

REVIEW ARTICLE

Differentiation in plant epidermal cells

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Abstract

The plant epidermis is a multifunctional tissue playing important roles in water relations, defence and pollinator attraction. This range of functions is performed by a number of different types of specialized cells, which differentiate from the early undifferentiated epidermis in adaptively significant patterns and frequencies. These various cells show different degrees of morphological specialization, but there is evidence to suggest that even the less specialized cell types may require certain signals to ensure their correct differentiation and patterning. Epidermal cells may potentially adopt certain fates through a cell lineage based mechanism or a cell interaction mechanism. Work on stomatal development has focused on the cell lineage mechanism and work on trichome differentiation has focused on the cell interaction model. Recent work on the *Arabidopsis* trichome suggests that interactions between neighbouring cells reinforce initial differences, possibly in levels of gene expression or cell cycle stage, to commit cells to different developmental programmes. In this review these mechanisms are explored in a number of specialized cell types and the further interactions between different developmental programmes are analysed. It is in these interactions between differentiating cells adopting different cell fates that the key to the patterning of a multifunctional tissue must lie.

Key words: Epidermis, differentiation, trichome, stomata, development.

Introduction

In the words of the old song, 'You gotta have skin—it keeps your insides in'. The epidermis of a plant does indeed keep its insides in, but it does a great deal more besides and it is in the multifunctionality of the plant

epidermis that the root of its developmental complexity lies. The interlocking epidermal cells of a plant provide mechanical strength while still allowing growth and flexibility. They provide a barrier which is almost totally impermeable to water, and yet can allow water to leave (and gas to enter) as effectively as if there were no epidermis at all. Certain epidermal cells protect the plant from herbivores while others attract the predators of those herbivores and still others are involved in attracting pollinating animals. In many organs these different functions are fulfilled simultaneously by the activities of different specialized cells, and for those cells to function effectively they must be distributed in a non-random pattern. The formation of this pattern has fascinated developmental biologists for many years, and the use of molecular genetic techniques focusing on individual specialized cell types has recently begun to provide information concerning the mechanism of this patterning. However, it has also become clear that the diversity of epidermal functions requires a balancing of the different cell types. It is thus apparent that their pattern-forming mechanisms have not evolved in isolation, but operate through a complex web of positive and negative interactions. This review will focus on the development of each of the specialized cells of the aerial parts of the plant and the interactions of each pattern-forming mechanism with those around it.

Pattern formation in any two-dimensional field of developing cells may potentially occur through either of two methods. The cell lineage mechanism of pattern formation relies on an ordered system of cell divisions, usually asymmetric, which autonomously divides cells into different categories which may correspond to different cell fates. Thus the terminal differentiated state of a cell is a predictable result of its status in a lineage of cells occupying a certain position. It is through this mechanism that the primary basic patterning of *Arabidopsis thaliana* stomatal complexes is determined (Yang and Sack, 1995;

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Larkin *et al.*, 1997; Serna and Fenoll, 1997). Alternatively, interactions between cells may reinforce any tiny initial differences to produce significantly different cells which may then adopt different fates. This cell interaction mechanism does not rely on cell lineage, and so cells contributing to the development of a single structure may be clonally unrelated. The patterning of *Arabidopsis* trichomes is controlled through a cell interaction mechanism, with the subsidiary cells of the trichome derived from different lineages to that of the trichome cell itself (Larkin *et al.*, 1996). The classic, although non-epidermal, example of this method of pattern formation is the recruitment of individual parenchyma cells to xylem vessels after wounding causes discontinuity in the primary xylem. The cell interaction mechanism may also rely on progress through the cell cycle to distinguish between cells, but it is not dependent on actual cell division in the way that the cell lineage mechanism is.

Pavement cells

The most frequently occurring cell type in the epidermal layers of all plant organs is the pavement cell. Pavement cells are relatively unspecialized morphologically, with no protrusions or gas exchange abilities. They fulfil the basic function of protecting the tissue layers underneath ('keeping the insides in') and ensure that morphologically more specialized cells are spaced out correctly. The pavement cells of different organs frequently adopt rather different shapes, possibly as a result of the different functions and growth forms of the organs. In dicot leaves pavement cells are usually shaped like the interlocking pieces of a jigsaw puzzle (Fig. 1A). This form gives the leaf a measure of mechanical strength which is important in the light of the large air spaces necessary in the underlying mesophyll layers to ensure rapid diffusion of carbon dioxide for photosynthesis. The jigsaw puzzle shape of leaf pavement cells also reflects the growth of a leaf which requires cell expansion in all directions within the plane of the lamina. The pavement cells of the stem and other elongated organs are often more rectangular in shape with their long axis parallel to the direction of organ expansion. However, these differences in basal shape and direction of expansion are the only distinctive morphological characteristics of a largely unspecialized cell type.

