Epidermal cell patterning and differentiation throughout the apical–basal axis of the seedling

Laura Serna*

Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Real Fábrica de Armas, Avda. Carlos III, s/n, E-45071 Toledo, Spain

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Abstract

The idea of common pathways guiding different fates is an emerging concept in plant development, and epidermal cell fate specification in Arabidopsis thaliana is an excellent example to illustrate it. In the root epidermis, both hair patterning and differentiation depend on a complex interaction between both negative (WER, TTG, GL3, EGL3, and GL2) and positive (CPC, TRY, and ETC1) regulators of hair cell fate. These regulators pattern and differentiate hairs through a bi-directional signalling mechanism. The same molecular components (WER, TTG, GL3, EGL3, and GL2) seem to be involved in the patterning of stomata in the embryonic stem. However, the possible role of CPC, TRY, and ETC1 on stomatal patterning and/or differentiation has not been studied, questioning whether they, and the underlying bi-directional mechanism, guide patterning formation and differentiation in the hypocotyl.

Key words: Bi-directional signalling, differentiation, epidermis, hypocotyl, lateral inhibition, patterning, root hair, stoma, WER/TTG/GL3/EGL3/GL2 and CPC/TRY/ETC1 pathways.

Introduction

The development of a plant depends on the generation of diverse cell types arranged in predictable patterns. The plant epidermis has been used as an excellent model system to study cell fate specification. In Arabidopsis thaliana, two specialized epidermal cell types can be observed at the seedling stage: hairs in the root and paired guard cells (stomata) in both the hypocotyl and the cotyledons. In spite of the obvious differences concerning the morphogenesis and function of these structures, a similar position-dependent mechanism patterns hairs in the root and stomata in the hypocotyl (Benfey, 1999; Larkin et al., 2003; Serna, 2004).

Epidermal cells in both organs, roots and hypocotyls, are arranged in columns running parallel to the long axis of the seedling. The fate of these cells depends on its position relative to the underlying cortical cell tissue: (i) all cells located outside two cortical cell files differentiate into hairs in the root and some of them enter into the stomatal pathway in the hypocotyl, and (ii) those located outside a single cortical cell file do not develop as hair cells in the root and they do not enter into the stomatal pathway (Dolan et al., 1993, 1994; Galway et al., 1994; Berger et al., 1998a; Hung et al., 1998). As a result, files with all cells being hair cells alternate with those consisting of non-hair cells in the root (Fig. 1A). In the hypocotyl, stoma cell files also alternate with non-stoma cell ones (Fig. 1B). However, stomata in a given file never make contact with other stomata, and they are surrounded by a full or partial complement of non-stomatal cells, which derive from the stomatal lineage (Berger et al., 1998a; Fig. 1B).

Prior to cell maturity, the epidermal cell files located above two cortical cell files can easily be distinguished from those located outside a single cortical cell file. Hair-forming cells in wild-type plants exhibit an increased cytoplasmic density (Dolan et al., 1994; Galway et al., 1994), a delay in vacuolation (Galway et al., 1994), a reduced cell length (Dolan et al., 1994; Masucci et al., 1996), unique cell surface ornamentation (Dolan et al., 1994), distinct cell wall epitopes (Freshour et al., 1996), and an increased cell division rate (Berger et al., 1998b) relative to non-hair-forming cells. In the hypocotyl, cells in epidermal files overlying two cortical cell files, which may enter into the stomatal pathway, are shorter and narrower than those located in files overlying a single cortical cell file (Gendreau et al., 1997; Hung et al., 1998).
Findings regarding the molecular mechanism regulating epidermal cell patterning and differentiation throughout the apical–basal axis of the Arabidopsis seedling are summarized here. The emerging picture reveals that a common set of genes (WER, GL3, EGL3, TTG, and GL2) triggers non-hair and non-stoma cell fate, but it is not clear how far this similarity extends. In particular, this review considers whether the molecules (CPC, TRY, and ETC1) and the mechanism regulating hair cell fate play any role in the repression of the non-stoma cell fate. This review also highlights the little-known genetic control of the early stages of epidermal cell differentiation in the hypocotyl, which contrasts with the abundant genetic information of such stages in the root.

