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Trichome morphogenesis in *Arabidopsis*

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Trichomes (plant hairs) in *Arabidopsis thaliana* are large non-secreting epidermal cells with a characteristic three-dimensional architecture. Because trichomes are easily accessible to a combination of genetic, cell biological and molecular methods they have become an ideal model system to study various aspects of plant cell morphogenesis. In this review we will summarize recent progress in the understanding of trichome morphogenesis.

Keywords: trichomes; morphogenesis; branching

1. INTRODUCTION

In plants, only few cell types are suitable to study how cells control their cell shape in a spatially defined manner (Hülskamp *et al.* 1998). The directionality of tip growth is studied in pollen tubes and in root hairs (Aeschbacher *et al.* 1994). Cell expansion is analysed genetically in cortex cells of the root and in trichomes (Hauser *et al.* 1995; Hülskamp *et al.* 1999). Among these cell types, trichomes are particularly well suited to study the spatial regulation of morphogenetic events because they develop a distinct, genetically defined shape that facilitates the identification of mutants affecting branching, cell expansion and cell size (Hülskamp *et al.* 1999; Marks 1997).

2. WILD-TYPE DEVELOPMENT OF TRICHOMES

Trichomes are the first epidermal cells that begin to differentiate in the epidermis of developing leaf primordia (Hülskamp *et al.* 1994; Larkin *et al.* 1996). While the surrounding epidermal cells continue to divide, protodermal cells committed to a trichome fate stop cell divisions and proceed with on average four DNA replication cycles (endoreduplication) (Hülskamp *et al.* 1994). After two or three endoreduplication cycles the incipient trichome expands out of the leaf surface and initiates two successive branching events (figure 1). The first branching is co-aligned with the proximal–distal leaf axis. The second branching is usually found only on the branch pointing to the leaf tip (main branch) with the new branching plane at a right angle relative to the first branching (Folkers *et al.* 1997; Hülskamp *et al.* 1994). After branch initiation is completed, a second growth phase begins that is characterized by rapid vacuolization concomitant with the rapid expansion of the three branches (Schwab *et al.* 2000). Mature leaf trichomes are *ca.* 200–400 µm tall and 40–60 µm wide at the trichome base. On average they have a DNA content of about 32 C suggesting that trichome cells have executed four endoreduplication cycles (Hülskamp *et al.* 1994).

3. GENETIC DISSECTION OF TRICHOME DEVELOPMENT

More than 40 genes have been identified that play an important role at distinct steps during trichome cell morphogenesis (Hülskamp *et al.* 1999). These genes can be grouped into four classes on the basis of the processes affected in the corresponding mutants (figure 2): (i) trichome differentiation mutants have reduced and not fully developed trichomes (Hülskamp *et al.* 1994; Schneider *et al.* 1997); (ii) endoreduplication mutants show a reduced or increased nuclear DNA content and mutant trichomes are smaller or larger and exhibit fewer or more branches, respectively (Hülskamp *et al.* 1994; Oppenheimer 1998; Perazza *et al.* 1999); (iii) branching mutants exhibit fewer or more trichome branches (Folkers *et al.* 1997; Hülskamp *et al.* 1994; Luo & Oppenheimer 1999); (iv) the directionality of extension growth is impaired in mutants of the *distorted* (*dis*) group (Feenstra 1978; Hülskamp *et al.* 1994; Marks & Esch 1992).

For an understanding of spatial aspects of trichome cell morphogenesis two aspects are particularly suited: the regulation of branching and the regulation of the directionality of cell expansion. In this review we will therefore focus on these two aspects.

4. TRICHOME BRANCHING

(a) Genetic analysis of branching

The genetic analysis of trichome branch formation has identified 18 genes that regulate trichome branch number. Generally, two classes of branching mutants can be distinguished. One class of mutants appears to primarily affect the number of endoreduplication cycles and probably as a consequence also cell size and branch number. In the second class of mutants trichome branch number is altered but endoreduplication is unchanged.

