Structural insight into nucleosome transcription by RNA polymerase II with elongation factors

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RNA polymerase II (RNAPII) transcribes chromosomal DNA that contains multiple nucleosomes. The nucleosome forms transcriptional barriers, and nucleosomal transcription requires several additional factors in vivo. We demonstrate that the transcription factors Elf1 and Spt4/5 cooperatively lower the barriers and increase the RNAPII processivity in the nucleosome. The cryo–electron microscopy structures of the nucleosome-transcribing RNAPII elongation complexes (ECs) reveal that Elf1 and Spt4/5 reshape the EC downstream edge and intervene between RNAPII and the nucleosome. They facilitate RNAPII progression through superhelical location SHL(–1) by adjusting the nucleosome in favor of the forward progression. They suppress pausing at SHL(–5) by preventing the stable RNAPII-nucleosome interaction. Thus, the EC overcomes the nucleosomal barriers while providing a platform for various chromatin functions.

NA polymerase II (RNAPII), a multisubunit protein factory (1), transcribes nucleosomal DNA to produce protein-coding mRNAs and many noncoding RNAs. A single nucleosome core particle includes a histone octamer, comprising two H2A-H2B dimers and an (H3-H4)2 tetramer, wrapped with ~1.7 turns of DNA (2). Nucleosomes are inherent roadblocks of transcription, and RNAPII stalls at multiple locations within a nucleosome (3–5). We previously observed by means of cryo–electron microscopy (cryo-EM) that RNAPII stalls near the entry [superhelical locations SHL(–6) and SHL(–5)] and before the dyad [SHL(–2) and SHL(–1)] of the nucleosome, where the stalled RNAPII is maintained by tight histone-DNA contacts and direct RNAPII-nucleosome contacts (6). On the other hand, in cells the nucleosomal barriers are overcome by the dynamic, combinatorial actions of transcription elongation factors, histone modifications, histone chaperones, and nucleosome remodelers (7, 8).

To understand how Elf1 and Spt4/5 facilitate efficient transcription through the nucleosome (9, 10), Elf1 and Spt4/5 are conserved basal elongation factors that are associated with transcribing RNAPII (11–13). Spt4/5, also known as DSIF in humans, is a heterodimer of Spt4 and Spt5 (14). Spt4/5 and its bacterial homolog NusG stimulate transcription elongation by suppressing RNAP pause or arrest (15–17). Spt4/5 modulates the RNAPII processivity on nucleosomal DNA transcription (18). Elf1 (Elof1 in humans) is a small zinc finger protein (19) that has genetic interactions with other elongation factors—including Spt4, Spt5, Spt6, Spt16, and TFIIS—implying their functional relationships (20). A genome-wide profi le study suggested their roles in gene-body transcription (II). Both Elf1 and Spt4/5 also play a role in chromatin structure maintenance in actively transcribed genes (20, 21). However, it remains unclear how these factors allow RNAPII to overcome the nucleosomal barriers while maintaining the chromatin structure.

We examined the effects of Elf1 and Spt4/5 on nucleosomal DNA transcription. Transcription was performed by the yeast Komagataella pastoris RNAPII on the human nucleosome reconstituted with a modifi ed Widom 601 DNA, as described previously (Fig. 1A and fig. S1) (6). RNAPII does not efficiently advance beyond the entry of the nucleosome [SHL(–5)] in the presence of TFIIS alone (Fig. 1B). By contrast, the addition of Spt4/5 allowed more efficient RNAPII progression to SHL(–1) or the DNA end (run-off). Elf1 exerted only a slight effect on the RNAPII progression on the nucleosome. However, the addition of both Elf1 and Spt4/5 exhibited a strong synergistic effect; together, they drastically reduced the pausing at SHL(–5) and dramatically increased the run-off product. Thus, Elf1 and Spt4/5 cooperatively lower the nucleosomal barriers, ensuring high elongation processivity on the nucleosome. This effect relies on TFIIS, which reactivates the stalled RNAPII (fig. S2) (22, 23).

To understand how Elf1 and Spt4/5 facilitate the nucleosome transcription, we analyzed the structure of the nucleosome-transcribing RNAPII elongation complex bound with these factors (hereafter called the EC) (Fig. 1). The reconstituted nucleosome was transcribed by RNAPII in the presence of Elf1, Spt4/5, and TFIIS. The nucleosomal DNA contained a T-less region to enrich the EC at SHL(–1) in the presence of 3′-deoxyadenosine triphosphate (3′-dATP) (fig. SIA) (6). The EC-nucleosome complexes were prepared by means of the GraFix method (24) and subjected to cryo-EM analyses (Fig. 1C, figs. S3 to S9, and tables S1 and S2). Three-dimensional classifications revealed the EC-nucleosome complexes at SHL(–1) and SHL(–5), with ~60 and 20 base pairs (bp) DNA torn off from the histone surface, respectively (Fig. 1, D and E). The former complex is the one stalled at an intrinsic site(s) because it was also observed by using ATP (fig. S10). In these complexes, RNAPII-bound Elf1 and Spt4/5 were clearly observed, whereas TFIIS was missing, probably because it dissociated during the purification step. No discernible structures were obtained for ECs at SHL(–6) and SHL(–2).

