Themes and variations in cell type patterning in the plant epidermis

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It has recently become evident that plant development, like animal development, has molecular patterning modules that are reused again and again to create different cell type patterns. Here we focus on three of these plant modules: (1) the MYB-bHLH-WD40 protein complex, (2) the transmembrane calpain protease DEFECTIVE KERNEL1 (DEK1), and (3) homeodomain leucine zipper (HD-ZIP) class IV transcription factors acting in concert with SIAMESE-related cyclin-dependent kinase inhibitors. These three modules initiate the patterning of multiple cell types in the plant epidermis: the regular spacing of trichomes (leaf hairs), the stripes of root hairs, diverse pigmentation patterns in petals, the scattering of giant cells, and the files of bulliform cells. Varied combinations of players and additional regulatory inputs partially account for the diversity of patterns that are generated by reusing the same molecular mechanisms.

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Introduction

During both plant and animal development, specialized cell types are formed in complex spatial and temporal patterns. In animal systems, it is well established that molecular patterning mechanisms are reused many times to arrange multiple different cell types. For example, in *Drosophila*, Notch-Delta signaling first generates the regular spacing pattern of sensory bristle precursor cells and later breaks the symmetry between bristle precursors' progeny, leading to the specification of shaft, socket, glia, and neuron cells [1]. Plants evolved multicellularity independently from animals [2], and plant genomes lack homologs of most animal patterning genes, including Notch/Delta, Wingless/Wnt, Hedgehog, SMAD/TGF-β, and

JAC/STAT [3,4]. Although plants' patterning modules evolved independently, it is becoming increasingly evident that both lineages have evolved multifunctional patterning modules that pattern multiple cell types [5].

In plant biology, patterns are discussed on two scales. The larger scale describes the arrangement of organs or of structures in a given organ: phyllotaxy (arrangement of leaves) and venation are patterns in this sense (for reviews see [6–8]). A second scale of pattern refers to the arrangement of cell types within a tissue [9]. The epidermis, the tissue covering the plant's outer surface, provides an ideal system in which to study this type of pattern. Land plants have a highly multifunctional epidermis, in which discrete cell types function in protection (trichomes), gas exchange (stomata, which have been covered extensively elsewhere and will not be discussed further here; [10,11]), uptake (root hairs), communication with pollinators (pigmentation), and curvature control (bulliform cells and giant cells). Each of these cell types occurs in a characteristic pattern; the mechanisms generating these patterns have been a subject of intensive study.

At least three modules are shared in the patterning of many plant epidermal cell types. The first module is a transcriptional activator complex consisting of a MYB family member, a bHLH family member, and a WD40 repeat protein (the MBW complex): MYB and bHLH proteins are transcriptional regulators, and the WD40 repeat protein is believed to facilitate protein–protein interactions within the complex [5]. Patterning occurs through the movement of components between cells, presumably through the plasmodesmata, channels connecting the cytoplasm of adjacent cells. Members of these three transcription factor families have been shown to function in the initiation of at least seven epidermal cell types in *Arabidopsis*, maize, *Antirrhinum*, and *Petunia* [5].

The transmembrane calpain protease DEFECTIVE KERNEL1 (DEK1) functions in the second patterning module. It is necessary for patterning multiple cell types in *Arabidopsis* and maize. DEK1 is thought to be activated by a signal received through the transmembrane and loop domains. The protease is then thought to cleave itself autocatalytically from the rest of DEK1 protein, and subsequently to cleave downstream targets [12••,13–19]. DEK1 functions with other components including CRINKLY4 (CR4); how these interact is still unclear, although feedback loops are likely to be involved [15–19].

A third module, acting downstream of either module 1 or 2, consists of a homeodomain leucine zipper (HD-ZIP) class IV transcription factor and a cyclin-dependent kinase inhibitor in the plant-specific SIAMESE-related (SMR) protein family (HD-ZIP IV endoreduplication module) [20,21]. Class IV HD-ZIP proteins have recently been shown to inhibit cell proliferation [22]. SMR proteins suppress normal mitotic cell divisions and trigger entry into endoreduplication. This module leads to the differentiation of the cell type, generally in concert with endoreduplication. In *Arabidopsis*, the HD-ZIP class IV family contains 16 genes and the SMR family contains six [20,21].

These modules' functions are well described in trichomes and root hairs, two of the first patterning systems studied. Studies are now revealing that they are also important in the patterning of other systems, and are also revealing the importance of diversity in the modules. Here, we will discuss the parallels and differences among these modules in established and emerging patterning systems, which include anthocyanin pigmentation, giant cells, and bulliform cells.

