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RNA polymerase II elongation factors use conserved regulatory mechanisms

Ying Chen[1] and Patrick Cramer

Abstract

RNA polymerase II (Pol II) transcription is regulated by many elongation factors. Among these factors, TFIIF, PAF-RTF1, ELL and Elongin stimulate mRNA chain elongation by Pol II. Cryo-EM structures of Pol II complexes with these elongation factors now reveal some general principles on how elongation factors bind Pol II and how they stimulate transcription. All four elongation factors contact Pol II at domains external 2 and protrusion, whereas TFIIF and ELL additionally bind the Pol II lobe. All factors apparently stabilize cleft-flanking elements, whereas RTF1 and Elongin additionally approach the active site with a latch element and may influence catalysis or translocation. Due to the shared binding sites on Pol II, factor binding is mutually exclusive, and thus it remains to be studied what determines which elongation factors bind at a certain gene and under which condition.

Addresses

Department of Molecular Biology, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, 37077 Göttingen, Germany

Corresponding authors: Chen, Ying (yingchen@email.sdu.edu.cn); Cramer, Patrick (pcramer@mpinat.mpg.de)
(Chen Y.), (Cramer P.)
[1] Present address: Department of Clinical Laboratory, Qilu Hospital of Shandong University, Jinan, 250012, Shandong, China.

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Introduction

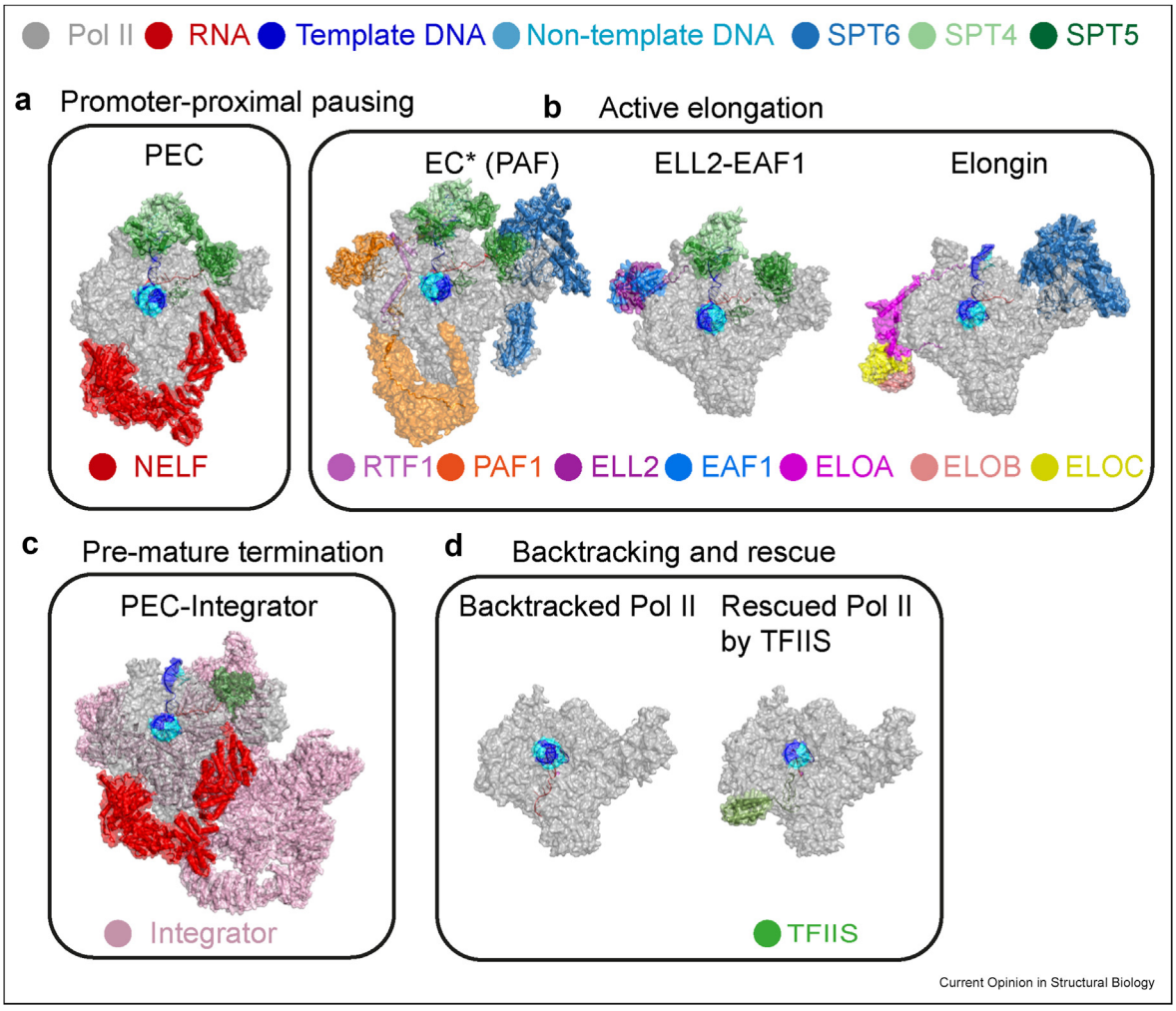
Transcription is the first step in gene expression and underlies development and cell function. Misregulation of transcription is related to cancerous cell growth and disease [1–3]. Therefore, interfering with the regulation of transcription has therapeutical potential [4]. Transcription of mRNAs is carried out by RNA polymerase II (Pol II) and regulated during initiation, elongation and termination, which require initiation, elongation and termination factors [5]. During initiation, Pol II and general transcription factors assemble on promoter DNA, forming a preinitiation complex, followed by DNA opening and initial RNA synthesis, resulting in an initial transcribing complex (ITC), as reviewed in Refs. [5,6]. Pol II then escapes from the promoter and forms a stable elongation complex.

