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IDENTIFICATION AND CHARACTERIZATION OF PLANT HOMOLOGS OF YEAST ELONGATOR AND OTHER TRANSCRIPTON ELONGATION FACTORS IN ARABIDOPSIS

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Ying Yan

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M.S. degree in Cell and Molecular Biology

Major Professor's Signature

7/17/04

Date

IDENTIFICATION AND CHARACTERIZATION OF PLANT HOMOLOGS OF YEAST ELONGATOR AND OTHER TRANSCRIPTION ELONGATION FACTORS IN ARABIDOPSIS

By

Ying Yan

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ABSTRACT

IDENTIFICATION AND CHARACTERIZATION OF PLANT HOMOLOGS OF YEAST ELONGATOR AND OTHER TRANSCRIPTION ELONGATION FACTORS IN ARABIDOPSIS

By

Ying Yan

Whereas much attention has been placed on characterizing the multitude of protein factors and sequence of events involved in recruitment of RNA polymerase II (Pol II) to eukaryotic gene promoters, relatively little is known about subsequent events associated with promoter clearance and transcriptional elongation. In cells of budding yeast, the hyperphosphorylated, elongation-competent form of PolII is associated with several factors that may facilitate transcription through chromatin. These include Elongator, a multisubunit histone acetyltransferase (HAT) that may act to disrupt chromatin packaging. The three subunits of 'core' Elongator (Elp1, Elp2 and Elp3) are highly conserved among eukaryotes. In humans, mutation in the Elp1 homolog IKAP is associated with a severe neurodegenerative disorder termed familial dysautonomia. We found that in Arabidopsis, each of the core subunits is represented by a single clear homolog. Mutations in either *AtELP1* and *AtELP2* lead to identical defects in vegetative and floral development. Double mutants of *Atelp1/Atelp2* were phenotypically similar to either single mutant, suggesting *AtELP1* and *AtELP2* have identical function. *AtELP1* and *AtELP2* functionally interact with genes encoding homologs of elongation factors TFIIS and Paf1. Transcriptional profiling experiments revealed that *AtELP1* is involved in regulation of a limited and diverse subset of genes, including the MADS-box flowering time regulator *FLC*.

To

My family

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LITERATURE REVIEW

Transcription initiation and elongation

Eukaryotic transcription by RNA polymerase II (Pol II), a ~600-kDa, 12-subunit enzyme highly conserved from yeast to mammals, is a remarkably intricate biochemical and multistage process that is tightly regulated at many levels. This process proceeds via five stages: preinitiation, initiation, promoter clearance, elongation, and termination. An enormous body of work generated over the past decades suggests that during initiation, Pol II assembles at promoters together with general transcription factors IIA, IID, IIB, IIF, IIE, and IIH into a pre-initiation complex (PIC) (Roeder, 1996). Apart from possessing intrinsic capabilities for basal transcription, these proteins are also believed to represent the ultimate targets of various gene specific DNA-binding activators or repressors. For example, TFIID, a multisubunit complex that consists of the TATA box-binding protein TBP and a number of TBP associated factors (TAFs), is regarded as the major, if not exclusive, target of transcriptional activator proteins (Muller and Tora, 2004). In *Drosophila,* some of these TAFs have been shown to directly bind activation domains of activators, and in turn activators can recruit TFIID by interacting with TFIID through TAFs (Dikstein, 2001). This interaction is essential for activator-dependent transcription in vitro. In addition, biochemical and genetic evidence showed that a protein complex termed Mediator (MedC) serves as an adaptor between activator proteins and the basal transcription machinery (Myers and Kornberg, 2000). Subunits of Mediator interact with the C-terminal domain of Pol II in vivo. The mammalian counterparts of Mediator also interact with transcriptional activator proteins and have an important role in modulating Pol II activity in promoter-dependent transcription (Myers and Kornberg, 2000).

Recently, a novel form of Pol II has been purified from yeast cells, which contains Paf1 (RNA polymerase II associated factor 1) and Cdc73, together with other proteins implicated in transcription initiation such as Gal11, TFIIB, and TFIIF, but lacks TBP, TFIIH, transcription elongation factor TFIIS, and the Srbs subunits of MedC (Shi et al., 1997). It is unclear why cells contain both the Srb/MedC-containing and the Paf1/Cdc73-containing RNA polymerase II complexes at the initiation stage.

Transcription elongation factors

Although the initiation stage of transcription has received much attention during the past decades, little is known about events associated with promoter clearance and transcriptional elongation.

Transcription elongation by RNA polymerase II in eukaryotes has blossomed into a broadly active area of investigation (Conaway et al., 2000; Orphanides and Reinberg, 2000). A major advance in the understanding of elongation has come with the recent determination of the high-resolution crystal structures of free and elongating forms of Pol II (Cramer et al., 2001; Gnatt et al., 2001). These structures revealed many of the key catalytic properties of the polymerase, leading to a better understanding of the enzyme's catalytic mechanism, the nature of the enzyme's interactions with its DNA template and nascent transcript, and the mechanisms underlying the enzyme's propensity to pause and arrest (Shilatifard, 2003).

More importantly, a diverse collection of nuclear proteins that regulate the activity of Pol II during the elongation phase of messenger RNA synthesis have been identified and biochemically characterized (Conaway et al., 2000). These so-called transcription

elongation factors typically display some common features: First, these proteins can be crosslinked with both the promoters and coding regions. Second, deletion of the genes encoding such proteins typically render cells sensitive to the drug 6-azauracil (6-AU), a transcription elongation inhibitor. This compound is believed to cause the depletion of intracellular GTP or UTP level by inhibiting their biosynthetic enzymes. Third, such proteins have copurified with the hyperphosphorylated, elongating form of Pol II. Finally, genes have been implicated in transcription elongation by genetic or physical interaction with other known elongation factors.

Transcription elongation factors have been classified into three distinct groups (Shilatifard, 1998): Class I, those involved in drug-induced arrest or sequence-dependent arrest of transcription, such as DSIF (Spt4, Spt5) and TFIIS; Class II, those that function to suppress transient pausing and increase the catalytic rate of elongation by altering the Km and /or the Vmax of the polymerase, such as ELL, TFIIF and Elongin; and Class III, those that facilitate passage of polymerase through chromatin, such as FACT (Spt16, Pob3) and Elongator.

Transcription elongation by Pol II is a dynamic process that does not occur at a constant rate. Throughout its elongation phase, Pol II can encounter constraints causing pause, arrest, and even termination (Uptain et al., 1997). Pausing results from reversible backtracking of the enzyme by 2 to 4 nucleotides. This leaves the mRNA transcript's misaligned 3'-OH terminus unable to serve as an acceptor for the incoming ribonucleoside triphosphate in synthesis of the next phosphodiester. In contrast, arrest is caused by irreversible backsliding of the enzyme by 7-14 nucleotides. An arrested elongation complex is unable to efficiently resume transcript synthesis without the aid of accessory

factors (Uptain et al., 1997).

The first class of transcriptional elongation factors plays an indispensable role to overcome arrest. For example, at some T-rich sites scattered along the DNA template, arrested polymerase can be reactivated only by the action of TFIIS (Wind and Reines, 2000). Elegant biochemical studies have revealed that TFIIS allows the passage of arrested Pol II by directly interacting with the catalytic site of the enzyme and creating a new 3'-OH terminus that can be reextended by the polymerase (Reines and Mote, 1993).

Rather than rescue polymerase from arrested state, Class II elongation factors stimulate the rate of elongation by interacting directly with elongating polymerase and suppressing transient pausing (Uptain et al., 1997). Although the exact mechanism of suppressing RNA polymerase II pausing is unknown, some evidence suggested that these proteins help to maintain the 3'-OH terminus of the nascent RNA chain in its proper position in the polymerase catalytic site, thereby preventing backtracking of the enzyme (Takagi et al., 1995).

