TRANSCRIPTION
2018, VOL. 9, NO. 2, 117–122
https://doi.org/10.1080/21541264.2017.1356902

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POINT-OF-VIEW



# The plant RNA polymerase II elongation complex: A hub coordinating transcript elongation and mRNA processing

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**ABSTRACT**
Characterisation of the *Arabidopsis* RNA polymerase II (RNAPII) elongation complex revealed an assembly of a conserved set of transcript elongation factors associated with chromatin remodellers, histone modifiers as well as with various pre-mRNA splicing and polyadenylation factors. Therefore, transcribing RNAPII streamlines the processes of mRNA synthesis and processing in plants.

**ARTICLE HISTORY**
Received 27 June 2017
Accepted 11 July 2017

**KEYWORDS**
chromatin; polyadenylation; RNA polymerase II; splicing; transcript elongation

## Introduction

In addition to controlling the initiation step, transcription by RNA polymerase II (RNAPII) is dynamically regulated during the elongation phase of transcript synthesis by various mechanisms. Accordingly, a range of so-called transcript elongation factors (TEFs) were identified that modulate the transcription process after initiation. Consequently, TEFs typically are detected along the transcribed part of active genes. The heterogeneous TEFs can be divided into functional groups including factors that (i) modulate the catalytic activity of RNAPII, (ii) facilitate progression of the enzyme through repressive chromatin or (iii) covalently modify histones of nucleosomes situated in the transcribed region of genes.[1,2] The requirement for TEF activities very much depends on the context and likely reflects the variety of challenges that elongating RNAPII encounters. In many cases in the absence of a certain TEF only a relatively small subset of genes is differentially expressed, while the majority of genes is ordinarily transcribed. In line with that, mutations in genes encoding TEFs commonly cause rather distinct developmental phenotypes.[3,4]

Isolation of various affinity-tagged TEFs from *Saccharomyces cerevisiae* cells demonstrated that several TEFs co-purified with each other and with RNAPII.[5,6] This set of TEFs comprised SPT4/SPT5 and TFIIS which can modulate RNAPII activity, the histone chaperones FACT and SPT6, the chromatin remodeller CHD1, PAF1C which can promote transcription-related histone modifications, and the SPT6-interactor IWS1. Therefore, these factors may associate with the twelve-subunit RNAPII to form the yeast elongation complex. Importantly, the heptapeptide repeats of the carboxy-terminal domain (CTD) of the largest subunit (NRPB1) of RNAPII are differentially modified during the transcription cycle, for instance, with Ser5 and Ser2 phosphorylation marking early and later transcript elongation stages, respectively.[7,8] Moreover, the RNAPII-CTD modifications are critical for the coordination of ongoing transcription and co-transcriptional pre-mRNA processing events.

## The *Arabidopsis* RNAPII transcript elongation complex (TEC)

Relatively little is known about the RNAPII elongation complex in plants. Compared to the yeast model, the situation in plants is complicated by the fact that several genes encoding TEFs are duplicated. The *Arabidopsis* genome, for instance, encodes alternative versions of SPT4, SPT5 and SPT6 that may be differentially expressed and/or could serve (partially) distinct functions.[9,10] A recent approach expressing tagged versions of various TEFs in *Arabidopsis* cells followed by affinity purification and mass spectrometry identified a network of interactions between different TEFs and

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RNAPII.[11] As exemplified by TFIIS and the PAF1C subunit ELF7 (orthologue of yeast PAF1), the Ser2 phosphorylated form of elongating RNAPII is enriched in the TEF eluates relative to the non-phosphorylated RNAPII. Like in yeast,[5,6] FACT, SPT6, TFIIS, SPT4/SPT5 and the six-subunit PAF1C co-purified with each other and with RNAPII, whereas P-TEFb was not among the interactors. Therefore, the core plant RNAPII elongation complex appears to resemble that of yeast cells (Fig. 1). In addition, various ATP-dependent chromatin remodelling factors,[12] NAP1 histone chaperones[13] and several enzymes involved in histone acetylation including Elongator[14] repeatedly co-purified with the *Arabidopsis* TEFs.[11] Remodelling factors may support elongation by facilitating the passage of RNAPII through nucleosomes.[15] NAP1 occurs in four versions in *Arabidopsis*[13] and three of them repeatedly co-eluted with the TEFs.[11] Therefore, perhaps in collaboration with the FACT and SPT6 histone chaperones NAP1 proteins could promote chromatin transcription.[13] Histone acetyltransferases (HATs, i.e. Elongator, SWR1/NuA4) and histone deacetylases (HDACs) co-purified repeatedly with *Arabidopsis* TEFs, while enzymes involved in histone methylation and ubiquitination were hardly detected. Whereas Elongator and different HDACs co-eluted with various TEFs, SWR1/NuA4 rather specifically was detected in the affinity purification of the P-TEFb subunit CDKC;2.[11] The HATs and HDACs may control dynamic histone acetylation within gene bodies to modulate the efficiency of transcript elongation, but generally HDACs (in collaboration with chromatin remodelling enzymes) maintain a low acetylation level to counteract deleterious, cryptic transcription within transcribed regions.[16,17] Interestingly, in contrast to studies in yeast,[5,6] the SPT6-interactor IWS1 was not found associated with *Arabidopsis* TEFs, although both SPT6 and SPT6L were robustly identified in the affinity purifications of various TEFs.[11] IWS1 interacts directly with the N-terminal region of SPT6,[18] and it was reported to regulate transcription in *Arabidopsis*.[19,20] Therefore, it remains unclear whether IWS1 is a component of the plant RNAPII elongation complex.