Before entering productive elongation, metazoan Pol II often pauses downstream of the transcription start site, termed promoter-proximal pausing [7]. DRB sensitivity inducing factor (DSIF) and negative elongation factor (NELF) bind to paused Pol II [7], forming a paused elongation complex (PEC) [8] (Figure 1a). Release of Pol II to active elongation requires binding of the PAF1 complex (PAF), SPT6 and the kinase activity of positive elongation factor b (P-TEFb, consisting of Cyclin-dependent Kinase 9 and Cyclin T1/T2), forming an active elongation complex (EC*) [9,10] (Figure 1b). Alternatively, paused Pol II could be bound by the Integrator complex, which sterically prevents PAF and SPT6 binding, cleaves RNA and leads to pre-mature termination [11–15] (Figure 1c).

During productive elongation, Pol II translocates on DNA and pauses transiently. Pol II sometimes also backtracks and arrests [16,17]. Various elongation factors are required for elongation, as reviewed in Ref. [18]. One class of factors directly regulates the elongation activity of Pol II, including TFIIS [18,19], TFIIF [19,20], eleven-nineteen lysine-rich leukemia (ELL) [21,22] and Elongin [23,24] (Figure 1b, d). TFIIS stimulates the endonucleic activity of Pol II (Figure 1d). TFIIF, ELL and Elongin stimulate Pol II elongation by repressing transient pausing [25] (Figure 1b). Additionally, the PAF subunit RTF1 also stimulates Pol II elongation [10,26]. Another class of elongation factors influences elongation by altering chromatin, and these include chromatin remodeling enzymes and the histone chaperones SPT6 and FACT [18].

Recent advances in cryo-electron microscopy (Cryo-EM) enabled the determination of many new structures of Pol II elongation complexes [10,27,28]. Here, we compare elongation factors that directly regulate the elongation activity of Pol II. We discuss the structural similarities and differences of these elongation

Figure 1



Overview of elongation complexes at different states (front view) a. Structure of the paused elongation complex (PEC). The structure of the mammalian PEC (PDB: 6GML) [8] is shown as surface representation. Elongation factors DSIF, NELF and the DNA-RNA scaffold are shown as cartoon representations and colored as indicated in figure. **b. Structures of active elongation complexes.** The structures of the EC* complex (Pol II-DSIF-PAF-SPT6, PDB: 6TED [10]), the Pol II-DSIF-ELL2-EAF1 complex (PDB: 7OKY [41]) and the Pol II-SPT6-Elongin complex (PDB: 8OF0, [28]) are shown as surface representation. Elongation factors are colored as indicated in figure. **c. Structure of the PEC-integrator complex.** The structure of PEC-Integrator (PDB: 7PKS) [11] is shown as surface representation and colored as indicated in figure. **d. Structures of backtracked Pol II and rescued Pol II at intermediate state.** The structures of yeast Pol II at backtracked (PDB: 3PO2) and rescued state (PDB: 3PO3) are shown to represent the function of TFIIS in rescuing Pol II [17].

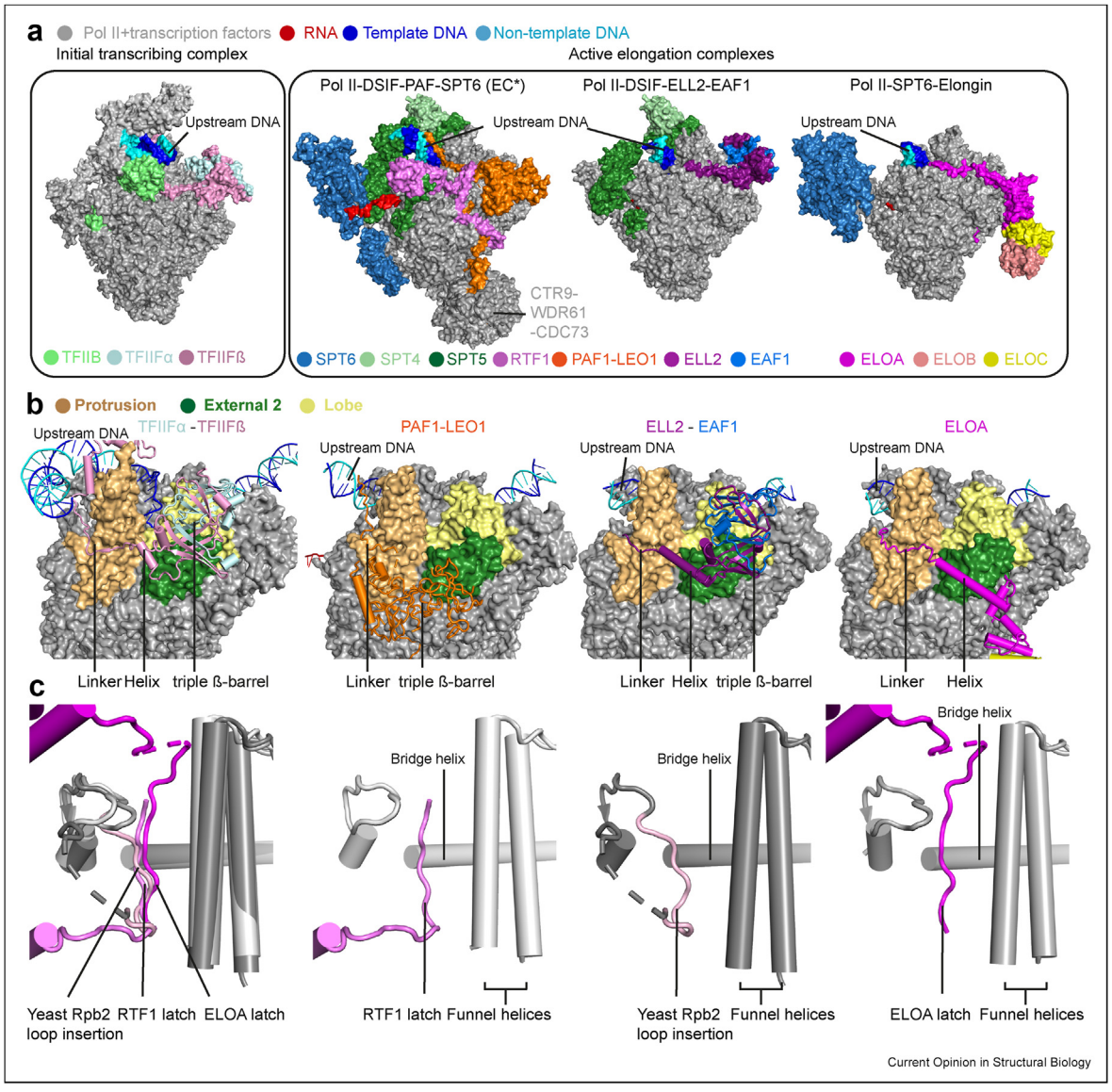
complexes, and the implications for understanding the mechanism of elongation regulation.

