AAO activity, but a lower amount of AA compared with the more rapidly dividing surrounding cells (Kerk and Feldman, 1995; Jiang et al., 2003). Because we were interested in knowing if redox could change overall root cell type identities, we investigated whether the expression of columella-specific markers also could be altered by AA. Here, our treatment with ascorbic acid completely abolished GUS expression (Fig. 2B) in UCR-27 (a columella initial cell-specific enhancer line; Geisler et al., 2002) and also partially weakened GFP expression (Fig. 2D) in the columella-specific marker line J2341 (Sabatini et al., 2003). The amount of starch granules in the columella cells also was reduced by this AA treatment (Fig. 2F).

We also tested if the expression of QC-specific markers, such as SCR::GFP (Di Laurenzio et al., 1996), would be changed by such treatment. In fact, AA weakened the SCR::GFP signal only at the QC, but not in the endodermal layer (data not shown).

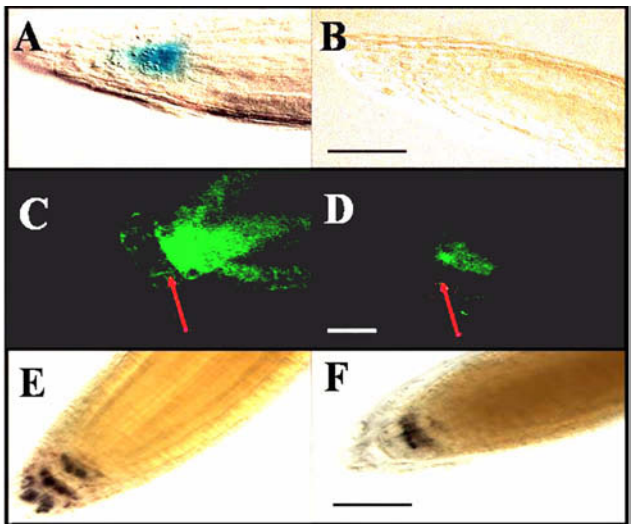

Figure 2. Expression of columella cell marker lines is altered by AA treatment. (**A**) UCR-27, untreated control, (**B**) UCR-27, 24 h after 5 mM AA treatment, (**C**) J2341::GFP, untreated control, or (**D**) J2341::GFP, 24 h after 5 mM AA treatment. (**E**) Staining of starch granule, untreated control, and (**F**) staining of starch granule, 24 h after 5 mM AA treatment. Red arrows in (**C**) and (**D**) indicate columella initials. Bar = 50 μm.

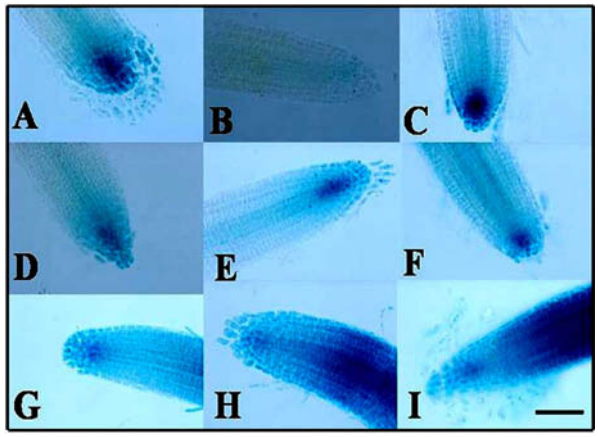

Figure 3. AA treatment disrupts auxin response, as determined by expression of DR5::GUS, probably by decreasing major auxin transport. (**A**) Untreated control; DR5::GUS signal is found in QC and columella initial cell region, i.e., an auxin maximum. Roots treated with: (**B**) 5 mM AA, (**C**) 10^{-10} M IAA, (**D**) 5 mM AA and 10^{-10} M IAA, (**E**) 5 mM AA and 10^{-8} M IAA, (**F**) 10^{-6} M IAA, (**G**) 5 mM AA and 10^{-6} M IAA, (**H**) 5 mM AA and 10^{-4} M IAA, or (**I**) 10^{-4} M IAA. Bar = 50 μm.

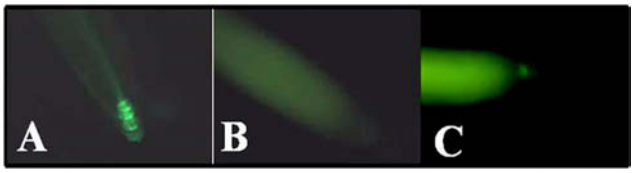

Figure 4. Copper treatment for 24 h disrupts auxin response, as determined by expression of DR5::GFP. (**A**) Untreated control, (**B**) 10 μM $CuCl_2$, or (**C**) 10 μM $CuCl_2$ and 1 μM IAA. Bar = 50 μm.

