Progress in the molecular genetic analysis of trichome initiation and morphogenesis in *Arabidopsis*

Daniel B. Szymanski, Alan M. Lloyd and M. David Marks

Arabidopsis **trichomes are large unicellular structures that develop on the surface of most shoot-derived organs. In leaves, the number, spacing and shape of trichomes is tightly regulated, and this process has been used as an experimental system to study the control of cell fate and pattern formation. The control of trichome initiation is complex: both the potential of a cell to adopt the trichome cell fate and an intricate signaling pathway determine the pattern of trichome initiation events. Several important new results suggest that trichome initiation and morphogenesis are redundantly regulated by both positive and negative factors. A testable model for the control of trichome initiation is presented.**

In plants, the control of cell fate is a central issue during embryo development, meristem function and the transition from vegetative to reproductive phases. The regulation of cell fate requires a balance of cell proliferation, differentiation, intercellular communication and morphogenesis control. All of these developmental processes are involved in the formation of unicellular trichomes in the shoot epidermis of *Arabidopsis*. The integration of these processes is complex, and progress in understanding control mechanisms has been slow. Trichome development has provided a simple model to gain mechanistic insight into the control of cell fate and morphogenesis. Because of their amenability to genetic analysis and the physical accessibility of the leaf epidermis, diverse molecular, pharmacological and cytological approaches have been used to study different aspects of trichome development. Here we summarize recent data on the molecular genetics of leaf trichome initiation and morphogenesis.

Trichomes, defined as hair-like structures that extend from the epidermis of aerial tissues, are present on the surface of most terrestrial plants[1]. Plant trichomes comprise a diverse set of structures, and many plants contain several types of trichomes on a single leaf. It has been difficult to demonstrate their function clearly, but several ideas have gained widespread acceptance. The presence of trichomes can increase the boundary layer thickness between the epidermal tissue and the environment, and can reduce heat and water loss. In many species, trichomes are thought to protect the plant against insect or pathogen attack, either by secreting chemical components or by physically limiting insect access to or mobility on vegetative tissues. The stellate trichomes of *Arabidopsis* do not have a secretory anatomy, but at a functional level, they might limit herbivore access to the leaf in the field[2].

Arabidopsis trichome initiation

Cellular determinants of the potential to enter the trichome pathway

Trichome differentiation is integrated with leaf development, hormone levels and the vegetative development phase. The potential to acquire the trichome cell fate is highly regulated[3–7]. For example, the first trichome at the leaf tip appears only after the leaf grows to ~100 μm in length[5]. Subsequent initiation events proceed basipetally as the leaf grows. As leaf development progresses, cell division patterns become less regular: islands of dividing cells can be observed among differentiated pavement cells with their characteristic lobed morphology. Trichome initiation in the expanding leaf occurs within these islands of cells and often defines points along the perimeter of a circle, with an existing trichome defining the center (Fig. 1). The developmental window during which cells have the potential to acquire trichome cell fate is further limited; cells beyond a certain developmental stage lose the ability to respond to trichome differentiation signals[4].

Genetic analysis of trichome initiation

Genetic screens for trichome initiation and spacing mutants have been useful in addressing two important questions:

(1) What genes control entry into the trichome pathway?
(2) What controls the spacing of initiation events?

