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SPECIAL ISSUE REVIEW

Transcript elongation by RNA polymerase II in plants: factors, regulation and impact on gene expression

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SUMMARY

Transcriptional elongation by RNA polymerase II (RNAPII) through chromatin is a dynamic and highly regulated step of eukaryotic gene expression. A combination of transcript elongation factors (TEFs) including modulators of RNAPII activity and histone chaperones facilitate efficient transcription on nucleosomal templates. Biochemical and genetic analyses, primarily performed in *Arabidopsis*, provided insight into the contribution of TEFs to establish gene expression patterns during plant growth and development. In addition to summarising the role of TEFs in plant gene expression, we emphasise in our review recent advances in the field. Thus, mechanisms are presented how aberrant intragenic transcript initiation is suppressed by repressing transcriptional start sites within coding sequences. We also discuss how transcriptional interference of ongoing transcription with neighbouring genes is prevented. Moreover, it appears that plants make no use of promoter-proximal RNAPII pausing in the way mammals do, but there are nucleosome-defined mechanism(s) that determine the efficiency of mRNA synthesis by RNAPII. Accordingly, a still growing number of processes related to plant growth, development and responses to changing environmental conditions prove to be regulated at the level of transcriptional elongation.

Keywords: RNA polymerase II, Chromatin, nucleosome, Arabidopsis, PAF1C, TFIIS, SPT4-SPT5, SPT6, FACT, PELF1.

INTRODUCTION

In eukaryotes, all protein-coding genes are transcribed by the 12-subunit RNA polymerase II (RNAPII) enzyme. Hence, RNAPII transcription plays a key role in differential gene expression to ensure that appropriate amounts of mRNAs are produced in a spatially and temporally coordinated manner. This is instrumental for growth, development and response to environmental conditions. The basis is established by controlling transcriptional initiation through a variety of transcription factors that bind to specific DNA elements of target genes. Diverse sets of transcription factors (and cofactors) bound to these *cis*-regulatory regions cooperate to determine in a combinatorial way the efficiency of RNAPII transcriptional initiation (Brkljacic & Grotewold, 2017; Reiter et al., 2017). For years, the following transcript synthesis was regarded as a simple polymerisation reaction, elongating the growing mRNA molecule. However, it became apparent that transcript elongation by RNAPII is a rather dynamic and discontinuous phase of the transcription cycle, which is highly regulated. Accordingly, a variety of functionally distinct transcript elongation factors (TEFs) modulate different aspects of RNAPII progression on chromatin templates (Chen et al., 2018; Kwak & Lis, 2013; Osman & Cramer, 2020; Sims et al., 2004). TEFs can be divided into various groups, such as modulators of RNAPII activity, facilitators of chromatin transcription (i.e. histone chaperones, ATP-dependent chromatin-remodelling complexes) and enzymes writing/erasing covalent histone modifications within transcribed regions (Kwak & Lis, 2013; Sims et al., 2004; van Lijsebettens & Grasser, 2014). In our review, we focus on the mechanism of chromatin transcription by RNAPII, but we largely omit the wide field of transcription-related post-translational histone modifications (i.e. acetylation, methylation, mono-ubiquitination), as that has been covered by a range of comprehensive review articles (Espinosa-Cores et al., 2020; Feng & Shen, 2014; Grasser et al., 2021; Jarosz et al., 2020; Leng, Thomas, et al., 2020; Xiao et al., 2016). Likewise, regarding the emerging research area on co-transcriptional mRNA processing in plants, we refer to

excellent recent reviews (Godoy Herz & Kornblihtt, 2019; Marquardt et al., 2023; Qin et al., 2022). In the course of our overviews of mRNA production by RNAPII, we present information derived from various organisms, most importantly plants (which means almost exclusively *Arabidopsis*), mammals and yeast. We would like to emphasise that one has to be aware that there are enormous differences between these organisms regarding important genomic parameters that undoubtedly influence the process of transcript elongation. To name but a few, the length of transcribed regions varies from typically a few thousand base pairs in yeast and *Arabidopsis* to tens or even hundreds of thousands in mammals, or the number/size of introns that increases substantially from yeast to *Arabidopsis* to mammals. In line with that, it is especially advantageous to comparatively consider the findings from different organisms, as despite these differences they still share many highly conserved TEFs. At the same time, it remains to be seen to which extent findings in *Arabidopsis* can be transferred to other plants such as maize that seem to share some genomic features (i.e. genome size) rather with mammalian systems.

RNAPII AND THE TRANSCRIPT ELONGATION COMPLEX (TEC)

From the largest subunit of RNAPII, termed NRPB1, extends the long, repetitive and basically unstructured carboxy-terminal domain (CTD). The CTD consists of tandem heptapeptide repeats with the consensus sequence $Y_1S_2P_3T_4S_5P_6S_7$. The number of repeats varies, for instance, 26 in *Saccharomyces cerevisiae* to 52 in *Homo sapiens* (Harlen & Churchman, 2017; Jeronimo et al., 2016), while the CTD of *Arabidopsis thaliana* RNAPII contains 15 consensus and 19 divergent repeats (Hajheidari et al., 2013). RNAPII is recruited to promoters with an unphosphorylated CTD to form the pre-initiation complex, but during the advancing transcription cycle the RNAPII-CTD is dynamically phosphorylated (and modified by other post-translational modifications), which influences the interaction with factors modulating the transcription process as well as co-transcriptional events (Harlen & Churchman, 2017; Jeronimo et al., 2016). Particularly well studied is the phosphorylation of residues S2 and S5 within the CTD repeats (referred to as S2P and S5P) during transcript elongation, which thus serve as marks for elongating RNAPII. Still the exact distribution of these marks over RNAPII transcribed regions differs somewhat between organisms such as yeast and human (Harlen & Churchman, 2017; Jeronimo et al., 2016). The distribution of RNAPII-S2P and -S5P in *Arabidopsis* is again different. The typical accumulation of S5P at the transcriptional start site (TSS) seen in yeast and human, depending on the detection method, is at least less pronounced in *Arabidopsis* (Antosz et al., 2020; Zhu et al., 2018) and this mark is rather distributed over the transcribed region (Figure 1). RNAPII-S2P coverage increases towards the transcriptional end site (TES) and exhibits a prominent peak downstream of the TES. Changes in the CTD phosphorylation state during the transcription cycle play important roles, determining the transitions between initiation, elongation and termination (Harlen & Churchman, 2017; Jeronimo et al., 2016). The CTD phosphorylation, for instance, contributes to the recruitment of certain TEFs to form the RNAPII elongation complex.