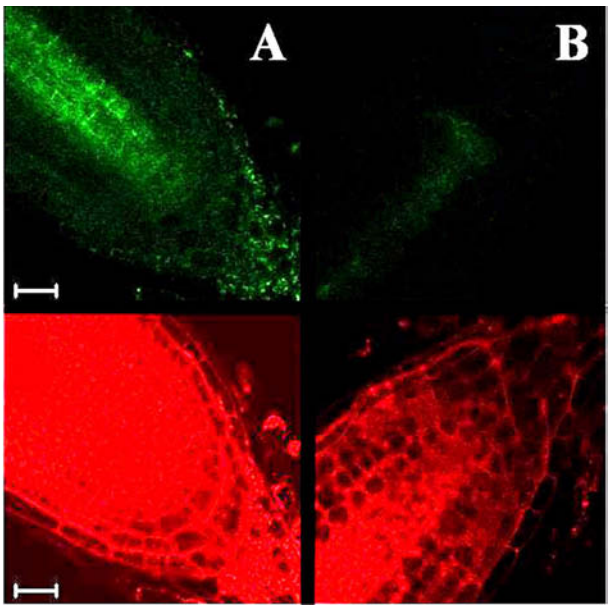

Figure 5. AA treatment down-regulates PIN1-GFP expression in roots. (**A**) Upper panel: PIN1-GFP signal, untreated control; lower panel: root cell walls stained with 5 μg mL^{-1} propidium iodide, untreated control; (**B**) Upper panel: PIN1-GFP signal after 5 mM AA treatment; lower panel: root cell walls stained with 5 μg mL^{-1} propidium iodide, root treated with 5 mM AA. Bar = 50 μm.

AA Treatment Disrupts the Auxin Response, Probably by Inhibiting Major Auxin Transport

Kerk and Feldman (1995) have proposed that QC formation in maize roots is associated with an auxin-regulated oxidizing environment. Thus, we reasoned that the exogenous AA-driven changes in marker gene expression might be due to shifts in auxin metabolism (homeostasis) in the roots and/or by alterations in auxin transport to the root tips. To test this idea, we first investigated the effect of AA treatment on the expression of the DR5-driven GUS protein (DR5::GUS) and on QC localization of the auxin maximum. The *Arabidopsis* DR5::GUS transgenic line carries the GUS reporter driven by an auxin-responsive synthetic promoter fused to a minimal CaMV35S promoter (Ulmasov et al., 1997).

In the untreated control, the auxin maximum was located, as expected, specifically at the QC cells and columella initials (Fig. 3A). This pattern of localization also was found in DR5::GFP transgenic plants that expressed the GFP reporter (data not shown; Ottenschläger et al., 2003). Treatment with 5 mM AA at the root tip disrupted the expression of this auxin maximum (Fig. 3B), whereas the application of 10^{-10} M IAA to these AA-treated roots completely restored the auxin maximum at the QC cells (Fig. 3D). These results suggest that AA-incurred ablation of the auxin maximum is due to the depletion of endogenously active auxin. Higher concentrations of IAA (10^{-8}, 10^{-6}, and 10^{-4} M) strengthened this GUS recovery (Fig. 3E, G, H), and similar levels of GUS expression were observed in tissues that had been treated with only the corresponding amount of IAA (Fig. 3C, F, I).

We also wanted to determine if the above effect of AA on expression of the auxin maximum was correlated with AAO (ascorbate oxidase) activity. AAO converts reduced AA to DHA, an oxidized form of AA. Copper is a cofactor of AAO, and the addition of Cu ions to the culture medium increases AAO transcription (Esaka et al., 1992; Kerk et al., 2000). Besides its regulation of redox homeostasis, copper is involved in auxin metabolism and the control of homeostasis. Here, treatment with 10 μM $CuCl_2$ for 24 h abolished this auxin maximum, similar to the result found from our AA treatment (Fig. 4B). Likewise, the auxin response originally ablated by $CuCl_2$ was recovered by the addition of 1 μM IAA (Fig. 4C).