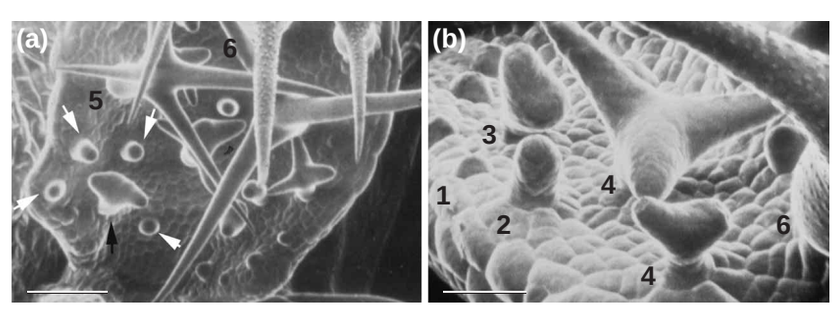

Fig. 1. Scanning electron micrographs of the adaxial surface of *Arabidopsis* leaves that illustrate aspects of trichome spacing and morphogenesis. (a) An example of secondary trichomes (white arrows) initiating around an existing central trichome (black arrow). Numbers to the left of each labelled trichome indicates its developmental stage: (5) trichome expansion with pointed branch tips; (6) mature trichome with a papillate cell wall. Scale bar = 100 μm. (b) Additional stages of trichome development: (1) isodiametric expansion in the plane of the epidermis; (2) stalk emergence and polar expansion; (3) branch initiation; (4) expansion of the stalk and branches with a blunt tip morphology; (6) see (a). Scale bar = 50 μm.

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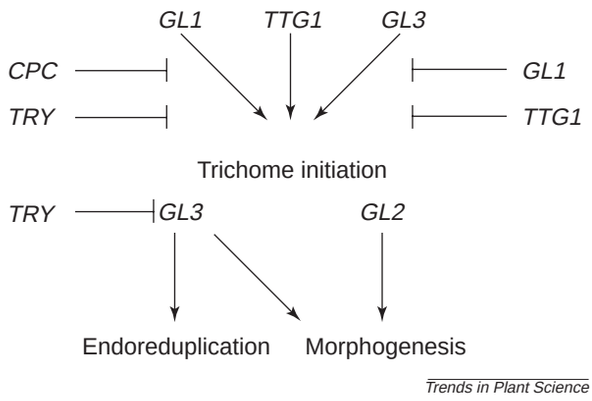

Fig. 2. A model summarizing the genetic control of trichome initiation and the early phases of trichome morphogenesis. *GLABROUS1* (*GL1*), *TRANSPARENT TESTA GLABRA1* (*TTG1*) and *GLABROUS3* (*GL3*) are required for normal trichome initiation. GL1 and TTG1 are also required for normal trichome spacing and have the capacity to limit trichome initiation. *CAPRICE* (*CPC*) and *TRIPTYCHON* (*TRY*) negatively regulate trichome initiation. *GL2* and *GL3* redundantly regulate aspects of trichome morphogenesis, and *GL3* also promotes endoreduplication in trichomes. *TRY* negatively regulates *GL3* activity in trichomes. In each example, arrows indicate a positive influence, blocked lines indicate negative regulation.

Loss-of-function mutations in the *GLABROUS1* (*GL1*) and *TRANSPARENT TESTA GLABRA1* (*TTG1*) genes result in a nearly complete loss of leaf trichome initiation[8]. The effects of mutations in *GL3* are less severe; trichomes are observed, but branching is either reduced or eliminated[9]. Genetic analyses of *gl1*, *gl3* and *ttg1* mutants have not indicated clear epistatic relationships between these genes. In addition, *GL1* and *TTG1* can negatively regulate trichome initiation. Weakly semi-dominant mutations in *TRIPTYCHON* (*TRY*) also result in a partial loss of negative regulation of trichome initiation, and give rise to limited trichome clustering[9–11]. The *try* trichomes also display an elevated branch number and DNA content. Mutations in *CAPRICE* (*CPC*) do not result in a trichome phenotype, but because overexpression of *CPC* reduces trichome initiation, this gene is also thought to negatively regulate trichome initiation[12] (Fig. 2).

