Steroids are required for epidermal cell fate establishment in *Arabidopsis* roots

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The simple structure of *Arabidopsis* roots provides an excellent model system to study epidermal cell fate specification. Epidermal cells in contact with 2 underlying cortical cells differentiate into hair cells (H cells; trichoblasts), whereas cells that contact only a single cortical cell differentiate into mature hairless cells (N cells; atrichoblasts). This position-dependent patterning, in combination with the constrained orientation of cell divisions, results in hair and nonhair cell files running longitudinally along the root epidermis. Here, we present strong evidence that steroid hormones called brassinosteroids (BRs) are required to maintain position-dependent fate specification in roots. We show that BRs are required for normal expression levels and patterns of *WEREWOLF* (*WER*) and *GLABRA2* (*GL2*), master regulators of epidermal patterning. Loss of BR signaling results in loss of hair cells in H positions, likely as a consequence of reduced expression of *CA-PRICE* (*CPC*), a direct downstream target of WER. Our observations demonstrate that in addition to their well-known role in cell expansion, BRs play an essential role in directing cell fate.

brassinosteroids | cell fate specification | positional cues | root epidermis

Molecular genetic studies in *Arabidopsis* have defined a number of genes that influence root cell fate patterning (1–3). A transcriptional complex composed of the MYB transcription factor WEREWOLF (WER), a WD-40 repeat protein called TRANSPARENT TESTA GLABRA (TTG), and 2 basic helix–loop–helix transcription factors called GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) promotes hairless cell (N cell) differentiation. This complex induces expression of *GL2*, encoding a homeodomain transcription factor, and *CA-PRICE* (*CPC*), encoding a single-repeat MYB protein. CPC moves laterally to the adjacent epidermal cells, where it inhibits *WER* and *GL2* expression, thus promoting hair cell (H cell) differentiation (4). Recent mathematical simulations of root epidermal cell specification lend support to a lateral inhibition mechanism relying on the lateral movement of CPC and GL3 (5). The identification of a receptor-like kinase, SCRAMBLED (SCM), has led to a model where an asymmetrically distributed positional signal activates SCM in epidermal cells located between 2 cortical cells (6). Activated SCM promotes hair cell fate by decreasing the abundance of WER (7). Laterally translocated CPC promotes the preferential accumulation of SCM in the H cell position, thus reinforcing hair cell fate in epidermal cells located between 2 cortical cells (8).

The role of hormones in the regulation of root epidermal cell identity program is not well established. Studies using auxin and ethylene have demonstrated that these 2 hormone pathways control root epidermis development at a relatively late stage, after the completion of the cell differentiation program (9). A recent study has demonstrated that auxin transport through the nonhair cells controls the elongation of root hairs in the hair cell position (10). Global transcriptome analysis of whole seedlings revealed that expression of *WER* can be induced by treatment with brassinosteroids (BRs) (11), a class of steroid hormones known to promote growth in diverse plant species (12). Although BRs are known to affect root elongation (13), no previous work has connected them to the root hair formation program. This study reveals a role for plant steroid hormones in the regulation of key transcription factors required for acquisition of cell fate during root hair development. Before this study, SCRAMBLED, a leucine-rich repeat receptor-like kinase, was the only known mediator of positional cues in the root epidermis (6). The present study points to a cell type-specific role for BRs in root hair formation and provides evidence for an additional mechanism for interpreting cell position.

