Environmantly Induced Plasticity of Root Hair Development in Arabidopsis

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Postembryonic development of plants is dependent on both intrinsic genetic programs and environmental factors. The plasticity of root hair patterning in response to environmental signals was investigated in the Columbia-0 wild type and 19 Arabidopsis mutants carrying lesions in various parts of the root hair developmental pathway by withholding phosphate or iron (Fe) from the nutrient medium. In the aging primary root and in laterals of the wild type, the number of root hairs increased in response to phosphate and Fe deficiency in a manner typical of each growth type. Although an increase in root hair density in –phosphorus plants was mainly achieved by the formation of extra hairs over both tangential and radial wall of underlying cortical cells, roots of –Fe plants were characterized by a high percentage of extra hairs with two tips. Root hair patterning and hair length was differentially affected by the presence or absence of phosphate and Fe among the genotypes under investigation, pointing to separate cascades of gene activation under all three growth conditions. Divergence in root hair patterning was most pronounced among mutants with defects in genes that affect the first stages of differentiation, suggesting that nutritional signals are perceived at an early stage of epidermal cell development. During elongation of the root hairs, no differences in the requirement of gene products between the growth types were obvious. The role of genes involved in root hair development in the aging primary root of Arabidopsis under the various growth conditions is discussed.

1 Root hairs are cylindrical, tubular structures perpendicular to the main cell axis derived from specialized epidermal cells, the trichoblasts. By greatly increasing the absorptive surface area, root hairs serve in water and nutrient uptake, anchoring the plant to the substrate, and are the site of interaction with nitrogen-fixing bacteria. Root hairs are widespread among vascular plants such as ferns, gymnosperms, and angiosperms, underlining their importance in plant/rhizosphere exchange processes. Similar to pollen tubes, root hairs are tip-growing structures formed by reorientation of cell extension. By maintaining a single growing point, new membrane is continuously added at the tip by secretory vesicle fusion. In Arabidopsis, root hairs are formed in a predictable, position-dependent pattern. Root hairs develop on the apical end of cells that overlie the clefs of underlying cortical cells, and non-hair cells are located over periclinal cortical walls. A number of genes that control epidermal cell patterning and root hair development have been defined by molecular genetic studies (Schiefelbein, 2000, 2003; Dolan, 2001b; Grierson et al., 2001). Epistasis analysis has shown that these genes do not act in a linear cascade but in a complex and not strictly hierarchical pathway. Cell fate specification is regulated by a transcription factor cascade early in the meristem, including the GL2 (GLABRA2) homeobox and the WEREWOLF MYB-type transcription factor, which are required for the non-hair fate. Another Arabidopsis gene, CPC (CAPRICE), encodes an MYB transcription factor mandatory for the hair fate (Wada et al., 1997, 2002). GL2 gene expression is regulated by the WD40 domain protein TTG (TRANSPARENT TESTA GLABRA) by activating an R-like bHLH protein (Scheres, 2000; Schiefelbein, 2000; Larkin et al., 2003). Expression of WER has been shown to be biased by positional cues and to regulate CPC and GL2 by transcriptional feedback loops (Lee and Schiefelbein, 2002). CPC and TRY (TRYPTYCHON) act redundantly in lateral inhibition of root hair patterning (Schellmann et al., 2002). Other specification genes with less well-defined functions have been identified by analysis of mutants harboring defects in root hair patterning. The recessive mutants erhl1 (ECTOPIC ROOT HAIR1), pom1 (POM-POM1), and erhl3 (ECTOPIC ROOT HAIR3) form additional root hairs in N positions normally occupied by non-hair cells (Schneider et al., 1997). ERH3 has been shown recently to encode a katanin-p60 protein (Webb et al., 2003). The rhl (ROOT HAIRLESS) mutants rhl1, rhl2, and rhl3 show disrupted differentiation of epidermal cells in the meristem and form very few root hairs (Schneider et al., 1997). Of the latter genes, only RHL1 has been cloned and was shown to encode a novel protein localized in the nucleus.

