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The mechanics of cell fate determination in petals

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The epidermal cells of petals of many species are specialized, having a pronounced conical shape. A transcription factor, MIXTA, is required for the formation of conical cells in *Antirrhinum majus*; in shoot epidermal cells of several species, expression of this gene is necessary and sufficient to promote conical cell formation. Ectopic expression has also shown *MIXTA* to be able to promote the formation of multicellular trichomes, indicating that conical cells and multicellular trichomes share elements of a common developmental pathway. Formation of conical cells or trichomes is also mutually exclusive with stomatal formation. In *Antirrhinum*, *MIXTA* normally only promotes conical cell formation on the inner epidermal layer of the petals. Its restricted action in cell fate determination results from its specific expression pattern. Expression of *MIXTA*, in turn, requires the activity of B-function genes, and biochemical evidence suggests that the products of *DEFICIENS*, *GLOBOSA* and *SEPALLATA*-related genes directly activate *MIXTA* expression late in petal development, after the completion of cell division in the petal epidermis.

A *MIXTA*-like gene, *AmMYBML1*, is also expressed in petals. *AmMYBML1* expression is high early in petal development. This gene may direct the formation of trichomes in petals.

In specifying the fates of different cell types in petals, regulatory genes like *MIXTA* may have been duplicated. Changes in the timing and spatial localization of expression then provides similar regulatory genes which specify different cell fates.

Keywords: petal; epidermis; conical cell; trichome; stomata; cell division

1. INTRODUCTION

The success of many flowering plants is associated with their ability to attract pollinators, usually insect pollinators. Petals and sepals have been developed to attract insects and to provide a landing platform during pollination (Martin 2000). Floral reproductive organs are often specialized to deliver pollen to, and to receive it from, the bodies of pollinators, but petals may also be specialized, often by developing long hairs, to facilitate pollen retrieval. In addition, petals are specialized to provide a visual attraction; they are brightly coloured over an expanded surface, often with bold and striking pigmentation patterns. In over 80% of angiosperm species examined, the epidermal cells of the petal also have a specialized form (Kay *et al.* 1981; Kay 1988). This form is usually conical (sometimes referred to as conical-papillate), with a thick cuticle which may be moulded into exquisite patterns by cuticular striations (figure 1). Occasionally, petal epidermal cells have a convex base which protrudes into the mesophyll below. These latter cells have been called 'reverse-papillate' (Kay *et al.* 1981; Kay 1988). The function of these specialized cell shapes is believed to be associated with the effects of conical cells on incident light (Kay *et al.* 1981). Conical form will tend to reflect light (especially light at a low angles of incidence) into the epidermal cells where it will be absorbed by the pigments (contained within the vacuoles or plastids of the cells). Hence, less white light will be reflected from corrugated compared with flat surfaces and the petals will appear more darkly pigmented. The properties of conical cells in dispersing light reflected back from the underlying mesophyll also give petals a brilliance and sheen to their appearance (Kay *et al.* 1981). Epidermal sheets carrying specialized conical cells tend to be free from epidermal hairs (trichomes) and relatively free from stomata, which are common in other epidermal surfaces in the shoot.

2. *MIXTA* AND THE CONTROL OF CONICAL CELL FATE

One gene, *MIXTA*, from *Antirrhinum majus*, has been shown to be necessary for conical cell formation (Noda *et al.* 1994). Mutations in the *MIXTA* gene give rise to flat epidermal cells instead of conical ones. The flat cells of the mixta mutant remain fully pigmented. Although in petal tissue they appear paler than conical cells, this is a facet of their shape (and light-reflective properties), because when the cell walls are removed, flat and conical cells appear equally pigmented. Although *MIXTA* activity is required for conical shape, it is not required for the for-