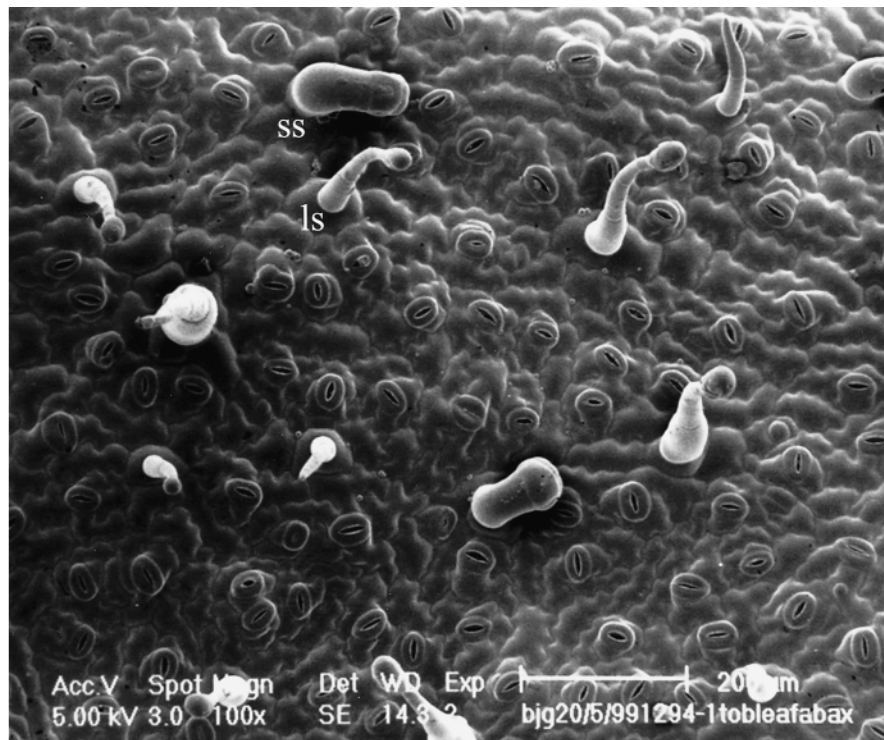
As a result of their relatively unspecialized morphology pavement cells have traditionally been regarded as rather insignificant in the patterning of the epidermis, filling a default role rather than arising as a result of an active patterning process. It is likely that this view arises partly from an intuitive bias towards positive rather than negative regulators in plant development. A mutation which results in reduced trichome number in the epidermis is usually interpreted as the loss of function of a gene whose product is a positive regulator of trichome cell differenti-

ation. However, such a mutation could equally well result from the loss of function of a gene whose product acts as a negative regulator of pavement cell differentiation, allowing more cells to adopt the default trichome state in the wild-type situation. While it is unlikely that many of the known trichome-regulating genes do behave in this way, it is possible that pavement cell development is not simply the default process it is usually assumed to be but requires the activity of specific genes. Genetic evidence from mutations affecting root hair formation has certainly suggested that atrichoblasts, the root epidermal equivalent of shoot pavement cells, are not the default state in the root epidermis (Galway *et al.*, 1994; Masucci *et al.*, 1996). The *Arabidopsis* genes *TTG* (*TRANSPARENT TESTA*, *GLABRA*) and *GL2* (*GLABRA 2*) have been demonstrated to be negative regulators of root hair formation and positive regulators of atrichoblast formation. *GL2* is expressed only in atrichoblasts, and the loss of function *gl2* mutant develops ectopic root hairs in non-hair files (Rerie *et al.*, 1994; Masucci *et al.*, 1996). The atrichoblast can therefore be viewed as the specialized cell type and the root hair cell as the default state (Galway *et al.*, 1994; Masucci *et al.*, 1996). Of course, it is also possible that there is no default state and that both cell types, and indeed all cell types in the more complex aerial epidermis, represent differently differentiated forms.

