Trichome morphogenesis: a cell-cycle perspective

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Arabidopsis leaf hairs (trichomes) are polyploid epidermal cells with a predictable branching pattern. More than 15 genes have been identified that are involved in the regulation of branching. The cloning of the *ZWICHEL*, *ANGUSTIFOLIA* and *STICHEL* genes points to two mechanistic aspects of branch formation: (i) a role of the microtubule cytoskeleton; and (ii) a link to the regulation of cell divisions. The latter aspect is supported by the recent identification of an *Arabidopsis* mutant with multicellular trichomes, the *siamese* mutant, suggesting that *Arabidopsis* trichomes are evolutionarily derived from multicellular forms. We speculate that the spatial information for branch formation is derived from mechanisms employed in cell divisions.

Keywords: trichomes; cell cycle; cytoskeleton

1. TRICHOMES AS A MODEL SYSTEM TO STUDY CELL MORPHOGENESIS

In *Arabidopsis*, trichomes are found on most aerial organs, including the rosette and cauline leaves, sepals and the stem. They are large single cells that display a predictable branching pattern (Hülskamp *et al.* 1999; Marks 1997; Oppenheimer 1998). On rosette leaves they have usually three branches, which are aligned with respect to the proximal–distal leaf axis (Hülskamp *et al.* 1994). Trichomes on cauline leaves, sepals and the stem are less branched or unbranched. Therefore, in particular branch formation of rosette leaf trichomes provides an excellent system to study cell morphogenesis: the first branching event is co-aligned with the proximal–distal leaf axis, the second branching event takes place only on the distal branch and is twisted by 90°, and the angle between primary (120°) and secondary (90°) branches is different (Folkers *et al.* 1997).

Trichomes develop from protodermal precursor cells. On rosette leaves, incipient trichomes are separated by about three or four epidermal cells (Hülskamp *et al.* 1994; Larkin *et al.* 1996). While the surrounding epidermal cells continue to divide, trichomes exit the mitotic cycle and enter an endoreduplication cycle in which DNA replication continues in the absence of nuclear and cellular divisions (Hülskamp *et al.* 1994). During development, trichomes appear to proceed through four endoreduplication cycles since the mature trichome has on average a DNA content of 32C. The first endoreduplication cycle is likely to be completed prior to outgrowth. The trichome cell then changes its polarity and starts to grow out from the surface. The second endoreduplication cycle takes place concomitantly with the first branching event, and

the third endoreduplication cycle occurs around the time the second branching event is initiated. During the last endoreduplication cycle the trichome cell expands giving rise to the mature three-branched trichome that may be up to 0.5 mm tall.

2. DRUG INHIBITOR STUDIES SHOW THAT MICROTUBULES AND ENDOREDUPLICATION CONTROL BRANCHING

Inhibitor studies have been carried out to determine which intracellular processes are involved in trichome branching. Treatments with drugs affecting the cytoskeleton have shown that microtubules, but not actin, are involved in branch formation (Mathur & Chua 2000; Mathur *et al.* 1999; Szymanski *et al.* 1999). Stabilization of and severing of microtubules cause reduced branching. Conversely, the stabilization of microtubules in the unbranched mutant *stichel* can trigger branch formation (Mathur & Chua 2000). Another study has shown that treatment with aphidicolin, which inhibits replication, results in under-replicated trichomes with a reduced branch number (B. Schwab & M. Hülskamp, unpublished data). Thus, it appears that the number of branches is controlled by the number of endoreduplication cycles.

3. GENETIC ANALYSIS OF BRANCHING

In an effort to dissect trichome branch formation genetically, more than 15 genes have been identified that affect branch number (figure 1); no mutations were described disturbing the branching pattern (Folkers *et al.* 1997; Hülskamp *et al.* 1994; Luo & Oppenheimer 1999). The systematic analysis of all double mutant combinations has shown that most branching mutants act in separate genetic pathways, indicating that they are also involved in different biochemical pathways (Luo & Oppenheimer 1999). Two observations support the findings obtained by the drug inhibitor experiments that microtubules and

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Figure 1. Branching mutants. (a–c) Scanning electron micrographs of wild-type and mutant trichomes. (a) Wild-type. (b) *stichel* mutant. (c) *zwichel* mutant.

endoreduplication are both important for branch formation. (i) One branching mutant, *fass/ton*, was shown to be affected in the organization of the cortical microtubules and the pre-prophase band during cell divisions (Torres-Ruiz & Jürgens 1994; Traas *et al.* 1995). (ii) Trichome endoreduplication mutants also show changes in branch number. Mutants in which trichomes have an increased DNA content have more branches and one mutant known to have a reduced DNA content has fewer branches (Folkers *et al.* 1997; Hülskamp *et al.* 1994; Perazza *et al.* 1999).

4. MULTICELLULAR TRICHOMES IN *ARABIDOPSIS*: IS BRANCHING A RUDIMENTARY FORM OF A CELL DIVISION PATTERN?

Two genetic situations have recently been described that result in a transformation of the unicellular *Arabidopsis* trichomes into multicellular trichomes. Mutations in the *Arabidopsis* gene *siamese* (*sim*) result in the formation of multicellular trichomes (Walker *et al.* 2000). *sim* mutants have on average three trichomes but up to 15 cells. Occasionally, trichomes occur in clusters but trichome form is usually normal. Multicellular trichomes are also formed when a mitotic B-type cyclin is ectopically expressed in trichomes (A. Schnittger & M. Hülskamp, unpublished data). These two findings show that endoreduplication cycles can be changed easily into mitotic cycles and suggest that trichomes in *Arabidopsis* are evolutionarily derived from multicellular forms (figure 2).

