Page 1

Running title:
A “selective” TAF in plants

Corresponding author:
Name, Tsuyoshi Furumoto; address, Department of Biological Science, Graduate School of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-Hiroshima 739-8526, Japan; telephone number, +81-82-424-7453; fax number, +81-82-424-7392; e-mail address, tfurumoto@hiroshima-u.ac.jp.

Subject areas:
(3) gene structure and expression

Number of black and white figures, color figures and tables:
Color figures, 7

Title:

Temporary expression of the *TAF10* gene and its requirement for normal development of *Arabidopsis thaliana*

Authors:

Yosuke Tamada [1, 3], Kazuki Nakamori [1, 4], Hiromi Nakatani [1], Kentaro Matsuda [2, 5], Shingo Hata [1, 2], Tsuyoshi Furumoto [1, 6, *] and Katsura Izui [1, 2, 7]

The names and addresses of the institutions:

[1] *Laboratory of Plant Physiology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

[2] *Division of Applied Biosciences, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

Abbreviations:

atTAF10, *Arabidopsis thaliana* TAF10; CaMV, cauliflower mosaic virus; dmTAF10, *Drosophila melanogaster* TAF10; ftTAF10, *Flaveria trinervia* TAF10; GA_3, gibberellin A_3; GTF, general transcription factor; GUS, β-glucuronidase; mmTAF10, *Mus musculus* TAF10; NAA, α-naphthalene acetic acid; NPA, N-(1-naphthyl)phthalamic acid; ORF, open reading frame; PIC, preinitiation complex; *pnh*, *pinhead*; RNAP2, RNA polymerase II; SAM, shoot apical meristem; scTAF10, *Saccharomyces cerevisiae* TAF10; TAF, TATA box-binding protein-associated factor; TBP, TATA box-binding protein; TFIID, transcription factor IID; *tfl2*, *terminal flower2*; VM, vegetative meristem; *wus*, *wuschel*

Footnotes:

[3]Present address: Department of Biochemistry, University of Wisconsin, 433 Babcock Dr., Madison, WI 53706, USA

[4]Present address: Yakult Central Institute for Microbiological Research, Yakult Honsha Co., Ltd., Kunitachi, Tokyo 186-0011, Japan

[5]Present address: Laboratory of Science Communication and Bioethics, Graduate

School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
[6]Present address: Department of Biological Science, Graduate School of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-Hiroshima 739-8526, Japan
[7]Present address: Department of Biotechnological Science, Kinki University, Nishimitani Kinokawa, Wakayama 649-6493, Japan
* Corresponding author: E-mail, tfurumoto@hiroshima-u.ac.jp; Fax, +81-82-424-7453.

Abstract:

TAF10 is one of the TATA box-binding protein (TBP)-associated factors (TAFs), which constitute a TFIID with a TBP. Initially most TAFs were thought to be necessary for accurate transcription initiation from a broad group of core promoters. However, it was recently revealed that several TAFs are expressed in limited tissues during animal embryogenesis, and are indispensable for normal development of the tissues. They are called “selective” TAFs. In plants, however, little is known as to thus “selective” TAFs and their function. Here we isolated the *Arabidopsis thaliana TAF10* gene (*atTAF10*), which is a single gene closely related to the *TAF10* genes of other organisms. *atTAF10* was expressed transiently during the development of several organs such as lateral roots, rosette leaves and most floral organs. Such an expression pattern was clearly distinct from that of Arabidopsis *Rpb1*, which encodes a component of RNA polymerase II, suggesting that atTAF10 functions in not only general transcription but also the selective expression of a subset of genes. In a knockdown mutant of *atTAF10*, we observed several abnormal phenotypes involved in meristem activity and leaf development, suggesting that atTAF10 is concerned in pleiotropic, but selected morphological events in Arabidopsis. These results clearly demonstrate that TAF10 is a “selective” TAF in plants, providing a new insight into the function of TAFs in plants.

Key words:

TAF10, general transcription factor, morphogenesis, T-DNA insertion mutant, *Arabidopsis thaliana*

Introduction

Transcription requires the assembly of a preinitiation complex (PIC), which consists of template DNA, RNA polymerase II (RNAP2) and several general transcription factors (GTFs). The assembly of PIC is initiated by a transcription factor IID (TFIID), one of the GTFs, by binding to the TATA box (Roeder 1996, Woychik and Hampsey 2002). A TFIID is composed of a TATA box-binding protein (TBP) and about fourteen TBP-associated factors (TAFs) (Dynlacht et al. 1991, Veenstra and Wolffe 2001, Sanders et al. 2002). The amino acid sequences of TAFs are conserved from yeast to man (Albright and Tjian 2000), and recently they were renamed TAF1 to TAF15 depending on the amino acid sequence similarity (Tora 2002). Studies on yeast and animal TAFs revealed multiple biochemical functions of TAFs. First of all, TAFs act as coactivators, mediating the transcriptional activation signal from a sequence-specific activator to RNAP2 (Jacq et al. 1994, Näär et al. 2001). Secondly, some TAFs mediate the interactions between a TFIID and other GTFs (Kraemer et al. 2001, Pan et al. 1999). Thirdly, TAFs function in the stabilization of PIC (Mohan et al. 2003).

Initially, it was thought that most TAFs were necessary for accurate transcription initiation from a broad group of core promoters (Roeder 1996), suggesting that the expression of TAFs was simply correlated to transcriptional activity. In fact, most *Saccharomyces cerevisiae* TAFs (scTAFs) are essential for the growth (Klebanow et al. 1996, Green 2000). However, it was recently revealed that each TAF is required for the transcription of a characteristic and limited subset of genes, ranging from 3% to 67% of the total expressed genes (Green 2000, Lee et al. 2000). Moreover, a subset of TAFs is expressed in limited cells and/or organs during embryogenesis in animals, and is indispensable for normal development of the tissues (Freiman et al. 2001, Hiller et al. 2001). These TAFs are called “selective” TAFs to distinguish them from other relatively “general” TAFs. Thus, transcription is regulated by not only sequence-specific activators but also “selective” TAFs in specific tissues (Verrijzer 2001).

Besides PIC, a group of TAFs is also found as components of other mediator complexes, such as the SPT-ADA-GCN5-acetyltransferase complex (Grant et al. 1998, Martinez 2002). These complexes exhibit histone acetyltransferase activity and are involved in transcriptional activation by establishing an euchromatic state (Agalioti et al. 2002). These facts suggest that TAFs are also involved in transcription through modulation of the chromatin structure.

TAF10 is one of the TAFs, which are components of TFIID and other histone acetylation complexes. In *Drosophila melanogaster*, two *TAF10*s, named *dmTAF10* (formerly *dTAF$_{II}$24*) and *dmTAF10b* (*dTAF$_{II}$16*), are expressed differentially during embryogenesis (Georgieva et al. 2000). F9 embryonal carcinoma cells lacking *Mus musculus TAF10* (*mmTAF10*) show G1 arrest and undergo apoptosis (Metzger et al. 1999). Although the accumulation of mmTAF10 protein is ubiquitously detected in a wide range of organs in adult mice (Perletti et al. 1999), *mmTAF10* mRNA is accumulated in limited cells during embryogenesis and essential for the normal development of these cells (Mohan et al. 2003). The results suggest that TAF10 is a "selective" TAF in these organisms. Further physiological studies on TAF10 and other TAFs were difficult because an organism lacking one of the TAFs is early embryogenetic lethal with a few exceptions. Recently, the functions of TAFs after early embryogenesis became clear with the use of conditional knockout systems (Indra et al. 2005).

Although there have been several reports related to plant general transcriptional machinery (Lagrange et al. 2003, Pan et al. 2000, Sieberer et al. 2003), there have only been a few papers about plant TAFs. TAFs can mediate the interactions between a TFIID and other GTFs (Pan et al. 1999). In wheat, TAFs can act as co-activators and have been identified as 15 proteins ranging in size from c.a. 250 to 30 kDa (Washburn et al. 1997). This profile is similar to that of human TAFs. HAF2, one of the putative Arabidopsis TAF1s, is required to integrate light signals to regulate gene expression through histone acetylation (Bertrand et al. 2005). With the completion of the Arabidopsis genome sequence (Arabidopsis Genome Initiative 2000), putative Arabidopsis TAFs were predicted *in silico* using the conserved amino acid sequences among several organisms as query sequences (Lago et al. 2004). This report indicated that all sets of TAFs function inherently in Arabidopsis, like in other organisms. Recently, two reports showed that there are several "selective" TAFs in plants. Lago et al. (2005) revealed that *atTAF6* specifically functions in pollen tube growth. Moreover, our group showed that *Flaveria trinervia TAF10* (*ftTAF10*) is expressed preferentially in vascular tissues and that over-expression of *ftTAF10* causes two types of abnormal morphologies in Arabidopsis, suggesting that ftTAF10 regulates the transcription of a subset of genes in vascular tissues (Furumoto et al. 2005). To analyze the function of TAF10 in plants in more detail and to confirm that TAF10 is a "selective" TAF in plants, we here isolated the *A. thaliana TAF10* gene (*atTAF10*, At4g31720), which is a single gene closely related to the *TAF10* genes of other organisms. The expression pattern of *atTAF10* in young seedlings and the phenotype in a transformant over-expressing it were similar to those of *ftTAF10*, indicating that *atTAF10* is an ortholog of *ftTAF10*. Detailed expression analyses revealed that *atTAF10* is transiently expressed during the

development of a number of plant organs, and its level is up-regulated on the exogenous auxin- or cytokinin treatment. In addition, a reduction of *atTAF10* expression causes a morphological abnormality involved in maintenance of the shoot apical meristem (SAM) and leaf development. These data demonstrate that atTAF10 is a “selective” TAF in plants.

