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Arabidopsis thaliana TBP-associated factor 5 is essential for plant growth and development

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Abstract The TATA binding protein-associated factor 5 (TAF5) is a subunit of TFIID and SAGA complexes involved in RNA polymerase II transcription initiation and histone acetylation. Although members of the putative SAGA complex in *Arabidopsis* such as GCN5 and ADA2b have important roles in plant development and abiotic stress responses, the function of other components of the *Arabidopsis* putative SAGA complex, like TAF5, is unknown. We used reverse genetics to elucidate the biological role of *TAF5* in *Arabidopsis thaliana*. The absence of homozygote *taf5* mutants indicated that *AtTAF5* is an essential gene for the plant viability. Genetic approaches also revealed that *AtTAF5* plays a critical role in regulatory mechanisms involved in male gametogenesis and pollen tube growth. Moreover, *Arabidopsis taf5* heterozygous mutants displayed terminal flower-like phenotype, suggesting that *TAF5* could be involved in molecular mechanisms that regulate indeterminate inflorescence meristems. Therefore, this work suggests that *Arabidopsis TAF5* is necessary and sufficient for a complete plant life cycle.

Keywords Transcription · TFIID · SAGA · Male gametophyte · Pollen tube

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Introduction

The general transcription factor TFIID plays a pivot role in the recognition of core promoter elements and is required for transcription initiation of the majority of eukaryotic genes by RNA pol II (Hahn 1998; Lee and Young 1998). TFIID is a multiprotein complex composed of *T*ATA *b*ox-binding *p*rotein (TBP) and 14 other *T*BP-*a*ssociated *f*actors (TAFs) that have been conserved throughout evolution (Tora 2002). TAFs subunits serve multiple functions within the TFIID holocomplex. For instance, TAF1 and TAF2 have been shown to bind to the transcription initiator, whereas TAF6 and TAF9 have been shown to interact with the promoter elements (Chalkley and Verrijzer 1999; Verrijzer et al. 1994). Furthermore, several TAFs have been shown to interact with transcription activators or other general transcription factors to stabilize the pre-initiation complex (Roeder 1996). In yeast, 13 TAFs are required for viability (Green 2000). In vitro experiments in humans and *Drosophila* show that TAFs are necessary for transcription

initiation in TATA-less promoters (Wright et al. 2006). In *Caenorhabditis elegans*, TAF5 protein is important for transcription during embryogenesis (Walker and Blackwell 2003), whereas in *Drosophila melanogaster* the TAF5 homolog protein, called cannonball, is found only in male germ cells and is required for normal transcription in primary spermatocytes (Hiller et al. 2001, 2004).

Several TAF_{II}s are also components of histone acetyltransferases (HAT) complexes such as Spt-Ada-Gcn5-Acetyltransferase (SAGA), which functions in nucleosomal histone acetylation and chromatin-associated transcriptional activation or repression (Grant et al. 1998; Pray-Grant et al. 2002). The catalytic subunit of the SAGA complex is the protein GCN5; the complex also include ADA proteins and many TAFs, like TAF5, 6, 9, 10, 12 (Daniel and Grant 2006). The SAGA complex is also required for transcription elongation, mRNA export and nucleotide excision repair (Baker and Grant 2007). In *Arabidopsis*, mutations in *GCN5/HAG1* and *ADA2b/PRZ1* cause pleiotropic alterations in pattern formation and organogenesis, also manifested as defects in the interpretation of signals relevant to plant development (Bertrand et al. 2003; Sieberer et al. 2003; Vlachonasios et al. 2003; Benhamed et al. 2006; Long et al. 2006; Cohen et al. 2009; Kornet and Scheres 2009; Anzola et al. 2010; Servet et al. 2010). Furthermore, ADA2b and GCN5 are implicated in abiotic stress response mechanisms (Vlachonasios et al. 2003; Hark et al. 2009).

TBP and TAF-like proteins have been identified in *Arabidopsis* (Gasch et al. 1990; Lago et al. 2004; Bertrand et al. 2005). An extensive yeast two-hybrid study was conducted to map the interactions of putative TFIID components in *Arabidopsis* (Lawit et al. 2007). The results indicated that plant TFIID might posses some unique features. Alterations in the levels of *Arabidopsis* TBP or specific TAFs are implicated in plant development affecting the organization of shoot apical meristems, leaf development, formation of floral organs and leaves, fertility, pollen tube growth and light responses (Gurley et al. 2006). The TAF1 family of histone acetyltransferases in *Arabidopsis* consists of two members, HAF1 and HAF2/TAF1 (Bertrand et al. 2005; Benhamed et al. 2006). While mutations in *HAF1* showed no visible phenotype, mutations in *HAF2/TAF1* regulate light-responsive genes by controlling acetylation of histones H3 and H4 at the target promoters (Bertrand et al. 2005; Benhamed et al. 2006). *Arabidopsis* has two *TAF6* genes that are distantly related (Lago et al. 2005). *AtTAF6*a is essential for plant viability since loss of function mutants are lethal, whereas heterozygous plants displayed a drastic reduction in pollen tube growth rate (Lago et al. 2005), suggesting that TAF6 plays an important role in pollen tube function. Arabidopsis mutants with reduced expression of TAF10 are more sensitive to salt stress, whereas overexpression of TAF10 increased seed germination rate upon osmotic stress (Gao et al. 2006), suggesting that TAF10 is involved in osmotic stress responses. When TAF10 from *Flavenia trivernia* was overexpressed in *Arabidopsis*, a terminal flower-like phenotype resulted, characterized by limited growth of an indeterminate inflorescence, chimeric floral organs and curled leaves (Furumoto et al. 2005). A mutation in an *Arabidopsis* TFIID-interacting transcription factor *TAF12B/EER4* results in failure to induce a subset of ethylene-regulated genes in etiolated seedlings (Robles et al. 2007).