Class III elongation factors do not affect polymerase activity directly, but instead modify the chromatin template and enhance movement through chromatin. In eukaryotes, DNA typically exists in vivo as a repeating array of nucleosomes, in which 146 bp of DNA are wound around a histone octamer (consisting of two each of histone proteins H2A, H2B, H3 and H4), which can be subject to higher-order packaging (Kornberg and Lorch, 1999). In this way, genomic DNA is compacted some 10,000-fold. Such condensation of DNA provides a considerable obstacle to transcription. A direct connection between chromatin alteration and transcriptional elongation has been demonstrated in recent years by the finding that Class III elongation factors have chromatin modification activity. For example,

FACT binds to the nucleosomes and promotes removal of nucleosomal histones H2A and H2B, hence facilitating elongation by RNA Pol II (Shilatifard et al., 2003). Chd1 is a member of a large class of ATP-dependent chromatin remodeling proteins. These proteins alter the distribution of nucleosomes along DNA (Björklund et al., 1999).

Other chromatin-altering elongation factors act by covalently modifying histone proteins. Each core histone consists a structured domain and an unstructured amino-terminal 'tail' of 25-40 residues extending through the DNA gyres and into the space surrounding the nucleosomes. These histone tails provide sites for a variety of posttranscriptional modifications, including acetylation, methylation, and phosphorylation (He and Lehming, 2004). It is becoming increasingly apparent that such modifications of histone tails determine the interactions of histones with other proteins, which may in turn regulate chromatin structure. For example, the Paf1 complex, which consists of seven subunits: Paf1, Cdc73 (Shi, 1997), Leo1, Rtf1, Ctr9 (Mueller and Jaehning, 2002), Ccr4 and Hpr1 (Wade et al., 1996), is required for the recruitment of Set1, Set2 and Dot1 methylases, which catalyze histone H3 methylation at lysine 4, 36 and 79 (Santos-Rosa et al., 2002). The evidence that hypermethylated H3-K4 is exclusively associated with active transcription, and that the association of Paf1C with elongating Pol II, suggests that this modification provides a molecular memory of recent transcriptional activity (Ng et al., 2003).

In contrast, Elongator functions in histone acetylation. The Elp3 subunit of Elongator is a member of GNAT (Gcn5-related N-acetyltransferase) superfamily (Sterner and Berger, 2000). Histone acetyltransferases (HATs) transfer an acetyl group from acetyl-coenzyme A (acetyl-CoA) to the ε-amino group of certain lysine side chains (Loidl,

1994). In particular, the highly conserved histone H3 lysines at amino acid positions 9, 14, 18 and 23, and H4 lysines at 5, 8, 12, and 16 are target sites for acetylation by HATs (Roth et al., 2001). It is theorized that Elongator may perform a function during elongation that is similar to the function of the related HAT Gcn5 during initiation. In yeast, Gcn5 is the catalytical subunit of two multi-subunit protein complexes, SAGA and ADA (Grant et al., 1997). Recruitment of SAGA to the nucleosomes by some transcriptional activators causes histone acetylation and concomitant activation of transcription in vitro (Utley et al., 1998). Acetylation of histones can be reversed by histone deacetylases (HDACs) (Khochbin et al., 2001). Acetylated histones are usually associated with transcriptionally active chromatin and deacetylated histones with inactive chromatin (Sterner and Berger, 2000). The interplay between HDACs and HATs results in dynamic transitions in chromatin structure, which may affect the efficiency of transcription elongation.

The Elongator complex was isolated by its ability to interact with the hyperphosphorylated CTD of the elongating form of Pol II in yeast (Otero et al., 1999). Elongator is composed of a tightly bound core of Elp1-Elp3, and an additional trimer of Elp4-Elp6. In vitro work using recombinant Elp3 demonstrated that Elp3 possesses intrinsic HAT activity (Wittschieben et al., 1999). Further investigation has been done to show Elp3 could physically interact with both naked and nucleosomal DNA, and predominantly acetylates lysine-14 of histone H3 and lysine-8 of histone H4 (Winkler et al., 2002). Chromatin immunoprecipitation experiments show that Elongator is important to keep the normal histone H3 and H4 acetylation levels in chromatin in vivo (Winkler et al., 2002). The ELP2 gene encodes a protein with eight WD repeats (Fellows et al.,

2000). WD repeats are thought to be a platform for protein-protein interaction (Smith et al., 1999), and thus, Elp2 might have a role in the assembly of the Elongator complex.

Mutant cells lacking any of the six *ELP* genes showed similar phenotypes, such as slow adaptation to new growth conditions and hypersensitivity to salt and temperature stress (Otero et al., 1999; Fellows et al., 2000; Wittschieben et al., 1999). These phenotypes might result from a delay in gene expression under rapidly changing conditions. Microarray experiments demonstrated that the gene expression profiles of strains carrying deletions of genes encoding any of Elp1, Elp2, Elp4 and Elp6 subunits are very similar (Krogan and Greenblatt, 2001). Different combination of *elp* double mutants, even triple mutants, do not enhance the phenotype (Otero et al., 1999, Wittschieben et al., 1999, Fellows et al., 2000), suggesting that these proteins function exclusively as part of Elongator.

Although Elp4, Elp5 and Elp6 do not have HAT activity by themselves, and do not exhibit homology with known HATs, they are required for HAT activity of Elp3 in the core Elongator complex (Winkler et al., 2002). The observation that Elongator subunits are not essential in yeast suggests that Elongator does not provide a crucial function, or that other HATs may replace the role of Elongator in the absence of Elongator function (Wittschieben et al., 1999).

Elongator subunits are highly conserved in humans. Human Elongator complex has been purified and was found to consist of six subunits (hElp1-hElp6) presenting as a six-subunit holo-Elongator complex (Hawkes, 2002). A mutation in IKAP, the human homolog of yeast Elp1, results in familial dysautonomia, a fatal disorder affecting the nervous system (Anderson et al., 2001), and causes bronchial asthma in children

(Takeoka et al., 2001). As in yeast, the human holo-Elongator complex has HAT activity targeted to histone H3 and H4 in vitro, and associates with the elongating form of Pol II (Hawkes, 2002). This suggests that Elongator in higher eukaryotes may also have a role in transcriptional elongation. However, researchers failed to detect an interaction between human Elongator and other transcription elongation factors (Kim et al., 2002). A role for Elongator in elongation has recently been put in doubt by the finding that the majority of Elongator Elp1-Elp3 subunits in yeast are excluded from the nucleus (Pokholok et al., 2002). In addition, it has been noted that in contrast to other elongation factors, Elongator subunits have not been found crosslinked with ORFs (Krogan et al., 2002). Thus, Elongator may play a unique and unanticipated role in transcription elongation.

INTRODUCTION

Whereas much progress has been made to discover transcription elongation mechanisms in yeast and humans, very little is known about transcription elongation in plants. Our interest in the role of transcriptional elongation in plant development came from the finding that mutations in the plant homologs of the yeast Paf1C subunits Leo1, Rtf1 and Ctr9 led to developmental pleiotropy, including reduced plant size, reduced apical dominance, male sterility, defects in floral organs, and early flowering (Zhang and van Nocker, 2002; Zhang et al., 2003; Oh et al., unpublished). Intriguingly, we found that the early flowering phenotype was associated with silencing of the MADS-box flowering inhibitor *FLC (FLOWERING LOCUS C)* and *MAF* genes (Bastow et al., 2004; Oh et al., unpublished). In natural, winter-annual ecotypes of Arabidopsis represses flowering until after an appreciable period of cold, a phenomenon called vernalization. Cold results in epigenetic silencing of *FLC*, associated with hypoacetylation and repressive methylation of *FLC* chromatin (Bastow et al., 2004).