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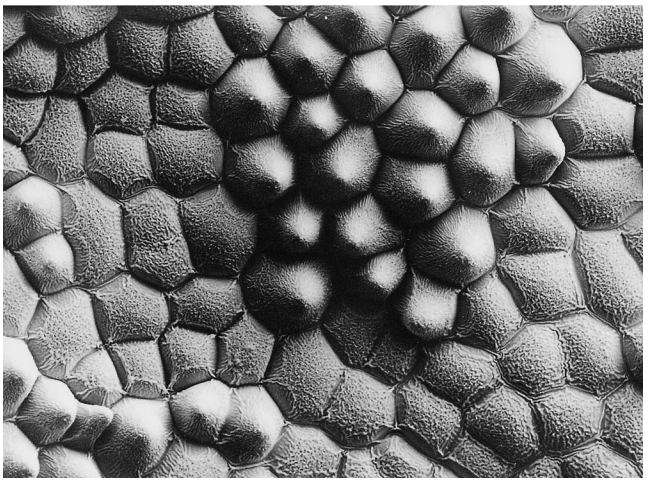

Figure 1. SEM of *Antirrhinum* petal epidermis from a plant mutated at the *MIXTA* locus because of a transposon insertion. A background of mutant, flat, epidermal cells is present due to the transposon insertion blocking the action of the *MIXTA* gene. Where the transposon has excised somatically from the locus, *MIXTA* gene function has been restored and sectors of wild-type, conical cells are seen. Comparison of these two cell types shows clearly the action of the *MIXTA* gene. It is required for the development of conical shape, but it is not required for the production of cuticular striations which are present on both conical and flat cells.

mation of the cuticular striations which are also produced in mutant cells (Noda *et al.* 1994).

MIXTA encodes a transcription factor of the R2R3 MYB family, which is large and diverse in plants. When *MIXTA* is placed under the control of a strong constitutive promoter (such as the 35S promoter of CaMV), it will drive the ectopic production of conical shape in shoot epidermal cells that are normally flat. This was first demonstrated in tobacco plants, where *MIXTA* will convert almost every leaf epidermal cell into a conical cell, promoting the formation of a single central outgrowth on the cuticularized surface of the cell (Glover *et al.* 1998; Payne *et al.* 1998). *MIXTA* is not expressed in *Antirrhinum* leaves and, by analogy, the functionally homologous gene is not expressed in tobacco leaves. Therefore *MIXTA* is not only necessary but also sufficient to induce cell shape change in epidermal cells.

3. *MIXTA* AND THE FORMATION OF MULTICELLULAR TRICHOMES

MIXTA does not induce the formation only of conical cells when expressed ectopically, however. In some lines of transgenic tobacco, and in some specific tissues, *MIXTA* promotes the formation of multicellular trichomes. There are two principal types of trichome produced on tobacco plant leaves (Glover *et al.* 1998). *MIXTA* will promote the formation of multicellular, glandular trichomes, but has no impact on the formation of the hydathode type of trichome that exudes nicotine. The conclusion from this observation is that although *MIXTA* is normally associated with the formation of conical cells only (in petals of *Antirrhinum*), it can promote the formation of both conical cells and multicellular glandular trichomes when

