

TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in *Arabidopsis*

S.Schellmann^{1,2}, A.Schnittger^{1,3}, V.Kirik^{1,4},
T.Wada⁵, K.Okada^{5,6}, A.Beermann⁷,
J.Thumfahrt¹, G.Jürgens¹ and
M.Hülkamp^{4,8}

¹ZMBP, Entwicklungsgenetik, Universität Tübingen, Auf der Morgenstelle 3, ⁷Abteilung Genetik der Tiere, Interfakultäres Institut für Zellbiologie, Auf der Morgenstelle 28, D-72076 Tübingen, ³Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné Weg 10, D-50829 Köln, ⁴University of Köln, Botanisches Institut III, Gyrhofstr. 15, D-50931 Köln, Germany, ²CSIRO Plant Industry, Adelaide Laboratory, Entry No. 2 Waite Campus, Hartley Grove, Urrbrae, PO Box 350, Glen Osmond, SA 5064, Australia, ⁵RIKEN Plant Science Center and ⁶Department of Botany, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

⁸Corresponding author
e-mail: martin.huelkamp@uni-koeln.de

Trichome patterning in *Arabidopsis* is a model for the generation of a spacing pattern from initially equivalent cells. We show that the *TRIPTYCHON* gene that functions in lateral inhibition encodes a single-repeat MYB-related transcription factor that lacks a recognizable activation domain. It has high sequence similarity to the root hair patterning gene *CAPRICE*. Both genes are expressed in trichomes and act together during lateral inhibition. We further show that *TRIPTYCHON* and *CAPRICE* act redundantly in the position-dependent cell fate determination in the root epidermis. Thus, the same lateral inhibition mechanism seems to be involved in both *de novo* patterning and position-dependent cell determination. We propose a model explaining trichome and root hair patterning by a common mechanism.

Keywords: MYB/pattern formation/root hairs/trichomes/*TRIPTYCHON*

Introduction

In animal systems, gene cassettes controlling pattern formation are often employed in several independent patterning processes, and the particular cell types generated depend on the developmental context. A well-studied example is the receptor–ligand-based Delta/Notch system in *Drosophila* that governs the epidermal versus neural precursor cell decision in the ectoderm (Campos-Ortega, 1993; Ghysen *et al.*, 1993) as well as the segregation of midgut epithelial cells in the endoderm (Tepass and Hartenstein, 1995).

In *Arabidopsis*, two patterning systems, trichome (leaf hair) and root hair formation, are known to involve a common set of genes. The underlying mechanisms,

however, appear to be different. The root hair pattern seems to be position dependent: root epidermal cells are arranged in adjacent files of root hair cells and non-hair cells. Root hair files are located over the intercellular space between underlying cortical cells; non-hair cells arise over single cortical cells (Masucci *et al.*, 1996). This implies that the underlying cortical cells provide positional cues for the epidermal root hair patterning system. In contrast, trichome patterning is thought to be generated *de novo*, i.e. all epidermal cells initially are equivalent, and single trichome cells are selected by a self-organizing system (Larkin *et al.*, 1996; Schnittger *et al.*, 1999).

The genetic analysis of trichome and root hair development has enabled the identification of genes that are required for proper pattern formation. Two genes, *GLABRA1* (*GL1*), encoding a MYB-related transcription factor, and *TRANSPARENT TESTA GLABRA1* (*TTG1*), encoding a protein containing a WD40 domain that is thought to mediate protein–protein interactions, function as positive regulators of trichome development (Koornneef, 1981; Marks and Feldmann, 1989; Walker *et al.*, 1999). The corresponding mutants produce no trichomes. In *glabra3* (*gl3*) mutants, only few trichomes are produced, suggesting that *GL3* is also a positive regulator of trichome patterning (Hülkamp *et al.*, 1994; Payne *et al.*, 2000). *GL3* encodes a basic helix–loop–helix (bHLH)-related transcription factor, and *GL3* overexpression causes an increase in trichome density (Payne *et al.*, 2000). The involvement of a bHLH-related transcription factor in this process was postulated previously because the *GL3*-homologous *R* gene from maize triggers trichome formation in *Arabidopsis* when overexpressed (Lloyd *et al.*, 1992, 1994). *TRIPTYCHON* (*TRY*) is the only known negative regulator of trichome development. In *try* mutants, ~5–10% of the trichomes are arranged in clusters, suggesting that *TRY* mediates lateral inhibition of cells surrounding an incipient trichome (Schnittger *et al.*, 1998, 1999). The mechanism by which these genes control trichome patterning is unknown. Because *GL3* physically interacts with both *GL1* and *TTG1* in the yeast-two hybrid assay, it is postulated that a multimeric complex of *GL1*, *GL3* and *TTG1* promotes trichome development and it is assumed that *TRY* counteracts their activity (Payne *et al.*, 2000). It is thought that the combinatorial action of the patterning genes regulates trichome-specific expression of *GLABRA2* (*GL2*). *GL2* encodes a homeobox transcription factor and is thought to control trichome cell differentiation (Rerie *et al.*, 1994).

Root hair patterning is regarded to be position dependent. During the establishment of cell files, trichoblasts or hair cells (overlying the clefts between cortex cells) and atrichoblasts or non-hair cells (overlying the cortex cells) differ from early on in several morphological features

including cell length, cytoplasmic density and vacuolization (Galway *et al.*, 1994; Masucci *et al.*, 1996; Berger *et al.*, 1998). Only two genes, *TTG1* and *WEREWOLF* (*WER*), a MYB-related transcription factor gene with high similarity to *GL1*, are known to be required for the initial establishment of these two cell files (Galway *et al.*, 1994; Lee and Schiefelbein, 1999). In *wer* and *ttg1* mutants, all cell files are root hair cell files. Thus both genes can be considered positive regulators of non-root hair file establishment. Similarly to the trichome system, the maize *R* gene also affects root hair patterning when overexpressed (Lloyd *et al.*, 1994). Overexpression results in a reduced root hair number and counteracts *ttg1* mutations but not *wer* mutations, suggesting that root hair patterning involves an endogenous, as yet unknown bHLH-related transcription factor (Lee and Schiefelbein, 1999). As in trichome development, *GL2* governs early differentiation processes. *GL2* is expressed exclusively in non-hair cells and suppresses the formation of root hairs (Masucci *et al.*, 1996). Its expression is controlled by *WER* and *TTG1*. The *CAPRICE* (*CPC*) gene, encoding a MYB-related transcription factor lacking a transactivation domain, is the only known negative regulator of non-root hair cells (Wada *et al.*, 1997). *CPC* is required for cell file-specific expression of *GL2* and genetically counteracts *WER* and *TTG1* (Wada *et al.*, 1997; Lee and Schiefelbein, 1999). Current models postulate that a complex consisting of *WER*, *TTG1* and an *R* gene-related transcription factor is active in non-hair cells where it promotes expression of *GL2*. In hair cells, the activity of this complex is inhibited by *CPC* by lateral inhibition (Lee and Schiefelbein, 1999, 2002). How the underlying cortex cells control this pattern is not known.

A partial overlap in the mechanisms controlling cell fate choice during root hair patterning and trichome patterning is assumed, as several genes function in both processes. In *ttg1* and *gl2* mutants, root hair differentiation and trichome differentiation are affected in opposite ways: root hair development is promoted whereas trichome development is abolished. In contrast, the mechanism for the actual determination of cell fate seems to be distinct since corresponding genes such as *GL1/TRY* (trichomes) and *WER/CPC* (root hairs) are specific for each system. However, the finding that overexpression of the root hair gene *CPC* abolishes trichome development suggests a genetic overlap with cell fate determination in the leaf epidermis, although this particular finding was interpreted as an unspecific competition with the trichome-promoting factor *GL1* (Wada *et al.*, 1997).

In this work, we report the positional cloning and molecular characterization of the *TRY* gene. *TRY* encodes a MYB-related protein with high sequence similarity to *CPC*. Both genes are expressed in young leaf primordia and developing trichome cells. We show that *TRY* and *CPC* act not only in trichome or root hair patterning, respectively, but also together in both processes. This suggests that epidermal cell type selection employs the same lateral inhibition mechanism in roots and shoots. Our results lead to a model that explains *de novo* pattern formation of trichomes and position-biased root hair file establishment by a common mechanism.