We also questioned whether the disappearance of an auxin response in AA-treated roots was caused by an interruption of auxin transport from the proximal roots. To test this possibility, we examined the effect of AA treatment on the expression of an auxin efflux carrier, PIN1, by using an *Arabidopsis* line that carries the PIN1-GFP reporter construct. PIN1 is normally localized at the basal membranes of the xylem parenchyma (Fig. 5A; Billou et al., 2005; Petrásek et al., 2006). It is primarily responsible for the polar transport of auxin through the vascular tissues to the root meristem, specifically to the QC region (Kerk and Feldman, 1995). The auxin-transporting activity of PIN1 is a prerequisite for the proper positioning of the auxin maximum in the root meristem (Sabatini et al., 1999). Interestingly, normal PIN1-GFP expression in our xylem parenchyma cells was effectively inhibited by 24 h of AA treatment, which implies that ascorbate disrupts the auxin response, partly by inhibiting major auxin transport (Fig. 5B).

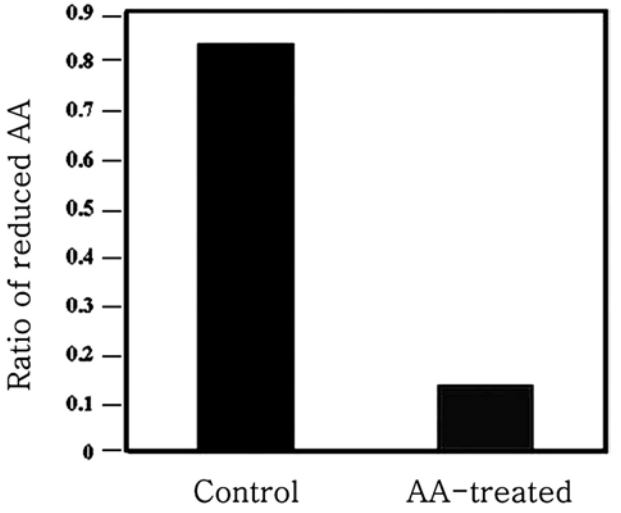

Figure 6. Ratio of reduced:total ascorbic acid in root tissues. Y-axis represents amount of reduced AA out of total AA, which is the sum of reduced AA plus DHA. (See Materials and Methods for chemical measurements.)

AA-Treated Roots Show a High DHA:AA Ratio

Because the exogenous application of AA triggered many changes in cell type identity and the maintenance of an auxin maximum in our study, we also investigated whether these effects were directed by AA itself or if they were caused by the DHA produced inside the root through endogenous AAO activity. Potter et al. (2000) have shown that exogenous AA is rapidly oxidized to DHA in the apoplast before being transported across the plasma membrane. We also found that the addition of $CuCl_2$, whose role as a cofactor of AAO should stimulate endogenous AAO activity, abolished the auxin maximum (Fig. 4B), but that this depletion could be rescued by auxin treatment (Fig. 4C).

We measured the levels of total AA and its oxidized form, DHA, in the root tissues after our standard period of 24 h. Here, the reduced form made up 83.4% of the total AA in the untreated roots, while it accounted for only 13.5% of the total in the treated roots (Fig. 6). This indicates that exogenously applied AA increases the concentration of DHA in the roots.

DISCUSSION

Different Genes in a Root Cell Type Might Show Differential Sensitivity to Changes in the Redox State

We have demonstrated here that treatment with ascorbic acid leads to the suppression of columella-specific promoter activity in two lines of *Arabidopsis* - UCR-27 and J2341 (Fig. 2). The number of starch granules in the columella also is reduced by AA treatment. Therefore, these results suggest that redox changes the physiological state of the tissue or its specificity.

Interestingly, it seems that different genes within the same cell type are differentially regulated by redox. For example, columella-specific UCR27 marker expression is completely suppressed by AA treatment while that of J2347 or the

starch granule counts show only partial suppression (Fig. 2). Application of ascorbate also completely inhibits the expression of a QC marker, SCR::GFP, but does not alter expression of another marker, QC46 (data not shown). Therefore, we might conclude that AA treatments change the maintenance of cell type identities in roots, and that cell type-specific genes are differentially influenced.

Alterations in Intracellular Redox Status May Influence Both the Level of Endogenous IAA and Cell Type Identity in Roots

Ectopic expression of AAO in tobacco has revealed that oxidation of AA is associated with the loss of auxin response and higher mitogenic activities (Pignocchi et al., 2006). We showed in the present study that AA also increases the cellular ratio of DHA to AA (Fig. 6), and eliminates the auxin response in root meristems (Fig. 3). The AA-induced disappearance of the auxin maximum is restored by adding IAA exogenously (Fig. 3). A similar inhibitory effect on this auxin response is observed when roots are treated with a copper ion, which is a cofactor of the auxin-degrading enzyme AAO (Fig. 4). However, the addition of IAA to the $CuCl_2$-treated roots partially recovers this IAA response. Therefore, we postulate that both an increase in DHA and an alteration of the auxin metabolism/homeostasis facilitated by the action of AA-activated cellular AAO are responsible for those ascorbate-induced root meristematic changes in cell type identities.

