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The *Arabidopsis* TFIID factor AtTAF6 controls pollen tube growth

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Abstract

Initiation of transcription mediated by RNA polymerase II requires a number of transcription factors among which TFIID is the major core promoter recognition factor. TFIID is composed of highly conserved factors which include the TATA-binding protein (TBP) and about 14 TBP-associated factors (TAFs). Recently, the complete *Arabidopsis* TAF family has been identified. To obtain functional information about *Arabidopsis* TAFs, we analyzed a T-DNA insertion mutant for *AtTAF6*. Segregation analysis showed that plants homozygous for the mutant allele were never found, indicating that inhibition of the *AtTAF6* function is lethal. Genetic experiments also revealed that the male gametophyte was affected by the *attaf6* mutation since significant reduced transmission of the mutant allele through the male gametophyte was observed. Detailed histological and morphological analysis showed that the T-DNA insertion in *AtTAF6* specifically affects pollen tube growth, indicating that the transcriptional regulation of only a specific subset of genes is controlled by this basal transcription factor.

Keywords: TAF6; TFIID; *Arabidopsis*; Pollen tube; Gametophyte; Transcription

Introduction

The life cycle of plants can be divided into two phases, the haploid gametophytic phase and the diploid sporophytic phase. The gametophytic phase begins at the completion of meiosis. Unlike the situation in animals, in which the meiotic products differentiate directly into gametes, in higher plants, the products of meiosis (named spores) develop into multicellular structures, the gametophytes, which bear the gametes. In spermatophytes, male gametophytes (or microgametophytes) are pollen grains, and the female gametophytes (or megagametophytes) are embryo sacs. Upon the fusion of sperm and egg to form a zygote, the gametophytic phase ends and the sporophytic phase begins, thus completing the alternation of generations that is characteristic of the sexual life cycle of plants.

Pollen development consists of several distinct stages (McCormick, 1993). A microspore mother cell (the microsporocyte) undergoes meiosis to give rise to a tetrad of four microspores which are encased in a callose wall. These uninucleate microspores are released upon the degradation of the callose wall. After quickly increasing in size, each uninucleate microspore undergoes an asymmetric mitotic division (the microspore mitosis) to give rise to two cells with distinct fates, the vegetative cell and the generative cell. The larger vegetative cell is transcriptionally active and is thought to provide most of the proteins to the pollen grain (Mascarenhas, 1990). Moreover, it forms the pollen tube during germination. The generative cell is completely enclosed within the cytoplasm of the vegetative cell, and it will divide one more time to produce two sperm cells (the pollen mitosis). In *Arabidopsis*, this second mitosis takes place before pollen is released from the anthers (Regan and Moffatt, 1990). Mature *Arabidopsis* pollen therefore contains three cells, one vegetative cell and two sperm cells enclosed within the cytoplasm of the vegetative cell. The chromatin of

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the sperm cells is highly condensed, and traditionally they have been regarded as transcriptionally inactive (McCormick, 1993). However, recently, it has been shown that sperm cells have a complement of mRNAs diverse from the vegetative cell (Engel et al., 2003). After the pollen grains are released from the anthers and reach a compatible stigma, they germinate and the pollen tubes grow rapidly (up to 35 mm per hour) into female tissues to reach the ovules where they release the two sperm cells into the female gametophyte. Subsequently, the two sperm cells fertilize the two female reproductive cells (Mascarenhas, 1990; Lord and Russell, 2002).

The male gametophyte performs a wide variety of developmental functions including cell specification and differentiation, cellular recognition, rapid polarized growth, chemotactic sensing and fertilization (Lord and Russell, 2002). All these developmental functions will require a specific regulation of a subset of genes. Recently, several studies have been performed to investigate expression profiles of pollen on a more global scale (Honys and Twell, 2003; Lee and Lee, 2003; Becker et al., 2003; and reviewed in da Costa-Nunes and Grossniklaus, 2003). These studies showed that the expression in pollen is significantly divergent from the diploid sporophyte. This difference is mainly due to changes in expression levels of single genes and of whole subsets of genes with related functions. High expression levels were found for genes that are probably involved in signaling, cell-wall metabolism and cytoskeletal dynamics which fit with the developmental functions of this male gametophyte. Furthermore, subsets of genes that are pollen-specific were identified, although the numbers of pollen-specific genes varied significantly between these reports (da Costa-Nunes and Grossniklaus, 2003).

Gene transcription is the first step in the regulation of gene expression. Initiation of transcription by RNA polymerase II (Pol II) in eukaryotic organisms requires the assembly of general transcription factors (GTFs) at the core promoter of genes. The TFIID complex is the prime sequence-specific DNA binding GTF and is thought to form the scaffold upon which the rest of the basal initiation complexes (TFIIA, TFIIB, TFIIE, TFIIF and TFIIH) and RNA polymerase II assemble (Roeder, 1996; Orphanides et al., 1996). The TFIID complex consists of the TATA-box binding protein (TBP) and more than 10 TBP associated factors (TAFs) ranging in size from 250 kDa to less than 20 kDa (reviewed in Albright and Tjian, 2000).

