The Composition of the *Arabidopsis* RNA Polymerase II Transcript Elongation Complex Reveals the Interplay Between Elongation and mRNA Processing Factors


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**Short title:** RNA Pol II transcript elongation complex

**One-sentence summary:** The RNA polymerase II transcript elongation complex is an interaction site for transcript elongation factors, facilitating chromatin transcription and its coordination with mRNA processing.

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**ABSTRACT**

Transcript elongation factors (TEFs) are a heterogeneous group of proteins that control the efficiency of transcript elongation of subsets of genes by RNA polymerase II (RNAPII) in the chromatin context. Using reciprocal tagging in combination with affinity-purification and mass spectrometry, we demonstrate that in *Arabidopsis thaliana*, the TEFs SPT4/SPT5, SPT6, FACT, PAF1-C, and TFIIS co-purified with each other and with elongating RNAPII, while P-TEFb was not among the interactors. Additionally, NAP1 histone chaperones, ATP-dependent chromatin remodelling factors, and some histone-modifying enzymes including Elongator were repeatedly found associated with TEFs. Analysis of double-mutant plants defective in different combinations of TEFs revealed genetic interactions between genes encoding subunits of PAF1-C, FACT, and TFIIS co-purified with each other and with elongating RNAPII, while P-TEFb was not among the interactors. Additionally, NAP1 histone chaperones, ATP-dependent chromatin remodelling factors, and some histone-modifying enzymes including Elongator were repeatedly found associated with TEFs. Analysis of double-mutant plants defective in different combinations of TEFs revealed genetic interactions between genes encoding subunits of PAF1-C, FACT, and TFIIS resulting in synergistic/epistatic effects on plant growth/development. Analysis of sub-nuclear localisation, gene expression and chromatin-association did not provide evidence for an involvement of the TEFs in transcription by RNAPI (or RNAPIII). Proteomics analyses also revealed multiple interactions between the transcript elongation complex and factors involved in mRNA splicing and polyadenylation, including an association of PAF1-C with the polyadenylation factor CstF. Therefore, the RNAPII transcript elongation complex represents a platform for interactions among different TEFs, as well as for coordinating ongoing transcription with mRNA processing.
INTRODUCTION

In eukaryotes, functional mRNA levels in the cell are precisely controlled in a spatially and temporally defined manner. To achieve this goal, transcript synthesis by RNA polymerase II (RNAPII), as well as mRNA processing, are regulated at several stages. Traditionally, the initiation of transcription is considered the crucial step controlling transcript synthesis. In recent years, it has become apparent that the elongation phase of RNAPII transcription is also dynamic and highly regulated. The differential phosphorylation of heptapeptide repeats within the RNAPII carboxy-terminal domain (RNAPII-CTD) characterize subsequent steps of the elongation phase (Buratowski, 2009; Hajheidari et al., 2013). In addition, a variety of so-called transcript elongation factors (TEFs) have been identified, reflecting the importance of regulating transcript elongation. TEFs facilitate efficient mRNA synthesis in the chromatin context, and accordingly they act as histone chaperones, modify histones within transcribed regions, or modulate the catalytic properties of RNAPII (Jonkers and Lis, 2015; Selth et al., 2010; Sims et al., 2004). Importantly, transcript elongation is also essential for coordinating transcript synthesis and co-transcriptional pre-mRNA processing (Perales and Bentley, 2009).

Genetic and biochemical studies have revealed that various TEFs regulate plant growth and development (Van Lijsebettens and Grasser, 2014). In Arabidopsis thaliana, the histone chaperone complex FACT (facilitates chromatin transcription), consisting of the SSRP1 (structure-specific recognition protein 1) and SPT16 (suppressor of Ty 16) proteins, is involved in the expression of the floral repressor gene FLC and thereby modulates the induction of flowering (Lolas et al., 2010). Likewise, the PAF1 (RNAPII-associated factor 1) complex (PAF1-C), which promotes transcription-coupled histone modifications (i.e., H3K4me, H3K36me, H2Bubi) (Tomson and Arndt, 2013), was found to regulate flowering by altering the expression of FLC and related MADS box factors (He et al., 2004; Oh et al., 2004). Another TEF that regulates FLC expression is P-TEFb (positive transcript elongation factor b, consisting of CDKC;2 and CYCT1), which mediates the phosphorylation of Ser2 residues within RNAPII-CTD repeats (Wang et al., 2014). The Elongator complex has histone acetyl-transferase activity and modulates developmental and immune response pathways (Woloszynska et al., 2016). TFIIS assists RNAPII progression of transcription through various obstacles and is involved in the expression of DOG1, a key regulator of Arabidopsis seed dormancy (Grasser et al., 2009; Mortensen and Grasser, 2014). Another factor altering
the performance of RNAPII is the SPT4/SPT5 heterodimer, which is thought to render the
is required for the efficient expression of genes involved in auxin signalling and therefore
contributes to auxin-related gene expression (Dürr et al., 2014).

In yeast, the assembly of the RNAPII transcript elongation complex (TEC) and the
tracking of TEFs with RNAPII along transcribed regions has been examined in quite some
detail. Additionally, genetic/biochemical interactions between various TEFs have been
studied (Jonkers and Lis, 2015; Selth et al., 2010; Sims et al., 2004). In higher eukaryotes and
particularly in plants, considerably less is known about the RNAPII TEC and the
collaboration of TEFs. Therefore, in this study, we used a reciprocal tagging approach to
affinity-purify various TEFs from Arabidopsis cells. Mass spectrometric analyses identified
interactions between transcript elongation-related proteins, but they also revealed interplay
with mRNA processing factors. Moreover, the examination of plants defective in different
combinations of TEFs demonstrated genetic interactions between distinct types of TEFs.

RESULTS
Components of the Arabidopsis RNAPII elongation complex
To identify proteins that form the Arabidopsis RNAPII elongation complex, we expressed bait
proteins fused to a GS tag (2x protein G domains and streptavidin-binding peptide) in PSB-D
suspension cultured cells (ecotype Landsberg erecta) (van Leene et al., 2015), an approach
that has been used to characterise other nuclear protein complexes (Dürr et al., 2014; Nelissen
et al., 2010; Pauwels et al., 2010). Employing cultured cells enables the production of
homogenous biomaterial to characterise the composition of protein complexes involved in
basic cellular functions out of a developmental context and in the absence of tissue-specific
influences (van Leene et al., 2015). For GS-tagged bait proteins, we selected the FACT
subunits SSRP1 and SPT16, the PAF1-C components ELF7 and CDC73, the SPT4-2 subunit
of SPT4/SPT5, TFIIS, and the CDKC;2 component of P-TEFb. For comparison, we analysed
the GS fusion protein harbouring the largest subunit (NRPB1) of RNAPII. To identify
proteins that interacted with the bait proteins, the GS-fusion proteins and the unfused GS
control were isolated from cell extracts by IgG affinity-purification. In pilot experiments, we
performed comparative affinity-purifications with cell extracts that were either untreated or
treated with the endonuclease Benzonase to degrade nucleic acids. According to SDS-PAGE
(Figure 1A) and mass spectrometry analyses, we observed only minor changes in protein
recovery, with a tendency towards enhanced protein detection upon nuclease treatment, which may be due to an improved solubilisation of chromatin-bound proteins (Lambert et al., 2014).