Initially, the composition of the RNAPII-TEC was elucidated in yeast by biochemical approaches that were supplemented by the analysis of genetic interactions between genes encoding various TEFs (Krogan et al., 2002; Lindstrom et al., 2003; Squazzo et al., 2002). Isolation of the TEC from *Arabidopsis* cells using reciprocal tagging of different TEFs in combination with affinity purification and mass spectrometry (AP-MS) essentially confirmed the findings regarding the yeast TEC, but identified also additional

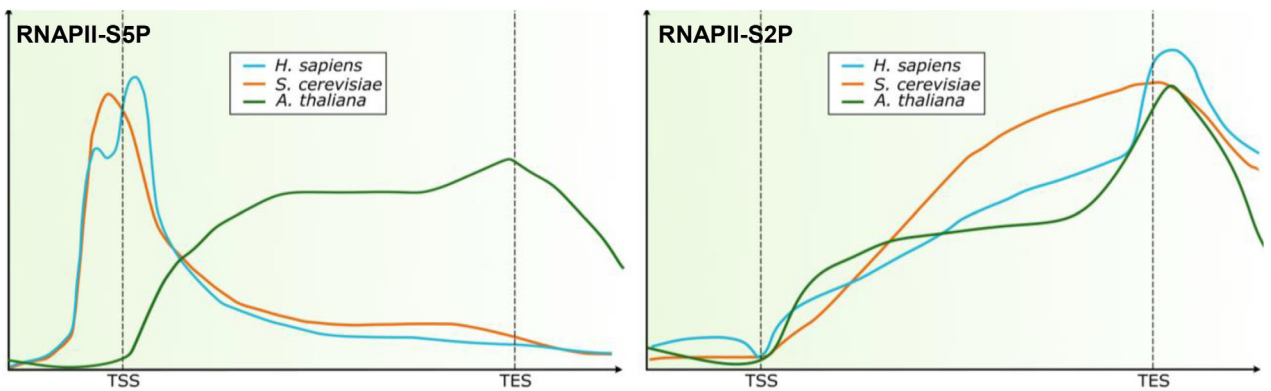

Figure 1. Chromatin immunoprecipitation-sequencing (ChIP-seq) profiles of phosphorylated residues of the RNAPII-CTD (S2P, S5P) across protein-coding genes in humans and budding yeast along with *Arabidopsis*.
Profiles for yeast and humans are depicted schematically as presented in Harlen & Churchman (2017). For *Arabidopsis* profiles, data plotted over highly expressed protein-coding genes (Antosz et al., 2020) have been superimposed. The second peak observed for RNAPII-S5P upstream of the transcriptional start site (TSS) in humans relates to divergent transcription, which seems to be a comparatively rare event in *Arabidopsis* (Hetzel et al., 2016; Kindgren et al., 2020; Thieffry et al., 2020; Zhu et al., 2018).

interactors (Antosz et al., 2017). RNAPII co-purified with the TEFs PAF1C, TFIIS, SPT4-SPT5, SPT6L and FACT, while P-TEFb appeared not to stably associate with the TEC in *Arabidopsis*. In addition, chromatin remodelling complexes, NAP1 histone chaperones and several histone-modifying enzymes including Elongator were found to associate with the TEC (Antosz et al., 2017). Recently, the TEF ELF1 was identified as another component of the *Arabidopsis* TEC, although it appears to associate only with a subpopulation of elongating RNAPII molecules (Markusch et al., 2023). During the past few years, advances in cryo-electron microscopy in combination with X-ray crystallography enabled enormous progress resolving the three-dimensional structure of the RNAPII-TEC of yeast and mammals. Thus, many details about the association of various TEFs with RNAPII along with the position of the DNA template and the nascent mRNA have been uncovered (Ehara et al., 2017; Vos et al., 2018, 2020). These studies demonstrated that the TEFs cover a major portion of the RNAPII surface, and that some TEFs are (tightly) connected with each other, such as SPT4-SPT5, SPT6 and PAF1C or SPT4-SPT5 and ELF1 (Figure 2). Some TEFs are placed at strategic positions, for instance, ELF1 at the 'DNA entry tunnel' for passage of the downstream DNA, and SPT4-SPT5 at the 'DNA exit tunnel'. Moreover, it became clear that many components of the TEC (i.e. TEFs) are mutually exclusive with components of the initiation complex (i.e. general transcription factors), indicating that the exchange of initiation factors with elongation factors is crucial for establishing a functional TEC and to block reassociation of initiation factors (Ehara et al., 2017; Vos et al., 2018, 2020). Further structural analyses provided also insight into the mechanism of how RNAPII with the assistance of the histone chaperone FACT and other TEFs can pass nucleosomes during transcript elongation (Ehara et al., 2019, 2022; Farnung et al., 2021, 2022; Liu et al., 2020). Initially, RNAPII (with SPT6, IWS1, PAF1C and ELF1 forming the downstream edge of the TEC) approaches the downstream nucleosome (Figure 2), starting to unwrap DNA from proximal histones and exposing the proximal H2A-H2B. FACT is successively engaged in histone and DNA contacts, eventually releasing the FACT-histone intermediate, which is then transferred presumably to the upstream edge of the TEC (formed by SPT4-SPT5, SPT6 and PAF1C). Finally, the nucleosome reassembles, explaining how nucleosomal histones and histone modifications are preserved in the wake of RNAPII passage (Ehara et al., 2019, 2022; Farnung et al., 2021; Filipovski et al., 2022). Multiple acidic regions occurring in SPT4-SPT5, SPT6, ELF1, PAF1C and FACT may contribute to binding basic nucleosomal regions exposed during transcript elongation, thereby facilitating histone transfer and nucleosome reassembly (Kujirai & Kurumizaka, 2020). Because nucleosomes in the path of RNAPII can cause backtracking of the TEC, TFIIS-stimulated cleavage of the backtracked RNA can promote nucleosome passage (Farnung et al., 2022; Kireeva et al., 2005; Nock et al., 2012). Hence, various TEFs assist RNAPII in transcribing nucleosomal templates that proceeds with an amazing rate of 1–3 kb min^{-1} *in vivo* (Muniz et al., 2021). Currently, there is no structural information regarding the TEC from plants but, in view of the evolutionary conservation of RNAPII and most of the TEFs, a marked resemblance to the findings on the yeast/mammalian TEC is expected.