After initiation, hairs begin to elongate by rapid polarized expansion. During tip growth, biosynthesis of new wall material, localized wall loosening, and polarized targeting of vesicles from the endomem-
brane system to the growing tip are regulated by localized ion influxes and by the cytoskeleton (Ryan et al., 2001). A number of mutants with altered root hair phenotypes such as RHD (ROOT HAIR DEFECTIVE), TIP GROWTH1, COW1 (CAN OF WORMS1), and KJK (KOJAK) have been isolated, but only in a few cases is the underlying mechanism understood at the molecular level (Schiefelbein, 2000; Grierson et al., 2001; Ryan et al., 2001). In addition, ethylene and auxin have been shown to affect patterning and initiation of root hairs, by acting either independently from or downstream of the WER/TTG/CPC/GL2 pathway (Masucci and Schiefelbein, 1996). Root hairs of mutants defective in ethylene and auxin signal transduction form shorter hairs than the wild type or no hairs at all, indicating that the ethylene and/or auxin signal transduction pathway is required for root hair elongation (Pitts et al., 1998; Dolan, 2001a). Blocking the endogenous ethylene pathway was shown, however, to be ineffective in affecting root hair formation (Cho and Cosgrove, 2002).

The developmental program of roots is greatly influenced by environmental factors and can be modified according to the prevailing conditions. In particular, the availability of essential nutrients can affect root development to allow for an increased uptake of these nutrients (trophomorphogenesis; Forde and Lorenzo, 2001; López-Bucio et al., 2003). These responses include alterations in growth rates, root growth angle, and architecture of the root system. Soil-immobile nutrients such as phosphate and iron (Fe) can induce an increase in the root surface by an increase in the density of root hairs when the supply is inadequate to meet the demand of the plant (Gilroy and Jones, 2000; Schmidt et al., 2000; Ma et al., 2001). The acquisition of both Fe and phosphate depends on a concerted action of an array of physiological and morphological adaptations (Raghothama, 1999; Schmidt, 1999, 2003; Curie and Briat, 2003; Hell and Stephan, 2003). Although the sensors and signal transduction mechanisms have not been identified so far, it appears that the induction of trophomorphogenetic and physiological responses to Fe and phosphorus (P) starvation run parallel but separate courses. Under both conditions, homeostatic compensation of the nutritional levels is systematically regulated, whereas morphological acclimations such as transfer cell formation and the development of extra root hairs are controlled by local signals (Raghothama, 2000; Schikora and Schmidt, 2001). This implies that external phosphate and Fe levels can be perceived by individual cells or tissues independent of the internal nutrient status. At which stage of root hair development nutrient supply modulates the fine-tuning of meristematic prepatter is unclear.

This study is an attempt to elucidate how environmental signals modify cell differentiation in the root epidermis of Arabidopsis. By analyzing mutants defective in different processes of root hair development, we show that root hair patterning, initiation, and elongation are variable under different growth conditions to adapt to changes in the availability or distribution of nutrients in the environment.