Results

Relationship among atTAF10s *in various species*

At4g31720 solely encodes a protein the amino acid sequence of which is closely related to those of TAFs in other organisms including hsTAF10, dmTAF10, dmTAF10b and scTAF10 (Lago et al. 2004). This protein exhibits 75.7% and 46.6% identity to the entire ftTAF10 and the C-terminal half of hsTAF10, respectively. A region similar to hsTAF10 contains a putative histone folding domain, which is conserved in the TAF10s of various organisms (Fig. S1, Gangloff et al. 2001, Lago et al. 2004). Thus we named the Arabidopsis gene *atTAF10*.

Vascular tissue-preferential expression of atTAF10:GUS *in young seedlings*

To investigate *atTAF10* expression in detail, we prepared transgenic plants, into which the 2-kb-upstream region from the 3’ end of the *atTAF10* first exon (from 15358196 to 15356197 of chromosome IV) coupled to the coding region of the *β-glucuronidase* (*GUS*) reporter gene (*atTAF10:GUS*) was introduced.

In 10-day-old vegetative seedlings, *atTAF10:GUS* showed vascular tissue-preferential expression (Fig. 1a). It was expressed preferentially in the vascular bundles and bundle sheath cells of cotyledons (Fig. 1b), the phloem of hypocotyls (Fig. 1c), and the central cylinders of roots (Fig. 1d). This expression pattern is closely similar to that of *ftTAF10* in *F. trinervia* observed on *in situ* hybridization (Furumoto et al. 2005). Strong GUS activity was observed in the upper portions of hypocotyls (Fig. 1a). This part consisted of stipules (Fig. 1f, arrowheads) and the region around the intersection of vascular bundles from the hypocotyl and rosette leaves, which were distinguishable from the SAM and leaf primordia (Fig. 1e). Such an expression pattern was clearly distinct from that of the cauliflower mosaic virus (CaMV) 35S promoter fused *GUS* reporter gene (*35S:GUS*), for which GUS activity was detected in almost all tissues (Fig. 1j-m).

Developmental stage- and tissue-specific expression of atTAF10:GUS *in lateral roots and rosette leaves*

atTAF10:GUS expression was observed transiently during the development of several organs. In the lateral roots of 16-day-old seedlings, the expression of *atTAF10:GUS* was strong in lateral root-primordia and developing lateral roots of which the length was less than ca. 1.5 mm, but later the expression was limited to the central cylinder and root caps (Fig. 1g).

In rosette leaves, *atTAF10:GUS* expression was detected in all tissues of young leaves of which the length was ca. 700 μm (Fig. 1h, arrowhead). Along with their maturation, the GUS activity in mesophyll cells became negligible, and it was only detected in vascular bundles and bundle sheath cells (Fig. 1i). At the reproductive stage, this expression in vascular tissues became weak, and sometimes the expression was only detected in hydathodes (Fig. 2d, arrowheads). The *atTAF10:GUS* expression in lateral roots and rosette leaves can be summarized as follows: In young and developing organs except for the SAM, it is expressed generally in all tissues, later it is expressed preferentially in vascular tissues, and then it becomes weak at the reproductive stage.

In 5-week-old plants at the reproductive stage, tissue-preferential expression of *atTAF10:GUS* was observed in inflorescences (Fig. 2a, 3) and stems (Fig. 2b). In stems, it was expressed in the cortices and vascular bundles limited to the side of branching (Fig. 2b, c).

Transient expression patterns of atTAF10:GUS *in developing floral organs*

Arabidopsis inflorescences comprise a number of flowers at various developmental stages (Smyth et al. 1990). *atTAF10:GUS* seemed to be expressed in several tissues of each flower (Fig. 3a). To investigate this tissue- and/or developmental specificity, we analyzed the *atTAF10:GUS* expression of inflorescences in detail.

Although *atTAF10:GUS* expression was slight in the primordia of floral organs at stage 7 (Fig. 3b, c), it was detected in most tissues at stage 9, preferentially in anther locules, which are composed of the tapetum and pollen tetrad (Fig. 3d). At stage 10, it was detected preferentially in petals, the tapetum and ovules (Fig. 3b). The expression was detected preferentially in petals, pistils and ovules at stage 11 (Fig. 3b, e), and in anther walls, pollen and enlarging ovules at stage 12 (Fig. 3f). Thus, expression in pollen and ovules disappeared after stage 15 (Fig. 3g) and stage 17 (Fig. 3h), respectively. At stage 17, *atTAF10:GUS* expression was limited to the podosperm (Fig. 3h), and this expression was diminished at stage 18 (Fig. 3i).

The expression pattern of *atTAF10:GUS* in inflorescences is summarized in Fig. S2. In most floral organs such as sepals, petals, stamens, carpels, anther stalks, the tapetum, pollens, pistils, ovules, embryo sacs and funicles (podosperm), *atTAF10:GUS* was expressed strongly in the middle stage but not in either the early or late stage of development. Such a transient expression pattern is

consistent with those observed in rosette leaves and lateral roots, as described above in Fig. 1 and 2.

Coincident accumulation of atTAF10 *transcripts with* atTAF10:GUS *activity*

To confirm that the expression pattern of *atTAF10:GUS* correlates with the histological localization of *atTAF10* transcripts, we carried out *in situ* hybridization using the upper portions of hypocotyls of vegetative seedlings and inflorescences of reproductive plants, in which the highest expression of *atTAF10:GUS* was detected (Fig. 1-3).

In the upper portions of hypocotyls, *atTAF10* transcripts were detected in the region around the intersection of vascular bundles from the hypocotyl and rosette leaves, and young rosette leaves (Fig. 4a, an arrowhead). Relatively strong signals were detected in stipules (Fig. 4b). In addition, slight signals were detected in other tissues, including leaf primordia. In inflorescences, the accumulation of *atTAF10* transcripts was detected in pollen tetrads of flowers at stage 9 (Fig. 4d), and in sepals and the tapetum at stage 11, but it was below the detection limit near the SAM and floral organ primordia at stages 5, 6 and 8 (Fig. 4c). Transcripts were also detected in all cell types, particularly in the tapetum and ovules in the flowers at stage 10, in all cell types, particularly in elongating petals, pollen and ovules in the flowers at stage 11, and in pollen and ovules at stage 12 (Fig. S3). With the control sense probe, the signal was negligible (Fig. 4e, f, S3).

These results are basically in accord with those for *atTAF10:GUS* (Fig. 1-3), indicating that *atTAF10:GUS* activity correctly reflects the histological distribution of *atTAF10* transcripts.

Distinct distribution of Rpb1 *transcripts from that of* atTAF10 *transcripts*

To confirm the characteristic expression of *atTAF10*, we examined the histological distribution of *Rpb1* transcripts. *Rpb1* (At4g35800) encodes subunit 1 of RNAP2, which is essential for general transcription, and is a single gene in the Arabidopsis genome (Cramer et al. 2000, Dietrich et al. 1990). Thus, we expected that the expression level of *Rpb1* is well related to total transcriptional activity.

In the upper portions of hypocotyls, Arabidopsis *Rpb1* was accumulated in all tissues, particularly near the SAM, leaf primordia, and the region around the intersection of vascular bundles from the hypocotyl and rosette leaves (Fig. 4g). In inflorescences, the accumulation of *Rpb1* transcripts was detected in almost all tissues, particularly in the SAM, flower primordia (flowers at stages 1 and 2), and primordia of floral organs at stages 5, 7 and 9 (Fig. 4h), as expected. These signals were obvious in comparison with those with the sense-control probes (data not shown). Such an expression pattern was clearly distinct from that of *atTAF10*. Based on these data, we conclude

that *atTAF10* expression is “selective” compared with that of *Rpb1* at least, and that atTAF10 is one of the “selective” TAFs in plants, which are involved in the transcription in limited stages during the development of several organs.

Up-regulation of atTAF10 *expression in specific tissues upon addition of exogenous auxin and cytokinin*

Using the PLACE program (http://www.dna.affrc.go.jp/htdocs/PLACE/, Higo et al. 1999), putative responsible elements for auxin were found in the 2-kb-*atTAF10* promoter region, which was used for the *atTAF10:GUS* analyses. This led us to investigate the effects of plant hormones including auxin on *atTAF10* expression, 3-week-old *atTAF10:GUS* transformants being treated with α-naphthalene acetic acid (NAA), kinetin or gibberellin A_3 (GA_3) at 10 μM for 24 h.

The expression pattern of *atTAF10:GUS* in seedlings treated with GA_3 was almost the same as that in non-treated control plants (data not shown). In contrast, on NAA treatment, stronger GUS activity was observed in the vascular bundles of rosette leaves (Fig. 5a, b), and central cylinders and lateral root primordia of roots (Fig. 5e, f). On kinetin treatment, *atTAF10:GUS* expression was also up-regulated in vascular bundles and mesophyll cells of young rosette leaves (Fig. 5c, d), and the central cylinder and root caps of roots (Fig. 5e, g). The up-regulation of endogenous *atTAF10* transcript levels was confirmed by Northern hybridization analysis using total RNA prepared from roots treated with NAA and shoots treated with kinetin (Fig. 5h). In these preparations, the transcript levels were approximately 2-fold higher than those without the treatments. In addition, an auxin polar transport inhibitor, N-(1-naphthyl)phthalamic acid (NPA), affected the *atTAF10* expression pattern (Fig. 5i, j). The expression of *atTAF10:GUS* was up-regulated in vascular bundles of cotyledons, young rosette leaves and roots, accompanying growth inhibition due to the inhibition of auxin efflux by NPA. These results suggest that the expression of *atTAF10* is up-regulated by auxin and cytokinin, but not gibberellin, at least under the conditions we used.

