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TAF13 interacts with PRC2 members and is essential for *Arabidopsis* seed development

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ABSTRACT

TBP-Associated Factors (TAFs) are components of complexes like TFIID, TFTC, SAGA/STAGA and SMAT that are important for the activation of transcription, either by establishing the basic transcription machinery or by facilitating histone acetylation. However, in *Drosophila* embryos several TAFs were shown to be associated with the *Polycomb* Repressive Complex 1 (PRC1), even though the role of this interaction remains unclear. Here we show that in *Arabidopsis* TAF13 interacts with MEDEA and SWINGER, both members of a plant variant of *Polycomb* Repressive Complex 2 (PRC2). PRC2 variants play important roles during the plant life cycle, including seed development. The *taf13* mutation causes seed defects, showing embryo arrest at the 8–16 cell stage and over-proliferation of the endosperm in the chalazal region, which is typical for *Arabidopsis* PRC2 mutants. Our data suggest that TAF13 functions together with PRC2 in transcriptional regulation during seed development.

Introduction

Transcription of protein encoding genes by RNA polymerase II (Pol II) requires the formation of the Preinitiation Complex (PIC), which is composed of Pol II and several General Transcription Factors (GTFs), such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH (Thomas and Chiang, 2006). The first step of PIC assembly is the recognition of core promoter elements, which is mainly driven by TFIID. This general transcription factor complex is composed of the TATA-box Binding Protein (TBP) and several highly conserved TBP-Associated Factors (TAFs). In *Arabidopsis thaliana* a total of 21 different *TAF* genes were identified (Lago et al., 2004; Lawit et al., 2007). Several TAFs share the presence of a Histone Fold Domain (HFD) (14 out of 21 in *Arabidopsis*), a fundamental motif for protein–protein and DNA–protein interactions that is important for TFIID structure and function. The TAF proteins facilitate TFIID binding to different core promoter elements not necessarily containing the TATA-box (Basehoar et al., 2004). Besides their ability to bind core promoter elements, TAFs also function as co-activators interacting with specific transcription factors to modulate basal transcription machinery activity (Thomas and Chiang, 2006).

According to their role in the basal transcription machinery, TAFs were expected to be required for accurate transcription of all genes. However, in yeast it was shown that each TAF regulates the expression of a limited subset of genes, ranging from 3% to 61% of the genes (Shen et al., 2003; Lee et al., 2000). Moreover, tissue-specific TAFs was were reported in animals (Voronina et al., 2007; Hiller et al., 2001). The specificity of TAFs in the regulation of distinct subsets of genes is in accordance with results obtained in *Arabidopsis*. For instance, the *taf1* mutant showed decreased levels of chlorophyll accumulation, light-induced mRNA levels, and acetylation of histone H3 in light-responsive promoters, suggesting an involvement of *TAF1* in light signal transduction (Bertrand et al., 2005). Moreover, in young *taf1* mutant leaves 9% of the genes showed an alteration in their expression. Other examples are *Arabidopsis TAF6*, which regulates pollen tube growth (Lago et al., 2005), and *TAF10*, which controls meristem activity, leaf development and osmotic regulation (Tamada et al., 2007; Gao et al., 2006).

TAFs are also components of other complexes different from TFIID, such as TFTC (TBP-free TAF_{II}-containing complex), TFTC-related PCAF/GCN5 complexes, the Spt–Ada–Gcn5 acetyltransferase (SAGA) complex, SAGA-like complexes (SLIK), the Spt3–TAF9–GCN5L acetylase (STAGA) complex, and the Small TAF complex

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(SMAT), all of which do not contain TBP (Wieczorek et al., 1998; Grant et al., 1998; Ogryzko et al., 1998; Martinez et al., 1998; Demeny et al., 2007). The majority of these complexes possess histone acetylase (HAT) activity, meaning that they are involved in gene activation (Thomas and Chiang, 2006). Surprisingly, six different TAFs were pulled-down with several components of *Polycomb* Repressive Complex 1 (PRC1) from *Drosophila* embryos (Saurin et al., 2001). *Polycomb* Group (PcG) proteins act in multi-protein complexes to maintain a repressive state of gene expression, controlling several important developmental processes. In particular, PRC1 is a well-characterized multi-protein complex able to mono-ubiquitinylate lysine 119 of histone H2A (H2AK119ub), generally recognized as a transcriptional repressive mark able to modify chromatin structure. In *Drosophila*, two other PcG containing complexes were identified named PRC2 (involved in histone H3 lysine 27 trimethylation, H3K27me3) and Pho Repressive Complex (PhoRC), which has sequence specific DNA-binding activity.

In *Arabidopsis*, most homologs of PRC1 components are absent but PRC1-like activities were recently demonstrated (Bratzel et al., 2010; Chen et al., 2010). In contrast, several variants of plant PRC2 are well characterized, and the core components of PRC2 are highly conserved in protein structure and function when compared to animals (reviewed in Schatlowski et al., 2008; Hennig and Derkacheva, 2009; Holec and Berger, 2012; Bemer and Grossniklaus, 2012). In *Arabidopsis*, three different variants of PRC2 were described, acting in different processes and developmental stages during the plant life cycle: the FERTILIZATION INDEPENDENT SEED (FIS) complex acting in the female gametophyte and during seed development, the VERNALIZATION (VRN) complex acting in the vernalization response, and the EMBRYONIC FLOWERING (EMF) complex preventing precocious flowering and regulating flower development. These complexes share common subunits, such as FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) and MULTICOPY SUPPRESSOR OF IRA1 (MSI1), and complex-specific components: FIS2 and MEDEA (MEA) or SWINGER (SWN) in the FIS-PRC2; VRN2 and CURLY LEAF (CLF) or SWN in the VRN-PRC2, and EMF2 and CLF or SWN in the EMF-PRC2 (Kohler et al., 2003a; Chanvivattana et al., 2004; Wood et al., 2006; De Lucia et al., 2008). Mutations in any of the components of the FIS-PRC2, with the exception of SWN, lead to autonomous seed development in the absence of fertilization, and to seed abortion with embryo and endosperm overgrowth when fertilization occurs (Ohad et al., 1996, 1999; Chaudhury et al., 1997; Grossniklaus et al., 1998; Luo et al., 1999; Kohler et al., 2003a).