RESULTS

Wild-Type Responses

In contrast to seedling roots, in which almost all epidermal cells that lie over the clefs of underlying cortical cells (H position) form root hairs, in the aging primary root and in laterals, the number of root hair files is considerably reduced (Table I). In cross sections of primary roots, only three epidermal cells in the H position develop into root hairs, resulting in 37% of the epidermal cells in this position being differentiated as root hairs. As previously reported, the formation of root hairs in Arabidopsis was sensitive to the availability of Fe and P in the growth medium (Schmidt and Schikora, 2001). Under the present conditions, the root hair frequency in the H position in the primary root of the Columbia-0 wild type was increased to 42% and 61% under Fe- and P-deficient conditions, respectively (Table I; Fig. 1, a–g). Hairs of P-deficient plants were markedly longer than those formed in response to Fe starvation or under control conditions; their length increased from 0.2 ± 0.01 up to 0.6 ± 0.02 mm (Fig. 1, a–c). The increase in root hair frequency in P-deficient plants is associated with the development of hairs in N positions normally occupied by non-hair cells (Table I; Fig. 1d). The formation of ectopic hairs was less pronounced in roots of −Fe plants (5.8% of the epidermal cells in the N position in −P plants versus 2.2% in −Fe plants). In Fe-deficient plants, about one-third of the hairs were branched (Table I; Fig. 1, h–j). Under consideration of the epidermal cell length (control, 121.9 ± 4.3 μm; −Fe, 113.3 ± 6.3 μm; and −P, 118.8 ± 2.1 μm), the number of root hairs per millimeter was 25, 34.4, and 52.2 in control, −Fe, and −P plants, respectively. Based on the number of tips, the frequency of hairs in −Fe plants is 45.9 per millimeter of root length. Thus, it appears that the increase in absorptive surface is realized by different developmental programs in −Fe and −P plants, either by the formation of branched hairs, as in the case of Fe-deficient plants, or by the development of hairs in both normal and ectopic positions and root hair elongation, as in the case of P-deficient plants.

In lateral roots, the number of epidermal cells was significantly lower under all growth conditions. The increase in the number of root hairs in response to Fe and P deficiency was similar to that of the primary roots, with the exception of the number of hairs in the N position in laterals of P-deficient plants, which was markedly reduced. Similar to roots of 3-week-old plants in which no hairs during the first 3 d after transfer to Fe-depleted
Plasticity of Root Hair Development in Arabidopsis

Table 1. Effect of Fe and P deficiency on root hair formation in Arabidopsis wild-type and mutant plants

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<td></td>
<td>col-0</td>
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<td>3.5 ± 0.1</td>
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<td>Primary root</td>
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<td>Seedling root</td>
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<td>rhl4</td>
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* Comparison with primary roots of the wild type under the respective growth condition results in a P value < 0.01 (in addition, these data are underlined).  
* Only small bulges appear that were not visible in the cross sections. The quoted no. represents elongated root hairs.  
* n.d., Not determined.  
* The rhd2 mutant develop also small bulges, but in the cross sections, distinct rhizodermal cells showed strong toluidine blue staining. These cells were counted as root hair cells.
conditions were formed (data not shown), 5-d-old seedlings germinated on Fe-free medium displayed a root-hairless phenotype (Table I). Thereafter, root hairs developed in both cases. When seedlings were germinated on P-deficient medium, the root hair patterning was not significantly different from control conditions, although some hairs in the N position were formed (Table I).

**Cell Fate Determination and Initiation**

To investigate whether defects in root hair development also affect the formation of hairs in response to nutrient starvation, plants of 19 mutants harboring defects in root hair development in the seedling stage were grown on P- or Fe-free medium. Mutations in several genes have been shown to disrupt the patterning of hair cells and non-hair cells in the meristematic zone of the roots or to affect the initiation of the hairs. Defects in the **TTG**, **GL2**, and **WER** genes result in roots producing hairs on almost all epidermal cells, and defects in the **CPC** gene cause the formation of excess non-hair cells (Scheres, 2000). **WER** encodes an MYB-related protein that is preferentially expressed in non-hair cells and has been shown to represent an early regulator of epidermal

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**Figure 1.** Effects of Fe and P status on root hair development of Arabidopsis wild-type roots. a, Control. b, Fe-deficient root. c, P-deficient root. d, Formation of ectopic root hairs (*) in roots of P-deficient Arabidopsis plants. Cross sections of a control root (e), a Fe-deficient root (f), and a root grown in the absence of phosphate (g). Cryo-scanning electron microscopies of a control root (h) and Fe-deficient roots (i and j). Bar = 250 μm (a–c), 100 μm (d, h, and i), 50 μm (j), and 20 μm (e–g).

