Transcription of the ribosomal RNA precursor by RNA polymerase (Pol) I is a prerequisite for the biosynthesis of ribosomes in eukaryotes. Compared to Pols II and III, the mechanisms underlying promoter recognition, initiation complex formation and DNA melting by Pol I substantially diverge. Here, we report the high-resolution cryo-EM reconstruction of a Pol I early initiation intermediate assembled on a double-stranded promoter scaffold that prevents the establishment of downstream DNA contacts. Our analyses demonstrate how efficient promoter-backbone interaction is achieved by combined re-arrangements of flexible regions in the ‘core factor’ subunits Rrn7 and Rrn11. Furthermore, structure-function analysis illustrates how destabilization of the melted DNA region correlates with contraction of the polymerase cleft upon transcription activation, thereby combining promoter recruitment with DNA-melting. This suggests that molecular mechanisms and structural features of Pol I initiation have co-evolved to support the efficient melting, initial transcription and promoter clearance required for high-level rRNA synthesis.
he transcription of the ribosomal RNA (rRNA) precursor by RNA polymerase (Pol I) is a prerequisite for ribosome biosynthesis in all known eukaryotes. As such, Pol I transcription is tightly regulated, mostly at the level of pre-initiation complex (PIC) formation. Whereas Pol II and Pol III use related initiation mechanisms, the processes underlying Pol I promoter recognition, PIC formation and DNA melting substantially diverge. In bakers’ yeast *Saccharomyces cerevisiae*, a basal initiation system required for Pol I activity consists of the promoter DNA core element (CE), specific initiation factor Rrn3 and heterotrimeric core factor (CF). CF binds a CE stretch promoter DNA core element (CE), specifying the basal initiation system required for Pol I activity consists of the initiation complex (PIC) formation.

During transcription initiation, Pols are recruited to their promoters by a set of general transcription factors, forming a ‘closed complex’ (CC). After melting of both DNA strands, an ‘open complex’ (OC) is established, transitioning into an ‘initially transcribing complex’ (ITC) with the beginning of RNA chain synthesis. In ITCs, a stable DNA/RNA hybrid is formed and the polymerase has initiated movement into the gene before establishment of a processive elongation complex (EC); for a review of initiation phases compare refs. 24,25. Previous structural analyses of Pol I initiation complexes by us and others relied on an artificially stabilized, mismatched bubble scaffold assembled with an initially transcribed RNA sequence and a double-stranded DNA (dsDNA) sequence extending to up to 24 bps downstream of the TSS.

This experimental approach originates from the analysis of Pol II elongation complexes (ECs), preventing heterogenic sample conformations and making use of the tight DNA/RNA hybrid association with the polymerase. In the case of the Pol I PIC a similar experimental strategy results in the visualization of late initiation intermediates. Consequently, an inconsistent occupancy of Rrn3 and divergent localization of the tandem-winged helix (twh) domain of Pol I subunit A49 and the C-terminal domain of subunit A12.2 have been observed, leaving room for speculation with regard to the functional roles and temporal classification of the analyzed conformations during initiation.

Therefore, we aimed at analyzing Pol I initiation mechanisms at an early initiation stage, allowing the visualization of promoter recognition, Pol I recruitment and DNA melting in a scenario as close to the native situation as possible. For this purpose, we assembled a complete initiation complex on double-stranded (ds) promoter DNA and performed single-particle cryo-EM analysis. The dsDNA scaffold was truncated on its downstream edge at

---

**Fig. 1 Cryo-EM reconstruction of a Pol I early intermediate PIC.** a Overview of the Pol I eIPIC cryo-EM reconstruction at 3.5 Å resolution (unsharpened; transparent gray envelope) overlaid with the PDB model (colored ribbon) and DNA (space filling). The right panel shows transparent density (gray) for protein components and solid density for the DNA path (template strand in blue and non-template in light blue). PAD promoter-associated domain (of Rrn11); PIR polymerase interacting region (of CF). b Schematic representation of promoter dsDNA used for PIC assembly, densities observed in the eIPIC reconstruction are highlighted in blue and light blue for template strand and non-template strand, respectively. c Atomic model of the bridge helix in subunit A190 overlaid with sharpened eIPIC density (gray mesh) indicates residue orientations.
position +8 relative to the TSS, thus preventing a contact with the clamp core and jaw domains of the polymerase. Three-dimensional particle reconstruction, cryo-EM density refinement and structural modeling allow the placement of basal PIC components and a comparative PIC analysis of the three eukaryotic Pols. Furthermore, structure-guided analysis indicates how Pol-I-specific ribosomal DNA (rDNA) promoter melting may be achieved.