In this report, we explore the biological role of *TAF5* in *Arabidopsis*. Our data suggests that *TAF5* is necessary and sufficient for plant viability.

Materials and methods

Plant material and growth conditions

Five mutants bearing a T-DNA insertion in the *Arabidopsis TAF5* gene were identified; *taf5-1* (SALK_021380), *taf5-2* (SALK_052284) and *taf5-3* (SALK_036395) were found from the Salk Institute collections (Alonso et al. 2003). while *taf5-4* (SAIL_274-A04) and *taf5-5* (SAIL_752-BO9) were found from Syngenta collections (Sessions et al. 2002). Seeds from those mutants were obtained from the Nottingham Arabidopsis Stock Center (NASC). Seeds were surface-sterilized with 20% sodium hypochlorite solution for 10 min, washed three times with sterile water and plated onto Petri dishes containing Gamborg's B5 medium (Duchefa) supplemented with vitamins, 1% (w/v) sucrose, and 0.8% agar, and adjusted to pH 5.7. Seeds were stratified for 3 days at 4°C in the dark and then grown in long-day conditions (16-h light/8-h dark at 21°C) for 14 days. The wild-type, ecotype Columbia-0

(Col), and the *taf5* mutant plants were then grown in soil at 18–22°C in long-day conditions (16-h light/8 h dark).

Molecular characterization of *taf5* mutants

The genotypes of mutant plants were confirmed by PCR using gene-specific and T-DNA border-specific primers (Electronic Supplementary Material Table 1S). PCR was carried out using EX-Taq™ DNA polymerase (Takara). *taf5-1* was genotyped using KB165-166 primers to test for the wild-type gene and KB112 and LBa1 to test for the T-DNA insertion. *taf5-2* was genotyped using KB165-166 primers to test for the wild-type gene and KB166 and LBa1 to test for the insertion. *taf5-3* was genotyped using KB163-164 primers to test for the wild-type gene and KB164 and LBa1 to test for the insertion. *taf5-4* was genotyped using KB163-164 primers to test for the wild-type gene and KB164 and KB162 to test for the insertion and *taf5-5* was genotyped using KB229-230 primers to test for the wild-type gene and KB230 and KB162 to test for the insertion.

Total mRNA from rosette leaves or from flower inflorescences was isolated using the Nucleospin Plant RNA kit (Macherey–Nagel, Duren, Germany). DNase treatment was carried out by adding 5 µl 10x buffer and 1 µl DNase (10 U) to 60 µl of RNA solution followed by incubation for 2 h at 37°C. Samples were extracted using an equal volume of phenol, chloroform, isoamylic alcohol (25:24:1 v/v) and RNA was precipitated using a half volume of 7.5 M ammonium acetate and two volumes of 100% ethanol. The resulted RNA pellet was washed with 75% ethanol and resuspended in 50 µl nuclease-free water. Levels of specific mRNAs were assayed using reverse transcription (RT) followed by PCR and quantitated using real-time PCR. cDNA was synthesized using the Reverse Transcription System Kit (Promega, Madison, WI, USA). RT reactions were carried out using 0.25 µg of DNAse-treated RNA. The final reaction volume was 40 µl. After the reaction the samples were diluted five times in nuclease-free water. Real-time PCR was carried out in reactions using 1 × buffer containing KAPA SYBR® FAST qPCR Master Mix ABI Prism™ (Kapa Biosystems, Boston, MA, USA). In each 10 µl reaction, 2 µl of cDNA sample was used. The gene-specific primers used for RT-qPCR are listed in Table 1S. Gene-specific primers for *At4g26410* or *ACT2* open reading frame were used as references. The Ct values obtained from *TAF5* or *AP1* genes were normalized to the values obtained from *At4g26410* or *ACT2,* respectively. The values were expressed as reference gene- normalized levels of the target genes. Student's *t* test was used to compare the expression of the target genes between wild-type and mutants plants to determine whether the difference was significant at $P \leq 0.05$. Semi-quantitative RT-PCR was performed to identify transcripts that terminate in the T-DNA of *taf5-1* heterozygous mutants. PCR was applied using KB112 and LBa1 primers and the products were subjected to gel electrophoresis and visualized via ethidium bromide staining. *PDF2* was used as reference gene.

Pollen development, germination and pollen tube growth

Sufficient mature pollen was obtained by placing 3–4 open flowers from wild-type and heterozygous *taf5-1* and *taf5-4* mutants in an Eppendorf tube containing 300 µl H_2O. After brief vortexing and centrifugation, the pollen pellet was diluted in 2-µl of 4′,6-diamino-2-phenylindole dihydrochloride (DAPI) staining solution (0.4 µg/ml DAPI in antifade solution which consists of PBS solution containing 50% glycerol and 2–7 mM phenylenediamine, pH 8.0, Millipore, USA) for at least 2 h in the dark and then transferred to a microscope slide. Pollen was viewed by a light and UV epi-fluorescent inverted microscope, Nikon Eclipse TE 2000-S (Nikon Corporation, Tokyo, Japan) and images were captured with a digital camera, Nikon DS-L1 (Nikon Corporation). For the analysis of spores at earlier stages, single anthers were dissected from isolated buds. Anthers were disrupted on microscope slides using dissecting needles and gently squashed in DAPI staining solution (0.4 µg/ml) under a coverslip.

