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SHORT COMMUNICATION

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Arabidopsis thaliana TCP15 interacts with the MIXTA-like transcription factor MYB106/NOECK

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ABSTRACT

MYB106 and MYB16 are MIXTA-like transcription factors that control trichome maturation and cuticle formation in *Arabidopsis*. In a recent study, we found that the TEOSINTE BRANCHED 1, CYCLOIDEA and PROLIFERATING CELL FACTORS (TCP) transcription factor TCP15 also acts as an important regulator of aerial epidermis specialization in *Arabidopsis* through the control of trichome development and cuticle formation. TCP15 and MYB106 regulate the expression of common groups of genes, including genes coding for transcription factors and enzymes of the cuticle biosynthesis pathway. In this study, we report that TCP15 physically interacts with MYB106 when both proteins are expressed in yeast cells or *Nicotiana bentamiana* leaves. Furthermore, we also observed interaction in leaves of *Arabidopsis thaliana*. Altogether, our findings raise the possibility that TCP15 and MYB106 bind together to the promoters of target genes to exert their action. Our data provide a base to investigate the role of TCP-MIXTA complexes in the context of cuticle development in *Arabidopsis thaliana*.

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The epidermis of plant aerial organs contains specialized cells such as trichomes and is covered by a layer of cutin and waxes, the cuticle, both of which perform protective functions. MYB16 and MYB106/NOECK are MIXTA-like R2R3-MYB transcription factors that control trichome maturation, including limitation of trichome branching, and act as positive regulators of cuticle formation in *Arabidopsis thaliana*.[1,2] MYB106 positively regulates cutin biosynthesis and wax accumulation through regulation of the expression of cutin and wax biosynthetic genes and WAX INDUCER1/SHINE1 (SHN1), an APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) transcription factor that also positively regulates cutin biosynthetic genes.[1,3,4]

TEOSINTE BRANCHED 1, CYCLOIDEA and PROLIFERATING CELL FACTORS (TCP) transcription factors constitute a plant-specific gene family and orchestrate numerous growth-related processes during *Arabidopsis* development in response to both environmental and endogenous cues.[5–9] TCP family members can be divided into two classes[10] and exert their biological functions through interaction with each other or with other proteins.[11,12] There are 24 members of the TCP protein family in *Arabidopsis* assigned to either class I (13 proteins) or class II (11 proteins).[5] Among them, the class I TCP proteins TCP14 and TCP15 have key roles during seed germination,[13–16] hypocotyl cell elongation,[17,18] leaf and stem growth,[6] flowering,[19] stamen filament elongation,[20] and gynecium development.[21] In addition, TCP14 and TCP15 play an important role in immune responses[22,23] and redox modulation of anthocyanin accumulation under high irradiation conditions.[24] Recently, we described an additional function of these class I TCPs in cuticle development.[25] We observed that *tcp14* and *tcp15* single loss-of-function mutants exhibited increased cuticle permeability and that expression of a TCP15 chimeric repressor fusion (TCP15-EAR) under the control of the *TCP15* promoter also induced cuticle deficiencies.[25] TCP14 and TCP15 regulate cuticle formation through the activation of the expression of genes encoding SHN1, SHN2 and MIXTA transcription factors, as well as CYP86A4, GPAT6 and CUS2 cuticle biosynthesis enzymes.[25] In addition, we found that TCP14 and TCP15 affect trichome branching by modulating nuclear endoreduplication of trichome cells and the expression of MIXTA transcription factors.[25] We identified *MYB106* as a direct target of TCP15 and determined that *MYB106* overexpression only partially rescued the overbranched trichome phenotype of a *tcp14 tcp15* double mutant. Reciprocally, overexpression of *TCP15* in the *myb106-2* mutant background also partially rescued the aberrant trichome phenotype of this mutant.[25] Moreover, we uncovered a significant overlap in genes regulated by TCP15 and MYB106, among them some that are direct targets of both transcription factors.[25] These results led us to analyze the possible existence of physical interactions between TCP15 and MYB106, which would be consistent with their involvement in the control of the expression of similar groups of target genes. For this purpose, we first performed a yeast two-hybrid assay using fusions of TCP15 and MYB106 to the GAL4 activation domain (AD) and DNA-binding domain (BD), respectively. We observed a strong increase in β-galactosidase activity relative to controls when both fusions were co-expressed (Figure 1a). In addition, the yeast strain expressing TCP15-AD and MYB106-BD, but not