**Results**

**Complex formation and cryo-EM analysis.** To study promoter recognition and DNA melting, we formed a complete Pol I initiation complex in vitro. UAF was assembled on a dsDNA promoter scaffold ranging from position −155 to +8 relative to the TSS together with TBP, CF, and a fragment of the protein Net1.22,28,29 (Methods). Endogenously purified Pol I13,30,31 was pre-incubated with recombinant Rrn332 to reconstitute a complete early PIC that was stable throughout size exclusion chromatography (Supplementary Fig. 1a; Methods). Accordingly, Pol I could be recruited to a UAF/TPB/Net1/CF-bound promoter scaffold lacking sequence stretches required for forming extended downstream contacts with the jaw- and clamp-head domains of the polymerase. Single-particle cryo-EM data was collected on a Titan Krios equipped with Gatan K2 summit direct electron detector basically as described12,13. Following pre-processing, two-dimensional (2D)- and three-dimensional (3D)-classification in RELION33, a total of 122,099 particles were selected from 4,088 micrograph movies (Methods; Supplementary Fig. 1). A final cryo-EM reconstruction exhibits an overall resolution of 3.5 Å and shows a Pol I early intermediate PIC (eiPIC; Fig. 1 and Supplementary Fig. 1). The cryo-EM density clearly reveals secondary structure features for the entire particle and side chain orientations in most regions (Fig. 1c and Supplementary Fig. 2a–f). Despite protein–protein crosslinking, TBP, UAF, and Net1-CTR remain flexible, although apparently stabilizing CF similar to the co-activator ‘mediator’ in context of a Pol II PIC34.

**An early intermediate PIC exhibits a well-defined architecture.** Initial assignment located template and non-template DNA strands, Pol I, CF subunits, and Rrn3, followed by manual model building and real-space refinement, resulting in a model of high quality (Methods, Table 1). Upstream DNA is well-ordered between CF-interacting regions and entry into the Pol I active center cleft. Following the canonical DNA-path further downstream, however, no density is visible around the active center itself, but ≥12 well-defined base-pairs can be placed on the downstream edge between bridge helix and the clamp-head/jaw domains, even though our scaffold should not extend this far. Most likely the conserved35 and highly charged region is bound by foreign DNA or the far upstream end of our scaffold. A similar effect was observed for patches of the nucleosome, after transcription by Pol II ‘peeled’ off supercoiled DNA36. Well in line, in vitro initiation assays previously showed a strong preference contrast to inactive Pol I30,31,40, the ‘expander’ and ‘connector’ subdomains are flexible and the central bridge helix is refolded in the eiPIC (Fig. 1c) as expected from EC structures41,42. The C-terminal domain of subunit A12.2 shows only residual density in funnel domain of subunit A190 (Supplementary Fig. 2b), but is not localized on the A135 lobe as observed in a 12-subunit EC43.