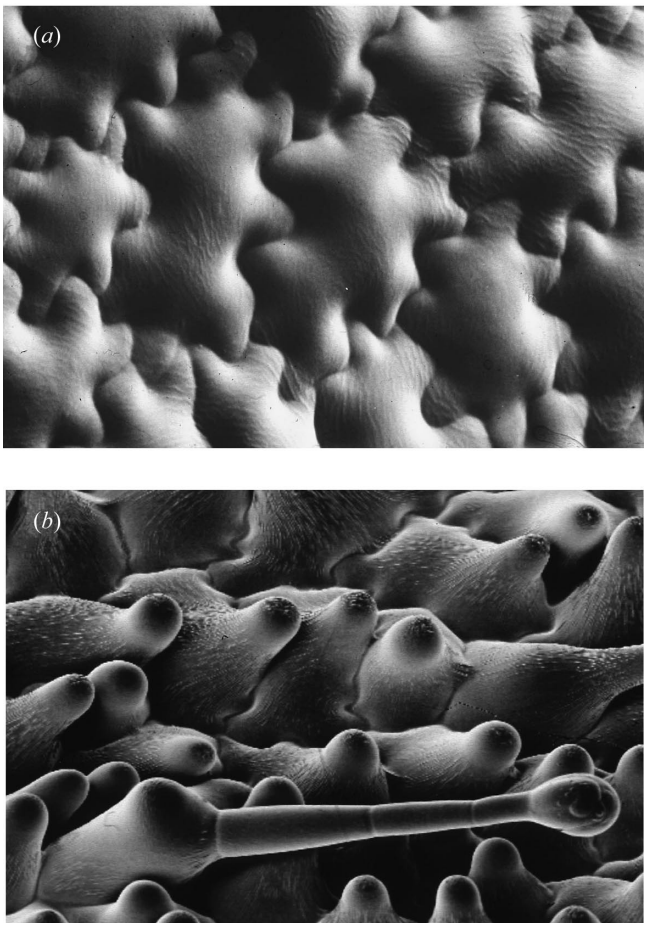

Figure 2. SEMs of leaf epidermal cells from *Antirrhinum majus*. (*a*) Wild-type leaf cells which form shapes similar to pieces of a jigsaw puzzle. (*b*) Surface of a leaf from a transgenic *Antirrhinum* plant expressing *MIXTA* ectopically under the control of the CaMV 35S promoter. The action of the *MIXTA* gene has converted most cells to a conical shape but some have formed multicellular trichomes.

expressed ectopically. Therefore, the developmental pathways leading to conical cells and multicellular, glandular trichomes in tobacco must share a common route. This same result has now been demonstrated for *MIXTA* in *A. majus*. Ectopic expression of *MIXTA* under control of the CaMV 35S promoter stimulates the production of conical cells and multicellular trichomes on *Antirrhinum* leaves (figure 2).

4. CELL FATE MAY BE DETERMINED BY THE RELATIVE TIMING OF *MIXTA* EXPRESSION

Why does *MIXTA* sometimes promote the formation of conical cells and sometimes promote the formation of trichomes? A clue to the distinction between these two cellular fates has come from the analysis of the timing of *MIXTA* expression in transgenic tobacco lines. In those lines producing predominantly conical cells on their leaves, ectopic *MIXTA* expression peaks relatively late during leaf development, whereas in plants producing predominantly multicellular trichomes on their leaves, *MIXTA* expression peaks relatively early. Therefore, the distinction between cell fate in transgenic tobacco may

