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Initiating inhibition

Control of epidermal cell patterning in plants

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A recent publication by Schellmann *et al.* (2002) in *The EMBO Journal* has provided important insights into the control of cell fate and pattern formation in plants. To study the underlying molecular mechanisms, the authors made use of the development of root hairs and trichomes in *Arabidopsis thaliana*. *A. thaliana* trichomes are large, unicellular structures that project out from the shoot epidermal surface and are thought to form a defence against herbivorous insects. Root hairs are specialized cells extending from the root epidermis and are important for water and mineral uptake. The positional cues that regulate the spacing of trichomes and root hairs are unique. In the root, positional information is derived from the location of the epidermal cells relative to underlying cortex cells (Fig. 1A). Epidermal cells that are located over a junction between two cortex cells become root hairs (H cells), whereas the other epidermal cells become non-root-hair cells (N cells). On the leaves, the trichome spacing pattern is determined largely by the position of the first trichome (Fig. 1B). After the leaf primordium reaches a length of ~100 μm, a single epidermal cell at the leaf tip becomes a trichome. Thereafter, a regulated spacing pattern evolves in which newly initiated trichomes are approximately equal distance from one another. As older trichomes become separated by epidermal cell divisions, new intervening trichomes emerge (Fig. 1B).

Despite the obvious differences between these systems, extensive mutational analyses of root hair and trichome development have identified a partly shared set of genes encoding transcription factors that regulate the patterning of both cell types (see Table 1) (Szymanski *et al.*, 2000; Dolan, 2001). These genes seem to act by promoting trichome development in the shoot, and inhibiting H-cell initiation in the root. They include the functionally equivalent Myb genes *GLABROUS1* (*GL1*) and *WEREWOLF* (*WER*), which are expressed in the shoot and root, respectively; the WD-40 repeat-encoding gene *TRANSPARENT TESTA GLABRA* (*TTG*), which probably acts as a transcriptional scaffold in both the shoot and root; and the basic helix–loop–helix (bHLH) gene *GLABRA3* (*GL3*). Although mutations in *GL3* affect only trichome initiation, a closely related bHLH gene in *A. thaliana* is predicted to have overlapping functions with *GL3* in both the root and shoot. Yeast interaction assays have shown that the GL3 protein can homodimerize and contains discrete domains that interact with the GL1 and TTG proteins (Payne *et al.*, 2000). It has been proposed that a protein complex composed of GL1, GL3 and TTG functions as an activator of genes required for trichome formation and that a similar complex containing WER, TTG and a bHLH protein is important for N-cell identity. The homeodomain encoding *GLABRA2* (*GL2*), which is required for both trichome differentiation and root hair repression, is likely to be a primary target of the shoot and root complexes.

The expression patterns of *WER*, *GL1* and *GL2* have been well characterized. In the shoot, *GL1* and *GL2* are diffusely expressed throughout young leaf primordia before the emergence of trichomes, and increase sharply in incipient trichomes (Szymanski *et al.*, 1998). In the root, the expression of *WER* and *GL2* is largely restricted to the N cells (Lee & Schiefelbein, 1999).

So how is the spacing of trichomes controlled? The gene *TRIPTYCHON* (*TRY*) is a key player, because mutations in *TRY* result in an abnormally high frequency of clustered trichomes (Hülskamp *et al.*, 1994). Now, in this recent paper, Schellmann *et al.* report that *TRY* encodes another Myb-like transcription factor; however, unlike *WER* and *GL1*, which encode proteins with two Myb DNA-binding domains and putative acidic transcriptional activation domains, the predicted TRY protein contains only one Myb DNA-binding domain and seems to lack an activator domain. The expression pattern of *TRY* in the shoot mirrors the expression of *GL1* and *GL2*. Given that the genes that positively influence trichome initiation (*GL1* and *GL2*)

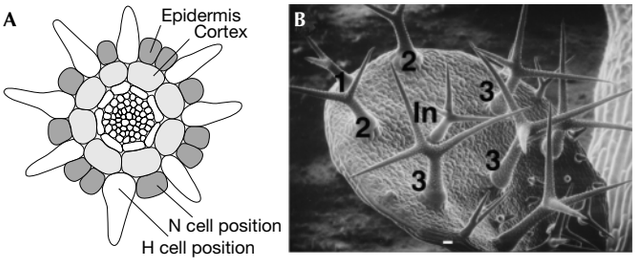

Fig. 1 | Epidermal patterning of roots and leaves. (**A**) Line drawing of a cross-section of a mature region of an *Arabidopsis* root. Epidermal and cortical cell layers are labelled. H, root-hair cell; N, non-root-hair cell. (**B**) Scanning electron micrograph of a young developing leaf. Trichomes are numbered according to their relative age. The mature trichome labelled 1 was the first to develop. The trichomes labeled In are intervening trichomes that have not fully developed.