Regulation of Pol II elongation by the PAF complex

Human PAF consists of PAF1, LEO1, CTR9, CDC73, WDR61 and RTF1 [1,9]. RTF1 was shown to stimulate Pol II transcription in nuclear extracts [26]. Cryo-EM structures of the Pol II-DSIF-SPT6-PAF complexes showed that PAF has at least two functions *in vitro*. First, it promotes the release of Pol II from the paused elongation complex by competing with NELF for Pol II binding at Pol II foot domain [9]. Second, PAF-RTF1 directly stimulates the activity of Pol II and the presence of PAF and a RTF1 'latch' element is required for the stimulation [10].

In the structure, the PAF1-LEO1 subcomplex contacts Pol II external 2 and protrusion domains via a TFIIF-like triple β-barrel structure (dimerization domain) and a LEO1 C-terminal extension, respectively (Figure 2a, b). RTF1 further locks PAF1-LEO1 onto the Pol II surface and extends the RTF1 'latch' to a groove formed by the Pol II funnel helices and the bridge helix [10] (Figure 2c). This region is also the binding site for a loop insertion in yeast Pol II Rpb2 (Figure 2c). Interestingly, the transcription elongation

Figure 2



Structures of the initial transcribing complex (ITC) and active elongation complexes. **a. Overview of the structures of ITC and active elongation complexes (back-top view)**. From left to right, the complexes are ITC (PDB: 5IYD) [52], Pol II-DSIF-PAF-SPT6 (PDB: 6TED [10]), Pol II-DSIF-ELL2-EAF1 (PDB: 7OKY [41]) and Pol II-SPT6-Elongin (PDB: 8OF0, [28]). The structures are shown as surface representation and shown side by side, so that the position of the complexes on Pol II can be compared. The color code of the factors is indicated in figure. **b. Interaction between elongation factors and Pol II lobe, external 2 and protrusion domains (closeup view).** The elongation factors are shown as cartoon presentations. Pol II is shown as surface presentation. Pol II lobe, external 2 and protrusion domains are colored in pale yellow, forest green and bright orange, respectively as indicated in the figure. The color code of the elongation factors is the same as that in panel a. RTF1 in the EC* complex is omitted for clarity. **c. Interaction between RTF1, Elongin and Pol II funnel helices.** Left panel, superposition of the Pol II-DSIF-PAF-SPT6 (PDB: 6TED [10]), Pol II-SPT6-Elongin (PDB: 8OF0, [28]) and yeast Pol II (PDB: 3PO3 [17]). Middle left panel, interaction between RTF1 latch and Pol II funnel helices. Middle right panel, interaction between yeast Pol II Rpb2 loop insertion and funnel helices. Right panel, interaction between ELOA latch and Pol II funnel helices. For clarity, only the yeast Rpb2 loop insertion, the RTF1 latch, the ELOA latch, the funnel helices and the bridge helix are shown in the panels. Other Pol II elements are omitted.

rate of yeast Pol II is about 5 times faster than mammalian Pol II [29]. The presence of the RTF1 latch may allosterically regulate the activity of Pol II by changing the dynamics of the bridge helix, a mobile element of the Pol II active center [10].

Regulation of Pol II elongation by ELL

ELL and its paralog ELL2 were discovered as elongation factors in the 1990s [21,30], followed by the discovery of the associated factors ELL associated factor (EAF)1 and EAF2 [31,32], which enhance the elongation activity of

ELL/ELL*2* [33]. In 2010, it was shown that ELL-EAF belongs to a multi-subunit complex, termed the super-elongation complex (SEC) [3,34,35] or Tatcom1 [36], which additionally contains the protein kinase complex P-TEFb (CDK9 and Cyclin T1/T2), ALL1-fused gene from chromosome 9 (AF9) or eleven nineteen leukemia (ENL) and a scaffold protein AF4/FMR2 family member 4 (AFF4) or its paralog AFF1. In 2011, ELL-EAF was identified as part of another complex, the little elongation complex (LEC), which additionally contains ICE (interacts with the C terminus of ELL) 1, ICE2, ZC3H8 and USPL1, but not P-TEFb [37–39].

The super elongation complex promotes the release of promoter-proximal paused Pol II by delivering P-TEFb [1,9] and also stimulates transcription elongation via its ELL-EAF subcomplex *in vitro* [21,29,31,33]. It regulates elongation of genes that encode heat shock and other stress response molecules, developmental regulators and enhancer RNAs [18]. The little elongation complex regulates the transcription cycle of snRNAs and snoRNAs [18,40]. Despite the importance of SEC and LEC in transcription regulation, how SEC and LEC interact with Pol II and stimulate elongation was unclear.

The recent cryo-EM structure of the Pol II-ELL*2*-EAF1 complex partially provides an answer to this question [41]. In this structure, ELL*2*-EAF1 binds to the Pol II lobe, external *2* and protrusion domains via their N-terminal dimerization domains, an ELL*2* helix and a linker extension (Figure 2a, b). The ELL*2*-EAF1 dimerization domains fold into TFIIF-like triple β-barrel structure and occupy the same binding site as TFIIF (Figure 2b, 3a, b). Biochemical assays further show that the ELL*2* protrusion-interacting linker is required for the elongation stimulation activity of ELL*2*. Structural analysis shows that binding of ELL*2*-EAF1 induces conformational change at Pol II lobe domain, resulting in a narrower DNA cleft (Figure 3c). We propose that the binding of ELL*2*-EAF1 complex allosterically stimulates transcription elongation. This study indicates that SEC could bind to Pol II via the ELL-EAF subcomplex and exert its function. As LEC also contains the ELL-EAF subcomplex, it is likely that LEC could bind to Pol II via ELL-EAF as well, although it is not clear whether other subunits of LEC also interact with Pol II. However, considering that SEC and LEC regulate different genes, additional mechanism must exist that render them gene-specific. Future structural studies involving more subunits and binding partners of LEC and SEC can provide further insights.