Once a cell enters the trichome pathway it undergoes an elaborate morphogenesis program that has been divided into different stages based on specific morphological hallmarks[13] (Fig. 1). The *GL3* and *GL2* genes regulate several aspects of trichome morphogenesis and function upstream from most other morphogenesis genes[9,14,15]. For example, mutations in *GL3* result in a reduction in endoreduplication (DNA synthesis cycles in the absence of cell division) levels and smaller, less branched trichomes[9]. Mutations in *GL2* alone do not affect trichome initiation or endoreduplication but do result in a loss of aerial expansion during trichome development. The genetic interactions between *GL2*, *GL3* and *TRY* provide some interesting results. Leaves of the *gl2 gl3* double mutant lack evidence of trichome morphogenesis, *gl3 try* double mutants produce a reduced number of trichomes, but the trichomes that do develop are often clustered and exhibit the same morphology as *gl3* trichomes[9] (D. Marks, unpublished). Interestingly, *try gl2* double mutant plants produce trichomes with a nearly wild-type morphology, but have the *try* clustered phenotype[9]. These genetic analyses have been used to make a model (Fig. 2), which proposes that *GL1*, *GL3* and *TTG1* are required for normal trichome initiation. The model also explains the suppression of the *gl2* trichome phenotype by *try*. *GL2* and *GL3* are proposed to have redundant functions to regulate genes needed for trichome morphogenesis, and relief of *TRY* inhibition of *GL3* in *try gl2* double mutant plants allows trichomes resembling those of the wild type to develop.

To understand trichome initiation control mechanisms it is essential to clone the regulatory genes and to study the function of the encoded protein products (Table 1). The key initiation genes encode a *myb* (*GL1*), a *bHLH* (*GL3*) and a *WD-40* repeat-containing protein (*TTG1*), which mirrors the triad of regulatory genes that control anthocyanin biosynthesis in petunia[16,17]. Thus, a model involving the regulated assembly of a multimeric complex composed of GL1, TTG and GL3, might have general applicability for predicting how pathways are regulated in other systems.

Trichome promoting complex: GL1, TTG and GL3

GL1 encodes a *myb*-type transcription factor that is initially expressed diffusely in young leaf primordia. As leaf development progresses, *GL1* expression persists transiently in developing trichomes, but is no longer detected in surrounding epidermal cells[18,19]. Mutations in *TTG1* are pleiotropic, but the leaf trichome phenotype is indistinguishable from that of *gl1*. Almost all of the *TTG1* coding sequence consists of *WD-40* repeats; therefore it is likely that the function of *TTG1* is to recruit and/or assemble additional regulatory factors[20]. Northern blot experiments and the variety of tissues affected by mutations in *TTG1* suggest that *TTG1* is expressed in most major plant organs.

Both *TTG1* and *GL1* are required for normal leaf trichome initiation. Overexpression of *GL1* using the cauliflower mosaic virus 35S promoter (*GL1*^{oe}) is not sufficient for substantial ectopic trichome formation or for bypassing the requirement for *TTG1* (Refs 13,21), although mutation of *TRY* can enhance initiation in *GL1*^{oe} *ttg1* plants[22]. Indeed, overexpression of *GL1* leads to a developmentally regulated decrease in trichome number[21,23], hinting at the dual role of *GL1* in the negative regulation of trichome initiation. Overexpression of *GL1* can cause widespread trichome initiation if the maize *bHLH*-containing *R* gene, which qualitatively compliments all aspects of *TTG1* function, is co-expressed with *GL1* (Ref. 21). GL1 and R physically interact *in vitro*, and it is possible that the R–GL1 complex forms a dominant, transcription-activating complex[13]. However, a stable DNA-binding activity of the R–GL1 complex has not been detected using recombinant proteins.

The recent cloning of *GL3* has shed some light on the relationship between the maize *R* gene and *Arabidopsis* trichome development. The *GL3* gene is essential for several aspects of normal trichome development. In the Landsburg *erecta* (L*er*) background, *GL3* mutations cause a reduction in trichome number in leaves one to four (A. Lloyd, unpublished.). In the Columbia (Col) background, mutations in *GL3* appear to have a minor effect on trichome number, but lead to an increase in clustering (J. Larkin, pers. commun.). It is possible that the effect of *GL3* on initiation is masked by the presence of additional *GL3*-like genes in *Arabidopsis*. In addition, *gl3* trichomes exhibit reduced branching and endoreduplication levels[9]. *GL3* is a member of the *bHLH* class of transcription factors and is closely related to the maize *R* gene (T. Payne, F. Zhang and A. Lloyd, unpublished). Unlike *R*, *GL3* overexpression does not cause widespread trichome initiation in the *ttg1* background. However, in a wild-type background, *GL3* overexpression leads to a dramatic increase in trichome number, similar to that observed in 35S::*R ttg1* plants (T. Payne, F. Zhang and A. Lloyd, unpublished). Therefore *TTG1* is required for full *GL3* over expression-dependent activation of trichome initiation. Based on western blots of fractionated petunia corolla cells, the AN11 protein (a *WD-40* repeat-containing protein with extensive amino acid sequence similarity to *TTG1*) was only