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Table 1 | Genes involved in trichome and root hair initiation

Locus	Gene product	Wild-type expression profile	Function
GL1	Myb transcription factor	Young leaf primordia and developing trichomes	Promotes trichome initiation
WER	Myb transcription factor	Non-root-hair (N) cells	Prevents root-hair (H)-cell initiation
GL3	Myc transcription factor	Not well characterized	Promotes trichome initiation
TTG	WD-40 repeat	Not well characterized	Promotes trichome initiation and prevents H-cell initiation
CPC	Myb transcription factor	Young leaf primordia and developing trichomes and N cells	Limits trichome initiation and promotes H-cell initiation
TRY	Myb transcription factor	Young leaf primordia and developing trichomes and weakly expressed in roots	Prevents trichome clustering

along with the negative regulator, *TRY*, are all initially expressed at high levels in young leaf primordia, it is difficult to understand how the pattern of trichome initiation is established. Schellmann *et al.* propose a model in which the activator complex (for example, GL1–GL3–TTG in the shoot) has the ability to stimulate the production of a transmitted inhibitory signal. Subtle differences in the levels of the activator complex would create a cell that is capable of sending a stronger inhibitory signal to its neighbours, and this signal would prevent those neighbouring cells from entering the trichome pathway. The prediction is that the TRY gene is a target of the activator complex and is responsible for transmitting the inhibitory signal. The exact nature of this inhibitory signal is still unknown, although it is possible that it is encoded by the *TRY* gene itself. The predicted TRY protein is sufficiently small for it to be able to move from cell to cell through the plasmodesmata, and it might act as an inhibitor either by disrupting the formation of the activator complex or by binding to regulatory elements of genes regulated by the activator complex (see Fig. 2 and Schellmann *et al.* (2002) for possible models).

Schellmann *et al.* also established that there is a relationship between *TRY* and *CAPRICE* (*CPC*), a closely related gene required for H-cell formation. *CPC*, like *TRY*, encodes a Myb-type factor that contains a single Myb domain and lacks an obvious activating domain (Wada *et al.*, 1997). Previously, Lee & Schiefelbein (2002) presented evidence that *CPC* acts as a lateral inhibitor in the root epidermis to prevent *WER* from activating *GL2* in H cells. Schellmann *et al.* extended these observations by providing evidence that *TRY* and *CPC* have overlapping functions in both the root and the shoot. They found that *CPC* expression overlaps *TRY* expression in the shoot and that the loss of both *CPC* and *TRY* activities greatly enhances trichome clustering. In addition, they showed that *TRY* is expressed at low levels in the root and that the *cpc/try* double mutant exhibits an even greater loss of H cells than the *cpc* mutant. Interestingly, *cpc* mutants do not have a trichome-clustering phenotype, nor do *try* mutants exhibit a root hair defect. Thus, we can speculate that the *TRY* and *CPC* genes, which probably arose from a fairly recent gene duplication event, are in the process of evolving distinct functions in *Arabidopsis*. It will be interesting to examine these genes in related species to compare the degree of this separation in function.

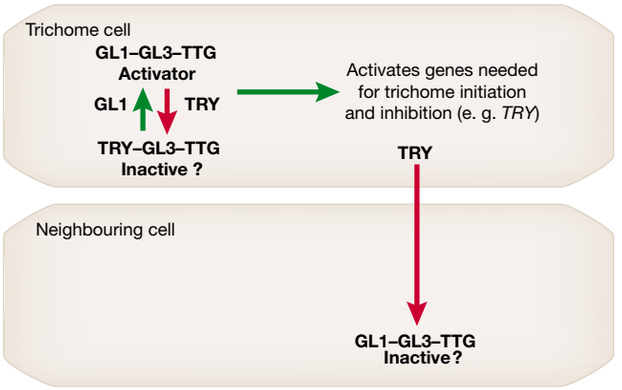

Fig. 2 | One possible model to explain the function of *TRY*. In the cell fated to become a trichome the proteins encoded by the *GL1*, *GL3* and *TTG* genes form a transcriptional complex capable of activating genes required for trichome differentiation (such as *GL2*) and gene(s) needed to mediate the lateral inhibition signal (such as *TRY*) that prevents neighbouring cells from becoming trichomes. In the simplest case, the TRY protein is able to modulate the level of activator complex in the developing trichomes and also moves into the neighbouring cell to disrupt the activator complex. A complex containing TRY, GL3 and TTG is here predicted to be inactive; however, as an alternative this complex could activate the inhibition genes. A similar model could exist in the root in which GL1 and TRY are replaced by WER and CPC, and the activator complex level is highest in N (non-root-hair) cells.

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