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Positional information and mobile transcriptional regulators determine cell pattern in the Arabidopsis root epidermis

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Abstract

The root epidermis is a model system for deciphering the mechanism underpinning the formation of cellular pattern in planar groups of cells. The epidermis comprises rows of hair cells (H) and non-hair-bearing epidermal cells (N). Laser ablation and clonal analysis have shown that the fates of epidermal cells are flexible through development and that positional information which may be located in the cell wall or extracellular matrix determines cell fate. A leucine-rich repeat protein called SCRAMBLED is required for the development of cell pattern which may be involved in the perception of positional information. It is proposed that positional signals then initiate the cell-specific expression of a number of transcription factors that complete the patterning process, resulting in the expression of hair-promoting genes in hair cells (H) and their repression in the hairless cells (N).

Key words: Arabidopsis, root epidermis, root hairs.

Cell types are arranged in a longitudinal striped pattern in the Arabidopsis root epidermis

The root epidermis in young primary roots is derived from a ring of 16 initials located to the outside of the columella initials (Dolan et al., 1993, 1994; Galway et al., 1994; Kidner et al., 2000). This ring of initials gives rise to 16 epidermal cell files although the number of cell files can increase as a result of longitudinal divisions in some epidermal cells. Of these 16 epidermal cell files 8 are located between the junctions of underlying cortical cells and 8 are located over a single cortical cell when viewed in transverse orientation. Those cells that are located over cortical junctions develop root hairs. These cells are referred to as H (hair) cells and their location relative to their neighbours as the H position (Fig. 1). Cells located over single cortical cells develop as non-hair-bearing epidermal cells (N) and this position will be referred to as the N position.

When viewed from the outside of the root the epidermal cells appear as longitudinal stripes: there are files of H cells and files of N cells (Dolan et al., 1994; Galway et al., 1994). This pattern of epidermal cell differentiation is found in Arabidopsis and related members of the Brassicales (Costa and Dolan, 2000). The majority of eudicots can develop hairs in any epidermal cell: there is no bias of cell position as there is in Arabidopsis. Nevertheless, the striped pattern has evolved independently at least three times (Costa and Dolan, 2000). Alternating stripes of hair and non-hair cells have been found in all families of the Caryophyllales that have been examined to date with the exception of the Cactaceae. The striped epidermis has also been observed in a number of species of the Boraginaceae. Given that these three groups of plants (Brassicaceae, Caryophyllales, and Boraginaceae) are unrelated, the development of the striped epidermis in each group indicates that the striped root epidermal pattern has evolved in the eudicots independently at least three times. Further sampling and more detailed morphological examination are required to determine if the striped pattern has evolved more than three times and to determine if, in each case, the developmental mechanism is likely to be the same.

Positional information controls the specification of cellular identity in the root epidermis

Cell specification in plants is generally considered to be controlled by positional information (Berger et al., 1998a), that is, the development of a cell is determined by its

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location relative to other cells. Such positional determination of fate is flexible, so that if a cell changes its position relative to its neighbours during development it also changes fate. This is, in contrast to lineage determination of cell fate in which a decision regarding the fate of a group of cells is made in a progenitor cell and is propagated through a cellular memory. While lineage-restricted cell determination has been observed during animal development it has not yet been observed in plant development.

Laser ablation has been used to distinguish between positional information and lineage-controlled cell specification during development in the epidermis (Berger et al., 1998a). The laser is used to kill selected groups of cells and the behaviour of the neighbouring cells is followed and their ultimate fates determined. In one set of experiments cells in the hair position (H) were killed and occasionally neighbouring non-hair cells (N) invaded the space previously occupied by the ablated cells. On each occasion when the invading cells made contact with the junction between two underlying cortical cells they differentiated as hair cells. That is, the hairless cell (N) cells changed to a hair cell (H) identity when they made physical contact with the cortical junction. This experiment suggested that positional information located near the cortical junction was responsible for the specifying H cell identity.