All subsequent affinity-purifications in this report were performed with nuclease-treated protein extracts and therefore, the observed associations of proteins were likely due to protein interactions rather than mediated by nucleic acids. Proteins in the eluates of the affinity-purifications were identified after tryptic digestion by mass spectrometry, and in some cases, the eluates were also examined by immunoblotting. NRPB1 was specifically detected in the NRPB1-GS purification, but not in the control GS purification (Figure 1B). Ten subunits of RNAPII (Ream et al., 2015) were detected by mass spectrometry in the NRPB1-GS eluate (Table 1; Supplemental Data Set 1), but no subunits specific for other RNAPs were observed. Only the two smallest RNAPII subunits (NRPB10/12; 8.3 and 5.9 kDa, respectively) were absent, likely due to our gel-based protein separation. Besides the RNAPII subunits, other transcription-related proteins were identified, including the two subunits of the heterodimeric transcription initiation factor TFIIIF and the TEFs SPT5, TFIIIS and SPT6, which are known to directly interact with RNAPII. TFIIIF and TFIIIS are high-affinity interactors of RNAPII (Sopta et al., 1985), and the prominent presence of initiation and elongation factors in the NRPB1-GS sample indicates that functionally different forms of RNAPII were isolated in this experiment.

To identify components of the elongation complex, GS-tagged versions of various TEFs were affinity-purified. Isolation and immunoblot analysis of the FACT subunits SSRP1 and SPT16 demonstrated that the two proteins robustly were purified together (Figure 1C-E), which is in agreement with their occurrence as a heterodimer (Duroux et al., 2004). As expected for a histone chaperone, various core histones (i.e., H2A, H2B, H2A.Z and H2A.X) co-purified with FACT (Supplemental Data Set 2), but since many of them, likely due to their abundance, appear in the list of common contaminants (van Leene et al., 2015), they are not further discussed here. In addition, various TEFs were found to co-purify with FACT, including SPT5 and subunits of PAF1-C (Table 1, Supplemental Data Set 2). Members of the NAP1 family of histone chaperones and histone deacetylases (HDACs) were also identified in the SSRP1-GS and SPT16-GS eluates.

*Arabidopsis* PAF1-C was isolated previously and the subunits VIP3, VIP4, VIP6/ELF8 and CDC73 were identified by immunoblotting (Oh et al., 2004; Park et al., 2010). Affinity-purification of ELF7-GS and CDC73-GS (Figure 1F) in combination with mass spectrometry analyses resulted (reproducibly) in the identification of six predicted
subunits of PAF1-C, with high Mascot scores, indicating a high probability of correct identification (Table 1, Supplemental Data Set 3). Therefore, Arabidopsis PAF1-C is likely a hexameric complex consisting of ELF7, VIP6/ELF8, VIP3, VIP4, VIP5 and CDC73, resembling the situation in human and Drosophila rather than in yeast, where PAF1-C is composed of five subunits lacking SKI8 (orthologue of Arabidopsis VIP3) (Jaehning, 2010; Tomson and Arndt, 2013). In line with a hexameric PAF1-C, according to publicly available mRNA expression data, the genes encoding the six subunits show a very similar expression profile in Arabidopsis (http://www.arabidopsis.org/). Additionally, other TEFs including SPT5, TFIIS, FACT, SPT6 and Elongator co-purified with PAF1-C. The SPT6 histone chaperone occurs in two versions in Arabidopsis (Gu et al., 2012), and according to publicly available microarray data, SPT6L appears to be commonly expressed, whereas the SPT6 transcript is barely detectable in most tissues (http://www.arabidopsis.org/). Consistently, only the SPT6L transcript was detected by RT-PCR analysis with seedling RNA, but both genes are expressed in PSB-D cells (supplemental Figure 1A-C). SPT6L, as well as SPT6, were isolated along with PAF1-C from PSB-D cells (Table 1). In addition, subunits of RNAPII, NAP1, and several proteins involved in ATP-dependent chromatin remodelling complexes (CRCs) were identified, primarily in the ELF7-GS eluates.

Along with GS-TFIIS (Figure 1G) diverse subunits of RNAPII and all subunits of PAF1-C were isolated (Table 1, Supplemental Data Set 4). Moreover, other TEFs including SPT5 and SPT6L, as well as NAP1 proteins and HDACs were identified in the GS-TFIIS eluates. We previously investigated SPT4-GS affinity-purifications (Dürr et al., 2014). Because the sensitivity of our mass spectrometric analyses has since been markedly improved, the experiment was repeated under comparable conditions (Figure 1H). This experiment confirmed the proteins that were found to co-purify with SPT4-GS before (Dürr et al., 2014), but with more robust Mascot scores, and several additional interactors were identified. Thus, in addition to SPT5, several subunits of RNAPII, as well as TFIIF and HDACs, were isolated along with SPT4-GS (Table 1, Supplemental Data Set 5). A number of TEFs including TFIIS, PAF1-C, FACT, SPT6L/SPT6 and Elongator were also found to co-purify. Interestingly, various proteins of ATP-dependent chromatin remodelling complexes (Gentry and Henning, 2014) were identified in the SPT4-GS eluates. Two components that are involved in RNAPV-mediated RNA-directed DNA methylation (RdDM), SPT5L and AGO4, were found to specifically associate with SPT4-GS, but with none of the other analysed GS fusion proteins.
The plant-specific protein SPT5L is a direct interactor of SPT4 and AGO4 (Bies-Etheve et al., 2009; Dürr et al., 2014; He et al., 2009), and SPT4 can modulate RdDM (Köllen et al., 2015).

Affinity-purification of the P-TEFb component CDKC2-GS (Figure 1I) demonstrated that it can interact with three different versions of CYCT1 (Table 1, Supplemental Data Set 6), which is consistent with recent results (Wang et al., 2014). Surprisingly, apart from SPT16, no other TEFs and no RNAPII subunits were found to co-purify with CDKC2-GS. However, various subunits of the NuA4/SWR1 chromatin remodelling complex, with combined histone acetyl-transferase and chromatin remodelling activity (Bieluszewski et al., 2015) (Table 1), as well as several BRD4 (bromodomain-containing protein4)-like proteins (Supplemental Data Set 6) were detected in the CDKC2-GS eluates. Since BRD4 proteins are involved in recruiting P-TEFb to chromatin containing acetylated histones at target genes in mammalian cells (Bisgrove et al., 2007; Jang et al., 2005), this mechanism may be conserved in plants. In conclusion, our proteomics analyses demonstrate that there is a considerable overlap in the interactions seen with FACT, PAF1-C, TFIIS and SPT4/SPT5, but the protein interactions of P-TEFb differ markedly from those seen with the other tested TEFs (Figure 2). Additional factors (e.g. NAP1, CRCs, Elongator) repeatedly co-purified with the TEC and may contribute to efficient transcript elongation in Arabidopsis. To examine which form(s) of RNAPII co-purified with the TEFs, we analysed affinity-purifications of GS-TFIIS and ELF7-GS by immunoblotting using antibodies directed against the non-phosphorylated RNAPII-CTD and against Ser2-phosphorylated CTD-repeats. Relative to the input samples in the affinity-purifications, the Ser2-phosphorylated form of RNAPII was enriched compared with the hypophosphorylated RNAPII (supplemental Figure 2A). Therefore, the elongating, Ser2-phosphorylated form of RNAPII predominantly co-purified with the TEFs.