**Figure 2.** Roots of the **wer** and **rhd1** mutants. a, Bulge formation in roots of the **wer** mutant under control conditions. Rhizodermal development of **rhd1** mutants in the primary root (b) and in laterals (c). Bar = 100 μm.
cell fate in the root and hypocotyl (Lee and Schiefelbein, 1999, 2002). The typical phenotype with nearly all rhizodermal cells differentiated into root hairs was only observed a few days after germination. Thereafter, the aging primary root and newly formed laterals formed only bulges under control conditions that did not elongate (Fig. 2a). When grown in media lacking either Fe or P, a high number of hairs were formed in wer plants (Table I). The number of hairs in the H position was nearly similar to that of the wild type under Fe-deficient conditions and was slightly reduced in P-deficient roots, but the frequency of hairs in the N position was considerably enhanced under both Fe and P deficiency (Table I). TTG encodes a small protein with WD40 repeats (Walker et al., 1999). The reported root hair number of ttg seedlings ranges between 94 and 98 hairs per millimeter of root length (Masucci and Schiefelbein, 1996; Wada et al., 1997). When the number of hairs in the H position is considered, the frequency of root hairs is not significantly different from the wild type under all three growth conditions (Table I). Similar to wer roots, the frequency of hairs in the N position was drastically enhanced in the absence of Fe and P. In P-deficient roots, 29.9% of the epidermal cells in the N position were developed into root hairs. In addition, roots of P-deficient ttg plants produced root hairs that were clearly longer than those of the wild type under similar conditions (1.0 ± 0.02 mm, Fig. 3f). GL2 encodes a homeobox-containing putative transcription factor and is preferentially expressed in atrichoblasts (Masucci et al., 1996; Wada et al., 1997). Root hair density of gl2 mutant plants was comparable with the ttg1 phenotype under the respective conditions, although statistically significant higher frequencies of hairs in the H positions were observed under control and −P conditions.

CPC encodes a protein with an MYB-like DNA-binding domain, negatively regulating the expression of GL2 and moving from the hairless cell to the hair cell (Wada et al., 1997, 2002; Lee and Schiefelbein, 2002). Under control conditions, roots of cpc mutants formed only a few hairs that were randomly distributed along the root. The appearance of the phenotypes of cpc roots under −Fe and −P conditions were similar to the wild type, although the number of hairs was lower (P < 0.01; Fig. 3, a–c; Table I). Ectopic root hairs were not produced in cpc roots.

Despite marked changes in root hair frequency in H and N positions among the wer, ttg, gl2, and cpc mutants with respect to the wild type under all growth conditions, the number of hairs was increased in response to P or Fe starvation. Thus, the nutritional signal can be perceived and translated in this group of mutants. Interestingly, in wer mutants, branched root hairs with a number typical of −Fe wild-type plants were induced by Fe deficiency, which stands in contrast to cpc, ttg, and gl2 plants in

Figure 3. Root tips of the cpc and ttg mutants grown under control conditions or in the absence of Fe or P. a, cpc control. b, cpc −Fe. c, cpc −P. d, ttg control. e, ttg −Fe. f, ttg −P. Bar = 250 μm.
which a significant lower number of branched hairs was observed.

The RHL1 gene encodes a nuclear protein that is required for root hair initiation (Schneider et al., 1998). rhl mutants show no cytoplasmic differentiation of epidermal cells, indicating that the RHL1 gene product is required for cell specification (Schneider et al., 1998). Under the present conditions, rhl1 roots remained hairless when grown on standard medium or under either P or Fe deficiency, suggesting an essential function of the RHL gene in the presence and absence of Fe and phosphate (Table I). rhl2 and rhl3 showed phenotypes similar to rhl1. All rhl mutants formed normal air-borne root hairs.