In multicellular trichomes, the branching pattern is due to a defined cell division pattern where branches originate from cell divisions generating two new cell files (Uphof 1962). It is therefore tempting to speculate that the spatial information underlying branching in the unicellular *Arabidopsis* trichomes is based on the cell division machinery. In this scenario, processes involved in rearranging a cell's polarity prior to cell division are still operating in trichomes. In the absence of nuclear and cellular divisions, only cell growth is seen.

5. ZWICHEL, STICHEL AND ANGUSTIFOLIA: MOLECULAR ASPECTS LINKING THEIR FUNCTION TO THE CELL CYCLE

ZWICHEL, or *ZWI*, was the first branching gene cloned and molecularly characterized (Oppenheimer *et al.* 1997). *ZWI* seems to be required for the establishment of proper trichome polarity because in *zwi* mutants the distally

Figure 2. Model to explain branching in a multicellular scenario. The schematic drawings show subsequent developmental stages of *Arabidopsis* trichomes in a multicellular scenario. The branching pattern originates from a standardized cell division pattern. Below, two branching mutants are shown. The model postulates that in a multicellular scenario branching mutants show an altered cell division pattern.

pointing branch that normally branches again is gradually reduced with increased strength of the *zwi* allele (Folkers *et al.* 1997; Oppenheimer *et al.* 1997; M. Hülskamp, unpublished data). As a consequence, the second branching event is also usually missing (figure 1c). *ZWI* encodes a kinesin-like Ca^{2+} /calmodulin-regulated microtubule motor protein that has been described independently before as KCBP (Reddy *et al.* 1996). Kinesin-like proteins function in various cellular processes, including intracellular transport of vesicles and organization of microtubules. *ZWI/KCBP* is expressed in all tissues in *Arabidopsis*. That

zwi mutants show a trichome-specific phenotype is probably due to a redundancy of ZWI/KCBP function in other tissues since a mutation in the *SUPPRESSOR OF ZWICHE1* gene, which has no phenotype by itself, in a *zwi-3* mutant background results in male sterility and defects in pollen germination and pollen tube growth (Krishnakumar & Oppenheimer 1999). Two observations point to a role of ZWI in cell divisions. First, immunolocalization studies have shown that ZWI/KCBP is localized at the pre-prophase band, the spindle apparatus and the phragmoplast in dividing tobacco BY-2 cells (Bowser & Reddy 1997; Vos *et al.* 2000). Second, injection of ZWI/KCBP antibodies directed to the Ca^{2+} /calmodulin domain of ZWI/KCBP into *Tradescantia* stamen hair cells led to a premature breakdown of the nuclear envelope and to metaphase arrest (Vos *et al.* 2000).

The *ANGUSTIFOLIA*, or *AN*, gene is important not only for trichome branching but also for morphogenesis of various organs. In *an* mutants, all leaves are narrower than in wild-type and the stem and the siliques are twisted (Redei 1962). It has been shown that the narrow leaf phenotype is due to a polarity defect of all leaf cells (Tsuge *et al.* 1996). Leaf epidermal cells are thicker and reduced in their lateral extension. Not only cell shape but also the number of cells is reduced, specifically in the width axis. In *an* mutants, trichomes are two-branched and still orientated with respect to the leaf axis, suggesting specifically that the secondary branching event is missing (Folkers *et al.* 1997). *AN* encodes a protein with sequence similarity to CTBPs/BARS and is expressed in all organs (U. Folkers & M. Hülskamp, unpublished data). The molecular function of CTBPs/BARS is still unclear. On the one hand CTBPs in *Drosophila* have been shown to bind transcriptional repressors such as the zinc-finger transcription factor Krüppel, suggesting that they act as transcription cofactors (Nibu *et al.* 1998). On the other hand BARS are proteins that become ribosylated after treatments with the Golgi-disturbing drug brefeldin A (Matteis *et al.* 1994). Recently it was demonstrated that BARS play a role in membrane fission and thereby Golgi dynamics (Weigert *et al.* 1999). Because BARS can bind to the same transcription factors as CTBPs, and CTBPs are ribosylated after brefeldin A treatment, they are considered to be homologous proteins (Spanfo *et al.* 1999).

How the AN protein is involved in cell morphogenesis is still unclear. A potential role of AN in cell divisions is suggested by two findings. First, the number of cell divisions in the width axis is reduced (Tsuge *et al.* 1996). Second, the AN protein contains a PEST sequence with a CDC2a phosphorylation site in its vicinity, suggesting a cell-cycle-regulated degradation of the AN protein (U. Folkers & M. Hülskamp, unpublished data).

Mutations in the *STICHEL*, or *STI*, gene specifically affect trichome branching; *sti* mutant trichomes are unbranched (figure 1b). The *STI* gene encodes a protein with sequence similarity to the γ DNA polymerase subunit from Eubacteria (H. Ilgenfritz & M. Hülskamp, unpublished data). Although endoreduplication levels are normal in *sti* mutants, this homology suggests that STI function is linked to the cell-cycle machinery.

6. PERSPECTIVE

Several lines of evidence have accumulated that are consistent with the hypothesis that the spatial information underlying trichome branching is mechanistically based on the cell division machinery. Since neither branching nor the regulation of the orientation of cell divisions is understood, this hypothesis is no more than exchanging one black box for another. It is, however, an exciting change of view since, in studying branching mutants at the molecular level, we may learn something about the general principles underlying the spatial regulation of cell divisions.

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GLOSSARY

- BARS: brefeldin A-ADP-ribosylated substrate
 CTBP: C-terminal binding protein
 KCBP: kinesin-like calmodulin binding protein
 PEST: domain named after the single-letter abbreviations for proline, glutamic acid, serine and threonine