ELF7 and SPT6L co-localise with RNAPII in euchromatin

Microscopic immune-fluorescence analyses have shown that the TEFs SPT5 and FACT are enriched within transcriptionally active euchromatin in Arabidopsis nuclei and that SPT5 co-localises with elongating RNAPII (Droux et al., 2004; Dürr et al., 2014; Lolas et al., 2010). In view of the mutual association of FACT and SPT4/SPT5 with PAF1-C, TFIIS and SPT6L, we raised antibodies against recombinant ELF7, TFIIS and SPT6L. While the TFIIS antibodies proved not to be useful, the ELF7 and SPT6L antibodies yielded specific staining patterns in pilot experiments and were used for super-resolution structured illumination microscopy (SIM). ELF7 and SPT6L fluorescent signals were analysed in flow-sorted 8C
nuclei of *Arabidopsis* leaves (Figure 3). To investigate the sub-nuclear distribution of ELF7 and SPT6L relative to RNAPII, cells were simultaneously labelled with the respective antibodies and counterstained with DAPI. We used antibodies specific for the elongating form (phosphorylated at Ser2 of the CTD repeats) and specific for the non-phosphorylated form of RNAPII. In *Arabidopsis* interphase nuclei, the majority of RNAPII is globally dispersed in the euchromatic part of the nucleoplasm (Schubert and Weisshart, 2015). Consistently, our SIM analyses revealed that ELF7/SPT6L and both forms of RNAPII comprised separate, dispersed, reticulate structures within the euchromatin, but they were absent from the nucleolus and heterochromatin (Figure 3). Further analysis of the degree of co-localisation between ELF7/SPT6L and the RNAPII signals revealed that both TEFs are more often associated with the elongating (CTD-Ser2P) than with the non-phosphorylated form of RNAPII. This tendency is visible in the magnified panels on the right (Figure 3) and in the comparable merged images (supplemental Figure 3), and it is also in line with the quantification of the co-localising fluorescence signals (supplemental Table 1). SPT6 from human and the fungus *Candida glabrata* binds preferentially to Ser2-phosphorylated RNAPII-CTD repeats (Sun et al., 2010; Yoh et al., 2007), and because of the co-localisation of *Arabidopsis* SPT6L with the elongating RNAPII, we examined its interaction with the RNAPII-CTD. The interaction of the putative CTD-interaction domain SPT6L(Phe1218-Asp1412) with RNAPII-CTD peptides was examined by microscale thermophoresis (MST). The fluorescently labelled peptides were phosphorylated at Ser2 or Ser5 or were unmodified. The MST measurements revealed a clear concentration-dependent interaction with the Ser2-phosphorylated peptides with a $K_D$ of 135 (±26.6) μM, whereas the affinity for the Ser5-phosphorylated and non-phosphorylated peptides was at least 10-fold lower (supplemental Figure 2B).

**Genetic interactions between genes encoding different TEFs**

Since several TEFs were found to co-purify efficiently and to co-localise with elongating RNAPII, we generated and analysed various double-mutants deficient in different combinations of TEFs. The double-mutants, along with the respective single-mutants and Col-0 wild-type plants, were primarily examined regarding growth and developmental phenotypes for which differences were observed in the parental lines. We generated plants deficient in *TFIIS* (which have an essentially wild-type appearance (Grasser et al., 2009)) and the FACT subunits (*ssrp1* and *spt16*), which express reduced amounts of the FACT subunits and
similarly display various developmental defects (Lolas et al., 2010)). While the tfIIIs srrp1
double-mutants are synergistically affected regarding rosette diameter and number of primary
inflorescences (Figure 4A-C, supplemental Figure 4A), the tfIIIs spt16 plants are comparable
to the spt16 single-mutants (Figure 4D-F, supplemental Figure 4B). The seed set of tfIIIs spt16
is clearly reduced relative to Col-0 and tfIIIs, but comparable to that of spt16 (and srrp1)
(supplemental Figure 4C), whereas tfIIIs srrp1 plants are sterile. In spt16 plants, the leaf vein
patterning is mildly altered and in srrp1 leaves, the venation is more strongly affected (Lolas
et al., 2010), while the leaf venation of tfIIIs is comparable to that of Col-0. Examination of the
vein pattern in cleared leaves demonstrated that the venation of tfIIIs srrp1 and tfIIIs spt16 is
similar to that of the respective single-mutants defective in the FACT subunits (supplemental
Figure 5). Therefore, concerning different phenotypes, the tfIIIs srrp1 double-mutants are
synergistically affected, while analysis of the tfIIIs spt16 plants indicated that SPT16 acts
epistatically to TFIIIS. However, regarding bolting time (supplemental Figure 4D,E) and leaf
vein patterning, both double-mutant combinations behave like the respective single-mutant
deficient in the FACT subunit. We also generated plants defective in TFIIIS and in a PAF1-C
subunit (elf7, which show reduced growth and early flowering (He et al., 2004)). The tfIIIs elf7
double-mutants display a synergistically reduced rosette diameter and increased number of
primary inflorescences (Figure 4G-I, supplemental Figure 6A). The elf7 single-mutant
showed reduced leaf venation, lacking most of the tertiary and higher-order veins. In the tfIIIs
elf7 plants, the defects in vein patterning were severely enhanced (supplemental Figure 5).
Similarly, elf7 plants have a reduced seed set and seed production in tfIIIs is comparable to that
of Col-0, but the double mutant is sterile. Regarding bolting time, tfIIIs elf7 is comparable to
elf7, but the number of leaves at bolting is reduced relative to elf7 (supplemental Figure
6B,C). The analysis of double-mutants defective in ELF7 and FACT subunits revealed that
the elf7 srrp1 combination is lethal, while elf7 spt16 plants are viable, but their growth is
strongly reduced (supplemental Figure 7). The examination of double-mutants defective in
different combinations of TEFs demonstrated distinct genetic interactions between the genes
encoding FACT, PAF1-C and TFIIIS, yielding synergistic/epistatic effects on the analysed
developmental traits.

Interaction of the RNAPII transcript elongation complex (TEC) with mRNA processing
factors
In view of the central role of the RNAPII TEC in coordinating the synthesis and processing of transcripts, as established in yeast and metazoa (Perales and Bentley, 2009), we examined our proteomics data obtained from the affinity-purification of various TEFs for the occurrence of factors involved in pre-mRNA processing. Since almost no interactions with mRNA processing factors were observed in the data obtained with P-TEFb, we did not include these in our analysis (Table 2). However, we performed additional affinity-purifications with the polyadenylation factors CstF64-GS and CstF77-GS (supplemental Figure 8) to explore possible interactions among the mRNA processing machineries and with the TEC. Many splicing factors were found to co-purify with SPT4, SPT16 and ELF7 and to a lesser extent with TFIIS, SSRP1 and CDC73 (Table 2, Supplemental Data Set 2-5). Various spliceosomal complexes including U1, U2, U5, Sm and NTC were identified, suggesting that spliceosomes of different assembly stages associate with the TEC. A few polyadenylation factors were also identified in the SPT4-GS and ELF7-GS eluates. The robust co-purification of CstF50, CstF64 and CstF77 in the CstF64-GS and CstF77-GS samples (Table 2, Supplemental Data Set 7) indicates that the composition of the Arabidopsis CstF complex resembles the situation in metazoa rather than in yeast (Yang and Doublé, 2011). In mammals, CstF is a trimeric complex consisting of CstF50, CstF64 and CstF77 and it is believed to dimerise, forming a functional hexameric complex that is critically involved in pre-mRNA 3´end processing (Shi and Manley, 2015). Other polyadenylation factors were also identified in the CstF77-GS eluates. In addition, multiple splicing factors (e.g. Sm, NTC) co-purified with CstF77-GS (Table 2), which is in agreement with recent studies reporting a close cooperation between mRNA splicing and polyadenylation (Kaida, 2016; Misra and Green, 2016).

