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JACKDAW controls epidermal patterning in the *Arabidopsis* root meristem through a non-cell-autonomous mechanism

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SUMMARY

In *Arabidopsis*, specification of the hair and non-hair epidermal cell types is position dependent, in that hair cells arise over clefts in the underlying cortical cell layer. Epidermal patterning is determined by a network of transcriptional regulators that respond to an as yet unknown cue from underlying tissues. Previously, we showed that JACKDAW (JKD), a zinc finger protein, localizes in the quiescent centre and the ground tissue, and regulates tissue boundaries and asymmetric cell division by delimiting SHORT-ROOT movement. Here, we provide evidence that *JKD* controls position-dependent signals that regulate epidermal-cell-type patterning. JKD is required for appropriately patterned expression of the epidermal cell fate regulators GLABRA2, CAPRICE and WEREWOLF. Genetic interaction studies indicate that *JKD* operates upstream of the epidermal patterning network in a *SCRAMBLED* (*SCM*)-dependent fashion after embryogenesis, but acts independent of SCM in embryogenesis. Tissue-specific induction experiments indicate non-cell-autonomous action of JKD from the underlying cortex cell layer to specify epidermal cell fate. Our findings are consistent with a model where JKD induces a signal in every cortex cell that is more abundant in the hair cell position owing to the larger surface contact of cells located over a cleft.

KEY WORDS: Epidermal patterning, Position-dependent signals, Root hairs, *Arabidopsis*

INTRODUCTION

The *Arabidopsis thaliana* root epidermis provides an attractive system to study position-dependant cell patterning and specification. Epidermal cell types at defined positions are specified in a predictable manner. Cells located in a cleft between two underlying cortical cells become trichoblasts (T cells) that differentiate into root-hair (H) cells, whereas cells positioned over a single cortical cell become atrichoblasts (A cells) that acquire the non-hair cell (N) fate (Dolan et al., 1994; Galway et al., 1994). A regulatory gene network controls this process through intracellular and intercellular transcriptional feedback loops (Lee and Schiefelbein, 2002; Schiefelbein, 2003; Ueda et al., 2005). Non-hair fate specification requires the homeodomain transcription factor protein GLABRA2 (GL2), the WD40-repeat protein TRANSPARENT TESTA GLABRA (TTG), the bHLH transcription factors GLABRA3, ENHANCER OF GLABRA3 (GL3 and EGL3) and a MYB transcription factor WEREWOLF (WER), all predominantly expressed in the non-hair cells. Mutations in these genes lead to an increase in the frequency of hair cells, implying that they are all required for the specification of non-hair cell fate (Rerie et al., 1994; Galway et al., 1994; Masucci et al., 1996; Walker et al., 1999; Lee and Schiefelbein, 1999; Bernhardt et al., 2003).

The MYB-like proteins CAPRICE (CPC), TRIPTYCHON (TRY) and ENHANCER OF CPC (ETC) act redundantly to promote H fate in the epidermis. The *cpc* mutant has only few hair cells, whereas *try* and *etc* single mutants do not show epidermal patterning defects; however, double mutants with *cpc* cause all the cells to adopt the N fate (Wada et al., 1997; Schellmann et al., 2002; Kirik et al., 2004).

Together these genes form an intercellular regulatory circuit to control epidermal fate specification. WER-GL3-EGL3-TTG1 form a complex that accumulates in A cells, where WER binds directly to the promoters of *GL2* and *CPC*, promoting their expression (Bernhardt et al., 2005). Induction of *GL2* then leads to specification of the N cell fate. CPC moves laterally to the neighbouring T cell where it competes with WER for binding to the GL3-EGL3-TTG1 complex (Bernhardt et al., 2003; Bernhardt et al., 2005). When CPC binds to the GL3-EGL3-TTG1 complex in T cells, *GL2* promoter activity is inhibited and GL2 protein is reduced. This results in specification of the H cell fate. CPC accumulation in T cells leads to reduction of *WER* expression and an increase in *GL3* and *EGL3* expression (Bernhardt et al., 2005). The GL3 protein acts then in a lateral feedback loop by moving to the neighbouring A cells where it generates more of the WER/GL3/EGL3/TTG complex, inducing additional *GL2* and *CPC* expression and also repression of *GL3* and *EGL3* expression (Bernhardt et al., 2005). These two lateral feedback loops lead to a mutual support mechanism that stabilizes alternating cell fate choices (Savage et al., 2008).