The examination of patterns of cell division in the root epidermis provided further support for the role of positional information in the control of cell fate in the epidermis (Berger et al., 1998a, b; Kidner et al., 2000). The majority of cell divisions in the root epidermis are transverse which means that the new wall is formed perpendicular to the long axis of the root. These divisions increase the number of cells in a single cell file. Occasionally longitudinal divisions occur in hair cell files (H) resulting in a pair of daughter cells side by side in a file where previously there had been a single mother cell. This appears as a local cell file duplication. Given that the mother cell had been located over the cortical junction one of the daughter cells is also located over the cortical junction. It was found that the cell that made physical contact with the cortical junction differentiated as a hair cell (H) and the daughter cell that did not make contact with the cortical cell junction differentiated as a non-hair cell (N). Therefore even though the progenitors of these two cells had been in the hair position (H) and expressed the genes necessary to differentiate as a hair cell, once the longitudinal division resulted in one daughter being located in the N position, it changed fate and developed as a non-hair (N) epidermal cell. Together these experiments indicate that positional signals determine the identity of a cell in the epidermis and that cellular memory (cell lineage) plays no role in the determination of cell fate (Berger et al., 1998a).

The molecular basis of positional information

Laser ablation experiments have suggested that positional information may involve a signal that acts at the cell surface (Berger et al., 1998a). Recently a major breakthrough has been made which indicates that a receptor protein located at the cell surface may be involved in the perception of positional information during epidermal development (Kwak et al., 2005). The Schiefelbein group has identified a leucine-rich repeat protein called SCRAMBLED (SCM) that is required for cell-specific gene expression in the developing epidermis. Plants homozygous for the $scm$ mutation develop random epidermal patterns of cell-specific gene expression. For example, the gene $GLABRA2$ ($GL2$) is exclusively expressed in the non-hair cells (N) in the wild type, but is expressed in random patches in both N and H positions in $scm$ plants, i.e. some N cells express $GL2$ and others do not and some H cells express $GL2$ and others do not. This indicates that SCM activity is required for pattern to develop.

SCM may act as a receptor for a ligand for an extracellular signal which may be a peptide or other macromolecule (Kwak et al., 2005). SCM is present in all cell types suggesting that the SCM distribution does not confer pattern. It is possible that the ligand has a discontinuous distribution in the epidermis and its distribution is what determines the patterned cell differentiation in the root.

A cascade of transcription factors controls the development of pattern

It is proposed that the SCM-mediated positional information confers an as yet undefined difference between cells in

Fig. 1. Organization of cells in the root epidermis. Blue cells develop hairs (H) and are located between the junctions of underlying cortical cells (grey). Orange cells remain hairless (N). Root cap cells are in white and cover the epidermis in the meristem.
the two positions. This difference then controls the cell-specific expression of genes required for the development of hair cells in the H position and hairless cells in the N position. Consistent with this model is the observation that SCM controls the expression of a group of transcription factors that promote hair cell development in the H position and repress hair cell development in the N position (Kwak et al., 2005). For example, one of the downstream targets of these transcription factors is the transcriptional repressor GL2 (Masucci et al., 1996) which is expressed in N cells. GL2 represses genes such as phospholipase D, which is required for root hair development (Ohashi et al., 2003).

The question then is how is GL2 expression restricted to cells in the N position? A transcription complex comprising two basic helix loop helix proteins GLABRA3 (GL3), ENHANCER OF GLABRA3 (EGL3), and the Myb protein, WEREWOLF (WER) are proposed to activate the transcription of GL2 in the hair cell (Bernhardt et al., 2003, 2005; Lee and Schiefelbein, 1999, 2002). In the non-hair cell it is proposed that a similar complex comprising GL3 and EGL3 and the transcriptional repressor CAPRICE (CPC) forms which is transcriptionally inactive (Wada et al., 1997, 2002). That is, it cannot activate GL2 transcription and hair cell development is derepressed and hair cell development proceeds.