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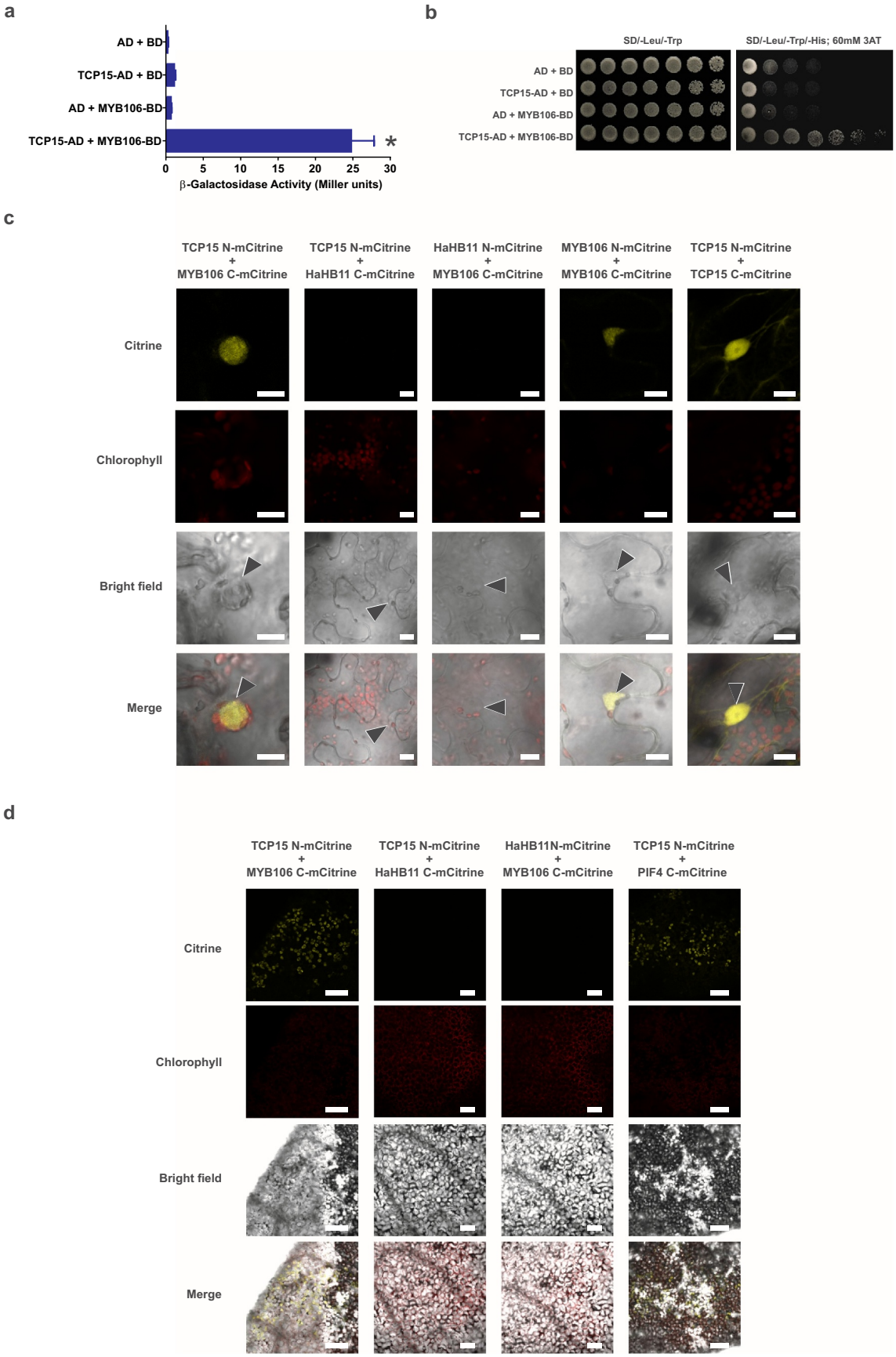