Pollen germination experiments were conducted according to the protocol of Boavista and McCormick (2007). The pollen grains, from flowers that had just opened from 1 week after bolting inflorescence, were applied to microscope slides with the pollen germination medium (5 mM KCl, 5 mM $CaCl_2$, 1 mM $MgSO_4$, 1.5 mM H_3BO_3, 10% w/v sucrose and 1% w/v agar) adjusted to pH 8. The slides were then placed onto moist paper in empty tip racks (Johnsson-

Brousseau and McCormick 2004) and incubated into a dark room at 22°C for 16 h. Approximately 650–7,100 pollen grains collected from at least three different flowers were examined from each mutant and wild-type plant. Pollen germination and pollen tube growth was examined under the Zeiss Axiostar Plus (Jena, Germany) microscope with DIC optics. Images were captured using a camera (Canon USA, Inc.). The pollen grains were counted with the ImageJ software (http://rsb.info.nih.gov/nih-image/index.html), using the cell counter application.

Bioinformatics tools

The amino-acid sequence of TAF5 proteins from different organisms were retrieved from the National Center of Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) and from Phytozone (http://www.phytozone.net/). The amino-acid sequence alignment was conducted using Clustal W2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) using default parameters. The alignment was graphically enhanced using ESPript software (Gouet et al. 1999). Phylogenetic relationships were analyzed using MEGA 4.1 (http://www.megasoftware.net/).

Results

Plants have one *TAF5* homolog

Although members of the putative SAGA complex in *Arabidopsis* such as GCN5 and ADA2b have important roles in plant development and abiotic stress responses (Bertrand et al. 2003; Vlachonasios et al. 2003; Benhamed et al. 2006), the function of other components of the *Arabidopsis* putative SAGA complex is unknown. Therefore we searched for an *Arabidopsis* ortholog of *TAF5*, a component of SAGA and TFIID complex in yeast and other organisms. A BLAST search using the yeast TAF5 amino-acid sequence identified one *Arabidopsis* ortholog, *At5g25150*, which encodes for a 74.4-kDa protein. Alignment of *Arabidopsis TAF5* with homologs from *Homo sapiens* (Hs), *Saccharomyces cerevisiae* (Sc) and *D. melanogaster* (Dm) are presented in Figure 1Sa. Two regions of AtTAF5 share a high degree of sequence identity with the other TAF5 proteins. The first conserved domain, the NTD2 domain, was found in the N-terminus of AtTAF5. This alpha-helical domain is homodimerized and could play an important role in organizing the TFIID structure (Bhattacharya et al. 2007). The other conserved domain was found in the C-terminus of AtTAF5, the WD40 motif, which is repeated five times and has 40% identity with the yeast TAF5 (Fig. 1Sa and 1Sb). The last four repeats are common in yeast, human and *Drosophila* but the first one is observed only in plants (Fig. 1Sb). The WD repeats form a closed beta-propeller structure (Smith et al. 1999). The beta-propeller domains are critical for the structural role of TAF5 in the TFIID complex (Leurent et al. 2004) and have been shown to mediate protein–protein interactions (Tao et al. 1997). Genes that encode TAF5 proteins from other plants are also found (Fig. 1Sc). Phylogenetic relationships between *Arabidopsis* TAF5 and the other plant TAF5 proteins show a distinct division between monocotyledonous and dicotyledonous plants as well as from mosses, Prasinophyceae and green algae lower plants (Fig. 1Sc). Interestingly, *Populus trichocarpa* and *Glycine max* have two and three orthologs of TAF5, respectively, that could arise from genome duplication.

Molecular characterization of *taf5* mutants in *Arabidopsis*

In order to study the biological function of the *Arabidopsis TAF5* gene, a search in *Arabidopsis* T-DNA insertion mutant collections was performed. Five mutants bearing a T-DNA insertion were identified. TAF5 mutants *taf5-1*, *taf5-2*, *taf5-3*, and *taf5-4* disrupt the predicted coding region at 838, 1,057, 3,271, and 3,962 bp in the genomic DNA and downstream of the initiation codon, respectively (Fig. 1). T-DNA insertion in the *taf5-5* mutant is located 641 bp upstream of the initiation codon (Fig. 1). The genotyping of the *taf5* mutants were performed by PCR using gene-specific primers and T-DNA primers (Table 1S). The analysis revealed the presence of *taf5-1*, *taf5-2*, *taf5-3*, and *taf5-4* heterozygotes and only the presence of *taf5-5* homozygote plants (Fig. 2S). The progeny of a self-pollinated for *taf5-1*$^{(+/-)}$, *taf5-2*$^{(+/-)}$, *taf5-3*$^{(+/-)}$, or *taf5-4*$^{(+/-)}$ mutants was analyzed by PCR using gene-specific and T-DNA primers. PCR analysis of approximately 100 T3 mutant plants showed that 24–32 of them were *taf5*$^{(+/-)}$ and 76–69 wild-type (+/+) (Table 1). The segregation ratio from each heterozygote plant