Results and Discussion

To investigate a potential role for BRs in cell fate specification, we examined the effect of brassinolide (BL), the most biologically active BR, on root tissue. RNA was extracted from roots of seedlings grown in mock or BL treatments. Quantitative RT-PCR experiments demonstrated that *WER* and *GL2* were induced ≈2 and 2.5 times, respectively, in response to 10 nM BL treatment (Fig. 1*A*), consistent with the results from a previous global transcriptome analysis using whole seedlings (11). *WER* is likely a BR early-response gene, because the effects of BL could still be detected in the presence of the protein synthesis inhibitor cycloheximide. Although cycloheximide treatment alone led to increased WER expression, an additional effect of BL treatment was clearly observable (Fig. 1*B*). The effects of reduced BR response were also examined by using the strong BR mutant *brassinosteroid insensitive 1* (*bri1*). *BRI1* encodes the BR receptor, a plasma membrane-localized, leucine-rich repeat receptor kinase (14). We found that *bri1* plants showed a significant reduction in both *WER* and *GL2* expression (Fig. 1*C*). Thus, both normal levels and response to BRs are required for the wild-type expression of *WER* and *GL2*.

Experiments using the *GL2::GUS* reporter, a central tool in studying root epidermal cell fate (6, 9, 15), revealed that BRs are required for the levels of expression and position-dependent patterning of *GL2*. In contrast to wild-type roots, *GL2::GUS* expression in *bri1* roots was significantly reduced and was no longer restricted to orderly files (Fig. 2 *A* and *B*). This aberrant patterning of *GL2::GUS* expression could be more easily observed if staining reactions were left to develop for approximately twice as long as those used for wild-type roots (Fig. 2*C*). Transverse sections made clear that in many cases, cells in contact with 2 cortical cells aberrantly expressed the *GL2::GUS* reporter (Fig. 2*H*). A loss of *GL2::GUS* expression in the nonhair files was never observed in these roots. Similar changes in expression levels and patterns of *WER* reporters were also seen in *bri1* mutants (Fig. S1). When BR levels were reduced by treatment with 100 nM brassinazole (BRZ), a BR biosynthetic inhibitor (16), *GL2::GUS* expression resembled what was seen in *bri1* mutants (Fig. 2*E*). In contrast, treatment with BL had no
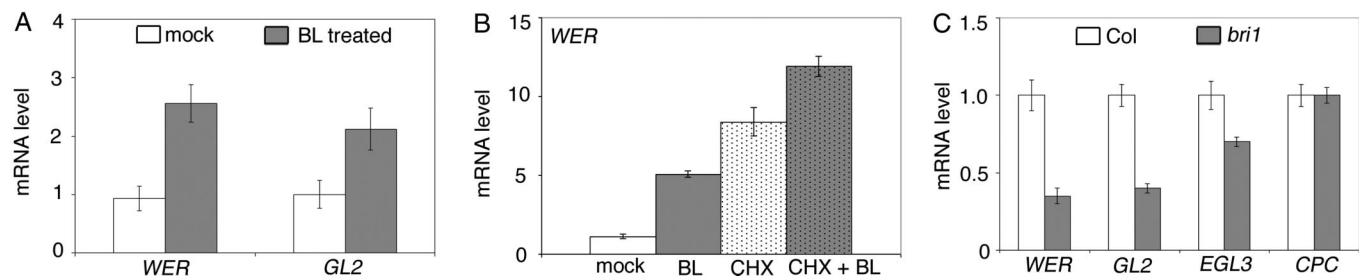
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Fig. 1. BRs regulate expression levels of root epidermal cell markers. (*A*) Quantitative RT-PCR using wild-type roots treated with 10 nM BL shows increased expression of *WER* and *GL2*. (*B*) Treatment with cycloheximide (CHX) does not inhibit the induction of *WER* by BL. (*C*) The *bri1* roots show decreased expression of *WER*, *GL2*, and *CPC*. Results are represented as mean ± standard error (*n* = 3 replicates).

effect on the patterning of *GL2* expression (Fig. 2*F*). Changes in expression levels were not seen consistently in BL-treated roots, likely reflecting an insufficiently large effect to influence the long-lived GUS reporter. In combination, these results suggest that BRs are necessary for proper epidermal patterning but are not sufficient when added exogenously to scramble the SCM-EGL3-CPC-specified program.