That endoreduplication levels control trichome branching is suggested by two lines of evidence. First, all mutants in which the DNA content is altered exhibit a branch phenotype. Trichome mutants with increased DNA levels such as *triptychon* (*try*), *kaktus* (*kak*), *rastafari*

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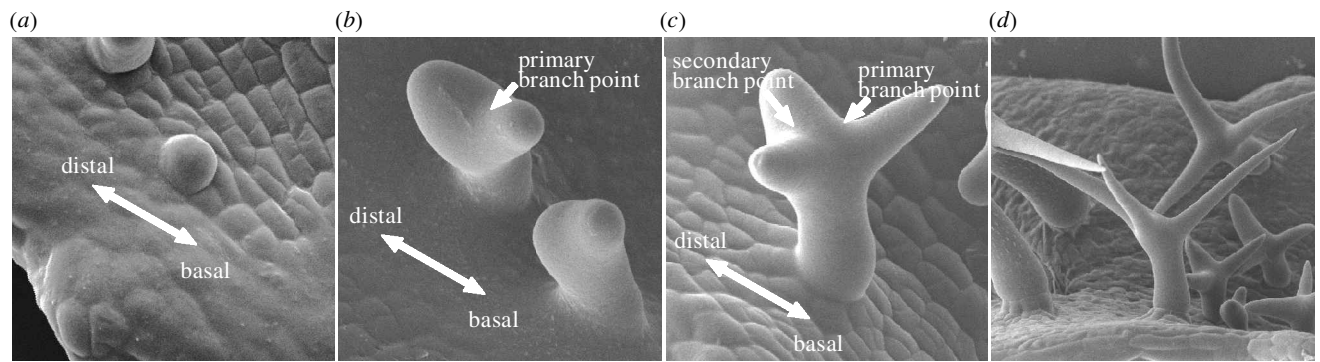


Figure 1. Scanning electron micrographs of developing wild-type trichomes. (a) Incipient unbranched trichome. (b) Trichome with primary branch point. Note orientation of the branches with respect to the basal–distal leaf axis. (c) Trichome with primary and secondary branch. (d) Mature trichome.

(*rfi*), *polychome* (*pym*) and *spindly* (*spy*) form up to eight branches, whereas trichomes with reduced DNA levels such as *glabra3* (*gl3*) mutants exhibit only one branch point or are unbranched (Hülkamp *et al.* 1994; Perazza *et al.* 1999). A second line of evidence comes from the observation that the same correlation is found in situations in which the DNA levels in trichomes are altered in a wild-type background. In tetraploid *Arabidopsis* plants the DNA level is doubled in all cells (Perazza *et al.* 1999). In these plants, trichomes have a DNA content of 64C and produce up to eight branches. Conversely, if DNA replication is inhibited by drug treatment (Aphidicolin), the DNA levels in trichomes are drastically reduced and trichomes form fewer branches (B. Schwab, unpublished data). These correlations between DNA content and branch number support the idea that branching is positively controlled by cell growth or cell size, which are thought to depend on the DNA content of a cell.

Twelve mutants have been identified that control branching without affecting endoreduplication. One gene, *NOECK* (*NOK*) is a negative regulator of branching, *nok* mutants have up to seven branches (Folkers *et al.* 1997). Eight genes, *STICHEL* (*STI*), *ANGUSTIFOLIA* (*AN*), *STACHEL* (*STA*), *ZWICHEL* (*ZWI*), *FURCA1* (*FRC1*), *FRC2*, *FRC3* and *FRC4* are positive regulators of branching (Folkers *et al.* 1997; Luo & Oppenheimer 1999). *sti* mutants show the strongest phenotype and trichomes are completely unbranched. The other seven mutants all display only one branch point. The detailed genetic analysis has revealed a complex genetic network in which several independent pathways are employed to regulate branching in a partially redundant manner (Luo & Oppenheimer 1999) (figure 3). Simultaneous mutations in any two pathways result in an unbranched trichome phenotype. Because of this redundancy, it is sufficient to assume that the negative regulation of branching by *NOK* or *TRY* targets only one branching pathway. For example if *NOK* inhibits *AN*, more *AN* activity in *nok* mutants could compensate a mutation in the *STI* gene.