Fig. 1 Molecular characterization of *taf5* mutants in *Arabidopsis*. Schematic diagram of *AtTAF5* gene. *Black boxes* indicate the exons and *white boxes* the introns. The *vertical lines* represent the T-DNA insertions

Table 1 Segregation analysis of T3 generation of *taf5-1*, *taf5-2*, *taf5-3* and *taf5-4* mutants

Mutant	Homozygous	Heterozygous	Wild-type	−/+ (%)	χ^2 analysis
taf5-1	0	24	76	24%	0.053, $P > 0.5$
taf5-2	0	25	75	25%	0, $P = 0.995$
taf5-3	0	31	69	31.25%	1.92, $P > 0.1$
taf5-4	0	22	78	22%	0.48, $P > 0.1$

was approximately 25% heterozygous *taf5*$^{(+/-)}$ and 75% wild-type (+/+) plants. No homozygous individuals were identified in *taf5-1, taf5-2, taf5-3*, and *taf5-4* mutants. This data strongly suggests that the *Arabidopsis taf5* mutants are lethal. Furthermore, the segregation of wild-type (+/+) and heterozygous (±) individuals was in a ratio of approximately 3:1, which is in disagreement with the expected 1:2:1 (−/−, ±, +/+) according to Mendelian genetics (Table 1). These segregation ratios suggest a haplo-insuffiency character where the single wild-type *TAF5* allele of the heterozygous locus is not sufficient to restore wild-type function and as a result wild-type *TAF5* has a semi-dominant character. The loss of homozygous *taf5* mutant plants could have occurred early during seed development or before fertilization, suggesting that *TAF5* might play important role in *Arabidopsis* embryo development and/or male/female gametophyte formation.

The level of *TAF5* gene expression was determined in *taf5* mutants using quantitative real-time RT-PCR. In *taf5-1*$^{(+/-)}$, *taf5-2*$^{(+/-)}$, *taf5-3*$^{(+/-)}$, and *taf5-4*$^{(+/-)}$ mutants the level of *TAF5* was reduced to approximately 50–60% in comparison to wild-type plants, since we predicted that only one copy of the *TAF5* transcript was made (Fig. 2a). In the homozygous *taf5-5* mutant the level of *TAF5* expression was reduced to 25% in comparison to wild-type plants (Fig. 2b), suggesting that *taf5-5* is a hypomorph. Moreover, RT-PCR analysis using primers from the N-terminus of the *TAF5* gene and the T-DNA revealed that several splice variant transcripts are present in the heterozygous *taf5-1* mutant plants (Fig. 2c).

Phenotypical analysis of *taf5* mutants

Although homozygous mutations in the coding region of *TAF5* were lethal, we checked whether the heterozygous *taf5*$^{(+/-)}$ mutants exhibited any developmental phenotypes. All the heterozygous *taf5*$^{(+/-)}$ mutants that disrupt the *TAF5* open reading frame displayed a terminal flower-like phenotype (Fig. 3a–d) in comparison to wild-type plants (Fig. 3e). This phenotype is characterized by suppressed growth of the main inflorescence and lack of indeterminate growth. Interestingly, the secondary inflorescences and the stem branches of the heterozygous *taf5* mutants developed normally without showing terminal flower-like phenotypes. Homozygous *taf5-5* plants showed no obvious morphological differences from wild-type plants at various stages of growth and development (Fig. 4f), suggesting that reduction of *TAF5* gene expression to 25% was not sufficient to affect developmental processes in *Arabidopsis*. These differences between *taf5-5* homozygous and the rest of *taf5* heterozygous mutants could be explained by the fact that several truncated *TAF5* transcripts are made at least in *taf5-1*$^{(+/-)}$ (Fig. 3c). The terminal flower-like phenotype observed in heterozygous *taf5-1*, *taf5-2*, *taf5-3*, and *taf5-4* mutants could be the result of the truncated transcripts that terminated inside the