FUNCTION OF RNAPII-TEFS

Polymerase-associated factor 1 complex (PAF1C)

The multifunctional PAF1C originally was discovered and characterised in *S. cerevisiae*, where it consists of five subunits, while in metazoa it is composed of six subunits

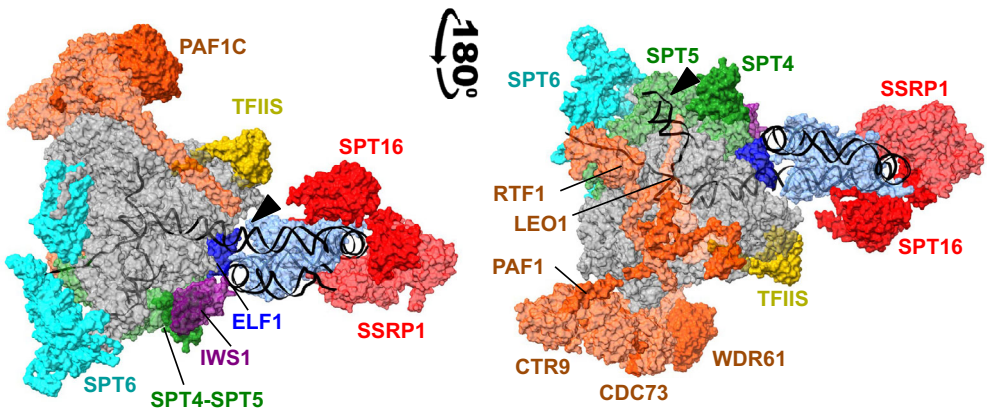

Figure 2. Structure of the RNAPII transcript elongation complex (TEC) engaged in nucleosome transcription.
The TEC consists of the 12-subunit RNAPII associated with several transcript elongation factors (TEFs). The shown structure is composed of an alignment of the PDB entries 7UNC (RNAPII core, PAF1C, TFIIS, SPT6, SPT4-SPT5, histones, DNA, RNA) (Filipovski et al., 2022) with 7NKY (SSRP1, SPT16) (Farnung et al., 2021) and 7XSE (ELF1, IWS1) (Ehara et al., 2022). The DNA entry- and exit-tunnels are indicated by arrowheads in the left and right views, respectively. RNAPII is indicated in grey, nucleosomal histones in light blue, DNA and RNA in black, and the colour of the respective TEFs is indicated.

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(PAF1, CTR9, LEO1, RTF1, CDC73 and WDR61/SKI8). PAF1C associates with elongating RNAPII stabilising the TEC and stimulating transcription. In addition, PAF1C links transcript elongation with post-translational histone modifications over transcribed regions, including various histone methylations (i.e. H3K4me2/3, H3K36me3, K3K79me2/3) as well as H2B mono-ubiquitination (Francette et al., 2021; Jaehning, 2010). Plant PAF1C was initially recognised because of its role in the transition from vegetative to reproductive development, and consequently the subunits were named after the *Arabidopsis* mutants (Table 1) – early flowering (*elf*) and/or vernalisation independence (*vip*): PAF1 (At-ELF7), CTR9 (At-ELF8, At-VIP6), LEO1 (At-VIP4), RTF1 (At-VIP5), WDR61/SKI8 (At-VIP3) and CDC73 (At-CDC73, At-PHP) (He et al., 2004; Oh et al., 2004; Park et al., 2010; Yu & Michaels, 2010; Zhang et al., 2003; Zhang & van Nocker, 2002). Co-immunoprecipitation experiments and AP-MS analyses demonstrated that as in metazoa, *Arabidopsis* PAF1C is composed of six subunits (Antosz et al., 2017; Oh et al., 2004). Mutant *Arabidopsis* plants deficient in PAF1C subunits share early flowering phenotypes that are associated with reduced expression of the floral repressor *FLC* (and paralogs) (He et al., 2004; Oh et al., 2004; Park et al., 2010; Yu & Michaels, 2010; Zhang et al., 2003; Zhang & van Nocker, 2002). Moreover, also the temperature-responsive transition to flowering depends on PAF1C (Nasim et al., 2022). Besides the regulation of flowering, the phyllotactic regularity of spatial auxin-dependent patterning at meristems requires the action of PAF1C (Fal et al., 2017). In addition, PAF1C proved to be involved in plant responses to abiotic stress conditions. Mutants deficient in PAF1C subunits did not react as wild-type plants to mechanical stimulation inflicted by repeated touch and failed to induce touch-responsive transcripts (Jensen et al., 2017). When exposed to elevated NaCl concentrations, the growth of *elf7* and *elf8* mutants was clearly reduced when compared with wild-type plants, whereas *cdc73* plants exhibited rather enhanced tolerance. In line with their susceptibility to salt stress, the transcriptional response of *elf7* plants upon exposure to salt was severely reduced, while the response of *cdc73* plants was hardly affected (Obermeyer et al., 2022; Zhang et al., 2022).

