From priming to plasticity: the changing fate of rhizodermic cells

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Summary

The fate of root epidermal cells is controlled by a complex interplay of transcriptional regulators, generating a genetically determined, position-biased arrangement of root hair cells. This pattern is altered during postembryonic development and in response to environmental signals to confer developmental plasticity that acclimates the plant to the prevailing conditions. Based on the hypothesis that events downstream of this initial mechanism can modulate the pattern installed during embryogenesis, we have developed a reaction diffusion model that reproduces the root hair patterning previously observed experimentally. Under all growth conditions, an almost equal spacing between root hair forming cells was observed both in vitro and in silico, indicating that long-range intercellular communication is crucial for the trichoblasts' decision to form a root hair. We assume that a hair growth promoter (HGP) is upregulated in root-hair-forming cells by a trichoblast-specific component. Once established, HGP production is self-enhancing. The autocatalytic regulation of HGP is antagonized by an HGP-produced hair growth inhibitor (HGI). HGI is exported from trichoblasts and diffuses to neighboring cells, where it inhibits further HGP production and promotes the non-hair cell fate. Under conditions of phosphate deficiency, we hypothesise that HGP production is increased and HGI diffusion rate is reduced, leading to a position-independent formation of extra root hairs. ***BioEssays*** **30:75–81, 2008.**

Introduction

The restricted possibilities of plants to escape from unfavorable conditions necessitate sophisticated mechanisms to perceive and convey environmental information and to modulate developmental programs. Due to their open morphogenesis (i.e. continuous enlargement of the body plan by addition of reiterative elements), the post-embryonic development of plants remains highly plastic throughout their life-cycle. Plasticity is crucial for acclimation to changing conditions and confers a competitive advantage by avoiding metabolic and nutritional misbalances. Acclimation of plants comprises morphological, physiological and biochemical responses, and is often associated with alterations in cell division rates and changes of cell differentiation programmes. In plants, regeneration of cells, tissues and organs can occur from either meristematic cells or from fully differentiated cells that are reprogrammed to acquire new fates. This transdifferentiation of cells is critical for the plant's survival in an ever-changing environment and a decisive component for evading stress exposure. Given the importance of phenotypic plasticity in the life cycle of plants, understanding of environment-dependent development may have wide application in crop improvement. We here present a testable hypothesis that may aid to direct research into the effects of environmental signals on developmental processes.

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Binary cell fate decisions in the root epidermis

The root epidermis represents a simple and well-explored model to elucidate the effects of environmental signals on changes in developmental decisions (Fig. 1). Root epidermal cells can enter one of two developmental pathways: they can differentiate as a root hair cell or develop into a non-hair cell. Depending on the species, root hairs form either in a random pattern or by asymmetric cell division along files of epidermal cells, or the cell fate can be determined by positional cues. In Arabidopsis roots, the fate of the epidermal cells is dependent on their position; cells that have contact with two underlying cortical cells (H position) develop into a hair cell, those that touch only one cortical cell (N position) enter the non-hair cell fate. The differentiation of epidermal cells is controlled by a network of patterning genes (Fig. 2, see Refs. 1–3 for recent reviews). Current thinking suggests that an internal apoplastic signal, deriving from underlying tissue(s), influences the root epidermal cell fate. The signal is perceived at the plasma membrane and conveyed into the cell by a leucine-rich repeat receptor-like kinase named SCRAMBLED (SCM).[4,5] The intensity of the signal is slightly greater for cells in the H position (lying over the cortical cleft), inducing a decrease in