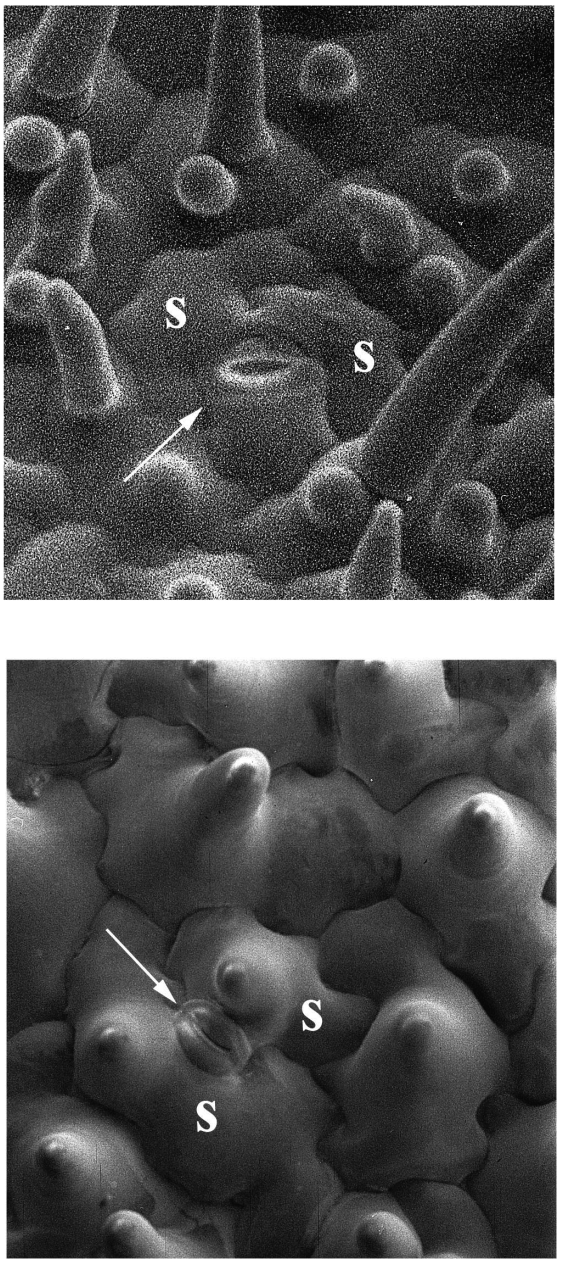

Figure 3. Effect of the 35S–*MIXTA* construct on stomatal development on leaves. SEMs of (*a*) tobacco and (*b*) *Antirrhinum*. In tobacco, outgrowths do not form on the subsidiary cells (S) of the stomatal meristemoid complex, nor on the guard cells (arrowed). In *Antirrhinum*, the cells adjacent to the guard cells (arrowed), which are presumably the subsidiary cells (S), do form outgrowths. These data suggest that the decision to form a stoma or an outgrowth (fates which appear to be mutually exclusive) are taken at different stages of epidermal development in the two species.

depend on the timing of *MIXTA* expression. The timing of expression is probably critical relative to the progression of the cell cycle. In most developing organs of the shoot, development involves a period of cell division followed by a period of extensive cell expansion. This applies to leaves and petals. Multicellular trichomes require cell division following cellular outgrowth in order to form. By contrast, conical cells only start to form after the completion of cell division in petal cells (Noda *et al.* 1994). Therefore, the timing of *MIXTA* expression relative to the progression of the division phase in epidermal cells will determine whether trichomes or conical cells result from its activity. By way of confirmation of these ideas, *MIXTA* promotes only the formation of conical cells in *Antirrhinum* petals where expression of the gene is restricted to the inner petal epidermal layer, to a time after the completion of cell division, as indicated by expression of a predictive marker of the cell cycle, cyclin D3b (Glover *et al.* 1998).

5. THE CONTROL OF *MIXTA* EXPRESSION DETERMINES CONICAL CELL FATE

If the timing and location of expression of the *MIXTA* gene is central to the determination of petal epidermal cell fate, the key to petal cellular specialization is the process by which *MIXTA* expression is controlled. Floral homeotic genes are required to determine the identity of floral organs (Coen & Meyerowitz 1991; Weigel & Meyerowitz 1994), but there is considerable evidence that some may also serve a later function in cellular specialization. In *Antirrhinum* petals, identity requires the interaction of A and B functions. Genes involved in determining A function in *Antirrhinum* have not yet been unambiguously identified, but two genes, *DEFICIENS* and *GLOBOSA*, make up the B function (Sommer *et al.* 1990; Trobner *et al.* 1992). Transposon insertions in some *deficiens* alleles inactivate the gene and cause conversion of the second-whorl floral organs to sepals. However, the transposon may excise somatically to restore gene function. Excisions late in second-whorl organ development cause the conversion of flat green sepal-type epidermal cells to conical, pigmented petal-like cells (Coen & Carpenter 1992). This demonstrates a late function of *DEFICIENS* in promoting petal cell fate and establishes that the relationship between *DEFICIENS* activity and *MIXTA* activity must be close. *DEFICIENS* and *GLOBOSA* encode MADS-box transcription factors. It seems likely that they act together with the products of one or more of the intermediate MADS genes (*DEFH72*, *DEFH84*, *DEF200*), to form a ternary complex to activate *MIXTA* expression (Gutierrez-Cortines & Davies 2000). In support of this idea, the *MIXTA* promoter region contains several good CArG boxes, the cognate binding sites for MADS-box proteins, and *DEFICIENS*, *DEFH72* and *DEFH200* are all expressed late in petal ontogeny, particularly in the inner epidermis where *MIXTA* expression is localized (Davies *et al.* 1996). Through this short transcriptional hierarchy, the chain of command from organ initiation to cellular specialization is realized.