TBP and most of the TAFs are highly conserved from yeast to human and are encoded by essential genes. TBP is responsible for the TATA-box binding activity of TFIID, whereas the DNA-binding TAFs recognize other core promoter elements like the initiator and the downstream promoter element (Albright and Tjian, 2000; Chalkley and Verrijzer, 1999). Furthermore, selected TAFs are believed to function as adaptors to bridge between DNA sequence specific activators and the basal transcriptional machinery and thus function as coactivators (Näär et al., 2001). In agreement with this specific coactivator function is the observation that mutations in TAFs affect the expression of subsets of genes (Näär et al., 2001).

Most TAF proteins contain a histone fold domain (HFD), a motif common to the core histones. Several structural studies have shown that these HFD-TAFs assemble into multimeric complexes with homology to the histone complex found in nucleosomes (Xie et al., 1996; Hoffmann et al., 1996; Selleck et al., 2001; Hahn, 2004). Furthermore, a three-dimensional model of yeast TFIID showed that the TFIID subunits are organized as a molecular clamp composed of three lobes, and immunolabeling experiments revealed that most of the HFD-TAFs are distributed over these lobes (Leurent et al., 2002).

TAF6 was the first histone fold TAF cloned from human and *Drosophila* (Weinzierl et al., 1993). It has been shown that TAF6 binds TAF9 and TAF1 in both organisms, and these interactions seem to be critical elements for integrity of TFIID (Michel et al., 1998). Furthermore, analyses in vitro showed that TAF6 mediates transcriptional activation and can be cross-linked to the downstream promoter element, suggesting that TAF6 may stabilize the interaction of TFIID with certain promoters, possibly in an activator-dependent way (Burke and Kadonaga, 1997; Guermah et al., 1998; Pham et al., 1999; Sauer et al., 1995; Thut et al., 1995). The TAF6 protein is highly conserved at the primary sequence level in all eukaryotic organisms examined to date (Aoyagi and Wassarman, 2000). In human and *Drosophila*, two proteins have been identified, named TAF6 and TAF6-like (Aoyagi and Wassarman, 2000). TAF6 is member of the TFIID complex, whereas TAF6-like is a component of the histone acetyltransferase complex PCAF (p300/CREB-binding protein-associated factor) that affects transcription by altering chromatin structure. In yeast, only one TAF6 protein has been identified, and it has been shown to take part in both the TFIID complex and the SAGA complex, the yeast homolog for PCAF (Grant et al., 1998).

A detailed study of *TAF6* mutants in *Drosophila* showed that TAF6 is required for a variety of developmental events (Aoyagi and Wassarman, 2001). Since homozygous *TAF6* mutants are lethal, the authors tried to rescue *TAF6* mutants with an inducible ubiquitously expressed *TAF6* transgene. Through this approach, they were able to show that *TAF6* expression is required during early developmental stages, but it is less required or dispensable for the post-mitotic and fully differentiated cells of the adult flies. Moreover, both male and female adult flies were sterile because of a broad range of spermatogenesis defects in males and polarity defects of the egg in females. The analysis of the sequences of the *Drosophila TAF6* mutant lines allowed identification of two regions of TAF6 critical for viability. Interestingly, these regions are evolutionarily conserved in TAF6 but not in TAF6-like proteins, suggesting different functional roles for the two members of the family. The finding that *TAF6* is an essential gene is consistent with the hypothesis of non-redundancy between TAF6 family members.

In contrast to the plethora of information about TAF factors in mammals, insects and yeast, the available information for plant TAFs is limited. One of the first reports addressed the question whether plants contain TAFs that have coactivator activity (Washburn et al., 1997). They reconstituted a heterologous in vitro transcription system containing wheat TFIID and the remaining GTFs from human cells. These experiments showed that transcription activation depends on TAFs present in wheat TFIID. Moreover, they identified 15 peptides that coimmunoprecipitated with wheat TBP which ranged in size from about 250 kDa to 30 kDa and which had a gel profile that was remarkably similar to that obtained with human TAFs. Recently, Bertrand et al. (2005) have studied the function of *Arabidopsis HAF2* which encodes TAF1. They showed that *HAF2* functions as a coactivator that integrates light signals and acetylates histones to activate light-induced gene transcription. Another report presents the analysis of a TAF10 factor of *Flaveria trinervia* (ftTAF10) (Furumoto et al., 2005). They investigated the expression profile of *ftTAF10* and showed that overexpression of *ftTAF10* in *Arabidopsis* affects leaf and inflorescence development, indicating that appropriate TAF10 levels are necessary for proper plant development. Recently, we presented an analysis of the complete TAF family in *Arabidopsis* (Lago et al., 2004). This study shows protein structures and the expression profiles for the TAF factors of *Arabidopsis*. Furthermore, it presents a phylogenetic analysis of TAF factors which indicates that most of the TAF factors of *Arabidopsis* are more related to those of yeast than to those of multicellular organisms.