The ECTOPTIC ROOT HAIR genes ERH1 and ERH3 are required for correct differentiation of rhizodermal cells. erh1 and erh3 mutants show a staining pattern that is not strictly correlated with the position of trichoblasts and atrichoblasts in the meristematic region (Schneider et al., 1997). Both mutants are characterized by an increase in radial cell expansion, but only in erh3 roots is the number of cortical cell files irregular, ranging from seven to nine (Schneider et al., 1997). Under all conditions, both mutants formed more root hairs than wild-type plants in H positions (Table I). This might in part be because of a significantly higher number of cortex cells in both mutants, increasing the epidermal cell number in the H position. The difference between the mutants is not evident when only the hairs in ectopic positions are considered, which are generally present in higher frequency independent of the growth type. An increase in root hair frequency in response to P and Fe deficiency was apparent, however, when compared with the control. The number of branched root hairs formed in response to Fe deficiency was significantly lower in erh plants.

The RHDr gene promotes root hair initiation (Mausucci and Schiefelbein, 1994). In roots of the rhdr mutant, no hairs were formed under all three growth conditions, but the mutant formed normal hairs when the roots were in contact with air.

Bulge Formation and Tip Growth

Defects in the RHD1 locus lead to larger bulges, presumably because of disturbed control of cell wall loosening (Schiefelbein and Somerville, 1990). Under the present conditions, the primary root of rhd1 plants was completely devoid of hairs (Fig. 2b), with the exception of –P plants, in which some hairs were observed occasionally (Table I). In laterals, root hair density of rhd1 roots was not significantly different from the wild type under all growth conditions, when only those trichoblasts were considered that succeeded in initiating root hairs. The non-hair cells were characterized by wide bulges that comprised the entire epidermal cell wall (Fig. 2c). Some laterals displayed the wild-type phenotype. Of all mutants under investigation, only in the rhd1 mutant were clear differences in root hair density between the primary and lateral roots observed (data not shown).

TRH1 is a potassium transporter that appears to be crucial for tip growth under standard growth conditions but not for overall plant K+ nutrition because the trh1 phenotype is not rescued by high external potassium concentrations (Rigas et al., 2001; Desbrosses et al., 2003). Plants lacking the TRH1 gene function formed only some bulges that did not elongate but did not form root hairs under control and –Fe conditions. trh1 plants produced normal root hairs under –P conditions in a frequency and positional pattern that differed not considerably from the wild type (Table I; Fig. 4, a–c). Thus, TRH1 is apparently not necessary for the formation of root hairs in response to P deficiency.

LRX1 encodes a chimeric Leu-rich repeat/extensin protein expressed in root hairs that is required for tip growth to proceed properly (Baumberger et al., 2001). lrx1 mutants show an irregular pattern of root hair development with hairs often being shorter and with swellings either at the basis or along the stalk. Hairs of lrx1 roots were often ruptured at their tips. In this mutant, fewer hairs than in the wild type were formed in response to P deficiency. Under –Fe conditions, most of the hairs (79.5%) in the lrx mutant were branched, and a significant higher number of branched hairs was also formed under control conditions.

In tip1 roots, only very short root hairs were formed, probably because of a defect in microtubule dynamics (Ryan et al., 1998). The frequency of these short root hairs did not differ markedly from the wild type under all growth conditions (Table I; Fig. 4, d–f). Hairs of –P plants are longer than those of the control plants. Under control and –P conditions, but not in medium lacking Fe, tip1 plants produce significantly more cortical and epidermal cells. Independent of the growth conditions, the majority of the hairs were branched. Under control conditions, nearly all hairs had two tips (87.5%), and the percentage of branched hairs was slightly lower in –Fe and –P plants.

In contrast, mutation in the KJK (KOJAK/AICSMD3) gene causes a burst of the hairs after swelling formation. KJK was isolated by positional cloning and was found to encode a cellulose synthase-like protein required during root hair elongation (Favery et al., 2001). Grown under either Fe or P deficiency, kjk mutants displayed a phenotype almost similar to the wild type with respect to the root hair number, but most of the hairs were ruptured at their tip and had irregular lengths. Only under control conditions were hairs in H positions formed in a considerably lower frequency. In contrast to the wild type, only very few branched root hairs developed in response to Fe starvation. The same gene was cloned independently by Wang et al. (2001). In contrast to the kojak mutation, bulges of csld3-1 mutants did not elongate.
The frequency of the bulges did not significantly deviate from the wild type under all growth conditions (data not shown).