| Table 1 Cryo-EM data collection, refinement, and validation statistics. |
|---------------------------------------------------------------|
| **eiPIC EMDB-10544** | **CF in eiPIC EMDB-10663** |
| **Data collection and processing** | | |
| Magnification (PDB code) | PDB 6TPS | PDB 5W66 |
| Voltage (kV) | 300 | 300 |
| Electron exposure (Å) | 56 | 56 |
| Defocus range (Å) | –1.5 to –3.1 | –1.5 to –3.1 |
| Pixel size (Å) | 1.09 (0.545 superres) | 1.09 (0.545 superres) |
| Symmetry imposed | C1 | C1 |
| Initial particle images (no.) | 311,557 | 311,557 |
| Initial particle images (no.) | 122,099 | 122,099 |
| Map resolution (Å) | 3.54 | 3.91 |
| FSC threshold | 0.143 | 0.143 |
| Map resolution range (Å) | 3.3 to 9.9 | 3.4 to 16.7 |
| Refinement | | |
| Initial model used | 6TPS | | |
| Model resolution (Å) | 3.5 | | |
| FSC threshold | 0.143 | | |
| Map sharpening B factor (Å²) | –75 | | |
| Model composition | | |
| Non-hydrogen atoms | 50,070 | | |
| Protein residues | 6,109 | | |
| Ligands | 8 (Zn and Mg) | | |
| B factors (Å²) | | |
| Protein | 65.6 | | |
| Ligand | 102.9 | | |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.009 | | |
| Bond angles (°) | 0.985 | | |
| Validation | | |
| MolProbity score | 1.85 | | |
| Clashscore | 5.96 | | |
| Poor rotamers (%) | 0.59 | | |
| Ramachandran plot | | |
| Favored (%) | 91.10 | | |
| Allowed (%) | 8.75 | | |
| Disallowed (%) | 0.15 | | |

**Core factor embraces the promoter DNA.** The cryo-EM density allows the construction of a CF model, which we found to resemble the overall ITC conformation. To define the structural changes that take place upon promoter recruitment, we compared the architecture of CF in free (PDB 5O7X) and promoter-engaged eiPIC conformation (Supplementary Fig. 3). This shows that CF module I and II retract from each other by up to 12 Å upon
binding of the CE promoter sequence. This retraction leads to the exposure of positively charged residues that are now free to engage the phosphate backbone (Supplementary Fig. 3a–c). These DNA-binding regions lie within the Rrn11 promoter-associated domain (‘PAD’) and the cyclin domains of Rrn7. The same regions engage the DNA in ITCs 15–17 and have been described in detail in late ITCs devoid of Rrn3 17. Remarkably, the Rrn7 residues involved in DNA-binding are not conserved within TFIIB or Brf1, which share a similarity in their overall fold 44–46 and would clash with TBP 15 in canonical TFIIB-TBP 47 or Brf1-TBP 48,49 complex.

Comparison of free and promoter-engaged CF also shows that the Rrn7-specific helix α4a in the N-terminal cyclin domain shifts and is inserted into the minor groove of the CE promoter DNA, while loop α7–α8 in cyclin II becomes well-structured and contacts the major groove further upstream upon ePIC formation (Fig. 2a). Thereby, the distal upstream DNA-path is modified towards the C-terminal domain of Rrn7 and the β-propeller-domain of Rrn6. Thus, promoter binding by Rrn7-specific regions on one face and by the TFIIB-unrelated CF subunit Rrn11 on the opposite face tightly squeeze the DNA. This may explain why the basal Pol I initiation system does not require TBP association opposite of the Rrn7 cyclins.

To address the importance of these residues, we constructed CF mutants with deletions in helix α4a and in loop α7–α8. Both can still associate with promoter DNA (Fig. 2b), but show defects in basal initiation in vitro (Fig. 2c). Engagement of these regions may therefore be important to induce a specific DNA conformation required for Pol I recruitment or promoter melting.

The Pol I ‘sandwich’ region is important for PIC formation. We have previously described a Pol-I-specific proximal upstream promoter-binding region consisting of loop α11a-α12 (residues 452–456) and the loop β28-β28 (residues 815–818) in the protrusion and wall domains of Pol I subunit A135, respectively 15. In the ePIC, a positively charged loop (892–895, wall domain of subunit A135) re-orientates towards the promoter DNA, contributing