SPT4-SPT5 heterodimer

SPT5 is the only elongation factor that is conserved across all domains of life from bacteria (there termed NusG) to mammals. In eukaryotes and archaea, SPT5 associates with SPT4. While yeast *spt4Δ* strains are viable, SPT5 is essential for life in various eukaryotes. SPT4-SPT5 influences pleiotropic functions during RNAPII transcription, including the regulation of pausing, promoting productive elongation and coordination of co-transcriptional mRNA processing (Decker, 2021; Hartzog & Fu, 2013; Song & Chen, 2022). In *Arabidopsis*, both SPT4 and SPT5 are encoded by two genes each. While *SPT5-1* seems to be expressed in pollen, *SPT5-2* is expressed ubiquitously throughout the plant. Homozygous individuals of three independent *spt5-2* mutant lines proved not viable, and SPT4-RNAi lines (depending on the degree of downregulation of the *SPT4*-mRNAs, which is paralleled by reduced amounts of the SPT5-2 protein) exhibited severe growth and developmental defects (Dürr et al., 2014). In line with the decreased expression of auxin-related genes (particularly *AUX/IAA* genes) in the SPT4-RNAi plants, various defects caused by impaired auxin signalling were observed, including decreased lateral root density and reduced leaf venation. In the SPT4-RNAi plants, elevated levels of RNAPII were detected over the transcribed regions (including those of downregulated genes) suggesting transcript elongation defects (Dürr et al., 2014). In *Arabidopsis*, in addition to the generally conserved RNAPII-associated SPT4-SPT5 heterodimer, SPT4 was found to directly interact with the plant-specific SPT5L/KTF1 protein that in collaboration with RNAPV modulates transcriptional silencing by RNA-directed DNA methylation (Dürr et al., 2014; He et al., 2009; Köllen et al., 2015).

RNA cleavage-stimulating factor TFIIS

TFIIS is a three-domain protein with the N-terminal domain I, the middle domain II and the C-terminal domain III. Domain II and the linker between domains II and III are required for RNAPII interaction. Domain III including the highly conserved acidic β-hairpin reaches to the enzyme active site, inducing extensive conformational changes to the TEC and stimulating the weak intrinsic RNA cleavage activity of RNAPII. Transcript cleavage is required to rescue backtracked/arrested RNAPII elongation complexes, thereby facilitating transcription through blocks to elongation including nucleosomes (Fish & Kane, 2002; Kettenberger et al., 2003; Noe Gonzalez et al., 2021). In accordance with the normal growth of yeast *tfIIsΔ* cells, the vegetative development of *Arabidopsis* plants lacking TFIIS is comparable to wild-type, but mutant seeds exhibit impaired dormancy (Grasser et al., 2009). The seed dormancy phenotype is caused by reduced expression of the *DOG1* gene in *tfIIs* mutant seeds (Liu et al., 2011; Mortensen & Grasser, 2014). Constitutive expression of a mutant TFIIS variant (termed TFIISmut) that efficiently inhibits the RNA cleavage activity of RNAPII proved lethal in *tfIIs* plants (Antosz et al., 2020; Dolata et al., 2015). Induced, transient expression of TFIISmut in *tfIIs* resulted in severe growth defects and transcriptomic changes. In addition, transcription-related redistribution of elongating RNAPII towards the TSSs was observed, predominantly to the position of the +1 nucleosome (first nucleosome following the TSS). Therefore, RNAPII backtracking/arrest appears to occur frequently *in planta* and TFIIS-mediated RNAPII-reactivation is essential for efficient transcription (Antosz et al., 2020). Despite their wild-