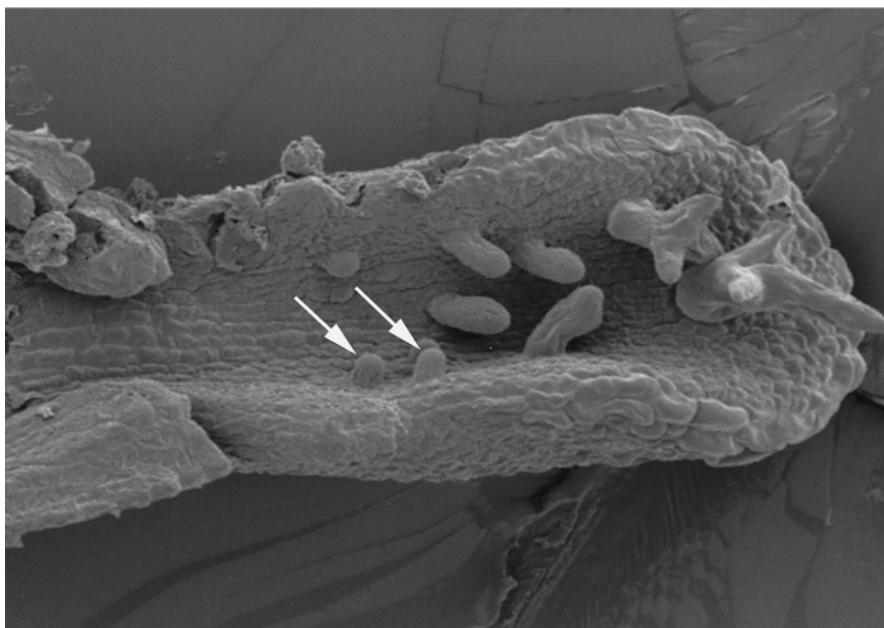
Stomatal guard cells

Stomatal guard cells are essential to keep one particular component inside the plant—water. However, they must also allow the gaseous exchange essential for photosynthetic activity. It is clear from theoretical considerations that the spacing of stomata should not be random. The optimal spatial pattern of stomata is dependent on the size of the cells and the size and shape of the air-space below. Through precise control of all these parameters it is possible for an epidermis to be almost as permeable to gases as no epidermis at all, but with the ability to become more or less impermeable to water vapour when environmental conditions demand it. It is also clear from observations of stomatal patterning that the process is not random. If guard cell pairs were distributed randomly throughout the epidermis then clustering would be observed at predictable frequencies. The actual frequency of guard cell clustering is virtually zero, indicating that a pattern generating mechanism must be involved (Larkin *et al.*, 1997).

The essential nature of stomatal guard cells has limited the extent to which mutations in their patterning and development have been identified. This has resulted in a more cellular than genetic understanding of the ways in which their pattern is determined. However, it is clear that a cell lineage-dependent mechanism is the primary force driving stomatal separation, at least in some dicots.



A



B

Fig. 1. Patterning of differentiated epidermal cells. (A) Abaxial leaf epidermis of tobacco showing four types of cells—pavement cells, stomatal guard cells, long-stalked trichomes (ls) and broader short-stalked trichomes (ss). The guard cells and trichomes are regularly spaced with regard to each other, with no pair of trichomes or stomata or trichome and stoma adjacent to each other. (B) Adaxial epidermis of young *Arabidopsis* leaf. The trichomes develop in a wave down the leaf, with even the very youngest trichomes (arrowed) separated by several pavement cells.

The separation of guard cell pairs from other guard cell pairs is achieved through a highly regulated series of asymmetric cell divisions. These place the two guard cells at the centre of a stomatal complex composed of three further cells, the subsidiary cells, which may serve a role in ion channel-mediated opening and closing of the stomatal pore. As a result of the development of these surrounding subsidiary cells the guard cell pair are always separated from other stomatal guard cells (Fig. 1A). This observed system of highly conserved cell divisions is, in principle, sufficient to account for the precisely regulated patterning of stomatal complexes, although it is possible that more complex mechanisms play a secondary role (Yang and Sack, 1995).

The series of cell divisions which differentiate the guard cells and the stomatal subsidiary cells occurs late in epidermal development, often after most cytokinesis has finished and the pavement cells are undergoing cell expansion. Two *Arabidopsis* mutants with lesions in stomatal patterning have been described and it is likely that the phenotypic consequences of both lesions are affected by the length and timing of the developmental window during which stomatal initials form. The *too many mouths* (*tmm*) mutant produces excess numbers of sometimes clustered stomata on some organs, but reduced numbers of stomata on other organs, notably the cauline leaves. The gene product is believed to be involved in determining precursor cell number and in regulating the division which generates guard mother cells from the dividing complex and ensures appropriate spacing of stomata (Yang and Sack, 1995; Geisler *et al.*, 1998). The *fourlips* mutant also generates clustered guard cells, as a result of lesion in a gene believed to regulate part of the ordered series of cell divisions (Yang and Sack, 1995). No *Arabidopsis* mutants with reduced numbers of stomata overall have yet been described.