Regulation of Pol II elongation by Elongin

Elongin was discovered as an elongation factor [23,24] consisting of subunits Elongin A (ELOA), Elongin B (ELOB) and Elongin C (ELOC) [23]. ELOA directly stimulates Pol II elongation *in vitro* [42]. ELOB is a ubiquitin-like protein and ELOC resembles the SCF ubiquitin ligase subunit Skp1 [23,42]. The ELOB-ELOC complex binds to the BC-box motif of ELOA and enhances the activity of ELOA [23,42]. Although the elongation stimulation activity of Elongin was dissected biochemically [18], the molecular basis for this activity was revealed only recently, by the cryo-EM structure of the Pol II-SPT6-Elongin complex [28].

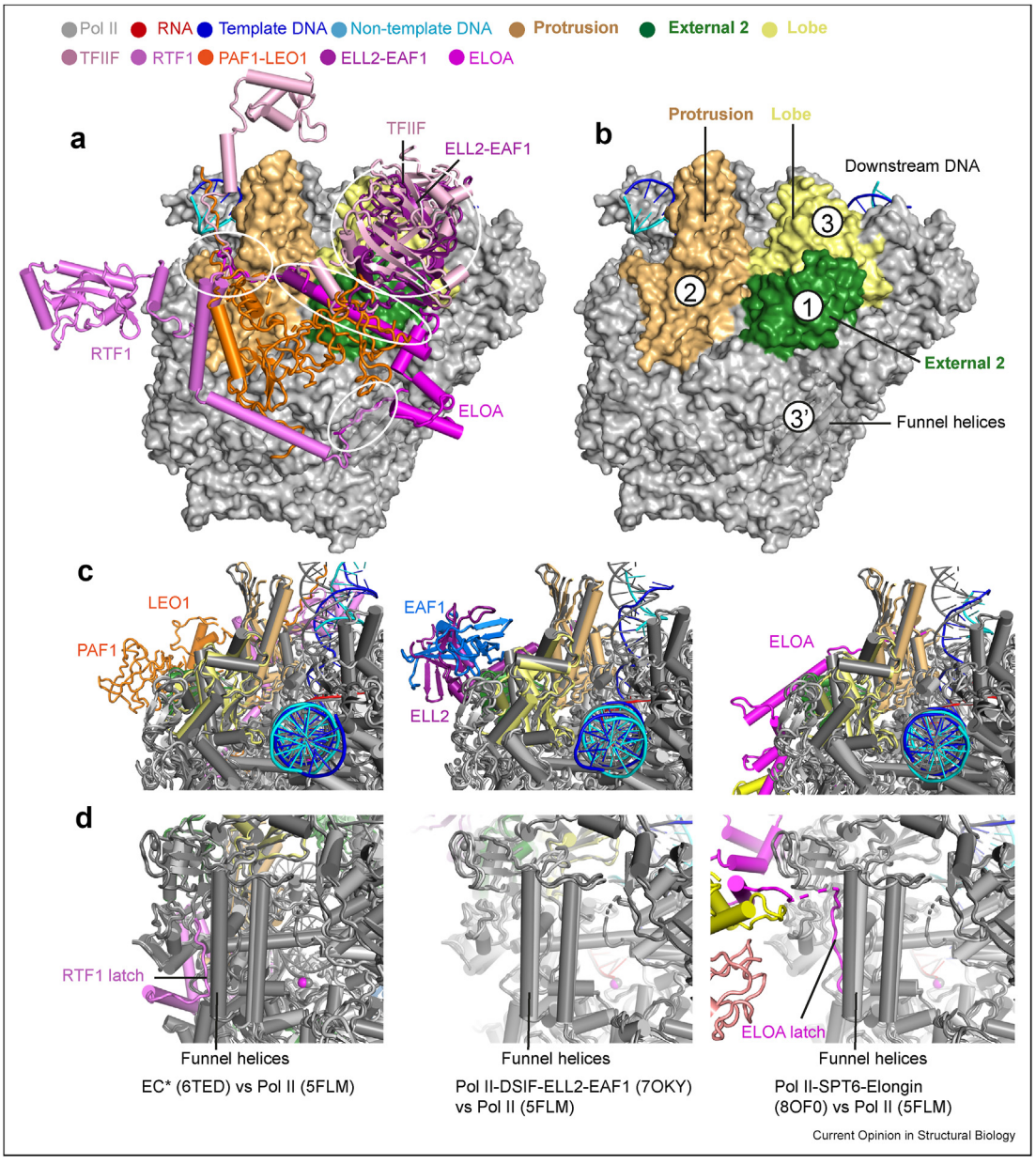
In the Pol II-SPT6-Elongin structure, Elongin interacts with the Pol II domains external *2* and protrusion via the helix α5 of the ELOA superfamily domain and an ELOA C-terminal linker, respectively (Figure 2a, b). Additionally, an ELOA 'latch' element binds near the Pol II funnel helices, in the same region as that of the RTF1 latch and the yeast Rpb*2* insertion (Figure 2c). Biochemical assays show that the ELOA latch and C-terminal linker are required for the elongation stimulation activity of ELOA. Importantly, binding of Elongin induces a conformational change at the Pol II funnel helices and the ELOA latch is required for this conformational change (Figure 3d). We propose that Elongin allosterically regulates Pol II activity, probably by promoting translocation or stabilizing Pol II at a more active conformation.

Elongin also interacts with the Cullin-RING E3 ubiquitin ligase CUL5-RBX2 complex, forming a pentameric ubiquitin ligase complex, under oxidative or other stresses or upon UV irradiation [18,43,44]. This pentameric Elongin complex promotes degradation of the RPB1 subunit of stalled Pol II [45,46]. The structure of the Pol II in complex with trimeric Elongin indicates that the 5-subunit Elongin could bind to Pol II via ELOA. However, whether there is an additional contact between Pol II and the 5-subunit Elongin ubiquitin ligase is unknown. How the 5-subunit Elongin ubiquitylates Pol II also remains to be investigated.