Results

***TRY* encodes a CPC-homologous MYB-related transcription factor**

In extensive screens for trichome patterning mutants to date, only one gene, the *TRY* gene, has been identified that appears to function in lateral inhibition and hence most probably in cell–cell signalling (Hülkamp *et al.*, 1994; Schnittger *et al.*, 1998, 1999). To understand the molecular mechanism underlying lateral inhibition during trichome patterning, we cloned the *TRY* gene by a positional cloning approach. Initially, the *TRY* gene was mapped to a 150 kb interval flanked by two newly developed CAPS markers on chromosome V (details are available on request). The annotated genomic sequence in this region revealed one gene that showed high homology to the *CPC* gene. To test whether this gene encodes *TRY*, we sequenced its genomic region from several *try* alleles. Four *try* alleles exhibited mutations leading to stop codons in the predicted open reading frame (ORF) of the gene. In one allele, the mutation affects the splice acceptor site before the third exon, leading to a stop in the next codon following the splice donor site (Figure 1A). To confirm that this gene encodes *TRY*, we introduced a 4.2 kb DNA fragment containing the *TRY* gene into *try* mutant plants, which rescued the *try* mutant phenotype.

To determine the genomic structure of the *TRY* gene, we isolated cDNA fragments by RT–PCR. A comparison of the cDNA with the genomic sequence revealed a structure different from the published annotation. The *TRY* gene has a 1 kb ORF and contains three exons and two introns (Figure 1A). The 5' and 3' ends of the cDNA were determined by RACE–PCR. The 5' end of the transcript was mapped 139 bp upstream of the start codon, and the 3' end 81 bp downstream of the stop codon.

The deduced amino acid sequence of *TRY* revealed a 106 amino acid protein that contains a MYB-related DNA-binding domain but lacks a recognizable activation domain. Overall, *TRY* shows highest homology to a group comprised of three MYB proteins in *Arabidopsis*, including *CPC*; the other two are of unknown function (Figure 1B).

TRY expression was analysed by RT–PCR. *TRY* mRNA accumulated in all organs analysed, including roots, leaves, siliques and inflorescences (Figure 1C). Thus, *TRY* is expressed not only in trichome-bearing organs but also in organs lacking trichomes, such as roots and siliques.

A paradoxical situation: ubiquitous expression of TRY suppresses trichome initiation although TRY is expressed in trichomes

Genetic analysis of *try* mutants suggests that *TRY* functions as a negative regulator of trichome development. To test this, we created transgenic lines expressing *TRY* ubiquitously. A construct containing the *TRY* coding sequence under the control of the cauliflower mosaic virus (CaMV) 35S promoter was introduced into Ler wild-type. These 35S::*TRY* plants were completely glabrous (Figure 2B), confirming that *TRY* is a negative regulator of trichome development.

The *TRY* expression pattern was determined by *in situ* hybridizations on paraffin-embedded rosette leaf sections

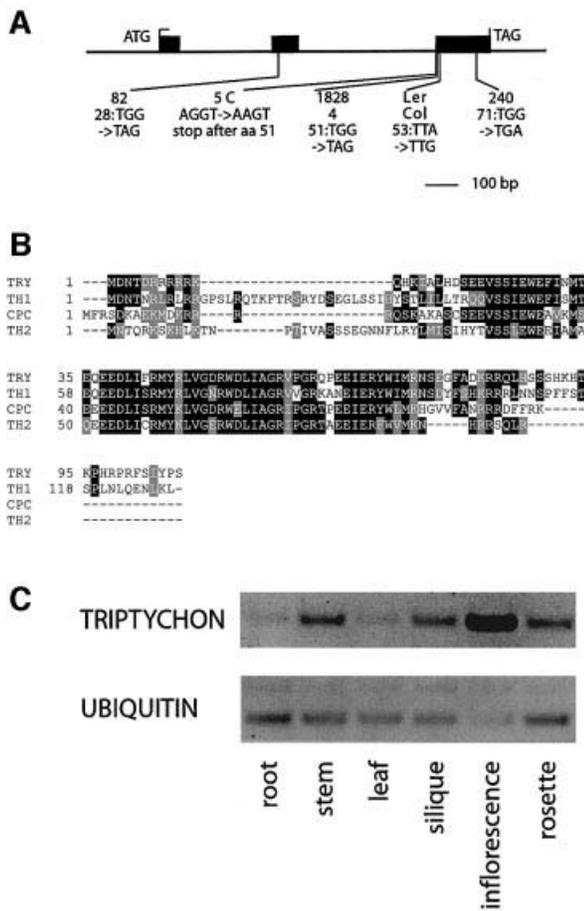


Fig. 1. Molecular characterization of the *TRY* gene. (A) Exon–intron structure of the *TRY* gene. The positions of mutations in the different *try* alleles are indicated. (B) Amino acid sequence comparison with the MYB-related proteins CPC, TRIPTYCHON HOMOLOG1 (TH1) and TRIPTYCHON HOMOLOG2 (TH2). (C) RT–PCR from total RNA extracts of different tissues. The minimal number of PCR cycles that allowed detection of *TRY* expression was used to enable a comparison of the expression levels between the tissues. RT–PCR amplification of ubiquitin was used as a control.

with digoxigenin-labelled sense and antisense probes of the *TRY* cDNA (see Materials and methods). Sections hybridized with the antisense probe revealed staining in developing trichomes (Figure 3D). The same expression pattern was observed in transgenic plants carrying a *GUS* fusion construct with the 1.4 kb *TRY* upstream region. Incipient trichome cells at stages when they were still indistinguishable from epidermal cells by their cell size exhibited already stronger *GUS* activity (Figure 3A). In addition, we observed ubiquitous expression in young leaves. Later, ubiquitous expression is only seen at the leaf base in the trichome initiation zone (Figure 3B). In mature leaves, only trichome cells were stained (Figure 3C).

The *TRY*-homologous root hair patterning gene *CPC* is involved in trichome patterning

Overexpression of the *TRY*-homologous root hair patterning gene *CPC* causes a suppression of trichome formation (Wada *et al.*, 1997). Initially, this was interpreted either to indicate that *CPC* plays also a role in trichome patterning or that it was caused by an unspecific interaction with the

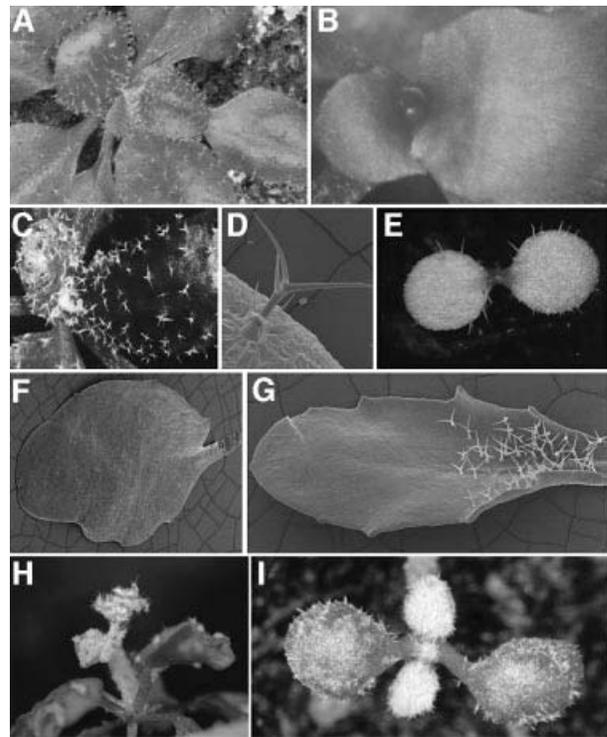


Fig. 2. Overexpression phenotypes of *35S::TRY* and *35S::CPC* lines. (A) Rosette leaves of a *try* mutant plant rescued with the genomic 4.2 kb fragment. (B) Glabrous *35S::TRY* plant. (C) Rosette leaves of a *try* mutant plant. (D) Scanning electron micrograph (SEM) of a *35S::GL1 cpc* leaf trichome with two accessory cells transformed to trichomes. (E) Cotyledons of a *35S::GL1 cpc* plant; many ectopic trichomes are formed. (F) SEM of a glabrous *35S::CPC* leaf. (G) SEM of a *35S::CPC 35S::R-GR* leaf after *R* gene induction with dexamethasone. Because in developing leaves only young cells at the base can respond to *R* gene-induced trichome induction, rescue is found only at the leaf base. (H) Inflorescence of a *35S::GL1 try cpc* mutant plant. (I) Primary leaves of a *35S::GL1 try cpc* mutant plant.