While it is clear that cell lineage determines the primary pattern of stomatal guard cells within an epidermis there is also a large body of evidence to suggest that this pre-pattern is modified by interactions between developing stomatal complexes (inter-stomatal interactions), interactions between stomata and other epidermal cells (inter-cell interactions) and by environmental inputs. In the monocot *Tradescantia* spp. some stomatal initials arrest later in development, apparently in response to signals from neighbouring initials. This suggests that lateral inhibition operating through cell interactions (inter-stomatal interaction) also plays a role in stomatal patterning. As many as 10% of the stomatal complexes produced by the initial pre-patterning mechanism fail to complete their development (Boetsch *et al.*, 1995). Interactions between stomatal complexes and other types of epidermal cells, notably trichomes, are usually competitive. Ectopic expression of the *MIXTA* gene from *Antirrhinum majus* in tobacco results in the formation of

excess numbers of multicellular trichomes in the leaf epidermis and a concomitant reduction in stomatal density. In the adaxial epidermis stomatal density was reduced up to 40-fold, while in the abaxial epidermis, where the majority of photosynthetic gas exchange occurs, stomatal density was unaffected by the increase in trichome numbers (Glover *et al.*, 1998). The *Woolly* mutant of tomato also develops excess trichome numbers and shows a corresponding reduction in stomatal density (BJ Glover, unpublished data). It is not clear whether this phenomenon represents lateral inhibition of developing stomatal initials by trichomes or a simple competition between the two developmental programmes for cells to recruit. However, it clearly reflects the importance of intercellular interactions in determining stomatal pattern. Because the frequency of developing stomatal initials is an adaptation to maximize favourable water and gas exchange regimes it is also under environmental control, with ethylene and cytokinin mediating environmental cues and affecting stomatal density (Boetsch *et al.*, 1996; Serna and Fenoll, 1997; Wang *et al.*, 1997).

It has recently been demonstrated that stomatal distribution on the hypocotyl is governed by the same patterning process as governs root hair distribution, indicating a developmental link between root and shoot epidermis. It has been shown that, on the hypocotyl, guard cells only developed on the cell files corresponding to the root epidermal cell files capable of generating root hairs, that is in those cell files overlying a cortical anticlinal cell wall (Berger *et al.*, 1998). This was the first direct demonstration that *Arabidopsis* stomatal development may be linked to the development of other cell fates. It was further shown that hypocotyl stomatal patterning is disrupted in both the *ttg* and *gl2* mutants, with ectopic stomata arising in other cell files, mirroring the effects of these two mutations on root hair patterning (Berger *et al.*, 1998). This correlation between root hair and stomatal distribution is specific to the hypocotyl, and is not seen on any other aerial organ, reflecting the common derivation of the two structures from the basal pole of the embryo (Scheres *et al.*, 1994).

Trichomes: *Arabidopsis*

The *Arabidopsis* trichome is the best studied of the epidermal cell types and has been extensively dissected by molecular genetic methods (Hulskamp *et al.*, 1994). It is a large single cell 200–300 µm in length with three branches, an elaborate cuticle and a suite of socket cells. In common with the trichomes of other species it is believed to play a role in protecting the plant from predators and the disease-causing organisms they carry. While many other plants carry trichomes which trap or poison predators the *Arabidopsis* trichome acts as a simple barrier on the leaf surface. The *Arabidopsis* trichome has

an enlarged nucleus and a ploidy level of 16C or 32C. During *Arabidopsis* trichome development the process of DNA synthesis is uncoupled from the process of cytokinesis. The trichome cell differentiates while epidermal pavement cells are still dividing, and it synthesizes more DNA at the same rate as they do, but cell division is arrested. After three rounds of endoreduplication, the cell expands outwards from the leaf surface as a single cone. This is followed by another round of endoreduplication after which branches are initiated (Oppenheimer, 1997).

The pattern of trichome development in the *Arabidopsis* leaf epidermis is not random, as demonstrated by the statistically improbable frequency of trichome clusters. However, in contrast to stomatal patterning, the spacing of trichomes does not appear to rely on a cell lineage-based pattern-generating mechanism. Trichome socket cells and the trichome cell itself are not necessarily clonally related (Larkin *et al.*, 1996) indicating that there is no ordered cell division system to provide intervening cells between trichomes. Further evidence against the cell lineage theory was recently provided (Schnittger *et al.*, 1999) when it was demonstrated that the cells within the clusters of trichomes which form in the *triptychon* (*try*) mutant line need also not be clonally related. It is therefore likely that interactions between developing epidermal cells determine which cells become committed to trichome development. These cells may then recruit a set of socket cells and inhibit neighbouring cells from adopting the trichome fate.