Perturbation of polar auxin transport by an auxin transport inhibitor, NPA, suggests a critical role for that phytohormone in establishing and maintaining the organization of the root meristem (Jiang et al., 2003). Many auxin transport mutants, such as *pin1* (Gälweiler et al., 1998), *pin3* (Friml et al., 2002a), and *pin4* (Friml et al., 2002b), show defects in their root development. Moreover, auxin-inducible PLT1 and PLT2, which are AP2-type transcription factors, are involved in QC specification and stem cell activity (Aida et al., 2004). Here, we showed that AA treatment reduces the acropetal transport of IAA to the QC region, probably by decreasing PIN1 protein expression in the root tips (Fig. 5). This means that such diminished auxin transport, which could cause an increase in the ratio of root DHA (Fig. 6), might be an additional determining factor of tissue cell type. Paradoxically, we also realized here that the DR5::GUS signal and the PIN1-GFP signal can be partially recovered by treatment with 1 mM DHA (data not shown). These observations suggest that the root tissue can act as a dynamic redox buffer in balancing redox potentials.

In summary, a redox change in the oxidized state can alter root cell type identities and decrease the level of endogenous auxin in that tissue. We now propose that redox potential in the root meristem regulates auxin homeostasis in cells, possibly resulting in a change in cell type identities led by unidentified signal transductions. More details must be obtained before we can make absolute conclusions about the roles of redox in determining and maintaining root cell type identity. Using recently developed redox-sensitive GFP (roGFP) at a high resolution (Jiang et al., 2006), we will now be able to conduct further analyses that test the effect of auxin and reducing agents. Such efforts will deepen our understanding about the roles of redox homeostasis in regulating root cell identities.

ACKNOWLEDGEMENTS

The authors thank Dr. Ottenschläger (Institut für Biologie II, Universität Freiburg, Germany) for kindly supplying the DR5::GFP transgenic *Arabidopsis* seeds. We also thank Dr. Ka, Hak-Hyun for our use of his Nikon Eclipse TE2000-U inverted microscope. We appreciate the assistance of Mr. Kwon, Seung-Hae for his confocal microscopy work at the Korea Basic Science Institute, Chuncheon, Korea. This research was supported by Korea Research Foundation Grants funded by the Korea Government, Basic Research Promotion Fund (KRF-2005-015-C00454 and KRF-2006-311-C00149).

Received December 28, 2006; revised April 30, 2007; accepted July 11, 2007.

LITERATURE CITED

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B (2004) The PLETHORA genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119: 109-120

Billou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* root. Nature 433: 39-44

de Pinto MC, Francis D, de Gara L (1999) The redox state of the ascorbate-dehydroascorbate pair is a specific sensor of cell division in tobacco BY-2 cells. Protoplasma 209: 90-97

Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN (1996) The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. Cell 86: 423-433

Esaka M, Fujisawa K, Goto M, Kisu Y (1992) Regulation of ascorbate oxidase expression in pumpkin by auxin and copper. Plant Physiol 88: 656-660

Foyer CH, Kiddle G, Verrier P (2006) Transcriptional profiling approaches to understanding how ascorbate regulates growth and the cell cycle, *In* S Baginsky, AR Fernie, eds, Plant Systems Biology. Birkenhauser, Basel

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling, a metabolic interface between stress perception and physiological responses. Plant Cell 17: 1866-1875

Friml J, Benkova E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002b) AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell 108: 661-673

Friml J, Winiewska J, Benková E, Mendgen K, Palme K (2002a) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415: 806-809

Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. Science 282: 2226-2230

Geisler M, Jablonsk B, Springer PS (2002) Enhancer trap expression patterns provide a novel teaching resource. Plant Physiol 130: 1747-1753

Jefferson RA (1987) Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol Biol Rep 5: 387-405

Jiang K, Feldman LJ (2003) Root meristem establishment and maintenance: The role of auxin. J Plant Growth Regul 21: 432-440

Jiang K, Feldman LJ (2005) Regulation of root apical meristem development. Annu Rev Cell Dev Biol 21: 485-509