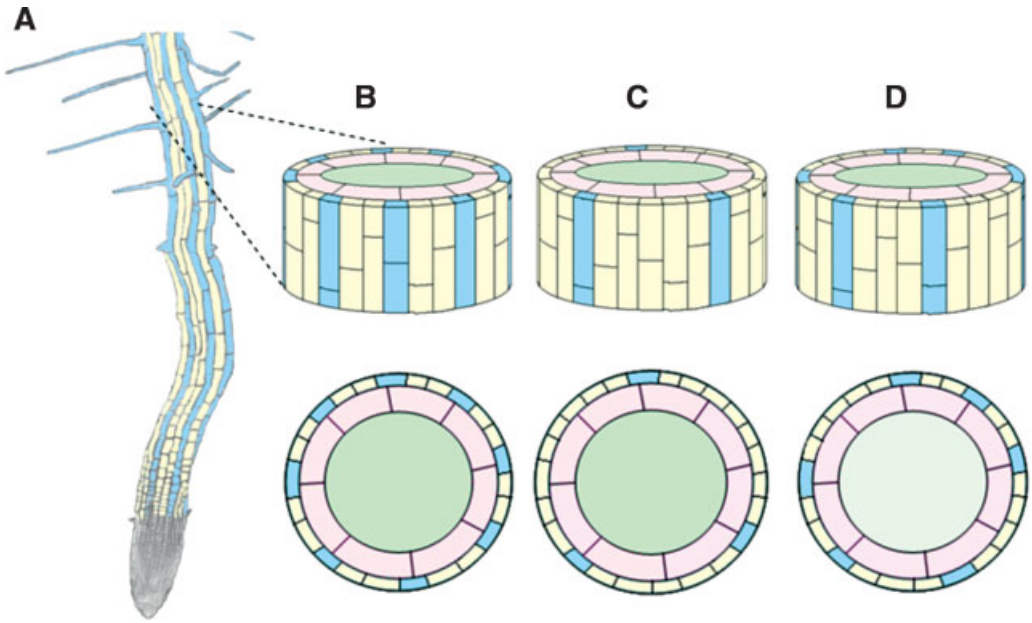

Figure 1. Arrangement of root hair and non-hair cell files in the epidermis of Arabidopsis roots. **A:** Tip of an Arabidopsis root. **B–D:** Cross sections from the root hair zone showing the position and number of root hair cells (blue) with respect to the cortical cells (pink) and non-hair epidermal cells (yellow) in the root epidermis. **B:** The root of a seedling, all the epidermal cells located over the clefts of underlying cortical cells enter the hair fate. **C:** The root of an adult plant in which the number of root hairs is reduced from eight to three, and **D:** the root of an adult plant grown under phosphate-deficient conditions in which the frequency of root hairs is increased and some hairs are formed in ectopic positions.