At least three genes, *TTG*, *GL1* (*GLABROUS 1*) and *TRY*, are involved in the patterning of *Arabidopsis* trichomes. *TTG* encodes a WD40 repeat protein (Walker *et al.*, 1999), *GL1* encodes a MYB transcription factor (Oppenheimer *et al.*, 1991) and *TRY* has not yet been cloned. *TTG* and *GL1* are necessary for the initial enlargement of the nucleus, and possibly for the uncoupling of DNA synthesis from cytokinesis. *TRY* is required for lateral inhibition of neighbouring cells and therefore the correct spacing of trichomes. *GL1* is initially expressed in all epidermal cells, but is later restricted to developing trichomes. By the time developing trichome cells are first visible they are already separated by three or four epidermal cells (Fig. 1B; Hulskamp *et al.*, 1994; Larkin *et al.*, 1996), indicating that patterning decisions are made before any morphological changes occur. It is not currently known how the decision as to which cells should continue to express *GL1* and thus differentiate into trichomes is made. It is possible that initial undetectable differences in expression levels play a role. It is also tempting, particularly in light of the wound xylem generation model (Dodds, 1981), to implicate the cell cycle and suggest that the initial decision may be a result of the stage in the cell cycle of an epidermal cell when *TTG* and *GL1* expression first occurs. Progress through the cell

cycle non-synchronously provides a mechanism to differentiate between neighbouring cells in a sheet of tissue and may also be associated with differential expression and activity of receptor molecules.

A model based on genetic analysis has recently been proposed to explain how events proceed to commit individual cells further to trichome differentiation while inhibiting neighbouring cells after loss of the initial balance through unknown mechanisms (Schnittger *et al.*, 1999). The model proposes that *TTG* and *GL1*, and possibly another factor analogous to the R protein of maize, positively regulate each other's activity in a feedback loop. The activity of these proteins within a cell is necessary for trichome differentiation. The activity of this feedback loop also activates the *TRY* protein, which acts over longer distances to inhibit the same positive feedback loop, but in different cells. Thus, once any one cell has become biased towards the trichome pathway relative to neighbouring cells it will have greater *TTG/GL1/R* activity and thus be more likely to differentiate. The increase in the activity of this loop will increase *TRY* activity which will in turn suppress the loop in neighbouring cells, inhibiting trichome differentiation. The inhibitory activity of the *TRY* protein is clearly only effective over distances of a few cells, as trichome differentiation may resume 3 to 4 cells away (Schnittger *et al.*, 1999). This model provides a very simple and satisfactory explanation of trichome spacing and differentiation, although it is unable to address the source of the initial bias within a trichome precursor cell.

Interactions between the differentiating *Arabidopsis* trichome and other types of epidermal cell have not been well documented, largely because studies of stomatal patterning have been conducted in glabrous lines. However, selection of traits modifying trichome density can be demonstrated under changing environmental conditions (Mauricio and Rausher, 1997) and it is therefore likely that trichome differentiation is also responsive to signals from the environment. Changes in trichome density as a result of environmental factors will also affect the number and distribution of other cell types, particularly pavement cells.

Two of the *Arabidopsis* trichome mutants also affect the development of other epidermal cell types, at least in the root. The *ttg* and *gl2* mutants, which do not develop trichomes, have greatly increased numbers of root hairs which form ectopically in what would normally be non-hair cell files. A more complex interaction demonstrating an evolutionary link between trichome and root hair differentiation has been described (Wada *et al.*, 1997). The *Arabidopsis* mutant *caprice* fails to produce root hairs, and the *CAPRICE* (*CPC*) gene product encodes a MYB DNA binding protein without an activation domain. It was therefore proposed (Wada *et al.*, 1997)

that CPC acts to repress GL2 activity by binding the promoter region of the gene, but failing to activate transcription. In this way *GL2* expression is prevented in certain cells which may then differentiate into root hairs. In the *cpc* mutant *GL2* activity is much higher and few cells are able to differentiate. It is predicted that in the non-hair cells of the root epidermis a similar MYB protein with an activation domain is required to activate *GL2* expression. However, constitutive expression of *CPC* in *Arabidopsis* results in a loss of trichomes on all aerial organs (Wada *et al.*, 1997). It was argued that this phenotype may result from CPC competing with GL1, the trichome inducing MYB protein, to bind the promoter region of *GL2* in committed trichome cells (Wada *et al.*, 1997). In the absence of an activation domain, CPC is unable to activate *GL2* expression and the plant fails to differentiate trichomes. This is an example in which, in the natural situation, GL1 and CPC do not interact in any way and trichome and root hair development are not normally interlinked. However, the genes may be evolutionarily related, and the two developmental pathways may therefore have ancestrally shared common components.

