**Efficient transcription of RNA polymerase II (Pol II) through nucleosomes requires the help of various factors. Here we show biochemically that Pol II transcription through a nucleosome is facilitated by the chromatin remodeler Chd1 and the histone chaperone FACT when the elongation factors Spt4/5 and TFIIS are present. We report cryo-EM structures of transcribing Saccharomyces cerevisiae Pol II—Spt4/5—nucleosome complexes with bound Chd1 or FACT. In the first structure, Pol II transcription exposes the proximal histone H2A—H2B dimer that is bound by Spt5. Pol II has also released the inhibitory DNA-binding region of Chd1 that is poised to pump DNA toward Pol II. In the second structure, Pol II has generated a partially unraveled nucleosome that binds FACT, which excludes Chd1 and Spt5. These results suggest that Pol II progression through a nucleosome activates Chd1, enables FACT binding and eventually triggers transfer of FACT together with histones to upstream DNA.**

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**Results**

**Chd1 and FACT promote nucleosome transcription.** To understand how Chd1 and FACT facilitate nucleosome passage by Pol II, we reconstituted factor-facilitated Pol II transcription through a nucleosome in vitro. We designed an extended nucleosome substrate for the biochemical and structural investigation of nucleosome transcription (Fig. 1a). The substrate consists of a single nucleosome, formed on a modified Widom 601 sequence, with a 40-base pair (bp) upstream DNA extension (Methods). The DNA extension has a 9-nucleotide (nt) 3′-overhang that enables annealing of a fluorescently labeled RNA oligonucleotide and allows for Pol II binding and catalytic RNA extension upon addition of nucleoside triphosphates (NTPs).

We then used a fluorescence-based RNA extension assay to determine the efficiency of nucleosome transcription in the presence of S. cerevisiae Pol II and various factors (Fig. 1b and Extended Data Fig. 1a). We formed a Pol II elongation complex on the extended nucleosome substrate, provided the elongation factors Spt4/5 (Spt 4 and Spt 5) and TFIIS, and initiated RNA elongation by the addition of 1 mM NTPs. Samples were removed at specific time points, and the RNA products were separated by denaturing gel electrophoresis and quantified. We observed that Pol II paused when its leading edge was located at SHL –5 (Pol II active site at bp 1 of the nucleosome) and again when reaching SHL –1 (Pol II active site at bp 42) (Fig. 1b). These pausing positions correspond to previously described sites where Pol II pauses during nucleosome passage. A small fraction of Pol II could overcome these barriers and transcribe through the nucleosome, consistent with published observations.

When Chd1 or FACT was added to the reactions containing Pol II, Spt4/5 and TFIIS, we observed a 5- or 7-fold increase in full-length product formation, respectively (Fig. 1b,c). The strong increase in full-length product in the presence of only Chd1 was dependent on the ATPase activity of Chd1, but a weak stimulatory effect was observed also with a catalytically inactive Chd1 variant (Fig. 1d and Extended Data Fig. 1b). Indeed, Chd1 binding to a nucleosome may facilitate transcription because it leads to detachment of two turns of DNA at the Pol II entry site.

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**Structure of Pol II—Spt4/5—nucleosome—Chd1 complex.** To investigate the structural basis for Chd1 and FACT function during Pol II nucleosome passage, we formed a Pol II—Spt4/5—nucleosome complex in the presence of Chd1, FACT and the transition-state analog ADP-BeF₃ (Extended Data Fig. 2a–d). Transcription was carried out in the presence of GTP, CTP and UTP, and the complex was purified by size exclusion chromatography followed by mild crosslinking with glutaraldehyde (Methods). We prepared cryo-EM grids, collected a total of 3.76 million particles and obtained a reconstruction at a nominal resolution of 2.9 Å (Table 1, Fig. 2, Extended Data Figs. 2b–e).
Fig. 1 | Chd1 and FACT stimulate nucleosome transcription. a, Schematic of nucleosome substrate used for formation of Pol II–nucleosome complexes and RNA extension assays. b, Nucleosome transcription assay shows an increase in full-length product in the presence of FACT, Chd1, or FACT and Chd1. RNA length and corresponding nucleosomal base pairs are indicated. c, Bar graph shows a significant increase in full-length product upon addition of FACT, Chd1, or FACT and Chd1 to Pol II–Spt4/5–TFIIS complexes after 30 min of transcription. n = 3 independent experiments with *P < 0.05, **P < 0.01 with two-tailed t test. d, Mutation of Chd1 to eliminate ATPase activity (D513N) strongly decreases production of full-length RNA product during nucleosomal transcription. n = 3 independent experiments with *P < 0.05, **P < 0.01 with two-tailed t test. Error bars represent ± standard deviation. Unprocessed gel images and derived values for plots are provided as source data and in Supplementary Data 1.