General mechanisms for elongation stimulation

Comparison of the structures of Pol II in complex with PAF-RTF1, Elongin, ELL-EAF and TFIIF reveals a general mechanism that regulates transcription elongation. It appears that at least three tethering sites between elongation factors and Pol II are required for their stimulatory transcription elongation activity. Pol II protrusion and external *2* domains are the two common binding sites for these elongation factors. The third binding site is at the Pol II lobe or funnel helices, which varies depending on the factors (Figure 3a, b). First, TFIIF, PAF1-LEO1, ELL-EAF and Elongin bind to the external *2* domain of Pol II RPB*2*. Second, a C-terminal linker interacts with the protrusion domain of Pol II. Third, RTF1 and Elongin, but not TFIIF and ELL-EAF, contain a latch that interacts with the funnel helices of Pol II. On the other hand, TFIIF and ELL-EAF contain a triple β-barrel structure that interacts with Pol II lobe

Figure 3



Comparison of the binding sites of TFIIF, PAF, ELL and Elongin on Pol II and the Pol II conformations in the presence of PAF, ELL and Elongin. **a. Superposition of the elongation factors on Pol II surface.** Clashes at Pol II lobe, external 2, protrusion and funnel helices are indicated. The color code is indicated in the figure and used in panel a and b. TFIIF, ELL2-EAF1 and PAF1-LEO1 are colored in light pink, deep purple and orange, respectively. RTF1 and ELOA are colored in violet and magenta, respectively. White circles indicate the overlapping regions on Pol II surface. Factors that are not within clashing regions are omitted for clarity. **b. The three binding sites of elongation factors on Pol II.** The two common binding sites are labeled as 1 and 2. The third variable binding sites are labeled as 3 and 3'. **c. Comparison of Pol II conformation at Pol II lobe region.** The structures of the elongation complexes (Pol II is colored as indicated in panel a. Elongation factors are in various colors.) are superposed onto the active site domain of Pol II elongation complex (PDB: 5FLM, in darker grey). The Pol II lobe and protrusion domains are shown in closeup view. The elongation complexes are Pol II-DSIF-PAF-SPT6 (PDB: 6TED [10]), Pol II-DSIF-ELL2-EAF1 (PDB: 7OKY [41]) and Pol II-SPT6-Elongin (PDB: 8OF0, [28]) from left to right. **d. Comparison of Pol II conformation at the funnel helices.** The region near funnel helices of the superposed complexes in panel c are shown in closeup view.

domain. The three tethering sites potentially synergize to stabilize an active Pol II conformation.

The conformational changes in Pol II upon binding of elongation factors are different, depending on the elongation factors bound (Figure 3c, d). In the case of ELL2-EAF1, the Pol II lobe domain moves towards the DNA cleft, forming a more compact cleft (Figure 3c, d, middle panel). In the case of Elongin, the funnel helices move towards the trigger loop, which might promote

translocation or changing the dynamics of Pol II conformation (Figure 3c, d, right panel). Notably, no conformational change was observed in the presence of PAF-RTF1, likely due to high flexibility of the interaction (Figure 3c, d, left panel). Whether TFIIF binding induces conformational change in Pol II remains unclear. In summary, the elongation factors seem to stimulate Pol II transcription by inducing conformational changes and stabilizing Pol II in a more active conformation.

Factor exchange during the transition from initiation to elongation

The exact mechanism for the transition from initiation to elongation is not entirely clear. Possible mechanisms are replacement of TFIIB by extending RNA at a certain length and competition between initiation factors and elongation factors for Pol II binding, based on structural comparisons between initiation complexes and the paused elongation complex [6]. Unlike other initiation factors, TFIIF may associate with early elongating Pol II and function as an elongation factor [18]. The overlap between the binding site of PAF, ELL and Elongin, and TFIIF on Pol II indicates that these factors cannot bind Pol II simultaneously (Figure 3a). This suggests that replacement of TFIIF by these elongation factors contributes to the transition from initiation to elongation. What determines which factor replaces TFIIF for a certain gene is unclear. PAF, Elongin and SEC appear to have distinct functions in vivo [18], indicating that they could regulate different sets of genes. The mechanism underlying the target selection of the elongation factors is of interest for future studies.

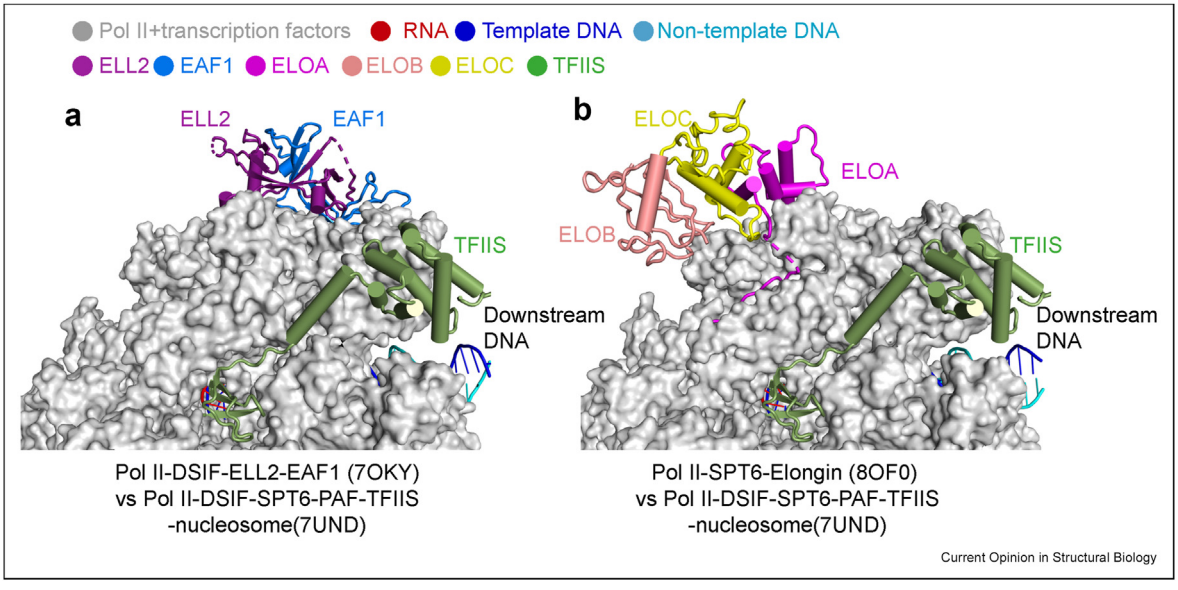
The PAF1-LEO1 in the EC* complex was shown to compete with the INTS1 subunit of Integrator [11]. Similarly, TFIIF, ELL-EAF and Elongin also clash with Integrator at the external 2 and protrusion domains. This suggests that they could also prevent pre-mature termination during processive elongation. What is intriguing is that LEC, which contains ELL-EAF as a subcomplex, recruits the Integrator complex to snRNAs [39]. How the two factors may co-exist in one complex requires further investigation.