GL2, TTG, and WER: negative regulators of both hair and stoma cell fate

Several genes controlling both stomata formation in the hypocotyl and hairs in the root have been identified (Table 1). Mutations in either GLABRA2 (GL2), TRANSPARENT TESTA GLABRA (TTG), or WEREWOLF (WER) genes disrupt epidermal pattern formation by increasing the number of both hairs and stomata due to the production of such cells in files overlying a single cortical cell file (Galway et al., 1994; Di Cristina et al., 1996; Masucci et al., 1996; Berger et al., 1998a; Hung et al., 1998; Lee and Schiefelbein, 1999).

GL2 encodes a homeodomain-leucine zipper protein (Rerie et al., 1994; Di Cristina et al., 1996), and its effect on root hair development depends on the modulation of phospholipid signalling (Ohashi et al., 2003). The GL2 expression, preferentially restricted to non-hair-forming cell files (meristematic and elongation region) and to the non-stoma-forming cell files (upper region) (Masucci et al., 1996; Hung et al., 1998), is positively regulated by both the WD repeat-containing protein TTG (Hung et al., 1998; Walker et al., 1999), and the R2R3 MYB protein WER (Lee and Schiefelbein, 1999). The expression pattern of WER overlaps with those of GL2 (Lee and Schiefelbein, 1999), and the protein encoded by WER not only increases the level of GL2 expression, but also it conveys information for the cellular localization of such as transcripts (Lee and Schiefelbein, 1999). At the moment, the TTG expression pattern and protein localization remain unknown.

WD domains function in protein–protein interactions (Neer et al., 1994), and accordingly, the yeast two-hybrid assay has shown that TTG physically associates with two basic helix–loop–helix proteins (BHLH) of Arabidopsis, GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) (see next section; Payne et al., 2000; Zhang et al., 2003). WER, as expected from its interaction with the maize R BHLH protein (Lee and Schiefelbein, 1999), also interacts with both GL3 and EGL3 (see next section; Bernhardt et al., 2003). These results suggest that a multimeric complex among TTG, WER, and BHLH proteins triggers both non-hair and non-stoma cell fate by regulating GL2 expression.

As stated above, mutations in these three genes disrupt pattern formation, by producing hairs and stomata in ectopic positions (Galway et al., 1994; Di Cristina et al., 1996; Masucci et al., 1996; Berger et al., 1998a; Hung et al., 1998; Lee and Schiefelbein, 1999). These genes also regulate cell differentiation. Interestingly, mutations in these genes do not produce a similar effect in all the stages.
of non-hair cell formation. The gl2 mutation only affects the final step of hair formation by inducing its outgrowth in cells overlying a single cortical cell file (Masucci et al., 1996; Berger et al., 1998b), whereas the wer and ttg mutations disrupt early aspects of non-hair cell differentiation (Galway et al., 1994; Berger et al., 1998b; Lee and Schiefelbein, 1999). The developing epidermal cells overlying a single cortical cell file in both wer and ttg mutants display a rate of vacuolation, cytoplasmic density, and cell division rate similar to those of the hair-forming cells in the wild type. Thus, WER and TTG are required at an earlier developmental stage than GL2 in the non-hair cell fate.

Although the vacuolation rate and the cytoplasmic density have not been studied in the hypocotyl epidermal cells, it is known that cells in files overlying a single cortical cell file are longer than those in files overlying two cortical cell files (Hung et al., 1998). These differences in cell length can be observed before stomatal development, and they are telling us that the epidermal cells in files overlying two cortical cell files exhibit a division rate higher that those of epidermal cells overlying a single cortical cell file. Mutations in either GL2 or TTG do not affect epidermal cell length (Hung et al., 1998), and the possible role played by the WER gene in such a process has not been studied. Thus, GL2 and TTG may participate only at the later stage of cell differentiation in the hypocotyl. The absence of a role for TTG in the early stage of hypocotyl cell differentiation differs from such as role during non-hair formation. It will be interesting to determine whether WER also regulates different differentiation stages in the embryonic stem (similar to that occurs in the root), or whether it acts only at a later developmental stage.

### The role of BHLH genes in hair and stoma pattern formation

The first evidence supporting a role for BHLH proteins in both root hair and stomatal patterning comes from the reduced number of root hairs and stomata in plants over-expressing the maize R BHLH gene under the control of the 35S promoter (Galway et al., 1994; Berger et al., 1998a). These findings support that an R-like BHLH protein in Arabidopsis represses both hair and stoma formation (Galway et al., 1994; Berger et al., 1998a).