Table 1 TEFs characterised in *Arabidopsis*

TEF	Synonyms	Complex	Molecular function	Mutant phenotype	Reference
TFIIS	Dst1	TFIIS	Modulates RNAPII properties Stimulates the intrinsic transcript cleavage activity of RNAPII	Seed dormancy Heat sensitivity	Grasser et al. (2009) Antosz et al. (2020) Szádeczky-Kardoss et al. (2022) Obermeyer et al. (2023)
ELF1	ELOF1	ELF1	Modulates RNAPII properties	Synergistic phenotypes with mutants lacking other TEFs	Markusch et al. (2023)
SPT4	DSIF	SPT4-SPT5	Modulates RNAPII properties	Growth and development, auxin response	Dürr et al. (2014)
SPT5	DSIF	SPT4-SPT5	Modulates RNAPII properties Phosphorylated by CDKD; 2	Growth and development, auxin response	Dürr et al. (2014)
IWS1	SPN1	IWS1	Modulates histone modifications	Brassinosteroid-/nitrogen-dependent gene expression ROS homeostasis	Li et al. (2010) Widiez et al. (2011) Bellegarde et al. (2019)
SPT6L	SPT6	SPT6	Histone chaperone Recruitment of chromatin remodellers	Embryo basal–apical polarity	Gu et al. (2012) Chen et al. (2019) Shu et al. (2022)
VIP3	SKI8/WDR61	PAF1C	Modulates histone modification	Reproductive development Touch response Phyllotaxis	Zhang et al. (2003) Fal et al. (2017) Jensen et al. (2017) Nasim et al. (2022)
VIP4	LEO1	PAF1C	Modulates histone modification	Reproductive development Reduced seed dormancy	Zhang & van Nocker (2002) Liu et al. (2011) Nasim et al. (2022)
VIP5	RTF1	PAF1C	Modulates histone modifications	Reproductive development Reduced seed dormancy	Oh et al. (2004) Liu et al. (2011) Nasim et al. (2022)
ELF7	PAF1	PAF1C	Modulates histone modifications	Reproductive development Reduced salt tolerance	He et al. (2004) Liu et al. (2011) Obermeyer et al. (2022) Zhang et al. (2022) Nasim et al. (2022)
ELF8/ VIP6	CTR9	PAF1C	Modulates histone modifications	Reproductive development Reduced seed dormancy Reduced salt tolerance	He et al. (2004) Oh et al. (2004) Liu et al. (2011) Fal et al. (2017) Obermeyer et al. (2022) Zhang et al. (2022) Nasim et al. (2022)
CDC73/ PHP		PAF1C	Modulates histone modifications	Reproductive development Enhanced salt tolerance	Park et al. (2010) Yu and Michaels (2010) Obermeyer et al. (2022) Nasim et al. (2022)
SSRP1	Pob3	FACT	Histone chaperone Suppression of cryptic transcription	Vegetative, reproductive development Reduced seed dormancy Anthocyanin synthesis upon light-induction	Lolas et al. (2010) Nielsen et al. (2019) Michl-Holzinger et al. (2019) Pfab et al. (2018) Michl-Holzinger et al. (2022)
SPT16		FACT	Histone chaperone Suppression of cryptic transcription Phosphorylation by CK2 modulates nucleosome occupancy at TSSs	Vegetative, reproductive development Anthocyanin synthesis upon light-induction	Duroux et al. (2004) Lolas et al. (2010) Pfab et al. (2018) Nielsen et al. (2019) Michl-Holzinger et al. (2022)

ROS, reactive oxygen species; TEF, transcript elongation factor; TSS, transcriptional start site.

type-like growth under standard conditions, *tflls* mutants were recently found to be highly sensitive to elevated temperatures. While the Col-0 wild-type and *TFIIS* overexpressing plants survived exposure at 37°C for 2 days, it proved lethal for *tflls* mutants (Obermeyer et al., 2023; Szádeczky-Kardoss et al., 2022). Further analyses demonstrated that particularly early heat stress response is dramatically impaired in *tflls* mutants along with altered alternative splicing pattern of hundreds of transcripts under heat stress conditions (Szádeczky-Kardoss et al., 2022). In the absence of TFIIS particularly heat-stress-induced genes are expressed at lower levels than in wild-type. At genes upregulated upon heat stress in *tflls* plants promoter-proximal accumulation of RNAPII occurred at +1 nucleosomes from which the histone variant H2A.Z was evicted in a temperature-dependent manner. Thus, the heat-stress-induced promoter-proximal accumulation of RNAPII in *tflls* conforms to that seen upon TFIISmut expression (Obermeyer et al., 2023). Therefore, these studies demonstrated that TFIIS is required for efficient reprogramming of gene expression to establish plant thermotolerance (Obermeyer et al., 2023; Szádeczky-Kardoss et al., 2022).

Zinc-finger protein ELF1

ELF1 is a small zinc-finger protein that is conserved in eukaryotes and some archaea. Originally, it was identified as a TEF in yeast by virtue of the synthetic lethality of the *elf1Δ* mutant in combination with mutations in genes encoding other known TEFs (Prather et al., 2005). ELF1 (mammalian orthologue termed ELOF1) localises to regions actively transcribed by RNAPII (Mayer et al., 2010; Prather et al., 2005; Rossi et al., 2021) and, because of its steady association with the yeast RNAPII elongation complex during *in vitro* transcription, it was designated core elongation factor (Joo et al., 2019). A putative orthologue of yeast ELF1 is encoded in plant genomes and, recently, *Arabidopsis* ELF1 was experimentally examined. Recombinant ELF1 interacted *in vitro* with DNA, histones and nucleosomes. ELF1 is a nuclear protein, and AP-MS analyses demonstrated that it co-purified with RNAPII and various TEFs including SPT4-SPT5, SPT6L, IWS1, PAF1C and FACT (Markusch et al., 2023). Plants lacking ELF1 basically have wild-type appearance, which is in agreement with the yeast *elf1Δ* mutation that caused no significant growth defect (Prather et al., 2005). Analyses of *Arabidopsis* double-mutants revealed distinct genetic interactions between *elf1* and mutants deficient in other elongation factors. Characteristic of TEFs, ELF1 associated with genomic regions occupied by elongating RNAPII, as evident from the coverage of the RNAPII-S2P chromatin immunoprecipitation signal. However, ELF1 occupied only one-third of the RNAPII transcribed loci with preference for inducible rather than constitutively expressed genes (Markusch et al., 2023). Taken together, these results indicate that *Arabidopsis* ELF1 represents a functional plant orthologue of ELF1/ELOF1 of other organisms.