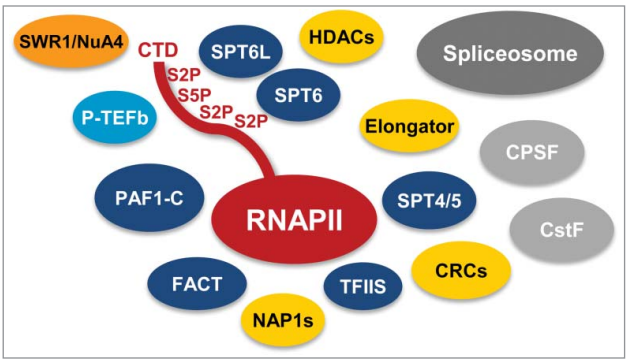


**Figure 1.** Scheme depicting the *Arabidopsis* RNAPII elongation complex based on affinity purification of TEFs in combination with mass spectrometry analyses.[11] The TEFs FACT, TFIIS, SPT4/SPT5, SPT6, SPT6L and PAF1-C (dark blue) robustly co-purified with each other and with RNAPII (red), while P-TEFb (light blue) was not enriched in these experiments. Moreover, further chromatin factors (yellow) also repeatedly co-purified with the TEFs, except for SWR1/NuA4 (orange), which was primarily isolated along with P-TEFb. In addition to the transcription related proteins, many spliceosomal components (dark grey) and polyadenylation factors (light grey) co-purified with the TEFs.

Mutant plants defective in TEFs are phenotypically affected to very different extents. Thus, plants lacking TFIIS or the PAF1C subunit CDC73 have essentially wild type appearance,[21-23] while plants lacking the PAF1C subunits ELF7, ELF8 are more severely affected[24,25] and the loss of SPT5-2, SPT6L or of the FACT subunit SSRP1 is lethal.[9,10,26] The analysis of double-mutant plants defective in different combinations of TEFs revealed various genetic interactions between genes encoding TEFs.[11] In line with the physical interaction of TFIIS and PAF1C in yeast and mammals[27,28] and synthetic growth defects of yeast cells lacking TFIIS and PAF1,[29] plants deficient in TFIIS and ELF7 exhibit (relative to the parental lines) severe synergistic defects, for instance, regarding plant size (Fig. 2) and the leaf vein patterning.[11] Both FACT and TFIIS can facilitate RNAPII transcription through nucleosomes,[30,31] and the analysis of double-mutant plants lacking TFIIS in combination with expressing reduced levels of the FACT subunits SSRP1 or SPT16 indicated that the genes encoding the FACT subunits are epistatic to *TFIIS* regarding bolting time and seed set.[11] Therefore, *Arabidopsis* double-mutants may prove a valuable tool for further studies addressing the functional interplay of TEFs in a higher eukaryote model, supplementing biochemical interaction studies.

## Cooperation of RNAPII-TEFs with other RNA polymerases?

TEFs were originally characterised as factors that regulate RNAPII-mediated transcription after the initiation stage.[1,2] However, several studies (primarily in yeast) suggested that RNAPII-related TEFs cooperate also

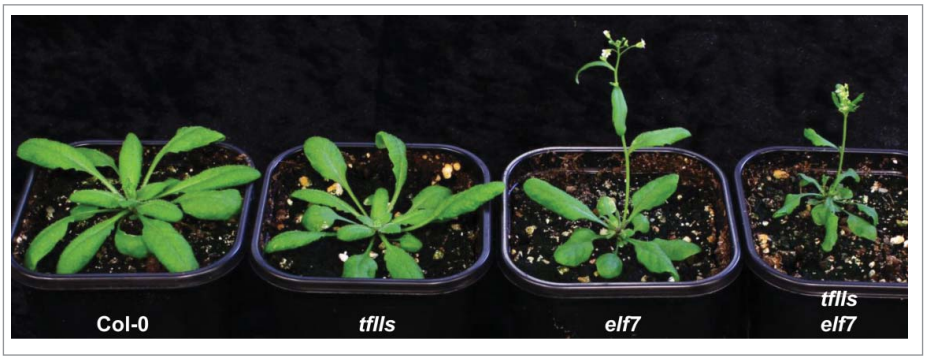


**Figure 2.** *tflls elf7* double-mutant *Arabidopsis* plants in comparison to the respective single-mutants and the Col-0 wild type. While *tflls* has essentially wild type appearance and *elf7* is relatively mildly affected, the growth of *tflls elf7* double-mutant plants is severely reduced, which is obvious from the rosette diameter. Plants at 28 days after stratification grown under long-day conditions are shown.