Fig. 2 Core Factor—promoter interactions in ePIC. a Model of promoter-bound CF in the ePIC. The same regions of Rrn7 and Rrn11 contribute to promoter phosphate backbone interactions compared to ITC reconstructions. b Electrophoretic mobility shift assay (EMSA) shows that wild-type CF interacts with double-stranded promoter DNA (0.25 pmol, 0.5 pmol, and 1 pmol CF added). Mutation of Rrn7 (Δα4a and Δloop α7–α8) does not impair promoter-DNA association. c In contrast to DNA binding, initiation efficiency of CF assembled with Rrn7 mutants Δα4a and Δloop α7–α8 is impaired (promoter-dependent in vitro transcription assay from a minimal scaffold).
additional phosphate-backbone interactions (Supplementary Fig. 4) similar to other ITC/PIC structures. These promoter interactions are all specific to Pol I, because the residues are not conserved in Pol II and III. Furthermore, DNA is occluded from the corresponding region in Pol II and III PICs by the N-terminal cyclin domains of TFIIH and Brf1/Brf2, respectively. Fittingly, this Pol I region was previously named ‘sandwich’. In the eiPIC, the sandwich region tightly holds the promoter in place between the wall and protrusion domains at the bottom of the cleft. sandwich elements contact both DNA strands, therefore rendering it specific for an un-melted duplex. Density for the DNA directly downstream of the sandwich is not observed, indicating a higher degree of flexibility. Consequently, the recruitment of the Pol-I-Rrn3 complex seems to mainly rely on (1) contacts between the promoter and the sandwich and (2) protein–protein contacts between CF and the Pol-I-Rrn3 complex. In contrast, further promoter contacts with the Pol I cleft or downstream elements and/or A49 appear not to be required for recruitment.

TFIIB-related elements in Rrn7 adopt divergent positions. The TFIIB-related ‘reader’ and ‘linker’ elements within Rrn7 are mostly ordered in the active center cleft of the eiPIC, with the exception of the residues 46–56 (B-reader homologous). The protein backbone extends from the N-terminal zinc ribbon into the Pol I cleft, apparently trapping the well-ordered ‘lid’ sub-domain of Pol I subunit A190 before forming two anti-parallel strands and exiting the Pol I upstream face on the side of the shelf module (Fig. 3a). The path of Rrn7 differs from a Pol I ITC and from TFIIH in complex with Pol II (Supplementary Fig. 5). During Pol II initiation, the TFIIH-reader-loop contacts the ‘rudder’ and the ‘fork loop I’ domains, while the TFIIH-linker binds the top of the rudder and forms a helix that interacts with the clamp core domain. In the eiPIC, rudder and fork loop I apparently interact neither with each other nor with the TFIIH-reader-homologous regions of Rrn7. Instead, rudder and fork loop I are oriented towards the bridge helix and an Rrn7 helix that is similar to the TFIIH linker contacts to CF module II.

In addition to a divergent path of Rrn7 compared to TFIIH, the residues contacting the template strand in a Pol I ITC and Pol II ITC are mostly flexible in the eiPIC, but not in Pol II CCs or in a Pol II–TFIIH complex. Furthermore, TFIIH reader-loop arginine residue 78, which is important for TSS selection by Pol II, does not exist in Rrn7. This adds to overall sequence and architecture differences between Rrn7 and TFIIH.

To clarify the importance of Rrn7 loop residues disordered in the eiPIC, we mutated the entire loop or smaller stretches and analyzed CF initiation activity in a basal assay (Fig. 3b). The loop-deletion Rrn7 mutant shows strongly reduced initiation efficiency, which can mainly be attributed to the residues 51–56, but not to residues 43–50. The Rrn7 version with loop-deletion still assembles well with Rn6 and Rn11 and is able to form a basal PIC in vitro (Supplementary Fig. 3d, e). Thus, the Rrn7-reader-loop is likely important for promoter melting.