Figure 1. TCP15 physically interacts with MYB106. (a,b) Yeast two-hybrid analysis of the interaction between TCP15 and MYB106. The MaV203 yeast strain, containing *LacZ* and *HIS3* reporters under the control of GAL4-responsive promoters, was transformed with constructs that allow the expression of TCP15 and MYB106 fused to the GAL4 AD and BD, respectively. As a control, vectors pGADT7 and pGBKT7 were used to express the AD and BD alone, respectively. (a) Average ß-galactosidase specific activity values obtained in three independent assays are shown. The asterisk indicates significant difference with the AD + BD control ($P < .05$; ANOVA). (b) Analysis of the capacity to grow in media lacking histidine and in the presence of 3-AT. Serial dilutions of yeast cultures expressing the indicated proteins were spotted onto plates

yeast strains expressing only one of the fusion proteins or the AD and BD domains alone, was able to grow on media lacking histidine (Figure 1b), indicating that the *HIS3* reporter gene was also induced. These results indicate the existence of physical interactions between TCP15 and MYB106 in yeast cells. To determine if the formation of the TCP15-MYB106 complex also occurs in plants, we performed a Bimolecular Fluorescence Complementation (BiFC) assay in *Nicotiana benthamiana*. Reconstitution of fluorescence was observed in cell nuclei when TCP15 and MYB106 fused to the N- and C-terminal moieties of mCitrine, respectively, were co-expressed (Figure 1c). The intensity of florescence was similar to that observed when fusions to both mCitrine moieties of TCP15, known to form homodimers,[17] where co-expressed (Figure 1c). However, no fluorescence was observed when the constructs expressing the fusions of either TCP15 or MYB106 were co-infiltrated with fusions to HaHB11 (Figure 1c), a HD-Zip transcription factor from *Helianthus annuus* used as a negative control,[26] suggesting that the observed reconstitution of fluorescence was effectively due to the interaction of MYB106 and TCP15. Notably, we also observed the possible formation of homodimers for MYB106 (Figure 1c). In experiments performed with the reciprocal fusions (i.e. TCP15 and MYB106 fused to the C- and N-terminal moieties of mCitrine, respectively), reconstitution of fluorescence was also observed (Supplementary Figure S1). In this case, however, a similar signal was observed with TCP15 and the negative control HaHB11 (Supplementary Figure S1), leading to a less conclusive result. Nevertheless, no signal was observed when MYB106 and HaHB11 were co-expressed (Supplementary Figure S1). Altogether, the results suggest that TCP15 and MYB106 are able to form protein-protein complexes in the nucleus of N. *benthamiana* cells. Next, we explored if the TCP15-MYB106 interaction also occurs in *Arabidopsis*. We performed a BiFC assay in 15-day-old *Arabidopsis* seedlings using the reported interaction between TCP15 and the transcription factor PIF4[17] as a positive control. We observed that, similar to the co-expression of TCP15 and PIF4 fused to the N- and C-terminal moieties of mCitrine, the co-expression of TCP15 and MYB106 fusions caused a reconstitution of fluorescence (Figure 1d). In turn, no fluorescence was observed when either TCP15 or MYB106 fusions were co-expressed with those of the negative control HaHB11 (Figure 1d). These results confirm that TCP15 and MYB106 form protein-protein complexes in *Arabidopsis thaliana*.