Histone chaperone SPT6L and IWS1

The RNAPII-associated histone chaperone SPT6 is involved in various steps of gene expression, including transcription and histone post-translational modifications. Initially, SPT6 was reported to bind the phosphorylated CTD of RNAPII, but more recently interactions with the RNAPII stalk region and the phosphorylated linker region were recognised (Sdano et al., 2017; Vos et al., 2018). SPT6 can facilitate RNAPII transcriptional elongation and suppresses aberrant intragenic transcriptional initiation by maintaining chromatin structure during ongoing transcription. In addition, by interaction with histone modifiers it influences post-translational histone modifications over transcribed chromatin (Duina, 2011; Kato et al., 2013). In the *Arabidopsis* genome, two potential orthologues are encoded, SPT6L (At1g65440) and SPT6 (At1g63210) (Gu et al., 2012), of which *SPT6L* appears to be widely expressed throughout the plant, whereas the *SPT6* transcript is barely detectable (or not expressed at all) in most tissues (Sullivan et al., 2019). The phenotype of *spt6* mutants was indistinguishable from wild-type, but *spt6l* mutants were characterised by embryos with defective apical-basal polarity, leading to embryo lethality (Gu et al., 2012). More recently, colleagues succeeded in growing *spt6l* mutant seedlings *in vitro* for molecular analyses, which revealed intriguing results (Chen et al., 2019). The genome-wide association of SPT6L correlated with that of RNAPII and, in *spt6l* plants, RNAPII occupancy was clearly reduced. Expression of a SPT6L-variant (SPT6LΔtSH2) that is defective in RNAPII interaction could partially rescue the *spt6l* phenotype and the SPT6LΔtSH2 protein accumulated at the TSS, suggesting an RNAPII-independent recruitment mechanism and a role of SPT6L during early elongation. Moreover, upon exposure to heat stress, SPT6L is rapidly recruited particularly to the TSS of heat-responsive genes, while RNAPII coverage increased over the transcribed region (Chen et al., 2019). In another study, it was uncovered that SPT6L can interact with SWI2/SNF2-type ATP-dependent chromatin remodellers SYD/BRM, linking them to the RNAPII transcription machinery (Shu et al., 2022). In line with that, SYD/BRM colocalise with SPT6L to TSS regions of many genes, and the chromatin-association of SYD/BRM is severely reduced in *spt6l* plants. Finally, SPT6L and SYD/BRM are involved in the regulation of nucleosome and RNAPII occupancy at TSS, thus contributing to early transcript elongation by RNAPII (Shu et al., 2022). The elongation factor IWS1 is a direct interactor of SPT6 that has been also characterised in plants. *Arabidopsis* IWS1 was found to interact with brassinosteroid-regulated transcription factor BES1. It has been proposed that BES1 recruits IWS1 to target genes to promote transcriptional elongation (Li et al., 2010). In this scenario, IWS1 may assist recruitment of the histone methyltransferase SDG8 to brassinosteroid-

regulated genes mediating gene expression (Wang et al., 2014). Another line of research connected IWS1 with the regulation of nitrate uptake. Under conditions of high nitrogen supply, IWS1 (there termed HNI9) mediated increased levels of H3K27me3 at the *NRT2.1* locus repressing its expression (Widiez et al., 2011). Moreover, under high nitrogen supply, IWS1 is required for the expression of detoxification genes to maintain reactive oxygen species (ROS) homeostasis (Bellegarde et al., 2019).

Histone chaperone FACT

The conserved heterodimeric histone chaperone FACT consists of the SPT16 and SSRP1 subunits, and is involved in various DNA-dependent processes in chromatin including transcription (Formosa & Winston, 2020; Grasser, 2020; Gurova et al., 2018). Details of FACT-nucleosome interactions have been further elucidated in a recent study, illustrating that the structure of FACT resembles a unicycle, consisting of a saddle and fork that is engaged in extensive interactions of SSRP1 and SPT16 with nucleosomal DNA and all histones (Liu et al., 2020). Both SPT16 and SSRP1 (Pob3 in yeast) co-purify efficiently with components of PAF1C and SPT4-SPT5, both from yeast and *Arabidopsis* cells (Antosz et al., 2017; Krogan et al., 2002; Lindstrom et al., 2003; Squazzo et al., 2002), but recent structural studies indicate that FACT interacts with the TEC more loosely/dynamically than the above-mentioned TEFs (Ehara et al., 2022; Farnung et al., 2021). *Arabidopsis* FACT is widely expressed throughout the plant and localises to the transcriptionally active euchromatin (Duroux et al., 2004; Ikeda et al., 2011; Pfab et al., 2018). It associates with transcribed regions of active, protein-coding genes in a transcription-dependent manner (Antosz et al., 2017; Duroux et al., 2004; Perales & Más, 2007). FACT is essential for viability in *Arabidopsis* (Frost et al., 2018; Lolas et al., 2010), and decreased *SSRP1*/*SPT16* expression levels cause a variety of vegetative and reproductive defects including increased number of leaves, early bolting, impaired circadian rhythm and reduced seed dormancy (Lolas et al., 2010; Ma et al., 2018; Michl-Holzinger et al., 2019). Upon exposure to high-light stress, several anthocyanin biosynthetic genes were induced in *ssrp1*/*spt16* mutants to a lesser extent than in the wild-type and, accordingly, the mutant plants depleted in FACT accumulated lower amounts of anthocyanin pigments (Pfab et al., 2018). Recently, detailed mass spectrometric analyses revealed that both FACT subunits isolated from *Arabidopsis* cells were post-translationally modified. Four acetylation sites were mapped in the basic C-terminal region of SSRP1 and phosphorylation of three Ser/Thr residues (catalysed by protein kinase CK2) were identified in the acidic C-terminal region of SPT16 (Michl-Holzinger et al., 2022). Mutational analysis revealed only mild effects for the SSRP1 acetylation sites, while a non-phosphorylatable version of SPT16 displayed reduced histone interaction and failed to complement growth and developmental phenotypes of *spt16* mutant plants. At a subset of genes, expression of the non-phosphorylatable SPT16 version resulted in enrichment of histone H3 upstream of TSSs in a region that usually is nucleosome-depleted. Therefore, SPT16 phosphorylation might be required to establish correct nucleosome occupancy at the TSS of active genes (Michl-Holzinger et al., 2022). NAP1 histone chaperones co-purified with the *Arabidopsis* RNAPII-TEC (Antosz et al., 2017), and the histone chaperones ASF1 and HIRA share some properties with FACT and SPT6L (Layat et al., 2021; Zhong et al., 2022), suggesting that beyond FACT and SPT6L additional histone chaperones may be involved in RNAPII chromatin transcription.