GL1 gene. As *TRY* shares high sequence identity with *CPC*, *CPC* might also be able to exert the same inhibitory function as *TRY* in overexpression studies. In order to test whether *CPC* is involved in trichome patterning, we analysed its expression pattern in leaves. Sections of paraffin-embedded rosette leaves were hybridized with ³H-labelled sense or antisense probes of the *CPC* gene. While the sense probe showed weak ubiquitous background staining, the antisense probe revealed a strong distinct signal in trichomes at all developmental stages (Figure 3E and F). The analysis of plants containing an expression construct where the *CPC* 5' regulatory sequence was fused to the *GUS* coding region (*CPC::GUS*) revealed an expression pattern indistinguishable from that of *TRY*. As shown in Figure 3H, *CPC* expression in mature leaves is confined to trichomes. Younger leaves show high levels of expression in trichomes at all developmental stages and, in addition, ubiquitous expression throughout the young leaf primordium (Figure 3G).

While the trichome-specific expression of *CPC* suggests a role in trichome development, no obvious trichome phenotype was observed in the initial studies (Wada *et al.*, 1997). In order to assess whether *CPC* has a role in trichome patterning, we carefully determined two aspects of trichome patterning: the frequency of trichome clusters and the total number of trichomes in *cpc* mutants. The

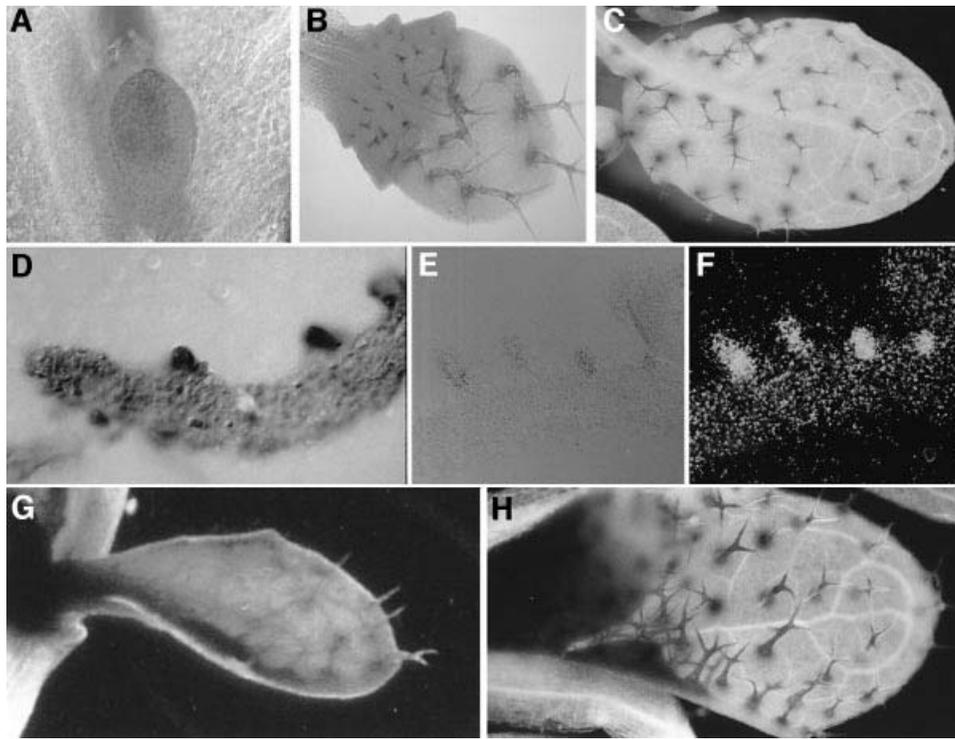


Fig. 3. Expression analysis of *TRY* and *CPC*. (A) Young leaf of a *TRY::GUS* line showing ubiquitous expression. (B) Slightly older leaf of a *TRY::GUS* line. Ubiquitous expression is seen at the leaf base. Note that early developmental stages of trichome cells exhibit elevated expression levels. (C) Mature leaf of a *TRY::GUS* line. *TRY* expression is limited to mature trichome cells. (D) *In situ* hybridization of a wild-type leaf section with antisense *TRY*. Trichome cells display high expression of *TRY*. (E) Light microscopy micrograph of an *in situ* hybridization of a wild-type leaf section with antisense *CPC*. (F) Dark field micrograph of (E). (G) Young leaf of a *CPC::GUS* line showing ubiquitous expression. (H) Mature leaf of a *CPC::GUS* line; *CPC* expression is seen only in trichomes.

cluster frequency in *cpc* mutants showed no deviation from wild-type. However, the total number of trichomes per leaf in *cpc* mutants was increased up to 2-fold as compared with the corresponding wild-type Ws-0 (Figure 4D and F). A statistical analysis using Wilcoxon rank sum test and Student's *t*-test showed that this difference is highly significant ($P < 0.01$). This was studied in more detail for leaf number four (Figure 4A and B). Here a difference of >30% in trichome number was found at all developmental stages of leaf development, indicating that the increase in the number of trichomes is due to an increased density rather than to an extended period of time of trichome formation or simply due to the formation of larger leaves (Figure 4A and B).

Another phenotypic difference between *cpc* mutants and *try* mutants was observed for the regulation of endoreduplication. Trichomes in *try* mutants contain on average twice the DNA (64C) content of wild-type (32C), overexpression of *GLI* results in an increased DNA content (64C) and trichomes in the *try 35S::GLI* double mutant contain four times the DNA content of wild-type (128C). To determine whether the *TRY*-homologous *CPC* gene is also involved in the control of endoreduplication, we studied the *cpc* single and several double mutants. Neither the single *cpc* mutant nor the *cpc 35S::GLI* double mutant showed an increased DNA content as compared with wild-type or *35S::GLI*, respectively (Table I). The DNA content in *try cpc* double mutant trichomes was not increased as compared with that in *try* mutants. These data

show that *CPC*, in contrast to *TRY*, is not involved in the regulation of trichome endoreduplication.

***TRY* and *CPC* play different roles in lateral inhibition during trichome patterning**

An increase in both cluster frequency in *try* mutants and trichome density in *cpc* mutants suggests that both genes act as negative regulators of trichome patterning. However, the different phenotypes also suggest separate functions. To assess this further, we generated the *cpc try* double mutant and analysed its phenotype by two criteria, cluster frequency and number of trichome initiation sites (TIS, defined as a location containing a single trichome or a trichome cluster).

The *try cpc* double mutant consisted of the strongest *try* allele available, *try-EMI*, and a *cpc* mutant in which the insertion of a T-DNA results in truncated protein that lacks part of the MYB domain. While clustering in *cpc* mutants was similar to wild-type (0.1%) and not very frequent in *try* mutants (4.4%, Table II), in the *try cpc* double mutant 76.3% of all TIS were clusters containing between 20 and 30 trichomes (Figure 5A–C). Cluster formation in *try cpc* mutants was initiated from single (66%, $n = 100$) or twin (34%, $n = 100$) trichomes. We used dental wax impressions to create replicas of subsequent stages of leaf development to follow the ontogeny of trichome clusters. During wild-type development, all trichome-surrounding cells start to elongate and to develop into trichome accessory cells. In *try cpc* double mutants, all cells in