Although the overall level of *WER* and *GL2* expression was reduced when the BR pathway was compromised, additional pathways must also be promoting N cell fate, because both reporters were clearly detectable in these cells. The effects of BRs were more pronounced in the H cells, where ectopic expression of both *WER* and *GL2* reporters was observed. To assess whether this ectopic expression was sufficient to change the fate of cells, we performed a quantitative analysis of epidermal cell fate relative to cell position. Experiments documenting the number of hair or nonhair cells in the N and H positions of *bri1* roots showed that 23% of cells in the H position were nonhair cells (Table 1) compared with 5% in wild-type roots. When wild-type plants were grown on 100 nM BRZ, 18% of cells in the H position were nonhair cells. In contrast, loss of BRs had only a modest effect on the number of hair cells formed in N positions (Table 1), suggesting that BRs are more important for suppressing nonhair fate in H cells than for promoting nonhair fate in N cells. To assess how early BRs may be exerting their effects on cell fate, we compared the number of cells in the N and H positions in wild-type and *bri1* root tips. As reported previously (17), we found that in wild-type root tips, there were ≈30% more cells in the H position. In *bri1*, the number of cells in H and N positions were nearly the same (H/N ratio for wild type, 1.3 ± 0.09; *bri1*, 1.01 ± 0.02). This indicates that BRs act at an early stage of root development.

Because epidermal cell fate is correlated with cortical arrangement, we tested the possibility that defective epidermal patterning in the *bri1* mutant is due to an abnormality in the root structure. Examination of the cross-sections of the wild-type and *bri1* mutant roots revealed no significant difference in the root structure. In wild-type roots, the epidermal cell number was

19.3 ± 1.2, whereas in *bri1* roots, it was 19.1 ± 1.0. Both wild-type and *bri1* roots displayed 8 cortical cell files and showed no obvious difference in the organization of the cell files.

The root epidermal phenotype observed in *bri1* and BRZ-treated plants was reminiscent of *cpc* mutants, where a large number of cells in the H position adopt the N cell fate (7, 18). Like *GL2*, *CPC* expression is WER-dependent (4, 19). Reduced WER expression caused by the loss of the BR pathway could lead to lower expression of *CPC*, which might allow ectopic expression of *WER* (and *GL2*) in H position cells. Consistent with this model, we found a decreased expression of *CPC* in *bri1* roots (Figs. 1*B* and 3 *A* and *B*). To assess whether these changes on gene expression reflected a general reorganization of the root epidermis, we examined the level and pattern of expression of *EGL3*. EGL3 is a component of the WER transcriptional complex that induces *GL2* but is not itself under the transcriptional control of WER (1, 2). Loss of BR signaling showed little to no effect on levels or pattern of expression of EGL3 (Figs. 1*B* and 3 *C* and *D*), strongly suggesting that BRs act specifically through regulation of WER and its downstream targets.

SCM activity is a major determinant of position-dependent expression of *WER* and *GL2* (6, 8). To determine the relationship between the SCM and BR pathways, *scm bri1* double mutants were analyzed (Table 1). In *scm bri1* mutants, there was a largely additive effect on conversion of H cells to nonhair cell fate. Approximately 43% of cells in the H position adopted nonhair fate compared with 24% and 23% in the *scm* and *bri1* single-mutant lines, respectively. In the N cell position, *scm bri1* double mutants showed a significant reduction in hair cell fate compared with the *scm* single mutant. This finding is consistent with a model for BR action via modulation of *CPC* expression. Genetic and molecular studies demonstrate that CPC plays a largely additive role with SCM in repressing *WER* expression and promoting hair cell fate in H cells (7). The conversion of N cells to hair cell fate observed in *scm* single mutants is almost completely eliminated in *scm cpc* double mutants (7), suggesting that ectopic hair cell fate requires CPC activity.
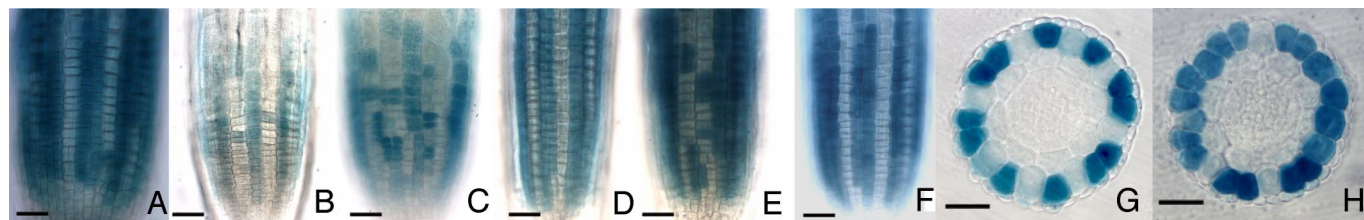