RESTRICTING TRANSCRIPTION

A fundamental challenge to gene regulation is preventing that transcription of a gene inappropriately influences expression of neighbouring genes. In metazoa, for instance, insulators ensure that transcriptional interference of that kind is suppressed (Chen & Lei, 2019; Schoborg & Labrador, 2014), while in plants such mechanism(s) are rather unclear. Interestingly, in *Arabidopsis* recently three BORDER proteins (BDR1-3) were identified that are enriched in intergenic regions and were reported to prevent interference between closely spaced genes (Yu et al., 2019). The BDRs contain a SPOC domain (found also in the SPEN family of transcriptional repressors) and a TFIIS-like domain. Single-mutants did not show clear phenotypes, whereas *bdr* triple-mutant plants exhibit severely reduced growth. BDRs apparently cause 3′ accumulation of RNAPII at the upstream gene and, in the absence of BDRs, RNAPII accumulation is reduced at this position and seems to be shifted into the promoter region of the downstream gene. The authors conclude that BDRs acting as negative elongation factors inhibit transcriptional interference by preventing RNAPII from intruding into the promoters of closely spaced tandem downstream genes (Yu et al., 2019). In view of the relatively small size and close spacing of *Arabidopsis* genes (mentioned above) in combination with the limited resolution of chromatin immunoprecipitation-sequencing (ChIP-seq) experiments, the functional principle of the BDRs remains to be clarified. Further analyses revealed that *bdr* triple-mutant plants are late flowering and fail to suppress the expression of the floral repressor *FLC*. Consistent with that compared with wild-type plants, in the *bdr* triple-mutants reduced levels of repressive histone H3K27me3 were detected over the *FLC* locus, while activating H3K4me3 was increased in the 5′ region (Yu et al., 2021). Over BDR-repressed genes high levels of RNAPII coverage were observed, albeit low levels of the corresponding mRNAs were produced. These findings imply that BDRs promote accumulation of paused or slowly elongating RNAPII over the body of BDR-repressed genes (Yu et al., 2021).

Another fundamental principle of eukaryotic transcription control is implemented by a general shutdown of transcription except that brought about by specific, positive regulatory mechanisms. This requires that TSSs of inactive genes as well as the vast number of intra- and intergenic TSSs are repressed, which is accomplished by wrapping DNA in nucleosomes (Kornberg & Lorch, 2020). As mentioned above, passage of RNAPII during transcript elongation displaces or disrupts nucleosomes along transcribed regions, and certain histone chaperones are involved in maintaining nucleosomal integrity over transcribed regions (Venkatesh & Workman, 2015). In this scenario, an important function of FACT in *Arabidopsis* chromatin was discovered by mapping thousands of intragenic TSS positions in *spt16* and *ssrp1* mutants that were not detected in wild-type plants (Nielsen et al., 2019). Hence, FACT is required for repression of aberrant intragenic transcript initiation at positions that in part are characterised by elevated histone H3K4me1 levels (Nielsen et al., 2019). Because a similar function of FACT was observed in other organisms (Formosa & Winston, 2020; Gurova et al., 2018), safeguarding transcriptional fidelity by restricting transcriptional initiation by RNAPII to promoters may be a key role of FACT (and possibly some other histone chaperones).

REGULATION OF EARLY ELONGATION

The elongation rates of RNAPII transcription vary between genes, but also within transcribed regions, and elongation is particularly slow during early elongation (Core & Adelman, 2019; Jonkers & Lis, 2015). RNAPII elongation rates influence also the mRNA synthesis in *Arabidopsis* under different experimental conditions through transcriptional as well as co-transcriptional mechanisms (Godoy Herz et al., 2019; Leng, Ivanov, et al., 2020; Wu et al., 2016). In metazoa, promoter-proximal pausing of engaged RNAPII plays an important role in shaping the transcriptome. RNAPII typically pauses within a narrow region of 25–50 nt downstream of the TSS and is released in a controlled manner into productive elongation. The interplay of SPT4-SPT5 and the four-subunit NELF complex is a critical determinant of establishment and release of the paused state (Core & Adelman, 2019; Dollinger & Gilmour, 2021). NELF orthologues are not encoded in yeast, nematode and plant genomes. The fact that the organisms that exhibit stable, regulated RNAPII pausing are those that encode NELF implies a functional connection (Core & Adelman, 2019). In line with that, various genome-wide studies in *Arabidopsis* failed to detect a (closely) related RNAPII pausing mechanism. However, recent GRO-seq, NET-seq and RNAPII ChIP-seq analyses are compatible with the option of a nucleosome-defined mechanism for promoter-proximal RNAPII stalling that may contribute to regulate RNAPII transcript elongation (Antosz et al., 2020; Hetzel et al., 2016; Kindgren et al., 2020; Liu et al., 2021; Zhu et al., 2018). Particularly, the position of the +1 nucleosome represents a critical site for RNAPII stalling in *Arabidopsis* upon exposure to low or high temperatures (Kindgren et al., 2020; Obermeyer et al., 2023), or upon inhibition of the RNAPII transcript cleavage activity (Antosz et al., 2020). Nucleosomes generally represent obstacles to RNAPII transcription, and their dynamics/stability is determined by a complex interplay of nucleosomal histone composition, histone post-translational modifications, nucleosome occupancy and positioning. The +1 nucleosome is a major barrier to RNAPII transcription that in a way controls the transition from initiation to productive elongation (Kujirai & Kurumizaka, 2020; Lai & Pugh, 2017). Correspondingly, +1 nucleosomes exhibit special features including that they

