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Plant Patterning: TRY to Inhibit your Neighbors Dispatch

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Two regulators of hair-cell patterning in *Arabidopsis* act in the cells where they are produced to inhibit primary cell fate in neighboring cells. Their relationship to the activators of the primary cell fate suggests a classical activator–inhibitor model for patterning that can now be analyzed in detail.

Plant epidermal cells specialize to produce hair-like projections separated by non-hair cells. The hair-like projections are formed by cells called trichomes (T) on leaves and stems, and by trichoblasts or H cells on roots. T cells appear to arise in random positions (Figure 1A), but H cells always overly clefts between cells of the underlying layer (Figure 1B). This arrangement suggests that T cells are specified by random fluctuations and subsequently inhibit neighbors from adopting the same cell fate, whereas H cells are specified through signals from underlying cells.

Despite these apparent differences, the two cell types are apparently patterned largely by the same gene products. Remarkably, T cells in the shoot and non-hair (N) cells in the root appear to be the primary defined fates, and closely related Myb-type transcription factors are positive regulators for both cell types (Figure 1B) [1,2]. Analysis of *triptychon* (*try*) mutant plants, which display clustered non-sister T cells, suggested that the affected gene encodes a candidate non-cell-autonomous inhibitor of the T- cell fate [3]. Schellmann *et al.* [4] have now reported that the *TRY* gene encodes a Myb protein lacking an activation domain — 'Δmyb' — and that its expression becomes restricted to T cells. They have also shown that ectopic *TRY* expression suppresses T-cell specification, suggesting that TRY indeed inhibits the T-cell fate in the neighbors of specified primary cells [4] (Figure 1C).

Inhibitory signals do not seem necessary in principle to explain the regular spacing of H cells in the root, but the observation that a gene implicated in this process, *CAPRICE* (*CPC*), also encodes a trunctated Myb protein suggested that inhibitory factors might indeed be involved in root epidermal fate decisions [5]. A recent study [6] on the interaction between N-cell activating factors and CPC has provided convincing support for this idea.

The new work by Lee and Schiefelbein [6] shows that a Myb factor which acts cell autonomously as a positive regulator of the N-cell fate is required for transcription of the inhibitory *CPC* gene in N cells, and that CPC acts non-cell autonomously to inhibit the positive Myb factor and generate H cells. [6] (Figure 1).

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In one elegant experiment, uncoupling of this transcriptional feedback loop was shown to yield random cell patterns. Schellmann *et al.* [4] performed an analysis of *cpc* single mutants and *cpc; try* double mutants which revealed that the two Δmyb factors, CPC and TRY, are both involved in patterning both T and N cells. In T cells, transcription of *TRY* and *CPC* could be clearly demonstrated. The *TRY* RNA level is very low in roots, but the *TRY* promoter was shown to be active in the root epidermis of a *cpc; try* double mutant — which lacks H cells — suggesting that *TRY* is indeed expressed in N cells of wild-type plants.

How, then, does a transcription factor-like inhibitor made in one cell act in the neighboring cells? Support for the view that CPC has a direct non-cell autonomous action has come from a localization study using fusion protein consisting of CPC linked to the green fluorescent protein (GFP). Wada *et al.* [7] found that, whereas *CPC* promoter directs expression of a linked GFP reporter gene exclusively to the N cell, the CPC–GFP fusion protein can also be detected in H cells. The implication is that CPC, and possibly also its close relative TRY, may move directly into neighboring cells.

How do the inhibitors interfere in the neighboring cells with the positively acting Myb factors? For this, two other genes involved in epidermal patterning have to be mentioned. The first, *GLABRA3* (*GL3*), encodes a putative basic-helix–loop–helix (bHLH) transcription factor required for appropriate T cell density. Indirect evidence suggests that a related, but as yet unidentified, bHLH factor acts in roots [8]. The second, *TRANSPARENT TESTA GLABRA1* (*TTG1*), encodes a WD40 domain protein required for both T-cell and N-cell fates [9].

Overexpression of *GL3* eliminates the requirement for *TTG1*, indicating that TTG1 may act by modulating the activity of GL3 [8]. The results of yeast two-hybrid assays suggest that GL3 binds the positive Myb factors and TTG1, bringing them together in a complex [8]. Several lines of evidence suggest that TRY interacts with TTG1, while the bHLH factors interact with CPC [3,7]. TRY and CPC may inhibit their respective complexes by direct inactivation or by competition for promoter binding sites with the positive Myb factor.