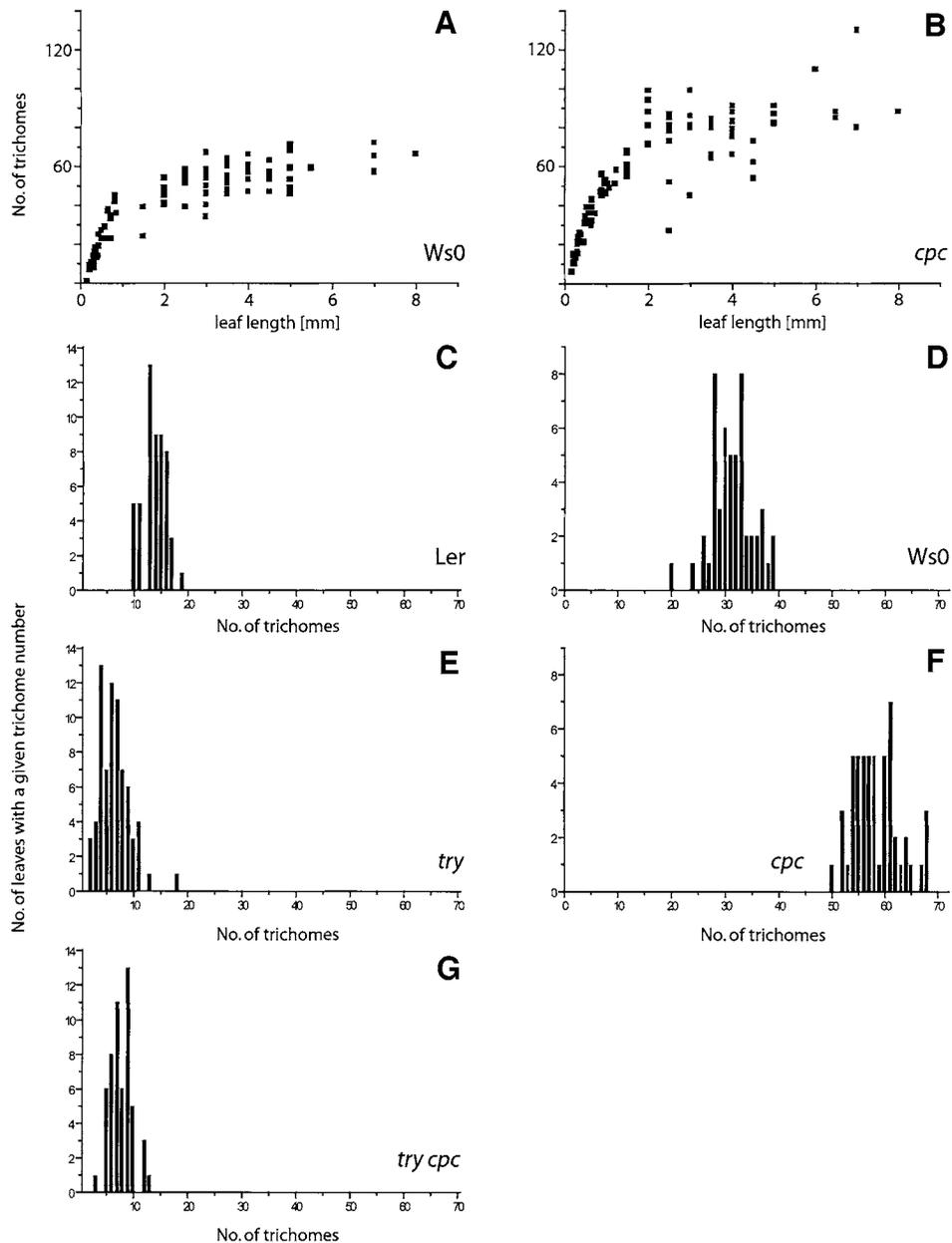


Fig. 4. Comparison of trichome number in different mutants. (A and B) Trichome number counts on the fourth leaf at all developmental stages of leaf development. (C–G) Number of trichome initiation sites on the first leaf. (A) In wild-type (Ws-0), trichome number increases until the leaf reaches a length of 1 mm, after which trichome initiation ceases but leaf growth continues. (B) A *cpc* mutant. Compared with wild-type, *cpc* mutants produce more trichomes at all developmental stages. (C) Wild-type ecotype Ler. (D) Wild-type ecotype Ws-0. (E) A *try* mutant. (F) A *cpc* mutant. (G) A *try cpc* double mutant.

immediate contact with an already existing trichome started to bulge out and developed into trichomes (Figure 5D–H). Several rounds of trichome induction are necessary to account for the large clusters seen on mature leaves.

The analysis of the number of TIS in the *try cpc* double mutant revealed a significantly ($P < 0.1$) reduced number of TIS as compared with the respective wild-types Ler and Ws. We also found a reduced TIS number in *try* mutants. Since *cpc* mutants show the opposite phenotype, these findings indicate that *try* is epistatic to *cpc* with respect to the number of TIS (Figure 4).

***TRY* and *CPC* act together in establishing atrichoblast and trichoblast cell files in the root epidermis**

The finding that *CPC* acts together with *TRY* in lateral inhibition during trichome formation raised the possibility that *TRY* might be involved in root hair patterning. Consistent with this, *TRY* expression was detected in roots by RT–PCR (Figure 1). In *try* mutants, root hair formation was indistinguishable from wild-type. Initial evidence for a role for *TRY* in root hair patterning came from the finding that *35S::TRY* lines produce extra root hairs (Figure 6B). In order to test whether a role for *TRY* during root hair

formation might be masked by a redundant function of *TRY* and *CPC* similar to that observed for trichome formation, we compared the root hair phenotype of the *cpc* mutant and the *try cpc* double mutant. In *cpc* single mutants, the number of root hairs is significantly reduced (Wada *et al.*, 1997) (Figure 6C). In the *try cpc* double mutant, we never found any root hairs (Figure 6D).

In wild-type development, root epidermal differentiation is carried out in two steps: First, a distinction between trichoblasts and atrichoblasts is established (patterning). Secondly, root hair differentiation is suppressed in atrichoblasts during further development

Table I. DNA content in wild-type and mutant trichomes

	Ws-0	<i>try</i>	<i>cpc</i>	<i>try cpc</i>	35S:: <i>GL1</i>	35S:: <i>GL1 cpc</i>
DNA content [C]	44.4	68	45.3	49.1	68.3	61.4
SD	15.6	22.3	18	21.1	38.1	33.1
<i>n</i>	100	50	100	100	50	100

Table II. Cluster frequency in wild-type and mutants

	Wild-type (Ler)	<i>try</i>	<i>cpc</i>	<i>try cpc</i>	
No. of trichome initiation sites	1009	707	1170	722	
No. of clusters	1	31	3	551	
Cluster frequency		0.1%	4.4%	0.25%	76.3%

(morphogenesis). In the wild-type, cell files overlying the longitudinal anticlinal cell walls of the cortical cells are trichoblasts (T) and contain more cells than atrichoblast (A) cell files that are overlying the cortex cells. We used the relative cell number, trichoblast to atrichoblasts (T/A) ratio, to determine whether the initial distinction between these two cell files is made. In wild-type, the T/A ratio is 1.3 in the root hair initiation and elongation regions. To enable a comparison between wild-type and mutants, cell files were defined according to their position relative to the cortex cells, and the T/A ratio was determined (Table III). The initial establishment of atrichoblast files and trichoblast files is normal in both the *try* and *cpc* single mutants as indicated by the T/A ratios of 1.27 and 1.25, which correspond to the T/A ratio of 1.27 found in wild-type roots. The *try cpc* double mutant revealed a T/A ratio of 1.07, indicating that all cell files have approximately the same number of cells. This indicates that *TRY* and *CPC* act together early during the initial establishment of atrichoblast and trichoblast cell file identity. Since *try cpc* double mutants do not form root hairs and 35S::*CPC* and 35S::*TRY* plants produce extra root hairs, it is conceivable that all cell files in *try cpc* mutants develop atrichoblasts whereas those in 35S::*CPC* and 35S::*TRY* plants develop trichoblasts.