In light of the strong effect of mutations in plant Paf1C genes on development, we explored the significance of other transcriptional elongation factors in plant development.

RESULTS AND DISCUSSION

Identification of potential components of the transcriptional elongation machinery in Arabidopsis.

As a first step to explore the significance of transcriptional elongation in plant development, we evaluated the Arabidopsis predicted proteome for proteins closely related to yeast transcription elongation factors (Table 1), because highly conserved proteins might have similar functions. We found that most, but not all, of the characterized yeast transcription elongation factors were represented in Arabidopsis, suggesting that mechanisms of elongation may be well conserved in plants. The Arabidopsis genome encodes for more than one homolog of Spt4/Spt5/DSIF and P-TEFB subunits, suggesting that their function might have evolved to mediate different aspects of elongation. However the Arabidopsis genome does not encode for obvious homologs of Tfg1 (a subunit of TFIIF), or the Elp4-Elp6 subunits of Elongator. In yeast, Elp4-Elp6 are not catalytic subunits, but they interact with the core subunits and have a regulatory role (Li et al., 2001). This suggested that plant Elongator might have a different structural composition. We also found that each of the core subunits of Elongator, Spt16, Pob3/FACT, Dst1/TFIIS, Soh1 or Chd1, is represented by a single clear homolog in Arabidopsis. We designated these AtElp1, AtElp2, AtElp3, AtSpt16, AtPob3, AtSoh1 and AtChd1, respectively.

To investigate the functions of these plant homologs of yeast transcriptional elongation factors in Arabidopsis, we obtained mutants for several of these genes from a large sequenced-indexed, T-DNA mutagenized population (Http://signal.salk.edu) (Table 1 and Fig.1). Because of the potential for paralogous genes to have redundant function,

Table 1. Arabidopsis homologs of elongation factors other than Paf1C subunits

Protein name in yeast	Arabidopsis homolog(s)	T-DNA lines	E value*
Elongator			
Elp1	At5g13680	Salk-004690	1.3e-67
		Salk-011529	
Elp2	At1g49540	Salk-106485	3.1e-66
Elp3	At5g50320		4.4e-204
Elp4	None		
Elp5	None		
Elp6	None		
Spt4, Spt5/DSIF			
Spt4	At5g63670		1.5e-74
	At5g08565		3.1e-13
Spt5	At2g34210		1.1e-43
	At4g08350		3.2e-42
Spt6	At1g63210		1.5e-74
	At1g65440		1.5e-71
Iws1	At1g32130		8.3e-23
	At4g19000		9.9e-15
Spt16,Pob3/FACT			
Spt16	At4g10710		5.1e-107
Pob3	At3g28730	Salk-058731	2.7e-46
P-TEFB			
CycT	~8 genes, overlapping with CycK		
Cdk9	> 100		
CycK	~8 genes, overlapping with CycT		
Dst1/TFIIS	At2g38560	Salk-056755	7.1e-30
		Salk-064316	
TFIIF			
Tfg1	None		
Tfg2	At1g75510, At3g52270		1.7e-14
			3.6e-09
Taf14/Tfg3	At2g18000		5.6e-10
Soh1	At5g19910	Salk-051025	1.2e-13
		Salk-035522	
Chd1	At2g13370	Salk-087283	3.8e-176
		Salk-020296	

* E value: false positive expectation value

we focused our efforts on those genes with a single, clear homolog in Arabidopsis.

No mutants were available for ATELP3, ATSPT16, or ATTFG3. We obtained two alleles for the *AtELP1* gene, designated *Atelp1-004690* and *Atelp1-011529.* Both of these contained a T-DNA insertion in the third of total five exons. For the *AtELP2* gene, the *Atelp2-106485* allele contains a T-DNA insertion in the third of total ten exons. The *AtTFIIS* gene had two alleles; *AttfIIs-064316* contains a T-DNA in the 5' UTR, and *AttfIIs-056755* contains a T-DNA insertion in the second of total two exons. *AtSOH1* had two alleles; *Atsoh1-051025* contains a T-DNA insertion in the second of total six exons, and *Atsoh1-035522* in the 3'UTR. *Atchd-020296* has a T-DNA insertion in the sixth of a total of thirty exons. Based on the location of the T-DNA insertion in these alleles, all of these mutations have the potential to significantly affect gene expression. We isolated homozygous mutants for all of these genes, and analyzed the phenotype of these mutants under standard growth conditions. However, we did not observe obvious defects in development for *AttfIIs, Atsoh1,* and *Atchd1* mutants. In yeast, none of these three proteins are essential (Giaever, *et al.,* 2002), and mutations in any of these genes do not obviously affect growth. Therefore, if these proteins have conserved functions in plants, the observed normal development of the mutant plants would not be surprising. In addition, because the VIP4, VIP5 and VIP6 genes, which encode homologs of Paf1C subunits, are required for the expression of *FLC/MAF* genes and proper timing of flowering, we were curious to see if *AtTFIIS, AtSOH1,* and *AtCHD1* influence flowering. *FLC* is not strongly expressed in the genetic background in which the T-DNA lines were isolated, due to a mutation in its activator *FRIGIDA*. Therefore, to assess the effect of

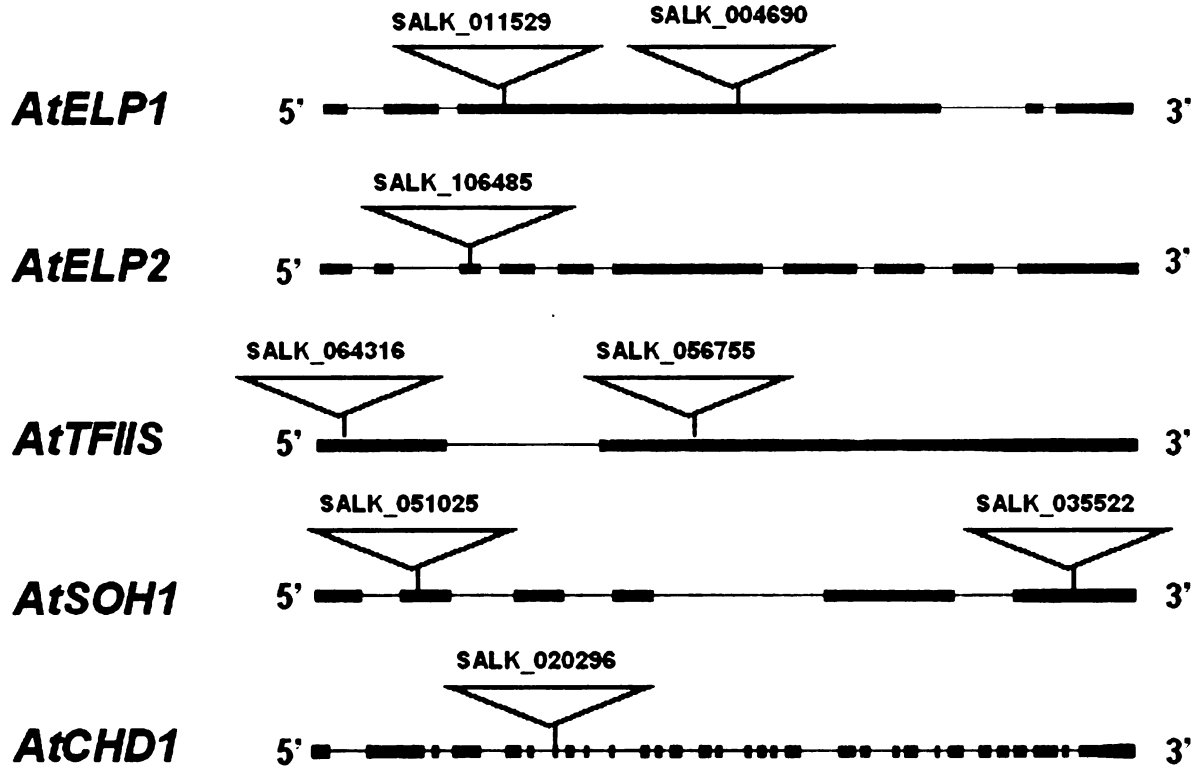


Figure 1. Position of T-DNA Insertions in the Arabidopsis *AtELP1, AtEPL2, AtTFIIS, AtSOH1* and *AtCHD1* genes. Indicated are the relative positions of exons (UTRs, black boxes; translated region, red boxes), introns (black line), UTRs (black boxes), T-DNA insertions, and the corresponding allele number.