Table 1. Summary of the key regulatory genes during trichome initiation and early morphogenesis

Locus	Gene identity	Affected tissues or cells	Trichome number	Trichome spacing	Trichome DNA synthesis effects	Expression pattern	Refs
GL1	MYB	Leaf epidermis	Glabrous	Clustering	ND	Developing leaf, transient trichome	18,19,25
GL1^{oe} [a]	MYB	Leaf epidermis	Reduced	Clustering	Reduced	Ubiquitous	11,21
TTG1	WD-40 repeat	Pleiotropic	Glabrous	Clustering	ND	Ubiquitous	20
GL3	bHLH	Leaf epidermis	Reduced	ND	Reduced	ND	[b]
GL2	HD-zip	Pleiotropic	Normal, (loss of aerial expansion)	Normal	No effect	Developing leaf, stable trichome	8,13
TRY	NC	Leaf epidermis, mesophyll	Normal[c]	Clustering	Enhanced	ND	9,11,25
CPC	MYB-like	Trichome root hair spacing	Normal[d]	ND	ND	ND	12
COT1	NC	Leaf epidermis	Normal[c]	Normal[c]	ND	ND	23

[a]Transgenic line in which *GL1* expression is under the control of the cauliflower mosaic virus 35S promoter.
[b]A. Lloyd, unpublished.
[c]Single mutant shows no significant affect, but in some double mutant combinations trichome number or spacing is affected.
[d]Overexpression leads to a glabrous phenotype, trichome number is not affected in the mutant.
Abbreviations: ND, not determined; NC, not cloned.

detected in cytoplasmic fractions[16]. The subcellular localization and mode of *TTG1*-dependent regulation in the *Arabidopsis* epidermis is not known.

Trichome-promoting activity appears to be specific to *R* and a subset of *R*-like *bHLH*-containing genes including *GL3*, EST AT146D23T7 (T. Payne, F. Zhang and A. Lloyd, unpublished) and the maize *B* gene (D. Marks, unpublished). Similar results have not been observed with some *R*-like *bHLH*-containing genes, such as *DELILA* (Ref. 24) and *AtMyc1* (F. Zhang and A. Lloyd, unpublished). Amino acid sequence analyses, domain swapping experiments and a comparison of the dimerization compatibilities of different *bHLH*-containing proteins might provide insight into the function of this class of proteins in the context of trichome initiation.

Recent experiments using the yeast two-hybrid assay have detected interactions between GL3 and both GL1 and TTG1 (T. Payne, F. Zhang and A. Lloyd, unpublished). An interaction between GL1 and TTG1 was not detected in these experiments. In addition to forming homodimers, GL3 has the potential to act as a bridging molecule between TTG1 and GL1: different N-terminal domains of GL3 can bind to GL1 and TTG1. These data and the genetic interactions between each of these regulatory genes suggest that the assembly of an active GL1–GL3–TTG1 complex is needed for normal trichome initiation (Fig. 3). However, complexes that lack any one of the components still have some trichome-