SCRAMBLED (SCM), a leucine-rich repeat receptor-like kinase (LRR-RLK), is required to bias this network for position-dependent epidermal cell patterning (Kwak et al., 2005). In *scm* mutants, distribution of H and N cell types is randomized. The SCM receptor has been proposed to perceive extracellular positional cues and influence the entire root hair network (Kwak et al., 2007). In this model, SCM negatively regulates WER in T cells, which enables these cells to adopt the H cell fate, whereas in the A cells, WER abundance becomes relatively high, resulting in activation of the non-hair fate differentiation pathway. However, the epidermal cell pattern is set up during embryogenesis (Costa and Dolan, 2003), where SCM action is not required. This suggests that other factors perceive the initial signals that pattern root epidermal cell types.

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Recently, we have shown that JACKDAW (JKD), a plant-specific zinc finger protein, controls radial pattern formation in the root meristem by restricting the range of SHORT-ROOT action. Mutation of *JKD* results in ectopic divisions in the cortex, leading to an extra cell layer in the ground tissue through broader activity of SCARECROW and SHORT-ROOT (Welch et al., 2007). Here, we show that JKD also regulates, from the underlying tissue layers, epidermal patterning in the *Arabidopsis* root meristem. In *jkd* mutants, the spatial distribution of epidermal cells is randomized and the expression pattern of the root-hair regulatory genes is misregulated already in the embryo, suggesting that JKD sets up epidermal patterning early in development. Analysis of double-mutant combinations of *jkd* with *wer*, *gl2*, *cpc* and *scm* indicates that the corresponding root-hair regulatory genes act downstream of JKD. Misexpression studies suggest that JKD acts from the underlying cortex layer to specify the pattern of epidermal cell types, which evokes a new explanation for the nature of the bias that leads to H cells over cortical clefts.

MATERIALS AND METHODS

Plant materials and growth conditions

The mutants *jkd-4*, *jkd-i mgpi* and *jkd-4 shr-2* used in this study were described in Welch et al. (Welch et al., 2007). Root-hair mutants *cpc-1*, *wer-1*, *gl2-1* and *try* were described in Lee and Schiefelbein (Lee and Schiefelbein, 2002), *try cpc* double mutants in Kirik et al. (Kirik et al., 2004) and the *scm-2* allele in Kwak et al. (Kwak et al., 2005). Combinations of *jkd-4* and *jkd-i* with all root-hair mutants and marker lines were generated by crossing. Homozygous lines were identified by phenotypic analysis, reporter gene expression and genotyping.

Phenotype analysis and microscopy

Preparation of plant material for light microscopy (Nomarski Optics) was carried out according to Willemsen et al. (Willemsen et al., 1998). Cell-type pattern analysis was done with at least 20 roots of 3-, 7- and 10-day-old seedlings. An epidermal cell was scored as a root-hair cell if any protrusion was visible, regardless of its length.

For quantitative analysis of root-cell type, the histochemical staining of plant material harbouring the *GUS* reporter gene was performed as described previously (Willemsen et al., 1998), followed with cross plastic sectioning and 0.05% Ruthernium Red (Sigma) staining as described in Scheres et al. (Scheres et al., 1994; Scheres et al., 1995). The histochemical staining of mature embryos containing the p*GL2::GUS* reporter gene was performed essentially as described in Hung et al. (Hung et al., 1998) and Masucci et al. (Masucci et al., 1996). Aniline Blue staining of mature embryos was performed according to Bougourd et al. (Bougourd et al., 2000).

Ectopic expression studies

Transcriptional *JKD* promoter fusion to a *CFP* reporter was described in Welch et al. (Welch et al., 2007). For translational fusion, the *JKD* coding sequence was placed under a 6 kb promoter and fused to the 3′ end of the GFP coding sequence. For ectopic expression analysis, *JKD* cDNA was driven by p*SCR*, p*CO2* (Heidstra et al., 2002), p*WOL* and p*WER* (Mahonen et al., unpublished data). The *JKD* promoter and cDNA were fused to *GFP;nost* and introduced into a p*Green* binary vector; the construct was transformed into wild type and *jkd* mutants as described in Cough et al. (Cough et al., 1998).