*rhd2* mutants produced short root hairs under control and Fe-deficient conditions. When grown in P-deficient medium, only bulges were produced that did not elongate. The number of these bulges in P plants was similar to the root hair frequency of the wild type (Table I). *RHD3* encodes a novel protein with putative GTP-binding motifs, which is required for regulated cell enlargement (Schiefelbein and Somerville, 1990). *rhd3* mutants produced also short hairs under all conditions (Fig. 4, g–i). The number of root hairs was similar to the wild type. *RHD4* is a further gene active in the maintenance of root hair elongation. Root hair density under Fe and P conditions in *rhd4* mutants did not deviate significantly from the wild type except for the number of hairs in H position under Fe deficiency, which was significantly higher than in the wild type. Hairs of the *rhd4* mutant were generally shorter than in the wild type. Interestingly, air-borne hairs of all mutants with defects in elongation resembled those of the wild-type (data not shown).

**DISCUSSION**

Developmental/Environmental Regulation of Root Hair Formation

The formation of extra root hairs in response to suboptimal availability of Fe or phosphate are well-
documented examples of morphologic acclimations aiding in the uptake of limiting nutrients from the soil (Forde and Lorenzo, 2001; Ma et al., 2001; López-Bucio et al., 2003). The number of root hair cells is markedly reduced in the aging primary root when compared with seedlings, in which all those cells form root hairs that lie over the intercellular spaces of underlying cortical cells (Schiefelbein, 2003; Table I). This number is increased in response to Fe and P deficiency, in a manner typical of each growth type.

In primary roots of P-deficient plants, 20% of the total root hairs are in the N position, whereas in laterals, only 10% of the hairs are ectopic. Because of the fact that in 3-week-old plants the majority of the root system consists of laterals, root hairs in N position are less important for the plant than the additional root hairs in H position. Ectopic root hairs in laterals of −Fe plants represent 5% of the total hairs; thus, ectopic hairs appear to be less important under Fe-deficient conditions.

Beside changes in root hair patterning, P-deficient conditions evoked a significant increase in root hair length (Figs. 1, 3, and 4), which is likely to improve P acquisition. In seedling roots, all epidermal cells in the H position developed into root hairs. When 5-d-old plants were germinated on Fe- or P-free medium, the roots showed a slight deviation from the control, indicating that the environmental stimulus can be perceived at an early developmental stage.

Mutants harboring defects in the WER/TTG/GL2 transcription factor cascade showed an increase in root hair density in response to Fe and P deficiency that resembled the wild type in most cases when the hairs in H position are considered. This stands in sharp contrast to the formation of ectopic hairs, which are dramatically increased in this group of mutants. Thus, it appears that in the aging primary root, this group of genes is more important for the fate of cells in N position than for adjusting the number of hairs in normal position. An increase in cortical cell number has been reported for P-deficient Arabidopsis roots and was suggested to represent a

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<th>Gene</th>
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<tr>
<td>wer1</td>
<td>Excessive root hairs</td>
<td>Lee and Schiefelbein (1999)</td>
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means of increasing the number of root hairs by providing a higher number of epidermal cells in the H position (Ma et al., 2001). Under the present conditions, an increase in cortical cell number was not observed in the primary roots or in laterals. A higher number of cortex cells relative to the wild type was formed in the two erh mutants. This can in part explain the increased frequency of hairs in the H position under P deficiency but not under control and −Fe conditions, where the increase in root hairs is higher than the increase in epidermal cell files in the H position.