Data Fig. 3 and Supplementary Video 1). We placed known structures and homology models of Pol II28, Spt4/5 (ref. 29) and the nucleosome core particle30 into the density, adjusted them and modeled the remaining DNA (Extended Data Fig. 4). Additional density was observed for Chd1, but not for FACT, and was fitted with the structure of Chd1 in its post-translocated state30 (Extended Data Figs. 4h,i and 5a). The structure was real-space refined and has good stereochemistry (Table 1).

The structure shows that Pol II adopts the active post-translocated state and has transcribed 27 bp into the nucleosome, as observed biochemically (Extended Data Fig. 2c). The Pol II front edge and active site are located around SHL ~3 and ~4.5, respectively (Fig. 2b,c and Extended Data Fig. 4a). At this stage, Pol II has unwrapped 45 bp of nucleosomal DNA, exposing the proximal histone H2A–H2B dimer (Extended Data Fig. 4a.g). Spt4/5 binds...
**Fig. 2 | Pol II—Spt4/5—nucleosome—Chd1 structure.**

**a,** Chd1 domain architecture. Residues at domain boundaries are indicated. Regions modeled in the Pol II—Spt4/5—nucleosome—Chd1 structure are indicated with a black bar. The same color coding is used throughout. **b,** Two views of the structure related by a 90° rotation. The same color code for Pol II, Spt4/5, histones, metal A, RNA, and template and non-template DNA is used throughout. Spt5N density is shown in surface representation. **c,** Schematic of the structure indicating key elements.

**Fig. 3 | Pol II—Spt4/5—nucleosome—FACT structure.**

**a,** Domain architecture of FACT subunits Spt16 and Pob3 (DD, dimerization domain; CTD, C-terminal domain). Residues at domain boundaries are indicated. Regions modelled in the Pol II—Spt4/5—nucleosome—FACT structure are indicated with a black bar. **b,** Two views of the structure related by a 90° rotation. **c,** Schematic of the structure indicating key elements.
Chd1 activation and Pol II progression. The structure shows that Chd1 binds the partially unwrapped nucleosome. Chd1 uses its double chromodomain and its ATPase motor domain to contact the nucleosome at SHLs +1 and +2, respectively (Fig. 2 and Extended Data Fig. 4a). In contrast, the DNA-binding region of Chd1 is not observed in our structure and apparently mobile (Fig. 2b). We previously observed that the DNA-binding region binds the second DNA gyre in a nucleosome–Chd1 complex6 (Extended Data Fig. 5c). However, in the structure we present here, the second DNA gyre is no longer available for Chd1 interactions because DNA corresponding to SHLs −7 to −5 has been transcribed by Pol II.

These observations explain how Chd1 is activated during transcription elongation (Extended Data Fig. 5c). As Pol II transcribes into the nucleosome, it displaces the DNA-binding region of Chd1 from DNA. The DNA-binding region is known to be an inhibitory domain that restricts Chd1 ATPase activity when engaged with nucleosomal DNA34. Displacement from DNA is predicted to release the inhibitory effect of the DNA-binding region7,31 and thereby activate Chd1. This results in DNA translocation toward the nucleosome dyad and into the Pol II cleft3, thereby facilitating Pol II progression.