Morphological abnormality caused by reduction of innate atTAF10 *expression*

To elucidate the “selective” function of atTAF10, we analyzed the phenotypes of an Arabidopsis transformant in which T-DNA was inserted into the *atTAF10* gene. In the SIGnAL database (Alonso et al. 2003, http://signal.salk.edu/), we found two transformant lines, SALK_105791 and GABI_406H09, in which T-DNAs, ca. 5 kb in size, are inserted at 470-bp (Fig. 6a) and 52-bp downstream of the putative transcription start site of the *atTAF10* gene, respectively.

In the SALK_105791 transformants homozygotic for the T-DNA insertion, the expression level of *atTAF10* was reduced to nearly 10% compared with that in WT in both vegetative seedlings and inflorescences (Fig. 6b and data not shown, respectively). On the other hand, in GABI_406H09 transformants homozygotic for the T-DNA insertion, the reduction of *atTAF10* expression was not so severe (ca. 60%) and no obvious phenotype was observed (data not shown). Therefore, we used this SALK_105791 transformant for further analyses, and named it *attaf10*.

Three types of abnormal morphology were observed in *attaf10* vegetative seedlings grown on soil. First of all, the size of 3-week-old *attaf10* seedlings was smaller than that of WT (Fig. 6c). However, the size of *attaf10* at the reproductive stage was comparable to that of WT (data not shown). Secondly, abnormal phyllotaxis was observed in 15.3% of the young *attaf10* seedlings (n=98; Fig. 6d), in which the first and/or second rosette leaves were lacking, although a morphologically third or second leaf developed at its original position (*i.e.* the position of third or second leaf, respectively). Thirdly, additional vegetative meristems (VMs) had emerged in 5.1% of the young seedlings (Fig. 6e). These meristems developed rosette leaves normally and bolted at almost the same time.

We additionally observed the abnormal morphology in the *attaf10* mutant grown on agar plates. The VMs had not developed in 39.7% of the mutant seedlings (n=63; Fig. 6f, g). Several primordia of rosette leaves, and sometimes a small radially symmetric pin-like structure (Lynn et al. 1999), arising from VMs were observed in half of these seedlings (Fig. 6g). These leaf primordia usually faced different directions, suggesting that multiple axillary VMs had developed in these seedlings. The leaf primordia had not developed normally and the mutant seedlings were infertile. On agar plates, roots of *attaf10* seedlings developed successfully, however, the length of the main root was only about 80% compared with that in the case of WT (Fig. 6h).

The frequency of defective VM and abnormal phyllotaxis in the *attaf10* mutant was reduced from 15.3% and 39.7% to 2.2% and 2.2%, respectively, by the introduction of a *Bam*HI-*Pst*I genomic fragment, ca. 7.0 kb in size, including the *atTAF10* gene on chromosome IV (n=89, Fig. 6a). Abnormal phyllotaxis was also observed on one seedling among total 25 transformants, in which the 7.0-kb-genomic fragment was introduced into the WT background. Thus, the transformation of the genomic fragment itself seems to cause the phenotype occasionally. These findings suggest that the abnormal morphology in the *attaf10* mutant was complemented well by the introduction of the 7.0-kb-genomic fragment including *atTAF10* gene, and that the above abnormal morphology was caused by the reduction of the *atTAF10* transcript level due to the T-DNA insertion in the *atTAF10* gene.

Thus, although the phenotypes of *attaf10* mutant were pleiotropic, they were found in meristem activity and normal leaf development rather than root or stem formation. These results indicate that atTAF10 functions “selectively” in multiple morphogenetic events.

Morphological abnormality caused by over-expression of atTAF10

To determine whether over-expression of *atTAF10* affects morphogenesis in Arabidopsis, we produced transformants in which *atTAF10* was expressed under the control of the CaMV 35S promoter (*35S:atTAF10*). In eight individual lines out of 32 transgenic *35S:atTAF10* T1 generations in total, a large number (ca. more than 20) of small and deformed leaves developed (Fig. 7a, right panel). There was no vascular tissue in these leaves and the seedlings could not survive after transplanting to soil. In all of the remaining 24 *35S:atTAF10* T1 seedlings, a terminal flower-like phenotype was observed (Fig. 7b). In these plants, several inflorescences per individual plant were terminated. These two phenotypes were observed in both the T1 and T2 generations. In the T2 generation, carpel-like structures were observed in the inflorescence of four out of the five *35S:atTAF10* lines (Fig. 7c). In these plants, a few inflorescences of individual plants were terminated and several flower buds had been converted to carpel-like structures. We rarely observed these phenotypes in *35S:GUS* and WT (Fig. 7a, b, left panels, respectively).

The over-expression of *atTAF10* in the transformant was confirmed by Northern analysis (Fig. 7d). The frequencies of terminal flower-like phenotypes and carpel-like structures were tightly correlated to the *atTAF10* expression, but that of over-production of deformed leaves was not. To trigger the last abnormal morphology, the ectopic expression of *atTAF10* in specific tissues, not entire seedlings, might be important.

Discussion

atTAF10 *is an ortholog of* ftTAF10

In the preceding paper (Furumoto et al. 2005), we revealed that *ftTAF10* shows vascular tissue-preferential expression in *F. trinervia* seedlings, and that the over-expression of *ftTAF10* causes two phenotypes in Arabidopsis, i.e., over-production of deformed leaves and a terminal flower-like phenotype. These results for *ftTAF10* are closely similar to those for *atTAF10* in Arabidopsis (Fig. 1, 7). Both *ftTAF10* and *atTAF10* are single genes in the *F. trinervia* and *A. thaliana* genomes, respectively (Furumoto et al. 2005, Lago et al. 2004). These results indicate that *atTAF10* is an ortholog of *ftTAF10* and that the function of TAF10 is conserved among a broad range

of dicotyledonous plants.

atTAF10 is one of the "selective" TAFs expressed preferentially and transiently during the development of several organs

In the case of mmTAF10, its necessity for normal development of certain limited tissues in early embryos is well known (Mohan et al. 2003). At subsequent developmental stages after early embryogenesis, its contribution has not been well analyzed except for in the case of establishment of the skin barrier function in foetal mice. In this case, mmTAF10 functions in the "terminal differentiation" of keratinocytes (Indra et al. 2005). We revealed the unique expression profile of *atTAF10* with the aid of both *atTAF10* promoter-GUS activity (Fig. 1-3, S2) and *in situ* hybridization (Fig. 4, S3). *atTAF10* was expressed preferentially and transiently in the middle stage of development of lateral roots, rosette leaves and most floral organs. The expression pattern of *atTAF10* suggests that atTAF10 is a "selective" TAF in plants. What is the function of atTAF10 in these tissues? One possibility is its involvement in the "terminal differentiation" of these organs as in the case of mmTAF10. This hypothesis is consistent with the phenotypes of *35S:atTAF10* transformants. Deformed leaves (Fig. 7a) may be caused by irregular development of the leaves. A terminal flower-like phenotype (Fig. 7b) and carpel-like structures (Fig. 7c) may also be due to unsuccessful development of inflorescences and flower buds, respectively. In these cases, ectopic and high expression of *atTAF10* led to abnormal development, indicating that its adequate expression is necessary for normal development. Given that each TAF including TAF10 is required for the transcription of a characteristic and limited subset of genes in yeast (Green 2000, Lee et al. 2000), we speculate that atTAF10 is also involved in the regulation of a limited subset of genes in Arabidopsis. To test this hypothesis, genome-wide expression analyses of WT and *attaf10* mutant or ChIP-on-chip analysis using atTAF10-specific antibody will be required.

The expression of *atTAF10* was up-regulated by exogenous auxin and cytokinin treatment (Fig. 5). The *atTAF10* promoter exhibits high activity in stipules, vascular bundles and hydathodes (Fig. 1f, a-e and 2d, respectively), in which free auxin is accumulated (Aloni et al. 2003). The phenotype of over-production of deformed leaves is similar to that of *35S:ARR1ΔDDK* transformants in which the cytokinin signal is constitutively activated (Sakai et al. 2001). These data suggest that atTAF10 might partly play a role in the auxin and/or cytokinin signal transduction pathways for morphogenesis.

atTAF10 is involved in the gene expression related to meristem activity and leaf development

On the *attaf10* mutant, we observed several abnormal phenotypes involved in meristem activity and leaf development, suggesting that atTAF10 is pleiotropically involved in morphological events in Arabidopsis (Fig. 6).

However, the abnormal phenotypes seem not always to be well correlated to the expression pattern and intensity of *atTAF10*. For instance, the expression of *atTAF10* was observed preferentially in young leaves and stipules, compared with the faint expression in the SAM and leaf primordia (Fig. 1e, f, h, and 4a, b). One hypothesis to explain these discrepancies is that weak- and basal expression of *atTAF10* around the SAM is important for the maintenance of SAM activity and leaf development. Another possibility is that atTAF10 functions at expressed tissues (Fig. 1e, f and 4b) and is indirectly involved in these morphogenetic events.