In order to assess whether *TRY* is expressed in atrichoblasts or trichoblasts, we analysed GUS expression in roots of the *TRY::GUS* lines. No GUS activity was detected in the wild-type background. A comparison of the *TRY* and *CPC* RNA levels by semi-quantitative RT-PCR showed that *TRY* expression in the root is reduced compared with that of *CPC*, suggesting that the expression

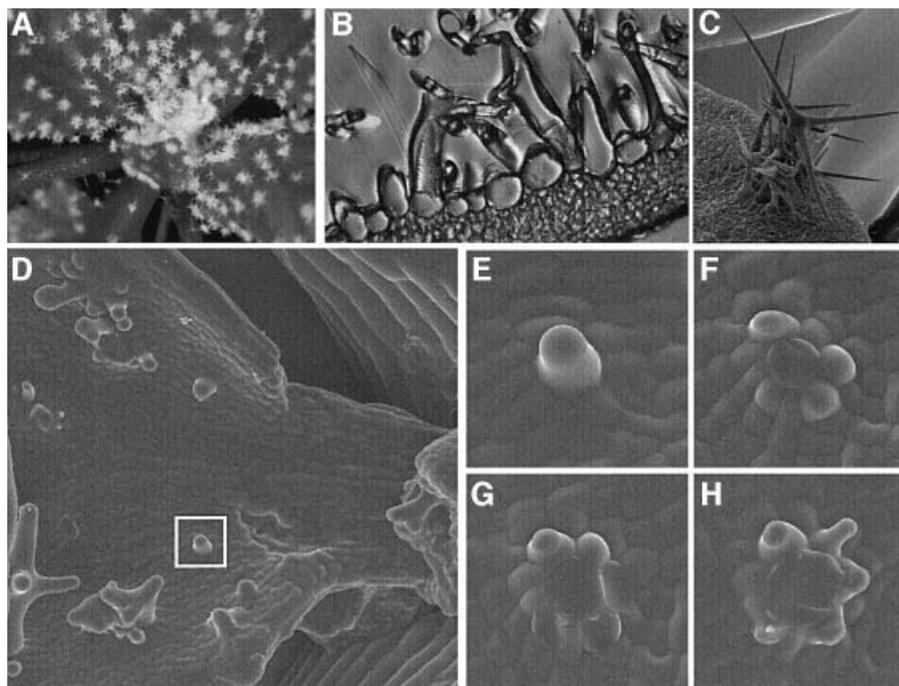


Fig. 5. *try cpc* double mutant phenotype. (A) Rosette leaf of a *try cpc* double mutant plant showing trichome clusters. (B) Section through a *try cpc* trichome cluster. (C) SEMs of a large *try cpc* cluster. (D–H) SEMs of wax replicas of developing *try cpc* trichome clusters. (D) Overview of a young leaf. The square marks the initial stage of the developing trichome cluster shown below. Replicas of the developmental stages were taken at 8 h intervals. Note that the central trichome has been torn away. (E) One initial trichome has emerged from the epidermis. (F) At least three immediately neighbouring epidermal cells appear as bulges. (G) Additional neighbouring cells show trichome development. (H) Surrounding trichomes are elongated.

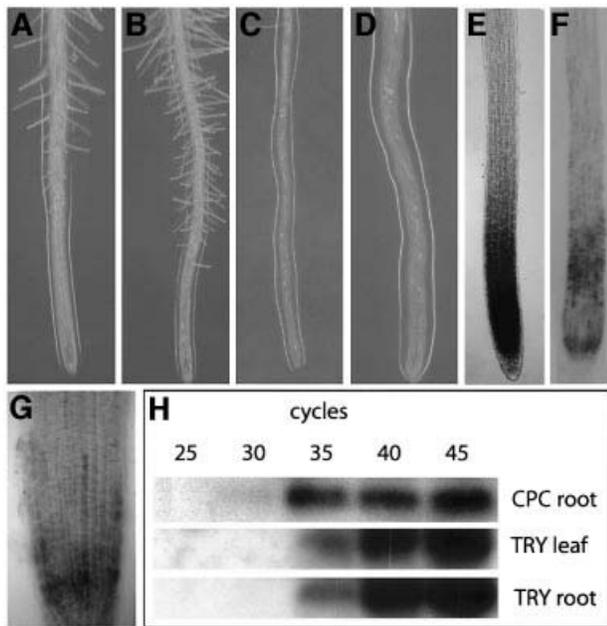


Fig. 6. Analysis of root hair phenotypes. (A–G) Light microscopy micrographs of wild-type and mutant roots. (A) Wild-type. (B) A *35S::TRY* root displaying extra root hairs. (C) A *cpc* mutant showing fewer root hairs. (D) A *try cpc* double mutant devoid of root hairs. (E) *TRY::GUS* expression in a *cpc try* background. (F) *TRY::GUS* expression in *35S::RGR* induced roots. (G) *TRY::GUS* expression in *35S::RGR* uninduced roots; expression is found in the tip region of the root. (H) Semi-quantitative RT-PCR of *TRY* and *CPC*. Note that *CPC* expression can be detected ~5 amplification cycles earlier than *TRY*.

levels of *TRY* are below the detection limit of the *TRY::GUS* construct (Figure 6H). However, strong expression of *TRY* was observed in *cpc try* double mutants and in a line overexpressing the *R* gene (Figure 6E–G). These data allow two conclusions. First, *TRY* levels are controlled by *TRY* and *CPC*. Secondly, since in both lines all cell files are atrichoblasts, *TRY* is likely also to be expressed in atrichoblasts in wild-type.

***TRY* and *CPC* exhibit different interactions**

Genetic results indicate that *TRY* acts as a negative regulator of *GLI*, as overexpression of *GLI* is counteracted by *TRY*, with respect to both trichome initiation and the control of endoreduplication. The overexpression phenotype of *GLI* was also enhanced in a *cpc* mutant background, as ectopic trichomes were found on cotyledons, and accessory cells were transformed into trichomes (Figure 2D and E). However, in contrast to the *35S::GLI try* combination, no ectopic trichomes were found in the subepidermis or in flower tissues, nor were the endoreduplication levels affected (Table I). The *35S::GLI cpc try* triple mutant showed essentially an additive phenotype, consistent with the view that both genes act as inhibitors of *GLI* (Figure 2H and I).

Although *try* and *cpc* act together in lateral inhibition, their phenotypes show differences in some important features, suggesting that they might interact with different partners, most probably the positive regulators *GLI* or *GL3*. In this case, one might expect that the overexpression phenotype of *TRY* and *CPC* can be rescued by overexpression of the specific partner. In an attempt to rescue

Table III. Trichoblast/atrichoblast ratio in wild-type and mutant roots

	Ws-0	Ler	<i>try</i>	<i>cpc</i>	<i>try cpc</i>	<i>ttg</i>	<i>gl2</i>
T/A	1.24	1.27	1.27	1.25	1.07	1.01	1.25
SD	0.06	0.05	0.07	0.05	0.1	0.14	0.06
<i>n</i>	6	10	9	9	9	10	8

the overexpression phenotypes of *35S::TRY* and *35S::CPC* by overexpression of the *GLI* or the *R* gene, we found a striking difference. Overexpression of *GLI* did not affect the *35S::TRY* and *35S::CPC* glabrous phenotype. In contrast, overexpression of the *GL3*-homologous *R* gene resulted in a complete rescue of the *35S::CPC* phenotype but not the *35S::TRY* phenotype (Figure 2G).

Discussion

The roles of TRY and CPC in lateral inhibition during trichome patterning

Our genetic model to explain the establishment of the trichome spacing pattern is based on a theoretical model (Meinhardt and Gierer, 1974). In this model, initially equivalent cells compete with each other to become a trichome cell. Only two components are required, an activator and a repressor. The activator stimulates its own activity and that of the negative regulator. The negative regulator represses the activator. Both factors may diffuse, though the negative regulator is predicted to be faster. As a result, cells start to compete and, because the activator is capable of autoactivation (a positive feedback loop), small fluctuations in the amount of one of the components are sufficient to break equivalence. Subsequently, the neighbouring cells are suppressed by the diffusible inhibitor and, eventually, a single cell is selected to become a trichome. Genetic data suggest that three genes, *GLI*, *GL3* and *TTG1*, together represent what is formally called the activator (Payne *et al.*, 2000). The *TRY* gene represents the negative regulator. Because mutations in *TRY* can strongly enhance the overexpression phenotype of *GLI*, it is thought that *GLI* is the target of the inhibitory effect of *TRY* (Schnittger *et al.*, 1998; Szymanski and Marks, 1998). The *TTG1* gene is likely to play an accessory role, e.g. in the postulated self-enhancing loop, because the *ttg1* mutant trichome phenotype can be rescued by overexpression of *GLI* in the absence of *TRY*. The *GL3* gene acts genetically downstream of *TTG1* since overexpression of *GL3* can partially rescue *ttg1* mutants. It is therefore conceivable that the activity of *GL3* is controlled by *TTG1*.

The *try cpc* double mutant phenotype described here revealed that the inhibitory function during trichome patterning consists of at least two components. Although these genes encode homologous proteins with indistinguishable expression patterns, the different phenotypes of the single mutants, such as cluster formation and reduction in trichome number in *try* mutants and an increased trichome number in *cpc* mutants, point to mechanistically different roles. One possibility to explain the single as well as the double mutant is that the *CPC* protein diffuses faster than the *TRY* protein. As a result, *TRY* would be important in short-range inhibition whereas the lack of *CPC* would

have predominantly a distant effect. This interpretation is consistent with the ontogenesis of the large clusters in the *try cpc* double mutant. Initial pattern formation is similar to that in *try* mutants. Only after trichomes become more and more separated due to cell divisions of epidermal cells does *CPC* become important and result in the formation of additional trichomes around an already existing trichome.

