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A novel upstream regulator of trichome development inhibitors

Much like the spikes that deter birds from sitting on fences, trichomes — hair like projections on the leaf surface — are the epidermis' first line of defence, discouraging insects and other pests (Levin, 1973). In addition to their role protecting the plant, trichomes are an excellent marker to study developmental biology: they arise from the same progenitor cells as stomata and pavement cells, are easily visible, and their patterning is under strict genetic control.

To better understand trichome development, we must understand two core processes: trichome patterning (or the fate determination of progenitor cells), and trichome differentiation, both of which have been most studied in *Arabidopsis thaliana*. The decision to adopt a trichome or other epidermal cell identity is regulated antagonistically by two protein complexes termed the initiator complex and the inhibitor complex (Figure 1A). The initiator complex consists of GLABRA1 (GL1), GLABRA3 (GL3) or ENHANCER OF GL3 (EGL3), and TRANSPARENT TESTA GLABRA1 (TTG1) (reviewed in Pattanaik et al., 2014). This initiator complex activates expression of *GLABRA2* (*GL2*), whose transcription factor product initiates trichome morphogenesis (Szymanski et al., 1998). Replacement of GL1 by an inhibitor renders the complex unable to activate *GL2* expression, preventing trichome morphogenesis. While the expression of most trichome inhibitors is regulated by the initiator complex, a small number are not, including *TRICHOMELESS1* and *2* (*TCL1* and *TCL2*). To date, the roles of *TCL1* and *TCL2* in trichome development in leaves have not been extensively studied, and their upstream regulators in this early stage of development are unknown.

In this issue of *Plant Physiology*, Vadde *et al*., 2019, from the Indian Institute of Science, present their comprehensive characterisation of the role of a class II TCP (a family named after the first four studied members: TEOSINTE BRANCHED1 (TB1; *Zea mays*), CYCLOIDIA (CYC; *Antirrhinum majus)* and PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR1/2 (PCF1/2; *Oryza sativa*)) in the regulation of *TCL1* and *TCL2*, and therefore its role in trichome development on *Arabidopsis thaliana* leaves. The authors hypothesised that upstream regulators of *TCL1* and *TCL2* would be transcription factors present for the duration of leaf morphogenesis. The TCPs are a family of plant-specific transcription factors that can be split broadly into two classes, which act antagonistically to promote (class I) or repress (class II) cell proliferation (reviewed in Martín-Trillo and Cubas, 2010). TCPs are known to regulate several developmental processes, including leaf shape determination, petal and stamen development, and circadian clock function (Li, 2015). In addition, TCP expression overlaps both temporally and spatially with factors involved in trichome initiation, making them a potential candidate in this process.

Plants with TCP4 (a class II TCP) gain-of-function have previously been observed to have fewer trichomes on their leaf surfaces compared to wild-type plants (Efroni et al., 2008).

Vadde and colleagues confirmed this observation through examining two TCP4 gain-of-function mutants: *TCP4:VP16*, in which *TCP4* is fused to a viral activation domain; and *pBLS::rTCP4:GFP*, which expresses a miR319-resistant form of TCP4 fused to GFP under an early leaf-specific promoter. Both plant lines showed reduced trichome density compared to the wild-type. Conversely, two TCP4 loss-of-function lines, the triple mutant *tcp2 tcp4 tcp10* and *jawD* (an over-expressor of miR319, whose targets include class II TCPs *TCP2, TCP3*, *TCP4*, *TCP10,* and *TCP24*), displayed an increased trichome density compared to the wild type (Figure 1B).

Publicly available gene expression data show that positive regulators of trichome initiation *GL1* and *GL2* are significantly up-regulated in *TCP* loss-of-function mutants and vice-versa in gain-of-function mutants. This was validated by Vadde and colleagues by qPCR time-course experiments and GUS assays (Figure 1C), demonstrating that TCP4 suppresses the expression of these two genes. As the class II TCPs are transcriptional activators, it was hypothesised that this down-regulation of *GL1* and *GL2* by TCP4 is an indirect effect.