In −Fe plants, the increase in absorptive surface area is mainly achieved by the formation of branched root hairs. Among the mutants with defects in cell specification genes, only wer shows this response, indicating that the WER product is not necessary for inducing the phenotype typical of Fe-deficient plants. Additional branched root hairs were also observed in mutants with defects in maintaining cell shape such as tip1 and lrx mutants, suggesting that this response is induced downstream of cell specification. This might be similar to trichome formation, where branching is controlled after cell specification by a microtubule-mediated multiple change in cell polarity (Ilgenfritz et al., 2003). Hairs with multiple tips were also described for the ethylene- and auxin-insensitive mutant axr2 (Wilson et al., 1990), pointing to a possible involvement of these hormones in their development. cDNA microarray analysis with Fe-deficient Arabidopsis plants has revealed that upregulation of genes involved in ethylene synthesis and signal transduction during Fe deficiency peaked together with the maximal density of root hairs, pointing to a possible involvement of ethylene in the induction of the −Fe root hair phenotype (Thimm et al., 2001). A further potential component in the signal cascade leading to branched hairs is auxin. Of the hormone-associated genes present in the same cDNA array, the auxin group was found to be the one in which the highest percentage of differentially expressed genes was observed.

The data presented above can be interpreted in terms of separate pathways induced in Fe- and P-deficient plants. This difference is most pronounced in the trh1 mutant, in which root hairs were only formed under P deficiency. Because all mutants harboring defects in root hair formation develop normally elongated air-born root hairs, it can be assumed that these root hairs have a different derivation.

### A Model of Root Hair Formation in the Aging Primary Root

Based on the phenotypical analysis of the mutants listed in Table II, a model that groups the known components involved in controlling epidermal patterning in Arabidopsis roots to the different pathways can be considered (Fig. 5). Independent cascade events are assumed to regulate the development of root hairs in response to P and Fe starvation. Differences concerning the required gene products are also evident concerning the formation of hairs in the H and N positions and for branched hairs. Functional products of WER and GL2 appear to be required in all three pathways for the development of hairs in both H positions and TTG for the formation of hairs in the N position. An exception is represented by roots of Fe-deficient plants, in which WER is not required for hairs in normal position. Interestingly, in contrast to all other genes involved in cell specification, the WER product is also not needed for the formation of branched hairs. CPC is required for the hair fate in H and N position, and a nonfunctioning CPC product completely inhibits the formation of ectopic hairs. CPC accumulates predominantly in non-hair cell files and acts together with TRY in lateral suppression of GL2 expression (Schellmann et al., 2002). Functional products of the ERH and RHL genes are required for the initiation of root hairs in the presence and absence of Fe and phosphate. In all growth types, root hair initiation is blocked by defects in RHD6.

Under control and −Fe conditions, proper regulation of the cellular events during bulge formation and tip growth is dependent on functional TRH1. Although sequence analysis and heterologous expression of this gene has shown that the TRH1 protein functions as a potassium transporter (Rigas et al., 2001), the physiological function of TRH1 is not entirely clear (Desbrosses et al., 2003). From the normal root hair development in −P trh1 mutants, it can be inferred that functional TRH1 protein is not required for tip growth of root hairs under −P conditions. Thus, we assume that TRH1 plays a regulatory role in root hair formation.

Concerning elongation of the hairs, no differences in the effect of mutations in the genes involved in this process were obvious between control, −Fe, or −P conditions. rhd2, rhd3, and rhd4 mutants produce short hairs under all conditions, indicating that the RHD genes are important under all three conditions. TIP1, CSLD, KJK, and LRX are further genes required for a proper control of the duration and rate of tip growth. Hence, it appears that after cell fate has been determined, similar components are involved in the further development of the hairs under all growth conditions. Defects in this group of genes also affect the formation of branched hairs in −Fe plants.