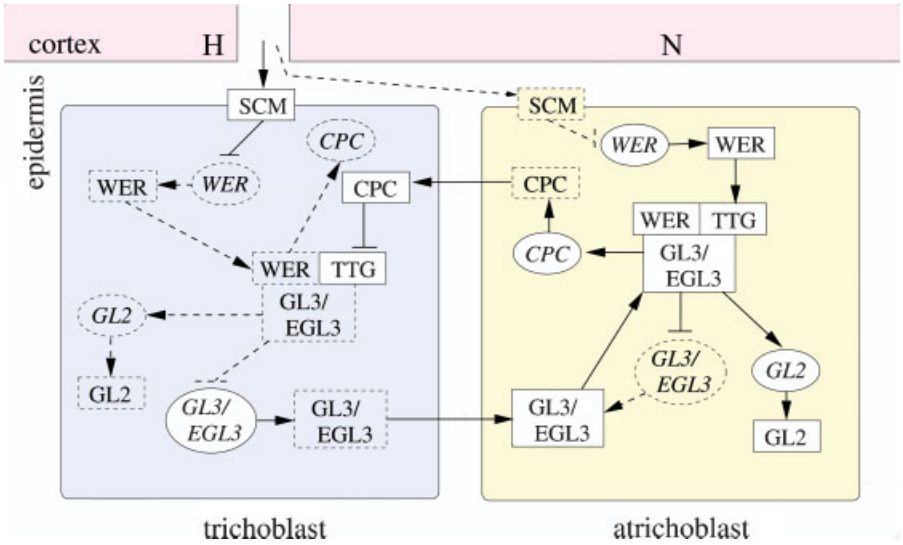

Figure 2. Interaction between patterning genes. Square boxes symbolise protein, oval boxes transcript abundance. Dotted boxes/ovals indicate low abundance or absence, dotted lines weak interactions. Sharp and blunt arrows represent upregulation and downregulation, respectively. Hair and non-hair positions are marked as H and N, respectively. See text for detailed explanation. Adapted from Bernhardt C, Zhao MZ, Gonzalez A, Lloyd A, Schiefelbein J 2005 Dev 132: 291–298.

the transcription of the MYB transcription factor WEREWOLF (WER). Transgenic plants ectopically expressing SCM under the control of the 35S promoter showed decreased *WER* RNA levels, supporting the assumption that SCM negatively regulates the transcription of the *WER* gene.[5] In epidermal cells that are in contact with only one cortical cell (that lie over the periclinal cortical cell walls), association of the R-like bHLH proteins GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) with WER and the WD40 protein TRANSPARENT TESTA GLABRA1 (TTG1), promotes the expression of the single-repeat MYB protein CAPRICE (CPC) and of the homeodomain leucine zipper protein GLABRA2 (GL2). GL2 acts as a positive regulator of the non-hair cell fate. The WER/GL3/EGL3/TTG complex further inhibits expression of the *GL3* and *EGL3* genes. Whereas the *CPC* promoter is active only in non-hair cells, the CPC protein moves from cells in the N position to cells in the H position where it (and possibly other related small MYB proteins such as TRY and ETC1[6]) suppresses the expression of *WER,* and a complex composed of CPC/GL3/EGL3 and TTG is formed. This complex prevents the expression of *CPC* and *GL2* in future hair cells. WER controls the transcription of *CPC* and *GL2* directly by binding to their promoter regions.[7] Expression of *GL2* is associated with changes in chromatin organization[8] and is affected by global changes in histone acetylation.[9] Recently, an additional link between chromatin modification and cell fate decisions of root epidermal cells has been presented by showing that overexpression of the DNA replication protein CDT1 increases the transcript level of *GL2*. A protein interacting with CDT1 was found to modulate the expression of *GL2* and *TTG1* by modifying histones upstream of the open reading frame of *GL2* and *CPC*.[10] A bidirectional signalling circuit is thought to be crucial for maintaining the cell differentiation defined by positional information (Fig. 2). The circuit is such that CPC moves from non-hair cells into hair cells where it suppresses the binding of WER to the GL3/EGL3/TTG complex, and GL3/EGL3 moves from hair cells to non-hair cells to form the WER/GL3/EGL3/TTG complex. Negative autoregulation of *GL3* and *EGL3* is dependent on bidirectional signaling between H and N cells.[11]

The root epidermis responds to environmental clues

As in animals, cellular plasticity in plants is often associated with cell cycle re-entry and changes in chromatin organization which increases the accessibility of transcriptional regulators to regulatory sequences (see Ref. 12 for an overview). However, in plants, changes in positional information are sufficient to induce fate switches in root epidermal cells that are not dividing.[13] Root epidermal cells receive information from neighbouring cells, from underlying tissues, and from the external environment. During postembryonic development, integration of these signals is crucial for plasticity which fine tunes the development. The epidermal cell layer is particularly responsive to the availability of immobile but essential mineral nutrients, such as iron, phosphate and manganese. Sub-optimal concentration of either mineral causes an increase in the absorptive surface area by altering the length, frequency and position of root hairs.[14–16] The resulting phenotype is typical of each growth type, suggesting different signalling cascades to be induced in response to the lack of a particular mineral ion.