Here, we present a functional characterization of *AtTAF6*. Screening the *Arabidopsis* genome sequence with known TAF6 protein sequences resulted in the identification of two *Arabidopsis* proteins with significant sequence homology to known TAF6 proteins from other species like yeast, *Drosophila* and human, which we called AtTAF6 and AtTAF6b (Lago et al., 2004). The putative proteins encoded by these two genes are quite divergent, showing that they might have different functions. Mutant analysis supports this idea since the *attaf6* mutation is lethal which further indicates that *AtTAF6* and *AtTAF6b* are not redundant. Segregation analysis of the *attaf6* mutant allele showed that the transmission of the mutant allele through the male gametophyte is significantly reduced. Subsequent detailed histological and morphological analyses demonstrated that, in the *attaf6* mutant, pollen tube growth is affected. This observation suggests that also in plants TAFs are important for the regulation of specific subsets of genes.

Materials and methods

Plant material and growth

Arabidopsis thaliana ecotype Wassilewskija was grown at 22°C in short-day (8 h light/16 h dark) or long-day (16 h light/8 h dark) conditions. For hand pollinations, flowers of developmental stage 12c (Smyth et al., 1990) were emasculated 24 h before pollination.

The *AtTAF6* insertion mutant CTI12 was obtained from the INRA collection (Ortega et al., 2002).

Bioinformatics tools

The protein sequences have been compared using the ClustalX program for protein sequence alignments (Jeanmougin et al., 1998). A search for conserved protein domains was performed using Motif Scan available on the site: http://hits.isb-sib.ch.

Expression analysis

Total RNA was extracted from *Arabidopsis* tissues as described by Verwoerd et al. (1989). Northern blotting was performed as described previously (Parenicova et al., 2003). For the *AtTAF6* probe primers, OL453 (5′ ATGAGCATTGTACCTAAGGAAACGGT) and OL454 (5′ AGCTCACCTCTTATTGGTTAGAGG) were used, and for the *AtTAF6b* probe primer, OL275 (5′ CGGGATCCTGACGAAAGAATCCATTGAAGTGATAGC) and OL276 (5′ CGCTCGAGTGTGGTACATACAATTACCATGTC) were used. Probes were labeled by random priming (Roche).

Complementation construct and Arabidopsis transformation

For the complementation test, we digested the BAC clone F13M7 (Mozo et al., 1998) with *Nhe*I and *Kpn*I and isolated and cloned a fragment of 7014 bp in the binary vector pCAMBIA 1300 digested with *Xba*I and *Kpn*I. This fragment contains locus At1g04950 encoding *AtTAF6*.

The binary vector was used to transform *Agrobacterium tumefaciens* C58C1/pMP90 (Koncz et al., 1984). *Arabidopsis* plants of line CT112 heterozygous for *attaf6* were transformed using the floral dip method described by Clough and Bent (1998).

Segregation and complementation analysis

Plants of line CTI12 were analyzed by PCR and Southern blot analysis. For the PCR analysis, we used primers specific for *AtTAF6* (OL453 and OL513, 5′ GTCAATAAGAGGTCCGTCTTTTTGTTCA 3′) and a T-DNA-specific primer (OL384, 5′ CTACAAATTGCCTTTTCTTATCGAC 3′). Southern blots were made using Hybond N+ membrane according to the manufacturer's instructions (Amersham). For the segregation and complementation analyses, 2 μg of genomic DNA was overnight digested with *Eco*RI and *Bam*HI, respectively. Blots for the segregation analysis were hybridized with the *AtTAF6* coding sequence amplified on cDNA using primers OL453 and OL513. Blots for complementation analysis were hybridized with a genomic

fragment comprising of 704 bp downstream of the T-DNA insertion site which we amplified with primers ATP253 (5′ CTAGAGTTTTGGTTGTGTAAGC) and ATP254 (5′ TCCGCAAAACAATAATACAGATGG). Probes were labeled by random priming (Roche).

Microscopy

Scanning electron microscopy was described previously (Favaro et al., 2003). Plant material was fixed, sectioned and stained with DAPI as described previously (Coleman and Goff, 1985).