Retention of the proximal H2A−H2B dimer. In our second structure, FACT additionally binds the exposed proximal H2A−H2B dimer using the C-terminal region of Spt16 (Fig. 3 and Supplementary Video 2). FACT embraces nucleosomal DNA near the dyad position at SHL +0.5 and contacts the template-strand DNA backbone with the middle domain of subunit Spt16 at SHL −0.5 (Fig. 3b,c). Compared to the structure of the *Homo sapiens* FACT–nucleosome complex5, the position of FACT appears shifted by one helical turn of DNA toward the side of the nucleosome that is distal to Pol II (Extended Data Fig. 8a). The previously observed position of FACT cannot be adopted in our structure because DNA is present on the distal side of the nucleosome but is absent in the isolated FACT–nucleosome complex structures that were reconstituted with shorter DNA to generate a subnucleosome without Pol II action21. In summary, our structure shows the position of FACT on a nucleosome that was partially unraveled by active Pol II transcription elongation.

Structure of Pol II–Spt4/5–nucleosome–FACT complex. To localize FACT during nucleosome transcription, we reconstituted a transcribing *S. cerevisiae* Pol II–Spt4/5–nucleosome–FACT complex by withholding Chd1 from the assembly (Extended Data Fig. 2e–h). We employed single-particle cryo-EM to determine the structure of the complex at a nominal resolution of 3.1 Å (Methods), with densities for FACT at lower local resolutions (Extended Data Fig. 6). The high-resolution density observed around the Pol II active site allowed us to unambiguously define the nucleic acid sequence register and revealed that Pol II had stalled with the active site located at bp 17 of the nucleosome (Extended Data Figs. 2g and 7a). As in the first structure, Pol II adopts the post-translocated state (Extended Data Fig. 7d).

In this structure, four turns of nucleosomal DNA (SHL −7 to SHL −3) are unwrapped from the histone octamer, and the proximal H2A−H2B dimer is exposed (Fig. 3 and Supplementary Video 2). FACT embraces nucleosomal DNA near the dyad position at SHL +0.5 and contacts the template-strand DNA backbone with the middle domain of subunit Spt16 at SHL −0.5 (Fig. 3b,c). Compared to the structure of the *Homo sapiens* FACT–nucleosome complex5, the position of FACT appears shifted by one helical turn of DNA toward the side of the nucleosome that is distal to Pol II (Extended Data Fig. 8a). The previously observed position of FACT cannot be adopted in our structure because DNA is present on the distal side of the nucleosome but is absent in the isolated FACT–nucleosome complex structures that were reconstituted with shorter DNA to generate a subnucleosome without Pol II action21. In summary, our structure shows the position of FACT on a nucleosome that was partially unraveled by active Pol II transcription elongation.

Superposition of our two structures results in a clash between Chd1 and the FACT subunit Pob3 (Extended Data Fig. 8b). This indicates that binding of Chd1 and FACT to the nucleosome is mutually exclusive, at least in the highly defined states we trapped in our structures. Nevertheless, Chd1 binds FACT with its flexible N-terminal region (Extended Data Fig. 9), allowing one factor to remain loosely associated with the complex while the other factor binds the nucleosome directly. Additionally, binding of the C-terminal domain of Spt16 to the H2A−H2B dimer is predicted to prevent re-association of DNA with the exposed histone octamer surface, as had been observed in previous cryo-EM studies of Pol II–nucleosome complexes that showed unassigned DNA fragments of unknown origin7. Taken together, these observations support a dynamic mechanism with Chd1 and FACT acting either subsequently or in an alternating manner.
Discussion
Based on our data and previously published data, we propose a model for how Chd1 and FACT mediate nucleosome transcription (Fig. 4 and Extended Data Fig. 10a). When the Pol II–Sppt4/5 complex transcribes into a Chd1-bound nucleosome, it would release the DNA-binding region of Chd1. This activates the Chd1 translocase\(^\text{20}\) and may facilitate Pol II progression. Pol II progression exposes the proximal H2A–H2B dimer, which is temporarily stabilized by Sppt5N binding. Further Pol II progression would then generate a binding site for FACT, which can then bind the partially unraveled nucleosome, leading to the displacement of Chd1 and Sppt5N. Modeling suggests a ~30-bp window for FACT binding during Pol II progression (Extended Data Fig. 10b). Further Pol II progression would displace FACT from downstream DNA and enable FACT to interact with upstream DNA. It remains to be studied whether at each nucleosome both Chd1 and FACT are essential for Pol II transcription. The order of events during nucleosome transcription also remain to be studied further, but it seems that Chd1 functions upstream of FACT because Chd1 can bind complete nucleosomes, whereas FACT can only bind partially unraveled nucleosomes. Chd1 may even recruit FACT because it is known to interact with FACT\(^\text{3,24,40}\). Indeed, we could confirm the Chd1–FACT interaction biochemically. This finding is consistent with the idea that FACT is recruited near the nucleosome by Chd1 but remains flexible and only binds the nucleosome once DNA is partially unwrapped by transcription. In conclusion, we provide molecular snapshots of the dynamic process of factor-mediated nucleosome transcription and a model for Pol II progression through the nucleosome.

