Review

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Transcript elongation factors: shaping transcriptomes after transcript initiation

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Elongation is a dynamic and highly regulated step of eukaryotic gene transcription. A variety of transcript elongation factors (TEFs), including modulators of RNA polymerase II (RNAPII) activity, histone chaperones, and histone modifiers, have been characterized from plants. These factors control the efficiency of transcript elongation of subsets of genes in the chromatin context and thus contribute to tuning gene expression programs. We review here how genetic and biochemical analyses, primarily in *Arabidopsis thaliana*, have advanced our understanding of how TEFs adjust plant gene transcription. These studies have revealed that TEFs regulate plant growth and development by modulating diverse processes including hormone signaling, circadian clock, pathogen defense, responses to light, and developmental transitions.

Transcript elongation by RNAPII

Eukaryotic gene expression is regulated at many consecutive stages, ensuring that precisely adjusted amounts of gene product are synthesized in a spatially and temporally defined manner. Controlling the initiation step of transcription by RNAPII therefore represents a crucial event. For years it had been assumed that, once mRNA synthesis was initiated, the polymerization reaction catalyzed by the polymerase simply continued until termination occurred. However, the elongation phase of the RNAPII transcription cycle (Box 1) has also been shown to be dynamic and highly regulated [1–3]. Studies performed primarily in yeast have revealed a still-growing number of TEFs (see Glossary) that act in a concerted manner to facilitate efficient mRNA synthesis. The TEFs are particularly important when chromatin templates are transcribed, which reflects the natural situation in the cell nucleus. TEFs serve diverse functions (Figure 1) including modulating the catalytic properties and processivity of RNAPII, assisting the progression of the enzyme through repressive chromatin, and covalently modifying nucleosomal histones within transcribed regions [1–3]. TEFs are often conserved in plants, but their crosstalk with environmental and developmental stimuli as well as their target genes and pathways have diverged [4]. Indeed, in the past few years, studies of mutant plants defective in different TEFs have revealed the crucial roles these factors play in development and various stress responses, suggesting that they are important for establishing the proper gene expression programs. Thus, TEFs were found to regulate plant genes involved in hormone signaling, developmental switches, pathogen defense, and abiotic stress tolerance (Table 1). In this Review, we provide an integrated view of these recent findings from different research areas, focusing exclusively on RNAPII-related TEFs that have been studied in plants.

Factors modulating RNAPII properties

Some TEFs directly influence the transcriptional properties of elongating RNAPII. The best-studied example of this type of RNAPII elongation factor is transcription factor IIS (TFIIS), which was recognized as a TEF early on [5]. TFIIS can promote elongation through various obstacles to transcription, including particular DNA sequences [5] and nucleosomes [6,7], by reactivating backtracked and arrested RNAPII molecules. After displacing the backtracked RNA in the RNAPII pore, conserved basic and acidic residues of the TFIIS C-terminal hairpin can induce cleavage of the RNA, creating a new RNA 3′ end at the RNAPII active site, which enables transcription to resume [8]. Given the important biochemical function of TFIIS, it was surprising that inactivation of the *Arabidopsis* (*A. thaliana*) single-copy *TFIIS* gene resulted in a differential expression of only ~2.3% of the genes and that the *tfIIs* mutant plants had an essentially wild type appearance [9], which is in line with the normal growth of yeast *tfIIsΔ* cells under ordinary conditions. However, seeds of *tfIIs* plants are affected at the end of the maturation phase, showing reduced dormancy [9,10]. Among the genes downregulated in *tfIIs* seeds, the *DELAY OF GERMINATION 1* (*DOG1*) gene has been identified [10,11]: *DOG1* codes for a seed-specific protein of unknown function and had previously been identified as a quantitative trait

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Glossary

Backtracking of RNAPII: under particular conditions RNAPII moves backwards, resulting in displacement of the RNA 3′ end from the polymerase active site.
ChIP-chip: chromatin immunoprecipitation coupled with microarray.
ChIP-seq: chromatin immunoprecipitation coupled with next-generation sequencing.
***ELF* (*EARLY FLOWERING*):** genes identified in an *Arabidopsis* genetic screen for plants flowering more rapidly than wild type.
ELO: ELONGATA mutations in *Arabidopsis* identified Elongator subunits.
ELP: subunit of the Elongator complex in yeast.
FACT (FACILITATES CHROMATIN TRANSCRIPTION): histone chaperone involved in transcript elongation.
FLC (FLOWERING LOCUS C): floral repressor of the MADS-box transcription factor family.
H2A.Z: variant of histone H2A that has an important function in transcription and other processes.
H2Bub: histone H2B monoubiquitination mark correlated with active genes.
H3K4me3: histone H3 lysine 4 trimethylation mark correlated with active genes.
H3K9ac: histone H3 lysine 9 acetylation mark correlated with active genes.
HUB: E3 ligase with histone H2B monoubiquitination activity.
IWS1 (INTERACTS WITH SPT6): factor that directly interacts with the histone chaperone SPT6.
JMJ: JUMONJI domain protein, some of which have histone demethylase activity.
mRNA: RNA molecule synthesized by RNAPII that contains the genetic information required for protein synthesis at ribosomes.
PAF1C (RNA polymerase II-associated complex): conserved protein complex containing the PAF1 subunit (ELF7 in *Arabidopsis*) that regulates transcription-related histone modifications.
Polyadenylation site: sequence signal on the pre-mRNA specifying 3′ cleavage and polyadenylation of most mRNAs.
P-TEFb (positive transcript elongation factor b): heterodimer consisting of cyclin T and a cyclin-dependent kinase that phosphorylates the RNAPII-CTD and some TEFs.
RNAPII (RNA polymerase II): enzyme catalyzing the synthesis of pre-mRNA and many small RNAs using DNA as a template.
RNAPII-CTD (C-terminal domain of RNAPII): contains multiple Y1-S2-P3-T4-S5-P6-S7 heptapeptide repeats that by the action of protein kinases and phosphatases are differentially phosphorylated during different stages of the transcription cycle.
SDG: SET DOMAIN GROUP proteins, some of which have histone methylation activity.
SET: *Su(var)3-9, Enhancer of zeste and trithorax, E(z),* and *absent, small, or homeotic discs1* (ash1), SET domain-containing proteins are histone methyltransferases (HMTs) consisting of the SU(VAR)3-9, E(z), TRX, and the ASH1 class.
***SPT* (*Suppressor of Ty*):** genes identified in a yeast genetic screen that are crucial for the suppression of the effects of insertion mutations caused by the transposable element Ty.
SSRP1 (structure-specific recognition protein 1): HMG-box protein that is a subunit of the FACT histone chaperone.
SWR1C (Swi2/Snf2-related complex): protein complex involved in the deposition of the histone variant H2A.Z.
TAFII250: TATA-BINDING PROTEIN-ASSOCIATED FACTOR II 250.
TEF (transcript elongation factor): member of a heterogeneous group of protein factors involved in transcript elongation.
TFIIS (transcription factor IIS): factor that stimulates the weak intrinsic RNA cleavage activity of RNAPII, facilitating paused or arrested enzymes to resume transcription.
UBC: E2 ubiquitin-conjugating enzyme.
Transcription start-site: the first nucleotide position (+1) of a transcribed DNA region.
***VIP* (*VERNALIZATION INDEPENDENCE*):** genes identified in an *Arabidopsis* genetic screen for maintenance of *FLC* activity in non-vernalized plants.