The phenotypes of the *attaf10* mutant are partially similar to those of *pinhead* (*pnh*) and *wuschel* (*wus*) mutants. In loss-of-function *pnh-8* seedlings, vegetative activity is also frequently diminished. Its emergency ratio is 44%, which is similar to that in the *attaf10* mutant, 39.7%. A small radially symmetric pin-like structure is also observed both in *attaf10* and *pnh* seedlings (Lynn et al. 1999, Fig. 6g). The SAM in the *wus* mutant is also frequently diminished, and then axially meristems develop (Laux et al. 1996). The weak mutant allele of *wus*, *jam*, show a lack of juvenile leaves like *attaf10* mutant, although the third leaf in the *jam* mutant develops at the first leaf position, in contrast to the *attaf10* mutant, in which the third leaf developed at its original position (Hamada et al. 2000, Fig. 6d). Additionally, the phenotypes on *35S:atTAF10* transformants are partially similar to those of *tfl2* mutant. The terminal flower-like phenotype and the emergence of carpel-like structures were observed both in *tfl2* mutant and *35S:atTAF10* transformants (Larsson et al. 1998, Fig. 7).

Here we established the transient expression of *atTAF10* in broad range of developing organs and its necessity for accurate development in Arabidopsis. Our results demonstrate that TAF10 is a “selective” TAF in plants as well as mammal selective TAFs. The genetic interactions between *attaf10* and above denoted mutants might be a clue for further detailed analysis of its “selective” function.

Addendum: After the submission of this paper, Gao et al. (2006) revealed that atTAF10 functions in the tolerance to saline stress during seed germination, but not during vegetative growth. Their results indicate that atTAF10 is temporarily involved in the response to environmental abiotic stress, and are consistent with our hypothesis of pleiotropic and selective function of atTAF10.

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana ecotype Columbia seeds served as the wild type. Plants were grown on vermiculite in a growth chamber as described by Furumoto et al. (2001). Alternatively, Arabidopsis seeds were sterilized and grown at 21°C with 16 h 60 μE light and 8 h darkness photoperiods on agar plates with 1x MS salt (Wako, Japan; Murashige and Skoog 1962), 1x Gamborg's B5 vitamin (Sigma, MO), 1% sucrose and 237μM carbenicillin (sodium salt, Wako, Japan). For Northern hybridization, a number of vegetative seedlings, which did not show any visible phenotypes, were used.

atTAF10:GUS *analysis*

The 2-kb-promoter region of *atTAF10* (15358196-15356197 of chromosome IV) was amplified by PCR with the following primer set, (5'-CCGTCGACCTACAACAACACATGAGTCA-3') and (5'-GGGGATCCCTAAACGAAGCAGCAACGGA-3'), which contained artificial *Sal*I and *Bam*HI sites, respectively. The PCR products were digested with these restriction enzymes, and then subcloned between the *Sal*I and *Bam*HI sites of the pBI101.2 vector. The nucleotide sequence was verified by sequencing with an ABI Prism dye terminator cycle sequencing reaction kit and ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA). The promoter-constructed pBI101 was transformed into Arabidopsis as previously described (Furumoto et al. 2005). After incubating the seedlings in staining buffer (100 mM phosphate buffer, pH 7.0, 0.3% Triton X-100, 1.9 mM 5-bromo-4-chloro-3-indoyl-β-D-glucuronide, and 0.5 mM potassium ferricyanide and ferrocyanide) for 24 h at 37°C, the plants were dipped in 70% ethanol (Weigel and Glazebrook 2002). Pictures were taken under a Nikon microscope, SMZ-U, with a Nikon digital camera, DXM1200 (Nikon, Japan). Then, each tissue was embedded in hydroxyethylmethacrylate (Technovit 7100, Kulzer, Germany) according to the manufacturer's protocol. Thin sections (6 or 12 μm thickness) were stained with 0.02% of safranin (Wako, Japan) for a few seconds, washed with distilled water, mounted with ENTELLAN (ENTELLAN new, Merck, Germany), and then photographed under a Nikon microscope, ECLIPSE E600 and DXM1200 (Nikon, Japan). All promoter-GUS-analyses were performed for three independent transgenic lines to confirm reproducible promoter activity.

Analyses of the attaf10 *mutant*

We found the *attaf10* mutant (SALK_105791) in the SIGnAL database, and obtained the seeds through the ABRC website (http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/abrchome.htm). For the genotyping of *attaf10* mutants, we followed the method described on the SIGnAL website (http://signal.salk.edu/tdnaprimers.html). The genomic fragments derived from the native *atTAF10* gene were amplified with an LP primer (5'-CCTAAATTTGGGCCATATGTGAAGA-3') and an RP primer (5'-CCAAATTTCAGTCATGTAGAGCCG-3'). The fragments derived from the T-DNA-inserted *atTAF10* gene were amplified with the RP and the LBb1 primers described on the SIGnAL website. Four homozygotes of *attaf10* were obtained from the original *attaf10* heterozygote seeds (homozygotes: heterozygotes: non-transformants = 4:10:4), and were analyzed. The proportion of the seedlings with an abnormal morphology was quite similar among their lines. Their morphology was recorded with a SMZ-U and DXM1200 (Nikon, Japan), or photographed with a digital camera, CAMEDIA E-10 (OLYMPUS, Japan).

Generation of 35S:atTAF10 *transgenic plants*

The *atTAF10* cDNA open reading frame (ORF) were amplified using a plasmid cDNA library prepared from Arabidopsis vegetative tissues by PCR with the following primer sets: *atTAF10* ORF, (5'-GGTCTAGAATGAATCACGGCCAACAATCT-3') and (5'-CCGAGCTCCTATTCGTCCCTTGTTGCA-3'), which contained artificial *Xba*I or *Sac*I sites, respectively. The PCR products were subcloned into the pGEM-T Easy Vector using pGEM-T Easy Vector System I (Promega Corporation, WI). The *atTAF10* ORF was digested with *Xba*I and *Sac*I, and then subcloned into a slightly modified pPZP211 vector (Furumoto et al. 2005). Constructs were verified by sequencing. These binary vectors were used for the transformation of Arabidopsis as described above. Thirty-two independent kanamycin-resistant *35S:atTAF10* T1 transformants were produced. Their successful transformation was confirmed by PCR-amplification of inserted cDNAs of expected sizes. Several T2 lines, showing a segregation ratio of 3:1 for resistance to kanamycin, were selected arbitrarily and used for subsequent analyses. Their morphology was recorded as described above.

Total RNA isolation and Northern hybridization

Sample tissues were immediately frozen in liquid nitrogen. Total RNA was prepared using TRIzol Reagent (Invitrogen Life Technologies, CA) according to the manufacturer's protocol. The hybridization procedure was the same as previously described by Tamada et al. (2003). Aliquots of

total RNAs were separated in 1.0% agarose gels containing 6% formaldehyde and then blotted onto Hybond-N^+ membranes (Amersham Biosciences, UK). The cDNA fragments of the *atTAF10* coding-region were labeled with [α-^{32}P] dCTP using a Megaprime labeling kit (Amersham Biosciences, UK). The *atTAF10* coding-region fragments were amplified using the *atTAF10* ORF primer set. The membranes were probed with these ^{32}P-labeled cDNA fragments.

In situ *hybridization*

The cDNA fragments of *Rpb1* were amplified with (5'-GCCGATTCATCAACAATGGA-3') and (5'-CTTTACCAATCGCCTCTGAA-3'). The PCR products were subcloned into the pGEM-T Easy Vector as described above. These plasmids were used for the synthesis of antisense and sense probes. *In situ* hybridization was carried out as described previously (Furumoto et al. 2005) using digoxigenin-labeled probes.

Acknowledgements

The generosity of Prof. F. Sato (Kyoto University, Japan) and Dr. K. Mise (Kyoto University, Japan) in providing us with the pBI101.2 vector and a plasmid library of Arabidopsis vegetative seedlings, respectively, is gratefully acknowledged. We would also like to thank Prof. R. M. Amasino (The University of Wisconsin-Madison, WI), Dr. K. Goto (Research Institute for Biological Sciences Okayama, Japan), and Dr. H. Kaya (Tokyo University of Science, Tokyo, Japan) for the fruitful discussions on the phenotypes of transformants, histochemical analyses, and the functions of plant chromatin modifying factors, respectively. Y. T. was supported by the 21st Century COE Program of the Ministry of Education, Culture, Sports, Science and Technology Agency of the Japanese Government.

References

Agalioti, T., Chen, G. and Thanos, D. (2002) Deciphering the transcriptional histone acetylation code for a human gene. *Cell* 111: 381-392.

Albright, S.R. and Tjian, R. (2000) TAFs revisited: more data reveal new twists and confirm old ideas. *Gene* 242: 1-13.

Aloni, R., Schwalm, K., Langhans, M. and Ullrich, C.I. (2003) Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and

leaf morphogenesis in *Arabidopsis*. *Planta* 216: 841-853.

Alonso, J.M., Stepanova, A.N., Leisse, T.J., et al. (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301: 653-657.

Arabidopsis Genome Initiative. (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796-815.