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Methods

No statistical methods were used to determine sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

Molecular cloning. S. cerevisiae Spt4 and Spt5 were cloned into vectors 438-A and 438-C, respectively, using ligation-independent cloning (LIC). Vectors 438-A and 438-C were a gift from S. Gradia (UC Berkeley). Addgene plasmids #55218 and #55220. Using LIC, the two genes were combined on a single 438-series vector. The construct contained Spt5 with an N-terminal 6x His tag followed by a maltose-binding protein tag, and a tobacco etch virus protease cleavage site. Spt4 did not contain tags. Each subunit in the combined vector was preceded by a N-terminal 6x His tag followed by a maltose-binding protein tag and a tobacco etch virus protease cleavage site.

Preparation of protein components. S. cerevisiae Pol II was purified as described previously. S. cerevisiae Chd1 and FACT were expressed and purified as described. All insect cell lines used for expression were purchased from Life Technologies (Sf9, Sf21) or from Expression Systems (Hi5) and used as identified by the vendor. Cell lines were not tested for mycoplasma contamination. S. cerevisiae Sp4 and Sp5 were co-expressed in insect cells using a similar approach as that reported previously. After harvest, cell pellets were resuspended in lysis buffer 500 (500 mM NaCl, 20 mM Na-HEPES, pH 7.4, 10% (v/v) glycerol, 1 mM DTT, 30 mM imidazole, pH 8.0, 0.284 μg ml⁻¹ leupeptin, 1.37 μg ml⁻¹ pepstatin A, 0.17 mg ml⁻¹ PMSF, 0.33 mg ml⁻¹ benzamidine). Cells were lysed by sonication. The cell lysate was subjected to centrifugation (18,000g, 4°C, 30min) and ultracentrifugation (235,000g, 4°C, 60min). The supernatant containing Sp4/5 was subsequently filtered using 0.2-μm syringe filters (Millipore). The filtered supernatant was applied to a GE HisTrap 5 ml HP (GE Healthcare), pre-equilibrated in lysis buffer 500, 3 CV high-salt buffer (1000 mM NaCl, 20 mM Na-HEPES, pH 7.4, 10% (v/v) glycerol, 1 mM DTT, 30 mM imidazole, pH 8.0, 0.284 μg ml⁻¹ leupeptin, 1.37 μg ml⁻¹ pepstatin A, 0.17 mg ml⁻¹ PMSF, 0.33 mg ml⁻¹ benzamidine). Bound protein was eluted by gradient over 9 CV to 100% nickel elution buffer (500 mM NaCl, 20 mM Na-HEPES, pH 7.4, 10% (v/v) glycerol, 1 mM DTT, 500 mM imidazole, pH 8.0, 0.284 μg ml⁻¹ leupeptin, 1.37 μg ml⁻¹ pepstatin A, 0.17 mg ml⁻¹ PMSF, 0.33 mg ml⁻¹ benzamidine) and 4.5 CV lysis buffer. Bound protein was eluted by gradient over 9 CV to 100% nickel elution buffer (500 mM NaCl, 20 mM Na-HEPES, pH 7.4, 10% (v/v) glycerol, 1 mM DTT, 30 mM imidazole, pH 8.0, 0.284 μg ml⁻¹ leupeptin, 1.37 μg ml⁻¹ pepstatin A, 0.17 mg ml⁻¹ PMSF, 0.33 mg ml⁻¹ benzamidine) and 4.5 CV lysis buffer. Bound protein was eluted by gradient over 9 CV to 100% nickel elution buffer (500 mM NaCl, 20 mM Na-HEPES, pH 7.4, 10% (v/v) glycerol, 1 mM DTT). Protein identity of E. coli TFIIIS was confirmed in Escherichia coli was cloned into LIC-compatible vector 1-O. Vector 1-O was a gift from S. Gradia (UC Berkeley), Addgene plasmid #29658. The construct contains an N-terminal 6x His tag followed by a maltose-binding protein tag and a tobacco etch virus protease cleavage site.