these mutations on *FLC* expression, we introduced these mutations into a synthetic introgression line containing a functional *FRI* allele (see Methods). Although we did not evaluate *FLC* expression directly, none of the resulting plants flowered abnormally early, suggesting that *FLC* was expressed to wild-type levels (not shown).

Phenotypic analysis of *Atelp1*, *Atelp2* and *Atelp1/Atelp2* double mutants

In contrast, mutations in *ATELP1* and *ATELP2* conferred moderate defects in development. Both *Atelp1*and *Atelp2* mutant plants showed indistinguishable pleiotropic phenotypes at multiple stages of development (Fig. 2). When the second pair of rosette leaves expanded, the leaf lamina of the mutant plants was narrower than that of wild type plants and exhibited obvious serrated edges (Fig. 2B and C). In addition, the leaves were curled downward, suggesting that the adaxial cells had divided or expanded faster than the abaxial cells. The leaf color was yellowish compared to that of the wild type (Fig. 2B, C and D). Moreover, *Atelp1* and *Atelp2* mutant plants showed significantly higher trichome density on the leaf surface, inflorescence stems and sepals (Fig. 2B, C, D and E). In addition, the *Atelp1* or *Atelp2* mutant plants grew much more slowly than the wild type plants (not shown), suggesting defects in cell division and/or cell expansion. Floral development was also affected in *Atelp1*and *Atelp2*mutant plants. Flowers exhibited abnormally small petals and large sepals, and the stamens were significantly shorter than the carpels (Fig. 2E and not shown). The flowers of both *Atelp1*and *Atelp2* showed a phenotypic gradient (not shown); whereas early-formed flowers were severely affected and sterile, later-formed flowers were less affected and produced shorter but thicker siliques compared to the wild type (Fig. 2F and not shown). In addition, seed size was

larger than that of the wild type (Fig. 2G). All of the seeds from heterozygous plants were wild type size, suggesting that seed size resulted from maternal affects.

We also found that *AtELP1* and *AtELP2* mutations did not obviously affect flowering time when introduced into a genetic background containing an active *FRI* allele, suggesting that these genes have little role in *FLC* expression (not shown). However, we later discovered through transcriptional profiling that *FLC* in fact was down-regulated in an *Atelp1* mutant (see below). The implications of this are discussed below.

To confirm that the mutant phenotypes were caused by T-DNA insertion in the *AtELP2* gene, we expressed antisense RNA for *AtELP2* in wild-type Col-0 plants. A subset of primary transformants showed all of the phenotypic defects seen in the *Atelp2* mutant plants (Fig. 2B), including narrower, yellowish and serrated leaves that curled downwards; high trichome density on the leaf surface, inflorescence stems and sepals; flowers with small petals covered by large sepals; short stamens, reduced fertility, and slow growth rate (not shown).

RT-PCR analysis using primers spanning the insertion sites did not detect the *AtELP1* transcripts in homozygous *Atelp1-004690* mutant plants or *AtELP2* transcripts in homozygous *Atelp2-106485* mutant plants (Fig. 2A). This suggests that these are null mutations, and these phenotypes (discussed below) reflect the absence of gene activity.

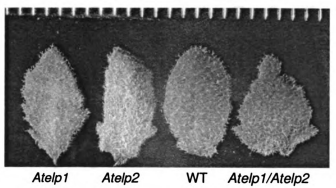
The apparently indistinguishable phenotypes exhibited by *Atelp1* and *Atelp2* mutant plants suggested that *AtELP1* and *AtELP2* have similar function. To explore this idea further, we constructed a *Atelp1/Atelp2* double mutant. If *AtELP1* and *AtELP2* have completely overlapping function, then we predicted that the *Atelp1/Atelp2* double mutant

Figure 2. Phenotypes of the *Atelp1-004690, Atelp2-106485,* and *Atelp1/Atelp2* double mutants.

(A) Expression analysis of the *AtELP1* and *AtELP2* genes in wild-type (WT), *Atelp1* and *Atelp2* mutant plants by RT-PCR. RNA was prepared from 14-d-old seedlings and subjected to RT-PCR as described in Methods, using primers specific for the *AtELP1* or *AtELP2* genes; *ACTIN* serves as a control **(B-G)** Phenotypes of *Atelp1* and *Atelp2* mutants, *Atelp1/Atelp2* double mutant, antisense *AtELP2* plants, and wild type plants. **(B)** whole plants; **(C)** rosette; **(D)** rosette leaf; **(E)** flower;**(F)** ripening silique and **(G)** seeds in the *Atelp1, Atelp2, Atelp1/Atelp2* double mutants, and wild-type (WT) Col-0 plants.

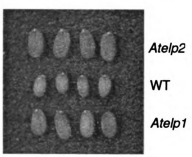

A
AtELP1
AtELP2
ACTIN
WT Atelp1-004690 Atelp2-106485

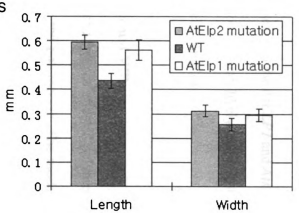

B
C
Atelp1 Atelp2 Atelp1/Atelp2 WT AtELP2-AS


D
Atelp1 Atelp2 WT Atelp1/Atelp2


E
Atelp1 Atelp2 Atelp1/Atelp2 WT


F
WT Atelp1 Atelp2


G
Atelp2
WT
Atelp1


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
mm
AtElp2 mutation
WT
AtElp1 mutation
Length
Width

would be phenotypically similar to the *Atelp1* or *Atelp2* single mutants. This was indeed the case; the *Atelp1/Atelp2* double mutant exhibited all the phenotypic defects of *Atelp1*or *Atelp2* single mutants (Fig. 2B, C, D and E). RT-PCR analysis (Fig. 2A) showed that neither the expression level of *AtELP1* in the *Atelp2-106485* mutant, nor the expression level of *AtELP2* in the *Atelp1-004690* mutant, was affected. This suggests that *AtELP1* and *AtELP2* are not regulated by each other. These findings reveal that the *AtELP1* and *AtELP2* genes are essential for proper development of Arabidopsis, and likely carry out an overlapping function, potentially as components of a protein complex.