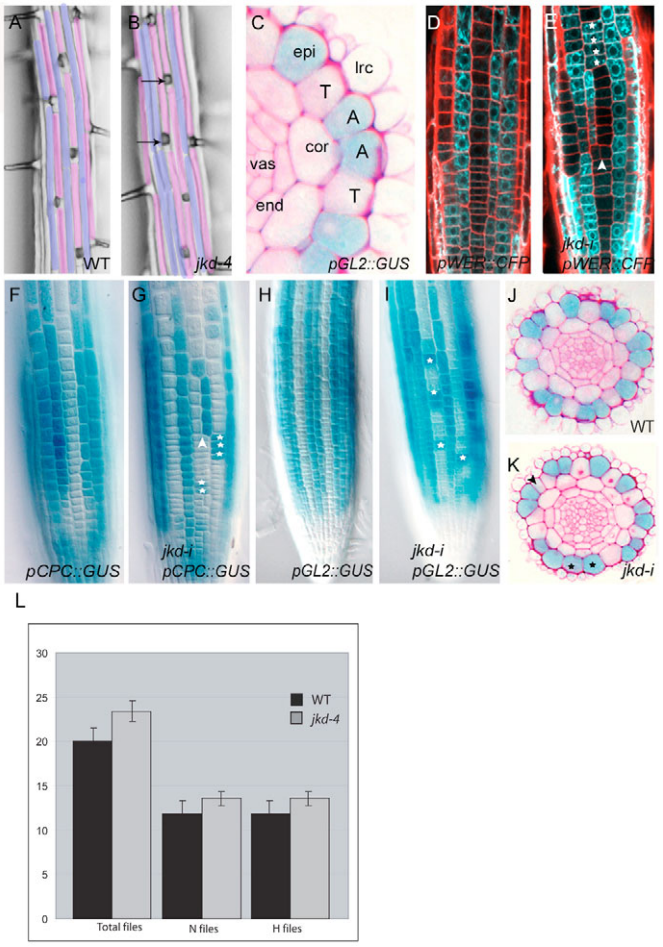

Fig. 1. Reduction of JKD activity causes mis-specification of cell types in root epidermis. (**A**,**B**) External view of roots of wild-type (A) and *jkd* (B) plants. Root-hair files are false-coloured in pink and non-hair files are in blue. Arrows indicate hairs emerging from consecutive hair files. (**C**) Expression pattern of the p*GL2::GUS* reporter gene in a transverse root section showing the organization of cells in the epidermis. Non-stained trichoblast cells (T) develop hairs, while blue-stained atrichoblasts cells (A) remain hairless. c, cortex; end, endodermis; epi, epidermis; lrc, lateral root cap; vas, vascular bundle. (**D**,**E**) Expression of root-hair regulatory genes. Confocal laser scanning microscope (CLSM) images of roots of wild type (D) and *jkd-i* mutants (E) expressing p*WER::CFP*. (**F-I**) DIC optic images of roots expressing p*CPC::GUS* in wild type (F) and *jkd-i* mutants (G), and p*GL2::GUS* in wild type (H) and *jkd-i* mutants (I). (**J**,**K**) Tissue sections of root expressing p*GL2::GUS* in wild type (J) and *jkd-i* mutants (K). (**L**) Comparison of cell file numbers between wild type and *jkd* mutants. Total cell number, as well as N file and H file numbers, are represented in the graph. Fifteen roots were counted. $P<0.05$ using the Mann-Whitney test. Stars mark examples of aberrant gene expression. Arrowheads point to ectopic divisions in the epidermis.

RESULTS

JKD regulates epidermal cell patterning in the root meristem

In wild-type roots, H and N cells are arranged regularly in alternating files (Wada et al., 2002), whereas in *jkd* root epidermis, H cell distribution was randomized (Fig. 1A,B). Examination of underlying cortex walls in the *jkd-4* mutant revealed that 17% of epidermal cells in the N position produced H cells, compared with less than 1% in the wild type. In addition, 11% of cells in the H position did not develop hairs in *jkd-4* mutant compared with 2% in the wild-type roots (Fig. 1A,B; Table 1), indicating that positioning of H and N cells in the epidermis requires *JKD* function.

In wild-type plants, the *GL2*/*WER*/*CPC* genes are preferentially expressed in the precursors of non-hair cells, the atrichoblasts (Masucci et al., 1996; Lee and Schiefelbein, 1999; Wada et al., 2002) (Fig. 1C). In *jkd-4* mutants and RNAi lines (*jkd-i*) (Welch et al.,