In vitro pollen germination

Pollen was germinated in a basic medium according to Azarov et al. (1990), but with a minor concentration of sucrose (20%). The information for this procedure described by Azarov et al. (1990) can be found at the website www.arabidopsis.org. Microscope slides were dipped in this medium to form a thin agar layer over the slides. Subsequently, pollen from flowers that just opened was applied to the agar layer, and slides were placed in a Petri dish on a layer of moist 3M paper. Dishes were incubated for 24 h in short-day conditions at 22°C and analyzed with an optical microscope.

Results

Protein structure and expression analysis of AtTAF6 and AtTAF6b

Since most of the TAF protein sequences are highly conserved between organisms, we used the TAF6 sequence from human, yeast and *Drosophila* for a BLAST search to identify similar proteins encoded by the *Arabidopsis* genome (Lago et al., 2004). This approach allowed us to identify two loci, At1g04950 and At1g54360, which encode the putative *Arabidopsis* proteins, AtTAF6 and AtTAF6b, respectively. Recently, several *AtTAF6* cDNA sequences have been submitted to the NCBI database including *NM_100373* for *AtTAF6* and *NM_104314* for *AtTAF6b* which allowed the correct annotation. The amino acid sequences of the predicted *Arabidopsis* TAF6 proteins share with those of human, *Drosophila* and yeast a level of similarity of about 40% on the full-length proteins and 55% on the N-terminal domains. The TAF6 proteins contain at the N-terminus a conserved histone fold domain which is followed by another highly conserved domain (Fig. 1) which for human TAF6 has shown to be involved in interactions with TBP, TAF1 and general transcription factors including TFIIEα and TFIIFα (Hisatake et al., 1995). The C-terminal parts of the TAF6 proteins are very divergent.

Functional studies of TAF6 proteins in yeast (21) and *Drosophila* (29) revealed some residues critical for the maintenance of protein functionality. We analyzed the *Arabidopsis* AtTAF6 and AtTAF6b sequences in order to check the presence of these critical residues (Fig. 1). In yeast, changing asparagine 151 to tyrosine (N to Y) causes a temperature-sensitive loss-of-function phenotype (Michel et al., 1998). Furthermore, in *Drosophila*, mutating the conserved tryptophan 128 (W) to arginine (R) causes lethality (Aoyagi and Wassarman, 2001). In both *Arabidopsis* TAF6 proteins, these residues are conserved. Interestingly, the N and W residues important for TAF6 function in yeast and *Drosophila*, respectively, are not conserved in the human and *Drosophila* TAF6-like proteins which are not part of TFIID.

The expression of *AtTAF6* and *AtTAF6b* was investigated by Northern blot analysis using RNA extracted from roots, leaves, inflorescences and siliques (Fig. 2). This analysis showed that both genes are expressed in all tissues analyzed.

The AtTAF6 mutant is lethal and affects male gametophytic development

Screening the flanking sequence database of the T-DNA insertion collection of INRA in Versailles (France), we identified a line (CTI12) containing an insertion in the second intron of *AtTAF6*, 352 bp downstream the ATG (Fig. 3A). The progeny (160 individuals) of a self-pollinated plant heterozygous for the *AtTAF6* insertion was analyzed by PCR using gene-specific and T-DNA primers, and the PCR results were confirmed by Southern blot analysis (Fig. 3B). These analyses showed that of these 160 individuals 72 were wild-type and 88 heterozygous for the T-DNA insertion. No homozygous individuals were identified, which suggests that the *AtTAF6* mutation is lethal. Furthermore, the segregation of wild-type and heterozygous individuals in a ratio of approximately 1:1 (72:88) indicates that the *AtTAF6* mutation affects gametophyte development.

A Southern blot that was used to analyze the progeny was also hybridized with a labeled T-DNA probe which revealed that line CTI12 contains a single T-DNA insertion (not shown).

To confirm that the absence of homozygous individuals is due to the T-DNA insertion in *AtTAF6* and not caused by a linked mutation, we performed a complementation test. A genomic fragment of 7 kb comprising the *AtTAF6*

Fig. 1. Amino acid sequence alignment of TAF6 proteins. Dashes represent gaps introduced to maximize the alignment; amino acids conserved in all the 7 proteins are indicated in red, amino acids conserved in AtTAF6 and others are indicated in yellow, amino acids that are conserved in others but not in AtTAF6 are indicated in green and blue. The HFD is indicated with + symbols. The included TAF6 and TAF6-like sequences are AtTAF6 and AtTAF6b from *Arabidopsis*; HsTAF6 from human; DmTAF6 from *Drosophila*; ScTAF6 from yeast; HsTAF6L from human; and DmTAF6L from *Drosophila*. Conserved amino acid residues essential for TAF6 function are indicated with Φ and Ψ.