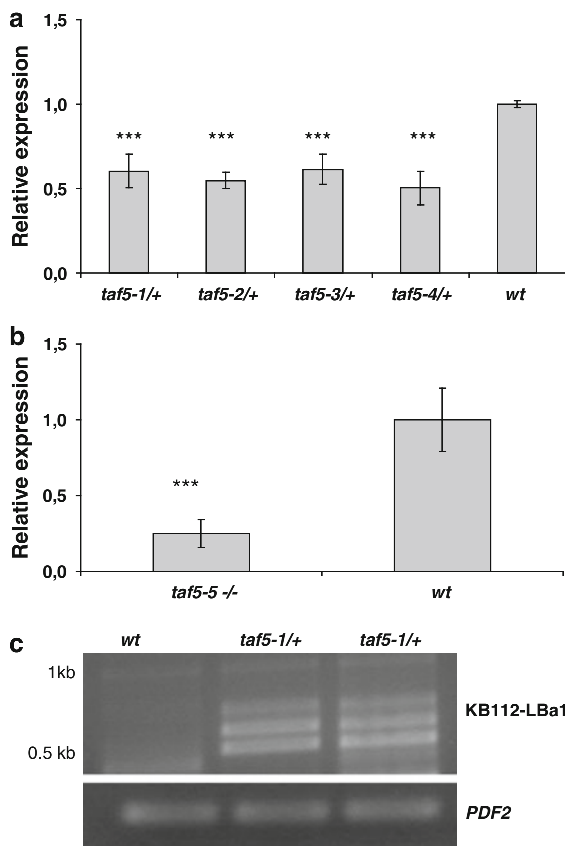

Fig. 2 *TAF5* gene expression in *taf5* mutants. Total mRNA was extracted from rosette leaves of wild-type and *taf5* mutants. **a** Reverse transcriptase-qRCP was used to quantitate *TAF5* expression in *taf5-1*$^{(+/-)}$, *taf5-2*$^{(+/-)}$, *taf5-3*$^{(+/-)}$ and *taf5-4*$^{(+/-)}$ mutant plants. The Ct values obtained for *TAF5* were normalized to the Ct values obtained for A*t4g26410*, and fold induction of *TAF5* was calculated by normalizing values from wild-type plants with those from *taf5* mutants. For each mutant and wild-type plants, triplicate samples in each experiment were assayed twice. Three independent experiments were performed; *error bars* represent SE, where $n = 4$. *Asterisks* mark values that are significantly different from the wild-type (*t* test, $P = 0.001$). **b** Reverse transcriptase-qPCR analysis to assess *TAF5* expression in *taf5-5* homozygous mutant plants; triplicates for *taf5-5* and duplicates for wild-type plants were used; *error bars* represent SE, where $n = 4$. **c** RT-PCR analysis to assess *TAF5* transcripts from the N-terminus of TAF5; wild-type and duplicates for *taf5-1* heterozygous plants were used. PCR was performed with KB112 and LBa1 primers and *PDF2* was used as control. Three splice variants ranging from approximately 500–700 bp were detected in the heterozygous *taf5-1* plants

T-DNA, at least in *taf5-1*$^{(+/-)}$ (Fig. 2c). These transcripts might generate peptides that could dimerize with the wild-type TAF5 protein, and this interaction could form a deleterious complex affecting the function of TAF5 during this developmental stage. The fact that *TAF5* is a single gene in *Arabidopsis*, and *taf5*$^{(+/-)}$ mutants have reduced expression of the *TAF5* gene and displayed developmental problems, further suggests that *taf5* mutants displayed a semi-dominant character.

In *Arabidopsis*, the terminal flower phenotype is known to arise from the function of two antagonist genes *TERMINAL FLOWER 1 (TFL1)* and *APETALA 1 (AP1)*. Overexpression of *AP1* is sufficient to convert the inflorescence meristem to a terminal flower (Mandel and Yanofsky 1995). Therefore, we examined *AP1* expression in the terminal flower of *taf5-1* and *taf5-3* heterozygote mutants in comparison with wild-type plants. The flowers were in stage 13. As we expected, in both *taf5*$^{(+/-)}$ mutants *AP1* expression was increased more than two-fold in comparison to wild-type flowers (Fig. 3g), suggesting that the terminal flower observed in the *TAF5/taf5* mutants could arise from the higher expression of *AP1*.

TAF5 is required for male gametophyte development

If only the *taf5* homozygous developing seeds were not viable, then the progeny of *taf5*$^{(+/-)}$ plants would segregate 2:1 for the heterozygous (±) and wild-type (+/+) locus, respectively. However, the percentage of heterozygous seedlings was much less than 67%, with only approximately 25% heterozygous at the *TAF5* locus (Table 1). This deviation from a standard inheritance pattern implies the death of more than just the double-null developing seed. The effect of *AtTAF5* mutations on male or female gametophytic development was further examined by back-crossing heterozygous *taf5-2* mutants to wild-type plants. When the stigma of the *taf5-2* mutant was pollinated with wild-type pollen, a normal Mendelian segregation ratio (1:1, wild-type:heterozygous) was observed. However, when pollen of *taf5-2* was used to pollinate wild-type plants, then only 11 out of 75 individuals were heterozygous, indicating a strong deviation from the expected 1:1 segregation. These results indicate that there is a defect in the transmission of the mutated *AtTAF5* allele through the male gametophyte, which suggests that pollen development is affected by the *taf5-2* mutation. Since male