At first glance, the expression pattern of *TRY* and *CPC* is surprising. As shown by the mutant phenotypes and the suppression of trichome initiation in overexpressing lines, both genes act as negative regulators of trichome development, and hence the finding that the highest expression is found in cells that are suppressed seems paradoxical. However, the expression patterns fit nicely with the dynamics of the inhibitor as predicted by the theoretical model of Meinhardt. According to the model, the activity of the negative regulator is tightly controlled by the activator. Hence, the expression of the inhibitor is always highest where the activator is highest. Because the diffusion rate of the inhibitor is higher than that of the activator, the inhibitor peak is broader than the activator peak. Therefore, at the point of highest activator activity, which eventually becomes the selected cell, the activity of the activator always exceeds the activity of the inhibitor. Consistent with this model, the spatial distribution of *GLI* is very similar to that of *TRY/CPC* such that initially *GLI* is expressed in all epidermal cells to become restricted only to trichome cells later (Larkin *et al.*, 1993). Whether expression levels or activity levels are different is unknown.

Which one of the positive regulators is the target of *TRY* and *CPC*? Our finding that overexpression of the *R* gene but not of *GLI* can rescue the glabrous phenotype of *CPC*-overexpressing lines indicates that *CPC* and the *R* gene can titrate each other's function. An attractive explanation for this finding is that *CPC* competes with the *R* gene homolog *GL3* in *Arabidopsis* and, depending on their balance, trichome cell fate is promoted when more *GL3* is available, while an excess of *CPC* promotes the epidermal cell fate. In contrast, *TRY* did not interact with either of the two overexpressor lines. It seems to interact with *TTG1*, as suggested by the genetic observation that double heterozygous *try/+ ttg1/+* plants showed an increased cluster frequency (Schnittger *et al.*, 1999).

Molecular interactions during trichome patterning

Except for the *TTG1* gene, all factors involved in trichome patterning code for putative transcription factors. Thus, it is conceivable that the relative balance or competitive interactions are important. The physical interactions observed in two-hybrid assays suggest that the factors involved can form multimeric complexes. As shown by Payne *et al.* (2000), *GL3* physically interacts with both *GL1* and *TTG1* in the yeast two-hybrid system, whereas no interaction was found between *GL1* and *TTG1*. Because *TRY* and *CPC* encode single MYB repeat proteins lacking a transactivation domain, an attractive scenario is that they compete with the positive trichome initiation factors for binding sites. The *CPC* gene is likely to counteract the *GL3* gene specifically. Competition of *TRY* or *CPC* with the positive regulators could occur either by protein-protein interactions resulting in inactive complexes or by a competition at the level of DNA binding.

Since the *ttg1* mutant trichome phenotype can be rescued in a *35S::GL1 try* background, it is likely that *TTG1* is not absolutely required for these interactions (Schnittger *et al.*, 1999). One possibility is that *TTG1* modifies the activity of the other factors probably by interacting with *GL3*, since molecular interactions between *GL3* and *TTG1* were observed in the yeast two-hybrid system (Payne *et al.*, 1999).

One major question is how cell-cell communication is mediated molecularly. Our finding that both genes involved in lateral suppression of trichome fate are eventually expressed exclusively in developing trichomes supports two alternative explanations. Either *TRY* and *CPC* switch on an unknown gene that can move into the neighbouring cells, or *TRY* and *CPC* proteins can move and act directly in the neighbouring cells. Generally, the transport of transcription factors between cells is well established in plants (Lucas, 1995). Because *TRY* and *CPC* proteins are very small and well below the exclusion size of 40 kDa found for plasmodesmata between epidermal cells in tobacco leaves (Oparka *et al.*, 1999), transport could even occur by diffusion. Genetic mosaics and a careful comparison between RNA and protein expression patterns are needed to clarify this.

Root hair cell file establishment by lateral inhibition

The current genetic and molecular data provide a detailed explanation for the control of cell differentiation in atrichoblasts and trichoblasts as well as for the atrichoblast-specific expression of the *GL2* gene. The genetic machinery involved is remarkably similar to that known in trichome development except that the logic is reversed such that atrichoblast cells correspond to trichome cells.

The expression of *GL2* in atrichoblasts depends on the *GLI* homolog *WER* that is expressed in atrichoblasts (Lee and Schiefelbein, 1999). Also, the *TTG1* gene controls the expression of *GL2* and, because the maize *R* gene can suppress the *ttg1* root hair phenotype, probably involves an as yet unknown bHLH-related transcription factor. In hair cells, *GL2* expression is down-regulated by the *CPC* gene. However, the finding that *GL2* expression during embryo development is not changed in *cpc* mutants suggested that at least one additional unknown factor is required (Lin and Schiefelbein, 2001). In both systems, a *TTG1*-controlled bHLH-related transcription factor (*GL3* in the shoot and an as yet unknown *R* gene homolog in roots) together with a MYB-related transcription factor (*GL1* in the shoot and its homolog *WER* in the root) determine epidermal cell differentiation via the *GL2* gene (Figure 7). This similarity of the involved factors raises the question of whether a lateral inhibition system is also involved during root hair patterning. Recently, an elegant series of genetic experiments has revealed that *CPC* is involved in the lateral suppression of *WER* and *GL2* (Lee and Schiefelbein, 2002). Our finding that in *try cpc* double mutants root hair patterning is completely abolished, leaving only atrichoblast cell files, strongly indicates that *TRY* and *CPC* act similarly to the behaviour observed during trichome patterning in a lateral suppression process. By analogy, *CPC* and *TRY* would be most strongly accumulated in non-root hair files and be involved in the lateral suppression of *GL2* expression in root hair files. Consistent with

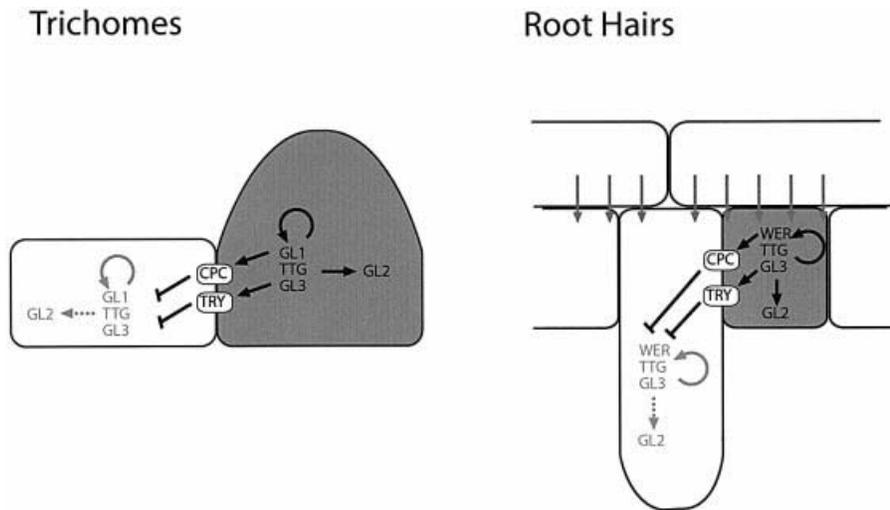


Fig. 7. Genetic model of trichome and root hair patterning. It is postulated that both trichome (left) and root hair patterning (right) are based on a competition mechanism mediated by *TRY* and *CPC*. In both systems, this competition results in the activation of a set of positive regulators consisting of TTG1, and a MYB-related (GL1 or WER) and a bHLH-related (GL3 or an unknown factor in root hairs) transcription factor. The biased decision in the root system with respect to the underlying cortex cells is explained by a positive signal from the cortex cells (arrows) which is slightly weaker in epidermal cells overlying a cleft. For clarity, only those regulation events are indicated here that are immediately relevant for the proposed model. These activate the *GL2* gene which in the leaf epidermis triggers trichome formation and in the root epidermis promotes non-root hair fate.

this idea, we found *TRY* expression only in genetic situations where root epidermal cells are atrichoblasts. Similarly, *CPC* was found to be expressed predominantly in atrichoblasts (Lee and Schiefelbein, 2002; T.Wada and K.Okada, unpublished). These data support an attractive model to explain the positioning of root hairs cells relative to the underlying cortex cells (Lee and Schiefelbein, 2002; Figure 7). If the same mutual inhibition mechanism operated during root hair file establishment, extremely small differences biasing the cell fate choice can easily be amplified to create a stable, predictable pattern. A positive cortex cell-derived signal would be slightly weaker in epidermal cells overlying a cleft between cortex cells but sufficient to bias the patterning system towards root hair cell fate.