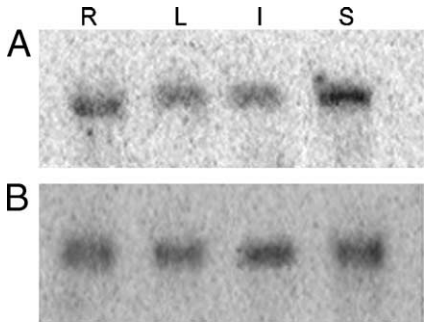

Fig. 2. Northern blot analysis of *AtTAF6* (A) and *AtTAF6b* (B). Total RNA was extracted from roots (R), leaves (L), inflorescences (I) and siliques (S). The coding sequences of *AtTAF6* and *AtTAF6b* were used as probes.

coding sequence and 2231 bp upstream the ATG start codon and 1211 bp downstream the stop codon was cloned by digesting BAC clone F13M7 with *Nhe*I and *Kpn*I. Subsequently, this fragment was cloned in a binary vector, and the resulting construct was used to transform *Arabidopsis* line CT112 which is heterozygous for the *AtTAF6* T-DNA insertion. From the 20 transgenic lines that we obtained, one transformant was selected, and the T1 generation of this plant was analyzed by Southern blot analysis (Fig. 3C). Of the 40 T1 plants that we analyzed, eight plants were homozygous for the *AtTAF6* allele, and these plants all contained the genomic *Nhe*I–*Kpn*I fragment. This result confirms that the additional copy of *AtTAF6* encoded by the genomic *Nhe*I–*Kpn*I fragment complements the lethal *attaf6* mutation. Furthermore, the fact that 25% of the plants were homozygous mutants indicates that the *AtTAF6* transgene also complements the gametophytic defect.

To investigate whether the *AtTAF6* mutation affects male or female gametophyte development, we performed backcrosses between the CTI12 heterozygous line and wild-type plants. When the CTI12 line was used as female and pollinated with wild-type pollen, a normal Mendelian segregation ratio was observed of 1:1 (wild-type:heterozygous). However, when pollen of the CTI12 line was used to pollinate wild-type plants, then only 9 out of 78 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 *AtTAF6* allele through the male gametophyte, which suggests that pollen development is affected by the *attaf6* mutation.

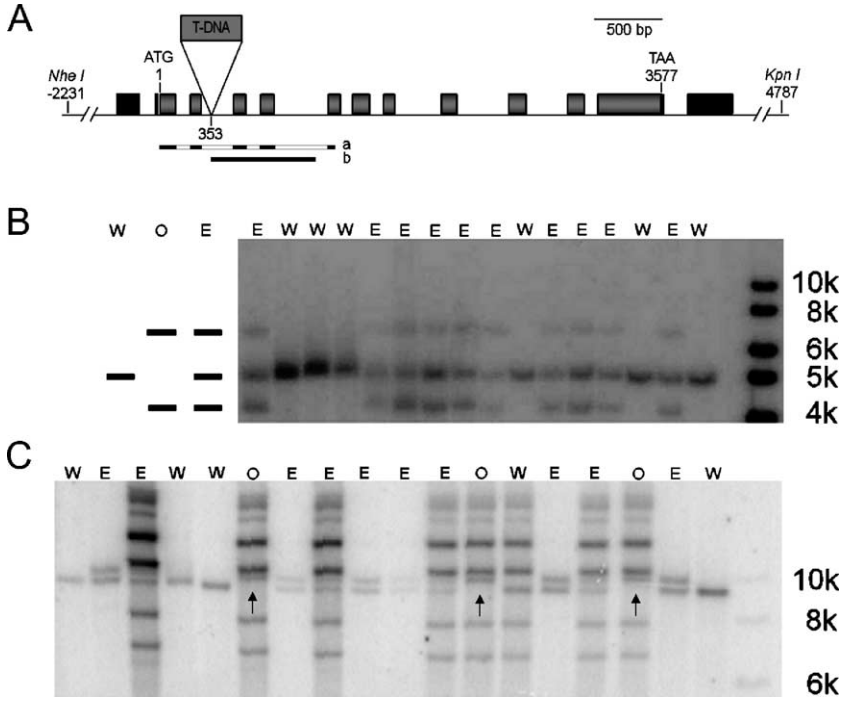

Fig. 3. Segregation and complementation analysis of the *AtTAF6* T-DNA insertion line CTI12. (A). Structure of the *AtTAF6* gene. The T-DNA insertion site is indicated. Bars marked with (a–b) indicate the regions used as probe for the Southern blots shown in panels (B, C) respectively. The positions of the *Nhe*I and *Kpn*I restriction sites that were used to clone the fragment for complementation are indicated relative to the ATG start codon. (B) Example of a Southern blot to analyze the progeny of self-pollinated plants heterozygous for the *attaf6* mutation. Bars at the left site of the panel indicate the expected band pattern for wild-type plants (W), plants heterozygous for the T-DNA insertion (E) and homozygous mutant plants (O). No homozygous plants were obtained. (C) Example of a Southern blot to analyze the ability to complement the CTI12 insertion line with a genomic fragment encoding wild-type *AtTAF6*. This analysis showed that plants homozygous for the *attaf6* insertion allele always contain extra T-DNA copies encoding the *AtTAF6* wild-type allele, showing that lethality is linked to the *attaf6* T-DNA insertion allele. CTI12 plants transformed with a wild-type *AtTAF6* genomic fragment contain multiple insertions (multiple bands). The indicated genotype wild-type (W), heterozygous (E) and homozygous (O) only reflect the T-DNA insertion allele. In the lanes of plants where the homozygous mutation is complemented, the location of the missing band corresponding to the wild-type allele is indicated with an arrow.