A candidate target for such regulatory complexes is *GLABRA2* (*GL2*), which encodes a homeodomain protein and is required for the differentiation of both T and N cells [10,11]. *GL2* may be a direct target, as Myb binding sites within the *GL2* promoter seem required for ectopic activation by Myb and bHLH proteins [12]. Interestingly, the *GL2* promoter coupled to the *CPC* gene rescues the *cpc* phenotype, suggesting that the native *CPC* and *GL2* promoters may be under similar control [7].

Although these interactions need to be rigorously proven, the current data suggest a minimal model for a lateral inhibition loop that patterns T and N cells (Figure 1E). In the case of T cells, the loop would be

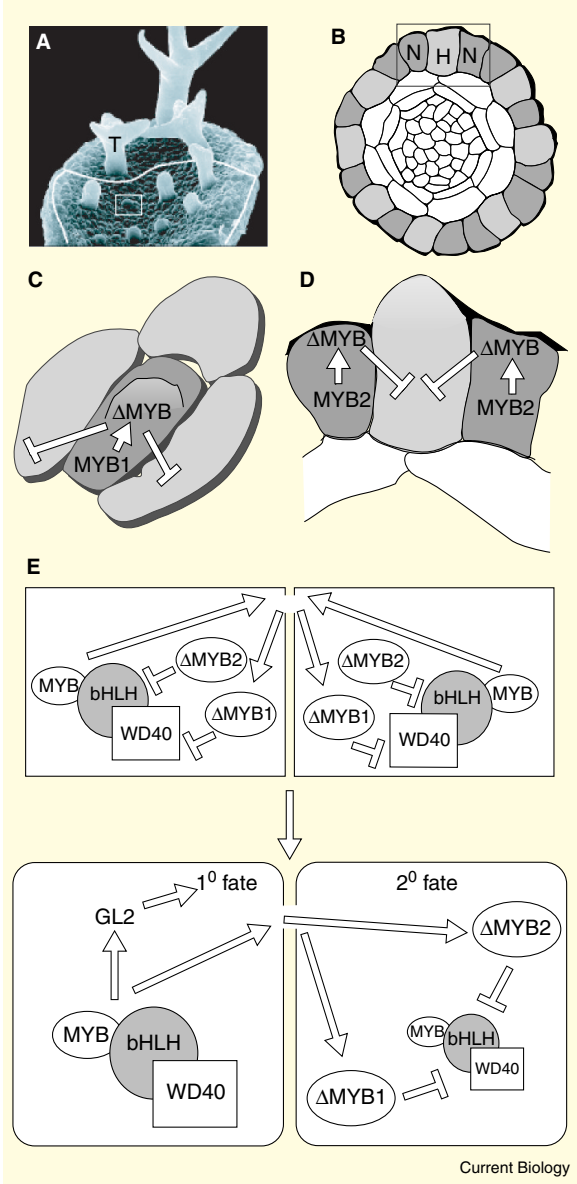

Figure 1. Epidermal patterning in *Arabidopsis*.

(A) Stochastic initiation of hair cells — trichomes (T) — on the leaf epidermis. (B) Initiation sites of hair cells — trichoblasts (H) — on the root epidermis over underlying cell wall. (C) Enlargement of the boxed region in (A) with a newly developing T cell (darker grey). (D) Enlargement of the boxed region in (B) with a newly developing H cell (paler grey). MYB1, GLABRA1; MYB2, WEREWOLF; ΔMYB1, TRY; ΔMYB2, CPC. (E) A model with an activator complex — consisting of MYB, a bHLH protein and a WD40 protein — which binds the promoter of the gene encoding the homeodomain protein GL2 to promote primary cell fate, and those encoding the ΔMYB factors for lateral inhibition. The ΔMYB factors inhibit the activator complex by interactions with two of its components and small initial differences resolve into a stable state in which one cell adopts the primary cell fate (bottom left) and the other the second cell fate (bottom right).

unbiased so that it generates a stochastic distribution. In the developing root, H cells would arise over an underlying cell cleft, because in this position they receive slightly less of an as yet unknown modulator from the underlying cells [6]. Alternatively, the cleft may slightly impair transport of inhibitor to underlying cells, leaving more inhibitor in the H cells.

The Delta–Notch pathway, which segregates neural and epidermal cell fates in the fruit fly *Drosophila*, is a well-studied example of lateral inhibition [13]. As in *Arabidopsis*, both activating and repressing transcription factors play a role in lateral inhibition during *Drosophila* development. Ligand proteins, such as Delta, act as inhibitory signals that can activate the Notch receptor on neighboring cells, resulting in transcriptional regulation that affects cell fate as well as ligand production in the neighbors.

