Trichome patterning on growing tissue

Pierre Barbier de Reuille [*†] Adam Runions[*] Richard Smith[*] Enrico Coen[†]
Przemyslaw Prusinkiewicz[*]

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Introduction

Leaves of *Arabidopsis thaliana* bear trichomes, or hairs, which are spaced in an approximately regular pattern on their adaxial side. Trichome cells differentiate in the fast-growing basal part of the leaf and do not divide. The regular spacing of trichomes is controlled by cell-to-cell interactions (Larkin et al., 1997). We created simulation models of these interactions to gain a better understanding of the patterning process. The models operate on a growing virtual leaf with dividing and differentiating cells. We show that, within the previously proposed class of reaction-diffusion processes (Hülskamp, 2004), trichome patterning is more readily generated by activator-inhibitor than activator-substrate models (Meinhardt and Gierer, 1974). We then report on current work, in which an activator-inhibitor model is being refined by taking into account present understanding of the genetic regulatory network that controls trichome differentiation in Arabidopsis.

Reaction-diffusion models

Growing leaf surface was specified by interpolating between a sequence of keyframe B-spline surfaces (Foley et al., 1996) that represented selected stages of leaf growth. These surfaces were defined interactively using a graphical editor according to the current qualitative understanding of the dynamics of early leaf growth. Specifically, the zone of maximum growth was first located at the tip of the leaf, then progressively displaced toward the leaf base. The leaf surface supported a layer of cells growing symplastically and dividing upon reaching a threshold size (Nakielski, 2000; Smith et al., 2006). An exception was made for the trichome cells, which stopped growing upon reaching a maximum size (their vertices have then "slided" with respect to the supporting surface). The entire model was implemented within the vv modeling environment (Smith and Prusinkiewicz, 2004).

We used the equations for activator-inhibitor model as given by Meinhardt and Gierer (1974):

$$\frac{\partial a}{\partial t} = c\frac{a^2}{1+jh^2} + \rho_a - a\mu_a + D_a\nabla^2 a \qquad \frac{\partial h}{\partial t} = ca^2 + \rho_h - \mu_h h + D_h\nabla^2 h$$

where a is the activator concentration, h the inhibitor concentration, c the autocatalysis constant of the activator, j the efficiency of the inhibition, ρ_a and ρ_h the production constants, μ_a and μ_h the degradation constants, and D_a and D_h are the diffusion constants.

It is known that, on a regular grid, activator-inhibitor models can produce a pattern of regularly spaced activation peaks when the diffusion rate of the inhibitor is much greater than the diffusion rate of the activator (Meinhardt and Gierer, 1974). We observed that the same conditions apply in models of cellular tissues. Moreover, as existing activated cells move apart due to the tissue growth, in-between cells may switch to the activated state. The resulting pattern closely resembles the pattern of trichome differentiation observed in nature (Fig. 1a-c).

The activator-substrate model was implemented using a slightly modified version of the equations proposed by Meinhardt (1982, chap. 5):

$$\frac{\partial a}{\partial t} = ca^2 s - \mu_a a + D_a\nabla^2 a \qquad \frac{\partial s}{\partial t} = \rho_s - ca^2 s - \mu_s s + D_s\nabla^2 s$$

where the same notation as for the activator-inhibitor model is used. On a regular grid and in a non-growing tissue the activator-substrate model can produce a pattern of activation peaks similar to that obtained with the activator-inhibitor model, although their spacing is less regular. When a tissue grows, however, the peaks spread over neighboring cells, forming clusters that move with the cellular structure, and eventually split (Fig. 1d). This behavior is consistent with Meinhardt's (1982, Chapter 5) observations of activator-substrate model operating on a growing linear array of cells, but is inconsistent with the dynamics of trichome patterning of nature. Thus, if trichome patterning is governed by a reaction-diffusion process, it is an activator-inhibitor rather than an activator-substrate process.

Biology of trichome patterning

Larkin et al. (1997) proposed a simple genetic network for trichome patterning involving three genes: Glabra1

[*]Department of Computer Science, University of Calgary, 2500 University Drive NW, Calgary, AB, Canada T2N 1N4
[†]Department of Cell and Developmental Biology, John Innes Centre, Colney Lane, Norwich NR4 7UH, United Kingdom