As was mentioned in the previous section, two genes named GL3 and EGL3 and encoding BHLH proteins closely related to R have been cloned (Payne et al., 2000; Zhang et al., 2003). Plants homozygous for mutations in both genes produce a hairy phenotype throughout the root due to the formation of hairs in ectopic positions, and plants over-expressing either gene exhibit a reduced number of hairs caused by the differentiation of non-hair cells in files overlying two cortical cell files (Bernhardt et al., 2003). In addition, each of the single mutants exhibits a small increase of the number of root hairs in the upper part of this organ caused by the formation of some root hairs in ectopic positions (Bernhardt et al., 2003). Taken together, these results indicate that GL3 and EGL3 function in a partially redundant manner to specify non-hair cell fate. Surprisingly, these BHLH genes are preferentially expressed in the

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**Table 1. Genes regulating epidermal cell patterning and differentiation in the root and hypocotyl of Arabidopsis discussed in this review**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell determination*</th>
<th>Early stages of cell differentiation*</th>
<th>Gene product</th>
<th>Key reference</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Root hair</td>
<td>Stoma</td>
<td>Root hair</td>
<td>Cells in stomatal files</td>
</tr>
<tr>
<td>TTG</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>No role</td>
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<tr>
<td>WER</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>?</td>
</tr>
</tbody>
</table>

*+, Positive regulator; –, negative regulator; ?, unknown function.

b GL3 and EGL3 act in a redundant manner.
c CPC, TRY, and ETC1 also act redundantly.
developing hair cells (meristematic and elongation region) (Bernhardt et al., 2005). In situ RNA hybridization has just shown that GL3 and EGL3 RNAs accumulate preferentially in developing hair cells (Bernhardt et al., 2005). However, the analysis of the YFP signal in GL3::GL3-YFP plants has revealed that the GL3 protein moves from hair-forming cells to the nucleus of non-hair-forming ones (Bernhardt et al., 2005). Thus, GL3 (and perhaps EGL3) guides hair cell patterning in a non-cell autonomous manner.

Analysis of interactions among transcriptional factors has revealed that GL3 and EGL3, redundantly, increase the level of both GL2 and CPC expression in non-hair cell files, but that they are not required for the position-dependent pattern of such genes (Bernhardt et al., 2003). In these developing non-hair cells, the expression of these BHLH genes is negatively regulated by WER, GL3, and EGL3 (Bernhardt et al., 2005). In developing hair cells, CPC and TRY positively regulate both GL3 and EGL3 expression (Bernhardt et al., 2005).

In the hypocotyl, it has just been shown that the gl3 eg3 double mutant exhibits an increased number of stomata due to its formation in files overlying a single cortical cell file, strongly suggesting that these BHLH genes together also pattern stomata in the embryonic stem (Bernhardt et al., 2005). The expression pattern of these genes, RNA accumulation, and protein localization in this plant organ have not been studied.

The yeast two-hybrid assay has shown that these BHLH proteins can physically interact with either WER or the single-repeat MYB proteins CAPRICE (CPC) and TRIPTYCHON (TRY) (see next section; Payne et al., 2000; Bernhardt et al., 2003; Zhang et al., 2003). Interestingly, three-hybrid analysis in yeast has shown that TRY prevents the interaction between GL3 and the R2R3 MYB GL1 (Esch et al., 2003). Because WER and GL1 share a high amino acid identity, it is likely that TRY also competitively competes with WER in the BHLH proteins binding. These BHLH proteins also mediate homo- and heterodimeric interactions, and they also physically interact with TTG (Payne et al., 2000; Zhang et al., 2003).

Like ttg and we r, the gl3 eg3 double mutant also disrupts early aspects of non-hair cell differentiation (Bernhardt et al., 2003). The epidermal cells overlying a single cortical cell file in the gl3 eg3 mutant exhibit a vacuolation rate, cytoplasmic density, and cell division rate similar to those of the hair-forming cells in the wild type. In addition, all epidermal cells in plants overexpressing the EGL3 gene exhibit the characteristics of non-hair-forming cells. Thus, GL3 and EGL3 control, in a redundant manner, the same stages regulated by WER and TTG. The epidermal cells overlying two cortical cell files in the R-expressing plants also exhibit the cellular characteristics of non-hair-forming cells (Galway et al., 2004). The effect of eg3 gl3 mutations in the early stages of differentiation in the hypocotyl epidermis has not been investigated.