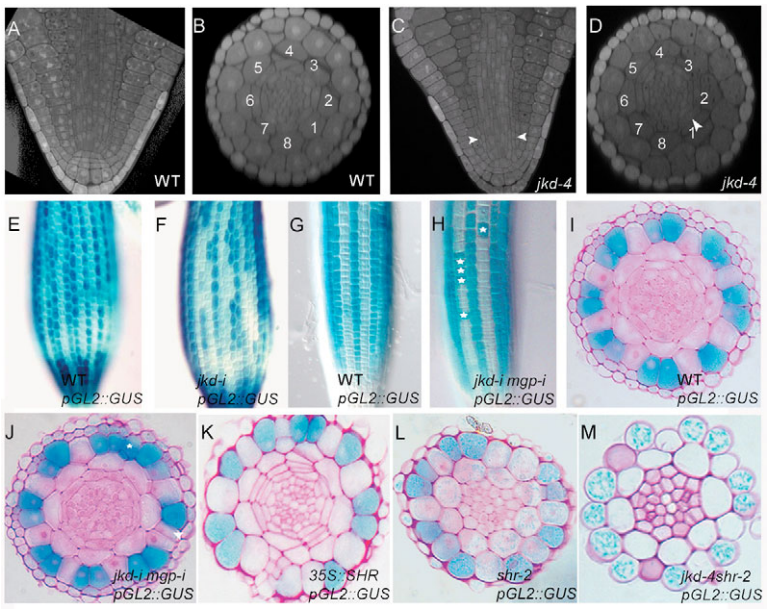

Fig. 2. JKD is required for proper *GL2* expression in embryos and its action is uncoupled from ground tissue defects. (**A-D**) Mature embryos stained with Aniline Blue showing normal cell number in the circumference in both wild type (A,C) and *jkd-4* mutants (B,D). (**E**,**F**) DIC optic images revealing expression of p*GL2::GUS* in mature embryos of wild type (E) and *jkd-4* mutants (F). (**G**,**H**) Three-day-old roots expressing p*GL2::GUS* in wild type (G) and *jkd-i mgp-i* double mutants (H). (**I-M**) p*GL2::GUS* expression in tissue sections of wild type (I), and *jkd-i mgp-i* (J), *35S:: SHR* (K), *shr-2* (L) and *jkd-4 shr-2* (M) mutants. Stars mark changes in epidermal cell pattern in relation to their position to the underlying cortical cells. Arrowheads mark ectopic divisions in the ground tissue.

2007), expression of *GL2*/*WER*/*CPC* in the root epidermis was no longer restricted to the A cells (Fig. 1D-I). We also noticed that T cells frequently divide and newly formed cells develop patches expressing atrichoblast fate markers (Fig. 1E,G, arrowheads). Cross-sections revealed that in *jkd-4* mutants, root cells located in the T position were now expressing *GL2*, while some located in the A position lacked *GL2* expression (Fig. 1J,K). Seventeen percent of the cells in the T position expressed p*GL2::GUS* in comparison with 3% in the wild type. Moreover, 2% of A cells in *jkd-4* mutants lacked *GL2* expression compared with 0% in the wild type (Table 2). We conclude that *JKD* is required for the appropriate expression of *GL2*/*WER*/*CPC* that leads to proper H and N cell patterning.

Wild-type root epidermis cells located at the T position in the meristematic region occasionally divide along the longitudinal axis to generate so-called 'T clones'. Cells in these clones are fated depending on their position relative to the underlying cortical cells, irrespective of where in the meristem the extra divisions take place (Berger et al., 1998a). In *jkd* mutants there is a slight increase in both N and H files (Fig. 1L) but, like the T-clones in wild type, these divisions do not enhance the number of H files (Fig. 1G). Consistent with this, daughter cells within *jkd* T-clones can switch fate.

Table 1. Root hair emergence in the root epidermis of 7 dpg wild type and *jkd* mutants

	Cells in the H position		Cells in the N position	
Genotype	Hair cells (%)	Non-hair cells (%)	Hair cells (%)	Non-hair cells (%)
Wild type	97.7±1.4	2±0.6	0	100
jkd4	94.8±2.9	5.2±1.1	17±1.5	83±2.8
jkdi	95.5±2.8	4.5±1.1	9.5±1.1	90.5±2.6

At least 20 roots of each line were analyzed (n=20). $P<0.05$ from Mann-Whitney test.

JKD-mediated epidermal patterning is independent of ground tissue organization and SHR action

Because epidermal cell fate correlates with cortical cell arrangement in the root meristem, we asked whether misplaced N and H cells in *jkd* mutants are a direct effect of JKD action on the epidermal layer or whether it might be a consequence of the extra divisions taking place in the cortex layer of *jkd* mutants (Welch et al., 2007).

In *jkd* mutant embryos, ectopic divisions in the cortex led to increased cell number in the radial axis, but not in the circumference, and hence the position of the cortical clefts relative to the embryonic epidermal cells was not changed (Fig. 2A-D). In the wild type, epidermal p*GL2::GUS* expression was restricted only to the A cells (Fig. 2E) but *jkd* mutant embryos displayed a patchy *GL2* expression pattern (Fig. 2F), suggesting that JKD acts early in development and not through alteration of the position of cortical clefts.