Trichomes: other

A trichome has been defined as any protrusion from the epidermis (Esau, 1953), although this review has focused on protrusions from the aerial epidermis. Following this definition stigmatic papillae, conical-papillate petal cells, both glandular and non-glandular hairs, thorns, and surface glands are all classified as trichomes. Few of these trichome varieties have been studied, and none as extensively as the *Arabidopsis* trichome. While the morphogenesis of the commercially important cotton (*Gossypium hirsutum*) trichome or fibre has been described extensively, little is known about the molecular or genetic control of its differentiation or patterning. However, some information on the differentiation of both multicellular glandular trichomes and petal conical-papillate cells exists, and it is clear from these data that these two differentiation pathways interact with each other and are themselves interactive with the stomatal development pathway.

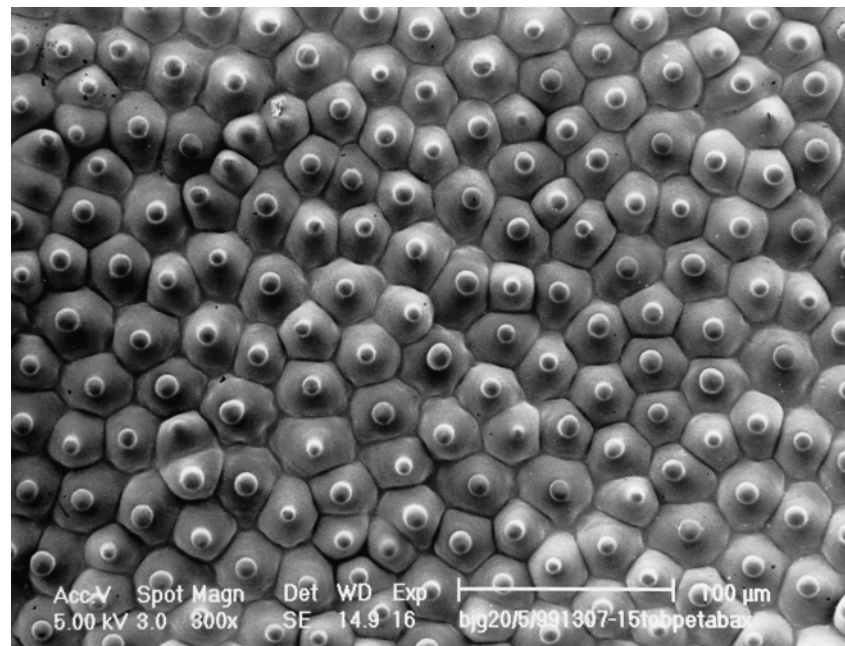
The majority of flowering plants produce multicellular trichomes. Although these trichomes must undergo processes of commitment, expansion and morphogenesis, which may, in principle, be similar to those of unicellular trichomes, they must also do this in the context of continuing cell division. Multicellularity allows trichomes to function as miniature organs, with differentiation within the trichome with regard to cell function. Many multicellular trichomes are glandular, developing a terminal gland which may secrete a variety of compounds, such as alkaloids to deter or poison predators.