**Interplay between PAF1-C and CstF**

In yeast and metazoa, PAF1-C has various functions related to transcription and mRNA processing, including interactions with the polyadenylation machinery (Jaehning, 2010; Tomson and Arndt, 2013). Thus, PAF1-C is involved in recruiting certain polyadenylation factors to transcribed regions and can modulate the 3´end processing of mRNAs (Nagaike et al., 2011; Nordick et al., 2008). Interestingly, several subunits of PAF1-C including CDC73 were identified in the CstF77-GS and CstF64-GS eluates (Figure 5A, Supplemental Data Set 7), albeit for some reason, CstF was not observed in the affinity-purification of ELF7/CDC73. The possible interaction of Arabidopsis PAF1-C and CstF is in accordance with the association of human CstF with CDC73 (Rozenblatt-Rosen et al., 2009). Arabidopsis mutants
deficient in CDC73 are early flowing, whereas mutants lacking CstF64 are late flowering, which is ultimately mediated by altered transcript levels of the floral repressor FLC (Liu et al., 2010; Park et al., 2010; Yu and Michaels, 2010). We generated double-mutants deficient in CDC73 and CstF64 to examine the induction of flowering in the cdc73 cstf64 combination. The cdc73 cstf64 plants bolted earlier than Col-0, but not as early as the cdc73 single-mutant (Figure 5B,C; supplemental Figure 9A). Consistent with the early flowering phenotype relative to Col-0, reduced FLC transcript levels were detected by RNA gel blot analysis and qRT-PCR in the double-mutant, but the amount was slightly higher than in cdc73 (Figure 5D; supplemental Figure 9B), indicating that in the double-mutant, the influence of cdc73 on flowering and FLC expression is more pronounced than that of cstf64.

Specificity of TEFs for RNAPII-mediated transcription

Certain TEFs including SPT5 and SPT6 were reported to play a role in RNAPI-catalysed transcription in yeast (Anderson et al., 2011; Engel et al., 2015). Additionally, there is evidence that mammalian FACT is involved in chromatin transcription by RNAPI and RNAPIII (Birch et al., 2009). Characteristic of TEFs, SPT5 and FACT (SPT16, SSRP1) were found to associate with transcribed regions of various genes actively transcribed by RNAPII in Arabidopsis (Duroux et al., 2004; Dürr et al., 2014; Perales and Más, 2007). Here, using chromatin immunoprecipitation with antibodies directed against SPT5 and SPT16, we investigated whether the two TEFs also associate with genomic regions transcribed by RNAPI and RNAPIII. As expected, SPT5 and SPT16 were detected at ACT2, UBQ5 and At3g02260, which are transcribed by RNAPII (Figure 6A,B), whereas only background levels were observed at the transcriptionally inactive retrotransposons TA2 and TA3. For both SPT5 and SPT16, background levels were also detected at three regions transcribed by RNAPI (18S rDNA, ETS). Similarly, no enrichment of the two TEFs was seen at three loci transcribed by RNAPIII (U6-1, U6-26, 7SL-1). For comparison, we analysed the association of RNAPIII with these loci using antibodies directed against RNAPII-CTD phosphorylated at Ser2 and Ser5 as well as an antibody against histone H3. Comparable to SPT5 and SPT16, both forms of transcribing RNAPII associated with the RNAPII-transcribed loci, but not with the other analysed regions (Figure 6C,D). As expected, H3 was detected at all tested genomic loci (Figure 6E), whereas no enrichment of these loci was observed in the controls without the addition of antibodies (Figure 6F). Therefore, according to our ChIP experiments, SPT5 and SPT16 do not associate with Arabidopsis loci transcribed by RNAPI and RNAPIII. In
addition, we determined the transcript levels of the 35S pre-rRNA that is synthesised by RNAPI in mutants deficient in different TEFs relative to Col-0. Using RNA gel blot analyses, no significant differences in the amounts of 35S pre-rRNA were detected in the mutant lines compared with Col-0 (supplemental Figure 10). Therefore, our data do not indicate that the analysed TEFs collaborate with RNAPI (and RNAPIII) in *Arabidopsis*.

**DISCUSSION**

Affinity-purification of various TEFs from yeast cells has revealed that several TEFs co-purified with each other and with RNAPII (Krogan et al., 2002; Lindstrom et al., 2003; Squazzo et al., 2002), and various TEFs were found to associate with the transcribed regions of all genes in the yeast genome that are actively transcribed by RNAPII (Mayer et al., 2010). Together, these experiments indicate that the assembly of certain TEFs with RNAPII promotes efficient chromatin transcription. Our reciprocal tagging approach using various TEFs demonstrated that elongating *Arabidopsis* RNAPII reproducibly co-purified with transcript elongation-related proteins. The set of TEFs that frequently co-purified comprised TFIIS, SPT4/SPT5, PAF1-C and FACT, and to a lesser extent Elongator, which are all implicated in the regulation of *Arabidopsis* transcript elongation (Dürr et al., 2014; Grassner et al., 2009; He et al., 2004; Lolas et al., 2010; Nelissen et al., 2010; Oh et al., 2008). SPT6L and SPT6 were also identified in several TEF eluates, which is consistent with the co-localisation of SPT6L with elongating RNAPII and its preferential binding to Ser2-phosphorylated CTD repeats. Therefore, it is likely that SPT6L and SPT6 also act as TEFs in plants. In contrast, the elongation factor P-TEFb (Quaresma et al., 2016) was not enriched in the affinity-purifications of the other TEFs and may interact with the TEC more dynamically, or its association with the complex may not be stable under our purification conditions. Other proteins that repeatedly co-eluted with TEFs include variants of the histone chaperone NAP1, suggesting that NAP1 may collaborate with other histone chaperones such as FACT and SPT6 to facilitate transcript elongation (Zhou et al., 2015). Interestingly, a variety of proteins involved in ATP-dependent chromatin remodelling (Gentry and Henning, 2014) were isolated along with ELF7 and SPT4. So far there is no evidence from plants, but in other organisms, CRCs are associated with the modulation of transcript elongation, probably by altering the structure of nucleosomes in transcribed regions (Murawska and Brehm, 2011; Subtil-Rodriguez and Reyes, 2011). Regarding histone modifying enzymes, only a few proteins were co-isolated with the TEFs. These include the histone methyltransferases SDG4 and WDR5A,
which co-purified with CDKC;2 and FACT, respectively, as well as the histone ubiquitinase
HUB1, which co-purified with CDC73 (Table 1). Compared to these proteins, enzymes
involved in histone acetylation (i.e. Elongator, SWR1/NuA4 and HDACs) were somewhat
overrepresented. In other organisms, histone acetyltransferases and histone deacetylases are
enriched along RNAPII-transcribed regions, although only relatively low levels of histone
acetylation are observed in the transcribed regions of active genes, suggesting that dynamic
histone acetylation is required for efficient elongation (Selth et al., 2010). The integration of
our proteomics analyses shows that similar to yeast (Krogan et al., 2002), in Arabidopsis,
TFIIS, SPT4/SPT5, SPT6, PAF1-C and FACT associate with elongating RNAPII (Figure 2).
Furthermore, additional chromatin factors such as NAP1, CRCs and enzymes involved in
histone acetylation (i.e. Elongator, HDACs) interacted with the complex in Arabidopsis cells
and may contribute to productive transcript elongation.