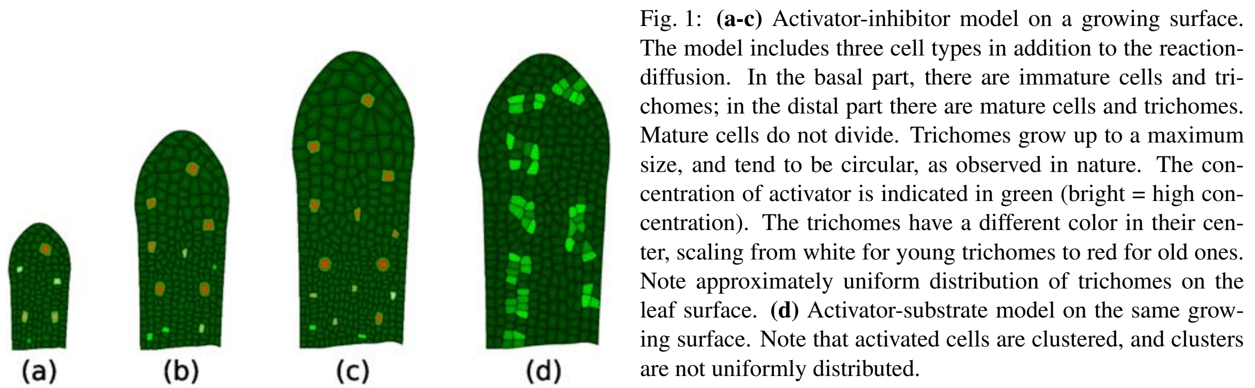

Fig. 1: **(a-c)** Activator-inhibitor model on a growing surface. The model includes three cell types in addition to the reaction-diffusion. In the basal part, there are immature cells and trichomes; in the distal part there are mature cells and trichomes. Mature cells do not divide. Trichomes grow up to a maximum size, and tend to be circular, as observed in nature. The concentration of activator is indicated in green (bright = high concentration). The trichomes have a different color in their center, scaling from white for young trichomes to red for old ones. Note approximately uniform distribution of trichomes on the leaf surface. **(d)** Activator-substrate model on the same growing surface. Note that activated cells are clustered, and clusters are not uniformly distributed.