RNA extension assays. RNA extension assays were performed on the same nucleosomal template substrate used for the structural studies. A 6-FAM 5′-labelled 11-nt RNA (5′-56-FAM/rUrArA rUrCrA rCrUrG rUrC-3′) was used for RNA extension reactions to prevent formation of overextended RNA hybrids and facilitate nucleosome passage. The position of Pol II pausing was assigned by indicating the position of the Pol II active site on the Widom 601 DNA. This provided an unambiguous assignment at nucleotide resolution. Therefore, our pausing sites at bp 1 and bp 42 correspond to the previously described pause sites with the Pol II leading edge at SHL—5 and SHL—1, respectively.

All subsequent concentrations refer to the concentration in the final reaction. The final concentrations of buffer components were 130 mM NaCl, 20 mM Na-HEPES, pH 7.4, 3 mM MgCl₂, 4% (v/v) glycerol, 1 mM DTT/TCEP. The final volume for each RNA extension reaction was 40 μl. RNA (80 nM), nucleosomal template (80 nM) and c. cerevisiae Pol II (100 nM) were combined in equimolar ratios and incubated for 5 min on ice. Sp4/5 (120 nM) and additional factors (500 nM each), 10% compensation buffer and water were added to achieve final assay conditions. The sample was incubated for 3 min at 30°C. Transcription elongation was started by the addition of ATP, CTP, GTP and UTP (1 μM each) and TFIIH (60 nM). Five microliters of the reactions were quenched after 5 min, 10 min and 30 min at 37°C in 6 M urea, 50 mM EDTA, pH 8.0, 1 X TBE buffer) if time courses were performed. Samples were treated with 4 μg proteinase K for 15 min at 37°C, denatured at 95°C for 3 min, and separated by denaturing gel electrophoresis (4% of sample applied to an 8% urea, 1 X TBE buffer, 12% acrylamide/bis-acrylamide 19:1 gel, run in 0.5X TBE buffer at 300 V for 30 min). RNA extension products were visualized using the 6-FAM label and a Typhoon 9500 FLA imager at an excitation wavelength of 472 nm and emission wavelength range of >520 nm.

Gels were subjected to linear contrast enhancement. Source data for all quantified RNA extension assays are provided in Source Data Fig. 1. All RNA extension assays were performed independently and at least three times. Full-length RNA extension products were quantified using Fiji 1.0. The products were normalized against the total intensity of the respective reaction lane to control for errors during gel loading. Bar charts show mean values and standard deviation as error bars. The following P values were applied: *P<0.05, **P<0.01.

Reconstitution of transcribing Pol II–nucleosome complexes. Complexes for cryo-EM were formed in a final buffer containing 130 mM NaCl, 20 mM Na-HEPES, pH 7.4, 3 mM MgCl₂, 1 mM DTT/TCEP, 4% (v/v) glycerol, RNA (480 pmol, same construct as used for RNA extension assays) and nucleosome (120 pmol) were incubated for 5 min on ice. Pol II (120 pmol), Sp4/5 (180 pmol) and Sp6 (180 pmol) were added and incubated for 5 min on ice. Water and compensation buffer were added to reach final buffer conditions, and the sample was incubated for 5 min. Transcription elongation was started by the addition of 1 mM each of GTP, CTP and UTP and 0.4 mM 3′-dATP in the case of the Pol II–Sp4/5–nucleosome–FACT complex. Instead of 3′-dATP, 1 mM ADP-BeF₃ was added to the Pol II–Sp4/5–nucleosome–Chd1–FACT complex. TFIIH (108 pmol) was added by diluting an Amicon Ultra-15 30K.