Ontogenetic changes in root hair patterning

The root epidermis derives from an initial ring of 16 cells.[17] The cortex of Arabidopsis roots consists of a single layer of eight cells, giving rise to equal numbers of trichoblasts (root-hair-forming cells) and atrichoblasts (non-hair cells) in the seedling root epidermis. All epidermal cells in the H position develop into hairs and so eight root hairs are formed per cross-section (Fig. 1B). Due to longitudinal cell divisions, the adult plant has an increased number of epidermal cells, 28 on average. Cortical files do not experience this division, thus the number of cortical clefts remains unchanged.[18] In three-week-old plants, the number of root hairs is reduced from eight to three, all of which are in the H position (Fig. 1C). Epidermal cells that develop into root hairs under these conditions are relatively equally spaced; in most cases, a maximum distance is approached. This means that the decision for a trichoblast to form a hair is unlikely to be independent of neighbouring trichoblasts. The probability of at least two neighbouring trichoblasts forming root hairs is close to once in every 7.3 trichoblasts; on average one pair of neighboring trichoblasts would be found in every cross section (based on experimental values of 8.1 cortical cells and three hairs per cross section).

We assume that under sufficient supply of all essential nutrients in the adult plant, fewer hairs are needed than predetermined by the initial trichoblast patterning. It may also be assumed that, at the stage between trichoblast fate and root hair growth, the root epidermal pattern becomes sensitive to environmental cues, adjusting the plant's absorptive surface area to the plant's demand for water and nutrients.

Phosphate deficiency alters the root hair pattern in a typical manner

Growing plants in media deprived of phosphate significantly increased the number of root hairs.[15,18] This increase is associated with an enlargement in root hair length, allowing for a larger soil volume to be explored. In roots of P-deficient plants, an average of six root hairs per cross section are formed.[18] Interestingly, under these conditions, the available free hair positions are not filled up in all cases; on average one of the extra hairs formed in response to P starvation is located over a tangential cell wall of the underlying cortical tissue, i.e. in ectopic (N) position (Fig. 1D). The formation of ectopic hairs is

highly significant and can be considered as typical of P-deficient Arabidopsis roots. Similar to roots of control plants, the hairs formed in P-deficient plants show an almost equal spacing, indicating that the mechanism conferring spatial awareness of epidermal cells is also active under P starvation conditions.

Activator–inhibitor models for pattern formation

The activator–inhibitor model was first proposed by the English mathematician Alan Turing during the 1950s.[19] According to this model, an activator produces both itself and an inhibitor, the inhibitor inhibits the activator. Self-activation of the activator allows for rapid amplification of subtle concentration differences. A crucial requirement for pattern formation is that the inhibitor diffuses faster than the activator from their origin, thereby causing an uneven distribution of the activator–inhibitor concentrations. This concept was taken up by Meinhard and Gierer[20] and since then extensively used to explain biological patterns, including patterning of epidermal cells in Arabidopsis roots and leaves.[21,22]

A model explaining the phenotype of adult plants under control conditions

We suppose that an activator–inhibitor mechanism acts downstream of the basic patterning mechanism executed by the WER-GL3-EGL3-TTG gene cascade. We further suggest that this downstream mechanism is responsible for controlling the number of hairs in the root of adult plants, and for conferring phenotypical plasticity into the epidermal patterning. Experimental support comes from the observation that the root hairs are almost equally spaced, suggesting communication between epidermal cells.

We consider a classical activator–inhibitor model in which an activator (hair growth promoter, HGP) triggers its own synthesis by autocatalysis and induces also the production of an inhibitor of the hair cell fate (hair growth inhibitor, HGI). The activator is also upregulated by a component specific to the trichoblast cell, i.e. the cells not expressing GL2, we denote this as T. While HGP remains in the cells, HGI is secreted into the apoplast. HGI diffuses through the apoplast, re-entering the cell from which it originated and neighboring cells. This hypothesis is illustrated schematically in Fig. 3. To illustrate how such a mechanism may manifest, we use an adaptation of the Meinhard-Gierer equations[20] (1) on a ring of 28 epidermal cells. Label each cell $i = 1,2,3,\ldots 28$. Let u_i be the concentration of HGP and v_i the concentration of HGI, in cell i

$$\begin{aligned} \frac{du_i}{dt} &= k_1 - k_2 u_i + k_3 \frac{u_i^2}{v_i} \\ \frac{dv_i}{dt} &= k_4 u_i^2 - k_5 v_i + \frac{D_v}{2}(v_{i-1} + v_{i+1} - 2v_i) \end{aligned} \qquad (1)$$