Our finding that *TRY* is expressed at very low levels in wild-type and derepressed in the *cpc try* double mutant indicates that the regulation of *TRY* expression involves an auto-repression by the two redundantly acting genes *TRY* and *CPC*. According to the model, this would be expected as in the absence of the repressor, the activator concentrations are increased and consequently also the transcription of the repressors.

Evolutionary divergence of epidermal cell fate specification

Epidermal cell fate specification in the root, the hypocotyl, the leaf and the seed coat involves a remarkably similar set of genes. The WD40 protein TTG1 and the homeobox transcription factor GL2 are required in all four epidermal tissues (Koornneef, 1981; Galway *et al.*, 1994; Walker *et al.*, 1999). In the root, hypocotyl and the leaf, a bHLH protein similar to the *R* gene product and functionally equivalent MYB transcription factors (WER and GL1) are important (Oppenheimer *et al.*, 1991; Lloyd *et al.*, 1992; Galway *et al.*, 1994; Hung *et al.*, 1998; Payne *et al.*, 2000). As shown in this work, in addition, two very similar MYB-

related transcription factors lacking a transactivation domain are involved in root, hypocotyl (data not shown) and leaf epidermal patterning. In the case of *WER* and *GL1*, it was shown that they are functionally equivalent, but do not share a functional overlap (Lee and Schiefelbein, 2001). In contrast, *CPC* and *TRY* function in a partly redundant manner. It is therefore tempting to speculate that in this system members of the same gene family such as *WER* and *GL1*, and *CPC* and *TRY* have evolved from gene duplications of common ancestor genes and that they have adopted new and different functions to variable degrees.

Perspectives

The analysis of trichome and root hair patterning has revealed a common underlying mechanism. The basic functions are carried out by the same genes or by a combination of highly homologous genes. This raises a number of interesting questions. What is the specificity of genes with equivalent functions such as *TRY* and *CPC*? Is their function interchangeable as found for *GL1* and *WER*? How can the patterning machinery be modulated by the cortex cells in the root or by environmental factors controlling trichome density in the shoot? The patterning genes identified seem to be responsible only for creating differences between cell types. How is the actual cell fate, root hair versus trichome, determined? Now that all genetically identified genes are cloned, the tools have become available to address these questions.

Materials and methods

Plant material, growth conditions and genetic analysis

Plants were grown on soil at 23 or 18°C under constant illumination. For the analysis of root hairs, plants were grown on 0.6% agar plates containing 1/2 MS medium and 2% sucrose. Wild-type strains used in this work were Landsberg *erecta* (Ler), Columbia-0 (Col) and Wasilewskaja (Ws-0). The *try* alleles *try*-EM1, *try*-EM2 and *try*-JC, the single mutants

gl2, *ttg1* and *cpc*, and the overexpressing lines 35S::*R-GR* 1857, 35S::*R-1435*, 35S::*GLI* and 35S::*CPC* were described previously (Hülkamp *et al.*, 1994; Larkin *et al.*, 1994; Lloyd *et al.*, 1992, 1994; Wada *et al.*, 1997). The *try* allele *try-1828* is a newly identified X-ray allele. The phenotypic analysis of plants overexpressing 35S::*TRY* or 35S::*CPC* together with 35S::*GLI*, 35S::*R-GR* 1857 or 35S::*R-1435* was carried out in the F₁ generation. Double mutants were created by crossing the parental lines, screening the F₂ generation for new phenotypes and verified by backcrosses to the parental lines. The number of TIS of *try*, *cpc*, *try cpc*, *Ws-0* and *Ler* was counted on the first four leaves of 25 different 21-day-old plants. For a comparison of trichome number in *cpc* and *Ws-0* in developing leaves, leaves smaller than 800 µm were stained with fluorescein diacetate. The trichome number was determined for various leaf stages as defined by their leaf lengths.

Microscopy and cytological methods

4',6-diamidino-2-phenylindole (DAPI) staining and DNA content measurements of trichome nuclei were done as described (Hülkamp *et al.*, 1994). For light microscopy, we used a Zeiss axiophot and for confocal laser scanning microscopy (CLS) a Leica CLS-microscope and the TCS-NT program. For SEM analysis, critical point dried leaves or Spurr impressions prepared as described below were mounted, sputtered and analysed at 10 or 15 keV.

Images were processed with Adobe Illustrator 9.0 and Adobe Photoshop 5.0 (Adobe Systems Inc., Mountain View, CA) software.

Analysis of root hair patterning

For each line, 7-day-old seedlings were stained with propidium iodide and analysed with a Leica laser confocal microscope. The cell length of at least four neighbouring root cell files was determined by counting the cell number for a defined root section. Each cell file was classified as hair cell files (T) or non-hair files (A) according to their position relative to the underlying cortex.

Wax impressions of leaf surfaces

For monitoring the formation of the large trichome clusters in *try cpc* double mutants, young developing leaves were covered with dental wax at 6 h intervals. Up to five wax impressions per leaf were filled with SPURR, hardened at 65°C and analysed by SEM as described above.

Molecular biology

New CAPS markers were designed by PCR amplification of random 2 kb fragments in the *TRY* interval. Fragments with a restriction enzyme polymorphism or a length polymorphism between the ecotypes *Ler* and *Col* were used as CAPS markers for further fine mapping. Details are available on request.

For sequencing mutant alleles, a 1.5 kb genomic fragment was amplified and sequenced using the primers 5'-AACGACAAGTCTACACAAAGGG-3' and 5'-CCTAACCGCATGGATTAAG-3'.

5' RACE was carried out with the Gibco-BRL 5' RACE kit using 5'-TGAATTCCTCAAACCTAAACAAGAAC-3' as GSPI primer and 5'-ACTAGTGACTAGGAAGGATAGATAGAAAAG-3' as nested primer. For 3' RACE, an oligo(dT) primer was used together with 5'-TCTAGATAGTAATGGATAACACTGACCGT-3' and 5'-CGTCGC-CGTCGTAAGCAACAC-3' as nested gene-specific primers. The PCR products were cloned into pGEM (Promega) and the clones sequenced (SP6/T7).

The analysis of *TRY* and *CPC* expression by RT-PCR was done as previously described (Kirik *et al.*, 2001). Hybridization experiments using gene-specific probes were included to show that the detected expression is specific for *TRY* or *CPC*.

In situ hybridizations with the *TRY* probe were done as described (Schoof *et al.*, 2000). For the analysis of *TRY* expression, the *TRY* coding sequence and the 5'-untranslated region were used to generate the sense and the antisense probe.

The fixation, embedding, sectioning and hybridization to analyse *CPC* expression were done as described by Drews *et al.* (1991).

Constructs and plant transformation

To rescue the *try* mutant phenotype, a 4.2 kb genomic *HindIII* fragment from the ecotype *Ler* was cloned into the binary vector pBARB.

For GUS analysis of *TRY* expression, 1.4 kb upstream untranslated sequence was PCR amplified using 5'-AAGCTTCGAAAGAAAACGCAAATCAGG-3' and 5'-CGTCGACTATTGAAGTAAGAAAAGAA-AAAATAG-3' as primers to introduce a 5' *HindIII* and a 3' *SalI* restriction site, subcloned into pGem and sequenced. This fragment was subcloned in front of the *GUS* marker gene and a terminator site in the vector pGUS

1. A *HindIII* and *EcoRI* fragment containing the *TRY* promoter::GUS fusion was subcloned further into the *HindIII* and *EcoRI* sites of pBARB.

