

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒ | ☐ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection Serial EM 3.8 beta 8

Data analysis RELION 3.0.5, UCSF ChimeraX 1.2.5, Coot 0.9, Warp 1.0.7, PHENIX 1.19, cryoSPARC 2.14.2, ImageJ 1.53

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The electron density reconstructions and final models were deposited with the EM Data Base (accession code: EMD-14927, 14928 and 14929) and with the Protein Data Bank (accession code 7ZS9, 7ZSA and 7ZSB). All mass spectrometry raw files were deposited to the ProteomeXchange Consortium (<https://www.proteomexchange.org/>) via the PRIDE52 partner repository with the dataset identifier PRIDE: PXD029840. Source data are provided with this paper.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Cryo-EM micrographs were collected until about 4-5 millions particles were autopicked by Warp for each dataset
Data exclusions	No data were excluded from the analyses.
Replication	All attempts at replication were successful. Cryo-EM single particle analysis inherently relies on averaging over a large number of independent observations. The transcription assays were carried out with at least 3 independent replicates.
Randomization	Samples were not allocated to groups.
Blinding	Blinding is not a common practice for cryo-EM studies.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field work?	☐ Yes ☐ No

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access & import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

Methods

n/a	Involved in the study	n/a	Involved in the study
☒	☐ Antibodies	☒	☐ ChIP-seq
☒	☐ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☒	☐ Human research participants		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		

Antibodies

Antibodies used	Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.
Validation	Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

Palaeontology and Archaeology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

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All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☐	☐ Public health
☐	☐ National security
☐	☐ Crops and/or livestock
☐	☐ Ecosystems
☐	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☐	☐ Demonstrate how to render a vaccine ineffective
☐	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐ Increase transmissibility of a pathogen
☐	☐ Alter the host range of a pathogen
☐	☐ Enable evasion of diagnostic/detection modalities
☐	☐ Enable the weaponization of a biological agent or toxin
☐	☐ Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session

(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

- Sample preparation *Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
- Instrument *Identify the instrument used for data collection, specifying make and model number.*
- Software *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
- Cell population abundance *Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
- Gating strategy *Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*
- ☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

- Design type *Indicate task or resting state; event-related or block design.*
- Design specifications *Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
- Behavioral performance measures *State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

- Imaging type(s) *Specify: functional, structural, diffusion, perfusion.*
- Field strength *Specify in Tesla*
- Sequence & imaging parameters *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
- Area of acquisition *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*
- Diffusion MRI ☐ Used ☐ Not used

Preprocessing

- Preprocessing software *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
- Normalization *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
- Normalization template *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
- Noise and artifact removal *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See [Eklund et al. 2016](#))

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a | Involved in the study

- ☐ ☐ Functional and/or effective connectivity
☐ ☐ Graph analysis
☐ ☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

Peer Review Information

Journal: Nature Structural & Molecular Biology

Manuscript Title: Structures of transcription preinitiation complex engaged with the +1 nucleosome

Corresponding author name(s): Professor Patrick Cramer

Editorial Notes:

Transferred manuscripts	This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments, rebuttal and decision letters for versions considered at Nature XX.
Transferred manuscripts (no peer review at Nature XX)	This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. The manuscript was considered suitable for publication without further review at Nature XX.
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Redactions – reviewer opt-out	Parts of this Peer Review File have been redacted as indicated as we could not obtain permission to publish the reports of reviewer no. XX.
Reviewer comments in marked-up manuscript	In their review of the [first/second/third/...] version of this manuscript, reviewer no. XX added their comments to the manuscript file. These comments, excluding minor textual revisions, have been copied into this Peer Review File.

Reviewer Comments & Decisions:

EA, duplicate for each version as needed, and then delete this instruction.

Decision Letter, initial version:
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Message: Our ref: NSMB-A46269-T

19th Jul 2022

Dear Dr. Cramer,

Thank you for submitting your revised manuscript entitled "Structures of transcription preinitiation complex engaged with the +1 nucleosome" (NSMB-A46269-T). It has now been seen by the original referees and their comments are below. The reviewers raise some pertinent concerns but overall they find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Structural & Molecular Biology, pending revisions to fully satisfy the referees' final requests and to comply with our editorial and formatting guidelines. No additional experiments are required but we think that some of the claims/interpretations need to be toned down. We may need to consult with the reviewers one final time to make sure that they are satisfied with the revised manuscript.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about two weeks. Please do not upload the final materials and make any revisions until you receive this additional information from us. In the meantime, however, we encourage you to start addressing the reviewers' comments.