Bertrand, C., Benhamed, M., Li, Y.F., Ayadi, M., Lemonnier, G., Renou, J.P., Delarue, M. and Zhou, D.X. (2005) *Arabidopsis* HAF2 gene encoding TATA-binding protein (TBP)-associated factor TAF1, is required to integrate light signals to regulate gene expression and growth. *J. Biol. Chem.* 280: 1465-1473.

Cramer, P., Bushnell, D.A., Fu, J., Gnatt, A.L., Maier-Davis, B., Thompson, N.E., Burgess, R.R., Edwards, A.M., David, P.R. and Kornberg, R.D. (2000) Architecture of RNA polymerase II and implications for the transcription mechanism. *Science* 288: 640-649.

Dietrich, M.A., Prenger, J.P. and Guilfoyle, T.J. (1990) Analysis of the genes encoding the largest subunit of RNA polymerase II in *Arabidopsis* and soybean. *Plant Mol. Biol.* 15: 207-223.

Dynlacht, B.D., Hoey, T. and Tjian, R. (1991) Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* 66: 563-576.

Freiman, R.N., Albright, S.R., Zheng, S., Sha, W.C., Hammer, R.E. and Tjian, R. (2001) Requirement of tissue-selective TBP-associated factor TAFII105 in ovarian development. *Science* 293: 2084-2087.

Furumoto, T., Tamada, Y., Izumida, A., Nakatani, H., Hata, S. and Izui, K. (2005) Vascular-tissue abundant expression of plant *TAF10*, an orthologous gene for TATA box-binding protein-associated factor 10, in *Flaveria trinervia* and abnormal morphology of *Arabidopsis thaliana* transformants on its over-expression. *Plant Cell Physiol.* 46: 108-117.

Furumoto, T., Teramoto, M., Inada, N., Ito, M., Nishida, I. and Watanabe, A. (2001) Phosphorylation of a bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphate 2-phosphatase, is regulated physiologically and developmentally in rosette leaves of *Arabidopsis thaliana*. *Plant Cell Physiol.* 42: 1044-1048.

Gangloff, Y.G., Romier, C., Thuault, S., Werten, S. and Davidson, I. (2001) The histone fold is a key structural motif of transcription factor TFIID. *Trends Biochem. Sci.* 26: 250-257.

Gao, X., Ren, F. and Lu, Y.T. (2006) The *Arabidopsis* mutant *stg1* identifies a function for TBP-associated factor 10 in plant osmotic stress adaptation. *Plant Cell Physiol.* 47: 1285-1294

Georgieva, S., Kirschner, D.B., Jagla, T., et al. (2000) Two novel *Drosophila* TAF(II)s have homology with human TAF(II)30 and are differentially regulated during development. *Mol. Cell.*

Biol. 20: 1639-1648.

Grant, P.A., Schieltz, D., Pray-Grant, M.G., Steger, D.J., Reese, J.C., Yates, J.R., 3rd and Workman, J.L. (1998) A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* 94: 45-53.

Green, M.R. (2000) TBP-associated factors (TAFIIs): multiple, selective transcriptional mediators in common complexes. *Trends Biochem. Sci.* 25: 59-63.

Hamada, S., Onouchi, H., Tanaka, H., Kudo, M., Liu, Y.G., Shibata, D., Machida, C. and Machida, Y. (2000) Mutations in the *WUSCHEL* gene of Arabidopsis thaliana result in the development of shoots without juvenile leaves. *Plant J.* 24: 91-101.

Higo, K., Ugawa, Y., Iwamoto, M. and Korenaga, T. (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res.* 27: 297-300.

Hiller, M.A., Lin, T.Y., Wood, C. and Fuller, M.T. (2001) Developmental regulation of transcription by a tissue-specific TAF homolog. *Genes Dev.* 15: 1021-1030.

Indra, A.K., Mohan, W.S., 2nd, Frontini, M., Scheer, E., Messaddeq, N., Metzger, D. and Tora, L. (2005) TAF10 is required for the establishment of skin barrier function in foetal, but not in adult mouse epidermis. *Dev. Biol.* 285: 28-37.

Jacq, X., Brou, C., Lutz, Y., Davidson, I., Chambon, P. and Tora, L. (1994) Human TAFII30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell* 79: 107-117.

Klebanow, E.R., Poon, D., Zhou, S. and Weil, P.A. (1996) Isolation and characterization of *TAF25*, an essential yeast gene that encodes an RNA polymerase II-specific TATA-binding protein-associated factor. *J. Biol. Chem.* 271: 13706-13715.

Kraemer, S.M., Ranallo, R.T., Ogg, R.C. and Stargell, L.A. (2001) TFIIA interacts with TFIID via association with TATA-binding protein and TAF40. *Mol. Cell. Biol.* 21: 1737-1746.

Lago, C., Clerici, E., Dreni, L., Horlow, C., Caporali, E., Colombo, L. and Kater, M.M. (2005) The Arabidopsis TFIID factor AtTAF6 controls pollen tube growth. *Dev. Biol.* 285: 91-100.

Lago, C., Clerici, E., Mizzi, L., Colombo, L. and Kater, M.M. (2004) TBP-associated factors in Arabidopsis. *Gene* 342: 231-241.

Lagrange, T., Hakimi, M.A., Pontier, D., Courtois, F., Alcaraz, J.P., Grunwald, D., Lam, E. and Lerbs-Mache, S. (2003) Transcription factor IIB (TFIIB)-related protein (pBrp), a plant-specific member of the TFIIB-related protein family. *Mol. Cell. Biol.* 23: 3274-3286.

Larsson, A.S., Landberg, K. and Meeks-Wagner, D.R. (1998) The *TERMINAL FLOWER2* (*TFL2*) gene controls the reproductive transition and meristem identity in *Arabidopsis thaliana*. *Genetics*

149: 597-605.

Laux, T., Mayer, K.F., Berger, J. and Jurgens, G. (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in Arabidopsis. *Development* 122: 87-96.

Lee, T.I., Causton, H.C., Holstege, F.C., Shen, W.C., Hannett, N., Jennings, E.G., Winston, F., Green, M.R. and Young, R.A. (2000) Redundant roles for the TFIID and SAGA complexes in global transcription. *Nature* 405: 701-704.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P. and Barton, M.K. (1999) The *PINHEAD/ZWILLE* gene acts pleiotropically in Arabidopsis development and has overlapping functions with the *ARGONAUTE1* gene. *Development* 126: 469-481.

Martinez, E. (2002) Multi-protein complexes in eukaryotic gene transcription. *Plant Mol. Biol.* 50: 925-947.

Metzger, D., Scheer, E., Soldatov, A. and Tora, L. (1999) Mammalian TAF(II)30 is required for cell cycle progression and specific cellular differentiation programmes. *EMBO J.* 18: 4823-4834.

Mohan, W.S., Jr, Scheer, E., Wendling, O., Metzger, D. and Tora, L. (2003) TAF10 (TAF(II)30) is necessary for TFIID stability and early embryogenesis in mice. *Mol. Cell. Biol.* 23: 4307-4318.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15: 473-497.

Näär, A.M., Lemon, B.D. and Tjian, R. (2001) Transcriptional coactivator complexes. *Annu. Rev. Biochem.* 70: 475-501.

Pan, S., Czarnecka-Verner, E. and Gurley, W.B. (2000) Role of the TATA binding protein-transcription factor IIB interaction in supporting basal and activated transcription in plant cells. *Plant Cell* 12: 125-136.

Pan, S., Sehnke, P.C., Ferl, R.J. and Gurley, W.B. (1999) Specific interactions with TBP and TFIIB in vitro suggest that 14-3-3 proteins may participate in the regulation of transcription when part of a DNA binding complex. *Plant Cell* 11: 1591-1602.

Perletti, L., Dantonel, J.C. and Davidson, I. (1999) The TATA-binding protein and its associated factors are differentially expressed in adult mouse tissues. *J. Biol. Chem.* 274: 15301-15304.

Roeder, R.G. (1996) The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem. Sci.* 21: 327-335.

Sakai, H., Honma, T., Aoyama, T., Sato, S., Kato, T., Tabata, S. and Oka, A. (2001) ARR1, a transcription factor for genes immediately responsive to cytokinins. *Science* 294: 1519-1521.

Sanders, S.L., Garbett, K.A. and Weil, P.A. (2002) Molecular characterization of Saccharomyces cerevisiae TFIID. *Mol. Cell. Biol.* 22: 6000-6013.

Sieberer, T., Hauser, M.T., Seifert, G.J. and Luschnig, C. (2003) *PROPORZ1*, a putative *Arabidopsis* transcriptional adaptor protein, mediates auxin and cytokinin signals in the control of cell proliferation. *Curr. Biol.* 13: 837-842.

Smyth, D.R., Bowman, J.L. and Meyerowitz, E.M. (1990) Early flower development in *Arabidopsis*. *Plant Cell* 2: 755-767.

Tamada, Y., Imanari, E., Kurotani, K., Nakai, M., Andreo, C.S. and Izui, K. (2003) Effect of photooxidative destruction of chloroplasts on the expression of nuclear genes for C4 photosynthesis and for chloroplast biogenesis in maize. *J. Plant Physiol.* 160: 3-8.

Tora, L. (2002) A unified nomenclature for TATA box binding protein (TBP)-associated factors (TAFs) involved in RNA polymerase II transcription. *Genes Dev.* 16: 673-675.

Veenstra, G.J. and Wolffe, A.P. (2001) Gene-selective developmental roles of general transcription factors. *Trends Biochem. Sci.* 26: 665-671.