Fig. 2. Disruptions in the BR pathway lead to aberrant levels and patterning of *GL2*. (*A–C*) *GL2::GUS* expression in roots of wild type (*A*) and *bri1* mutants (*B* and *C*). (*B*) The *bri1* roots were stained for 20 min, the same duration as shown for wild type. (*C*) The *bri1* roots stained for 40 min. (*D* and *E*) *GL2* shows an increased randomness of expression pattern on roots treated with BRZ (*E*) compared with the control roots (*D*). (*F*) BL treatment does not affect the pattern of *GL2* expression. (*G* and *H*) Cross-section of *GL2::GUS* plants in either wild-type (*G*) or *bri1* background (*H*). (Scale bars: *A–F*, 50 μm; *G* and *H*, 25 μm.)

Table 1. Distribution of root hair and nonhair cells in the root epidermis

	H position		N position	
	Hair, %	Nonhair, %	Hair, %	Nonhair, %
Wild type (Col)	95 ± 2	5 ± 2	0 ± 0	100 ± 0
bri1	77 ± 4	23 ± 4	4 ± 2	96 ± 1
Col and BRZ	82 ± 3	18 ± 3	3 ± 2	97 ± 2
scm	76 ± 4	24 ± 4	41 ± 7	59 ± 7
scm bri1	57 ± 6	43 ± 6	9 ± 4	91 ± 4

Values are expressed as the mean ± standard error of at least 40 roots for each line.

In summary, we have shown that: (*i*) *WER* is an early BR response gene, (*ii*) normal levels and patterning of both *WER* and its downstream target *GL2* are dependent on BR production and response, and (*iii*) ectopic expression of these master regulators in H cells when BR synthesis or response is compromised is correlated with a change of H cell fate. These results, in combination with previous findings, suggest that 2 leucine-rich receptor-like kinases, SCM and BRI1, convey positional information to root epidermal cells. In such a model, BR-activated BRI1 induces *WER* expression in cells overlying 1 cortical cell, resulting in the accumulation of CPC. CPC would then translocate to neighboring epidermal cells, where it would inhibit WER and GL2 and promote SCM accumulation. The preferential accumulation of SCM further represses WER activity in these cells, reinforcing the hair cell identity. Earlier studies have indicated that BR pathway components, such as BRI1, BIN2, and BZR1, are localized uniformly to cells within the root epidermis (20–22). Detailed temporal analysis as to the cellular and subcellular localization of these components is required to determine the mechanism of cell type-specific responses.

Recent studies have demonstrated that BRs acting in the epidermis are essential for growth of aerial tissues (23, 24). In this study, we show that BRs are required for normal expression and patterning of master regulators of epidermal cell fate. Unlike any other hormone examined, BRs affect early stages of cell fate by manipulating expression of WER, GL2, and CPC. Collectively, these studies suggest that BRs play an essential role in morphogenesis throughout the plant.

Methods

Fig. 3. *BRI1* regulates expression of *CPC* but not *EGL3*. (*A* and *B*) *CPC::GUS* expression is reduced significantly in *bri1-116* (*B*) in comparison with wild type (*A*). (*C* and *D*) The patterning expression of *EGL3*, a marker of H cell fate, is similar in wild type (*C*) and in *bri1* (*D*). (Scale bars: 50 μm.)

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