Thank you again for your interest in Nature Structural & Molecular Biology. Please do not hesitate to contact me if you have any questions.

Sincerely,

Tiago

Tiago Faial, PhD
Consulting Editor
Nature Structural & Molecular Biology

Reviewer #1 (Remarks to the Author):

The revised paper from Wang et al. contains two additional structures that give more

context to the structure (now Complex B) from the earlier version. This is helpful in that it shows the +1 nucleosome is not fixed relative to the PIC, validating the concern I raised in my first review. I have no technical issues with the work here, which lives up to the high standards of the Cramer lab. However, I still think the authors' interpretations go beyond what the data really show.

They still claim they've shown "intimate interactions" (abstract) between TFIID and the +1 nucleosome that "stabilize the PIC" (line 199). However, complexes A and B have completely different orientations and contact points (really just proximities, given the low resolutions). Complexes B and C only form after TFIID is already incorporated into the PIC and beginning to translocate. What effect would any PIC stabilization have at this late point? If anything, the inhibition of transcription suggests collision with the nucleosome destabilizes the PIC to ITC conversion by impeding progression to an open complex. The +1 nucleosome might have a positive effect via TFIID bromodomain interactions (which is the mechanism suggested in reference 11), but in the context shown here it clearly has a negative effect.

Multiple reviewers suggested that mutagenesis of the proposed TFIID contact points could help test the authors' model, but the authors say those experiments aren't feasible. Without functional validation, the contact points could just be where TFIID and the nucleosome collide as the translocase begins to pull in the DNA. In their reply, the authors seem to be moving in this direction ("we do not think that TFIID specifically recognizes the nucleosome, rather, it serves as a docking site that restricts nucleosome orientation"), but this doesn't come across in the paper, which seems to suggest a stable interaction.

The observation that Complex C, with ten extra base pairs, shows a similar TFIID-nucleosome orientation as Complex B could be significant, or it might just be because the non-native Widom 601 positioning sequence is only easily unraveled up to this point (as the authors suggest in the last sentences of the discussion). With only the current data, I don't think you can claim this is "a stable intermediate state that gets populated during the initiation phase". It could be one of many transient intermediate rotational positions of the nucleosome that we don't see, artifactually stabilized by the 601 sequence and crosslinking.

Line 201. I don't know of any data suggesting that TFIID activity is affected by histone modifications. Is there any basis for this speculation? Would this be a direct or indirect effect?

Is the slight TFIID rotation in Complex B similar to that reported by Murakami in the PIC species leading to ITC (Yang et al., Mol Cell 2022)? If so, that would be worth noting.

In sum, I think the work here is technically impressive, but the authors are stuck on a model of a positive TFIID-nucleosome interaction. There's no functional data to support this positive model, and the *in vitro* transcription inhibition even argues against it. The structures are interesting and worth publishing if the authors were more careful in their interpretation.

Reviewer #2 (Remarks to the Author):

The authors addressed in a satisfactory way all my comments and suggestions. They provide additional structures that confirm their hypothesis and strengthen the manuscript. In my opinion this work is ready to be published in NSMB.

Reviewer #3 (Remarks to the Author):

In this paper, Wang et al. present the structure of the PIC with an adjacent +1 nucleosome. They determined three structures that are potential intermediates in the collision of the PIC with the +1 nucleosome. The results are interesting and thought provoking, but the authors' many claims need further studies to determine. To dissect this, I will give comment on several of the major claims in the discussion of their paper first. Then go through some the major issues I see in the results.

We report here the first structures of Pol II PIC-nucleosome transcription complexes.

- True, but this is only a partial PIC. Though the reason for this is provided by the author was that TFIID was too flexible in their attempts generate a complex. This could be interesting data to show in the paper.

The structures show that TFIID interacts with the nucleosome and that a preferred orientation of the nucleosome with respect to the PIC may exist that is characterized by multiple TFIID-nucleosome contacts.

- First the structure does appear to show multiple contact between TFIID and the nucleosome however it would seem that each structure appears to show different contacts and as such a "preferred orientation" would not appear to be true.

- Additionally, the contacts they do observe I question the importance of, they are not observed at high enough resolution to show any meaningful interaction (no specific interacting residue pairs can be seen).