Verrijzer, C.P. (2001) Transcription factor IID--not so basal after all. *Science* 293: 2010-2011.

Walker, A.K., Rothman, J.H., Shi, Y. and Blackwell, T.K. (2001) Distinct requirements for *C.elegans* TAF(II)s in early embryonic transcription. *EMBO J.* 20: 5269-5279.

Washburn, K.B., Davis, E.A. and Ackerman, S. (1997) Coactivators and TAFs of transcription activation in wheat. *Plant. Mol. Biol.* 35: 1037-1043.

Weigel, D. and Glazebrook, J. (2002) Arabidopsis: a Laboratory Manual. pp. 243-245. Cold Spring Harbor Laboratory Press, NY.

Woychik, N.A. and Hampsey, M. (2002) The RNA polymerase II machinery: structure illuminates function. *Cell* 108: 453-463.

Legends to figures

Fig. 1 The expression of *atTAF10:GUS* in vegetative seedlings.

(a-f) The expression of *atTAF10:GUS* in 10-day-old seedlings. A whole seedling (a), transverse sections through a cotyledon (b), a hypocotyl (c), and a root (d), and a longitudinal section (e) and a transverse section (f) through the upper portion of a hypocotyl near the SAM. Arrowheads in (f) indicate stipules.

(g-i) The expression of *atTAF10:GUS* in 16-day-old seedlings. Lateral roots and a lateral root primordium (g), young rosette leaves (h), and an expanding rosette leaf (i). The arrowhead in (h) indicates a young rosette leaf whose size was ca. 700 μm.

(j-m) The expression of *35S:GUS* in 10-day-old seedlings. A whole seedling (j), and transverse

sections through a cotyledon (k), a hypocotyl (l), and a root (m).

BSC, bundle sheath cell; E, endodermis; Lp, leaf primordium; M, SAM; MC, mesophyll cell; Pe, pericycle; Vb, vascular bundle. Scale bars: 2 mm in (a, j); 50 μm in (b-f, k-m); 500 μm in (g-i).

Fig. 2 The expression of *atTAF10:GUS* in 5-week-old reproductive plants.

(a) A whole plant.

(b) The branching point of the first stem.

(c) A transverse section through the first stem.

(d) A rosette leaf.

C, cortex; Cl, cauline leaf; Vb, vascular bundle. Arrowheads in (d) indicate hydathodes. Scale bars: 2 mm in (a, d); 500 μm in (b); 100 μm in (c).

Fig. 3 The expression of *atTAF10:GUS* throughout the development of flowers.

(a) Overall view of an inflorescence.

(b) Flowers at stages 7, 10 and 11.

(c-i) Flowers at various developmental stages. Stage 7 (c), stage 9 (d), stage 11 (e), stage 12 (f), stage 15 (g), stage 17 (h), and stage 18 (i).

A, anther; O, ovule; Pe, petal; Pi, pistil; Ps, podosperm. Tissues were stained with 0.02% safranin, a red dye, before preparation of the sections (b-f). The numbers indicate the developmental stages of these flowers, as described (Smyth et al. 1990). Scale bars: 1 mm in (a); 100 μm in (b-f); 200 μm in (g-i).

Fig. 4 Histological detection of *atTAF10* and *Rpb1* transcripts on *in situ* hybridization.

Photographs were taken under dark field conditions. The signals appear as orange to dark purple, and tissues appear white. Thin sections were hybridized with an *atTAF10* antisense-RNA probe (a-d) or *atTAF10* sense RNA probe (e, f) generated from the entire *atTAF10* cDNA, or were hybridized with an *Rpb1* antisense RNA probe (g, h). Longitudinal sections through the upper portions of hypocotyls (a, b, e, g) and inflorescences (c, d, f, h) are shown. Panels (b) and (d) show higher magnifications of panels (a) and (c), respectively. M, SAM; Pt, pollen tetrad; S, stipule. The arrowhead in (a) indicates a young rosette leaf in which strong expression of *atTAF10* was detected. The numbers around flowers indicate the developmental stages. Scale bars: 200 μm in (a-c, e-h); 50 μm in (d).

Fig. 5 Induction of *atTAF10:GUS* expression on treatment with exogenous auxin or cytokinin, or

NPA.

(a, c, e) Three-week-old transformants were grown without hormones. A juvenile rosette leaf (a), a young adult rosette leaf (c), and roots (e).

(b, f) Three-week-old transgenic seedlings were treated with 10 μM NAA for 24 h. A juvenile rosette leaf (b) and roots (f).

(d, g) Three-week-old transgenic seedlings were treated with 10 μM kinetin for 24 h. A young adult rosette leaf (d) and roots (g).

(h) Northern hybridization analyses using the labeled *atTAF10* coding-region fragments. Five μg each of total RNA prepared from roots without hormone treatment (lane 1) and treated with 10 μM NAA (lane 2), and 10 μg of RNA from shoots without hormone treatment (lane 3) and treated with 10 μM kinetin (lane 4) were loaded. The lower panels show ethidium bromide-stained ribosomal RNAs as loading controls.

(i, j) The transformants were grown for 3 weeks on agar plates with (j) or without (i) 10 μM NPA.

Scale bars: 1 mm in (a-d); 500 μm in (e-g); 2 mm in (i, j).

Fig. 6 Characterization of the *attaf10* mutant.

(a) The insertion site of the T-DNA in *attaf10* mutants. The filled and open boxes indicate the exons of At4g31720 (*atTAF10*), and parts of At4g31710 and At4g31730, respectively. *Bam*HI and *Pst*I sites were used for the complementation experiments. The genomic fragment between *Bam*HI and *Pst*I includes the complete 2-kb promoter region used in the *atTAF10:GUS* analyses and the whole gene of *atTAF10*.

(b) Northern analysis of *atTAF10* transcripts in WT and four independent lines of the *attaf10* mutant. Two-week-old vegetative seedlings were used. Ten μg of total RNA was loaded in each lane.

(c) The appearance of WT and the *attaf10* mutant grown on soil.

(d, e) Representative morphology on abnormal phyllotaxis (d, right panel) and emergence of additional VMs (e) of the *attaf10* mutant grown on soil. That for WT is shown as a control (d, left panel). Arrows in (d) and (e) indicate cotyledons (some of them are hidden under rosette leaves) and visible VMs, respectively.

(f, g) Representative morphology with the lack of vegetative meristem activity of the *attaf10* mutant grown on agar plates. A seedling with several primordia of rosette leaves and a small radially symmetric pin-like structure derived from the VM are shown in (g). The right panels show higher magnifications of the left panels. Arrows indicate the direction of the adaxial surface of two visible rosette leaf primordia.

(h) The appearance of WT and the *attaf10* mutant grown on agar plates.

Scale bars: 10 mm in (a, b, h); 4 mm in (d); 1 mm in (e, f, g).

Fig. 7 Abnormal morphology caused by over-expression of *atTAF10*.

(a-c) Representative morphology on over-production of deformed leaves in 3-week-old seedlings (a, right panel), a terminal flower-like phenotype (b, right panel), and carpel-like structures in inflorescences of 10-week-old plants (c) of *35S:atTAF10* transformants. In *35S:GUS* transformants (a, left panel) and WT (b, left panel), these phenotypes were rarely observed. Arrowheads and arrows in (c) indicate carpel-like structures and ovule-like primordia, respectively. Scale bars: 4 mm.

(d) Northern analysis of WT and *35S:atTAF10* transformants in the T2 generation. Three-week-old vegetative seedlings of three independent transformant lines, in which the transgene was single, were used. Ten μg of total RNA was loaded in each lane.

Supplemental Data

Fig. S1 Amino acid sequence alignment of atTAF10 and TAF10s of other organisms.

Fig. S2 Schematic representation of *atTAF10:GUS* expression in inflorescences.

Fig. S3 Histological detection of *atTAF10* transcripts in flowers on *in situ* hybridization.