6. *MIXTA* MAY INFLUENCE THE SPECIFICATION OF OTHER CELL TYPES

In transgenic tobacco lines ectopically expressing *MIXTA*, the frequency of stomatal complexes on both leaf surfaces is dramatically reduced (Glover *et al.* 1998). This result suggests that commitment to form cellular outgrowths (either conical cells or trichomes) is mutually exclusive with the formation of stomata. Examination of transgenic tobacco lines in greater detail reveals that the decision between an outgrowth and a stoma probably occurs at an earlier stage than guard-cell differentiation. Even in those transgenic lines where outgrowth density

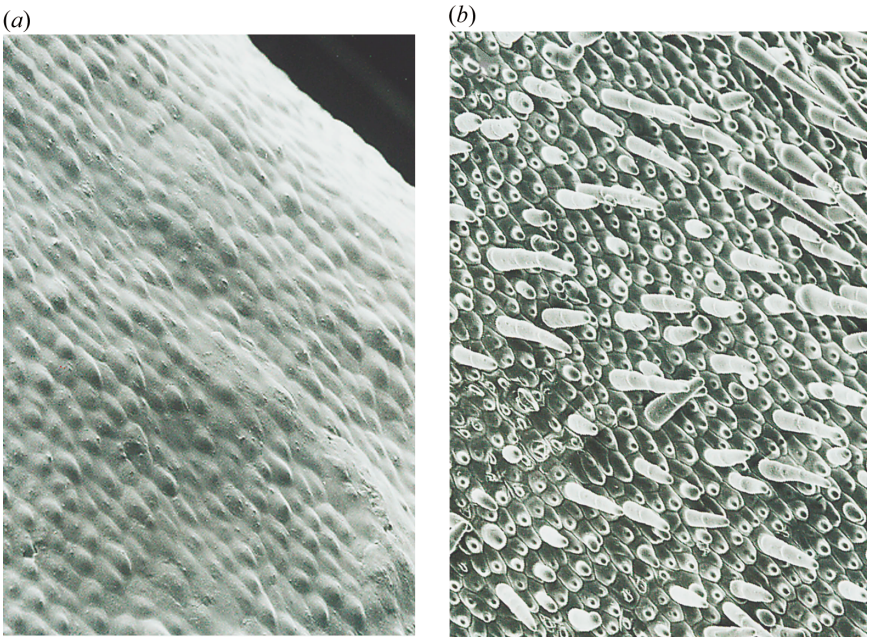

Figure 4. Effect of ectopic expression of *AmMYBML1* on cell morphogenesis in tobacco. SEMs of (*a*) untransformed (control) carpel tissue from tobacco and (*b*) carpel tissue from tobacco expressing *AmMYBML1* under the control of the CaMV 35S promoter. *AmMYBML1* induces the production of conical cells and multicellular trichomes.

is so high that almost every leaf epidermal cell shows an outgrowth, a few stomata are formed. In these cases, not only the guard cells, but also the two surrounding subsidiary cells, are free from outgrowths (figure 3*a*). This suggests that the decision in tobacco is taken at the stage of stomatal meristemoid initiation, and if the route of stomatal development is decided, cells on the route to stoma formation will not develop outgrowths. Generally, during leaf ontogeny trichomes form early and stomata later. Therefore, in transgenic tobacco lines, if most epidermal cells commit early to form outgrowths, then fewer stomata will form.

Interestingly, in *Antirrhinum* leaves, the decision between outgrowth and stoma appears to occur later. Although guard-cell numbers are reduced in *Antirrhinum* lines expressing 35S–*MIXTA* and outgrowths do not form on the guard cells themselves, the subsidiary cells, or cells adjacent to the guard cells, do exhibit outgrowths (figure 3*b*). This is quite a striking difference in development between two species that are considered relatively closely related and share many common developmental features.

The mutual exclusivity between stomatal formation and cellular outgrowths may explain the lack of stomata amongst the inner epidermal cells of petals—all the cells undertake an earlier commitment to conical cell formation.