D_v is the diffusion coefficient of the HGI (v_i), $k_1 \ldots k_5$ are reaction constants. Let $K_H = \{k_1,\ldots,k_5\}$ for cells in the H position and $K_N = \{k_1,\ldots,k_5\}$ for cells in the N position. Fig. 4 illustrates equations (1) schematically. By solving equations (1) with various initial concentrations of HGP and HGI, we can record the number and position of HGP peaks in each solution for comparison with experimental data.[18] The numerical solution of (1) tracks the evolution of u_i, v_i and hence the competition between trichoblasts to initiate root hair growth. Initially cells in both positions have a positive value for u_i and v_i, assigned

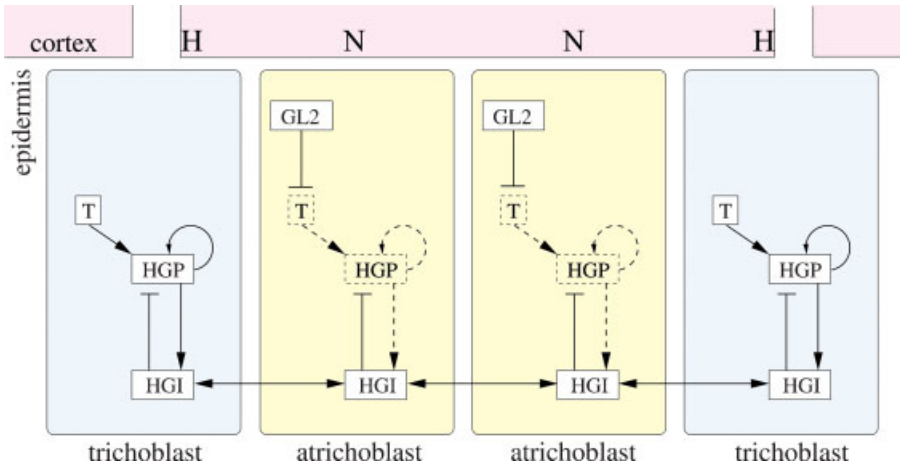

Figure 3. Illustration showing the hypothesized locations of HGP and HGI. Dotted boxes indicate low abundance or absence, dotted lines weak interactions. Sharp and blunt arrows represent upregulation and downregulation, respectively. Hair and non-hair positions are marked as H and N respectively. The component T, specific to cells not expressing *GL2* (trichoblasts), upregulates HGP, which positively self-regulates as well as promoting its inhibitor, the diffusible HGI. The lack of T in the N position and the presence of diffused HGI makes it difficult for HGP to become established, thus cells in the H position battle for HGP dominance.

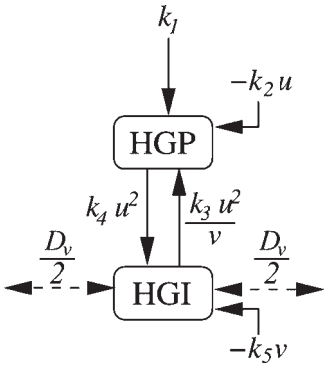

Figure 4. Reaction diagram showing interaction between HGP and HGI in one epidermal cell: k_1 is T upregulation of HGP, $-k_2u$ is degradation of HGP, k_3u^2/v is HGP self-regulation with HGI inhibition, $k_4\ u^2$ is HGP upregulation of HGI, $-k_5v$ is HGI degradation and $D_v/2$ is the diffusion of HGI in each direction.

randomly between 0 and 1. Thus, while the battle for hair growth takes place predominantly between cells in the H position, the positive self regulation of HGP allows some HGP peaks in the N position.