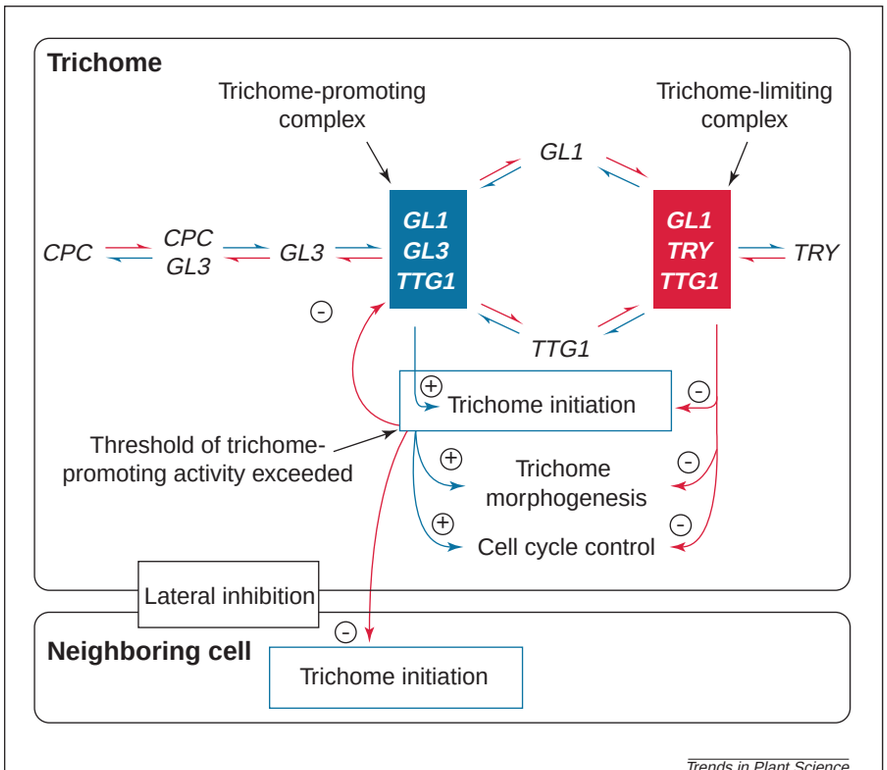

Fig. 3. A model proposing the interactions and equilibrium that affect trichome initiation and spacing. The upper box symbolizes a cell that has exceeded a threshold of trichome-promoting activity and is inhibiting an adjacent cell symbolized by the lower box. The equilibria between individual components and the hypothesized multimeric complexes that assemble are symbolized with opposite arrow sets. Blue arrows indicate the direction of the reactions that favor trichome formation. Red arrows indicate reactions that limit trichome initiation. The complexes highlighted in filled blue and red boxes are hypothesized to be the key complexes in the positive and negative regulation of trichome initiation, respectively. Encircled + symbols indicate positive regulation. Encircled − symbols indicate negative regulation.

promoting activity. Genetic experiments have shown that either *GL1* or *TTG1* is sufficient for limited trichome initiation, but the *gl1 ttg1* double mutant is completely glabrous[25]. *GL1* and *TTG1* have similar effects on the transcription level of the *GL2* promoter[13]. Loss of function mutations in either *GL1* or *TTG1* yield higher *GL2* transcription levels compared to the *gl1 ttg1* double mutant.

It is likely that *GL1*, *TTG1* and *GL3* are expressed in fields of cells that have the potential to become trichomes. If this is true, then it is possible that:

(1) The gene targets for trichome initiation requires some threshold concentration or activity of the complex to be activated.
(2) The composition or activity of the complex is regulated by local fluctuations in positively and negatively acting factors.

Pattern formation: negative regulation, dual function genes and redundancy

Given that the GL1–GL3–TTG1 complex regulates genes needed for trichome initiation, the regulated assembly of the proposed trichome-promoting complex does not adequately explain the control of trichome initiation. Trichomes are evenly spaced over the leaf surface and this spacing appears to be the result of cell–cell communication and an inhibitory pathway[5]. Fluctuations in the levels of trichome-promoting factors in fields of cells that have the potential to enter the trichome pathway cannot explain the non-random pattern of trichome initiation. Additional parameters that limit trichome initiation are likely to affect the pattern formation on the leaf surface.