Theoretical models of patterning in biological systems have involved mobile signals, some of which act as repressors and others which act as activators (Meinhardt and Gierer, 1974). During the maintenance of epidermal pattern in the root meristem there are not only transcriptional activators and inhibitors, as mentioned above (WER is an activator and CPC is a repressor), but some of these proteins also move from cell to cell. While CPC activity is required in the H cells for the development of the hair cells it is transcribed in the N cells (Wada et al., 2002). The protein then moves from N cell to H cell where it becomes active. Furthermore, WER (a positive regulator of N development) which acts in the N cell promotes the transcription of CPC (a negative regulator if N development) which acts in the H cell (Lee and Schiefelbein, 1999, 2002). Another way of looking at this is that while WER promotes N cell development in the N position it also promotes H cell development in the H position because it positively regulates CPC expression and then CPC moves from the N cell to the H cell where it promotes H cell identity.

GL3 is a positive regulator of N cell development which also moves from cell to cell (Bernhardt et al., 2005). GL3 is transcribed in the H cell where GL3 protein forms an inactive complex with CPC consequently repressing GL2 expression in hair cells which, in turn, allows hair cell development to proceed. GL3 also moves to N cells where it promotes GL2 transcription by forming a complex with EGL3 and WER. GL2 expression in this cell type then repressed the expression of genes, such as phospholipase D, which are required for root hair development.

Therefore there are at least two proteins that move from cell to cell during the patterning of the cells in the seedling root meristem; one moves from N cell to the H cell (CPC) and the other moves from H cells to the N cells (GL3). It is proposed that this movement of these activators and inhibitors of cell fate maintains the asymmetry that is defined by positional information resulting in the spatially regulated cell differentiation of H and N cells. This model indicates that the difference between H and N cells is established before these genes are expressed. Consequently, it is not known when the pattern, or the asymmetry defined by positional information, first develops nor is it known how this pattern develops. Given that the root anlagen (cells which give rise to the root) forms on the embryo, it is likely that the answer will be found there.

**Genes controlling the development of epidermal pattern are expressed and active in the embryo**

Root epidermal pattern formation occurs in the embryo but its mechanism is not yet understood. Nevertheless, it is known when epidermal cells are differentiated from each other and it is also known that genes involved in the maintenance of pattern in the post-embryonic seedling are also involved during embryogenesis.

When does epidermal pattern develop in the embryo? GL2 is first expressed throughout the epidermis anlagen at the developing root pole in the late heart stage (Costa and Dolan, 2003). At this stage it is expressed in all epidermal cells equally and then becomes progressively restricted to the N cells. By the end of embryogenesis the GL2 expression pattern is the same as that observed in the seedling root. That is, it is expressed exclusively in the N cells. Given that it is expressed throughout the epidermis at the late heart stage and is progressively restricted to N cells, it suggests that epidermal patterning occurs some time between the late heart stage and the mature stage of embryogenesis.

What genes are required for the formation of epidermal pattern in embryos? GL2 is expressed in the late heart stage but in situ hybridization does not detect the expression of WER or CPC at the heart stage (Costa and Dolan, 2000; Lin and Schiefelbein, 2001), in fact they are first expressed at the late torpedo stage (Costa and Dolan, 2003). The restriction of GL2 transcription to non-hair cells during embryogenesis requires WER and CPC activity, indicating that these genes are active in the process of pattern formation. Nevertheless, it is not known what activates the transcription of GL2 at the late heart stage (before WER and CPC are transcribed). This suggests that other components in this pattern formation network remain to be identified.

**Perspectives**

There is now a mechanistic framework to explain elements of the development of pattern in the root epidermis. It
suggests that that the movement of proteins from cell to cell may be a general mechanism by which patterning of cells occurs in plants. There are two outstanding questions which will shed light on pattern formation in the root epidermis. (i) What is the mechanism of pattern formation in the embryo and what genes are involved? (ii) What is the signal that conveys positional information and how does the SCM proteins mediate the positional signal?

References