In addition to the branching mutants discussed above, three mutants, *suppressor of zwichel-1* (*suz1*), *suz2* and *suz3*, were identified that show suppression of the branching phenotype of the *zwi-3* allele (Krishnakumar & Oppenheimer 1999). While *suz1* and *suz3* display no obvious trichome phenotype in the absence of the *zwi-3* mutation, *suz2* mutants exhibit more branches than

wild-type. Rescue of the branching phenotype by all three suppressor mutations is allele specific suggesting that these genes specifically interact with the *ZWI* gene product. The finding, however, that one of the suppressor mutants, *suz2*, can also rescue *frcl*, provides genetic evidence for a functional link between *ZWI* and *FRC1*.

(b) Cellular principles underlying branching

In plants, two mechanistically different growth modes of cells are distinguished (Hülkamp *et al.* 1998): (i) tip growth—tip-growing cells such as root hairs and pollen tubes are cells that have ceased cell divisions and grow exclusively at the extreme tip; (ii) elongation—cell elongation is typically found in dividing cell populations after cell division activity has ceased. During cell elongation growth is not restricted to the ends of the cell.

To understand the cellular basis of branching it is very important to determine the growth mode of trichome cells. If trichomes were tip-growing cells, branching would require the cell to define two new growth centres. If trichomes were elongating cells the regulation of trichome branching could be based on mechanisms related to cell divisions. One way to monitor the growth of single cells is to apply small latex beads to the cell surface and to follow their distribution over time (figure 4). In root hairs, this type of experiment revealed rapid growth exclusively at the extreme tip (Galway *et al.* 1997). By contrast, trichome cell growth was detected everywhere with slightly higher growth rates in tip regions (B. Schwab, unpublished data) (figure 4). These data indicate that trichome cells exhibit a growth mode similar to that of most dividing and elongating cells. One interesting possibility is that the positional information used for trichome branching is derived from the cell division machinery. This view is supported by two observations. First, trichomes in many plant species are multicellular (Uphof 1962) raising the possibility that unicellular trichomes in *Arabidopsis* represent hairs that lack the final step of cell division, cytokinesis, but still proceed through the corresponding cell morphogenesis. Second, the *fass* mutant, which affects the formation of the preprophase band, also has unbranched trichomes (McClinton & Sung 1997; Torres-Ruiz & Jürgens 1994; Traas *et al.* 1995). Because the preprophase band marks the future division plane in dividing cells, the observed *fass* mutant trichome