Pol I is primed for initiation at the eiPIC stage. Modeling of the active center based on our eiPIC density indicates, that aspartate 629 in subunit A190 (Asp483 in Pol II subunit Rpb1) has apparently changed its orientation with respect to the dimeric crystal structures. Assuming its active orientation in the eiPIC, Asp629 now allows coordination of the catalytic magnesium ion (‘metal A’), together with Asp627 and Asp631 for which we observe a clear cryo-EM density peak (Fig. 4b). In addition, the hybrid-binding domain of subunit A135 re-arranges to form a one-turn helix in the eiPIC. This helix also resembles the active Pol I, II, and III EC conformations and its formation exposes histidine 1038 to the bottom of the cleft, which is now free to contact the hybrid upon initial transcription as observed in ITCs. Furthermore, the previously buried lysines 462 and 463 in subunit A190 become exposed in the eiPIC (Supplementary Fig. 2f), now resembling the active Pol-II-fold and contacting the first visible downstream DNA base pair. This may contribute to a high affinity for foreign DNA and to the Pol I preference for initiation from ends of dsDNA. With the described structural changes upon eiPIC formation, Pol I enters a conformation that is primed for initial transcription via a conserved mechanism in the presence of NTPs.

We also observe, that the Pol I cleft continues to contract downstream of the sandwich region, adapting an intermediate conformation between the Rrn3-bound and ITC/actively elongating states (Fig. 4a). This adds an additional intermediate to the set of Pol I structures, but is in line with the suggestion, that cleft modulation is a major regulatory mechanism of Pol I.
transcription\textsuperscript{14,30,61}. At the stage of DNA-melting during the transition from CC to OC states, dsDNA cannot be accommodated between clamp core and protrusion domains any longer\textsuperscript{15}. Hence, simultaneous promoter loading and cleft contraction allosterically destabilize the upstream duplex at the position of the clamp core and may foster spontaneous melting at this position. Notably, the initially melted region shows the highest conservation among rDNA promoters identified thus far\textsuperscript{62}. Thus, the eiPIC apparently represents a trapped CC-OC transition intermediate conformation, which is important for spontaneous DNA-melting to take place during promoter association of the polymerase.

**Discussion**

Within this work, we describe an early intermediate initiation complex. The structure enables the independent discussion of promoter recruitment and DNA-melting in a sequential manner. Apparently the polymerase is recruited to its dsDNA promoter but cannot complete the melting process due to a lack of fixed downstream DNA. We described the eiPIC reconstruction in the context of PIC formation and continue to update our model of Pol I recruitment and DNA-melting in light of these findings. Our interpretation is well in line with the idea that targeting of the initiation machinery to the rDNA promoter depends mostly on UAF, and TBP serves to position CF downstream of the UE, while interacting with the promoter using a divergent interface\textsuperscript{63}. Recruitment of the Pol-I-Rrn3 complex then relies on a specific DNA architecture\textsuperscript{64}, namely a bendability that allows interactions of the Rrn11 TPR domain with the Pol I protrusion\textsuperscript{15} and binding of a promoter element to the Pol I sandwich region (Supplementary Fig. 4). Since our assembly originally comprised UAF and TBP, and only a single reconstruction was obtained from 39% of all recorded particles, it is likely that we capture a physiologically relevant conformation, while factors were artificially positioned by DNA/RNA hybrid scaffolds simulating initial transcription in previous analyses\textsuperscript{15–17}, even though RNA was lost in one case\textsuperscript{16}.

Within the eiPIC structure, re-arrangements between CF module I and II enable Rrn7 and Rrn11 to bind promoter DNA, mainly by phosphate backbone interactions of basic loops. This explains the (low) sequence specificity of DNA-binding by CF and thus the overall similar eiPIC architecture compared to ITCs and late PIC reconstructions. Likely, Rrn7-specific
DNA-interacting loops contribute to DNA-conformational modulation (compare Fig. 2). We further confirm cleft contraction between the protrusion and clamp core domains and exposure of basic residues bottom of the cleft during DNA-melting by Pol I in the eiPIC. While our findings do not oppose the idea of an upstream ratcheting mechanism to open Pol I promoter DNA, we also see no evidence to support such a mechanism deduced from shifts in CF-positions observed in ITC reconstructions.