locus controlling seed dormancy [12]. Introduction of an additional copy of *DOG1* into *tfIIs* plants restored the wild type *DOG1* transcript level in *tfIIs* seeds as well as normal seed dormancy [13]. This observation indicated that misregulation of a single gene was responsible for the seed dormancy phenotype of the *tfIIs* mutants.

Another factor that modulates RNAPII properties is a heterodimer consisting of the small zinc-finger protein SUPPRESSOR OF Ty4 (SPT4) and the multidomain protein SPT5, which in yeast and metazoa were found to regulate transcript elongation. In mammalian cells, SPT4–SPT5 collaborates with the multi-subunit complex termed negative elongation factor (NELF). However, whereas SPT4–SPT5 occur in all eukaryotes, NELF is not encoded in plant, yeast, or nematode genomes [14]. The exact mechanism of SPT4–SPT5 action is unknown; however, structural studies have revealed that the positioning of the heterodimer at the clamp domain of RNAPII places the heterodimer on the elongation complex, closing the DNA-binding cleft over the template DNA. This may prevent dissociation of RNAPII from the DNA, rendering the elongation complex stable and processive [15]. Downregulation of *SPT4* (and *SPT5*) expression in *Arabidopsis* results in severely reduced growth as well as defects in vegetative and reproductive development. Relative to wild type, ~5% of the genes in these plants were found to be differentially expressed, and genes involved in auxin signaling were overrepresented (Table 1). Most strikingly, *AUXIN/INDOLE-3-ACETIC ACID* (*AUX/IAA*) genes encoding repressors of auxin response factors were downregulated, which agrees with the finding that plants depleted in SPT4 have various phenotypes known to be regulated by auxin [16]. SPT5 has been detected in the euchromatin of *Arabidopsis* nuclei in association with genes transcribed by RNAPII, but not with non-transcribed regions. In plants with reduced amounts of SPT4, elevated levels of RNAPII and SPT5 associated with transcribed regions were indicative of transcript elongation defects such as reduced elongation rates [16].

In yeast and metazoa, positive transcript elongation factor b (P-TEFb) is a well-characterized heterodimeric complex consisting of cyclin T and cyclin-dependent kinase 9. Its major role is to phosphorylate Ser2 of the RNAPII C-terminal domain (CTD) and TEFs including SPT5 [1,3]. A recent study demonstrated that P-TEFb in *Arabidopsis* influences the phosphorylation level of the heptapeptide repeats of the RNAPII-CTD (Table 1). Moreover, it is involved in the transcription of a *FLOWERING LOCUS C* (*FLC*) antisense RNA, designated *COOLAIR*, thereby indirectly regulating *FLC* expression [17].

Facilitators of chromatin transcription

The second class of TEFs regulates transcription by RNAPII on chromatin templates by promoting transcript elongation through nucleosomes. Basically two groups of TEFs belong to this class: histone chaperones, which assist the disassembly of nucleosomes in the path of RNAPII [18], and ATP-dependent chromatin-remodeling complexes, which modulate nucleosome stability and positioning [19]. The histone chaperone FACILITATES CHROMATIN TRANSCRIPTION (FACT) is a heterodimer consisting of the STRUCTURE-SPECIFIC RECOGNITION PROTEIN 1 (SSRP1) and SPT16 subunits. It facilitates chromatin transcription in part by removing an H2A–H2B dimer from nucleosomes and by reassembling nucleosomes after polymerase passage, maintaining chromatin structure and histone modifications [20,21]. The SPT16 subunit is conserved among eukaryotes, whereas SSRP1 is different. In higher eukaryotes, including plants, the SSRP1 subunit contains a C-terminal high-mobility group (HMG)-box domain that mediates interaction with DNA and nucleosomes, whereas the yeast counterpart POB3 has