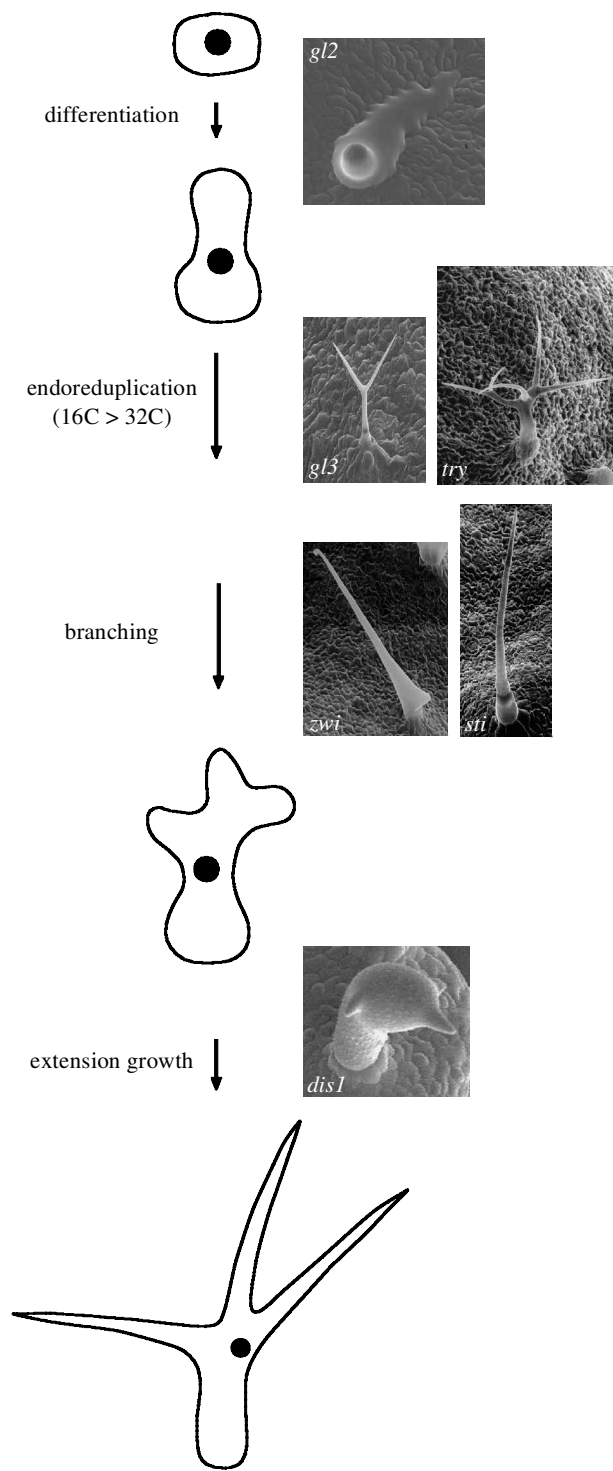


Figure 2. Developmental steps of trichome cell morphogenesis. The wild-type sequence of morphogenetic events is shown on the left. Examples of mutant phenotypes defining the corresponding regulatory steps are shown on the right. Scanning electron microscopy images are from Folkers *et al.* (1997).

phenotype supports a link between the orientation of cell divisions and branching.

(c) Molecular analysis of branching

Some light is shed on the molecular mechanisms underlying branching by the recent cloning of two branching genes: *ZWI* and *STI*. In the following we will discuss the

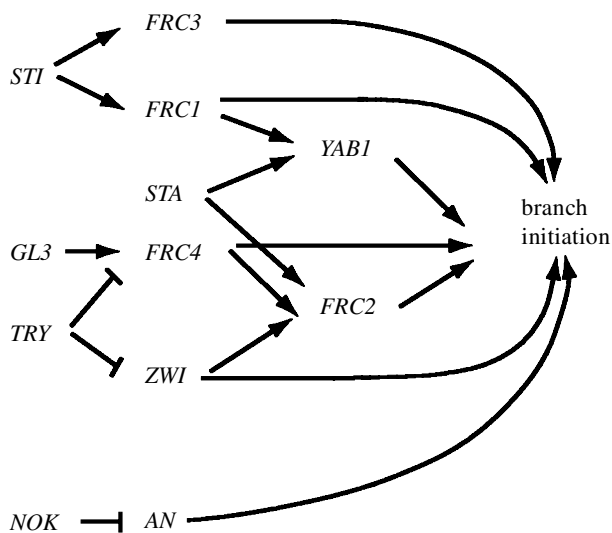


Figure 3. Genetic model for the regulation of trichome branch formation. Positive regulatory interactions are indicated by lines ending in arrows. Negative regulatory interactions are indicated by blunted lines.

molecular results in the context of genetic and morphological data available for these two genes.

Mutations in the *ZWI* gene result in a strong reduction of the main branch and probably as a consequence also in reduced branching (Folkers *et al.* 1997; Oppenheimer *et al.* 1997). Although the trichome specificity of the *zwi* phenotype suggests that *ZWI* is required only in trichome morphogenesis, the finding that the double mutant of *zwi-3* and *suz1*, single mutants of which are perfectly fertile, exhibits a synthetic pollen tube growth phenotype points to a more general and redundant role of *ZWI* (Krishnakumar & Oppenheimer 1999). The *ZWI* gene was cloned by a T-DNA tagging approach and encodes a kinesin-related protein containing a calmodulin-binding domain (Oppenheimer *et al.* 1997). *In vitro*, the ZWI protein binds to microtubules in a Ca^{2+} /calmodulin-dependent manner suggesting that its activity or the spatial regulation of its activity is regulated by Ca^{2+} /calmodulin also *in vivo* (Deavours *et al.* 1998; Reddy *et al.* 1996a,b). In synchronized *Arabidopsis* and tobacco cell cultures ZWI localizes to the preprophase band, the mitotic spindle and the phragmoplast (Bowser & Reddy 1997). Whether ZWI is involved in the formation of the corresponding microtubule arrays or whether ZWI uses them to transport cellular components is not known. Neither is it understood at the biochemical level how ZWI controls morphogenetic events during trichome branching.