In conclusion, our data show that the capacity to respond to environmental information is achieved in the aging primary root after intrinsic and positional cues have determined cell fate in a largely invariant way. Different pathways are evidently involved in installing the phenotype typically of −Fe and −P plants. The isolation of genes involved in the trophomorphogenetic signal transduction cascade will help
to elucidate the processes leading to the fine tuning of epidermal cell differentiation.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The rhd1, rhd2, rhd3, rhd4, and wer mutants were kindly provided by John Schiefelbein (University of Michigan, Ann Arbor); the rhl1 mutant was obtained from Polydektis Hatzopoulos (Agricultural University of Athens); tip-1 and loki-k were obtained from Eoin Ryan (University College, Dublin); cold-1 was obtained from Mieke Van Lijsebettens (University of Gent, Belgium); rhl1, rhl2-1, rhl3-1, erh1, and erh3 were obtained from Katharina Schneider (Max-Planck Institut für Züchtungsforschung, Köln, Germany); rhd6 was obtained from Hyung-Taeg Cho (Pennsylvania State University, University Park); and lzo1 was obtained from Nicolas Baumberger (University of Zurich). The other genetic stocks were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus). All mutants have been described elsewhere (see Table II).

Plants were grown in a growth chamber on an agar medium as described by Estelle and Somerville (1987). The seeds were surface sterilized by immersing them in 5% (v/v) NaOCl for 5 min and 96% (v/v) ethanol for 7 min, followed by four rinses in sterile water. The medium was composed of: 5 mM KNO₃, 2 mM MgSO₄, 2 mM Ca(NO₃)₂, 2.5 mM KH₂PO₄, 70 μM H₃BO₃, 14 μM MnCl₂, 1 μM ZnSO₄, 0.5 μM CuSO₄, 10 μM NaCl, 0.2 μM Na₂MoO₄, and 40 μM FeEDTA, and solidified with 0.55% (w/v) agar. Suc (43 mM) and 4.7 mM MES were included, and the pH was adjusted to 5.5. Seeds were placed onto petri dishes containing agar medium and kept for 3 d at 4°C in the dark before the plates were transferred to a growth chamber and grown at 21°C in continuous light (50 μmol m⁻² s⁻¹), TL lamps, Philips, Eindhoven, The Netherlands. Eleven days after sowing, plants were transferred to fresh agar medium (control plants), medium without P (–P) plants or without Fe, and medium with 100 μM 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulfonate (–Fe plants). The lower concentration of K because of the absence of KH₂PO₄ in the –P medium was corrected by the addition of KCl. Plants were analyzed 9 d after replanting to the different growth conditions. To prepare the seedling seeds, plants were sown on either control, –Fe, or –P medium and exposed to light after a stratification for 1 d at 4°C in the dark. The epidermal cell patterning was determined 5 d after sowing.

Microscopy

Root hair patterns were analyzed in cross sections of 10 primary root apical segments per genotype and treatment. The apical first cm of the root tip was excised, washed in 0.5 μM CaSO₄, and fixed in 3% (w/v) agarose solution. Hand-cut sections from the root hair zone were stained with toluidine blue, and one cell layer each was analyzed using an Eclipse E600 microscope (Nikon, Tokyo). The number of cortical and epidermal cells and the rate of root hairs in the H and N positions and branched root hairs were counted in five sections per root segment. If not otherwise marked, an epidermal cell was scored as a root hair cell, if any protrusion was visible, regardless of its length. Epidermal cell patterning of the seedling roots was analyzed on the basis of two cross sections per root between the beginning of the root hair zone and the collet region. In cold mutants, root hair frequency was estimated under the stereomicroscope (see below) by counting the bulges in apical segments of 20 roots in the 2nd mm behind the apex. The data were compared with the root hair number of the wild type calculated by the same way. Statistical significance of differences between mean values was determined using Student’s t test. Therefore, every mutant was compared with the wild type under the respective growth condition.

To quantify the epidermal cell length in the root hair zone, dark-field microscopy was performed on a Stermi 2000-CS stereomicroscope (Zeiss, Jena, Germany). For cryo-scanning electron microscopy, root tips were fixed on the probe plate with carbon adhesive and frozen in liquid nitrogen at ~175°C. After sublimation at ~95°C in an Oxford-Cryo chamber/-Transforsystem CTS 1500C (Oxford Cryosystems Ltd, Oxford, United Kingdom), roots were located in an S-3200N scanning electron microscope (Hitachi, Tokyo).

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LITERATURE CITED