Arabidopsis mutants defective in TEFs exhibit a great variety of alterations in
growth/development, ranging from mildly affected, for instance, in the case of tfIIs and cdc73
(Grasser et al., 2009; Park et al., 2010; Yu and Michaels, 2010), to severe/lethal phenotypes,
for instance, in the case of spt5-2 and spt6l (Dürr et al., 2014; Gu et al., 2012). We examined
double-mutants deficient in different combinations of TEFs (supplemental Table 2) to
elucidate the consequences on plant growth and development as well as possible interactions
between the genes encoding the TEFs. Mutant plants that express reduced amounts of the
FACT subunits SSRP1 and SPT16 are phenotypically similar (Lolas et al., 2010), but when
these mutations are combined with tfIIs or elf7, regarding most phenotypes, the ssrp1 version
of the double-mutant is clearly more severely affected than the spt16 version, suggesting that
under these conditions, the SSRP1 subunit is more critical for plant growth and development.
Consistently, in mammals, SSRP1 has additional, SPT16-independent roles in RNAPII
transcription (Antosch et al., 2012). Thus, SSRP1 can act as a co-activator of sequence-
specific transcription factors (Spencer et al., 1999; Zeng et al., 2002), and transcript profiling
revealed SSRP1-specific targets (Li et al., 2007). Moreover, Arabidopsis SSRP1, but not
SPT16, is required for the action of the DNA-demethylase DME in the central cell of the
female gametophyte (Ikeda et al., 2011). FACT and TFIIS can help RNAPII transcribe
through nucleosomes (Belotserkovskaya et al., 2003; Bondarenko et al., 2006; Nock et al.,
2012), and they might collaborate regarding this function, which may explain the impaired
growth of the tfIIs ssrp1 mutants relative to the respective single-mutants. In metazoa, PAF1-
C and FACT can apparently recruit each other to sites of transcription (Adelman et al., 2006;
Pavri et al., 2006), and yeast cells lacking both PAF1 and SPT16 exhibit synthetic growth defects (Squazzo et al., 2002). In a highly reconstituted mammalian chromatin transcription system, the establishment of H2B mono-ubiquitination, which is associated with transcriptional activity, is dependent on FACT and PAF1-C (Pavri et al., 2006). Genetic interactions were observed between *Arabidopsis SSRP1/SPT16* and the *HUB1* gene (encoding an H2B mono-ubiquitinase) (Lolas et al., 2010). In view of these findings, the severe growth defect of the *elf7 spt16* plants and the lethality of *elf7 ssrp1* suggest that there is also a close cooperation of FACT and PAF1-C in plants, possibly involving transcription-related histone ubiquitination. Human PAF1-C and TFIIIS physically interact and cooperatively bind to RNAPII, promoting activated chromatin transcription (Kim et al., 2010), and yeast cells lacking PAF1 and TFIIIS exhibit severe synthetic growth defects (Squazzo et al., 2002). *tfIIs elf7* double-mutant plants show strong synergistic defects in growth and development (e.g. number of primary inflorescences, leaf vein pattern) relative to the parental lines. The increased number of inflorescences, which may be due to reduced apical dominance and decreased leaf venation, could be associated with defects in auxin signalling (Benjamins and Scheres, 2008; Chapman and Estelle, 2009). Several TEFs including SPT4/SPT5 and Elongator are involved in auxin-mediated gene expression (Dürr et al., 2014; Nelissen et al., 2010), but to date, PAF1-C and TFIIIS have not been linked to auxin signalling. Our study of selected *Arabidopsis* double-mutants defective in different combinations of TEFs contributes to an emerging interaction network among factors involved in transcript elongation. The type of genetic interactions (epistatic, synergistic) detected in our comparative analysis of the single-/double-mutants depends on the respective trait and is likely determined by distinct changes in gene expression brought about by the deficiencies in the different TEF(s). Generally, in plants (and other organisms) lacking individual TEFs, a relatively small number of genes is differentially expressed, and currently, it is still unclear what determines the requirement of subsets of genes for TEF action to achieve proper expression (Van Lijssebettens and Grasser, 2014). Our experiments also illustrate that the examination of *Arabidopsis* double-mutants is a promising approach to elucidating the complex interplay of TEFs in higher eukaryotes, although further analyses are required to fully exploit the opportunities that the generated plant lines present.

Originally, TEFs were identified as factors modulating transcript elongation by RNAPII (Jonkers and Lis, 2015; Selth et al., 2010; Sims et al., 2004). A few studies yielded the finding that TEFs (i.e. SPT5, SPT6, FACT) known to cooperate with RNAPII also play a
role in RNAPI (and RNAPIII)-mediated transcription (Anderson et al., 2011; Birch et al.,
2009; Engel et al., 2015). Our proteomics experiments did not provide conclusive evidence
for the interaction of the seven analysed TEFs with RNAPI/RNAPIII, as no RNAPIII-specific
subunits co-purified with the TEFs and three RNAPI subunits were only isolated along with
SPT4 (Supplemental Data Set 5). In view of the structural differences between RNAPI and
RNAPII (Engel et al., 2013), it is unlikely that the TEFs that directly bind RNAPII (i.e. SPT5,
SPT6) interact in a similar manner with RNAPI. Consistently, sub-nuclear localisation
analyses demonstrated that several TEFs, including SPT5 (Dürr et al., 2014) as well as SPT6L
and ELF7 (this work), are excluded from nucleoli in Arabidopsis. Furthermore, using ChIP,
SPT5 and SPT16 were found to associate with RNAPII-transcribed genes, but only
background levels were detected at sites transcribed by RNAPI and RNAPIII. In agreement
with these findings, relative to wild-type plants, no altered levels of RNAPI-transcribed 35S
pre-rRNA were detected in mutants deficient in different TEFs. Therefore, our combined
results do not support a role for the analysed TEFs in RNAPI (and RNAPIII)-mediated
transcription in Arabidopsis, which does not exclude the possibility that certain TEFs may
collaborate with RNAPs other than RNAPII under specific conditions.