The equations were solved for 500 initial conditions. H positions were chosen randomly with the constraints—at least one N position between two H positions and eight H positions in each simulated epidermis. Initial values for u_i, v_i were random on the unit interval. $K_H = \{0.1, 0.35, 0.06, 0.06, 0.45\}$, $K_N = \{0, 0.35, 0.06, 0.06, 0.45\}$ and $D_v = 8$. Note k_1 in K_N is greater than zero reflecting the positive regulation of HGP by the trichoblast-specific component, T. From the 500 solutions we calculated an average 3.042 u_i peaks in the H position and 0.108 in the N position, corresponding to biological data (3.0 ± 0.2 and 0.1 ± 0.03 hairs in the H and N positions respectively[18]).

Fig. 5A tracks a typical solution of equations (1) for u_i at various times, increasing from left to right. The leftmost graph shows the initial values for u_i from here a number of peaks form, predominantly in the H position (centre graph), which then compete for HGP dominance resulting in three peaks situated in H positions (rightmost graph).

Phosphate starvation affects the activator-inhibitor mechanism

We hypothesise that in phosphate-deficient plants plasticity is conferred by an increase in HGP self regulation, and a

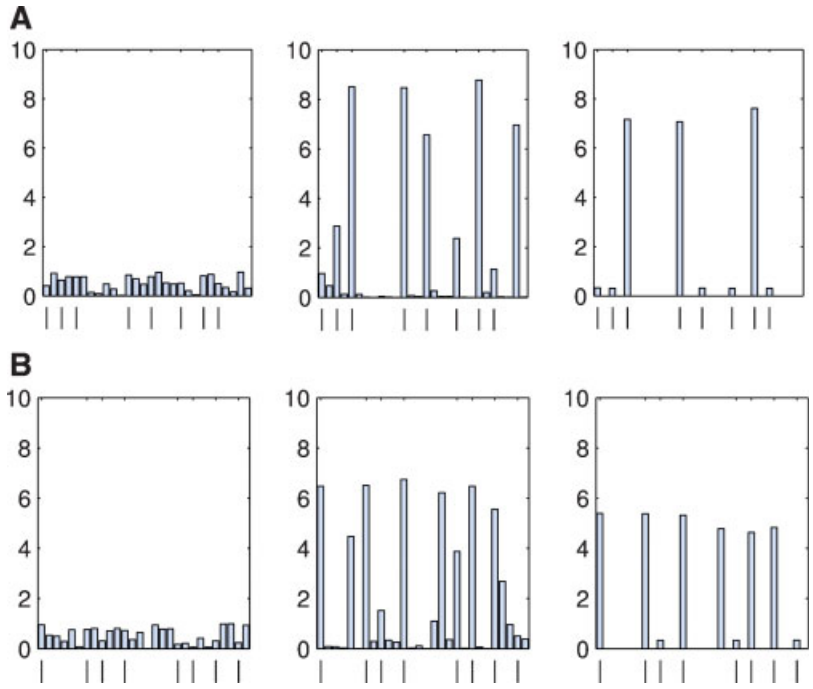

Figure 5. Solution of equations (1) for u_i with **A:** control parameters, $K_H = \{0.1, 0.35, 0.06, 0.06, 0.45\}$, $K_N = \{0, 0.35, 0.06, 0.06, 0.45\}$ and $D_v = 8$ and **B:** with phosphate-deficient parameters, $K_H = \{0.1, 0.35, 0.08, 0.06, 0.45\}$, $K_N = \{0, 0.35, 0.08, 0.06, 0.45\}$ and $D_v = 2$.The leftmost graphs show the initial values for u_i, the rightmost graphs show the final values and the middle graph shows an intermediate stage. Epidermal cells are represented along the x-axis with H positions marked using a vertical line. With control parameters (**A**), the final values of u_i show three HGP peaks in H positions, corresponding to biological data and with phosphate-deficient parameters (**B**) the final values of u_i show five HGP peaks in H position and 1 in N position, corresponding to biological data.