Figures

Fig. 1

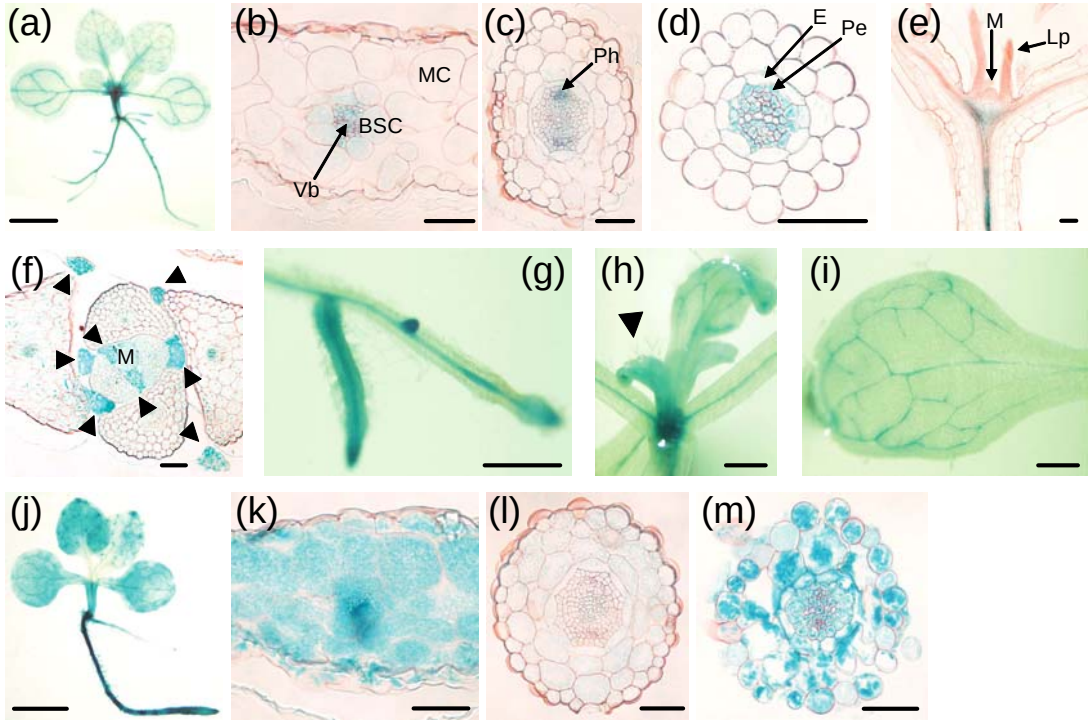

Fig. 2

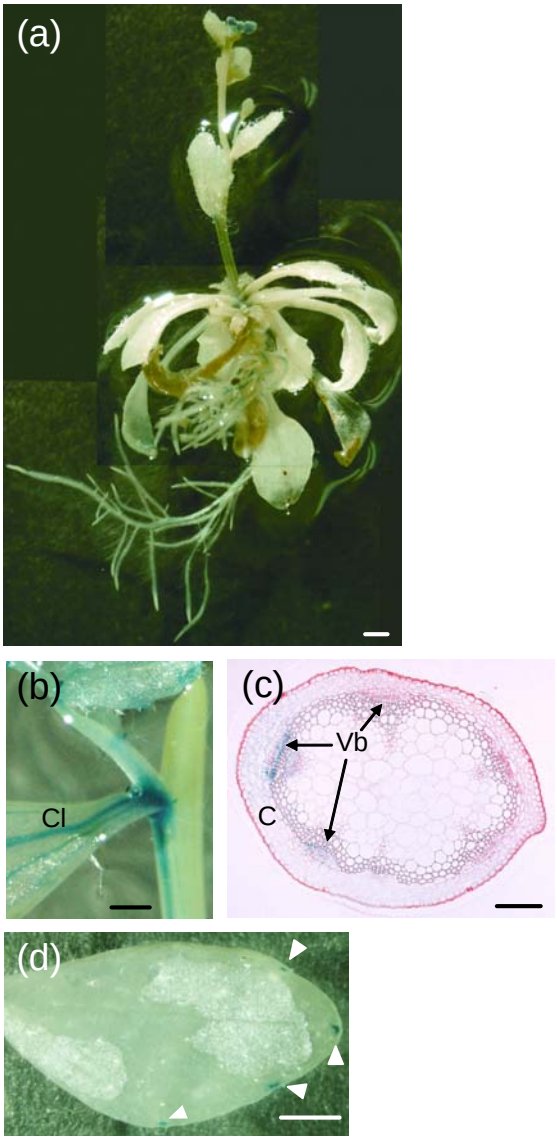

Fig. 3

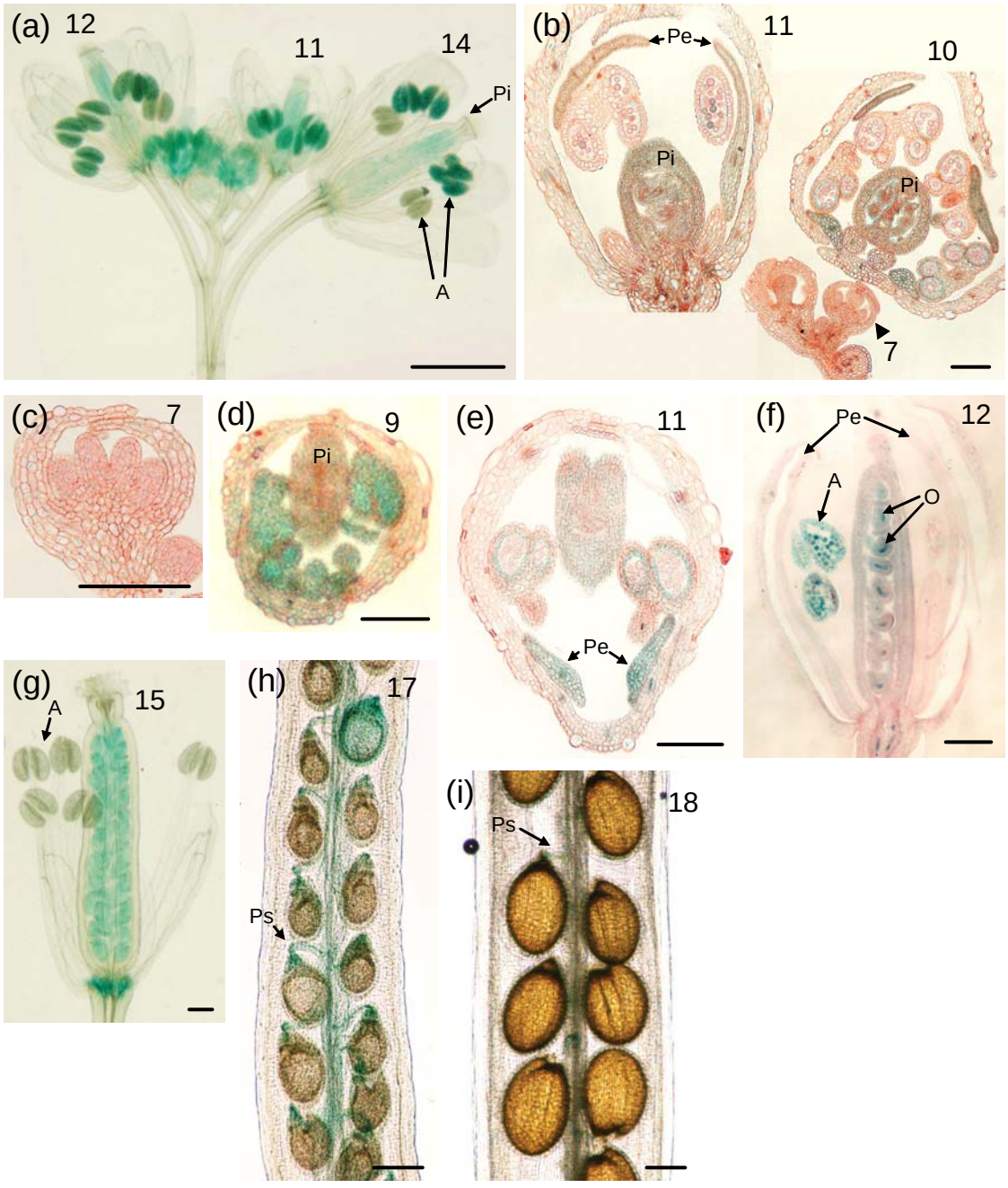
(a)
12
11
14
Pi
A
(b)
Pe
11
10
Pi
Pi
7
(c)
7
(d)
9
Pi
(e)
11
Pe
(f)
Pe
12
A
O
(g)
A
15
(h)
17
Ps
(i)
18
Ps