To identify which genes act downstream of TCP4 in GL2 transcriptional regulation, transcript levels of known trichome initiator and trichome inhibitor complex genes were compared between a TCP4 gain-of-function line and the wild type. Four genes, *TCL1*, *TCL2*, *ENHANCER OF TRY AND CPC2* (*ETC2*), and *ETC3*, were up-regulated in the TCP4 gain-of-function line. Comparison to microarray data previously produced in the Nath lab revealed that only *TCL1* and *TCL2* are activated soon after TCP4 induction (Challa et al., 2016). *TCL1* and *TCL2* activation is associated with down-regulation of *GL1*, supporting the model that TCP4 prevents trichome morphogenesis via activation of *TCL1* and *TCL2*, which constitute part of the trichome inhibition complex.

Vadde and colleagues go on to show that TCP4 directly targets *TCL1* and *TCL2*. In the absence of protein synthesis, induction of TCP4 increased *TCL1* and *TCL2* transcript levels by at least 2-fold. Putative TCP4 binding sites were identified in the regulatory regions of both *TCL1* and *TCL2*. These *in silico* predictions were verified by *in vitro* electrophoretic mobility shift assays (EMSAs), which demonstrated that TCP4 binds specifically to these sequence motifs, and chromatin immuno-precipitation (ChIP) showed that TCP4 is recruited to these sites in the genome. Formaldehyde-assisted isolation of regulatory element (FAIRE) assays confirmed that the presence of TCP4 increases chromatin accessibility in the promoters of *TCP1* and *TCP2*. Furthermore, an *in vivo* luciferase assay in isolated protoplasts and an *in planta* GUS assay both confirmed that TCP4 binds to and activates the *TCL1/2* genes, supporting the model that trichome morphogenesis is inhibited by TCP4 through direct activation of *TCL1* and *2* (Figure 1D).

Further genetic experiments showed that TCL1 is required for the reduction in trichome number observed in the leaves of TCP4 overexpression lines. Here, examination of trichomes on the inflorescence stems and older leaves could have been included to determine whether TCP4 regulation of *TCL1* is restricted to young leaves or is ubiquitous throughout plant development.

The thorough work presented in this paper is an important step forward in our understanding of trichome development, summarised in Figure 1E. For the first time, an upstream transcription factor controlling inhibitory regulators of trichome morphogenesis in leaves has been identified. Excitingly, TCP4 is also implicated in repression of cell proliferation (Schommer et al., 2014) and in fundamental leaf patterning (Koyama et al., 2017), and thus may play an important role in the co-ordination of development. As there is some degree of functional redundancy within the TCP family (Danisman et al., 2013), whether further TCPs are involved in trichome initiation and patterning must await further study. TCPs are ubiquitous across plant species; thus, advances in our understanding of their role in co-ordinating development will also be beneficial when considering economically important crop species.

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Figure 1: Graphical summary of the paper
A – Previous work has shown that GL2 positively regulates trichome initiation.
B – This paper uses several lines with altered *TCP4* expression. Lines where *TCP4* function is lost are *tcp2;4;10* and *jaw-D*. Lines where *TCP4* is overexpressed are *TCP4:VP16, pBLS::rTCP4:GFP*, inducible mTCP4 *jawD*;GR, inducible mTCP4 Col-0;GR, and p35S::mTCP4:GR. The wild-type Col-0 is used as a control.
C – Several techniques were used to study the indirect effect of TCP4 on GL2 and thus trichome number, including use of existing databases, qPCR, and GUS expression in stably transformed lines.
D – Several techniques were used to analyse TCP4 binding and activity, including genetic screens, binding motif prediction, EMSA, ChIP, FAIRE, and transient luciferase assays.
E – The authors propose that TCP4 affects trichome initiation through directly binding to the *TCL1/2* promoter, which in turn inhibits *GL2*. Adapted from Figure 6E of Vadde *et al.*, 2019. Figure created with biorender.com

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