Pollen tube development is affected in attaf6 mutant pollen

To understand the effect of the *attaf6* mutation on male gametophyte development, we performed a detailed analysis of pollen development. Since the *attaf6* mutation is lethal, only heterozygous individuals were available for this analysis. In these heterozygous mutant plants, we expect that 50% of the pollen is wild-type. For all our analysis, we also analyzed wild-type plants derived from the segregating population as a reference. By light microscopy, we analyzed different stages of anther development (Fig. 4). This analysis showed that pollen development is normal in line CTI12. Tetrads are formed, indicating that meiosis occurred normally (Fig. 4A). Subsequently, pollen development in the stage of microgametogenesis can be observed (Fig. 4B). Finally, in mature pollen, the three nuclei (one vegetative and two generative nuclei) can be clearly observed, showing that pollen mitosis is normal in this heterozygous mutant (Fig. 4C). Scanning electron microscopy (SEM) analysis of mature pollen of line CTI12 evidenced that also the shape of all the pollen grains was uniform (Fig. 4D). These analyses show that there is no visible effect on pollen development in heterozygous *AtTAF6* mutant plants.

Since the T-DNA insertion in *AtTAF6* did not seem to affect microsporogenesis and microgametogenesis, we analyzed whether the observed segregation distortion is due to a defect in pollen tube development. For these experiments, pollen was collected from wild-type and heterozygous *AtTAF6* young floral buds and germinated in vitro. All the plants that were used for this experiment were from a segregating population and grown next to each other under exactly the same conditions. After 24 h, pollen tube growth was analyzed using an optical microscope. The germinated pollen was classified in 4 groups (Fig. 5A): (I) germinated pollen that developed a pollen tube that has a length of more than 3 times the diameter of the pollen grain; (II) germinated pollen that has a tube with a length of 1–3 times the grain diameter; (III) germinated pollen with only a pollen tube primordia that is smaller than the diameter of the pollen grain; (IV) non-germinated pollen. We analyzed 10 wild-type and 10 heterozygous *AtTAF6* mutant plants and for each of these plants at least 100 pollen grains were germinated and analyzed. The quantity of pollen that did not germinate is rather high in these experiments (±50%), but there is no significant difference between the pollen of wild-type and heterozygous *AtTAF6* mutant plants. Interestingly, a clear difference is observed in the other three classes (Fig. 5B). The number of class I pollen tubes is significantly reduced in pollen samples taken from heterozygous *AtTAF6* mutant plants (23.1%) compared to those taken from wild-type plants (40.7%). In contrast, class II and III pollen tubes are increased in number in mutant samples (II = 8.3%, III = 17.0%) compared to wild-type (II = 2.4%, III = 9.4%). The reduction of about 50% in class I pollen tubes is expected when considering that 50% of the pollen taken from heterozygous *AtTAF6* mutant plants carries the wild-type allele. This analysis shows that *AtTAF6* mutant pollen develops tubes that are signifi-