The molecular genetic analyses of trichome spacing suggest that redundant negative regulation and an antagonistic function involving genes that promote trichome initiation are important for trichome pattern formation. Recent data have been summarized in a simple model (Fig. 3) with two unique, but functionally redundant components for negative regulation:

(1) Antagonistic activities of a *GL1–TTG1*-dependent complex that limits trichome initiation in fields of cells that have the potential to enter the trichome pathway (a sorting mechanism).
(2) A trichome-derived diffusible inhibitory signal that arises from cells that obtain a threshold of trichome promoting activity. Both activities contribute to the fine spatial control of trichome initiation.

GL1 and TTG1 are dual function genes

It has been well documented that both *GL1* and *TTG1* have the capacity to limit trichome initiation. For example, overexpression of *GL1* leads to reduced trichome initiation in the leaf. This phenotype can be partially suppressed by mutations in the *TTG1*, *TRY* and *COTYLEDON TRICHOME1* (*COT1*) genes[10,11,21,23]. Recently, it has been reported that hypomorphic alleles of *GL1* and *TTG1* yield clusters of aborted trichomes, and that allele-specific interactions between *gl1* and *ttg1* cause a non-additive increase in clustering[25]. In combination, these results strongly suggest that both *GL1* and *TTG1* can limit trichome initiation and that they function as a complex. Perhaps a balance of trichome-promoting and -limiting activities regulates the initiation in fields of cells that have the potential to enter the trichome pathway. Fluctuations in the levels of positive and negative factors in fields of cells that have the potential to enter the pathway, and the requirement for a threshold of trichome-promoting activity could contribute to the spatial control of initiation. In this model a clustering phenotype does not necessarily define a component of the intercellular communication and lateral inhibition pathway. Any mutation that pushes the equilibrium towards trichome-promoting activity could give rise to clusters. In the case of *gl1*, *ttg1* and *gl3*, clusters of abortive trichomes could reflect the inability to attain or maintain a sufficient threshold of trichome-promoting activity to trigger normal spacing control pathways.

We propose that GL1 and TTG1 partner with different proteins that affect their trichome promoting activity. TRY is a good candidate as an interacting protein that alters the activity of GL1 and TTG1-containing complexes. Like *gl1* and *ttg1*, *try* and *ttg1* display non-additive, allele-specific interactions with respect to trichome clustering[22,25]. In addition, mutations in both *TRY* and *TTG1* can suppress the reduced trichome number phenotype of $GL1^{oe}$ plants in a dose-dependent manner[11,21]. These genetic data are consistent with the idea that a GL1–TRY–TTG1 complex negatively regulates trichome formation. In this model, the clusters of trichomes on *try* leaves are caused by the misregulated and enhanced activity of the initiation complex. Based on the clustering phenotype on *try* leaves, previous models have defined *TRY* as a component of an intercellular inhibition pathway[21,26]. The possibility that *TRY* is a component of intercellular inhibition cannot be ruled out, and the models are not mutually exclusive. Future experiments must address the alternative mechanisms of *TRY*-dependent control of trichome initiation.

The model of initiation control (Fig. 3) mirrors the antagonistic activity of *GL3* and *TRY* in trichome DNA replication[9]. The trichome DNA content in $GL1^{oe}$ trichomes is reduced compared with that of the wild type. This reduction requires *TRY* function because $GL1^{oe}$ *try* trichomes contain a greatly elevated DNA content. The activity of an additional gene was proposed for $GL1^{oe}$-dependent promotion of DNA replication because the DNA content in $GL1^{oe}$ *try* plants was greater than *try* alone. Because mutations in *GL3* suppress the $GL1^{oe}$ *try* phenotype, it might be required for enhanced endoreduplication in $GL1^{oe}$ plants (D. Szymanski and D. Marks, unpublished).