Reduction of the activity of MAGPIE (MGP), a member of the JKD family, suppresses the ectopic ground tissue divisions in *jkd* mutants (Welch et al., 2007). To further confirm that the root-hair phenotype in *jkd* is independent from extra cortex divisions, we examined *GL2* expression in *jkd-i mgp-i* double mutants. Despite the normal ground tissue organization in *jkd-i mgp-i* mutants, mislocalization of *GL2* expression still occurred (Fig. 2G,H). In addition, these roots also showed ectopic divisions in epidermal cells

Table 2. Reporter gene expression in root epidermal cells of wild type and *jkd* mutants

	Cells in the H position		Cells in the N position	
Genotype	Cells lacking reporter expression (%)	Cells with reporter expression (%)	Cells lacking reporter expression (%)	Cells with reporter expression (%)
p*GL2::GUS*	97±3.6	3±0.6	0	100
jkd-4 p*GL2::GUS*	82.7±2.7	17.2±3.2	2.3	97.6±2.3
jkd-i mgp-i p*GL2::GUS*	86.7±4.5	13.3±3.5	0.27	99.7±4

Quantification of GL2 expression in *jkd*-4 and *jkd-i mgp-i* roots at 7 dpg. At least 15 roots of each line were analyzed (n=15). $P<0.05$ from Mann-Whitney test.

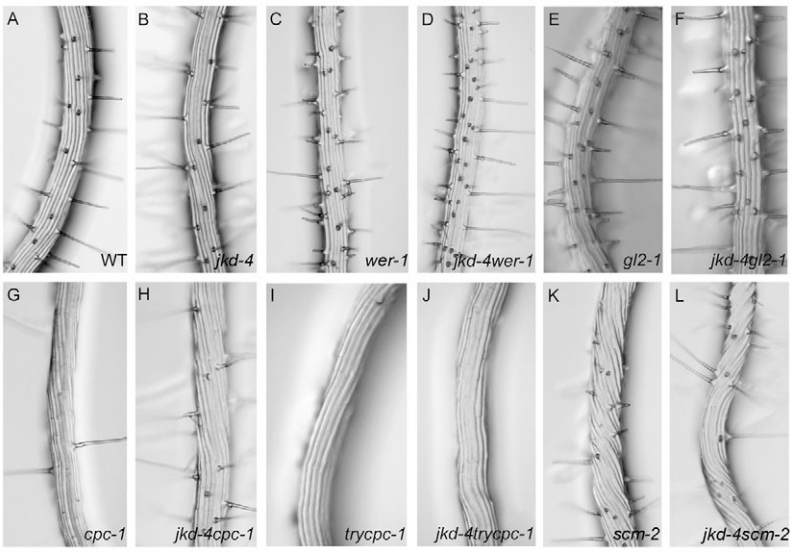

Fig. 3. Genetic interaction between *jkd* and epidermal fate mutants. (**A-L**) Binocular views of root-hair distribution in wild type (A), *jkd-4* (B), *wer-1* (C), *jkd-4 wer-1* (D), *gl2-1* (E), *jkd-4 gl2-1* (F), *cpc-1* (G) and *jkd-4 cpc-1*. Note that in *jkd-4cpc-1*, root-hair cells still develop owing to the redundant activity of CPC partner TRY (H), *trycpc-1* (I), *jkd-4 try cpc-1* (J), *scm-2* (K) and *jkd scm-2* (L).

within the H files and these cells again expressed N fate markers, indicating a switch to non-hair cell fate (Fig. 2I,J). These data suggest that epidermal defects in *jkd* mutants do not result from cell arrangement in the ground tissue but from a direct JKD action in epidermal cell specification.

We independently checked whether extra layers of ground tissue induced by other means would affect epidermal cell fate and thereby the pattern of *GL2* expression in the non-hair cells. Overexpression of *SHR* results in additional endodermal layers (Helariutta et al., 2000). However, analysis of *GL2* expression showed no changes, indicating that supernumerary ground tissue cell layers in this background are not sufficient to perturb epidermal patterning (Fig. 2K).

Collectively, these observations demonstrate that JKD is required to set up epidermal patterning early in development and that epidermal fate changes in *jkd* mutants are independent of disturbed division patterns in the ground tissue layer.

Ectopic periclinal divisions in the ground tissue of the *jkd* mutants require the activity of the transcription factor SHR, with which JKD protein genetically and physically interacts (Welch et al., 2007). We asked whether JKD could also act together with SHR in a transcriptional complex to control root-hair patterning. In *shr-2* p*GL2::GUS*, *GL2* expression was detected only in cells at the N position (Fig. 2L), whereas it was expressed in both N and H positions of *jkd-4 shr-2* double mutants (Fig. 2M). These results, together with our SHR overexpression experiments, indicate that JKD does not act through SHR to specify epidermal patterning.