concomitant decrease in HGI diffusion rate, leaving the pre-patterning established by the WER–GL3–EGL3–TTG gene cascade unchanged. We suggest that the HGP-HGI mechanism is spatially distant from the sensing of available P, which is probably perceived in the root cap.[23]

The above hypothesis is supported by equations (1) with $K_H = \{0.1, 0.35, 0.08, 0.06, 0.45\}$, $K_N = \{0, 0.35, 0.08, 0.06, 0.45\}$ and $D_v = 2$. K_3 is now *0.08* rather than *0.06* representing the increase in HGP production and $D_v = 2$ rather than *8* representing the decrease in HGI diffusion rate. Again the equations were solved 500 times and an average of *5.134* u_i peaks in the H position and *1.204* u_i peaks in the N position was calculated. This is in close correspondence with biological data where 5.0 ± 0.2 hairs were observed in the H position and 1.2 ± 0.2 hairs in the N position.[18]

Fig. 5B shows a typical solution of (1) for u_i at various times, increasing from left to right. The initial values for u_i (leftmost graph) develop into a greater number of competing peaks as a direct result of the reduced HGI diffusion rate and the increased self regulation of HGP (centre graph), this results in 5 HGP peaks in H positions and one in N position (rightmost graph).

Conclusions

Genetically determined developmental programmes allow cells to acquire different fates according to their position in the plant body. A sophisticated exchange of information between neighbouring cells is necessary to establish and to strengthen the identity of individual cells in the root epidermis. The unique life style of plants allows deviations from the default pathway to compensate for the lack of possibilities to get away from unfavourable conditions. This holds particularly true for the root epidermis, which represents the interface of the plant to the soil and which is highly responsive to environmental cues. This plasticity necessitates additional patterning mechanisms that allow for fine-tuning of the distribution and characteristics of root epidermal cells to secure highest functionality. Our concern has been plasticity as a consequence of phosphate deficiency. Wet experiments show a reduced number of hairs in the adult root, under control conditions, when compared to seedlings in the same environment, three rather than eight. These hairs are relatively evenly spaced implying that long-distance (across a number of cells) cell–cell communication, between cells in the H position, is used to compete for hair growth. These data imply an activator–inhibitor mechanism. Thus we put forward the hypothesis that an activator–inhibitor mechanism, acting downstream of the regulatory mechanism mediated by the WER–GL3–EGL3–TTG gene cascade, confers plasticity to the system and aids in generating a pattern that optimally suits for a given developmental stage. The hairs observed on an adult plant under phosphate-deficient conditions are more in number (than control plants) and equally spaced. This indicates that long-distance cell–cell communication is altered but not destroyed by phosphate deficiency. We hypothesize that, under phosphate deficiency, self regulation of the activator (HGP) is increased and the ability of the inhibitor (HGI) to move is decreased. The effect of phosphate deficiency on the activator and inhibitor is a prediction of the model. The success of the simulations to reproduce biological data is a proof of principle for our hypothesis but wet experiments are needed for verification. This could be achieved by forward genetic screening for mutants that are unable to increase the root hair number in response to phosphate starvation and subsequent isolation of the gene(s) involved in this process. This phenotype is supposed to be caused either by a defect in the upregulation of HGP or a defect in the movement of HGI. In the case of iron deficiency, which also leads to the formation of extra root hairs, a mutant with an altered root hair pattern has recently been described,[24] which suggests that non-redundant genes involved in environmentally induced changes can be identified in this way. An alternative (or supplementary) approach is a comparison of the transcriptional profile of single root-hair-forming and hairless epidermal cells under control and phosphate-deficient conditions. Cell-type-specific marker lines are available for a variety of tissues and the feasibility of single-cell-type-specific expression analysis has been demonstrated recently.[25]

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