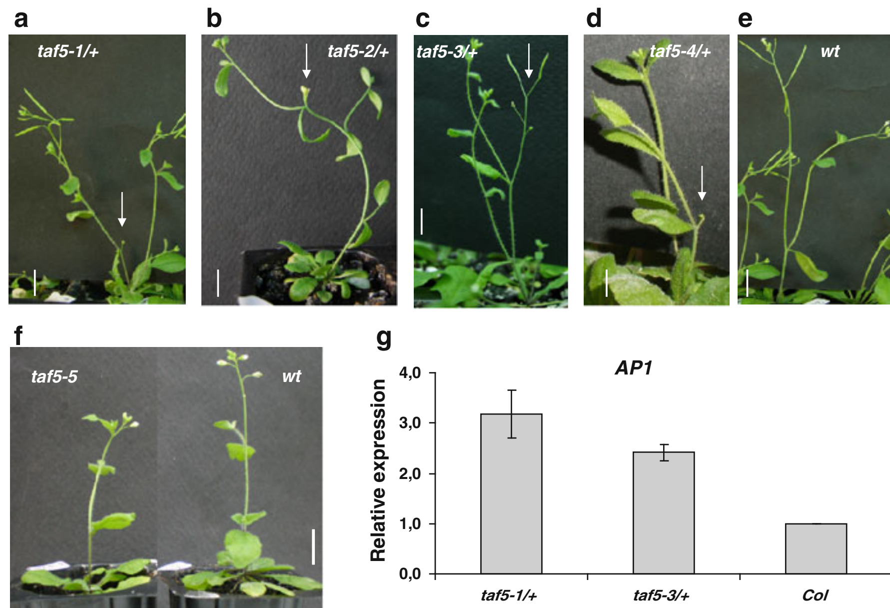

Fig. 3 Phenotypic characterization of *taf5* mutants. **a** Heterozygous *taf5-1* mutant plant after 30 days of growth. *Bar* = 1 cm. **b** Heterozygous *taf5-2* mutant plant after 30 days of growth. *Bar* = 1 cm. **c** Heterozygous *taf5-3* mutant plant after 30 days of growth. *Bar* = 1 cm. **d** Heterozygous *taf5-4* mutant plant after 30 days of growth. *Bar* = 1 cm. **e** Wild-type (Col-0) plants after 30 days of growth. *Bar* = 1 cm. The *white arrows* indicate the terminal flower phenotype observed. **f** Homozygous *taf5-5* and wild-type (Col-0) plants after 25 days of growth. *Bar* = 1 cm. **g** *AP1* expression in flower of *taf5* mutant in comparison to wild-type plants. Total mRNA was isolated from the first flower of *taf5-1*$^{(+/-)}$, *taf5-3*$^{(+/-)}$ mutants, and wild-type plants. RT-qPCR was used to quantitate the expression of *AP1* gene. The Ct values obtained for *AP1* were normalized to the Ct values obtained for *ACT2*, and fold induction of *AP1* was calculated by normalizing values from wild-type plants with those from *taf5* mutants. For each mutant and wild-type plant, triplicate samples in each experiment were assayed twice. Three independent experiments were performed; *error bars* represent standard error of mean, where $n = 3$

gametophyte formation could be affected in *taf5* mutants, we examined the viability of pollens of the *taf5-2* mutants in comparison to wild-type plants. Pollens collected from wild-type and *taf5-2* mutants were stained with Alexander solution. The pollens examined from wild-type and *taf5-2* mutant plants were viable (data not shown).

We further analyzed male gametophyte development in *taf5-1* and *taf5-4* heterozygous mutants by fluorescence microscopy. In *Arabidopsis*, the male gametophyte is a three-cell organism that is derived by stereotypical cell divisions (McCormick 2004). During microsporogenesis, meiosis of the microspore mother cell produces a tetrad of cells. After release from the tetrad, during microgametogenesis, each microspore goes through an asymmetric mitosis, to produce a bicellular pollen grain containing a generative cell and a much larger vegetative cell. The larger vegetative cell does not divide again but eventually will form the pollen tube. The smaller generative cell undergoes a second round of cell division, pollen mitosis, to form the two sperm cells (McCormick 1993, 2004). Pollen grain collected from open flowers of both wild-type and heterozygous *taf5-1* and *taf5-4* mutants were examined by staining with DAPI that specifically stains DNA. When wild-type flowers were open, the mature pollen is characterized by two brightly stained sperm cell nuclei and a faintly stained vegetative cell nucleus (Fig. 4b). Similar images were obtained in heterozygous *taf5-1* and *taf5-4* pollen (Fig. 4d, f). However, in *taf5-1* heterozygous plants the percentage of non-DAPI-stained pollen was approximately double that of wild-type, whereas the percentage of bicellular and tricellular pollen in *taf5-1* heterozygous plants was lower in comparison to wild-type pollen. Nevertheless, this observation was not found in *taf5-4* heterozygous pollen. In the early stages of