Repression of TFIIS-stimulated cleavage of non-arrested Pol II transcripts by TFIIF, Elongin and ELL

TFIIS is the first elongation factor identified and increases the Pol II elongation rate [47]. During elongation, Pol II translocates and sometimes backtracks. Extensive backtracking leads to arrest of Pol II and extrusion of the 3′-end of the RNA into a pore beneath the active site [48]. TFIIS rescues backtracked Pol II by stimulating the endonuclease activity of Pol II [18]. After cleavage, the 3′-end of RNA is re-aligned in the active site [17].

Elongation factors TFIIF, ELL and Elongin were shown to repress transient pausing and TFIIS stimulated cleavage of transiently paused but not stalled Pol II transcripts [25]. However, it is unclear whether elongation factors counteract the activity of TFIIS by competitive binding to Pol II or by changing the accessibility of TFIIS to 3′-end of the RNA transcript. As revealed by the structure of the Pol II-DSIF-PAF-SPT6-TFIIS-nucleosome structure [48], PAF and TFIIS bind

Figure 4



ELL and Elongin are compatible with TFIIS binding on Pol II surface. a. Superposition of the Pol II-DSIF-ELL-EAF1 structure (PDB: 7OKY) [41] **and the Pol II-DSIF-SPT6-PAF-TFIIS-nucleosome structure (PDB: 7UND)** [49]**.** The color code of the structures is indicated in the figure. Pol II from 7OKY is shown as surface representation. **b. Superposition of the Pol II-SPT6-Elongin structure and the Pol II-DSIF-SPT6-PAF-TFIIS-nucleosome structure (PDB: 7UND)** [49]**.** The color code of the structures is indicated in the figure. Pol II from 8OF0 is shown as surface representation.

to Pol II simultaneously. Superposing the structures of the elongation complexes containing TFIIF, ELL2-EAF1 or Elongin onto the TFIIS containing Pol II elongation complexes also showed no physical clash between these elongation factors and TFIIS (Figure 4a, b). This may suggest that TFIIF, ELL and Elongin might change the accessibility of the 3′-end of the RNA for TFIIS.

Conclusions and future research directions

In summary, cryo-EM analysis enabled the determination of many structures of Pol II elongation complexes with bound elongation factors. This greatly increased our understanding of the regulation of transcription elongation. However, the new results also raise new questions. First, how do elongation factors cooperate with each other? Despite the overlapping binding site on the Pol II surface, Elongin was shown to interact with PAF and the Integrator complex [50,51] and LEC interacts with Integrator subunits [40]. There seems to be an intricate functional network among elongation factors in vivo. Dissecting this interaction network structurally will advance our understanding of elongation regulation. Second, how do SEC, LEC and Elongin define their target elongation complexes? PAF seems to be a general elongation factor for pause release, but Elongin also functions in pause release. SEC and LEC share the same ELL-EAF interface with Pol II, but they regulate different sets of genes. It would be interesting to study how additional factors facilitate elongation factor recruitment. Third, how does the 5-subunit Elongin ubiquitin ligase ubiquitylate Pol II? Elongin and the 5-subunit Elongin ubiquitin ligase share the same ELOA-Pol II interaction interface, however, their functions differ. What is the mechanism that induces the switch of Elongin function from an elongation factor to a ubiquitin ligase? Fourth, how do SEC, LEC and Elongin function in the context of chromatin and in response to cellular signals and stress? Answering these questions would advance our understanding of transcription regulation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* * of outstanding interest

1. Chen FX, Smith ER, Shilatifard A: **Born to run: control of transcription elongation by RNA polymerase II**. *Nat Rev Mol Cell Biol* 2018, **19**:464–478.

2. Dabas P: **Transcription elongation factors in health and disease**. In *Gene Expression*. Edited by Uchiumi F, IntechOpen; 2022. https://doi.org/10.5772/intechopen.103013.

3. Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, Washburn MP, Conaway JW, Conaway RC, Shilatifard A: **AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia**. *Mol Cell* 2010, **37**:429–437.

4. Fisher RP: **Cdk7: a kinase at the core of transcription and in the crosshairs of cancer drug discovery**. *Transcription* 2018, **10**:47–56.

5. Hantsche M, Cramer P: **The structural basis of transcription: 10 Years after the nobel prize in chemistry**. *Angew Chem Int Ed Engl* 2016, **55**:15972–15981.

6. Farnung L, Vos SM: **Assembly of RNA polymerase II transcription initiation complexes**. *Curr Opin Struct Biol* 2022, **73**, 102335.

7. Core L, Adelman K: **Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation**. *Genes Dev* 2019, **33**: 960–982.

8. Vos SM, Farnung L, Urlaub H, Cramer P: **Structure of paused transcription complex Pol II-DSIF-NELF**. *Nature* 2018, **560**: 601–606.

9. Vos SM, Farnung L, Boehning M, Wigge C, Linden A, Urlaub H, Cramer P: **Structure of activated transcription complex Pol II-DSIF-PAF-SPT6**. *Nature* 2018, **560**:607–612.