AtELP1 and AtELP2 genetically interact with AtTFIIS and VIPs

In yeast, TFIIS promotes transcription elongation and rescues arrested polymerase by creating a new 3'-OH group that can be extended by the polymerase. The moderate sensitivity to the transcription elongation inhibitor 6-AU exhibited by *tfIIs* mutants was greatly enchanced when combined with an *elp1* mutant (Otero et al., 1999). This suggested a role for Elongator in transcription elongation in vivo. As a transcription elongation factor, Elongator in plants might interact with other hypothetical elongation factors. To test this possibility, we evaluated genetic interaction between AtELP and AtTFIIS by combining *Atelp1* and *Atelp2* mutations with the *AttfIIs-056755* allele. Although the *AttfIIs* mutant plants developed normally (see above), both *Atelp1/AttfIIs-056755* and *Atelp2/AttfIIs-056755* double mutant plants exhibited more severe phenotypic defects than seen in the *Atelp* single mutants: leaves were narrow and serrated, growth was extremely slow (not shown), inflorescence development was delayed, formation of sepal, petal, and stamen was defective, and fertility was further reduced (Fig. 3A). The enhancement of the

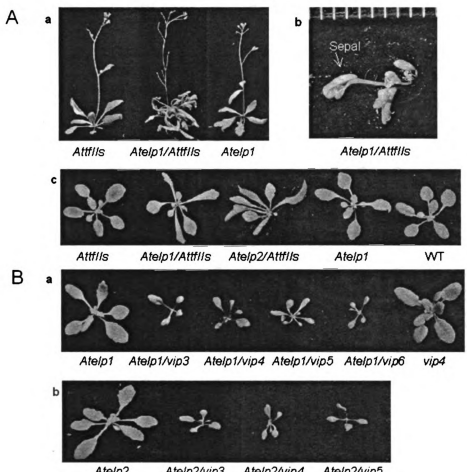


Figure 3. Phenotypes of *Atelp/Attfl1s* and *Atelp/vip* double mutants.

(A) Phenotypes of *Attfl1s*, *Atelp1/Attfl1s* and *Atelp2/Attfl1s* double mutants.**a.** Whole plant. **b**. Flower. **c**. Rosette. **(B)** Phenotypes of *Atelp/vip* double mutants. **a**.Phenotypes of *Atelp1/vip3, Atelp1/vip4, Atelp1/vip5,* and *Atelp1/vip6* double mutants. **b**. Phenotypes of *Atelp2/vip3, Atelp2/vip4, Atelp2/vip5,* and *Atelp2/vip6* double mutants.*vip3, vip4, vip5* and *vip6* have identical phenotypes, represented here by *vip4.*

Atelp phenotypes by loss of TFIIS activity suggests that *AtELP1* and *AtELP2* may carry out a function that is identical with that of *AtTFIIS,* such that when the *AtTFIIS* is absent, the plants developed normally. AtELP and AtTFIIS might be in parallel pathways that perform a non-essential function. The absence of this function gives rise to an enhanced phenotype. In addition, if AtTFIIS functions in transcription elongation, the genetic interaction between AtELP and AtTFIIS suggests that the AtELP1 and AtELP2 in plants have a role in transcription elongation.

In a similar manner, we tested genetic interaction between AtELP and plant homologs of subunits of Paf1C. The previously identified *VIP4, VIP5,* and *VIP6* genes in Arabidopsis encode the counterparts of Leo1, Rtf1, and Ctr9 subunits of the yeast Paf1 complex, respectively (Zhang and van Nocker, 2002, Zhang et al., 2003, Oh et al., unpublished). Mutation in *AtELP1* or *AtELP2* and in *VIP4-VIP6* showed similar defects in petal development, suggesting that Elongator and VIPs have some overlapping genetic targets. Therefore, we postulated that there might be functional interaction between the plant homolog of Elongator and Paf1 proteins. To test our hypothesis, we combined the *Atelp1* and *Atelp2* mutations with *vip3-vip6* mutations, and carried out a phenotypic analysis of the resulting double mutants. Although the *VIP3* protein does not show homology to any subunit of yeast Paf1 complex, this gene is functionally identical to *VIP4, VIP5*, and *VIP6* (Oh et al., unpublished). We found that all of these double mutants were similar and showed a remarkably enhanced phenotype as compared with either *Atelp* or *vip* single mutants: the plants were tiny and eventually died after producing only four to six rosette leaves (Fig. 3B). This piece of evidence revealed that plant homologs of Elongator and Paf1 proteins functionally interact. They may not encode the same biochemical

function, but act in parallel pathways (Guarente, 1993). If their functions are essential, then loss of both pathways will result in synthetic lethality.

Gene expression profiling of *Atelp1* mutants

The pleiotropic phenotypes of *Atelp1* and *Atelp2* mutants suggested that *AtELP1* and *AtELP2* function in multiple aspects of development through regulation of a variety of genes. To identify such genes, we used transcriptional profiling to detect differences in gene expression between the *Atelp1* mutant and its wild-type Col-0 genetic background (Fig.5A). We used the Arabidopsis ATH1 Genome Array representing 22,747 different Arabidopsis genes (Affymetrix, Santa Clara, CA). For this study, we considered only flowers and inflorescence apices, because of the striking effect of *Atelp1* on floral morphology, and because transcriptional and genetic networks contributing to floral development are widely studied (Buzgo et al., 2004). We employed two independent biological replicates, with each replicate composed of three independent samples, and with each sample composed of 10 inflorescences. Wild-type samples were harvested when the first flower was fully opened and stigmatic papillae were fully developed (stage 12). Because *Atelp1* has defects in petal development, we use stigmatic papillae development as a benchmark to coordinate developmental stages between mutant and wild type flowers. Using both replicates of *Atelp1* and wild-type, four pair-wise comparisons were created. Genes were considered to be down- or up-regulated if differences in hybridization signals were threefold or more in at least three of the four comparisons (see Methods). Based on these criteria, 196 genes were affected (159 were down-regulated, and 37 were up-regulated).

To confirm the Affymetrix array data, we performed relatively quantitative RT-PCR analysis for a subset of the affected genes (Fig. 4B). We chose these genes for further study, because they encode putative transcription factors, and they may represent more direct targets of *AtELP1* and play a role in the developmental defects that we observed in *Atelp1* mutants.

The *At1g71770* and *At1g22760* genes encode PAB5 and PAB3, respectively, two members of the polyadenylate-binding proteins (PABPs) family. Poly(A)-binding proteins are multifunctional proteins that play important roles in mRNA stability and protein translation (Wang and Grumet, 2004). Although *PAB3* and *PAB5* are not essential for viability, they are important for post-transcriptional regulation in plant sexual reproduction. *At1g71770 (PAB5)* expression in Arabidopsis is restricted to pollen and ovule development and early embryogenesis, and PAB5 protein is capable of rescuing a PABP-deficient yeast strain by partially restoring both poly(A) shortening and translational initiation functions of PABP (Belostotsky and Meagher, 1996). *At1g22760 (PAB3)* expression is restricted to late pollen development in immature flowers, and PAB3 is capable of promoting partial shortening of poly (A) tails in yeast (Belostotsky and Shaw, 1997). We confirmed that the PAB3 and/or PAB5 gene expression was significantly repressed in *Atelp1* mutant flowers. Potentially, the striking defects that we observed on floral development could be due to loss of activity of these genes. Alternatively, because PAB3 and PAB5 are expressed in stamens, under-representation of PAB3 and PAB5 in *Atelp1* mutant samples might merely be explained by failure of stamen development.

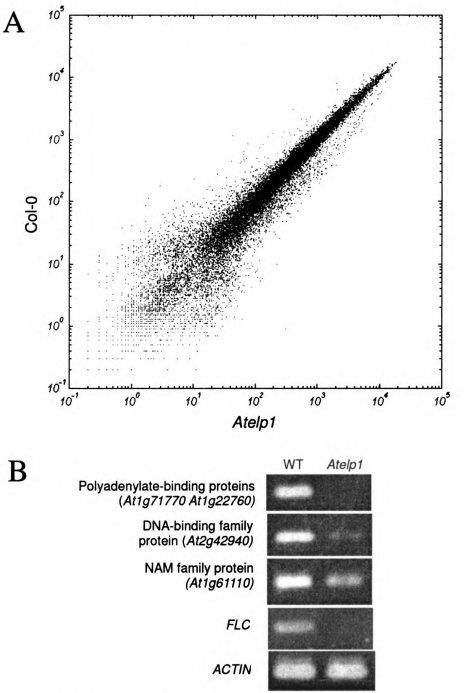


Figure 4. Characteristics of microarray data derived from wild-type Col-0 and the *Atelp1* mutant.