Jiang K, Meng YL, Feldman LJ (2003) Quiescent center formation in maize roots is associated with an auxin-regulated oxidizing environment. Development 130: 1429-1438

Jiang K, Schwarzer C, Lally E, Zhang S, Ruzin S, Machen T, Remington SJ, Feldman LJ (2006) Expression and characterization of a redox-sensing green fluorescent protein reduction-oxidation-sensitive green fluorescent protein in *Arabidopsis*. Plant Physiol 141: 397-403

Juliano RL (2003) Regulation of signaling and the cell cycle by cell interactions with the extracellular matrix, *In* J Boonstra, ed, G1 Phase Progression. Kluwer Academic/Plenum, New York, pp 110-119

Kang KS, Lim CJ, Han TJ, Kim JC, Jin CD (1998) Activation of ascorbate-glutathione cycle in *Arabidopsis* leaves in response to aminotriazole. J Plant Biol 41: 155-161

Kerk NM, Feldman LJ (1995) A biochemical model for the initiation and maintenance of the quiescent center, implications for organization of root meristems. Development 121: 2825-2833

Kerk NM, Jiang K, Feldman LJ (2000) Auxin metabolism in the root apical meristem. Plant Physiol 122: 925-932

Liso R, Innocenti AM, Bitonti MB, Arrigoni O (1988) Ascorbic acid-induced progression of quiescent centre cells from G1 to S phase. New Phytol 110: 469-471

Matamoros MA, Loscos J, Coronado MJ, Ramos J, Sato S, Testillano PS, Tabata S, Becana M (2006) Biosynthesis of ascorbic acid in legume root nodules. Plant Physiol 141: 1068-1077

Neil SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002) Hydrogen peroxide and nitric oxide as signaling molecules in plants. J Exp Bot 53: 1237-1247

Ottenschläger I, Wolff P, Wolverton C, Bhalerao RP, Sandberg G, Ishigawa H, Evans M, Palme K (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. Proc Natl Acad Sci USA 100: 2987-2991

Park KN, Sa JH, Han BD, Park EH, Lim CJ (1998) Activities of sulfhydryl-related and phenylpropanoid synthesizing enzymes during the germination of *Arabidopsis thaliana* seeds. J Plant Biol 41: 247-254

Petrásek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertová D, Wisniewska J, Tadele Z, Kubes M, Covanová M, Dhonukshe P, Skupa P, Benková E, Perry L, Krecek P, Lee OR, Fink GR, Geisler M, Murphy AS, Luschnig C, Zazímalová E, Friml J (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. Science 312: 914-918

Pignocchi C, Kiddle G, Harnandez I, Foster SJ, Asensi A, Taybi T, Barnes J, Foyer CH (2006) Ascorbate oxidase-dependent changes in the redox state of the apoplast modulate gene transcript accumulation leading to modified hormone signaling and orchestration of defense process in tobacco. Plant Physiol 141: 423-435

Potters G, Horemans N, Bellone S, Caubergs RJ, Trost P, Guisez Y, Asard H (2004) Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. Plant Physiol 134: 1479-1487

Potters G, Horemans N, Caubergs RJ, Asard H (2000) Ascorbate and dehydroascorbate influence cell cycle progression in a tobacco cell suspension. Plant Physiol 124: 17-20

Rouhier N, Gelhaye E, Jacquot JP (2004) Plant glutaredoxins, still mysterious reducing systems. Cell Mol Life Sci 61: 1266-1277

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Wiesbeck P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99: 463-472

Sabatini S, Heidstra R, Wildwater M, Scheres B (2003) SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Gene Dev 17: 354-358

Sanchez-Fernandez R, Fricker M, Corben LB, White NS, Sheard N, Leaver CJ, van Montagu M, Inze D, May MJ (1997) Cell proliferation and hair tip growth in the *Arabidopsis* root are under mechanistically different forms of redox control. Proc Natl Acad Sci USA 94: 2745-2750

Santos CV, Rey P (2006) Plant thioredoxins are key actors in the oxidative stress response. Trends Plant Sci 11: 329-334

Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants, biosynthesis and function. Crit Rev Plant Sci 19: 267-290

Smith J, Ladi E, Mayer-Pröschel M, Noble M (2000) Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proc Natl Acad Sci USA 97: 10032-10037

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9: 1963-1971

van den Berg C, Willemsen V, Hendricks G, Weisbeek P, Scheres B (1997) Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature 390: 287-289

Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S (2000) The ROOT MERISTEMLESS1/ CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. Plant Cell 12: 97-109