Transcription factors are known to form protein-protein complexes to exert their action. For TCP proteins, it has been reported that they are able to form complexes with each other or with other transcription factors.[8,11,12] Recently, MYB106 has been identified as a substrate for CRL3^{BPM} E3 ligases,[27] but no interactions with other transcription factors have been reported for MIXTA proteins in *Arabidopsis*. In this study, we found that TCP15 physically interacts with MYB106 *in vivo* in yeast and plant cells. Since these transcription factors modulate epidermis development through the regulation of the expression of common genes, our results indicate that TCP15 and MYB106 would bind together to the promoters of target genes to regulate their expression. In addition, we observed that most of the TCP15 and MYB106 co-regulated genes were regulated in the same direction by both transcription factors.[25] In the previous work, we found that TCP15 binds to TCP boxes present in the promoters of the *SHN1* transcription factor and the *CUS2* cuticle biosynthesis genes.[25] MYB106 directly regulates the activity of the *SHN1* promoter[1] and plants with defects in *TCP15* and *MYB106* expression showed reduced levels of *SHN1* and *CUS2* transcripts.[25] Then, it would be interesting to analyze if TCP15 and MYB106 bind together to the *SHN1* and *CUS2* promoters to regulate their expression and, thus, the synthesis of the cuticle. Moreover, the evaluation of the role of TCP15-MYB106 transcription factor complexes in the regulation of gene expression may provide valuable information not only on the regulatory mechanism of cuticle development but also on other processes in *Arabidopsis*.

Material and methods

Yeast two-hybrid assay

To express MYB106 fused to the GAL4 BD, the full-length *MYB106* coding sequence was amplified with specific primers (Supplemental Table S1) and inserted into the *Eco*RI/*Sal*I sites of the pGBKT7 vector (Clontech). The pGADT7-TCP15 (TCP15-AD) construct was previously described.[28] Yeast transformation and yeast two-hybrid assays were conducted as described in the Yeast Protocols Handbook (Clontech). Briefly, *Saccharomyces cerevisiae* MaV203 (Invitrogen) cells were co-transformed using the lithium acetate transformation method[29] and positive colonies were selected on dropout medium without Trp and Leu. The β-galactosidase activity from the *LacZ* reporter was analyzed as described in Viola et al.[30] using *o*-nitrophenylgalactoside as the substrate. To analyze the activity of the *HIS3* reporter, 10 µl of serial dilutions of transformed yeast cells were spotted onto plates containing dropout medium without Trp and Leu (positive growth control) or without Trp, Leu and His and with 60 mM 3-AT. The plates were incubated for 3 d at 30°C.

Bimolecular fluorescence complementation (BiFC) assay

To generate the constructs for BiFC assays, a full-length *MYB106* cDNA fragment without the stop codon was cloned into vector pENTR3C (Invitrogen) and then recombined into pAS-054 and pAS-059[31] to produce C-terminal fusions to the N- and C-terminal moieties of mCitrine. The

containing minimal medium supplemented as necessary and in the presence or absence of histidine (left and right panels, respectively) and incubated for 3 d at 30°C. (c,d) TCP15 and MYB106 interact in plant cells. BiFC assay showing the interaction of TCP15 with MYB106 in *Nicotiana benthamiana* (c) and *Arabidopsis thaliana* cells (d). Full-length TCP15 and MYB106 were fused to the N- or C-terminal fragments of mCitrine. HaHB11 and PIF4 fused to the N-terminal or C-terminal fragments of mCitrine were used as negative and positive controls, respectively. The panels show the mCitrine fluorescence, the chlorophyll fluorescence, the differential interference contrast in light microscope mode (Bright field) and the merged images. Arrows indicate nuclei. The images are representative of 40 images analyzed on 6–8 different leaves. Additional controls for the BiFC are presented in Supplemental Figure S1. Scale bars = 20 µm (C) and 100 µm (D).

constructs used for the expression of TCP15 as a fusion to the N- and C-terminal moieties of mCitrine and PIF4 as a fusion to the C-terminal moiety of mCitrine were previously described.[17] Fusions of mCitrine to the HD-Zip transcription factor HaHB11 were kindly provided by Dr. Raquel Chan (Instituto de Agrobiotecnología del Litoral, Argentina). The different constructs were introduced into *Agrobacterium tumefaciens* LB4404 and used in different combinations to transiently transform *Nicotiana benthamiana* leaves and *Arabidopsis thaliana* 15-day old seedling as described by de Felippes and Weigel[32] and Viola et al.,[33] respectively. Transformed plants were kept in the greenhouse under long-day conditions (16 h light/8 h dark) at 23°C for 48 hours. The fluorescence of mCitrine was examined using a confocal laser scanning microscope (TCS SP8, Leica Microsystems).

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Disclosure statement

The authors declare no conflict of interest.

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