Cell fate and cell morphogenesis in higher plants

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The differentiation of plant cells depends on the regulation of cell fate and cell morphogenesis. Recent studies have led to the identification of mutants and the cloning of genes that influence these processes. In several instances, the genes encode products with homeodomains or Myb or Myc DNA-binding domains.

Current Opinion in Genetics and Development 1994, 4:647–651

Introduction

In higher plants, the formation and differentiation of cells occurs in an orderly manner, often near meristems, which are groups of dividing cells that act like permanent stem cell populations. Cell differentiation in plants differs from that in animals because, for the most part, of a lack of the relative cell movements that are characteristic of animal development. Also, the rigid plant cell wall makes the control of cell shape a critical aspect of cell differentiation in plants.

The fate of undifferentiated plant cells is influenced primarily by cell position rather than cell lineage [1,2]. Thus, one of the current goals in studies of plant cell differentiation is to determine how cells acquire and respond to positional cues during development. Another goal is to understand how, once a particular fate is adopted, cell morphogenesis is regulated to generate a cell of the appropriate shape. The application of molecular genetic approaches have led recently to an understanding of some of the genes that participate in cell fate specification or cell morphogenesis in plants. This review will principally focus on these recent molecular and genetic experiments, including a summary of the rapidly growing number of homeobox genes that influence plant cell development and some of the model systems that are being used to study cell fate and morphogenesis in plants.

Maize homeobox genes associated with cell fate

The homeodomain is an approximately 60 amino acid DNA-binding domain found in a superfamily of eukaryotic proteins, some of which regulate cell fate [3,4]. The first plant homeobox gene to be isolated, *KNOTTED1* (*KN1*), is associated with dominant neomorphic mutations that alter leaf development in maize and cause excess cell proliferation (knots) along lateral veins of the leaf blade ([5]; reviewed in [6]). The phenotype of *Kn1* mutants led to the early notion that *KN1* influences the fate of leaf cells. Recent studies have shed light on the normal role of *KN1*. In localization experiments, KN1 is undetectable in leaves, but it is abundant in the apical meristem and the immature axes of vegetative and floral shoots [7]. Also, the dominant *Kn1-N2* mutation has been found to cause ectopic expression, such that *KN1* transcripts and KN1 protein are detected in lateral veins of developing leaves (in addition to the normal accumulation in the meristem), which correlates well with the alterations in leaf cell fate and knot formation associated with *Kn1* mutants [7]. When the *KN1* cDNA is expressed in tobacco under the control of the strong cauliflower mosaic virus (CaMV) 35S promoter, the plants display a reduction in shoot stature with rumpled or lobed leaves and (in some plants) a loss of apical dominance and severe dwarfism with meristematic tissue forming in inappropriate places [8•]. These results indicate that the *KN1* product is associated with indeterminate growth and cell divisions, although the lack of recessive (loss-of-function) alleles of *KN1* prevents a complete understanding of the role of this gene.

In addition to the *Kn1* mutations, other dominant maize mutations affect leaf cell fate, including mutations of the *Rough sheath1* (*RS1*) and *Liguleless* (*Lg3* and *Lg4*) genes [9]. A detailed examination of the *Rs1-0* mutant has shown that it is similar to *Kn1* mutants; each possesses dominant neomorphic mutations that act non cell autonomously to cause a transformation of blade tissue to sheath, although the *Rs1* mutant differs in that it affects the entire lateral dimension of the ligular region of the leaf, causes affected tissue to display attributes of both sheath and auricle, and does not show a dose response [10•]. Although the normal role of *RS1* cannot be determined directly by analyzing the neomorphic mutant phenotype, the *RS1* gene has recently been found to encode a KN1-like homeodomain protein (P Becraft, M Freeling, unpublished data cited in [9,10•]). Coincidentally, the *RS1* gene was one of several maize homeobox genes cloned by members of the Hake laboratory

Abbreviations

CaMV—cauliflower mosaic virus; *GL1—GLABRA1*; *KN1—KNOTTED1*; *RS1—Rough sheath1*; *TTG—TRANSPARENT TESTA-GLABRA*.

[5,11••] by virtue of their sequence similarity to *KN1*. The expression of four of these maize homeobox genes (*KN1*, *RS1*, *KNOX3*, and *KNOX8*) has been examined in detail, and in wild-type plants, transcripts from each gene have been shown to accumulate in shoot meristems and the developing stem, but not in determinate lateral organs such as leaves or floral organs, nor in root meristems [11••]. Overall, the pattern of expression of *KNOX8* is similar to that of *KN1*, whereas the *RS1* and *KNOX3* genes are expressed in only a subset of *KN1*-expressing cells. The expression of these four genes in the vegetative shoot apical meristem appears to predict the site of leaf initiation, as well as portions of the segmentation unit of the shoot ('phytomer') [11••]. The distinct regions of homeobox gene expression are suggestive of roles for the gene products in defining regional identities within the meristem which influence the fate of differentiating cells.