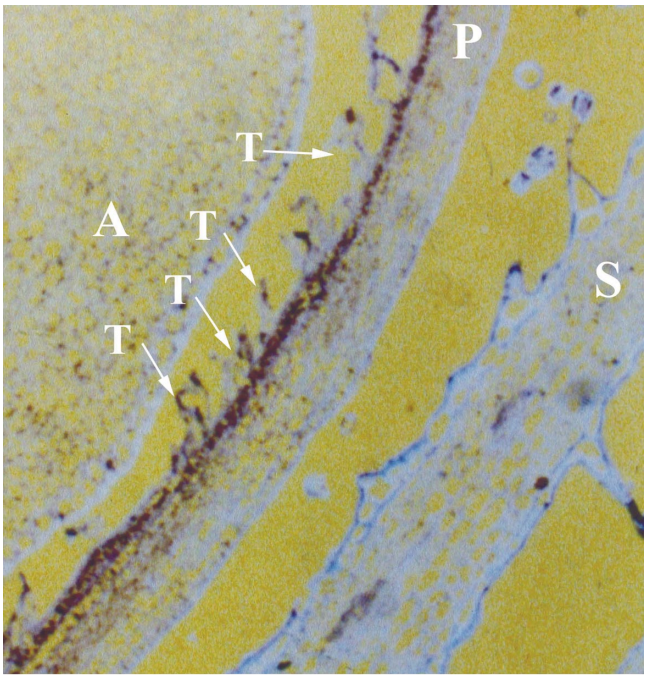

Figure 5. *In situ* hybridization of an *AmMYBML1* antisense probe to floral tissues of *Antirrhinum*. The brown staining shows where the gene is expressed (A, anther head; P, petal; S, sepal; T, trichome). The strongest labelling of trichomes was observed in the corolla tube.

7. *MIXTA*-LIKE GENES MAY CONTROL OTHER FORMS OF CELL SPECIALIZATION IN PETALS

Although *MIXTA* can promote multicellular trichome formation in *Antirrhinum*, and there are trichomes in the corolla tube and on the outer petal epidermis of *Antirrhinum* flowers, the *mixta* mutation affects only the formation of conical cells. This may be due to the timing and location of *MIXTA* expression. Our understanding of *MIXTA* function suggests that there may be other *MIXTA*-like genes that control trichome formation in flowers. One such gene is *AmMYBML1*, which encodes a protein with a DNA-binding domain almost identical to that of MIXTA (Glover *et al.* 1998). Ectopic expression of *AmMYBML1* in tobacco demonstrates that it can promote trichome formation in floral tissues (figure 4). The gene is expressed much earlier in petal ontogeny than *MIXTA*, before the cessation of cell division. We have also

detected *AmMYBML1* expression in developing trichomes (figure 5). This suggests that cellular specialization in *Antirrhinum* petals has developed through gene duplication. Two genes with very similar structures, *MIXTA* and *AmMYBML1*, can promote very similar morphological pathways in epidermal cells. The specific nature of the fate determined by these genes resides in the way that the genes are expressed: the spatial location and the timing of expression relative to cell division and other morphological influences. We are currently seeking an insertion mutation of *AmMYBML1* to confirm its role in trichome formation.

There is evidence to suggest that changing the expression of a regulatory gene may be used to switch from trichome to conical cell production. For example, amongst *Polygonum* species, some produce conical cells on their leaves (often an adaptation to shade growth), whereas others produce trichomes. Trichome differentiation normally occurs early in leaf development, whereas commitment to conical cell formation occurs relatively late in related species. These observations suggest that a complex change in epidermal cell type may be achieved through a relatively simple change in the timing of expression of a common regulatory gene (Lersten & Curtis 1992).

8. ADAPTIVE SIGNIFICANCE OF SPECIALIZED PETAL EPIDERMAL CELLS

It is clear that conical cell shape can influence the perceived intensity of petal pigmentation. Conical shape is also occasionally used in leaves of shade-adapted plants to enhance their capture of light for photosynthesis (for example, in *Oxalis*). Experiments to test the efficacy of conical shape of petals on bee pollinators of *Antirrhinum* have indeed revealed that this is a significant influence on choice. However, the preference of bees for conical cells is exhibited whether or not the flowers are pigmented (Glover & Martin 1998). Petals that produce no flavonoids at all are preferred if they have conical cells over those that have flat cells. This suggests that conical cells offer attractions in addition to enhanced pigmentation to prospective pollinators. Recently it has been demonstrated that fragrance in *Antirrhinum* petals is produced via enzymes expressed in the conical cells of the epidermis (Kolosova *et al.* 2001). It may well be that conical cell shape facilitates fragrance emission and acts as an added attraction of specialized cell morphology to prospective pollinators.

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GLOSSARY

CaMV: cauliflower mosaic virus
SEM: scanning electron micrograph