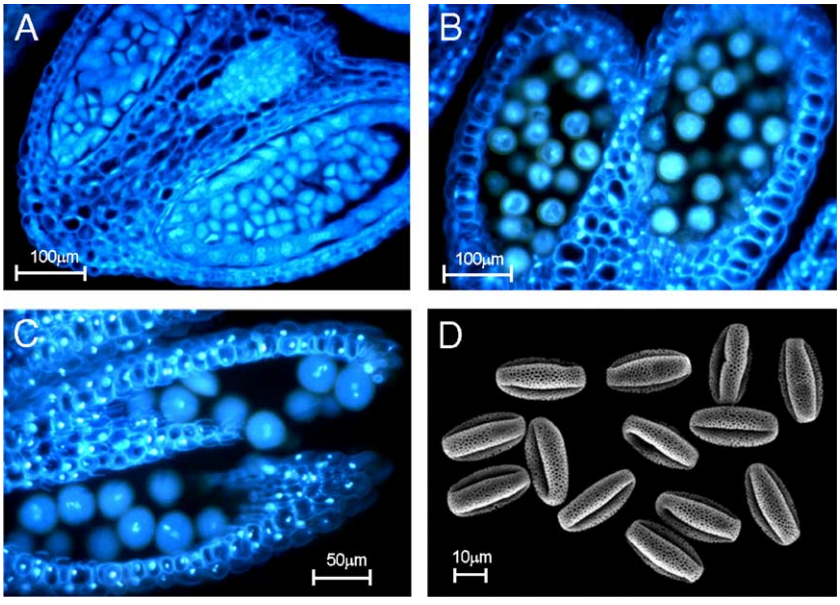

Fig. 4. Analysis of pollen development in *Arabidopsis* line CTI12. (A–C). Anther tissue of plants heterozygous for the T-DNA insertion in *AtTAF6* was fixed, and sections were stained with DAPI. This analysis shows that tetrads are formed (A), indicating that meiosis occurs normally. In panel (B), developing pollen is in the phase of microgametogenesis, and in panel (C), mature pollen grains can be observed that have three nuclei (2 generative and 1 vegetative). Panel (D) shows SEM analysis on pollen from plants heterozygous for the T-DNA insertion in *AtTAF6*. This SEM analysis revealed that all pollen grains are normal in shape. These analyses showed that the *attaf6* mutation has no effect on pollen development.

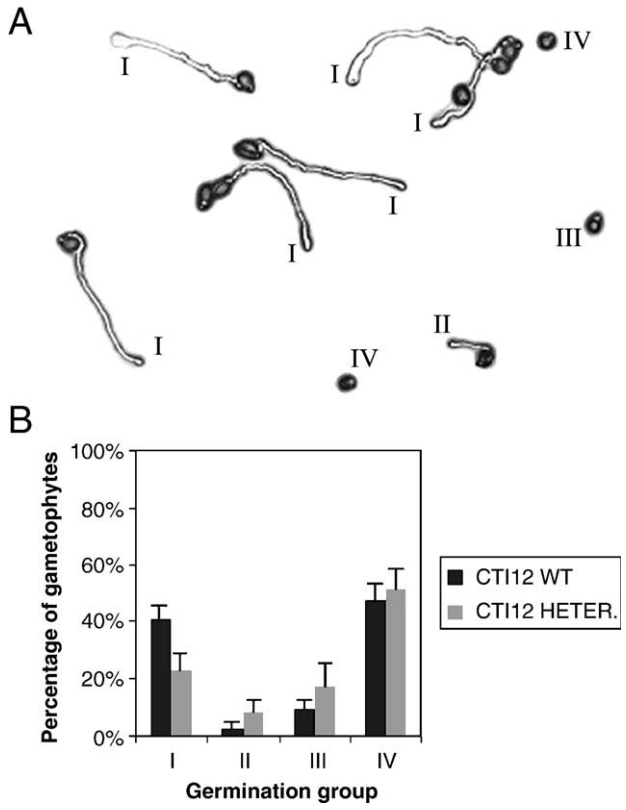

Fig. 5. (A) Example of in vitro germinated pollen. Pollen tubes longer than 3 times the pollen grain diameter were assigned to group I; pollen tubes with a length of 1–3 times the pollen diameter were assigned to group II; pollen tubes with a length that is less than the pollen diameter constitute group III; group IV contains ungerminated pollen grains. (B). This graphic shows the differences observed in pollen germination among wild-type and heterozygous plants of line CTI12. The number of pollen tubes from heterozygous plants that can be assigned to group I is about half the amount of those compared to wild-type plants, with a corresponding increase in pollen tubes belonging to classes II and III. The standard deviation is indicated with bars on top of the columns.

cantly reduced in size, which indicates that their growth rate is significantly reduced.

Discussion

The TAF6 proteins of Arabidopsis

Gene expression is often tightly controlled at the level of transcription. Therefore, studies that focus on the regulation of transcription are important in order to understand how organisms develop and function. For this reason in plant sciences, importance has been given to the functional analysis of transcription factors that regulate processes like those related to biotic and abiotic stress responses, metabolic pathway control, photosynthesis and development. In comparison to the attention that has been given to these specific transcription factors, the basal transcription machinery, important for the initiation of transcription, has been given much less consideration. This is in contrast to mammals, flies and yeast where general transcription factors have been intensively studied (Albright and Tjian, 2000). In these organisms, one of the best studied basal transcription factor is TFIID which is composed of TBP and 12–14 TAFs. Recently, as a starting point for the functional analysis of TAFs in plants, we identified all members of this family encoded by the *Arabidopsis* genome (Lago et al., 2004). The identification of these TAFs was possible due to the high level of sequence conservation between these factors in different organisms.