Instead, we propose a simplified melting-mechanism based on sterically DNA-distortion and electrostatic single-strand trapping which, in this combination, is only possible in Pol I, but not in Pol II and III. Firstly, Pol I recruitment relies on DNA-duplex binding to the sandwiching region and DNA-positioning within the expanded cleft of the Pol-I-Rrn3 complex (Fig. 4a and Supplementary Fig. 4). Sequence specificity is determined by proximal upstream bendability and distal upstream recognition by UAF, which is linked to the PIC via CF and TBP. Divergent TFIIIB reader-loop elements within Rrn7 are placed in the Pol I cleft, may play a role in duplex-destabilization, and bind the melted template strand similar to observations in ITCs.

In addition, allosteric duplex-destabilization resulting from a cleft contraction between the clamp and protrusion domains observed in the eiPIC likely contributes to melting (Fig. 4a). This contraction primes Pol I for initial transcription by re-ordering previously inactivated regions (Figs. 1 and 4 and Supplementary Fig. 2). Exposed basic residues can then contribute to stabilization of the initially melted template strand and ultimately the DNA/RNA hybrid at the bottom of the cleft. Furthermore, the non-template strand may be bound by the A49 linker (as observed in ref. 1), thereby preventing collapse of the early bubble similar to the σ-factor in bacterial Pol66,67. Only after initial transcription, the growing RNA chain can interact with Rrn7 and would finally clash with reader/linker elements, freeing the exit channel and expelling Rrn7 from the polymerase. This is probably concerted with the association of the flexible A49 twh domain at the back of the clamp core domain, leading to dissociation of CF and Rrn3 and preventing re-association, thereby fostering promoter escape.

In Pol II and Pol III initiation complexes, TFIIIB/Brf1 cyclin domains occlude the sandwiching region and reader/linker domains diverge from Rrn7, preventing a similar mechanism. Arguing for a model of combined adaptations, a number of CF-mutations impaired in vitro initiation rates, but only large deletions completely abolished functionality15,45. Furthermore, a 12-subunit Pol I lacking A49/A34.5 is still able to initiate from its native promoter (although the lack of A49 linker-positioning strongly impaired the process)13,37. TBP is not necessary for basal transcription11,24 and single A49 mutations have only minor effects on Pol I function69. Thus, the overall functionality of the system is robust and highly adaptive to conditional variations. However, full initiation rates required for physiological growth depend on the combined action of all Pol-I-specific elements that have accumulated throughout evolutionary adaptation and are basically conserved throughout eukaryotic organisms10,70,71. These adaptations increase initial transcription to such efficiency, that formation of a stable closed complex under physiological conditions appears unlikely. While such a state may be transiently established, the instant cleft contraction and Rrn7-dependent duplex-destabilization by the combined action of Pol I and CF elements directly lead to melting and prime the polymerase for initial transcription and hybrid stabilization.

During the final stages of revision of this work, a related study was published72. Sadian et al. provide an excellent description of CF-promoter contacts in detail and investigate the role of an acidic loop in Rrn3, based on higher resolution reconstructions. Compared to our results, interpretation relies on a minor subset of 0.7% or 0.5% of particles from two datasets indicating a transient nature of CCs. In our UAF/TBP-containing samples, however, 39% of initial particles contribute to the final reconstruction and divergent CF-positions are not observed. This may be due to a lack of available particles in our datasets, or due to stabilization of a more ‘native’ CF-orientation in the presence of UAF/TBP. Detailed structure-function analysis of UAF- and TBP-contributions are now instrumental to understanding the process of Pol I initiation in its entirety.