If the model for an antagonistic role of TTG1–GL3–GL1 and TTG1–TRY–GL1 is correct, anything that pushes the equilibrium towards the assembly of the GL1–TRY–TTG1 complex will inhibit trichome formation. For example, if GL3 activity is limiting, more TTG1–GL1 complex will associate with TRY, leading to a reduced capacity for initiation. The *CPC* gene encodes a *myb*-like protein that inhibits trichome initiation when overexpressed[12]. CPC has been reported to physically interact with the maize R protein (T. Wada, T. Tatsuhiko, Y. Shimura and K. Okada, unpublished). If CPC interacts with GL3 in a similar manner, and excludes GL1 or TTG1 binding, it would limit trichome initiation by removing GL3 from the equilibrium (Fig. 3).

The extent to which the equilibrium can be pushed to promote trichome initiation might be limited. Plants that over express the maize *R* gene, *GL1*, and that are homozygous for *try*, do not display universal conversion to the trichome fate in the leaf epidermis[22]. Perhaps the redundant negative regulation by genes such as *CPC* or *COT1* or by cell positional information influences trichome initiation early in leaf development.

Redundant negative regulation: a diffusible inhibitor?

Stochastic variation in the levels of positive and negative factors and competition for a threshold of trichome-promoting activity in a two-dimensional field of cells would yield some level of spatial control of initiation. However, this scheme does not include a mechanism for inter-cellular communication or for inhibiting neighboring cells from adopting the trichome fate. It is logical that the inhibition signal would be tightly coupled to the activity of the trichome-promoting complex, and upregulated or stabilized in cells that have a threshold of trichome-promoting activity. Defects in the inhibition signal should yield nests of trichomes.

Conventional genetic screens have not identified a mutation that causes severe trichome clustering. It is likely that trichome

spacing is redundantly regulated. For example, the *COT1* gene appears to limit trichome initiation in the *GL1^{oe}* background. Trichome initiation is dramatically enhanced if both *COT1* and *TRY* function are altered[11,23], implying that at least two distinct pathways limit trichome initiation. It is likely that enhancer and suppressor screens in sensitized genetic backgrounds, such as *GL1^{oe}*, in which trichome initiation control is altered, will identify important genes that limit trichome initiation.

The proposed model provides a robust redundant regulatory scheme to regulate trichome initiation events. The model is speculative, but it is consistent with the genetic and molecular data regarding the control of trichome initiation. Specific interactions are predicted and can be studied biochemically and *in vivo*. However, the difficult task of understanding how the balance of antagonistic activities is regulated during a dynamic process remains. For example, is there cross talk between antagonistic pathways, or does competition for limiting factors and DNA-binding sites regulate function? Does the TTG1–GL3–GL1 complex autoregulate its activity once a threshold of trichome-promoting activity is reached? What regulates the longevity of the complex? *GL1* expression occurs only transiently in developing trichomes. Perhaps there is a window of trichome development during which *GL1* is required, and other *myb* regulatory proteins, such as the trichome-specific *Atmyb5*, propagate regulatory cascades[27].

Trichome morphogenesis

Trichome morphogenesis is a unique experimental system to study how transcription factor function and cell cycle parameters affect the cytoskeleton and cell shape. Many genes required for trichome morphogenesis have been identified[9,14,15,28,29]. This is to be expected as many different cellular processes are involved. These include mechanisms to identify and maintain specific cell expansion sites, to maintain cell turgor, to promote cell-wall loosening and wall synthesis, and to control the rate and duration of cell expansion[30].

During trichome morphogenesis, a protodermal cell ceases to divide. Instead it expands according to a complex developmental program into a single cell that is composed of a stalk and two to four branches (Fig. 1). The first visible sign of trichome initiation is the radial expansion of a protodermal cell. During this phase of expansion, the nucleus is enlarged and situated at the cell center, which is proposed to be the first of several specific endoreduplication events[9]. However, the precise relationship between DNA content and trichome morphogenesis is not clear. The mature trichome nucleus contains as little as 8 C or as much as 64 C DNA (Refs 11,31). There is a loose correlation between trichome size and number of endocycles, because most mutants with larger trichomes have increased amounts of DNA, but in some cases enlarged cells do not have an elevated DNA content[28].