(A) Signal intensity was plotted to compare single replicates of WT with *Atelp1*.

(B) Genes identified by microarray analysis as down-regulated in *Atelp1* mutants relative to WT plants were monitored by RT-PCR; the constitutively expressed gene *ACTIN* served as a control.

The *At2g42940* gene encodes a potential DNA-binding protein containing AT-hook motifs, which preferentially bind to AT-rich regions in double-stranded DNA. Although its function is not well known, the study of other members of this family of proteins, such as HMG (High Mobility Group) proteins, revealed that AT-hook-containing proteins may regulate nucleosome phasing and 3' end processing of mRNA transcripts, and transcriptional regulation of genes containing AT-rich regions (Reeves and Nissen, 1990).

The *At1g61110* gene encodes a member of the NAM (No Apical Meristem) protein family. In Petunia, *NAM* mRNA accumulates in cells at the boundaries of meristems and primordia, implicating a role for NAM in determining positions of meristems and primordia (Souer et al., 1996). Another member of this family in Arabidopsis, NAP, is a target gene of *APETALA3/PISTILLATA (AP3/PI;* Sablowski and Meyerowitz, 1998*).* AP3 and PI are homeotic proteins belonging to the MADS-box family of transcription factors, and are involved in petal and stamen formation in the Arabidopsis flower (Jack et al, 1992). If *At1g61110* has a function similar to that of NAP, then down-regulation of *At1g61110* could help explain the defects in petal and stamen formation in *Atelp1* mutant plants.

We also found that *FLC* expression was down-regulated about 4-fold in *Atelp1* mutant flowers compared to the wild-type Col-0 flowers, revealing that *AtELP1* directly or indirectly activates *FLC*. This was surprising, because we did not observe early flowering after we transferred the homozygous *Atelp1* and *Atelp2* mutations into Col:*FRI*^{SF2} background, and because antisense *AtELP2* plants in the Col:*FRI*^{SF2} background did not flower early (not shown). Potentially because *FRI* is epistatic to *AtELP1*, or *AtELP1* regulates *FLC* only in floral phase.

Induction of cold-response genes and heat shock genes is not affected by *Atelp1* and *Atelp2* mutations.

In Arabidopsis and other plants, a set of genes referred to as COR genes are induced upon cold (Uemura et al., 1996). Previous studies demonstrated that in Arabidopsis, low-temperature induction of *COR* genes was delayed, and the final levels of transcripts were reduced, in *gcn5* mutants (Vlachonasios et al., 2003). The ELP3 subunit of Elongator and Gcn5 subunit of SAGA and ADA complexes both belong to the GNAT class of HATs, and in yeast these genes have an overlapping function (Wittschieben et al., 2000). Therefore, we tested whether induction of *COR* genes was affected in *Atelp1* and *Atelp2* mutants. Using RNA gel-blot analysis, we found that *COR6.6* and *COR47* transcripts became detectable about 4h after cold treatment in *Atelp1* and *Atelp2* mutants, and reached a plateau by 24 hours (Fig. 5A). This response was not obviously different from the wild type, suggesting that *AtELP1* and *AtELP2* do not have a crucial role in *COR* gene induction.

We also evaluated expression of the heat response pathway gene *HSP70* in *Atelp1* and *Atelp2* mutant plants. The heat shock (HS) response is a conserved cellular defense mechanism, which is activated by elevated temperature and a number of chemical stresses. During heat shock, transient reprogramming of gene expression occurs which results in accumulation of heat shock proteins (HSPs), most of which act as molecular chaperones and bind substrate proteins that are in an unstable, inactive state (Lohmann et al., 2004).

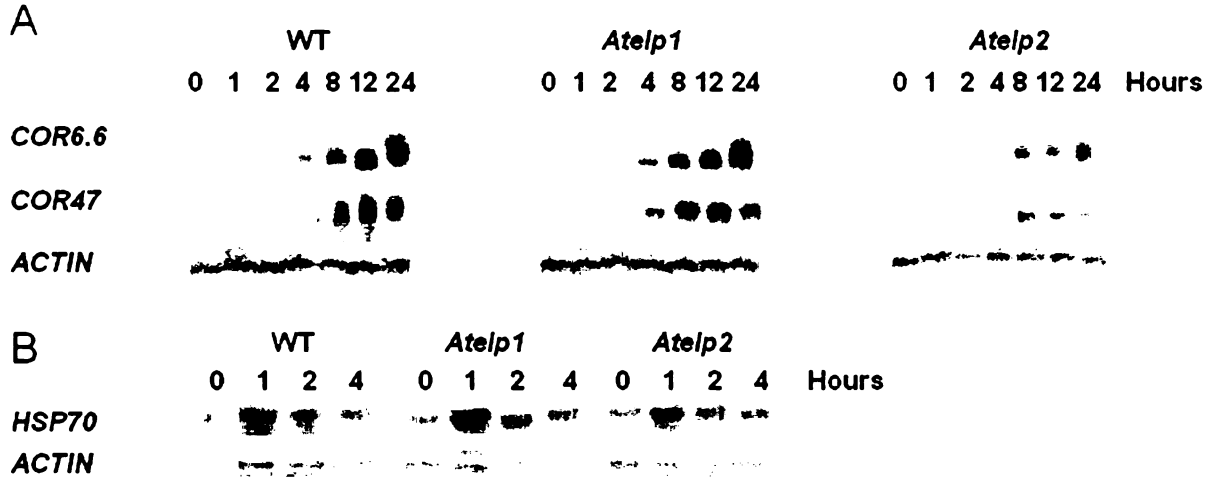


Figure 5. RNA gel-blot analysis of *COR* and *HSP70* gene expression in *Atelp1*and *Atelp2* mutant plants. **(A)** *COR* gene expression in *Atelp1*and *Atelp2* mutant plants. Total RNA was prepared from whole seedlings of wild-type (WT), *Atelp1* and *Atelp2* mutant plants. Eleven-day-old plants grown at 22C° under standard growth condition were cold treated at 4°C for the times indicated. The RNA gel blots were hybridized with *COR6.6* and *COR47* cDNA probes. Hybridization with a probe for constitutively expressed gene *ACTIN* served as a control. **(B)***HSP70* gene expression in *Atelp1* and *Atelp2* mutant plants. Total RNA was prepared from whole seedlings of wild-type (WT), *Atelp1* and *Atelp2* mutant plants. Fourteen-day-old plants grown at 22C° under standard growth conditions were heat treated at 37C° for the times indicated. The RNA gel blots were hybridized with a *HSP70* cDNA probe. Hybridizations with a probe for constitutively expressed gene *ACTIN* served as a control.

The expression of the heat-shock genes encoding HSPs in plants and other eukaryotes is primarily regulated at the transcriptional level (Schöffl et al., 1998). We tested the induction of *HSP70* gene expression by heat treatment in *Atelp1*, *Atelp2* and wild-type plants. For both wild-type and *Atelp* mutant plants, *HSP70* was detected after 1 hour heat treatment at 37°C. There was no remarkable difference in *HSP70* transcript levels between mutant plants and wild-type plants, suggesting that *AtELP1* and *AtELP2* do not have a crucial role in *HSP70* induction.