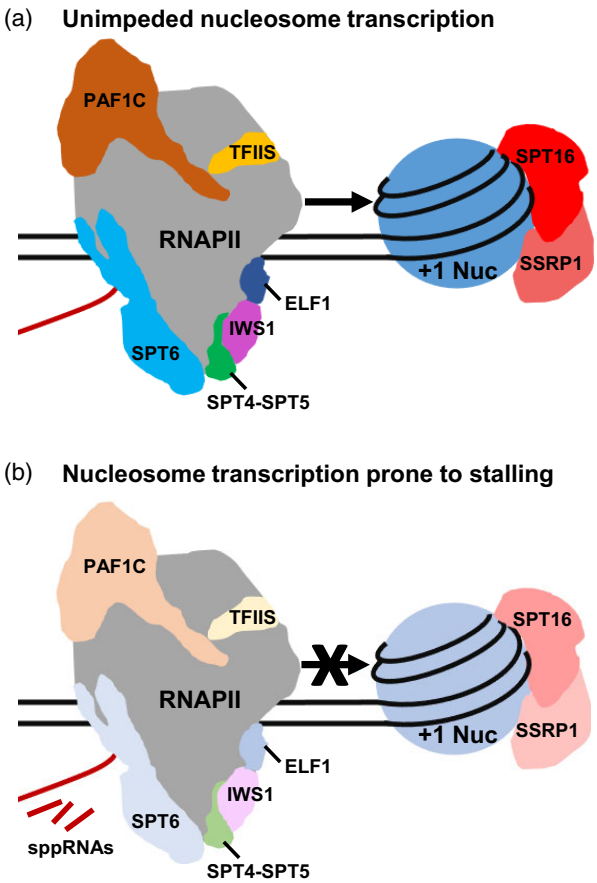

Figure 3. RNAPII-transcript elongation complex (TEC) approaching a +1 nucleosome under different conditions.
(a) RNAPII associated with all required transcript elongation factors (TEFs) during early elongation encounters a +1 nucleosome, which assisted by the TEFs is successfully passed and mRNA is synthesised.
(b) RNAPII associated with an incomplete set of TEFs (indicated by faded colours) encounters an altered version of the +1 nucleosome (e.g. differing in the presence/absence of a histone variant or modification, indicated by faded colour). The absence of one or several TEFs and/or the altered +1 nucleosome causes stalling of the elongating RNAPII and incomplete nucleosome passage. A reduced amount of mRNA (or none at all) is synthesised, instead sppRNAs could be produced, a process that may influence the synthesis of the corresponding mRNA.

are well-positioned and enriched in the histone variant H2A.Z (Lai & Pugh, 2017; Talbert & Henikoff, 2017). While H2A.Z incorporation into gene body nucleosomes is associated with gene repression, the correlation of H2A.Z incorporation at TSSs and plant gene expression is rather unclear and may depend on additional chromatin features (Jarillo & Piñeiro, 2015; Lei & Berger, 2020; Probst et al., 2020). In case of *Arabidopsis* genes that are upregulated upon heat stress, assistance of TFIIS is required for RNAPII to transcribe through +1 nucleosomes that are depleted in H2A.Z, suggesting that eviction of H2A.Z in this scenario results in a higher barrier for RNAPII passage (Obermeyer et al., 2023), which is in line with the lower *in vitro* stability of *Arabidopsis* H2A.Z containing nucleosomes (Osakabe et al., 2018). Intriguingly, in another study using GRO-seq, engaged RNAPII was found to accumulate downstream of TSSs upon heat stress during the immediate transcriptional response in *Arabidopsis* (Liu et al., 2021). Various studies provided evidence that additional TEFs including SPT4-SPT5, SPT6, ELF1 and FACT as well as ATP-dependent chromatin remodellers facilitate nucleosome transcription in yeast and metazoa (Gamarra & Narlikar, 2021; Kujirai & Kurumizaka, 2020). Consistently, SWI/SNF-type chromatin remodelling complexes were found recently to be targeted to nucleosomes at TSSs of *Arabidopsis* genes, likely contributing to tune early RNAPII transcript elongation (Diego-Martin et al., 2022; Shu et al., 2022). The identification of short promoter-proximal RNAs (sppRNAs) at ~14% of transcribed *Arabidopsis* genes, whose length is essentially defined by the +1 nucleosome, suggests that RNAPII stalling at this position can result in transcriptional termination (Thomas et al., 2020). Transcription events initiating at the same TSS can form full-length mRNAs or alternatively sppRNAs. At some genes increased mRNA production at the expense of sppRNAs was observed, but with most genes mRNA synthesis appears to be independent of sppRNA production (Thomas et al., 2020). In summary, it can be stated that early RNAPII transcript elongation in plants is a subject of extensive regulation (Figure 3), albeit likely by mechanisms (partially) distinct from those implemented in mammals. Depending on conditions, different molecular mechanisms including TEFs, chromatin remodellers, histone modifications and variants, and possibly sppRNAs presumably act in a concerted manner at TSS/+1 nucleosome to determine the efficiency of mRNA synthesis by RNAPII.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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