To create the 35S::*TRY* construct, the *TRY* cDNA was PCR amplified from a *Col* root cDNA library with 5'-TCTAGATAGTAATGGATAACACTGACCGT-3' and 5'-ACTAGTGACTAGGAAGGATAGATA-GAAAAG-3' primers containing a 5' *XbaI* and a 3' *XmaI* restriction site. This fragment was subcloned into the pGem vector, sequenced and introduced between the CaMV 35S promoter and a terminator site into the *XbaI* site of the vector pRT103, for which the *NcoI* site was deleted by digestion with *SmaI* and *BalI* and religation. A *HindIII* fragment containing the 35S promoter::TRY cDNA::terminator fusion was subcloned into the *HindIII* site of the binary vector pBARB.

Plant transformations and the selection of transformed plants were done as previously described (Clough and Bent, 1998).

Acknowledgements

We thank the members of our laboratory for critically reading the manuscript, Jürgen Berger (SEM) and Paul Grini (microscopy and computers) for expert advice, Ulrike Schöbinger for excellent technical assistance, Wolfgang Lukowitz, Ulrike Mayer and John Larkin for providing us with new *try* alleles, and Mandy Walker and John Larkin for sharing unpublished results. This work was supported by the Volkswagen Stiftung and the SFB446.

References

- Berger, F., Hung, C., Dolan, L. and Schiefelbein, J. (1998) Control of cell division in the root epidermis of *Arabidopsis thaliana*. *Dev. Biol.*, **194**, 235–245.
- Campos-Ortega, J.A. (1993) Early neurogenesis in *Drosophila melanogaster*. In Bate, M. and Martinez, A. (eds), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1091–1129.
- Clough, S. and Bent, A. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.*, **16**, 735–743.
- Drews, G.N., Weigel, D. and Meyerowitz, E.M. (1991) Floral patterning. *Curr. Opin. Genet. Dev.*, **1**, 174–178.
- Galway, M.E., Masucci, J.D., Lloyd, A.M., Walbot, V., Davis, R.W. and Schiefelbein, J.W. (1994) The *TTG* gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. *Dev. Biol.*, **166**, 740–754.
- Ghysen, A., Dambly-Chaudière, C., Jan, L.Y. and Jan, Y. (1993) Cell interactions and gene interactions in peripheral neurogenesis. *Genes Dev.*, **7**, 723–733.
- Hülkamp, M., Misera, S. and Jürgens, G. (1994) Genetic dissection of trichome cell development in *Arabidopsis*. *Cell*, **76**, 555–566.
- Hung, C., Lin, Y., Zhang, M., Pollock, S., Marks, M.D. and Schiefelbein, J. (1998) A common position-dependent mechanism controls cell-type patterning and *GLABRA2* regulation in the root and hypocotyl epidermis of *Arabidopsis*. *Plant Physiol.*, **117**, 73–84.
- Kirik, V., Bouyer, D., Schöbinger, U., Bechtold, N., Herzog, M., Bonneville, J.M. and Hülkamp, M. (2001) CPR5 is involved in cell proliferation and cell death control and encodes a novel transmembrane protein. *Curr. Biol.*, **11**, 1891–1895.
- Koornneef, M. (1981) The complex syndrome of *ttg* mutants. *Arabidopsis Inf. Serv.*, **18**, 45–51.
- Larkin, J.C., Oppenheimer, D.G., Pollock, S. and Marks, M.D. (1993) *Arabidopsis GLABROUS1* gene requires downstream sequences for function. *Plant Cell*, **5**, 1739–1748.
- Larkin, J.C., Oppenheimer, D.G., Lloyd, A.M., Pappozzi, E.T. and Marks, M.D. (1994) The roles of the *GLABROUS1* and *TRANSPARENT TESTA GLABRA* genes in *Arabidopsis* trichome development. *Plant Cell*, **6**, 1065–1076.
- Larkin, J.C., Young, N., Prigge, M. and Marks, M.D. (1996) The control of trichome spacing and number in *Arabidopsis*. *Development*, **122**, 997–1005.
- Lee, M.M. and Schiefelbein, J. (1999) WEREWOLF, a MYB-related protein in *Arabidopsis*, is a position-dependent regulator of epidermal cell patterning. *Cell*, **99**, 473–483.
- Lee, M.M. and Schiefelbein, J. (2001) Developmentally distinct MYB genes encode functionally equivalent proteins in *Arabidopsis*. *Development*, **128**, 1539–1546.
- Lee, M.M. and Schiefelbein, J. (2002) Cell patterning in the *Arabidopsis*

- root epidermis determined by lateral inhibition with feedback. *Plant Cell*, **14**, 611–618.
- Lin, Y. and Schiefelbein, J. (2001) Embryonic control of epidermal cell patterning in the root and hypocotyl of *Arabidopsis*. *Development*, **128**, 3697–3705.
- Lloyd, A.M., Walbot, V. and Davis, R.W. (1992) *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators R and C1. *Science*, **258**, 1773–1775.
- Lloyd, A.M., Schena, M., Walbot, V. and Davis, R.W. (1994) Epidermal cell fate determination in *Arabidopsis*: patterns defined by steroid-inducible regulator. *Science*, **266**, 436–439.
- Lucas, W.J. (1995) Plasmodesmata: intercellular channels for macromolecular transport in plants. *Curr. Opin. Cell Biol.*, **7**, 673–680.
- Marks, M.D. and Feldmann, K.A. (1989) Trichome development in *Arabidopsis thaliana*. I. T-DNA tagging of the *glabrous1* gene. *Plant Cell*, **1**, 1043–1050.
- Masucci, J.D., Rerie, W.G., Foreman, D.R., Zhang, M., Galway, M.E., Marks, M.D. and Schiefelbein, J.W. (1996) The homeobox gene *GLABRA 2* is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. *Development*, **122**, 1253–1260.
- Meinhardt, H. and Gierer, A. (1974) Applications of a theory of biological pattern formation based on lateral inhibition. *J. Cell Sci.*, **15**, 321–346.
- Oparka, K.J. *et al.* (1999) Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell*, **97**, 743–754.
- Oppenheimer, D.G., Herman, P.L., Sivakumaran, S., Esch, J. and Marks, M.D. (1991) A *myb* gene required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. *Cell*, **67**, 483–493.
- Payne, T., Zhang, F. and Lloyd, A. (1999) *GLABROUS3* is a bHLH transcription factor that interacts with other regulators of trichome development. 10th International Conference on *Arabidopsis* Research, Melbourne, Australia.
- Payne, C.T., Zhang, F. and Lloyd, A.M. (2000) *GL3* encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with *GL1* and *TTG1*. *Genetics*, **156**, 1349–1362.
- Rerie, W.G., Feldmann, K.A. and Marks, M.D. (1994) The *glabra 2* gene encodes a homeo domain protein required for normal trichome development in *Arabidopsis*. *Genes Dev.*, **8**, 1388–1399.
- Schnittger, A., Jürgens, G. and Hülskamp, M. (1998) Tissue layer and organ specificity of trichome formation are regulated by *GLABRA1* and *TRIPTYCHON* in *Arabidopsis*. *Development*, **125**, 2283–2289.
- Schnittger, A., Folkers, U., Schwab, B., Jürgens, G. and Hülskamp, M. (1999) Generation of a spacing pattern: the role of *TRIPTYCHON* in trichome patterning in *Arabidopsis*. *Plant Cell*, **11**, 1105–1116.
- Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F.X., Jürgens, G. and Laux, T. (2000) The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. *Cell*, **100**, 635–644.
- Szymanski, D.B. and Marks, M.D. (1998) *GLABROUS1* overexpression and *TRIPTYCHON* alter the cell cycle and trichome cell fate in *Arabidopsis*. *Plant Cell*, **10**, 2047–2062.
- Tepass, U. and Hartenstein, V. (1995) Neurogenic and proneural genes control cell fate specification in the *Drosophila* endoderm. *Development*, **121**, 393–405.
- Wada, T., Tachibana, T., Shimura, Y. and Okada, K. (1997) Epidermal cell differentiation in *Arabidopsis* determined by a *myb* homolog. *CPC. Science*, **277**, 1113–1116.
- Walker, A.R., Davison, P.A., Bolognesi-Winfield, A.C., James, C.M., Srinivasan, N., Blundell, T.L., Esch, J.J., Marks, M.D. and Gray, J.C. (1999) The *TTG1* (*transparent testa, glabra1*) locus which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis* encodes a WD40-repeat protein. *Plant Cell*, **11**, 1337–1350.

Received July 1, 2002; revised August 12, 2002;
accepted August 14, 2002