with RNAPI (and RNAPIII).[32] The affinity purification of various TEFs from *Arabidopsis* cells did not yield conclusive evidence for the association of the TEFs with RNA polymerases other than RNAPII,[11] which could be due to structural differences between RNA polymerases as well as the decoration with polymerase-specific assistant factors.[33] Moreover, according to immunofluorescence analyses several TEFs including SPT5, SPT6 and ELF7 are excluded from the nucleoli of *Arabidopsis* nuclei,[9,11] arguing against an involvement in RNAPI transcription. In line with that in various *Arabidopsis* mutants defective in TEFs the levels of the 35S pre-rRNA were unaffected and chromatin immunoprecipitation experiments revealed that the analysed TEFs associated with RNAPII-transcribed genes, but not with sites transcribed by RNAPI and RNAPIII.[11] These findings suggest that in *Arabidopsis* the RNAPII-TEFs are not involved in RNAPI/III-mediated transcription. Interestingly, the plant-specific protein SPT5L and AGO4 that are components of the RNAPV-mediated RNA-directed DNA methylation (RdDM)[34,35] robustly co-purified with SPT4, but not with the other analysed TEFs.[9,11] SPT4 directly binds SPT5L[9] and it can modulate SPT5L-specific RdDM.[36] Therefore, *Arabidopsis* SPT4 occurs in SPT4/SPT5 (which is conserved among eukaryotes) regulating RNAPII transcript elongation and in SPT4/SPT5L modulating RNAPV-mediated transcriptional silencing.

## Association of mRNA processing factors with the RNAPII TEC

It is well established in yeast and metazoa that the RNAPII TEC is pivotal in coordinating synthesis and co-transcriptional processing of pre-mRNAs by 5′ capping, splicing and 3′ end processing.[37] In plants, relatively little is known about the functional coupling of ongoing transcription and pre-mRNA processing. Accordingly, it was of great interest that many spliceosomal components[38] were identified that co-purified with TEFs (i.e. SPT4, SPT16, ELF7) from *Arabidopsis* cells. These splicing factors included multiple constituents of U1, U2, U5, Sm and NTC sub-complexes,[11] suggesting that spliceosomes of various assembly stages associate with the TEC. In addition, several components of the CPSF polyadenylation complex[39] co-eluted with SPT4 and ELF7.[11] In a reverse experiment, various polyadenylation and splicing factors co-purified with the CstF77 subunit of the CstF polyadenylation complex,[11] illustrating the interplay of polyadenylation and splicing factors that recently was also reported in plants.[40] Interestingly, several *Arabidopsis* PAF1C subunits co-purified with the CstF complex.[11] In line with that human PAF1C can recruit CstF to transcribed genes[41] and PAF1C modulates mRNA 3′end processing.[42] Transcript elongation can modulate the exact outcome of alternative splicing and polyadenylation events. Thereby the RNAPII elongation rate may influence the selection of splice/polyadenylation sites and/or the recruitment of splicing/polyadenylation factors to the transcription machinery couples mRNA synthesis and processing.[43,44] According to a recent pilot study in *Arabidopsis*, similar mechanisms appear to be relevant also for plant gene expression,[45] but future work will show to which extent plant transcriptome diversity is influenced by transcript elongation, which may depend on genome complexity. Surprisingly, the targeted proteomics approaches with several *Arabidopsis* TEFs[11] did not provide evidence for an interaction of the RNAPII

TEC with the 5′ mRNA capping machinery and the THO/TREX complex that plays a central role in linking ongoing transcription with the different mRNA processing events and mRNA export. Consistently, a study addressing protein interactions of the *Arabidopsis* THO/TREX complex also did not reveal a clear association with the RNAPII TEC.[46] In other systems, both the capping enzymes[47] and THO/TREX[48] associate co-transcriptionally with the TEC. However, in order to favour the detection of protein interactions rather than (indirect) interactions mediated by nucleic acids, the *Arabidopsis* protein extracts used for the isolation of TEC and THO/TREX were extensively treated with the nuclease benzonase.[11,46] Since efficient association of the capping complex[49] and of THO/TREX[48] with the TEC in addition to protein interactions requires RNA, it is possible that degradation of RNAs resulted in dissociation of these complexes from the TEC, which needs further clarification. In conclusion, the biochemical characterisation of the *Arabidopsis* RNAPII TEC in combination with the initial analysis of double-mutant plants defective in TEFs has demonstrated that transcribing RNAPII serves not only as a platform for interactions between TEFs, but also as a centre coordinating transcript synthesis and processing. Studying the *Arabidopsis* model in this context has the potential to extend the findings obtained with yeast and metazoa particularly regarding developmental aspects, as well as to discover plant peculiarities of which to date presumably only few have been unveiled.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Acknowledgments

We thank Alex Pfab and Wojtek Antosz for the image documenting *Arabidopsis* mutant phenotypes and for critically reading the manuscript.

## Funding

KDG acknowledges funding by the German Research Foundation (DFG) through grants SFB960/A6 and Gr1159/14-1.

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