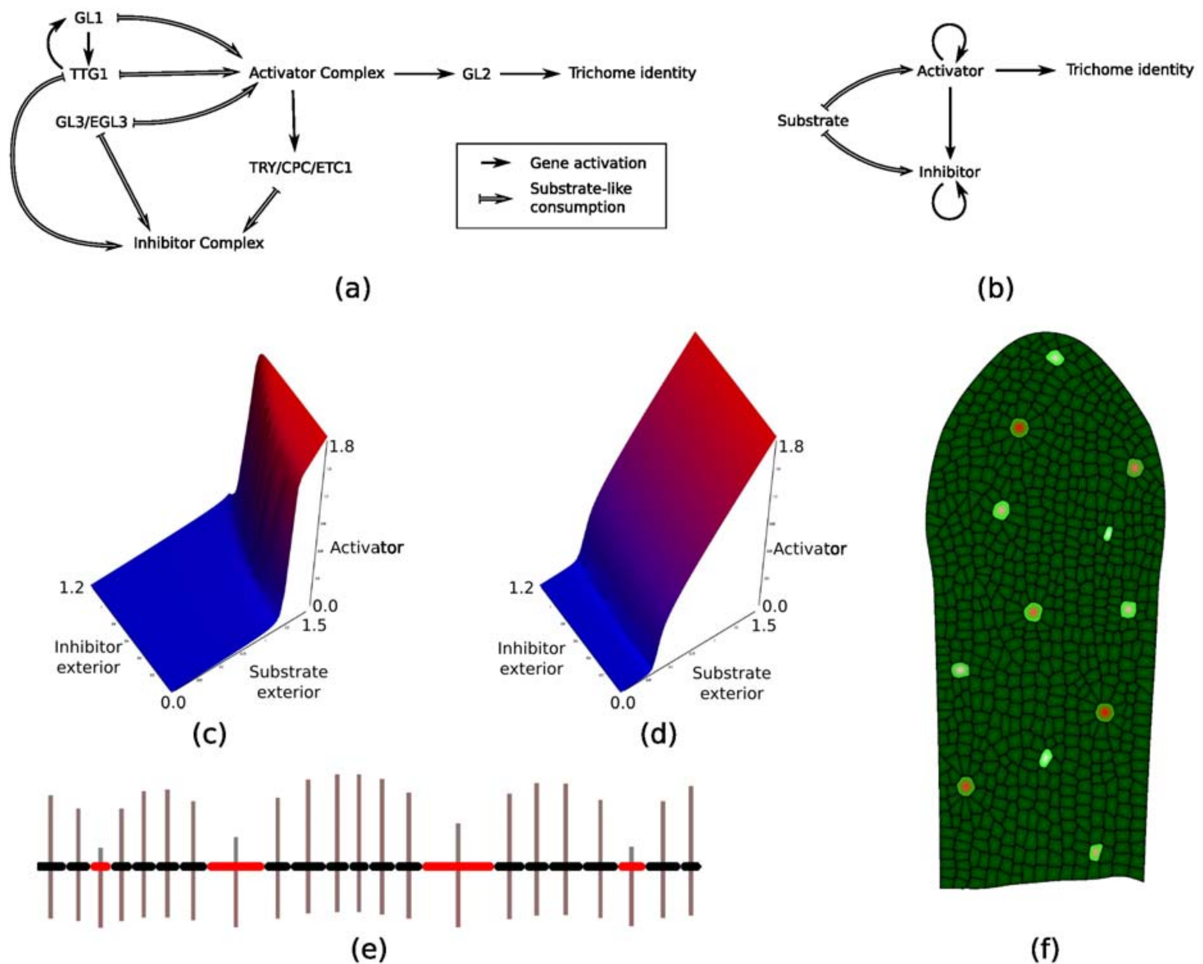

Fig. 2: **(a)** Genetic network for trichome positioning as described by Hülskamp (2004). **(b)** Substrate-activator-inhibitor (SAI) model. **(c,d)** Sample studies of the behavior of a single cell. The three-dimensional plots show the concentration of the activator as a function of the concentrations of the inhibitor and substrate, external to the cell, and the initial concentration of the activator, which is low for (c) and high for (d). The difference between plots c and d reflects different cell histories (hysteresis), and represents an essential component of cell differentiation. **(e)** Simulation of trichome differentiation in a filament. Trichome cells are shown in red. The upper and lower bars indicate concentrations of the substrate and the inhibitor, respectively. **(f)** Cell tissue simulation result with parameter values $\rho_a = 0.01, \rho_s = 1, \rho_h = 0, D_a = 0, D_h = 10, D_s = 5, \alpha = 1.6, \beta = 0.6, \lambda = 4.0, \mu_a = 0.2, \mu_h = 1.0, \mu_s = 0.3$. The color scheme is as described in Fig. 1. Note regular spacing of trichomes generated by this model.

(GL1), Transparent Testa Glabra (TTG) and Tryptychon (TRY). The first two genes form an autocatalytic activator of the trichome identity while the third one is an inhibitor of this identity. The model proposed by Larkin et al. can thus be viewed as an elaboration of the mechanism of activation-inhibition.

Since 1997, the understanding of the genetic network behind trichome patterning has evolved to include more genes and a more precise view of gene-proteins interactions. According to Hülskamp (2004), the patterning involves seven genes. Three of the resulting proteins (TTG1, GL3 and Enhancer of GL3 (EGL3)) form a complex that can further bind to either GL1 or TRY, Caprice (CPC) and Enhancer of TRY and Caprice 1 (ETC1). The complex with GL1 activates the trichome identity of a cell. The binding of TRY, CPC and ETC1 proteins to this complex prevents GL1 from binding to it, and thus indirectly inhibits trichome identity. We can describe the resulting mechanism as a competition between GL1 on one hand, and TRY, CPC and ETC1 on the other hand, for binding to the complex of TTG1, GL3 and EGL3. In addition, the complex including GL1 promotes the expression of the genes TRY, CPC and ETC1 (Fig. 2a). Of all the proteins, only CPC is known to diffuse (Hülskamp, 2004).

Substrate-Activator-Inhibitor (SAI) model

We modeled the network of gene and protein interactions, described above, as competition for a substrate s (representing TTG1, GL3 and EGL3) between a slowly diffusing activator a (representing the complex with GL1), and highly diffusive inhibitor h (representing the complex with TRY, CPC and ETC1, Fig. 2b). The concentration of the three morphogens in each cell is governed by the following system of ordinary differential equations:

$$\frac{\partial a}{\partial t} = \alpha a^2 s + \rho_a s - \mu_a a + D_a \nabla^2 a \qquad \frac{\partial h}{\partial t} = \beta h^2 s + \lambda a - \mu_h h + D_h \nabla^2 h$$

$$\frac{\partial s}{\partial t} = -\alpha a^2 s - \beta h^2 s - \lambda a + \rho_s - \mu_s s + D_s \nabla^2 s$$

where α and β are the autocatalytic constants for the activator and inhibitor, λ characterizes the upregulation of the inhibitor production by the activator, ρ_s, ρ_a, ρ_h are the production constants, μ_s, μ_a, μ_h are the degradation constants and D_s, D_a, D_h the diffusion constants.

We have developed three software tools to study this system. The first tool is a simulator of individual cells, implemented using NumPy (Oliphant, 2006), a scientific computing library for Python, and VTK (Schroeder et al., 2006), a 3D visualization toolkit. It allows for the exploration of the parameter space, considering a large number of individual cells simulated with different parameter values (Fig. 2c,d). The second tool is a simulator of a filament of cells, written using L+C (Karwowski and Prusinkiewicz, 2003). We use it to study the interaction between cells in an idealized, one-dimensional setting (Fig. 2e), which is simpler than a two-dimensional layer of growing and interacting cells. The third tool is the full simulator of the leaf, obtained by extending the activator-inhibitor model described earlier (Fig. 2f).

With this model, we were able to show that a reaction-diffusion based on competition over substrate can produce regularly spaced peaks. According to present knowledge, this is the most likely mechanism used by the plant to position the trichomes.

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