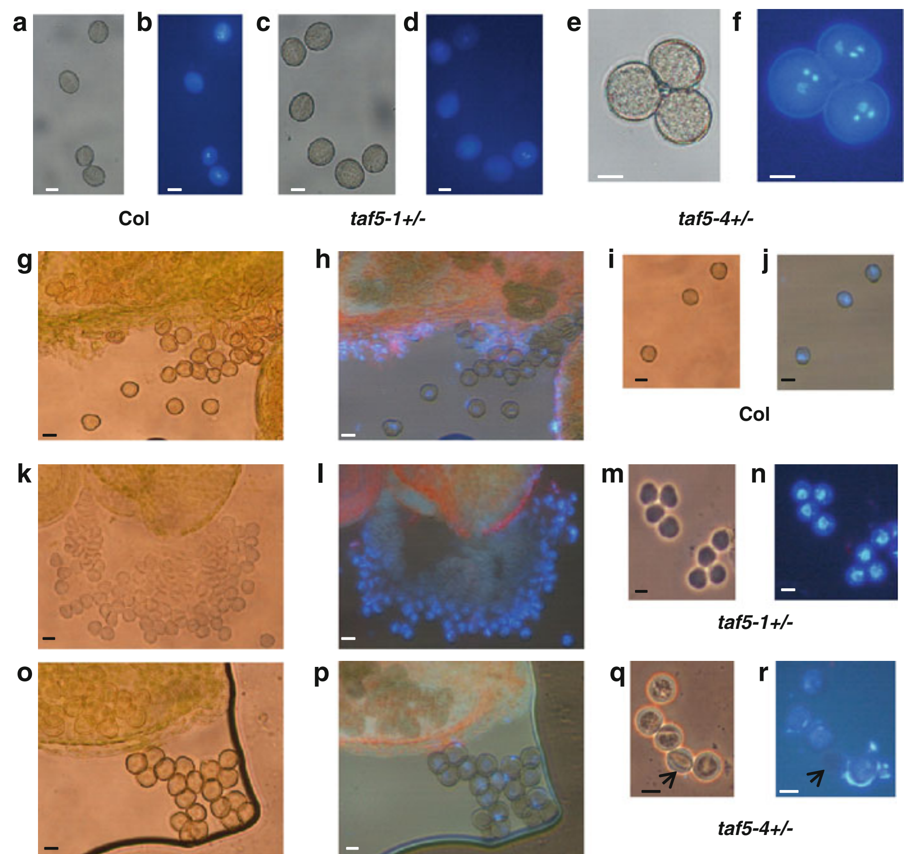

Fig. 4 Analysis of pollen development in *taf5*$^{(+/-)}$ mutants. Light (**a**, **c**, **e**) and fluorescence images (**b**, **d**, **f**) of DAPI-stained mature pollen from wild-type (**a**, **b**), heterozygous *taf5-1* (**c**, **d**) and heterozygous *taf5-4* (**e**, **f**) plants. Light (**g**, **i**, **k**, **m**, **o**, **q**) and fluorescence images (**h**, **j**, **l**, **n**, **p**, **r**) of DAPI-stained microspores at uninucleate stage from wild-type (**g**–**j**), heterozygous *taf5-1* (**k**–**n**), and heterozygous *taf5-4* (**o**–**r**) plants. Arrows indicate non-DAPI-stained pollen in *taf5-4* heterozygous pollen. *Bar* = 10 µm

microsporogenesis, microspores of wild-type plants (Fig. 4g–j) were indistinguishable from *taf5-1* and *taf5-4* heterozygous microspores (Fig. 4k–p). Moreover, tetrads are formed indicating that meiosis takes place normally (Fig. 4m–p). In a few cases, and only in *taf5-4* heterozygous plants, we observed aborted and non-DAPI-stained pollen grains (Fig. 4q, r). These results indicate that, at least in *taf5-1* heterozygous mutants, microspores are arrested in the bicellular and tricellular pollen stages.

We then studied the effect of *TAF5* in pollen tube development. Pollen was collected from *taf5-2*, *taf5-4*, and *taf5-5* mutants as well as from wild-type flowers from secondary inflorescence after 1 week of bolting, and germinated in vitro. At least three flowers per genotype and more than 200 pollen grain per flower were analyzed. The germinated pollen grains were classified into four groups, according to Lago et al. 2005: (1) germinated pollen that developed a pollen tube with length of more than three times the diameter of the pollen grain; (2) germinated pollen that has a pollen tube with a length of 1–3 times the pollen grain diameter; (3) germinated pollen that developed only a pollen tube primordia smaller than the diameter of the pollen grain; and (4) non-germinated pollen (Fig. 5a). In these experiments, the pollen germination percentage was rather low (15–25%) but there was no significant difference between the genotypes. However, differences were observed between the three classes (Fig. 5b). In the wild-type plants the majority of pollen tube observed belong to group I followed by the pollen tube of group II and group III (74, 17, and 9% respectively). In contrast, the number of class I pollen tubes was significantly reduced in the heterozygote *taf5-2* and *taf5-4* plants (19 and 38%, respectively) when compared to wild-type plants, whereas the number of pollen tubes of class II and III was significantly increased in the heterozygote *taf5-2* (45 and 36%) and *taf5-4* plants (35 and 27%) in comparison to wild-type plants. The homozygote *taf5-5* plants exhibited a similar pattern of pollen tube classes to

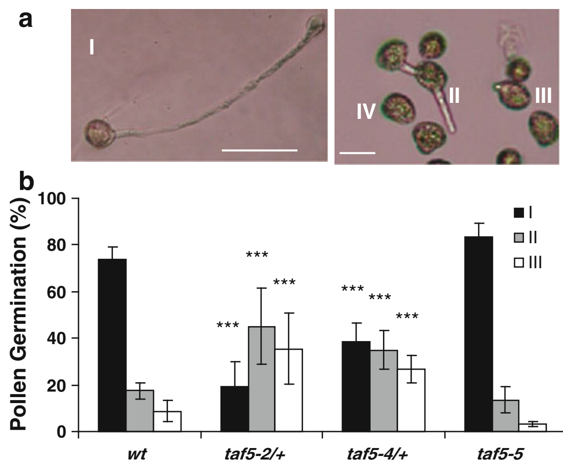

Fig. 5 Pollen tube growth of *taf5* mutants. **a** Four classes of germinated pollen grains were examined: (*I*) germinated pollen that developed a pollen tube with length more than 3 times the diameter of the pollen grain; (*II*) germinated pollen with pollen tube length of 1–3 times the pollen grain diameter; (*III*) germinated pollen with a pollen tube primordia smaller than the diameter of the pollen grain; (*IV*) non-germinated pollen. *Size bars* are 100 µm for (*I*) and 50 µm for (*II*) (*III*), and (*IV*). **b.** The effect of *taf5* mutants on pollen tube growth. Pollen from *taf5-2*$^{(+/-)}$, *taf5-4*$^{(+/-)}$ and *taf5-5* homozygous mutants as well as from wild-type (Col-0) was collected from flowers 1 week after bolting and germinated on solidified media at 22°C. *Bars* represent means ± SE ($n = 3$). *Asterisks* mark values that are significantly different from the wild-type (*t* test, $P = 0.001$)

the wild-type plants. The highest percentage was in group I (83%) followed by group II (14%) and group III (3%). The reduction of class I pollen tubes is expected when considering that 50% of the pollen taken from heterozygous *taf5-2* and *taf5-4* mutant plants carries the wild-type allele These results suggest that the pollen tube growth rate was reduced in the heterozygous *taf5* mutants, indicating that the pollen from wild-type *TAF5* was germinated and formed a pollen tube that was more competent to reach the ovule.