In the EC-nucleosome structures, Elf1, the NGN domain of Spt5, and Spt4 form a domain array, which intervenes between RNAPII and the nucleosome (Fig. 1, D and E). Although no notable change was observed in the RNAPII structure, the relative RNAPII-nucleosome positions changed in the presence of the elongation factors as compared with those in their absence. The domain array occupies the double-stranded DNA-binding site used in the pre-initiation complex (25, 26), preventing the nucleosome interaction to the site (fig. S11). Elf1 intervenes between the Rpb1 clamp-head domain, the Rpb2 lobe domain, the downstream DNA, and the nucleosome, affecting the RNAPII-nucleosome interaction. The domain array also intervenes between the upstream DNA and the downstream nucleosome, becoming a separator.

As for SHL(–1), classification yielded three EC-nucleosome structures: SHL(–1), SHL(–1)αA and SHL(–1)αB (Fig. 2A and figs. S5 and S6). According to the EC progression, the nucleosome rotates on the downstream DNA axis in front of RNAPII (~30°/bp because of the DNA helical pitch). Judging from the RNAPII-nucleosome distances and the nucleosome rotation angles, the ECs in SHL(–1)αA and SHL(–1)αB are advanced downstream by 1 bp with no obvious change in the histone-DNA contacts, relative to the SHL(–1) complex. The SHL(–1)αA and SHL(–1)αB complexes differ in their nucleosome orientations. These three structures may reflect the nucleosome mobility ahead of RNAPII (movie S1). As compared with the SHL(–1) complex without elongation factors (6), the nucleosome in the current SHL(–1) complex is rotated around the downstream DNA axis, and shifted away from the Rpb2 lobe, to avoid steric clashes with Elf1 and Spt5 NGN (Fig. 2A, and figs. S12, A to D, and S13). This direction of the nucleosome rotation is consistent with the forward EC translocation, suggesting that the elongation factors may help EC shift forward.

In the SHL(–1), SHL(–1)αA and SHL(–1)αB complexes, the DNA is torn off from one of the
two H2A-H2B dimers and its adjacent H3-H4, but the EC retains the intact histone octamer (Fig. 1D and fig. S6). Elf1 is located at the pivot point of the nucleosome rotation around the downstream DNA axis, and the Elf1 β sheet (the β3 and β4 strands) directly contacts the DNA-peeled parts of H3-H4 in SHL(−1) and SHL(−1)A (Fig. 2, B to D, and fig. S12E). The NGN domain of Spt5 is close to the nucleosomal DNA [SHL(−1)] or H2A-H2B [SHL(−1)A and SHL(−1)B]. Thus, Elf1 and Spt4/5 serve as a separator between the downstream DNA and the nucleosome, preventing the DNA reassociation to the exposed histone.

The histone-contacting amino-acid residues in Elf1 β3 and β4 are not well conserved, and their mutations do not substantially affect nucleosome transcription (fig. S14). The absence of specific interactions may be favorable for the nucleosome rotation in front of RNAPII.

In SHL(−1)B, the nucleosome is brought in closer proximity to RNAPII than that in SHL(−1)A without elongation factors (Fig. 2E). Specifically, the Rpb1 clamp head is adjusted at the contact site between the DNA and H3-H4 to act as a “wedge” between them (Fig. 2F and fig. S12F). This is not the case in the absence of the elongation factors (Fig. 2G). Moreover, the conserved N-terminal basic tail of Elf1 could interact with the DNA near the histone-DNA contact site and may compete with H3-H4 for the DNA to facilitate the separation of the histone-DNA contact (Fig. 2C). The deletion of the Elf1 N-terminal tail impaired the transcription processivity beyond SHL(−1) (Fig. 2H). Thus, Elf1 helps dissociate of the histone-DNA contact, and this is favorable for the progression of the RNAPII wedge upon separation of the contact.

At SHL(−1), the DNA-peeled parts of the histones tend to associate with another DNA segment (a “foreign” DNA) to generate a nucleosome-like structure (6), which could become an intermediate for the cis or trans histone transfer to other DNA regions and might perturb the chromatin structure and epigenetic information. However, the foreign DNA binding was strongly suppressed in the presence of the elongation factors. Classifications revealed that the binding of the foreign DNA and Elf1 is virtually mutually exclusive (figs. S5 and S6). Elf1 contacts the DNA-peeled H3-H4 (Fig. 2C), thus blocking the foreign DNA binding. In addition, although they are disordered, Elf1 and Spt5 have a C-terminal acidic tail and an N-terminal acidic tail, respectively, which could cover and hold the exposed H2A-H2B (Fig. 2C and fig. S12G). Spt16 (FACT) also conserves an acidic tail (fig. S15), which interacts with H2A-H2B (27). These findings are consistent with the previous observations that Elf1 and Spt4/5 are implicated in the chromatin structure maintenance (20, 21). Because Elf1 and Spt4/5 are genetically or physically linked to histone chaperones Spt6 and FACT, they might cooperate for the nucleosome reassembly in the wake of the EC passage.