Methods

Protein expression and purification

Partially purified endogenous Saccharomyces cerevisiae Pol I is a by-product of Pol III purification via a TAP-tag on subunit A4960. The Pol-I-containing MonoQ fractions were a gift from A. Vannini and G. Abascal-Palacios. Fractions were pooled, diluted fivefold in buffer A (20 mM HEPES/KOH pH 7.8, 10% glycerol, 1 mM MgCl2, 10 mM ZnCl2, 5 mM dithiothreitol (DTT)) and loaded onto a MonoS HR 5/5 column (GE Healthcare). Separation was performed with a gradient from 10–37.5% buffer B (buffer A with 2 M KAc) to a 2 CV plateau at 17.5% B. Pol I eluted at 470 mM KAc59, peak fractions were pooled, flash frozen in liquid nitrogen and stored at –80 °C. A supernatant was further purified by anion exchange chromatography (Mono Q 5/50, GE Healthcare). The column was equilibrated in MonoQ buffer 1 (50 mM HEPES at pH 7.8, 5 mM DTT, 10% glycerol), and proteins were eluted with a linear gradient of 20 column volumes from 0.1 mM to 1 M NaCl. After concentration (Amicon, 35 kDa cutoff), the sample was applied to a Superdex 200 increase 10/300 size exclusion column (GE Healthcare), equilibrated with buffer Rrn3-SEC (20 mM HEPES at pH 7.8, 300 mM NaCl 5 mM DTT).

CF subunits were co-expressed in E. coli BL21-CodonPlus(DE3)-RIL cells (Agilent) from two plasmids. A 41 culture was grown in LB medium at 37 °C until OD600 reached 0.5–0.7. Cultures were cooled on ice for 20 min and expression was induced with 0.1 mM IPTG. Cells were grown at 18 °C overnight. Cells were harvested by centrifugation, washed with phosphate-buffered saline (PBS) at 4 °C, flash frozen in liquid nitrogen and stored at –80 °C. One pellet was suspended in buffer CF-A (20 mM imidazole, 350 mM NaCl, 10 mM MgCl2, 10% (v/v) glycerol, 20 mM HEPES pH 7.8, 1 mM DTT, 1x protease inhibitor). Cells were lysed by sonication using a Branson Digital Sonifier, the lysate was centrifuged and the supernatant was filtered with a 0.22 μm filter (Millipore) to remove cell debris. Cell lysate was then applied to a Ni-NTA column (3 mL, GE Healthcare) and bound CF washed with 5 CV of buffer CF-B (25 mM imidazole, 200 mM NaCl, 10 mM MgCl2, 10% (v/v) glycerol, 20 mM HEPES pH 7.8, 1 mM DTT) at 4 °C. The column was then transferred to room temperature, washed with buffer CF-C (50 mM imidazole, 200 mM NaCl, 10 mM MgCl2, 10% (v/v) glycerol, 20 mM HEPES pH 7.8, 1 mM DTT, 5 mM ATP, 2 mg/ml denatured protein), incubated for 10 min, and washed again with 2.5 CV buffer CF-C. The column was transferred to 4 °C and washed with 5 CV buffer CF-D (50 mM imidazole, 200 mM NaCl, 10 mM MgCl2, 10% (v/v) glycerol, 20 mM HEPES pH 7.8, 1 mM DTT). Elution was performed with 5 CV of buffer CF-E (350 mM imidazole, 200 mM NaCl, 10 mM MgCl2, 10% (v/v) glycerol, 20 mM HEPES pH 7.8, 1 mM DTT). Protein was then loaded on a 5 ml heparin column (GE Healthcare) in buffer CF-F (200 mM NaCl, 1 mM MgCl2, 10% (v/v) glycerol, 20 mM HEPES pH 7.8, 1 mM DTT) and eluted with 30 CV of buffer CM from a Superase 12 column (GE Healthcare) at 80 °C. CF-containing fractions were concentrated using a 100 kDa cutoff centrifugal filter (Millipore). Size exclusion chromatography was carried out with a Superose 6 increase 10/300 column (GE Healthcare) in buffer CF-G (200 mM NaCl, 1 mM MgCl2, 5% (v/v) glycerol, 10 mM HEPES pH 7.8, 10 mM ZnCl2, 1 mM DTT). CF-containing fractions were concentrated using a 100 kDa cutoff centrifugal filter (Millipore) and directly used or flash frozen in liquid nitrogen for storage at –80 °C.