Cytoskeletal organization is essential for normal trichome morphogenesis[32]. Based on pharmacological data, polarized growth and branch initiation during stages two and three (Fig. 1) require normal microtubule-dependent function[33,34]. This was clearly shown using a dexamethose-regulated form of the maize *R* gene to induce trichome formation in the presence or absence of the microtubule-depolymerizing agent oryzalin. Application of oryzalin before initiation causes isotropic cell expansion without branch formation[34]. The *ZWICHEL* (*ZWI*) gene encodes a kinesin-like motor protein[35]. The reduced branching and cell expansion phenotype of *zwi* trichomes might reflect defects in either the transport of cell expansion components or in motor-dependent cortical microtubule organization. The cloning of genetic modifiers of *ZWI* should clarify its function during morphogenesis[29].

The actin cytoskeleton is not essential for polarity establishment during trichome formation, but the maintenance of cell shape control after stage three requires a precisely organized F-actin-cytoskeleton[34]. Interestingly, agents that disrupt actin organization phenocopy the trichome defects in the 'distorted' class of trichome mutants[33,34]. The distorted trichome morphology mutants have been previously identified[9,36], but recent data suggest that an analysis of the 'distorted' genes might be useful in understanding the relationship between actin organization and localized cell expansion.

The genetic control of branch formation has been examined closely. Mutations have been identified that either reduce or increase the number of branches. Several recent reports have used pair wise crosses between branch mutants to study the genetic relationship between these genes. Trichomes usually contain three branches. Previous models have attempted to separate the formation of the first two branches and the formation of the second and third branch into two genetically distinct steps[9,14]. This hypothesis was developed on the basis of the interaction of two reduced-branching mutants, *angustifolia* (*an*) and *stachel* (*sta*), with two branch-enhancing mutants, *try* and *noeck*. However, the analysis of four new branch mutants (*furca1–4*) and their genetic interactions with each other, with *an* and with *sta* indicate that most of these genes influence the formation of all branches[15]. A remarkable finding is that mutations in five different genes, *frc1, frc3, frc4, an* or *sta*, allow the formation of two branched trichomes. However, any of the ten pair-wise combinations of double mutants, such as *frc1 frc3*, results in the complete loss of branch formation. It appears that four of the genes, in any combination, are sufficient for branching. Because the branching mutants behave in a quantitative fashion, it is likely that increasing the activity of one can compensate for the loss of another; a model for branching control based on compensation has been proposed[15]. The isolation and altered expression of these genes will allow these predictions to be tested. In addition, the characterization of these genes will provide important information on how localized cell expansion is initiated in plants.

Conclusion

The control of trichome initiation in *Arabidopsis* leaves is surprisingly complex: cell cycle status, transcriptional control and cytoskeletal function comprise an integrated hierarchy of regulation. A basic description of signal transduction and regulation is beginning to emerge. Probabilistic events, based on the balanced activity of positive and negative regulation and a trichome-derived inhibitory signal are proposed to control trichome cell fate in fields of cells that have the potential to transmit differentiation signals. Although this model provides a testable framework to study genes that affect trichome initiation, it is highly speculative and lacks supporting biochemical data. For example, many questions about the composition, regulation and activity of the hypothesized multimeric trichome-promoting complex are unanswered. As more of the players in trichome development are identified and studied, a more mechanistic understanding of cell fate and morphogenesis control will follow.

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Dan Szymanski* is at Purdue University, Dept of Agronomy, 1150 Lilly Hall of Life Sciences, W. Lafayette, IN 47907, USA; Alan Lloyd is at the University of Texas at Austin, Molecular Cell and Developmental Biology, 1.448 Molecular Biology Building, Austin, TX 78712, USA; David Marks is at the University of Minnesota, Dept of Plant Biology, 220 Biosciences Center, St. Paul, MN 55108, USA.

*Author for correspondence (tel +1 765 494 8092; fax +1 765 496 7255; e-mail dszyman@purdue.edu).

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