CPC, TRY, and ETC1: positive regulators of hair cell fate

In addition to these negative regulators of hair and/or stoma cell-fate specification, three genes, CPC, TRY, and ENHANCER OF TRY AND CPC1 (ETC1), encode positive regulators at least of root hair formation (Wada et al., 1997, 2002; Schellmann et al., 2002; Kirik et al., 2004). These positive regulators act in a redundant manner patterning hairs in the root (Schellmann et al., 2002; Kirik et al., 2004): the cpc mutant exhibits a reduced number of hairs, and although mutations in either TRY or ETC1 have little effect, roots of the cpc try double mutant are hairless and those of the cpc etc1 reduce the number of root hairs over roots of cpc alone.

CPC, TRY, and ETC1 encode single-repeat MYB proteins that share high sequence identity (Wada et al., 1997, 2002; Schellmann et al., 2002; Kirik et al., 2004). Examination of the CPC expression pattern, RNA accumulation, and protein localization shows that CPC is preferentially expressed in the developing non-hair cells, but that the protein moves to the nucleus of the neighbouring hair-forming cells (Wada et al., 2002). Although the cellular localization of TRY and ETC1 proteins remains unknown, ETC1 (and perhaps TRY) is also preferentially expressed in the non-hair cell files (Schellmann et al., 2002; Kirik et al., 2004).

In non-hair-forming cells, CPC expression is positively regulated by WER and redundantly by GL3 and EGL3, while CPC into hair-forming cells negatively regulates WER, GL2, and its own expression (Lee and Schiefelbein, 2002; Schellmann et al., 2002; Wada et al., 2002; Bernhardt et al., 2003). In a redundant manner, TRY and CPC negatively regulate TRY expression in hair-forming cells (Schellmann et al., 2002). As stated in the previous section, both CPC and TRY physically interact with GL3 and EGL3 (Payne et al., 2000; Bernhardt et al., 2003; Zhang et al., 2003).

The possible role of these single-repeat MYB proteins in the hypocotyl has not been studied in detail, but it is known that the stomatal pattern in the try mutant is indistinguishable from that of the wild type (Berger et al., 1998a). Two explanations might justify this fact: (i) TRY plays no role in stomatal pattern formation, or (ii) TRY plays a role, but a redundant factor masks its effect in the try mutant. CPC and ETC1 are obvious candidates to mask a hypothetical TRY function in the embryonic stem. Interestingly, the expression patterns of CPC, TRY, and ETC1, preferentially restricted to non-stoma-forming cell files, suggest that a similar genetic mechanism guides root hair and stomatal patterning (Kirik et al., 2004). However, because the etc1 try cpc triple mutants exhibit trichomes in their hypocotyls (Kirik et al., 2004), these expression patterns may only reflect their roles in repressing trichome cell fate specification.

Although some cellular events occurring in cpc, try, and/or cpc try at the early stage of hair cell-fate specification
such as vacuolation rate or cytoplasmic density have not been studied, it is known that CPC and TRY act together at an early stage to increase the cell division rate in the hair-forming cell files relative to the non-hair-forming ones (Schellmann et al., 2002). The possible effect of ETC1 in such a stage has not been studied. This suggests that CPC and TRY (and perhaps ETC1) regulate together the same stages disrupted in wer, ttg, and gl3 egl3. The role of these single-repeat MYB genes in early stages of cell differentiation in the hypocotyl epidermis has not been addressed.

A model for epidermal cell patterning and differentiation throughout the apical–basal axis of the seedling