JKD acts through the root epidermal patterning network

Previous studies on cell patterning of the root epidermis showed that the relative activities of two competing MYB transcription factors (WER and CPC) are crucial for determining whether an epidermal cell activates the N or the H differentiation pathway (Lee and Schiefelbein, 1999). We investigated whether the effect of JKD on epidermal pattern is mediated by this mechanism.

First, we analyzed genetic interactions between *JKD* and *WER*. Similar to the phenotype observed in *wer-1* mutants, the *jkd-4 wer-1* double mutant developed ectopic root-hairs emerging from the N position in 91% of the cells (Fig. 3C,D; Table 3). The fact that the mutation in JKD does not have an additive effect on the *wer* phenotype indicates that JKD acts through WER to pattern epidermal cell fate.

In agreement with this, our analyses of *jkd-4 gl2-1* double mutants showed no additive effect of *jkd* on the phenotype observed in the *gl2* single mutant. Roots of both single and double-mutant plants showed a similar increase in the frequency of hair cells developed in the N position (6.83% in *jkd-4*, 67% in *gl2-1* and 65.30% in *jkd-4 gl2-1*; Fig. 3B,E-F; Table 3). These results indicate that *GL2* acts downstream of *JKD* in the N file position.

We next analyzed the *jkd-4 try cpc-1* double and triple mutants and found that the triple mutant was indistinguishable from *try cpc* and completely lacked H cells (Fig. 3I,J; Table 3). These results reveal genetic epistasis between *JKD* and the H-fate-promoting regulators, indicating that *CPC* and *TRY* act downstream of *JKD*.

SCRAMBLED (SCM) is proposed to mediate the action of positional cues that bias the transcription factor network to determine N and H cell fates (Kwak et al., 2007; Savage et al., 2008). Similar to *jkd*, plants homozygous for the *scm* mutation show changes in both H and N cell fate: cells in the A position might inappropriately adopt H fate and cells located in the T position might become N cells so that their distribution is no longer correlated with their position with respect to the underlying cell layer (Kwak et al., 2005). The *jkd-4 scm-2* double mutant reveals epistasis of the *scm-2* root-hair, indicating that *SCM* acts downstream of *JKD* (Fig. 3K-L;

Table 3. Percentage of root-hair emergence in the epidermis of 3-day-old roots of wild type and mutants

	Cells in the H position		Cells in the N position	
Genotype	Hair cells (%)	Non-hair cells (%)	Hair cells (%)	Non-hair cells (%)
Wild type	97.7±1.4	2±0.6	0	100
jkd-4	90±2.3	10±1.1	6.8±1.1	93±3
jkd-i	95.5±2.8	4.5±1.1	9.5±1.1	90.5±2.6
wer-1	92.4±3.3	7.5±0.9	90.2±1	9.8±0.7
jkd-4 wer-1	92.5±1.3	7.5±0.9	91.6±1.6	8.4±0.7
gl2-1	100	0	67.4±3.5	32.6±1.3
jkd-4 gl2-1	100	0	65.3±2.8	34.7±5.2
cpc-1	15.4±1.1	81.5±3	0	100
jkd-4 cpc-1	62.5±3.4	37.5±2	0	100
try cpc-1	0	100	0	100
jkd-4 try cpc-1	0	100	0	100
scm-2	48.4±1.6	51.6±0.8	15.6±1.0	84.4±1.5
jkd-4 scm-2	53.8±1.4	46.9±2	17.8±1.1	82.2±2.8

At least 10 roots of each line were analyzed (n=10). P<0.05 from Mann-Whitney test.