The trichomes of tobacco (*Nicotiana tabacum*) are the

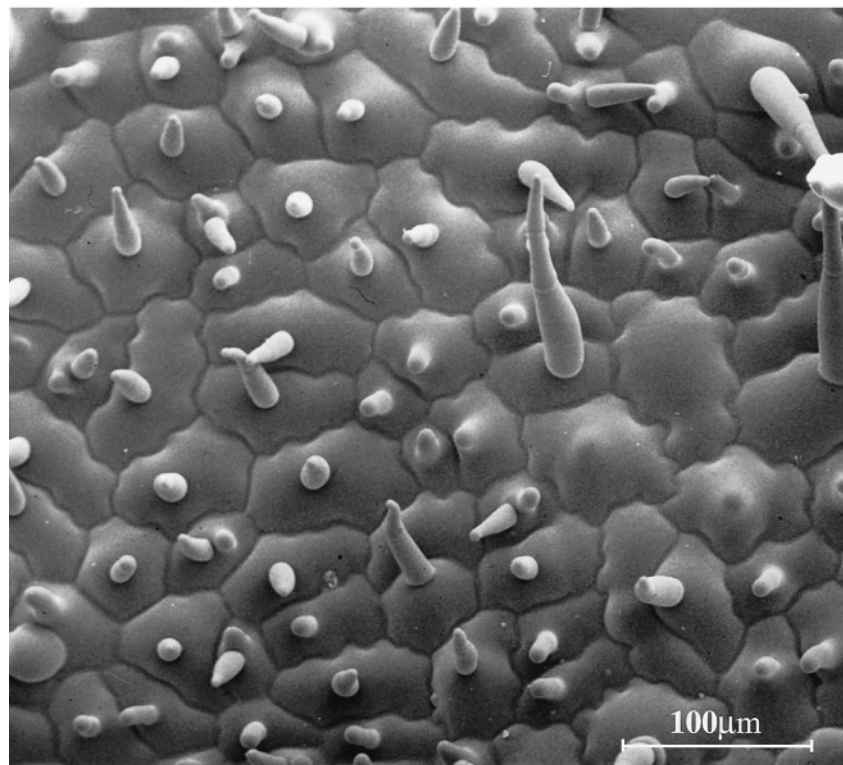
most extensively studied multicellular trichomes, but little is known of the molecular control of their differentiation. Tobacco leaves develop two discrete types of multicellular trichome, described as short and long-stalked (Fig. 1A). The short-stalked trichomes have an eight-celled glandular head, and exude nicotine, while the long stalked trichomes also have a gland, which is believed to produce and secrete diterpenoids (Akers *et al.*, 1978; Meyberg *et al.*, 1991; Nielsen *et al.*, 1991). There is substantial evidence that the two types of trichome are under the control of separate developmental programmes, as at least two mutations which affect the development of the gland on the long-stalked trichomes do not affect the short-stalked trichome (Burk *et al.*, 1982).

Differentiation of long-stalked trichomes may be initiated by the activity of a MYB transcription factor. Ectopic expression of the *MIXTA* gene from *Antirrhinum majus* in tobacco resulted in the development of excess long-stalked trichomes, both on epidermal surfaces that normally produce trichomes and on non-trichome producing surfaces (Glover *et al.*, 1998). The *MIXTA* gene encodes a transcription factor from the same family as GL1, although the two proteins show considerable sequence divergence. In *Antirrhinum* *MIXTA* is not required for multicellular trichome development, but is both necessary and sufficient for the differentiation of conical-papillate petal cells (Fig. 2A; Noda *et al.*, 1994; Glover *et al.*, 1998). It is likely that another *Antirrhinum* gene with strong sequence identity to *MIXTA* plays a role in the development of the trichomes of *Antirrhinum*, which are very similar to the trichomes of tobacco (Glover *et al.*, 1998). However, it is not yet clear how the product of this gene interacts with other factors to produce the regular spacing of trichomes observed on tobacco leaves. Until more molecular data become available it is only possible to conclude that the developmental steps leading to the later stages of multicellular trichome development must be significantly different to those of unicellular trichomes, and it is therefore likely that the molecular mechanisms may also be distinct. However, it is possible that spacing of multicellular trichomes acts through a mechanism similar to that proposed for the unicellular *Arabidopsis* trichomes.

The conical-papillate petal cells of *Antirrhinum*, which are specified by the action of *MIXTA*, are an adaptation which enhances attractiveness to pollinators through manipulation of light entering the petal (Glover and Martin, 1998). The *PHMYB1* locus in *Petunia hybrida* is similarly required for the establishment of conical cell form (Avila *et al.*, 1993; van Houwelingen *et al.*, 1998). The interaction between this developmental programme and that specifying long-stalked trichome differentiation was demonstrated by the ectopic expression of *MIXTA* in tobacco. Some of the transgenic lines produced conical cells, resembling petal epidermal cells, on their leaves as



A



B

Fig. 2. Epidermal differentiation without spacing. (A) Abaxial epidermis of tobacco petal lobe, with all cells differentiated as conical-papillate cells. This cell form increases light capture by the petal, making it more attractive to pollinators. As pollinator attraction is the primary role of the petal no other specialized cell types arise on the epidermis and there is therefore no need for an intricate pattern forming mechanism. (B) Adaxial epidermis of tobacco leaf ectopically expressing the *MIXTA* gene from *Antirrhinum*, which is sufficient for the specification of conical-papillate petal cells. Almost all cells on the leaf epidermis are converted to the conical-papillate or trichome fate, demonstrating that the spacing mechanisms normally seen in the leaf epidermis can be broken.