Cloned homeobox genes associated with developmental abnormalities

The role of plant homeobox genes in cell differentiation has also been studied in other plant species. A *KN1*-like homeobox gene isolated from rice (*OSH1*) produces alterations in leaf morphology reminiscent of those in *Kn1* mutants when introduced (in multiple copies) into rice [12•]. When the *OSH1* cDNA is expressed in *Arabidopsis* (under CaMV35S control) or tobacco (under the control of various promoters), plant morphology is altered dramatically, with abnormalities in shoots, leaves, and flowers, and the formation of excess meristematic tissue [12•,13]. The *OSH1* gene appears normally to be expressed in vegetative shoot apices [12•] which, when taken together with the cDNA data, implies that it (like *KN1*) may be a regulator of cell differentiation in/near the shoot meristem.

Using reduced-stringency DNA hybridization, a large number of homeobox genes have been identified from *Arabidopsis*, and one subfamily has been shown to be characterized by a leucine zipper motif adjacent to the homeodomain (denoted HD-Zip proteins) [14–16]. One HD-Zip gene, *HAT4*, may play a general role in plant development. Its transcripts accumulate in all tissues examined, with highest levels in stems and leaves [17•]. Transgenic plants expressing an antisense CaMV35S–*HAT4* gene construct display a general delay in development, whereas plants expressing a CaMV35S–*HAT4* sense construct are tall and develop rapidly [17•]. It is not clear whether cell fates are altered in these plants or whether the only effect is on the rate of cell formation and differentiation. A member of another *Arabidopsis* homeobox gene subfamily has been identified because its product (HAT3.1) binds to a portion of the photoregulated *cab-E* promoter [18]. HAT3.1 lacks the leucine zipper motif found in the HD-Zip proteins and has an amino-terminal region similar to metal-binding domains (denoted the PHD-finger), but no direct evidence for a role in plant development has (yet) been obtained [18].

Control of epidermal cell fate in *Arabidopsis*

The formation of hair-bearing and hairless cells in the shoot and root epidermis of *Arabidopsis* provides a useful model for the study of cell fate specification. In the shoot epidermis, some of the cells differentiate into trichomes (leaf hairs), and genetic studies have shown that two loci, *GLABRA1* (*GL1*) and *TRANSPARENT TESTA-GLABRA* (*TTG*), are required for the specification of a trichome cell. The *GL1* gene encodes a product with similarity to the DNA-binding domain of the *myb* family of transcriptional regulators [19], and it appears to act locally in trichome precursor cells, as evidenced by *in situ* RNA hybridization [20•] and genetic mosaic analyses [21•]. Interestingly, two pieces of evidence indicate that *GL1* is not only required for specifying cell fate, but also to maintain trichome cell differentiation. First, a weak allele of the *gl1* gene (*gl1-2*) which is associated with the loss of 27 amino acids from the carboxyl terminus of the protein generates a small number of trichomes, with some aborted trichomes that cease expanding before reaching full size [22]. Second, *GL1* transcripts persist in the differentiating trichome cells after expression in the surrounding leaf primordia has decreased [20•]. These results imply that *GL1* may be needed to maintain the expression of genes involved in cell morphogenesis.

The other gene required for trichome cell specification, *TTG*, may encode or activate a homolog of the maize *R* gene product [23]. Trichome (and anthocyanin) production is restored to *ttg* mutant plants when a cDNA from the *Lc* gene of the maize *R* family is introduced under the control of the CaMV35S promoter [23]. The *R* gene family members encode proteins with a basic domain containing a helix-loop-helix (HLH) motif, similar to that found in the *myc* class of DNA-binding proteins, and an acidic domain characteristic of transcriptional activators [24,25]. In maize, the *myc*-related *R* gene product and the *myb*-related *C1* product are required to activate transcription of genes encoding flavonoid biosynthetic enzymes (reviewed in [26]). Thus, an attractive possibility is that the *TTG* gene may encode a *myc*-related product that interacts with the *myb*-related *GL1* to activate trichome cell differentiation in the developing shoot epidermis. The *TTG* gene may also play a role in trichome spacing, as the *R*-expressing *Arabidopsis* plants produce excess trichomes over the leaf surface [23]. Trichome spacing is also affected in a newly isolated mutant, *TRIPTYCHON* (*Try*), which exhibits nests or clusters of trichomes on the leaf, implying that the affected gene may normally prevent cells adjacent to a trichome precursor from differentiating in the same manner [21•].