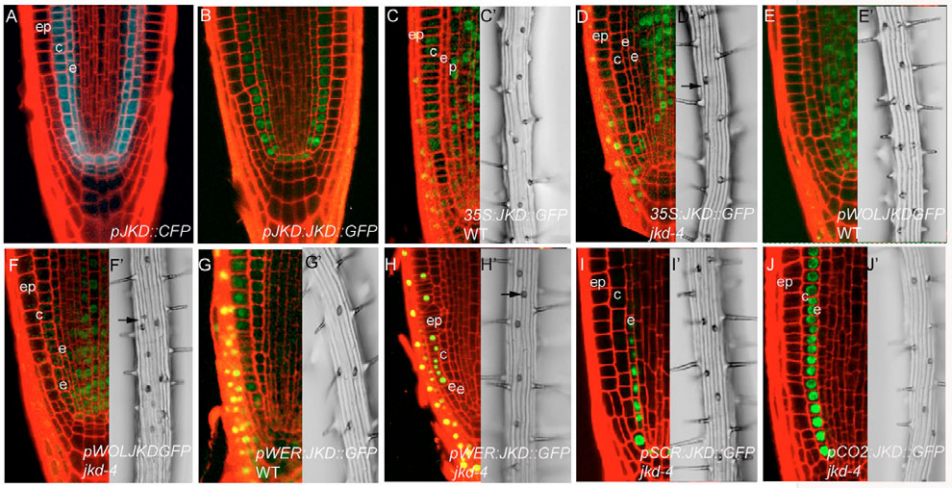

Fig. 4. JKD is required in the cortex layer for appropriate patterning of H and N cells. (**A-J**) Longitudinal CLSM images of: p*JKD::CFP* showing the location of JKD transcript (A) and p*JKD::JKD::GFP* for JKD protein localization (B); 35S::*JKD::GFP* in wild type (C) and *jkd-4* mutants (D); p*WOL::JKD::GFP* in wild type (E) and *jkd-4* mutants (F); p*WER::JKD::GFP* in wild type (G) and *jkd-4* mutants (H); p*SCR::JKD::GFP* in *jkd-4* mutants (I); and p*CO2::JKD::GFP* in *jkd-4* mutants (J). Note the rescue of the ground tissue phenotype in the *jkd-4* root mutant when expressing JKD in the endodermis and/or cortex layer (I,J). c, cortex; e, endodermis; ep, epidermis; p, pericycle. Arrows point to ectopic root hairs.

Table 3). From these data, we concluded that, during postembryonic development, *JKD* acts upstream of the canonical WER pathway through SCM.

JKD acts non-cell-autonomously to regulate epidermal patterning

Both *JKD* transcript and encoded protein are expressed in the ground tissue and the quiescent centre (QC) (Welch et al., 2007) (Fig. 4A,B) but not in the epidermis, suggesting a non-cell-autonomous action of JKD to control epidermal patterning. To determine from which tissue layer JKD might produce a signal necessary for correct epidermal cell type specification, we expressed JKD in wild type and *jkd-4* mutants using different cell-type-specific promoters. For constitutive expression, we used the 35S promoter for epidermis (*WEREWOLF*; p*WER*), cortex (*CORTEX2*; p*CO2*), endodermis (*SCARECROW*; p*SCR*) and vascular tissue (*WOODEN LEG*, p*WOL*) and then analyzed root hair distribution (Fig. 4).

In the wild-type background, we found no changes in cell type specification or root-hair pattern when JKD was ubiquitously activated or when it was ectopically expressed under these different promoters (Fig. 4 C,E,G,I; Table 4).

In *jkd-4* mutants, overexpression of a complementing JKD:GFP fusion under the 35S promoter, as well as ectopic expression in the epidermis under the WER promoter or in the vasculature using the *WOL* promoter, failed to rescue any aspects of the *jkd-4* phenotypes (Fig. 4D,F,H-J; Table 4).

When JKD:GFP was reintroduced in the ground tissue using p*SCR*, p*CO*, the aberrant divisions observed in ground tissue of *jkd-4* mutants disappeared (Fig. 4I,J). Interestingly, the root-hair phenotype was only partially rescued in plants expressing JKD in the endodermis even though expression levels were comparable with those in other tissue-specific drivers (Fig. 4I′; Table 4). By stark contrast, JKD:GFP expression in the cortex using p*CO* was sufficient to fully restore the root-hair patterning defects in *jkd-4* (Fig. 4J′; Table 4).

Together, these data indicate that JKD most effectively functions in the cortex layer to non-cell-autonomously control epidermal patterning.

Table 4. Percentage of root-hair emergence in roots expressing JKD under different tissue promoters in wild type and *jkd-4* mutants

	% of abnormal root-hair distribution	
Genotype	WT	*jkd-4*
Control	8.5 (*n*=35)	90 (*n*=50)
p*WER::JKD::GFP*	10	85.7 (*n*=70)
p*SCR::JKD::GFP*	6	57 (*n*=60)
p*CO2::JKD::GFP*	5	13 (*n*=55)

n=number of roots analyzed.

DISCUSSION

JKD is required for epidermal patterning from embryogenesis onward

JKD has been reported to control radial patterning by limiting SHR action (Welch et al., 2007). Here, we provide evidence that *JKD* has a second, genetically separated role from controlling cell division in the ground tissue and it is required for patterning of epidermal cell types in a SHR-independent fashion. Our data show that proper *GL2* expression in the embryo requires JKD action. *GL2* expression in the embryo depends on WER and CPC (Costa and Dolan, 2003), but not on the action of the receptor kinase SCM because no changes in *GL2* expression were found in either *scm* embryos or in triple mutants of *scm* with *srf1* and *srf2*, encoding the two members most closely related to SCM (Kwak and Schiefelbein, 2006). Therefore, the embryonic function of JKD must be independent from SCM. Some clues have been obtained for other factors involved in SCM-regulated processes: *scm*, also known as *strubbelig* (*sub*), was shown to be involved in ovule and flower development (Chevalier et al., 2005), and three mutants displaying SCM/SUB-like phenotypes might play additional roles in epidermal patterning (Fulton et al., 2009). One of the corresponding genes, *QUIRKY*, encodes a transmembrane protein with unknown function (Fulton et al., 2009). It is therefore possible that JKD acts through a yet unidentified signalling pathway during embryogenesis.