Canonical patterning systems

Trichomes

In the model species *Arabidopsis thaliana*, an evenly distributed pattern of single-celled hairs, called trichomes, forms on the leaf blade. Trichomes are initiated three cells apart, on average, and almost never directly neighbor one another [23]. This pattern is believed to be generated by a lateral inhibition mechanism, somewhat similar to a Turing-style reaction diffusion network, in which transcriptional activators and inhibitors diffuse from cell to cell [9,24–27•]. Trichome cell fate is initiated by a MYB-bHLH-WD40 complex in which the R2R3 MYB protein is GLABRA1 (GL1), the bHLH protein is GLABRA3 (GL3) or its redundant partner ENHANCER OF GL3 (EGL3), and the WD40 protein is TRANSPARENT TESTA GLABRA 1 (TTG1) (Figure 1, Table 1; [9,28,29]). The members of the GL1–GL3–TTG1 complex are initially expressed at low levels in all epidermal cells, but are thought to activate a positive feedback loop in a few initial cells in which random fluctuations above the average level occur [24–26,27•,30•]. The GL1–GL3–TTG1 complex also transcriptionally activates several R3 MYB-encoding genes: *TRIPTYCHON* (*TRY*), *CAPRICE* (*CPC*), *ENHANCER OF TRY AND CPC 1* (*ETC1*), and *TRI-CHOME-LESS* (*TCL*) [26,27•,29,31,32]. The R3 MYBs bind GL3–TTG1 in competition with GL1, but lack a transcriptional activation domain, creating an inactive version of the complex [26,27•,33,34]. TRY and CPC proteins move out of incipient trichome cells and into neighboring cells, presumably by diffusing through plasmodesmata, creating a zone of inhibition surrounding the localized peak of activation [25,26,29]. The WD40

protein TTG1 also moves between cells, but GL1 and GL3 are cell-autonomous in the leaf [29,35–37]. TTG1 is trapped in the trichome nucleus through interaction with GL3, leading to a depletion of TTG1 in the surrounding cells, which in turn inhibits surrounding cells from becoming trichomes [36,37]. New methods for the statistical analysis of the trichome distribution will allow more detailed analysis of trichome patterning in the future [38,39].

The active GL1–GL3–TTG1 complex initiates trichome differentiation by directly upregulating the HD-ZIP class IV gene *GLABRA2* (*GL2*) and the cell cycle gene *SIA-MESE* (*SIM*) — *SIM* controls endoreduplication, a process essential for trichome development (Figure 1, Table 1; [21,31,32,40–44]). Endoreduplication is required for the maintenance of trichome identity: some *sim* mutant cells initiate trichome development, but then revert back to epidermal pavement cells [44].

Recent evidence suggests that subfunctionalization of HD-ZIP class IV genes has occurred in trichome differentiation. GL2 is required for trichome differentiation on the leaf blade, but GL2 and HD-ZIP IV transcription factor HOMEODOMAIN GLABROUS11 (HDG11) have redundant roles in trichome formation at the leaf margins in *Arabidopsis* [30•].

Root hair patterning

The molecular mechanisms of root hair patterning in *Arabidopsis* largely parallel those of trichome patterning, but generate a very different pattern: striped files of hair and non-hair cells (Figure 1, Table 1; [45]). Root hair patterning is initiated by a MBW complex that shares two members — GL3/EGL3 and TTG1 — with the trichome MBW complex, but includes the R2R3 MYB *WEREWOLF* (*WER*) instead of GL1 [45–48]. This complex directly and indirectly activates the same R3 MYB inhibitors (e.g. *TRY* and *CPC*) as in trichomes, and these move to neighboring cell files to form inactive complexes with GL3 and TTG1 [49–53,54•]. Root hair patterning differs from trichome patterning in two respects. First, root hairs are patterned based on positional information: hair cells occur only over a junction between two underlying cortex cells, and non-hair cells occur over single cortex cells [55]. The position-sensing mechanism is still not entirely clear, but is known to include the receptor-like kinase SCRAMBLED (SCM), expressed in the epidermis, and the transcription factor JACKDAW (JKD), expressed in the underlying cortex layer [27•,56,57]. SCM inhibits expression of *WER* in hair cells. Second, root hair differentiation occurs in cells expressing the inactive version of the MBW complex. The inactive R3 MYBs TRY and CPC accumulate in hair cells, whereas the active R2R3 MYB WER accumulates in non-hair cells [54•,58]. The active complex upregulates expression of the HDZIP IV transcription factor GL2 in non-hair