10. ** Vos SM, Farnung L, Linden A, Urlaub H, Cramer P: **Structure of complete Pol II-DSIF-PAF-SPT6 transcription complex reveals RTF1 allosteric activation**. *Nat Struct Mol Biol* 2020, **27**: 668–677.
This paper describes the structure of the Pol II-DSIF-PAF-SPT6 complex and reveals how RTF1 may allosterically regulate Pol II elongation.

11. ** Fianu I, Chen Y, Dienemann C, Dybkov O, Linden A, Urlaub H, Cramer P: **Structural basis of Integrator-mediated transcription regulation**. *Science* 2021, **374**:883–887.
This paper describes the structure of the mamalian paused elongation complex with the Integrator-PP2A complex bound. The study shows how Integrator binds to Pol II and positions the nuclease near the nacent RNA for cleavage, resulting in premature termination of transcription.

12. Lykke-Andersen S, Žumer K, Molska EŠ, Rouvière JO, Wu G, Demel C, Schwalb B, Schmid M, Cramer P, Jensen TH: **Integrator is a genome-wide attenuator of non-productive transcription**. *Mol Cell* 2021, **81**:514–529.e516.

13. Elrod ND, Henriques T, Huang K-L, Tatomer DC, Wilusz JE, Wagner EJ, Adelman K: **The integrator complex attenuates promoter-proximal transcription at protein-coding genes**. *Mol Cell* 2019, **76**:738–752.e737.

14. Tatomer DC, Elrod ND, Liang D, Xiao M-S, Jiang JZ, Jonathan M, Huang K-L, Wagner EJ, Cherry S, Wilusz JE: **The Integrator complex cleaves nascent mRNAs to attenuate transcription**. *Gene Dev* 2019, **33**:1525–1538.

15. Beckedorff F, Blumenthal E, daSilva LF, Aoi Y, Cingaram PR, Yue J, Zhang A, Dokaneheifard S, Valencia MG, Gaidosh G,

et al.: **The human integrator complex facilitates transcriptional elongation by endonucleolytic cleavage of nascent transcripts**. *Cell Rep* 2020, **32**, 107917.

16. Wang D, Bushnell DA, Huang XH, Westover KD, Levitt M, Kornberg RD: **Structural basis of transcription: backtracked RNA polymerase II at 3.4 angstrom resolution**. *Science* 2009, **324**:1203–1206.

17. Cheung AC, Cramer P: **Structural basis of RNA polymerase II backtracking, arrest and reactivation**. *Nature* 2011, **471**: 249–253.

18. Conaway RC, Conaway JW: **The hunt for RNA polymerase II elongation factors: a historical perspective**. *Nat Struct Mol Biol* 2019, **26**:771–776.

19. Ishibashi T, Dangkulwanich M, Coello Y, Lionberger TA, Lubkowska L, Ponticelli AS, Kashlev M, Bustamante C: **Transcription factors IIS and IIF enhance transcription efficiency by differentially modifying RNA polymerase pausing dynamics**. *Proc Natl Acad Sci U S A* 2014, **111**:3419–3424.

20. Schweikhard V, Meng C, Murakami K, Kaplan CD, Kornberg RD, Block SM: **Transcription factors TFIIF and TFIIS promote transcript elongation by RNA polymerase II by synergistic and independent mechanisms**. *Proc Natl Acad Sci USA* 2014, **111**:6642–6647.

21. Shilatifard A, Lane WS, Jackson KW, Conaway RC, Conaway JW: **An RNA polymerase II elongation factor encoded by the human ELL gene**. *Science* 1996, **271**:1873–1876.

22. Shilatifard A, Haque D, Conaway RC, Conaway JW: **Structure and function of RNA polymerase II elongation factor ELL. Identification of two overlapping ELL functional domains that govern its interaction with polymerase and the ternary elongation complex**. *J Biol Chem* 1997, **272**: 22355–22363.

23. Aso T, Lane WS, Conaway JW, Conaway RC: **Elongin (SIII): a multisubunit regulator of elongation by RNA polymerase II**. *Science* 1995, **269**:1439–1443.

24. Aso T, Haque D, Fukudome K, Brewer CS, Conaway JW, Conaway RC: **A human cDNA encoding the 110-kDa A subunit of RNA polymerase II transcription factor elongin**. *Gene* 1996, **168**:277–278.

25. Elmendorf BJ, Shilatifard A, Yan Q, Conaway JW, Conaway RC: **Transcription factors TFIIF, ELL, and Elongin negatively regulate SII-induced nascent transcript cleavage by non-arrested RNA polymerase II elongation intermediates**. *J Biol Chem* 2001, **276**:23109–23114.

26. Cao QF, Yamamoto J, Isobe T, Tateno S, Murase Y, Chen Y, Handa H, Yamaguchi Y: **Characterization of the human transcription elongation factor Rtf1: evidence for nonoverlapping functions of Rtf1 and the Paf1 complex**. *Mol Cell Biol* 2015, **35**: 3459–3470.

27. Mohamed AA, Vazquez Nunez R, Vos SM: **Structural advances in transcription elongation**. *Curr Opin Struct Biol* 2022, **75**, 102422.

28. ** Chen Y, Kokic G, Dienemann C, Dybkov O, Urlaub H, Cramer P: **Structure of the transcribing RNA polymerase II-Elongin complex**. *Nat Struct Mol Biol* 2023.
This paper describes the structure of mammalian Pol II in complex with Elongin. It shows how Elongin binds to Pol II and allosterically stimulates Pol II elongation.

29. Palangat M, Larson MH, Hu X, Gnatt A, Block SM, Landick R: **Efficient reconstitution of transcription elongation complexes for single-molecule studies of eukaryotic RNA polymerase II**. *Transcription* 2012, **3**:146–153.

30. Shilatifard A, Duan DR, Haque D, Florence C, Schubach WH, Conaway JW, Conaway RC: **ELL2, a new member of an ELL family of RNA polymerase II elongation factors**. *Proc Natl Acad Sci U S A* 1997, **94**:3639–3643.