JKD is an upstream regulator of epidermal patterning

Our genetic interaction data reveal epistasis of all root-hair regulators over JKD, which is most parsimoniously explained by assuming that WER and GL2 promote the N fate and TRY and CPC promote the H fate downstream of JKD. Other evidence indicating that JKD operates upstream of the root-hair regulatory network comes from the observation that *scm* mutants are epistatic to *jkd* mutants after embryogenesis. These data indicate that, post-embryonically, JKD modulates cell fate decisions upstream of the entire currently known root-hair patterning network.

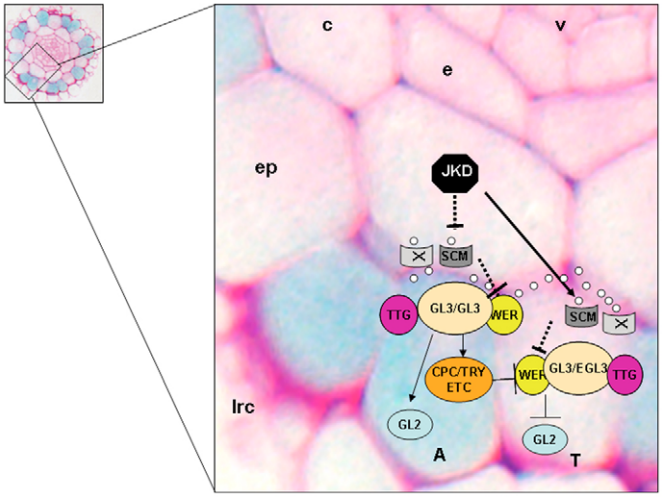

Fig. 5. Model illustrating JKD action on epidermal patterning. JKD (black octagon) will trigger a signal (white circles) that will either bind to the receptor kinase SCM (grey) and or to an unknown factor (X). Owing to its larger contact area, the cell located at the cleft will receive more input, leading to SCM activation, partial WER repression and GL2 inhibition. Cells located over the cortical cell will receive relatively less JKD-mediated input; JKD might also act by inhibiting SCM. This repression results in WER release and thus GL2 activation.

In *jkd* mutants, the major effect is in the H position, where patches of cells adopt the N fate. The effect in the N position is milder than in *scm* mutants. Although these data imply that JKD acts predominantly to prevent the N cell fate in the H position, the *JKD* gene clade comprises many related members and there is evidence for redundancy and ground tissue expression of several members (Welch et al., 2007; Cui et al., 2007). It will be interesting to investigate whether combinations of mutants in the *JKD* clade fully randomize root-hair patterning and whether the root regulatory network can be positioned downstream of JKD clade activity.

JKD action from the cortex suggests a novel bias mechanism for epidermal patterning

We show here that activation of JKD in the cortex layer is sufficient to correctly bias epidermal patterning. This finding suggests that a positional signal might be produced in all underlying cortex cells, where JKD protein is located in the wild type. This signal should then reach the epidermal cells and bias cell fate choices. Surprisingly, JKD expression in the endodermis only partially rescues the root-hair phenotype, which indicates that positional information provided by JKD in the endodermis does not effectively reach the epidermal cells and contradicts classical ideas that the biasing signal moves apoplastically through the cortical cleft (Dolan and Roberts., 1995).

Previously, it has been shown that distance to the anticlinal wall between cortex cells determined cell fate (Berger et al., 1998b). In agreement with this, we propose the following model to explain all available data: a T cell located over the cortical cleft has a larger contact surface spanning two cortical cells. Thus, it is expected to receive more signals from the cortex when compared with A cells located over a single cortical cell. This leads to more SCM-mediated inhibition of transcriptional regulation of WER in the T cells (Fig. 5). Accordingly, these cells surrender to the CPC/TRY/ETC-dependent lateral inhibition and adopt the H fate. This could explain the predominant effect of JKD in the H cell position. Reduction of the JKD (and possibly other JKD-clade member)-dependent signal releases WER repression, which then affects the relative abundance of WER and CPC leading to the N fate in the H position and, as a more rare secondary effect, the H fate in the N position. It will be interesting to test this model by determining the molecular nature of the positional signal that is expected to operate downstream of JKD and be perceived by SCM.

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Competing interests statement

The authors declare no competing financial interests.

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