In the root epidermis, cells differentiate to form either root hair cells or hairless cells. In *Arabidopsis*, the fate of immature epidermal cells is related to their position; cells located over an anticlinal wall separating underlying cortical cells differentiate into root hair bearing cells, and the other epidermal cells differentiate into mature hairless cells. Recently, the *TTG* gene has been found to participate in the specification of root epidermal cell fate, as *ttg* mutants produce root-hair cells in all epidermal positions, and *Arabidopsis* expressing the maize *R* gene generate hairless cells in all positions [27]. This indicates that *TTG* normally acts at an early stage in root epidermis differentiation to generate or respond to a position-dependent signal which influences cell fate. This also demonstrates overlap in the genetic control of cell differentiation in the shoot and root epidermis, although, interestingly, alterations in *TTG* action affect the root and shoot epidermis in opposite ways. Recessive *ttg* mutations cause excess root hair production and a lack of trichomes, whereas *R* expression results in excess trichomes and a reduction in root hair formation [23,27]. It may be that the ground state is different in these two tissues, with a hairless cell the ground state for the shoot epidermis and a hair-bearing cell the ground state for the root epidermis.

Genes controlling plant cell morphogenesis

Once cell fate is specified, the differentiating plant cell initiates a program of cell morphogenesis. The complexity of this process is reflected by the finding of at least 18 genes that are required for the morphogenesis of a trichome cell in *Arabidopsis* [21•,28]. The genetic analysis of trichome formation indicates that it is the result of many independent events, rather than a sequential stepwise process, with some of the events being dependent on the cell reaching a certain size [21•]. One of the trichome morphogenesis genes, *GLABRA2* (*GL2*), has been cloned recently [29••]. Plants homozygous for a *gl2* mutation produce trichomes with a laterally expanded shape, indicating a defect in trichome outgrowth [21•,29••]. The product of the *GL2* gene is a novel type of plant homeodomain protein which lacks the leucine zipper motif of the *Arabidopsis* HD-Zip proteins and the PHD-finger motif of the HAT3.1 protein [29••]. The *GL2* transcripts are detected in individual developing trichome cells of the emerging leaf primordia [29••], consistent with the results of genetic mosaic analysis [21•], indicating that *GL2* may activate downstream genes associated with the expansion of trichome cells.

Many investigators interested in plant cell morphogenesis have been attracted recently to the study of the *Arabidopsis* root. Major reasons are its small size and remarkably simple structure [30•,31•]. The *Arabidopsis* root apical meristem is composed of three tiers of putative initials that divide regularly to generate the longitudinal files of cells in the various tissues [31•]. Cell morphogenesis can be analyzed along individual cell files, with 'younger' cells present near the root apex and 'older' (more differentiated) cells further from the apex. Several *Arabidopsis* root morphogenesis mutants have been isolated and characterized recently [32,33•]. Three mutants display excessive cell expansion that is limited primarily to a single cell layer in each mutant (*cobra* mutant—expanded epidermis, *sabre* mutant—expanded cortex, and *lion's tail* mutant—expanded stele), indicating that the affected genes regulate cell expansion in a cell type specific manner [33•]. Mutants in which morphogenesis of *Arabidopsis* root hair cells is affected have also been identified and subdivided into those in which either root hair emergence or root hair tip growth is affected [34]. One of these mutants has been found to display abnormal pollen tube growth as well, which suggests that the affected gene (*TIP1*) is required for the morphogenesis of both of these tip-growing cell types [35]. Root hair morphogenesis mutants have also been identified recently in maize [36].

Conclusions

Molecular genetic approaches have led to new insights into the control of plant cell differentiation. One of the themes that is beginning to emerge from these studies is that plant cell differentiation appears to be governed, at least in part, by regulatory molecules with putative DNA-binding domains related to those present in some vertebrate and *Drosophila* proteins (e.g. homeodomains, Myb, and Myc domains). In these instances, it will be important to determine how the distribution of these regulatory molecules is controlled during plant development, and what the downstream targets of these regulatory molecules might be. There are many possible types of such downstream targets. Because cell position is known to be important in specifying fate, one class of targets may be genes encoding components of the cell wall (plant extracellular matrix) involved in cell–cell communication. In vertebrates and *Drosophila*, cell adhesion and substrate adhesion molecules have been implicated recently as targets of some of the homeodomain proteins [37], and the influence of the cell wall in algal cell fate has recently been documented during early development in *Fucus* [38•]. It is also possible that a class of downstream target genes may tie into pathways of plant hormone action, as hormones can markedly affect plant cell morphogenesis. Finally, another class of targets may be genes encoding regulators of the plant cytoskeleton, as microtubule and microfilament systems reorganize during cell differentiation and influence the shape of plant cells.

Acknowledgement

I thank members of the laboratory and colleagues in the field for helpful suggestions and for sharing unpublished data and manuscripts.

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