Mutations in the *STI* gene cause the strongest branching defect. In weak *sti* alleles most trichomes have one branch point and some trichomes are unbranched, in strong *sti* alleles all trichomes are unbranched. The finding that the gradual loss of STI activity in different alleles results in a gradually stronger phenotype suggests that STI functions in a dose-dependent manner (H. Ilgenfritz, unpublished data). The *STI* gene was cloned by a positional mapping approach and encodes a large protein of *ca.* 135 kDa (H. Ilgenfritz, unpublished data). The STI protein contains three distinct putative functional domains. A large domain with sequence

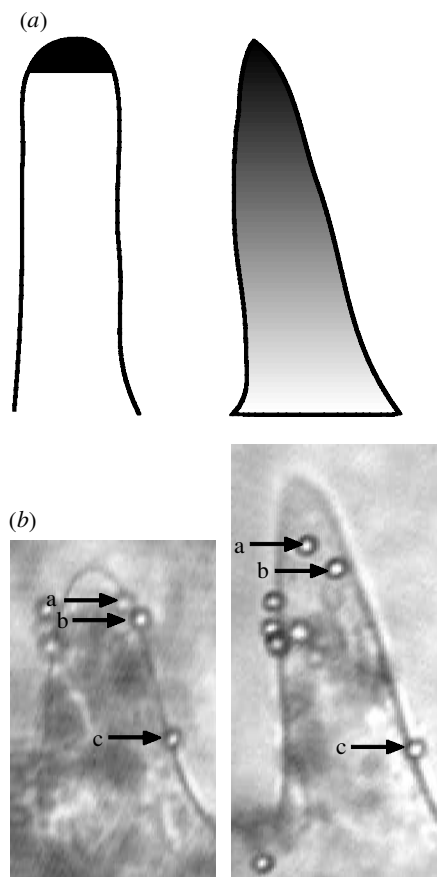


Figure 4. Tip growth versus cell elongation. (a) The left drawing shows a tip-growing cell. Cell growth is restricted exclusively to the tip (shown in black). The right drawing shows a trichome cell. Here, growth is found in all cell regions. (b) Bead experiment to determine where cell growth occurs. Latex beads are applied to the cell surface (left image) and their position is mapped after 12 h (right image). Three representative beads are marked by arrows and a, b and c.

similarity to the DNA-polymerase III γ -subunit of bacteria is found in the mid-region of the protein. This homology could suggest a functional link between the DNA replication and cell morphogenesis. Therefore STI should be expected to be a tightly regulated nuclear protein. These assumptions are supported by the presence of three putative bipartite nuclear targeting sequences (one in the N-terminal region, the other two as a tandem at the very C-terminus) and two presumptive PEST sequences preceding the γ -subunit-homology domain. Although the molecular mechanism by which factors of the replication machinery might be involved in the control of branching is elusive, it is likely that STI provides a new tool to unravel a novel mechanism of the regulation of cell morphogenesis.

5. DIRECTIONALITY OF TRICHOME EXPANSION

(a) Genetic analysis of expansion mutants

After branch initiation is completed all branches begin rapid expansion. Eight genes of the *DISTORTED* group control the directionality of expansion growth (Feenstra 1978; Hülskamp *et al.* 1994; Marks & Esch 1992). Mutations in any one of these genes result in irregular trichome growth, mature trichomes exhibit a twisted or distorted