After 15 min of incubation at 30°C, Chd1 (180 pmol) and FACT (180 pmol), preincubated with H₂A–H₂B dimer (180 pmol), or FACT alone (180 pmol), preincubated with H₂A–H₂B dimer (180 pmol), were added. The transcription reactions were allowed to proceed for an additional 30 min at 30°C and quenched
into two subsets and subsequently 3D classified using cryoSPARC. Particles were picked using an x-hose trained instance of the neural network BoxNet2 as implemented in Warp, yielding 3,227,093 particles for the Pol II–Chd1 dataset and 3,755,390 particles for the Pol II–nucleosome–Chd1 model. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The cryo-EM reconstructions and final models for the Pol II–Spt4/5–nucleosome–Chd1 complex were deposited with the Electron Microscopy Data Base (EMD-12449) and the Protein Data Bank (PDB 7NXY). The cryo-EM reconstructions and final models for the Pol II–Spt4/5–nucleosome–FACT complex were deposited with the Electron Microscopy Data Base (EMD-12450) and with the Protein Data Bank (PDB 7NXY). For the Pol II–Spt4/5–nucleosome–Chd1 complex, maps A–C were deposited as EMD-12666, EMD-12667 and EMD-12668, respectively. For the Pol II–Spt4/5–nucleosome–FACT complex, maps 1–3 were deposited as EMD-12669, EMD-12670 and EMD-12671, respectively. Source data are provided with this paper.

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52. Both atomic models were real-space refined using PHENIX, with secondary structure restraints against map D (Pol II–Spt4/5–nucleosome–Chd1 model) and map F (Pol II–Spt4/5–nucleosome–FACT model).

**Model building and refinement.** For the Pol II–Spt4/5–nucleosome–Chd1 structure, structures of S. cerevisiae Pol II (PDB 3F03), X. laevis nucleosome (PDB 3ZLO), Chd1 with ADP-BeF3 (PDB S0G9) and Spt4/5 (PDB 2XEU) were rigid-body docked into map D and refined using Coot. DNA from the elongation complex and nucleosomal DNA were connected using Coot. Density in the active site of Pol II allowed unambiguous assignment of DNA register. Surprisingly, the complex had transcribed over the T-less cassette that should stall further elongation. The ADP (Sigma-Aldrich) used in the formation of ADP-BeF3 was reported to be contaminated with up to 2.76% ATP, possibly providing the required substrate to transcribe past the end of the T-less cassette. Identification of DNA–RNA register was aided by map B. For the Pol II–Spt4/5–nucleosome–FACT structure, the refined Pol II part of the Pol II–Spt4/5–nucleosome–Chd1 structure was rigid-body docked into the density. Additionally, X. laevis nucleosome (PDB 3ZLO), H. sapiens FACT (PDB 6UPK), and Spt4/5 (PDB 2XEU) were rigid-body docked into map 4 using Coot.

**Data availability**

The cryo-EM reconstructions and final models for the Pol II–Spt4/5–nucleosome–Chd1 complex were deposited with the Electron Microscopy Data Base (EMD-12449) and the Protein Data Bank (PDB 7NXY). The cryo-EM reconstructions and final models for the Pol II–Spt4/5–nucleosome–FACT complex were deposited with the Electron Microscopy Data Base (EMD-12450) and with the Protein Data Bank (PDB 7NXY). For the Pol II–Spt4/5–nucleosome–Chd1 complex, maps A–C were deposited as EMD-12666, EMD-12667 and EMD-12668, respectively. For the Pol II–Spt4/5–nucleosome–FACT complex, maps 1–3 were deposited as EMD-12669, EMD-12670 and EMD-12671, respectively. Source data are provided with this paper.

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**Author contributions**

L.F. designed and conducted experiments and interpreted data, unless stated otherwise. M.O. assisted with the purification of Spt4/5 and RNA extension assays. L.F. and M.O. prepared the Pol II−Spt4/5−nucleosome−Chd1 and Pol II−Spt4/5−nucleosome−FACT complexes for cryo-EM. M.E. conducted initial FACT−nucleosome binding experiments. P.C. supervised research. L.F. and P.C. wrote the manuscript with input from all authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41594-021-00578-6.

**Supplementary information**

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**Extended Data Fig. 1 | Additional information on RNA extension assays.**

**a.** SDS-PAGE of purified proteins. Purified proteins were run on 4-12 % Bis-Tris SDS-PAGE gels in 1X MES Buffer, stained with Coomassie Blue. Asterisk (*) demarcates degradation products of Spt5. 