In *Arabidopsis*, there are two genes encoding TAF6, which we named *AtTAF6* and *AtTAF6b*. The proteins encoded by these TAF6 genes are not very similar (identity 35%, similarity 53%), although they both show significant homology with TAF6 proteins of other organisms. The *Arabidopsis* amino acid residues that have shown to be essential for TAF6 function in human, *Drosophila* and yeast are also conserved in these *Arabidopsis* proteins. Interestingly, also in human and *Drosophila*, there are two *TAF6* genes, although the extra copies are encoding TAF6-like proteins which do not seem to be part of the TFIID complex but form part of histone acetyltransferase (HAT) complexes (Ogryzko et al., 1998; Aoyagi and Wassarman, 2000). In these TAF6-like proteins, the residues essential for TAF6 function are however not conserved.

AtTAF6 and AtTAF6b are not redundant

TAF6 proteins have DNA binding activity and by forming an HFD-mediated interaction with TAF9 sequence-specific binding is stimulated (Shao et al., 2005). Since AtTAF6 and AtTAF6b are not very similar, it could well be that they have preferences for different sequences or are included in different complexes, and therefore they might be controlling the expression of different subsets of genes. This has also been reported for the *Arabidopsis* TAF1 proteins, although these are encoded by two highly homologous genes: *HAF1* and *HAF2* (Bertrand et al., 2005). Mutations in *HAF2* affect the expression of light-regulated genes and acetylation of histone H3 in light-responsive promoters. Microarray analysis on the *haf2* mutant indicated that an even wider range of genes are affected. The phenotypic effects observed in the *haf2* mutant (decrease in chlorophyll accumulation) also suggest that these genes are probably not fully redundant, although the *haf1* mutant does not show any obvious phenotype. The partial redundancy between these genes might be explained by expression differences.

That *AtTAF6* and *AtTAF6b* are not completely redundant is clear from the fact that the *AtTAF6* mutant is lethal. Furthermore, the *attaf6* mutant phenotype indicates that *AtTAF6* regulates specific subsets of genes since in the *attaf6* mutant only male gametes are affected whereas female gametophyte development is unaffected. Furthermore, the mutation in *AtTAF6* only affects pollen tube growth which strengthens the idea that *AtTAF6* is specific for the regulation of genes that are important for this

process. The hypothesis that the activation of specific subsets of genes is needed for pollen tube germination is further supported by recent microarray analyses (Honys and Twell, 2003; Becker et al., 2003).

Microarray analysis also revealed that in germinating pollen *AtTAF6b*, in contrast to *AtTAF6*, is not expressed (personal communication J.D. Becker and J.A. Feijó). This difference in expression profile could be another explanation for the fact that these two TAF6 encoding genes are not redundant.

AtTAF6 pollen tubes grow slowly

Pollen tube growth is an active process which requires the expression of a specific subset of genes. Microarray analysis showed that the expression profile of pollen is most divergent from all other tissues tested (Becker et al., 2003; Honys and Twell, 2003). Pollen has in respect to other tissues a higher expression level of genes with proposed functions in signaling, cell-wall metabolism and cytoskeletal dynamics. This observation fits with the requirements for pollen function: interaction with the stigma, rapid pollen tube growth and pollen tube guidance towards the ovule.

Low expression levels, compared to sporophytic tissues, were found for those genes linked to photosynthesis and protein synthesis. This is to be expected since pollen is not photosynthetically active and pollen grains have shown to accumulate large amounts of ribosomes and tRNAs during their development (Mascarenhas, 1989). Mature pollen grains already contain most of the transcripts needed for germination and tube growth (Mascarenhas, 1989; Honys and Twell, 2003). However, it was also shown that several transcription factors were expressed in germinating pollen, indicating that de novo synthesis of RNA takes place in germinating pollen (Becker et al., 2003). Our results sustain these data since they show that the basal transcription factor AtTAF6 is necessary for a proper pollen function which implies that de novo synthesis of RNA is important during pollen tube growth. Our results also indicate that *AtTAF6* activity is not absolutely essential for pollen tube growth since we observe a low transmission of the mutant allele to the progeny. Probably, mutant pollen tubes grow slower since in the in vitro germination tests we observe an increased number of shorter pollen tubes. This might be due to the lower, or absence of, expression of genes that are essential for rapid pollen tube growth. An explanation could be that *attaf6* mutant pollen tubes depend on the use of transcripts that were accumulated at a pre-meiotic stage.

The observed segregation distortion is probably due to the fact that slower growing mutant pollen tubes are less competitive than those carrying the wild-type allele. This means that by the time that the mutant pollen tubes reach the ovules most of these are already fertilized. This idea is supported by the observation that when we used very low quantities of pollen from the heterozygous *AtTAF6* mutant no aberration to Mendelian segregation was observed (not shown).

Studies to understand the function of TAFs are difficult since most mutations that inhibit the function of these factors are lethal. However, the data shown for TAF1 by Bertrand et al. (2005) and our data for TAF6 reveal that plants like *Arabidopsis* might provide a perfect model system to study these basal transcription factors in more detail.

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