Summary

The Arabidopsis genome encodes most of the homologs of yeast transcription elongation factors. This suggests that the mechanism of elongation may be well conserved in plants. The strong homology between yeast Elongator subunits and Arabidopsis counterparts, similar phenotypes we observed in *Atelp1* and *Atelp2* and *Atelp1/Atelp2* double mutants suggest that Arabidopsis has an Elongator. To further support this point, we need to perform co-immunoprecipitation assay using the tandem affinity purification (TAP) or direct antibodies to each subunits of AtELP1-AtELP3, to see if all these three subunits physically interact with each other.

The abnormal phenotypes in *Atelp1* and *Atelp2* and *Atelp1/Atelp2* double mutants suggest that Elongtor participates in various aspects of plant development, and this is also supported by the microarray analysis revealing that *AtELP1* is involved in regulation of a diverse subset of genes.

The fact that AtELP1 and AtELP2 genetically interact with AtTFIIS and plant homologs of subunits of yeast Paf1C suggests that Arabidopsis Elongator posses the

ability of interacting with other potential transcription elongation factors. Chromatin immunoprecipitation experiment is needed to be done to test whether Arabidopsis Elongator binds to the open reading frames of genes. In addition, co-immunoprecipitation experiments to test the interaction between Elongator and elongating form of Pol II may provide additional evidence to qualify Elongator as a transcription elongation factor in Arabidopsis.

METHODS

Strains and identification of T-DNA mutants

Introgression line Col:*FRI*^{SF2} consists of the *FRI* locus from ecotype San Feliu-2 (*FRI*^{SF2}) introgressed into the Columbia (Col) ecotype through six successive backcrosses and made homozygous by self-pollination (Lee et al., 1994). The *Atelp1-1, Atelp1-2, Atelp2, AttfIIs, Atsoh1 and Atchd1* mutant plants were obtained from a sequence-indexed, T-DNA-mutagenized population of Arabidopsis (http://signal.salk.edu). Mutant lines obtained were segregating for the T-DNA insertion. The primers used to detect T-DNA insertions into the *AtELP1, AtELP2, AtTFIIS, AtSOH1* and *AtCHD1*genes are listed in Table 2. The genotypes of isolated mutants were confirmed by PCR with two gene-specific primers or with one gene-specific primer and the T-DNA border–specific primer. In the case of homozygous mutant plants, only one PCR product, representing the T-DNA insertion, was detectable, whereas in the case of heterozygous plants, two products were detected, corresponding to the T-DNA insertion and the non-altered gene. The PCR products were separated by electrophoresis on 1% agarose gel.

An *Atelp1/Atelp2* double mutant was created by first crossing the *Atelp1-004690* mutant with the *Atelp2-106485* mutant. An *Atelp1/Atelp1, AtELP2/Atelp2* plant was identified in the respective F2 population and allowed to self-pollinate, and double mutants were identified in the corresponding progeny. *Atelp1/AttfIIs and Atelp2/AttfIIs* double mutants were created by first crossing *Atelp* with *AttfIIs-056755*. An *Atelp/Atelp, AtTFIISAt/AttfIIs* plant was identified in the respective F2 population and allowed to self-pollinate, and double mutants were identified in the corresponding progeny. *Atelp1/vip3, Atelp1/vip4-2, Atelp1/vip5, Atelp1/vip6-2, Atelp2/vip3, Atelp2/vip4-2* and

Atelp2/vip5 double mutants were created by first crossing *Atelp* with *vip* mutants in the Col:FRI^{SF2} background. An *Atelp/Atelp,* fri^{Col}/ fri^{Col}, *VIP/vip* plant was identified in the respective F_2 population and allowed to self-pollinate, and double mutants were identified in the corresponding progeny. PCR-based molecular markers were utilized to discriminate between wild-type and mutant alleles of *VIP3, VIP4, VIP5, VIP6* and *FRI* (Zhang and van Nocker, 2002; Zhang et al., 2003; Oh et al., unpublished; Johanson et al., 2000).

Growth conditions

Arabidopsis seeds were surface-sterilized, allowed to imbibe in sterile water for 2d at 4°C to ensure uniform germination, and planted directly into artificial soil mix (2 : 1 : 1 peat : vermiculite : perlite; Fafard Inc., Springfield, MA, USA). Standard growth conditions were 60–100 μmol m^{-2} s^{-1} of fluorescent lighting in a 16-h-light/8-h-dark photoperiod at 22°C, with approximately 50% relative humidity. For cold treatments, plants were grown under standard growth condition for 11 days, and then transferred to 4°C under continuous light (20-40 μmol m^{-2} s^{-1}) as described (Gilmour et al., 1998). Tissue was harvested at 0, 1, 2, 4, 8, 12, and 24 hours after the beginning of the cold treatment. For heat treatments, plants were grown under standard growth conditions for 14 days, and then transferred to a 37°C incubator in darkness. Tissue was harvested at 0, 1, 2, 4 hours after the beginning of the heat treatment.

Identification of potential transcription elongation factor components in Arabidopsis

To identify Arabidopsis proteins potentially involved in transcriptional elongation,

we evaluated the predicted Arabidopsis proteome for homologs of yeast proteins with known roles in transcriptional elongation, as listed in Table 1. Protein sequences were retrieved from the Saccharomyces Genome Database (http://www.yeastgenome.org/) and used as queries for homology searches of predicted Arabidopsis proteins as cataloged by The Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org/), using Washington University BLAST version 2.0 as maintained by TAIR. An Arabidopsis protein or paralogous group was designated as homologous to the yeast query if it met the following three criteria:1) it was the most closely related protein(s) 2) the Expectation (E) value for the match was less than 10E-8, and 3) the protein or all members of the paralogous group were more closely related than the next most significant match by a factor equal to or greater than 10E5.

Transcription profiling

Microarray analysis involved two replicates for each genotype, with each replicate composed of three independently derived samples. Each sample included 10 plants. Flowers (to stage 12) and inflorescence apices were utilized to make total RNA, using Qiagen RNAeasy columns (Qiagen, Valencia, CA). Synthesis of cDNA employed the SuperScript™ Double-Stranded cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) and 100 pmol of oligo$(dT)_{24}$ primer (Proligo, Boulder, CO), following the manufacturers' instructions. Synthesis of biotinylated cRNA utilized the BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Inc., Farmingdale, NY). The Arabidopsis ATH1 Genome Array (Affymetrix, Santa Clara, CA) was used for hybridization. Hybridization and scanning of microarrays was performed at the Genomics Technology Support Facility at Michigan State University.

Microarray data was analyzed using the statistical algorithms within the Affymetrix Microarray Suite (MAS) 5.0 Software. We employed pairwise comparisons of the independent biological replicates to identify genes that exhibited a marked change in transcript abundance according to a 'stringent' or 'relaxed' definition. In addition, the MAS software must have observed a statistically significant change in expression (i.e., called 'decrease', 'marginal decrease', 'increase', or 'marginal increase') in at least three of the four pair wise comparisons, and the mean difference in signal intensity must have been three-fold or greater. To evaluate the extent of background "noise" in these experiments, we compared replicates for each sample. The number of genes that were detected and exhibited a significant change in expression of at least three-fold in any one comparison of replicate sample pairs (i.e. Col vs. Col, *Atelp1* vs. *Atelp1*) was, at most, 196 (0.8% of microarrayed genes).

RT-PCR and RNA gel blot hybridization analysis

Relatively quantitative RT-PCR analysis of *AtELP1, AtELP2, FLC, At1g71770, At1g22760, At2g42940* and *At1g61110* gene expression was carried out using the following cycle: 94°C for 30s, 55 °C for 30s, and 72 °C for 1min, and using primer sets and number of cycles as listed in Table 3.