The results described above support a multimeric complex among WER, TTG, GL3, and EGL3 that induces GL2 expression and triggers non-hair and non-stoma cell fate in epidermal files placed above a single cortical cell file (Larkin et al., 2003; Serna, 2004; Bernhardt et al., 2005; Fig. 2A). This complex also positively regulates CPC expression in the future non-hair cells (Bernhardt et al., 2003; Larkin et al., 2003; Serna, 2004; Fig 2A). CPC (and perhaps TRY and ETC1) acts in a non-cell autonomous manner (Lee and Schiefelbein, 2002; Schellmann et al., 2002; Wada et al., 2002; Kirik et al., 2004). The CPC protein moves from differentiating non-hair cells to neighbouring cells placed above two cortical cell files (Wada et al., 2002), where it physically interacts with GL3 and EGL3 leading to the formation of an inactive complex (single-repeat MYB/GL3/EGL3/TTG). This inactive complex negatively regulates GL2 expression, which triggers hair cell fate specification (Fig. 2A). At the same time, while the increment of CPC in the future hair cells leads to a reduction in WER, CPC, and TRY expression, it also leads to an increase in GL3 and EGL3 expression. The GL3 protein (and perhaps EGL3) moves from the hair-forming

Fig. 2. Model for cell patterning and differentiation in the root and hypocotyl epidermis of Arabidopsis. (A) In the root, a complex consisting of WER, TTG, GL3, and EGL3 promotes non-hair cell fate by inducing both GL2 and CPC expression. The CPC protein moves to neighbouring cells placed above two cortical cell files, where it induces GL3 and EGL3 expression. In such cells, the complex among TTG, GL3, EGL3, and CPC (or perhaps TRY or ETC1) negatively regulates GL2 expression, which triggers hair cell fate. The GL3 protein (and perhaps EGL3) moves from the hair-forming cells to the neighbouring non-hair forming cells, where it physically interacts with WER and TTG. In the hypocotyl, the same complex consisting of WER, GL3, EGL3, and TTG negatively regulates stoma formation by inducing GL2 expression. The mechanism that represses GL2 expression in stoma-forming cell files is unknown. (B) The bi-directional mechanism that guides hair patterning extends to the early stages of hair differentiation regulating vacuolation rate, cell division, and cytoplasmic density. The downstream gene(s) of the multimeric complexes is unknown. Note that the epidermal cells overlying two cortical cells differentiate into hairs in the root, and they can develop stomata in the hypocotyl. Those overlying a single cortical cell remain as non-hair/non-stoma. See text for details on the transcriptional regulation among factors.
cells to the neighbouring non-hair forming cells (Bernhardt et al., 2005), where it physically interacts with WER and TTG. In the future non-hair cells, the active complex promotes both GL2 and CPC expression, and it negatively regulates expression of GL3 and EGL3. Thus, a bi-directional signalling mechanism accounts for root hair patterning in the Arabidopsis root.

The same genes (with the exception of GL2) and the same bi-directional signalling mechanism participate in the early stages of hair/non-hair differentiation (Masucci et al., 1998). Mutant is indistinguishable from wild-type plants in these early stages of hair/non-hair specification by events taking place in the early stages of differentiation outgrowth in the Arabidopsis root. In addition, the root-hair stream gene(s) of these multimeric complexes exist which specifically control such events. In other downstream gene(s) of these multimeric complexes exist which specifically control such events. In addition, the root-hair outgrowth in the gl2 mutant indicates that the cellular events taking place in the early stages of differentiation (vacuolation, cell division rate, etc) are not required for those that occur in the later ones (hair outgrowth).

Present data support the active complex (WER/GL3/EGL3/T TG) triggering non-stoma cell fate specification by positively regulating GL2 expression (Larkin et al., 2003; Serna, 2004; Bernhardt et al., 2005; Fig. 2A). Although it has not been studied, GL3 (and perhaps EGL3) may act in a non-cell autonomous manner by moving from stoma-forming cell files to non-stoma forming ones. The possible role of CPC, TRY, and ETC1 on stoma cell fate specification has not been addressed (Larkin et al., 2003; Serna, 2004). The fact that, in a given file, only a few cells enter into the stomatal pathway, while in a hair file all cells differentiate as root hair, illuminates substantial differences in the general mechanism that regulates epidermal cell patterning in both organs.

Very little is known about the process that controls the early stages of epidermal cell differentiation in the embryonic stem. The hypothetical role of WER, GL3, EGL3, CPC, TRY, and ETC has not been studied. However, the available data suggest that the parallelism between hair and stoma cell pattern may not extend throughout the cell differentiation stages; although TTG controls early stages of non-hair cell differentiation, it seems that this gene plays no role in cell differentiation in non-stoma cell files (Hung et al., 1998). Other gene(s) should be involved in regulating early events of cell differentiation in the hypocotyl.

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**References**