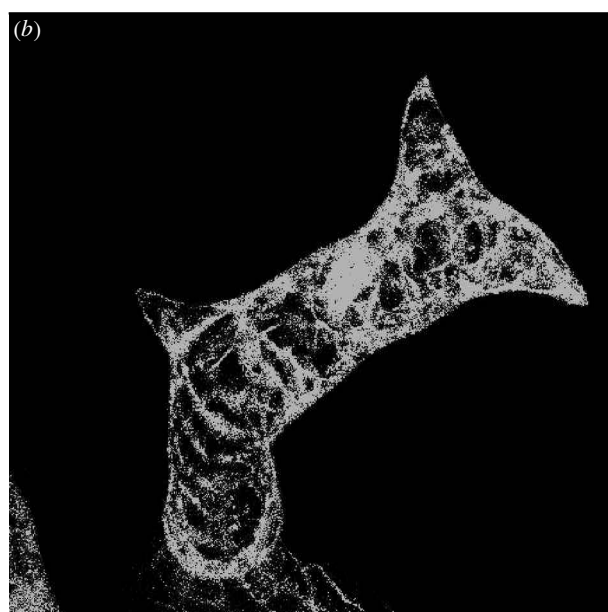
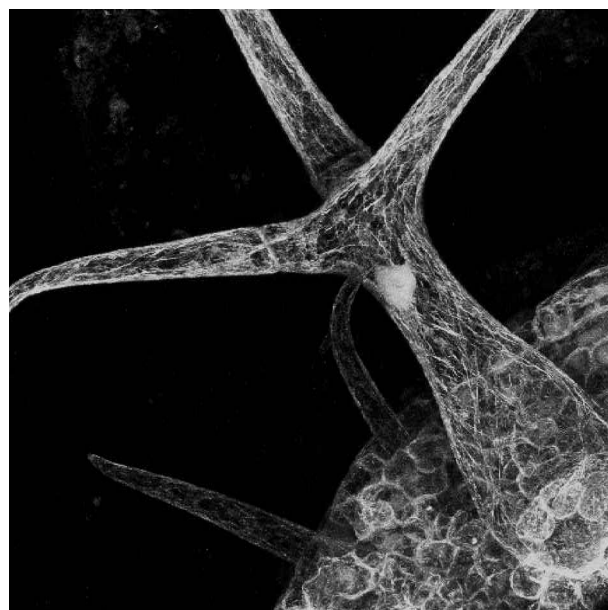


Figure 5. The F-actin organization in wild-type and *dis* mutants. (a) F-actin distribution in living wild-type trichomes as monitored by GFP-talin. Actin bundles are organized and run parallel to the long axis of the trichome cell. (b) F-actin distribution in one of the *dis* mutants, *crooked*. The F-actin is less organized.

phenotype. By morphological criteria, *dis* mutant trichomes develop normally until all branches are initiated and growth aberrations become apparent when the trichome cell expands (B. Schwab, unpublished data). This suggests that the *DIS* genes act specifically during expansion growth. Although no molecular data are available for any of the *DIS* genes, the analysis of genetic mosaics has revealed some insight into how the *DIS* genes might function. While *DISTORTED1* (*DIS1*) appears to act in a cell non-autonomous manner, all other *DIS* genes act cell autonomously (Hülskamp *et al.* 1994; B. Schwab, unpublished data). Thus either the *DIS1* protein or some metabolic components in the production of which *DIS1* is involved can move between cells, whereas all other *DIS* genes appear to function in intracellular morphogenetic processes.

(b) Cell biological analysis of expansion mutants

Some light is shed on the question of which intracellular mechanisms control the directionality of expansion growth by an elegant series of experiments by Mathur *et al.* (1999). Using various drugs affecting the actin or the tubulin cytoskeleton the authors demonstrated specific trichome phenotypes. Tubulin-interacting drugs lead to an unbranched trichome phenotype indicating that the tubulin cytoskeleton is involved in branch formation. By contrast, treatment with actin-interacting drugs causes a distorted trichome phenotype reminiscent of that found in *dis* mutants. This implies that the actin cytoskeleton plays a major role in coordinating the directionality of expansion growth. Using a transgenic GFP-talin line, which decorates F-actin *in vivo*, the authors demonstrated that six of the *dis* mutants tested, *alien*, *crooked*, *distorted1*, *gnarled*, *klunker* and *wurm*, show severe disorganization of the actin cytoskeleton in mature trichomes (figure 5). These observations strongly suggest that the actin cytoskeleton but not the tubulin cytoskeleton is a central regulatory target of the *DIS* genes.

6. CONCLUSIONS

Although the detailed genetic model of trichome morphogenesis and new cell biological tools such as a transgenic GFP-talin line that have been developed during the last couple of years provide a solid framework, the key question of what molecular mechanisms are employed is largely unresolved. The recent cloning of the first two trichome morphogenesis genes has revealed entry points into the analysis of poorly understood functions of known proteins (e.g. how a kinesin motor molecule (ZWI) is involved in cell morphogenesis) and the identification of new proteins (e.g. STI) that are likely to allow the discovery of new molecular pathways.

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