Total RNA from Arabidopsis tissues was isolated with the RNeasy Plant Mini Kit (Qiagen, Valencia, CA). RNA gel blot analysis was performed with 10 μg (per lane) of total RNA. After electrophoresis and blotting to a Zeta-Probe blotting membrane (Bio-Rad Lab, Hercules CA), RNAs were hybridized with radiolabeled cDNA probes that encode *COR6.6*, or *COR47* for cold treatment experiment (kindly provided by M. F. Thomashow).

HSP70 (amplified by primers HSP70F: 5'-ATGTCGGGTAAAGGAGAAGG-3' and HSP70RR: 5'-GAGACATCAAAAGTGCCACC-3') for heat shock experiment, and *FLC* (amplified by primers FLCF and FLCR listed in Table 3). Subsequently, the membranes were stripped and rehybridized with probes for *ACTIN* (amplified by primers ACTINF and ACTINR listed in Table 3). The probes were radiolabelled with α-[^{32}P] dATP using the Random Primers DNA Labeling System (Invitrogen, Carlsbad, CA). Hybridization was performed in IMAGE buffer (1.0mM EDTA, 1.25M Na_2HPO_6, SDS 7%, adjusted to pH7.4 with H_3PO_6) at 65°C overnight, and the blots were washed twice in low salt wash buffer (0.04M $NaHPO_4$, 1% SDS, and 1mM EDTA) at 65°C for 15 min before autoradiography.

Antisense *AtELP2*

For production of *AtELP2* antisense plants, a ~2.5-kb fragment corresponding to a portion of the translated region was amplified from apex cDNA using the primers At1g49540F (5'-CGGGATCCATGTCAGAAAACACAAAAGTCG- 3') and At1g49540R (5'- CGGGATCCAACTCTCACACAATTATCATCC-3') and inserted into vector pPZP201:BAR:35S. The constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 and used for plant transformation by the floral-dip method (Clough and Bent, 1998).

Table 2 Oligonucleotides used as PCR primers for the identification and characterization of T-DNA disruption mutants of *AtELP1, AtELP2, AtTFIIS, AtSOH1* and *AtCHD1* genes.

Gene	Primer name	Sequence
AtELP2	SALK_106485LP	5'- ATTTTGTTGTCCAATCCGCCC -3'
	SALK_106485RP	5'- CCCACTTCCAAGTTTGCCTTCA -3'
AtELP1	SALK_004690LP	5'- TTTCCAAGTGCCACATCTCCA -3'
	SALK_004690RP	5'- GCAGGTGTCTGTTCGCCACTT -3'
AtELP1	SALK_011529LP	5'- ATTTTTGCTGCTTCCGCTGGT -3'
	SALK_011529RP	5'- TGGGTGGCAGGCATTTCTACA -3'
AtSOH1	SALK_035522LP	5'- GTGCCAGGTCTGCCAAACAGT -3'
	SALK_035522RP	5'- TAGCTGTCACATTTTGCGGGG -3'
AtSOH1	SALK_051025LP	5'- GGCAGAAGCAATCTCTGCCAA -3'
	SALK_051025RP	5'- TGTGATTCTGCGTTTATGCCCA -3'
AtCHD1	SALK_020296LP	5'- GCTGCTTCAACAACACCACCC -3'
	SALK_020296RP	5'- TGAAATCTTCTTTCAAGTGGCTGC -3'
AtTFIIS	SALK_064316LP	5'- TGCGATCAATCCTTCTAATTTCCG -3'
	SALK_064316RP	5'- CCAACAATATCAACGGGGACG -3'
AtTFIIS	SALK_056755LP	5'-ACGATACCCTCGTTGCGACTC-3'
	SALK_056755RP	5'-ACGCCTCCACAAGCAACTCAC-3'
T-DNA border	LBb1	5'- GCGTGGACCGCTTGCTGCAACT -3'
	LBa1	5'- TGGTTCACGTAGTGGGCCATCG -3'

Table 2 Oligonucleotides used as PCR primers for the identification and characterization of T-DNA disruption mutants of *AtELP1*, *AtELP2*, *AtTFIIS*, *AtSOH1* and *AtCHD1* genes.

Gene	Primer name	Sequence
AtELP2	SALK_106485LP	5'- ATTTTGTTGTCCAATCCGCCC -3'
	SALK_106485RP	5'- CCCACTTCCAAGTTTGCCTTCA -3'
AtELP1	SALK_004690LP	5'- TTTCCAAGTGCCACATCTCCA -3'
	SALK_004690RP	5'- GCAGGTGTCTGTTCGCCACTT -3'
AtELP1	SALK_011529LP	5'- ATTTTTGCTGCTTCCGCTGGT -3'
	SALK_011529RP	5'- TGGGTGGCAGGCATTTCTACA -3'
AtSOH1	SALK_035522LP	5'- GTGCCAGGTCTGCCAAACAGT -3'
	SALK_035522RP	5'- TAGCTGTCACATTTTGCGGGG -3'
AtSOH1	SALK_051025LP	5'- GGCAGAAGCAATCTCTGCCAA -3'
	SALK_051025RP	5'- TGTGATTCTGCGTTTATGCCCA -3'
AtCHD1	SALK_020296LP	5'- GCTGCTTCAACAACACCACCC -3'
	SALK_020296RP	5'- TGAAATCTTCTTTCAAGTGGCTGC -3'
AtTFIIS	SALK_064316LP	5'- TGCGATCAATCCTTCTAATTTCCG -3'
	SALK_064316RP	5'- CCAACAATATCAACGGGGACG -3'
AtTFIIS	SALK_056755LP	5'-ACGATACCCTCGTTGCGACTC-3'
	SALK_056755RP	5'-ACGCCTCCACAAGCAACTCAC-3'
T-DNA border	LBb1	5'- GCGTGGACCGCTTGCTGCAACT -3'
	LBa1	5'- TGGTTCACGTAGTGGGCCATCG -3'

Table 3 RT-PCR primers and cycle numbers

Gene	Primer Name	Primer sequence	number of cycles
AtELP1	SALK_004690LP	5'- TTTCCAAGTGCCACATCTCCA -3'	35
	SALK_004690RP	5'- GCAGGTGTCTGTTCGCCACTT -3'	
AtELP2	SALK_106485LP	5'- ATTTTGTTGTCCAATCCGCCC -3'	35
	SALK_106485RP	5'- CCCACTTCCAAGTTTGCCTTCA -3'	
At1g22760	At1g22760F	5'-GCACGCTCTTGACGTCTGATG-3'	
	At1g22760RR	5'- ATCAAACCCACCCGAACTCG-3'	35
At2g42940	At2g42940F	5'-ATGGCTGGAGGTACAGCTC-3'	35
	At2g42940RR	5'-GACCAGATGCGATTAGCCCAC-3'	
At1g71770	At1g71770F	5'-GGATCAGAGTGGGAACTCGA-3'	35
	At1g71770RR	5'- AGACATCGGTGATGGAACTG-3'	
At1g61110	At1g61110F	5'-TAGTCACAAGGTTGGTGTCAAGA-3'	35
	At1g61110RR	5'-CTCTGAGGAATCTCACTAAGC-3'	
FLC	FLCF	5'-GGATCATCAGTCAAAAGCTCTG -3'	40
	FLCR	5'- AGTATCACACACAAAGTCTCTTGG -3'	
ACTIN	ACTINF	5'- AGAGATTCAGATGCCCAGAAGTCTTGTTCC-3'	25
	ACTINR	5'-AACGATTCCTGGACCTGCCTCATCATACTC -3'	

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