In the SHL(−5) complex, the downstream DNA is slightly bent because of the Elf1 intervention, and the nucleosome is located more distant from RNAPII, as compared with those in the previous SHL(−5) complex without the elongation factors (Fig. 3) (6). In the absence of the elongation factors, the nucleosome is trapped between the Rpb1 clamp head and the Rpb2 lobe (6, 28). By contrast, in the presence of the elongation factors, Elf1 occupies the same place, and consequently, the RNAPII-nucleosome contacts are lost. The nucleosomal DNA is ~7 Å apart from Elf1, and there is no apparent EC-nucleosome interaction that could trap the nucleosome (fig. S16A). Consistently, modeling of a 1-h advanced EC [SHL(−5)] suggests that the nucleosome can rotate in front of the EC without clashing with the Rpb1 clamp head, whereas there is a steric clash in the absence of the elongation factors (fig. S16B). Thus, the elongation factors minimize the RNAPII-nucleosome interaction at SHL(−5) and prevent the EC from being trapped in a stable paused state. Similar mechanisms may be applicable to the suppression of the SHL(−6) and SHL(−2) barriers because the nucleosome is trapped between the Rpb1 clamp head and the Rpb2 lobe in the absence of elongation factors (6) and sterically incompatible with Elf1 and/or Spt4/5 (fig. S16C).

**Fig. 1.** Cryo-EM analyses of the EC-nucleosome complexes. (A) The experimental setup. The template DNA is colored yellow, and the nontemplate DNA is colored orange. Histones H2A, H2B, H3, and H4 are colored dark red, light red, light blue, and dark blue, respectively. (B) The effects of Elf1 and Spt4/5 on nucleosomal transcription. The elongated RNAs were analyzed by means of urea polyacrylamide gel electrophoresis (PAGE). The concentrations of Spt4/5 are 0.1 and 0.4 μM, and those of Elf1 are 0.25 and 1.0 μM. The experiment was performed in triplicate. (C) Urea PAGE of the sample used for the cryo-EM analyses, after purification by Grafix. (D) Cryo-EM structure of the EC-nucleosome complex at SHL(−1). RNAPII is shown as a gray cartoon model. Elf1, Spt4, and Spt5 are shown with magenta, green, and blue surfaces, respectively. (E) Cryo-EM structure of the EC-nucleosome complex at SHL(−5).
We elucidated how the conserved elongation factors facilitate efficient RNAPII passage through a nucleosome (Fig. 4). This may better represent the transcription of gene-body nucleosomes because Spt4/5 and Elf1 associate with elongating RNAPII (11). In the cellular context, EC cooperates with the other elongation factors, histone chaperones, and nucleosome remodelers, which could help relieve the nucleosomal barriers. The observed partially DNA-peeled nucleosome at SHL(−1) or SHL(−5) should provide a platform for various chromatin functions associated with these factors. By contrast, the promoter-proximal, first (+1) nucleosome serves as a strong transcriptional barrier in many eukaryotic genes (5, 29). In this case, RNAPII stalls owing to DNA-binding factors or negative factors probably near the entry of the nucleosome, and this paused complex differs from that involved in gene-body transcription (30). Further studies will clarify the mechanism of the promoter-proximal regulation.
Fig. 4. Roles of Elf1 and Spt4/5 in the nucleosome transcription. Elf1 and Spt4/5 modify the RNAPII surface to intervene with the RNAPII-nucleosome interaction. At SHL(–6), SHL(–5), and SHL(–2), the elongation factors facilitate the EC passage by preventing the direct RNAPII-nucleosome interaction that causes pausing. They facilitate the EC passage through SHL(–1), not only by changing the RNAPII-nucleosome interactions to adjust the RNAPII wedge to the histone-DNA contact site but also by weakening the histone-DNA contacts via the Elf1 basic tail.

REFERENCES AND NOTES

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Getting over nucleosomal barriers

In eukaryotic cells, RNA polymerase II (RNAPII) transcribes DNA within nucleosome-coated chromatin. The nucleosomes can provide major roadblocks for transcription. Cells solve this problem by using transcription elongation factors. Ehara et al. solved the cryo-electron microscopy structures of the nucleosome-transcribing RNAPII with elongation factors Elf1 and Spt4/5. Elf1 and Spt4/5 cooperatively suppress RNAPII pausing at multiple super helical locations [SHL(−6), SHL(−5), and SHL(−2)] and facilitate RNAPII progression through SHL(−1) by adjusting the nucleosome position to favor forward progression.

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