might be predicted from a gene required for petal conical cell formation. Other lines produced an excess of long-stalked trichomes (Fig. 2B). The formation of either conical cells or trichomes was strongly correlated to the timing of expression of *MIXTA* in relation to the developmental stage of the tissue and particularly its competence for further cell divisions. Leaf tissue expressing *MIXTA* relatively late (maximum expression when the cells had finished dividing) differentiated cones, while epidermal leaf tissue that had high *MIXTA* expression while the cells were still mitotically active, differentiated predominantly long-stalked trichomes. Therefore trichome and conical cell development in *Antirrhinum* and tobacco appear to share parts of a common developmental programme. Separation of the two cellular differentiation programmes may have arisen by gene duplication. Differentiation of expression of two genes with a common function, particularly in relation to the progress of cell division, may thus result in different forms of cellular morphogenesis.

Development of either conical cells or excess trichomes in the transgenic tobacco leaves was also correlated with a dramatic reduction in stomatal density. This suggests that the conical cell/trichome developmental programme acts competitively with the guard cell developmental programme. This idea is supported by the observation that the petal epidermis, a tissue composed entirely of conical cells, contains no stomata and on cotton ovules the cells surrounding guard cells remain free of the late-forming trichomes. It is clear that cells which have been recruited to a conical or trichome cell fate cannot then differentiate into guard cells. Observation of both the 35S-*MIXTA* lines and of wild-type plants indicates that the converse is also true, the cells associated with the stomatal complex do not differentiate into trichomes or cones, no matter how many of the ordinary pavement cells have been converted to the trichome or cone fate (Glover *et al.*, 1998).

Conclusions

The differentiation of plant epidermal cells clearly operates through mechanisms of both pattern formation and morphogenesis. While many of the genes involved in both steps have been characterized at the molecular level, particularly those involved in the differentiation of the *Arabidopsis* trichome, it is apparent that the means by which individual cells choose between a variety of potential fates is still some way from being understood. The genetic model for trichome initiation and lateral inhibition of neighbouring cells (proposed by Schnittger *et al.*, 1999) goes some way towards explaining the later stages of pattern formation, but still relies on a so far uncharacterized initial difference between neighbouring cells. It is this initial discrimination between apparently identical cells at

the same stage of development that is at the heart of understanding all epidermal cell patterning. Individual cells must be marked as guard cell meristemoids, trichome precursors or pavement cells. In animals such cell fate is primarily governed by cell lineage and position, but in the developmentally plastic plants some other mechanism operates to identify and recruit appropriate cells into differentiation pathways. A possible contender for this role is the cell cycle. While neighbouring cells may appear identical in age, morphology and developmental state, as long as the cell cycle is not synchronous between neighbouring cells, at any given timepoint individual cells can be identified as a result of their cell cycle stage. Differentiation of stalk and spore cells in the slime mold *Dictyostelium* depends on the asynchrony of the cell cycle, with terminal cell fate depending on the cell cycle stage an amoeba is at when it first experiences starvation (Weijer *et al.*, 1984; Gomer and Firtel, 1987). Wound xylem regeneration is only possible because cells which have progressed from maturity through to callus formation pass through a cell cycle stage at which a putative receptor allows them to respond to the influx of auxin, inducing differentiation (Dodds, 1981). It is certainly clear from ectopic expression of *MIXTA* in tobacco that the state of a cell with regard to the cell cycle when a differentiation pathway is initiated can determine which of two alternate fates it adopts. It is therefore likely that other epidermal differentiation decisions also operate through the cell cycle, which may provide the initial differences necessary for complex patterns to arise.

The differentiation of plant epidermal cells is a complex process. The environmentally responsive nature of plant development means that cell lineage does not play the same role in plant cell patterning as it does in animals. Instead, signalling between differentiating cells is more important. Variations on the basic plant cell type, developed in response to different environmental inputs, may mean that discrete cell types share components of the same developmental programme, responding to that programme differently as the plant grows. The final shape of a plant epidermal cell is therefore determined by an extremely complex set of potentially conflicting signals, all interpreted with regard to the growth phase of the tissue and the competence of the cells for further division and differentiation. In the next few years many more of the genes specifying epidermal cell differentiation will be cloned and characterized. The identification of mutants with lesions in stomatal and trichome development will allow a better interpretation of the interactions between these pathways in the developing plant. As the pathways and their interactions become more clearly understood, it should be possible to develop a greater appreciation of the mechanisms by which individual cells interpret the conflicting signals of the various pathways and adopt their final differentiated form.

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