In addition to revealing interplay between various TEFs, our proteomics analyses
demonstrated interactions between the RNAPII TEC and mRNA processing factors. Many
splicing factors (e.g. components of U1, U2, U5 RNPs, Sm, NTC) and some polyadenylation-
related proteins co-purified with the TEFs. It is likely that a portion of the mRNA processing
factors that is enriched in the affinity-purifications of the TEFs physically interacts with the
elongating RNAPII (Elkon et al., 2013; Perales and Bentley, 2009; Saldi et al., 2016). Several
subunits of PAF1-C also co-purified with the polyadenylation factor CstF. This is in line with
the finding that mammalian TEFs including the PAF1-C component CDC73 contribute to the
recruitment of CstF to transcribed genes (Martincic et al., 2009; Rozenblatt-Rosen et al.,
2009) and that PAF1-C can modulate mRNA 3´end processing events (Nagaike et al., 2011;
Nordick et al., 2008). The analysis of the cdc73 cstf64 double-mutant revealed that the bolting
time and the transcript level of the floral repressor FLC are in between the values of Col-0 and
the cdc73 single-mutant, indicating that the effect of cdc73 clearly exceeds that of cstf64.
CDC73 regulates FLC expression by modulating histone H3 methylation (H3K4me3,
H3K27me3) (Park et al., 2010; Yu and Michaels, 2010), while CstF64 is required for
3´processing of FLC antisense transcripts, which influences the level of the FLC sense
transcript (Liu et al., 2010). In the cdc73 cstf64 double-mutant, both effects are potentially
combined and, in view of the observed interaction between CDC73 (PAF1-C) and the CstF complex, additionally the recruitment of CstF to the FLC locus might be affected. In recent years, it turned out that both splicing and polyadenylation are linked to transcript elongation and that there is a close interrelationalship between ongoing transcription and mRNA processing (Perales and Bentley, 2009). For example, the RNAPII elongation rate, which is under control of TEFs, influences the efficiency of splicing and polyadenylation events (Elkon et al., 2013; Saldi et al., 2016). In plants, the interplay between transcript elongation and mRNA processing has only recently become apparent, as exemplified by the connection of TFIIS and PAF1-C with splicing (Dolata et al., 2015; Li et al., 2016). We also observed that a range of splicing factors co-purified with CstF77. This is in accordance with recent studies showing a close cooperation of mRNA splicing and polyadenylation, particularly at the last exons (Kaida, 2016; Misra and Green, 2016). In conclusion, our proteomic and genetic experiments underscore the important role of the plant RNAPII elongation complex in the production of mRNAs, representing an interaction site for different TEFs and mRNA processing factors.

METHODS

Plasmid construction

The required gene or cDNA sequences were amplified by PCR with KAPA DNA polymerase (PeqLab) using Arabidopsis thaliana genomic DNA or cDNA as template and the primers (also providing the required restriction enzyme cleavage sites) listed in supplemental Table 3. The PCR fragments were inserted into suitable plasmids using standard methods. For affinity-purification, the coding sequences were fused to a C-terminal GS-tag under the control of the 35S promoter (except for TFIIS, where for steric reasons an N-terminal fusion was generated and for technical reasons it was expressed under the control of its native promoter). All plasmid constructions were checked by DNA sequencing. Details about the plasmids generated in this work are summarised in supplemental Table 3.

Plant material and documentation

Arabidopsis thaliana (Col-0) was grown at 21°C and 60% relative humidity in a growth chamber under long-day (LD) conditions (16 h photoperiod per day, 120 μmol m⁻² s⁻¹ using a combination of Osram Lumilux cool-white and Sylvania Lumiline plus warm-white fluorescent tubes) on soil, while for segregation analyses plants were grown on MS medium
Seeds of the T-DNA insertion lines elf7-2 and elf7-3 (He et al., 2004), cdc73-2 (Yu and Michaels, 2010), and cstf64-3 (Liu et al., 2010) were obtained from the European Arabidopsis stock centre (http://www.arabidopsis.info/), and those of ssrp1-2, spt16-1 and tfIIIs-1 were previously reported (Grasser et al., 2009; Lolas et al., 2010). By crossing the parental lines as previously described (Lolas et al., 2010), the following double mutant lines were generated: tfIIIs-1 elf7-3, tfIIIs-1 ssrp1-2, tfIIIs-1 spt16-1, ssrp1-2 elf7-2, spt16-1 elf7-2, cdc73-2 cstf64-3. After sowing, the seeds were stratified in darkness for 48h at 4°C prior to incubation in the plant growth chamber. All phenotypic analyses were independently performed at least twice and representative examples of the reproduced experiments are shown. Plant phenotypes including leaf vein patterning were documented as previously described (Dürr et al., 2014; Lolas et al., 2010).

Recombinant proteins, peptides and antibodies

For the MST experiments, SPT6L(Phe1218-Asp1412) fused to GST was expressed in E. coli and purified by glutathione-sepharose affinity chromatography as previously described (Kammel et al., 2013; Krohn et al., 2002). Using the pQE-plasmids described in supplemental Table 3, full-length TFIIIS, ELF7(D401-E589), SPT6L(V1121-M1430) and NRPB1(L1746-P1839) were expressed as 6xHis-tagged proteins in E. coli and purified by metal-chelate chromatography as previously described (Kammel et al., 2013). Purified recombinant proteins were analysed by SDS-PAGE and mass spectrometry and used for commercial immunisation (Eurogentec), and the obtained antisera were tested as previously described (Kammel et al., 2013; Launholt et al., 2006). Antibodies against SPT5, SPT16 and SSRP1 were previously described (Duroux et al., 2004; Dürr et al., 2014). For ChIP the following commercial primary antibodies were used: RNAPII-CTD-S2P (rabbit, ab5095, Lot: GR124547-2, Abcam, diluted 1:100), RNAPII-CTD-S5P (rabbit, ab1791, Lot: GR193750-4, Abcam, diluted 1:100) and H3 C-terminal region (rabbit, ab1791, Lot: GR176735-2Abcam, diluted 1:250), as well as the previously described antibodies against Arabidopsis SPT16 (Duroux et al., 2004) and SPT5 (Dürr et al., 2014). For immunostaining, we used primary antibodies against non-phosphorylated RNAPII-CTD (mouse, ab817, GR81285-5, AbCam, diluted 1:200) and RNAPII-CTD-S2P (rat, 04-1571, LOT: 2377414, Millipore, diluted 1:100), as well as the secondary antibodies anti-rabbit-rhodamine (LOT: 81183, Jackson Immuno Research, diluted 1:300), anti-rat-Alexa488 (Cat: 112–545–167, LOT: 103333, Jackson Immuno Research, diluted 1:200) and anti-mouse-Cy5 (Cat: 715–175–151, LOT: 112–545–167, LOT: 103333, Jackson Immuno Research, diluted 1:200) and anti-mouse-Cy5 (Cat: 715–175–151, LOT: 103333, Jackson Immuno Research, diluted 1:200).
109542, Jackson Immuno Research, diluted 1:50). N-terminally FITC-labelled peptides corresponding to RNAPII-CTD repeats were commercially synthesised (Biomatik): CTD-noP (PSYSPTSPSYSP), CTD-Ser2P (TSPSY(pS)PTPSY) and CTD-Ser5P (SYSPT(pS)PSYSPT).