**b.** 12 % denaturing urea gel of RNA extension assay with Chd1 D513N mutant. RNA is visualized using 6-FAM label on 5’ end of RNA.

**c.** 12 % denaturing urea gel of RNA extension assay with different factor combinations reveals dependence of Spt4/5 for increase in full-length product in the presence of FACT and Chd1. RNA is visualized using 6-FAM label on 5’ end of RNA.

**d.** Bar plot with quantification of c. Mean normalized intensity is shown as bar plot. Error bars represent standard deviation. Quantification from n = 3 independent experiments with *P < 0.05, ** P < 0.01 with two-tailed t-test. Uncropped gel images in a, b, c and data for the plots in d are available as Source Data Extended Data Fig. 1 and Supplementary Data.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Formation of Pol II-nucleosome complexes with bound Chd1 and FACT. a, Chromatogram of Pol II-Spt4/5-nucleosome-Chd1 complex formation using size exclusion chromatography. Fractions used in further analysis are indicated. b, SDS-PAGE of Pol II-Spt4/5-nucleosome-Chd1 complex formation. SDS-PAGE shows presence of FACT in the complex. c, 12 % denaturing urea gel of Pol II-Spt4/5-nucleosome-Chd1 complex formation. RNA is visualized using 6-FAM label on 5' end of RNA. d, Step-by-step flowchart of complex formation for the Pol II-Spt4/5-nucleosome-Chd1 complex. e, Chromatogram of Pol II-Spt4/5-nucleosome-FACT complex formation using size exclusion chromatography. f, SDS-PAGE of Pol II-Spt4/5-nucleosome-FACT complex formation. g, 12 % denaturing urea gel of Pol II-Spt4/5-nucleosome-FACT complex formation. RNA is visualized using 6-FAM label on 5' end of RNA. h, Step-by-step flowchart of complex formation for the Pol II-Spt4/5-nucleosome-FACT complex. Uncropped gel images for panels b, c, f, g are provided in Source Data Extended Data Fig. 2.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Data acquisition, processing, and data quality metrics for the Pol II-Spt4/5-nucleosome-Chd1 structure. a, Representative denoised micrograph of data collection with scale bar (50 nm). b, Sorting and classification tree of Pol II-Spt4/5-nucleosome-Chd1 dataset. c, 2D classes of final refinement show RNA polymerase II and nucleosome-like shape with additional density (Chd1) with scale bar of 10 nm. d, FSC curves of maps A–C and map-to-model. Resolutions at FSC threshold criterions 0.143 or 0.5 are indicated. e, Angular distribution of particles employed to reconstruct map A. f, Local resolution of composite map D.
Extended Data Fig. 4 | Cryo-EM densities of Pol II-Spt4/5-nucleosome-Chd1 complex. a, Protein-nucleosomal DNA contacts of Pol II-Spt4/5-nucleosome-Chd1 complex. Nucleotides are depicted as solid spheres (modelled) or empty spheres (not modelled). SHLs are indicated. b, Cryo-EM map (map D) of Pol II-Spt4/5-nucleosome-Chd1 complex. c, Pol II-Spt4/5-nucleosome-Chd1 structure with corresponding cryo-EM map (map D). Cryo-EM map is shown in grey. d, Active site of Pol II-Spt4/5-nucleosome-Chd1 structure with corresponding density (map D). Metal A is shown as a pink sphere. e, Rpb1 funnel helices with corresponding density (map D). f, Histone octamer with corresponding density (map D). g, Nucleic acids in Pol II-Spt4/5-nucleosome-Chd1 structure with corresponding densities (map D). h, Chd1 with corresponding density (map D). i, Active site of Chd1 with bound ADP-BeF3. ADP is shown in stick representation, BeF3 as green spheres. Density from map D.