S. cerevisiae TBP was cloned into vector pET28b via NheI/NotI restriction sites (compare Supplementary Table 1). Recombinant His6-TBP protein was expressed in BL21(DE3) pRIL (Agilent) cells, by autoinduction in TB medium (1.2% tryptone; 2.4% yeast extract; 0.5% glycerol; 1/10 volume of a sterile solution containing 0.17 M KH2PO4 and 0.72 M K2HPO4 and 1/50 volume of a sterile solution containing 25% glycerol; 10% lactose and 1% glucose were added. A culture was grown at 37 °C to an OD600 of 0.6, after cooling the culture on ice, 1x induction was continued at 16 °C overnight. Cells were harvested (6000 g, 10 min), resuspended in lysis buffer (50 mM HEPES/KOH, 10% glycerol; 10 mM MgAC2; 200 mM KCl; 10 mM imidazole; 5 mM β-mercaptoethanol; 1 mM DTT).
phenylisothiocyanate (PMTS); 2 mM benzamidine), and lysed by sonication (Branson Sonifier 250 macrotip, cooling in ice-water). The cell extract was loaded on a pre-run 6% native acrylamide gel in 0.5x TBE buffer and run at 200 V. The gel was stained with Coomassie blue R250 macrotip, cooling in ice-water). The cell extract was loaded on a pre-run 6% native acrylamide gel in 0.5x TBE buffer and run at 200 V. The gel was stained with Coomassie blue.
was refined using the real-space refinement tool of the Phenix suite and evaluated using MolProbity. Figures were prepared with UCSF Chimera or PyMOL.
46. Naidu, S., Friedrich, J. K., Russell, J. L. & Zomerdijck, J. C. B. M. TAF1B is a TFII B-like component of the basal transcription machinery for RNA polymerase II. Science 313, 1640–1642 (2001).

47. Nikulov, D. B. et al. Crystal structure of TFII B-TBP-TATA-element ternary complex. Nature 377, 119–128 (1995).

48. Vorländer, M. K., Khatter, H., Wetzel, R., Hagen, W. J. H. & Müller, C. W. Molecular mechanism of promoter opening by RNA polymerase III. Nature 553, 295–300 (2018).

49. Abascal-Palacios, G., Ramsay, E. P., Beuron, F., Morris, E. & Vannini, A. Structural basis of RNA polymerase III transcription initiation. Nature 553, 301–306 (2018).

50. Cramer, P., Bushnell, D. A. & Kornberg, R. D. Structural basis of transcription: RNA polymerase II at 2.8 Ångstrom resolution. Science (N. Y., N. Y.) 292, 1873–1876 (2001).

51. Hoffmann, N. A. et al. Molecular structures of unbound and transcribing RNA polymerase III. Nature 528, 231–236 (2015).

52. Dieremann, C., Schwalb, B., Schilbach, S. & Cramer, P. Promoter distortion and opening in the RNA polymerase II clef. Mol. Cell 73, 97–106.e4 (2019).

53. He, Y. et al. Near-atomic resolution visualization of human transcription promoter opening. Nature 533, 359–365 (2016).

54. Gouveia, J. et al. Redox signaling by the RNA polymerase III TFII B-related factor Brf2. Cell 163, 1375–1387 (2015).

55. Han, Y., Yan, C., Fischbain, S., Ivanov, I. & He, Y. Structural visualization of RNA polymerase III transcription machineries. Cell Discov. 4, 40 (2018).

56. Kostrewa, D. et al. Crystal Structure of RNA Polymerase II in Complex with TFII B (RCSB PDB, 2009).

57. Sainsbury, S., Niesser, J. & Cramer, P. Structure and function of the initially sharing their by-product polymerase I. Data were collected at the IGBMC cryo-EM facility (Strasbourg, France). M.P. and C.E. were supported by Deutsche Forschungsgemeinschaft SFB 960 and the Emmy-Noether Programm (DFG grant no. EN 1204/1-1 to C.E.).

58. Author contributions M.P. carried out experiments and data analysis. C.E. designed and supervised research. M.P. and C.E. carried out model building and prepared the manuscript.

59. Competing interests The authors declare no competing interests.

60. Additional information Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-15052-y.

61. Correspondence and requests for materials should be addressed to C.E.

62. Peer review information Nature Communications thanks Dong Wang and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

63. Reprints and permission information is available at http://www.nature.com/reprints

64. Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020