Discussion

Arabidopsis TAF5 is important for plant viability

Disruption mutations of the *Arabidopsis ADA2b* and *GCN5* genes resulted in striking phenotypes that affect plant growth and development (Vlachonasios et al. 2003). In this study we explore the biological role of another component of the putative SAGA complex in *Arabidopsis*, *TAF5*. Unlike *ada2b* and *gcn5* mutants, loss-of function mutation in *Arabidopsis TAF5* affects plant viability, suggesting that TAF5 is required for the complete plant life cycle. These differences could arise from the fact that TAF5 is not only a component of the SAGA complex but also a component of the TFIID complex which is involved in RNA polymerase II transcription initiation (Burley and Roeder 1996). Similarly, yeast TAF5 is also required for yeast viability (Poon et al. 1995) and in particular for cell cycle progression through G2/M (Apone et al. 1996). Site-directed mutations in the WD40 repeats of yeast *TAF5* cause a defect in the ability of TAF5 to interact with the TFIID and SAGA complexes and as a result TAF5 is required for the transcription of a large number of genes in yeast (Durso et al. 2001).

Alternative forms of the TAFs have been also proposed to play a role in selective activation of cell-type-specific gene expression programs during cellular differentiation (Freiman 2009). In *Arabidopsis*, our results suggest that *TAF5* is required for male gametophyte development, probably by affecting transcription of genes involved in male gametophyte differentiation. The importance of *TAF5* in spermatogenesis implies that it is conserved throughout evolution. For instance, in *C. elegans TAF5* is essential during early development since the development of taf-5 (RNAi) embryos was arrested at the first 100 cells without signs of differentiation, suggesting a broad defect in zygotic transcription (Walker and Blackwell 2003). The *cannonball* (*can*) gene of *Drosophila* encodes a homolog of a dTAF5 protein expressed only in spermatocytes which is required for normal transcription of genes involved in spermatid differentiation (Hiller et al. 2001).

A particularly interesting aspect of our findings is the remarkable similarity between the *taf5* mutant phenotype and the previously described effects of inhibiting expression of *TAF6* in *Arabidopsis* (Lago et al. 2005) The null *taf6* in *Arabidopsis* is also lethal, affecting male gametophyte development and pollen tube growth (Lago et al. 2005). This phenotype similarity supports the model that TAF5 and TAF6 are functionally linked. Further support of this model comes from the yeast two-hybrid experiments where AtTAF5 interacts with the histone-fold domain of TAF6 (Lawit et al. 2007).

Gametogenesis depends on coordination of sporophytic and gametophytic gene expression (McCormick 2004; Yadegari and Drews 2004). Expression profiling of male gametophytes has provided a list of genes that may be under gametophytic selection (Honys and Twell 2004; Pina et al. 2005). Studies on the sperm cell transcriptome (Borges et al. 2008), transcriptome changes associated with pollen germination and tube growth (Wang et al. 2008; Qin et al. 2009), and the recent discovery of several male gametophytic regulators of cell cycle progression and differentiation, together with the collection of male gametophyte development *Ds* insertional mutants (Kim et al. 2008; Boavista et al. 2009; Chen et al. 2009), serve as a foundation to decipher the regulatory mechanisms of male gametogenesis and delivery. It would be interesting to learn which of these regulatory mechanisms are dependent on the action of TAF5. The reduced expression of these genes in *taf5* mutants could be responsible for male gametophyte defects and reduced pollen tube growth.

TAF5 is involved in the gene expression related to inflorescence meristem identity

One of the striking phenotypes observed in the heterozygous *taf5* mutants is the terminal flower-like phenotype with characteristic determinate inflorescence. A similar phenotype was observed in the plants that overexpress *TAF10* (Furumoto et al. 2005; Tamada et al. 2007), a gene that could be functionally linked with *TAF5*. The terminal flower-like phenotype is mediated by the function of several genes including *TFL1* and *TFL2* (Shannon and Meeks-Wagner 1991; Takada and Goto 2003). To maintain the indeterminate inflorescence meristem, *AP1/CAL* and *LFY* must be activated only in floral primordia (Mandel and Yanofsky 1995; Weigel and Nilsson 1995). In the *tfl* mutant, ectopic expression of *LFY* and *AP1* transforms the whole inflorescence meristem into a floral meristem (Bradley et al. 1997). In *taf5*$^{(+/-)}$ mutants, *AP1* is overexpressed, suggesting that TAF5 could function directly or indirectly in the regulatory mechanisms involved to prevent *AP1* expression and the consequent termination of the inflorescence meristem.

In conclusion, our data indicates that *TAF5* is an essential gene in the *Arabidopsis* life cycle. Moreover, *TAF5* is required for male gamentogenesis and the regulatory mechanisms of pollen tube growth. Finally, *TAF5* is also required in transcriptional mechanisms involved in maintenance of indeterminate inflorescence.

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