Here, we characterize *TAF13* of *Arabidopsis* and show that, like mutants of the *fis* class, *taf13* mutants have endosperm overgrowth, suggesting a possible link between TAF13 and FIS-PRC2.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana accession Columbia was used as the wild type and grown at 22 °C in short-day (8 h light/16 h dark) or long-day (16 h light/8 h dark) conditions. Mutations disrupting *TAF13* (*At1g02680*), *taf13-1* (SALK_119642), *taf13-2* (SALK_016938), *taf13-3* (SALK_024774), *taf13-4* (SALK_078709), and the *atfh5-2* mutant (SALK_044464) were obtained from the European Arabidopsis Stock Centre (NASC) (Alonso et al., 2003). The *mea-8* mutant was supplied by NASC (SAIL_55_B04). The *mea-2* mutant was described by Grossniklaus et al. (1998), the *pMEA*::*GUS* transgenic line by Baroux et al. (2006), the *pPHE1*::*GUS* line by Kohler et al. (2003b), the *pFUS3*::*GUS* line by Makarevich et al. (2006). Plants carrying *pFIS2*::*GUS* were kindly provided by A. Chaudhury (Luo et al., 2000). The enhancer trap line KS117 was kindly provided by F. Berger (Sorensen et al., 2001).

Nicotiana benthamiana plants were grown for 4 weeks in a greenhouse under controlled conditions prior to agroinfection.

Genotyping, segregation and complementation analysis

Genotyping of *TAF13*, *taf13-1*, *taf13-2*, *taf13-3*, *taf13-4*, *MEA*, *mea-8*, *AtFH5* and *Atfh5-2* plants was done by PCR using the primers reported in Supplementary Table 1.

For complementation experiments the *TAF13* genomic region plus 2.5 Kb upstream the transcription start site was cloned into the pDONOR207 vector (Life Technologies; for primers see Supplementary Table 1) and then recombined into pGW::EGFP (pGreenII; Hellens et al., 2000). The obtained construct was used to transform *taf13-2*/*TAF13* plants using the floral dip method (Clough and Bent, 1998) through the *Agrobacterium tumefaciens* strain GV3101 pMP90. Transformed seeds were selected with BASTA.

GUS assay and microscopic analysis

The GUS assay was performed as described (Liljegren et al., 2000).

To analyze seed development in wild-type and mutant plants, seeds at different developmental stages were cleared overnight using a solution composed of 160 g chloral hydrate (C-8383; Sigma-Aldrich), 100 ml water, and 50 ml glycerol. The samples were observed using a Zeiss Axiophot D1 microscope equipped with DIC optics. Images were captured on an Axiocam MRc5 camera (Zeiss) using the Axiovision program (version 4.1).

For *pTAF13*::*TAF13-GFP* and KS117-GFP analysis, siliques at different developmental stages were dissected in water and observed using the Leica DM 6000 microscope.

In situ hybridization and expression analysis

In situ hybridization analysis was performed as described in Dreni et al. (2011). The antisense probe corresponds to a 321-bp fragment at the 5' end of the *TAF13* cDNA. The accumulation of *TAF13*, *MEA*, and *mea-2* transcripts were measured using quantitative real-time RT-PCR as described by Baroux et al. (2006). Quantitative analysis of transcript levels were carried out using Sybr Green or Taqman real-time PCR assays (Applied Biosystem). Three technical replicates were performed for each independent cDNA sample ($n=3$), and the specificity and amount of the unique amplification product were determined according to the manufacturer's instructions (Applied Biosystems). *MEA* and *mea-2* transcripts were measured to confirm the wild-type or mutant background of the samples. In all experiments, transcript levels were normalized to the level of *ACTIN1*.

Protein interaction and pull-down assays

The yeast two-hybrid assays were performed at 28 °C in the yeast strain AH109 (Clontech), using the co-transformation technique (Egea-Cortines et al., 1999). The interactions were tested on selective YSD medium lacking leucine, tryptophan, adenine or/and histidine, supplemented with different concentrations of 3-amino-triazole (5, 10 mM 3-AT). *TAF13*, *MEA*, *VRN2* and *MSI1* were cloned in the pGBKT7 and pGADT7 vectors (Clontech), passing through pENTR/D-TOPO (Life Technologies). *CLF* lacking the SET-domain and *EMF2* constructs were kindly provided by Dr. Chanvivattana (Chanvivattana et al., 2004). *FIE*, *SWN*, and *TFL2* were cloned into the pGBKT7 vector (Clontech), passing through the pENTR223 vector (Life Technologies), whereas the *SWN* lacking the SET-domain and *TFL2* lacking the chromodomain constructs were cloned into pCR8-GW-Topo (Life Technologies). The pull-down