**Affinity-purification and characterisation of GS-tagged proteins from *Arabidopsis* cells**

*Arabidopsis* suspension cultured PSB-D cells were maintained and transformed as previously described (van Leene et al., 2015). Protein isolation, purification and mass spectrometric analyses were essentially performed as previously described (Dürr et al., 2014). In brief, after sonicating 15 g cells in extraction buffer (25 mM HEPES-KOH pH 7.4, 0.05 % IGEPAL CA-630, 1 mM DTT, 100 mM NaCl, 2 mM MgCl₂, 5 mM EGTA, 10 % glycerol, proteinase inhibitor cocktail, 1 mM PMSF), the nonspecific endonuclease Benzonase (50 U/ml extract) was added to degrade DNA and RNA. GS-tagged proteins were affinity-purified using IgG-coupled magnetic beads (Hamperl et al., 2014), and eluted proteins were analysed by SDS-PAGE and digested with trypsin. Peptides were separated by reversed-phase chromatography on an UltiMate 3000 RSLCnano System (Thermo Scientific) using a Reprosil-Pur Basic C18 nano column (75 µm i.d. × 250 mm, Dr. Maisch GmbH) and applying a linear 90 min gradient of 4% to 40% acetonitrile in 0.1% formic acid. The LC-system was coupled on-line to a maXis plus UHR-QTOF System (Bruker Daltonics) via a nanoflow electrospray source (Bruker Daltonics). Data-dependent acquisition of MS/MS spectra by CID fragmentation was performed using a dynamic method with a fixed cycle time of 3 s (Compass 1.7, Bruker Daltonics). Protein Scape 3.1.3 (Bruker Daltonics) in connection with Mascot 2.5.1 (Matrix Science) facilitated database searching of the NCBInr database. Search parameters were as follows: trypsin, 1 missed cleavage allowed, deamidation (N,Q), oxidation (M), carbamidomethyl (C), propionamid (C) as variable modifications, precursor tolerance 5 ppm, MS/MS tolerance 0.04 Da, significance threshold p<0.05. Mascot peptide ion-score cut-off was set to 25. A protein score of minimum 80 and at least 2 peptides found with an individual ion-score of ≥25 were considered as criteria for reliable protein identification. The experimental background of contaminating proteins that were isolated with the unfused GS-tag or that co-purify non-specifically independent of the used bait protein was subtracted. The list of 760 known non-specific *Arabidopsis* proteins is based on 543 affinity-purifications with 115 bait proteins, mainly from PSB-D cells (van Leene et al., 2015).
**Immunostaining and super-resolution microscopy**

Nuclei of differentiated rosette leaves were fixed in 4% paraformaldehyde in Tris buffer and flow sorted as described (Weisshart et al., 2016). Immunostaining with different antibodies was performed as previously described (Weisshart et al., 2016). Nuclei were counterstained with 4’, 6-diamidino-2-phenylindole (2 µg/ml) in Vectashield (Vector Laboratories). To analyse the substructures and spatial arrangement of immunosignals and chromatin beyond the classical Abbe/Raleigh limit (super-resolution), spatial Structured Illumination Microscopy (3D-SIM) was applied using a Plan-Apochromat 63×/1.4 oil objective of an Elyra PS.1 microscope system and the software ZEN (Zeiss). The images were captured using 405, 488, 561 and 642 nm laser lines for excitation and the appropriate emission filters and merged using ZEN software (Weisshart et al., 2016). Imaris 8.0 (Bitplane) software was used to measure the degree of co-localisation between RNAPII and TEFs. Briefly, after loading SIM image stacks, the Imaris co-localisation tool was applied. An automatic threshold defined by the point spread function was calculated and used to establish a new co-localisation channel originating from the ELF7/SPT6 and RNAPII channels. This resulting channel contains the channel statistics including the degree of co-localisation (in %) and the Pearson’s and Mander’s coefficients. Imaris 8.0 was also applied to measure the amount of ELF7, SPT6 and RNAPII in flow-sorted 8C rosette leaf nuclei via voxel intensities and to produce 3D movies.

**PCR-based genotyping and quantitative RT-PCR (qRT-PCR)**

To distinguish between plants that were wild type, heterozygous, or homozygous for the T-DNA insertions, genomic DNA was isolated from leaves. The genomic DNA was used for PCR analysis with *Taq* DNA polymerase (PeqLab) and primers specific for DNA insertions and the target genes (supplemental Table 3). For RT-PCR, total RNA was extracted from ~100 mg of frozen plant tissue using the TRIzol method (Invitrogen) before the RNA samples were treated with DNase. Reverse transcription was performed using 2 µg of RNA and Revert Aid H minus M-MuLV reverse transcriptase (Thermo Scientific). The obtained cDNA was amplified by PCR using *Taq* DNA polymerase (PeqLab), or qPCR with KAPA SYBR FAST Universal reagents (PeqLab) and a Mastercycler ep realplex2 (Eppendorf), as previously described (Dürr et al., 2014).

**RNA gel blot analysis**
Total RNA (20 μg) isolated from plants at 14 days after stratification (DAS) was separated by agarose gel electrophoresis for RNA gel analysis as previously described (Grasser et al., 2009). Hybridisation probes were generated by PCR (supplemental Table 3) and 32P-labelled, including the antisense-probe that was used to detect the FLC sense transcript.

**Fluorescent MicroScale Thermophoresis (MST) binding assay**

MST binding experiments were carried out with 40 nM FITC-labelled peptide in 10 mM sodium phosphate (pH 7.0), 1 mM EDTA, 1 mM DTT, and 0.5 mM PMSF with various concentrations of GST-SPT6L(Phe1218-Asp1412) at 40% MST power, 80% LED power in premium capillaries on a Monolith NT.115 device at 25°C (NanoTemper Technologies). The Thermophoresis + TJump was used to analyse the data. The recorded fluorescence was normalised to fraction bound (0 = unbound, 1 = bound), processed using the software KaleidaGraph 4.5, and fitted using the Kd fit formula derived from the law of mass action. Technical duplicates were performed for each experimental setup.

**Chromatin immunoprecipitation (ChIP)**

ChIP assays using 21 DAS Col-0 plants and different antibodies were performed as previously described in detail (Dürr et al., 2014; Fiil et al., 2008). 5 μl precipitated DNA (diluted 1:200 for input and 1:10 for H3, SPT5, SPT16, RNAPII-CTD-S2P, RNAPII-CTD-S5P and the control without antibody) was analysed by qPCR with locus-specific primers (supplemental Table 3). Data were normalised to the input, and p-values were calculated with the software R version 3.2.3.

**Accession numbers**

Sequence data from this article can be found in the GenBank/EMBL libraries under accession numbers: NRPB1: AT4G35800; SPT4-2: AT5G63670; SPT5-2: AT4G08350; TFIIIS: AT2G38560; CDC73: AT3G22590; ELF7: AT1G79730; SSRP1: AT3G28730; SPT16: AT4G10710; SPT6L: AT1G65440; SPT6: AT1G63210; CDKC;2: AT5G64960.

**Supplemental Data**

**Supplemental Figure 1.** Expression of SPT6L and SPT6.

**Supplemental Figure 2.** Enrichment of Ser2-phosphorylated RNAPII in TEF affinity purifications and interaction of SPT6L with Ser2-phosphorylated RNAPII-CTD repeats.
Supplemental Figure 3. ELF7 and SPT6L co-localise with RNAPII in euchromatin.

Supplemental Figure 4. Analysis of tfIIs ssrp1 and tfIIs spt16 double-mutants in comparison to the respective single-mutants and the Col-0 wild type.

Supplemental Figure 5. Leaf vein patterning of Col-0, single- and double mutants.

Supplemental Figure 6. Analysis of tfIIs elf7 double-mutants in comparison to the respective single-mutants and the Col-0 wild type.

Supplemental Figure 7. Phenotype of spt16 elf7 double-mutant plants in comparison to the respective single-mutants and Col-0.

Supplemental Figure 8. Isolation of the CstF complex.

Supplemental Figure 9. Early bolting phenotype of cdc73 cstf64 double-mutant plants.

Supplemental Figure 10. 35S pre-rRNA levels in mutants deficient in different TEFs.

Supplemental Table 1. Degree of colocalisation of ELF7 and SPT6L with RNAPII (phosphorylated at Ser2 or non-phosphorylated).