31. Simone F, Polak PE, Kaberlein JJ, Luo RT, Levitan DA, Thirman MJ: **EAF1, a novel ELL-associated factor that is delocalized by expression of the MLL-ELL fusion protein**. *Blood* 2001, **98**:201–209.

32. Simone F, Luo RT, Polak PE, Kaberlein JJ, Thirman MJ: **ELL-associated factor 2 (EAF2), a functional homolog of EAF1 with alternative ELL binding properties**. *Blood* 2003, **101**: 2355–2362.

33. Kong SE, Banks CA, Shilatifard A, Conaway JW, Conaway RC: **ELL-associated factors 1 and 2 are positive regulators of RNA polymerase II elongation factor ELL**. *Proc Natl Acad Sci U S A* 2005, **102**:10094–10098.

34. He N, Liu M, Hsu J, Xue Y, Chou S, Burlingame A, Krogan NJ, Alber T, Zhou Q: **HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription**. *Mol Cell* 2010, **38**:428–438.

35. Yokoyama A, Lin M, Naresh A, Kitabayashi I, Cleary ML: **A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription**. *Cancer Cell* 2010, **17**: 198–212.

36. Sobhian B, Laguette N, Yatim A, Nakamura M, Levy Y, Kiernan R, Benkirane M: **HIV-1 tat assembles a multifunctional transcription elongation complex and stably associates with the 7SK snRNP**. *Mol Cell* 2010, **38**:439–451.

37. Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CAS, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP, *et al.*: **Human mediator subunit MED26 functions as a docking site for transcription elongation factors**. *Cell* 2011, **146**:92–104.

38. Smith ER, Lin C, Garrett AS, Thornton J, Mohaghegh N, Hu D, Jackson J, Saraf A, Swanson SK, Seidel C, *et al.*: **The little elongation complex regulates small nuclear RNA transcription**. *Mol Cell* 2011, **44**:954–965.

39. Hu DQ, Smith ER, Garruss AS, Mohaghegh N, Varberg JM, Lin CQ, Jackson J, Gao X, Saraf A, Florens L, *et al.*: **The little elongation complex functions at initiation and elongation phases of snRNA gene transcription**. *Mol Cell* 2013, **51**: 493–505.

40. Takahashi H, Ranjan A, Chen S, Suzuki H, Shibata M, Hirose T, Hirose H, Sasaki K, Abe R, Chen K, *et al.*: **The role of mediator and little elongation complex in transcription termination**. *Nat Commun* 2020, **11**:1063.

41. ** Chen Y, Vos SM, Dienemann C, Ninov M, Urlaub H, Cramer P: **Allosteric transcription stimulation by RNA polymerase II super elongation complex**. *Mol Cell* 2021, **81**: 3386–3399.
This paper describes the structure of the Pol II elongation complex with the ELL2-EAF1 subcomplex of the super elongation complex bound. This study shows how ELL2-EAF1 may stimulate Pol II transcription elongation allosterically.

42. Aso T, Haque D, Barstead RJ, Conaway RC, Conaway JW: **The inducible elongin A elongation activation domain: structure, function and interaction with the elongin BC complex**. *EMBO J* 1996, **15**:5557–5566.

43. Kamura T, Burian D, Yan Q, Schmidt SL, Lane WS, Querido E, Branton PE, Shilatifard A, Conaway RC, Conaway JW: **MUF1, a novel Elongin BC-interacting leucine-rich repeat protein that can assemble with Cul5 and Rbx1 to reconstitute a ubiquitin ligase**. *J Biol Chem* 2001, **276**:29748–29753.

44. Weems JC, Slaughter BD, Unruh JR, Hall SM, McLaird MB, Gilmore JM, Washburn MP, Florens L, Yasukawa T, Aso T, *et al.*: **Assembly of the elongin A ubiquitin ligase is regulated by genotoxic and other stresses**. *J Biol Chem* 2015, **290**: 15030–15041.

45. Harreman M, Taschner M, Sigurdsson S, Anindya R, Reid J, Somesh B, Kong SE, Banks CAS, Conaway RC, Conaway JW, *et al.*: **Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation**. *Proc Natl Acad Sci USA* 2009, **106**:20705–20710.

46. Yasukawa T, Kamura T, Kitajima S, Conaway RC, Conaway JW, Aso T: **Mammalian Elongin A complex mediates DNA-damage-induced ubiquitylation and degradation of Rpb1**. *EMBO J* 2008, **27**:3256–3266.

47. Sekimizu K, Kobayashi N, Mizuno D, Natori S: **Purification of a factor from Ehrlich ascites tumor cells specifically stimulating RNA polymerase II**. *Biochemistry* 1976, **15**: 5064–5070.

48. Gonzalez MN, Blears D, Svejstrup JQ: **Causes and consequences of RNA polymerase II stalling during transcript elongation**. *Nat Rev Mol Cell Biol* 2021, **22**:3–21.

49. Filipovski M, Soffers JHM, Vos SM, Farnung L: **Structural basis of nucleosome retention during transcription elongation**. *Science* 2022, **376**:1313–1316.

50. ** Wang YT, Hou LM, Ardehali MB, Kingston RE, Dynlacht BD: **Elongin A regulates transcription in vivo through enhanced RNA polymerase processivity**. *J Biol Chem* 2021:296.

This paper describes the role of the Elongin A subunit of Elongin during transcritpion in vivo. The results suggest that Elongin may regulate pause release of Pol II and identified its interaction partners.

51. ** Ardehali MB, Damle M, Perea-Resa C, Blower MD, Kingston RE: **Elongin A associates with actively transcribed genes and modulates enhancer RNA levels with limited impact on transcription elongation rate in vivo**. *J Biol Chem* 2021:296.

This paper describes the role of the Elongin A during transcritpion in vivo. This study suggests that Elongin A regulates the transcription of ehancer RNAs and identified interaction partners of Elongin A in vivo.

52. He Y, Yan C, Fang J, Inouye C, Tjian R, Ivanov I, Nogales E: **Near-atomic resolution visualization of human transcription promoter opening**. *Nature* 2016, **533**:359–365.