Extended Data Fig. 5 | Details of the Pol II-Spt4/5-nucleosome-Chd1 structure. a, Comparison of Chd1 (grey, PDB code 5O9G) with Chd1 (this study). The ATPase motor adopts the post-translocated state in both structures. b, Density for Spt5 N-terminal region (Spt5N) (low-passed filtered to 9 Å, map D) next to the proximal H2A-H2B dimer (surface representation). c, Comparison of a poised nucleosome-Chd1 structure26 with the structure of Chd1 bound to the transcribed nucleosome reveals displacement of the Chd1 DNA-binding region (DBR, pink) upon transcription.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Data acquisition, processing, and data quality metrics for the Pol II-Spt4/5-nucleosome-FACT structure. a, Representative denoised micrograph of data collection with scale bar (50 nm). b, Sorting and classification tree of Pol II-Spt4/5-nucleosome-FACT dataset. c, 2D classes of final refinement show RNA polymerase II and nucleosome-like shape with additional density (FACT) with scale bar of 10 nm. d, FSC curves of maps 1-4 and map-to-model. Resolutions at FSC threshold criterions 0.143 or 0.5 are indicated. e, Angular distribution of particles employed to reconstruct map 1. f, Local resolution of composite map 4.
Extended Data Fig. 7 | Cryo-EM densities of Pol II-Spt4/5-nucleosome-FACT complex. **a**, Protein-nucleosomal DNA contacts of Pol II-Spt4/5-nucleosome-FACT complex. Nucleotides are depicted as solid spheres (modelled) or empty spheres (not modelled). SHLs are indicated. **b**, Cryo-EM map 4 of Pol II-Spt4/5-nucleosome-FACT complex. **c**, Pol II-Spt4/5-nucleosome-FACT structure with corresponding cryo-EM map (map 4). Cryo-EM map is shown in grey. **d**, Active site of Pol II-Spt4/5-nucleosome-Chd1 structure with corresponding density (map 4). **e**, Rpb1 funnel helices with corresponding density (map 4). **f**, Histone octamer with corresponding density (map 4). **g**, Nucleic acids in Pol II-Spt4/5-nucleosome-FACT structure with corresponding densities (map 4). **h**, Nucleosome with bound FACT and corresponding density (map 4). Density corresponding to the Spt16 CTD is highlighted in purple. **i**, Spt16 CTD density (map 4) contacts the proximal H2A/H2B dimer.
Extended Data Fig. 8 | Details of the Pol II-Spt4/5-nucleosome-FACT structure. **a**, Superposition of a subnucleosome-FACT complex (PDB code 6UPL; FACT, pale green) on the Pol II-Spt4/5-nucleosome FACT structures reveals sliding of FACT by one superhelical location. FACT-transcribed nucleosome and the subnucleosome structure were aligned using the histone octamer. Transcribed nucleosome is shown in grey. **b**, Superposition of Chd1 structure with the FACT structure reveals a steric clash between the ATPase lobe 2 and double chromodomain of Chd1 and the Pob3 subunit of FACT.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Interaction between Chd1 and FACT. a, Domain architecture with different Chd1 constructs; full-length Chd1, Chd1 ΔN (residues 118-1468), Chd1 ΔC (residues 1-1274) and Chd1 ΔNC (residues 118-1274). b, Chromatogram of size exclusion chromatography runs to determine regions of Chd1 that interact with FACT. Fractions analysed with SDS-PAGE (c) are indicated with grey numbering. c, SDS-PAGE analysis of size exclusion chromatography runs (b) reveals interaction of Chd1 with FACT via the N-terminus of Chd1. Uncropped gel images are available as Source Data Extended Data Fig. 9. d, Summary of interaction results.
Extended Data Fig. 10 | Extended model for Pol II passage through a nucleosome. a, Extended model for Pol II progression through the proximal part of a nucleosomal substrate. b, Structural modeling reveals a ~30 bp window for FACT binding during transcription through the proximal part of the nucleosomal substrate.
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The cryo-EM reconstructions and final models for the Pol II–Spt4/5–nucleosome–Chd1 complex were deposited with the Electron Microscopy Data Base (EMD-12449) and the Protein Data Bank (PDB 7NXX). The cryo-EM reconstructions and final models for the Pol II–Spt4/5–nucleosome–FACT complex were deposited with the Electron Microscopy Data Base (EMD-12450) and with the Protein Data Bank (PDB 7NXY). For the Pol II–Spt4/5–nucleosome–Chd1 complex, maps A–C were deposited as EMD-12666, EMD-12667 and EMD-12668, respectively. For the Pol II–Spt4/5–nucleosome–FACT complex, maps 1-3 were deposited as EMD-12669, EMD-12670 and EMD-12671, respectively. Source data are provided with this paper.
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