Supplemental Table 2. Overview of phenotypes observed for single- and double mutants defective in various TEFs.

Supplemental Table 3. Oligonucleotide primers used in this study and construction of plasmids.

Supplemental Data Set 1. Proteins that co-purified with NRPB1-GS identified by mass spectrometry.

Supplemental Data Set 2. Proteins that co-purified with SPT16-GS and/or SSRP1-GS identified by mass spectrometry.

Supplemental Data Set 3. Proteins that co-purified with ELF7-GS and/or CDC73-SGS identified by mass spectrometry.

Supplemental Data Set 4. Proteins that co-purified with TFIIS-GS identified by mass spectrometry.

Supplemental Data Set 5. Proteins that co-purified with SPT4-GS identified by mass spectrometry.

Supplemental Data Set 6. Proteins that co-purified with CDKC2-GS identified by mass spectrometry.

Supplemental Data Set 7. Proteins that co-purified with CstF77-GS and/or CstF64-GS identified by mass spectrometry.

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**AUTHOR CONTRIBUTIONS**

M.G. and K.D.G. designed the research and wrote the manuscript. W.A., A.P., H.F.E., P.H., K.K., S.A.M., T.S., V.S. and M.G. performed the research. W.A., A.P., H.F.E., P.H., K.K., S.A.M., A.B., T.S., G.L., J.G., V.S., M.G and K.D.G. analysed the data. A.B., G.L., J.G., V.S., M.G. and K.D.G. provided the reagents and the tools for the analysis. All authors commented on the manuscript and contributed to the writing.

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### Table 1. Chromatin- and transcription-related proteins co-purifying with RNAPII and TEFs.

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1 The numbers indicate in which affinity purifications the interactors were identified and the respective average Mascot scores.
are given as well the number of times the interactor was detected in three independent affinity purifications – only proteins are listed that were detected at least twice in three experiments.

\(^2\)It is indicated to which protein complex or to which protein family the interactors belong.
Table 2. mRNA processing factors co-purifying with TEFs and CstF.

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2It is indicated to which protein complex or to which protein family the interactors belong.

3It is listed in which process, splicing or polyadenylation (PA), the interactors are primarily involved according to literature.
FIGURE LEGENDS

Figure 1. Isolation of components of the transcript elongation complex. The indicated GS-fusion proteins were purified from *Arabidopsis* cells by IgG affinity chromatography from protein extracts treated with the nuclease Benzonase (except right lane in (A)) to degrade DNA and RNA. Isolated proteins were separated by SDS-PAGE and stained with Coomassie brilliant blue (CBB) followed by mass spectrometric analyses or used for immunoblotting with the indicated antibodies. The unfused GS-tag (GS) served as the control in these experiments. (A) Eluates of the NRPB1-GS affinity-purification with (+Bz) and without (-Bz) Benzonase treatment. (B) Detection of NRPB1 in the NRPB1-GS sample, but not in the GS control, by immunoblotting using an NRPB1 antibody. (C-E) Analysis of the FACT subunits SSRP1-GS and SPT16-GS. Arrowheads indicate the efficient co-purification of SPT16 with SSRP1-GS (in D) and of SSRP1 with SPT16-GS (in E). In addition to the specific bands that react with the respective primary antibodies, the bands of the GS-fusion proteins are detected due to the affinity of the antibodies towards the protein G moiety of the tag. The bands are also detected in the input samples (in), but not in the GS control affinity-purifications (AP). (F-I) Analysis of PAF1-C (CDC73-GS, ELF7-GS), GS-TFIIS, SPT4-GS and CDKC;2-GS. Asterisks indicate the different GS-fusion proteins (that migrate in the gels according to their expected masses) and the unfused GS.

Figure 2. Scheme depicting the *Arabidopsis* RNAPII elongation complex based on the targeted proteomics results. The TEFs FACT, TFIIS, SPT4/SPT5, SPT6, SPT6L and PAF1-C (dark blue) robustly co-purified with each other and with RNAPII (magenta), while P-TEFb (light blue) was not enriched in these experiments. However, additional chromatin factors (orange) also repeatedly co-purified with the TEFs, except for SWR1/NuA4 (yellow), which was primarily isolated along with P-TEFb. Magenta characters of the oval symbols indicate the proteins that were affinity-purified as GS-tagged fusion proteins (for details, see Table 1).

Figure 3. ELF7 and SPT6L co-localise with RNAPII in euchromatin. Co-localisation analysis of ELF7 and SPT6L (magenta) with elongating RNAPII (Ser2P, green) and RNAPII (non-phosphorylated, white) within euchromatic regions of flow-sorted 8C nuclei of leaf cells visualised by SIM. Nuclei were counterstained with DAPI (blue). Fluorescent protein signals
are not detected in the nucleoli (n) and within heterochromatic chromocenters (arrows). In addition to the individual fluorescent signals, merges are shown in the three lower panels. The preferential association of ELF7 and SPT6L with RNAPII Ser2P rather than the non-phosphorylated form of RNAPII is visible from the magnified images (on the right of each panel).

**Figure 4.** Analysis of double-mutants defective in different combinations of TEFs. tfIIIs ssrp1(A-C), tfIIIs spt16 (D-F) and tfIIIs elf7 (G-I) mutants were examined relative to the respective single mutants and the Col-0 wild type. Representative images of the plants at 42 days after stratification (DAS) under long-day conditions are shown (A,D,G) with size bars representing 5 cm. The rosette diameter (∅ in B,E,H) and the number of primary inflorescences (C,F,I) were determined (n=13). Data were analysed by two-way ANOVA and error bars indicate standard deviation calculated from the measurements of 13 individual plants for each line. The letters above the histogram bars indicate the outcome of a multi comparisons Tukey’s test (p < 0.05).

**Figure 5.** Interaction of PAF1-C and the CstF complex. (A) Co-purification of PAF1-C subunits with CstF77-GS and CstF64-GS. In the first two columns, the average Mascot scores are given as well as the number of times the interactor was detected in three independent affinity-purifications. (B) Representative images of the cdc73 cstf64 double-mutant as well as the respective single-mutants and Col-0 at 35 DAS under long-day conditions are shown. The size bar represents 5 cm. (C) Quantification of the number of leaves at bolting (n=15). Data were analysed by two-way ANOVA (significance level p<0.05) and error bars depict standard deviation calculated from the measurements of 15 individual plants for each line. (D) RNA gel blot analysis of RNA isolated from the different genotypes using an antisense probe that detects the FLC sense transcript (top panel). For comparison, an ethidium bromide stain of the RNA in the agarose gel prior to membrane transfer is shown (bottom panel).

**Figure 6.** SPT5 and SPT16 are enriched at genes transcribed by RNAPII (orange), but not at loci transcribed by RNAPI (blue) and RNAPIII (green), or at non-transcribed regions (grey). ChIP analyses of various loci transcribed by different RNA polymerases. The antibodies used are directed against SPT5 (A), SPT16 (B), RNAPII-CTD-S2P/S5P (C,D), histone H3 (E) or without antibody (F). For the ChIP experiments, percentage input was determined by qPCR, and P-values for all qPCR data
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The Composition of the Arabidopsis RNA Polymerase II Transcript Elongation Complex Reveals the Interplay Between Elongation and mRNA Processing Factors


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This information is current as of October 20, 2017

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