Delta–Notch signaling involves receptor–ligand interactions, proteolytic processing, vesicle transport and large families of transcription factors. Why are the recent findings on hair-cell patterning in plants so exciting, if lateral inhibition scenarios have already been described? I believe that the main promise is the apparent simplicity of the interactions in this system, which may allow one to go beyond intuitive qualitative understanding and study patterning in a quantitative manner. Here, mathematical models that capture the essence of spacing patterns become important.

In a pioneering paper, Alan Turing [14] imagined diffusing chemicals that influenced each other's production rates and showed that slight deviations of a homogeneous distribution may resolve in regular patterns. In the context of the molecular components in our lateral inhibition scenario, it is appropriate to use formalisms in which inhibitors reduce activator production, as originally proposed by Gierer and Meinhardt [15]. A wide variety of patterns can be produced by such activator–inhibitor equations, as perhaps best illustrated in their tantalising ability to explain complex shell pigmentation patterns [16]. Unlike other regulatory networks that appear to display little parameter dependence [17], a stochastic patterning mechanism must be sensitive to parameter values.

To prove the existence of an activator–inhibitor model as postulated in these models, and to use it to make predictions, we need to know the *in vivo* concentrations of the various components, the rates of transcription, translation and diffusion, and the binding constants for their interactions. In *Arabidopsis*, this challenge may now be met by performing the biochemistry on a single activator complex, a few inhibitors and the DNA of a few promoters. Are the concentrations in the right range to pattern hairs by stochastic fluctuations? What is the autocatalytic process that amplifies the initial changes? Does the production rate keep the concentrations of inhibitors below those of activators in a producing cell — a stringent demand on activator–inhibitor systems? Do the diffusion ranges of different inhibitors match and explain differences in their phenotypes? Calculations may replace intuition to find the answers to these questions. Does TRY inhibit the neighbours? See PC!

References

1. Oppenheimer, D.G., Herman, P.L., Esch, J., Sivakumaran, S. and Marks, M.D. (1991). A *myb*-related gene required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. Cell *67*, 483–493.

2. 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.
3. 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.
4. Schellmann, S., Schnittger, A., Kirik, V., Wada, T., Okada, K., Beermann, A., Thumfahrt, J., Jürgens, G. and Hülskamp, M. (2002). *TRIPTYCHON* and *CAPRICE* mediate lateral inhibition during trichome and root hair patterning in *Arabidopsis*. EMBO J. *21*, 5036-5046.
5. 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.
6. 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.
7. Wada, T., Kurata, T., Tominaga, R., Koshino, Y., Tachibana, T., Goto, K., Marks, M.D., Shimura, Y. and Okada, K. (2002). Role of a positive regulator of root hair development, *CAPRICE*, in epidermal cell differentiation of the *Arabidopsis* root. Development, in press.
8. 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 TTG. Genetics *156*, 1349–1362.
9. Walker, A.R., Davison, P.A., Bolognesi-Winfield, A.C., James, C.N., Srinivasan, N., Blundell, T.L., Esch, J.J., Marks, M.D. and Gray, J.C. (1999). The *TRANSPARENT TESTA GLABRA1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis*, encodes a WD40 repeat protein. Plant Cell *11*, 1337–1350.
10. Rerie, W.G., Feldmann, K.A. and Marks, M.D. (1994). The *GLABRA2* gene encodes a homeodomain protein required for normal trichome development in *Arabidopsis.* Genes Dev. *8*, 1388–1399.
11. 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.
12. Szymanski, D.B., Jilk, R.A., Pollock, S.M. and Marks, M.D. (1998). Control of *GL2* expression in *Arabidopsis* leaves and trichomes. Development *125*, 1161–1171.
13. Artavanis-Tsakonas, S., Rand, M.D. and Lake, R.J. (1999). Notch signaling: cell fate control and signal integration in development. Science *284*, 770–776.
14. Turing, A. (1952). The chemical basis of morphogenesis. Philos. Trans. B. Biol. Sci. *237*, 37–72.
15. Gierer, A. and Meinhardt, H. (1972). A theory of biological pattern formation. Kybernetik *12*, 30–39.
16. Meinhardt, H. (1998). The algorithmic beauty of sea shells. Springer-Verlag Berlin Heidelberg New York.
17. von Dassow, G., Meir, E., Munro, E.M. and Odell, G.M. (2000). The segment polarity network is a robust developmental module. Nature *406*, 188–192.