Fig. 4

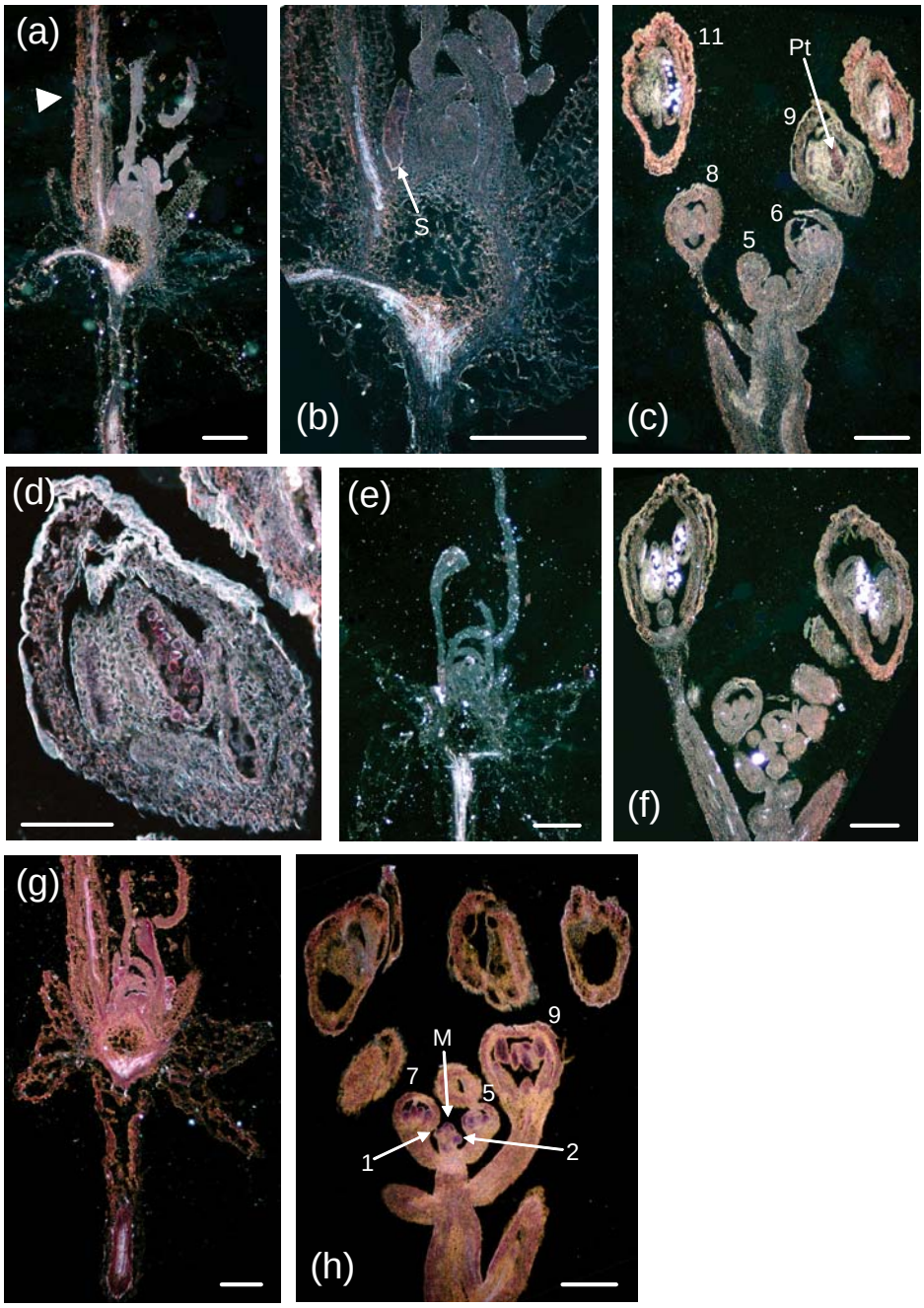

Fig. 5

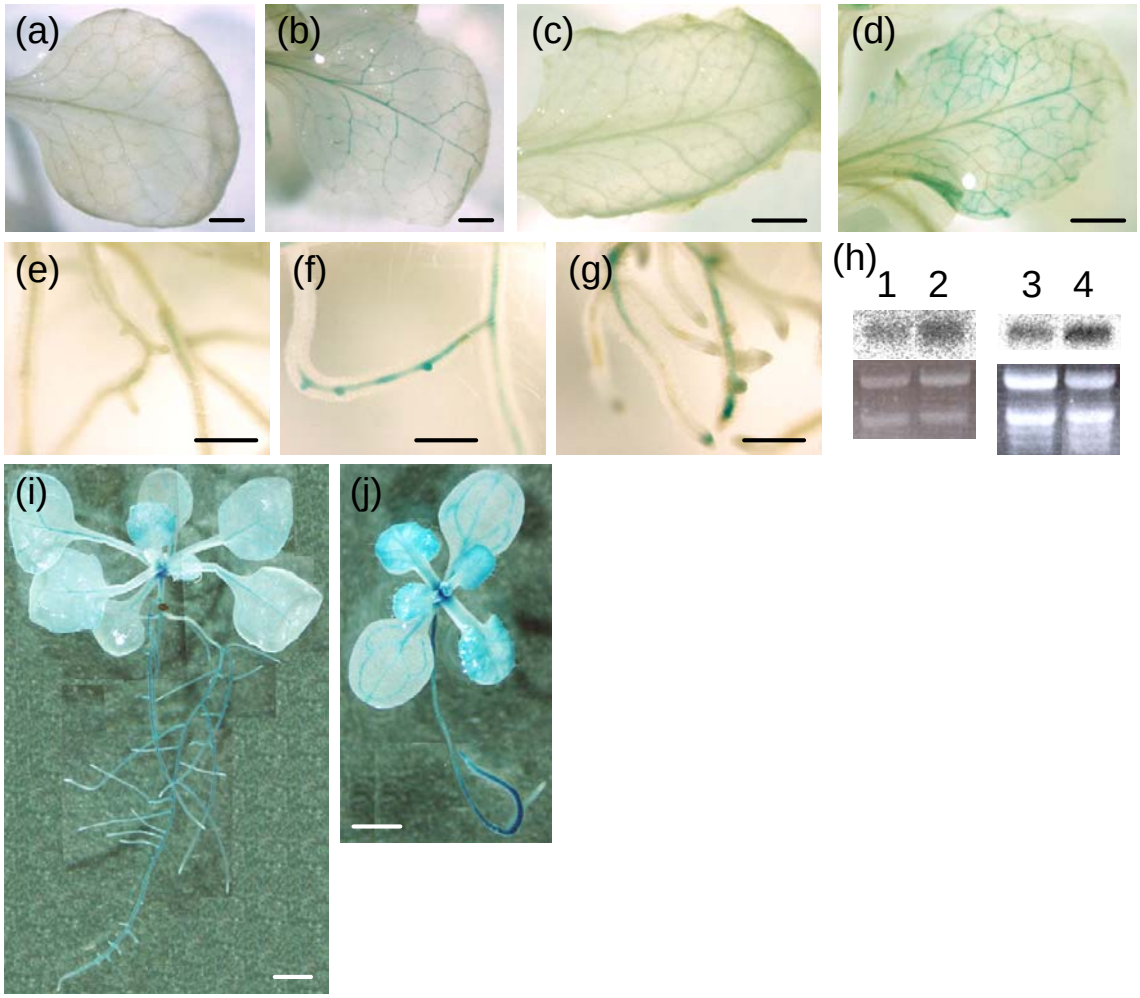

Fig. 6

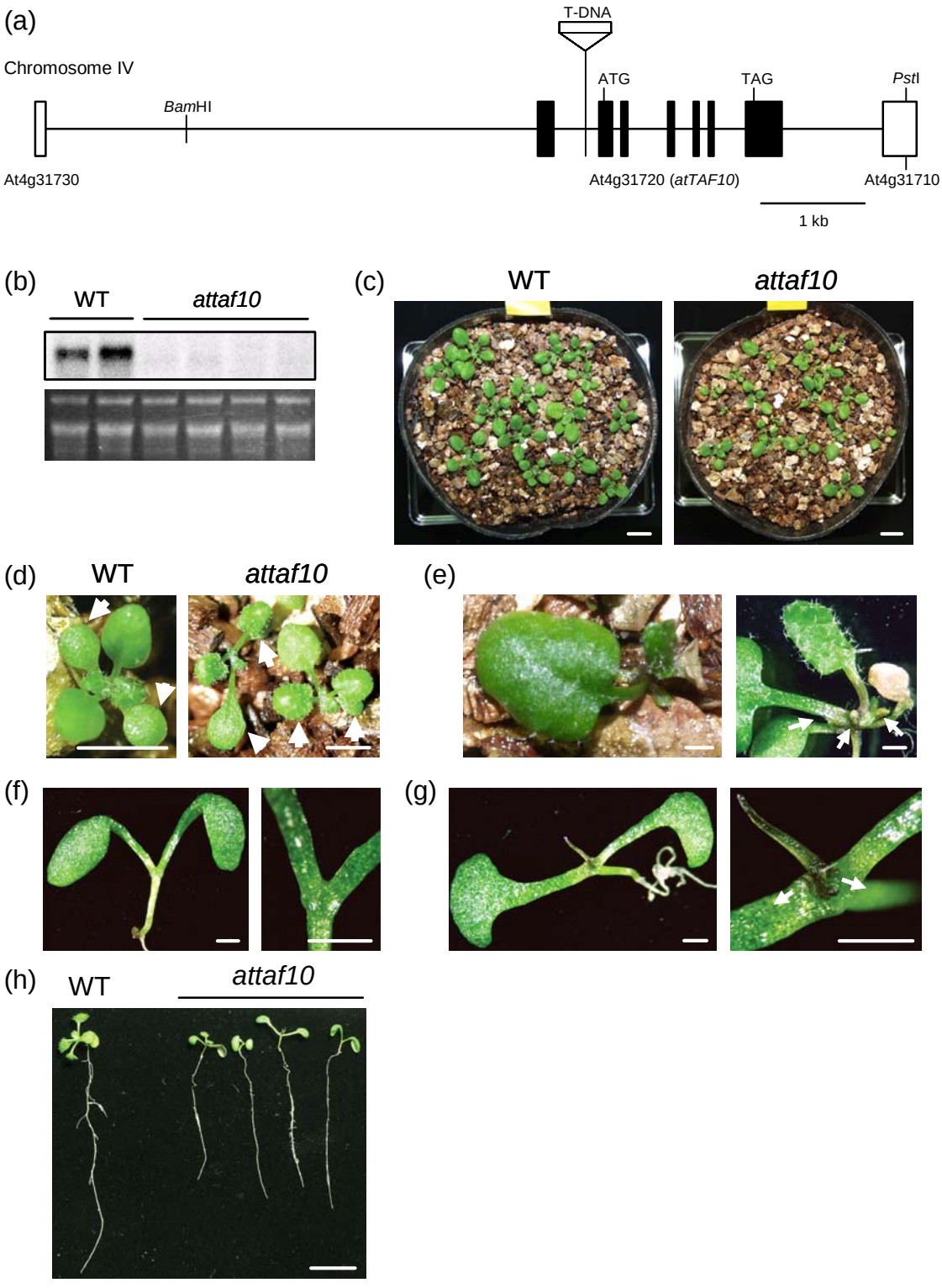
(a)
Chromosome IV
T-DNA
ATG
TAG
PstI
BamHI
At4g31730
At4g31720 (atTAF10)
At4g31710
1 kb
(b)
WT
attaf10
(c)
WT
attaf10
(d)
WT
attaf10
(e)
(f)
(g)
(h)
WT
attaf10

Fig. 7