These contacts suggest that the +1 nucleosome can help to stabilize the PIC via TFIID interactions and may explain how PIC formation can be influenced by the +1 nucleosome.

- Is the nucleosome-PIC interaction stable? The three states show different interactions. It would seem that the template used for the assembly of these complexes are a bigger factor for how the nucleosome will interact with the PIC.

The location of TFIID in the vicinity of histone tails might allow the regulation of TFIID activity by histone modifications.

- Is this a known phenomenon? Please give reference. I think this is the first time in the paper this is mentioned.

Although the nucleosome may initially be found at different rotational states with respect to the PIC, it is likely that action of the Ssl2 translocase leads to a preferred nucleosome orientation that allows for multiple TFIID contacts as observed here.

- Would it not be possible that if a differently spaced template was used that TFIID could also stabilize this interaction? Complex C used a 10 bp extended template that would give the nucleosome the same rotational state as complex B. Even still it appears that TFIID interact with the nucleosome in a different manner.

The observation that TFIID stops unravelling nucleosomal DNA at SHL -5 indicates that the translocase activity of TFIID is not enough to enable PIC to pass through the strong histone-DNA interacting sites (such as SHL -5 and -1) within nucleosome.

- Is this true in the both the A/B and C template containing structures? The comparison of Complex B and C needs to be expanded in the text and figures. It appears in Figure 5 that

the TFIIDH-nucleosome interactions are different.

Passage of PIC through the +1 nucleosome may require the help from chromatin remodelers and chromatin modifying complexes.

- While not a major point are the authors stating that a chromatin modifier directly helps the passage of the PIC through the +1 or that having a modified nucleosome would help?
- Secondly, would not the acetylation (if this is the type of modification the authors are thinking about) of the +1 typically precede the assembly of the PIC? This would lead to the bigger question as to whether a modified nucleosome would stall the PIC, given that acetylated nucleosome is thought to be slightly less stable than unmodified ones.

Together with modeling, the structures also provide an explanation for why the transcription start site (TSS) is often located ~60 bp upstream of the dyad of the +1 nucleosome in yeast.

- This is the first sentence of the second and last paragraph of the discussion. This claim is meant to be supported by the rest of this paragraph. I do not see how this claim is at all supported by data.

To initiate RNA chain synthesis, the yeast PIC scans downstream DNA for the TSS.

- While this may seem obvious, the how has never been determined. No one has yet shown the nature of this scanning and how the PIC can start at the TATA box and make its way to the TSS.
- What the authors capture is the beginning of this scanning, and to explain "why the transcription start site (TSS) is often located ~60 bp upstream of the dyad of the +1 nucleosome in yeast" they would need to show the TSS in the active site of the polymerase.

Modeling based on complex B indicates that scanning requires further progression of the PIC into the +1 nucleosome and detachment of three additional turns of DNA (Extended Data Fig. 10). This may be achieved by the ATP-dependent Ssl2 translocase that is required for scanning.

- Clearly this is not enough, or else you would see the promoter open.

We speculate that scanning may be impaired at the major nucleosomal barrier just upstream of the nucleosome dyad, which then may trigger TSS usage and RNA chain initiation as suggested.

- What do the authors mean by "major nucleosomal barrier"? The nucleosome itself or a specific DNA-octamer interaction (e.g. SHL -1)?

This model of nucleosome defined TSS usage may explain why TSSs in yeast are generally located at a distance of ~60 bp from the dyad of the +1 nucleosome, whereas the distance from the TSS to the TATA box varies in the range of ~40-120 bp.

- Yes, the model laid out can explain "why TSSs in yeast are generally located at a distance of ~60 bp from the dyad of the +1 nucleosome," but it does not explain why "the TSS to the TATA box varies in the range of ~40-120 bp." Like I stated before the "No one has yet shown the nature of this scanning and how the PIC can start at the TATA box and make its way to the TSS"

In our experimental system, the high stability of the nucleosome on the Widom-601 positioning sequence may have prevented scanning and led to a stable intermediate amenable to structure determination.

- Possibly, and could also have prevented the authors from explaining "why the transcription start site (TSS) is often located ~60 bp upstream of the dyad of the +1 nucleosome in yeast"

Future studies should focus on the roles of Mediator and TFIID in nucleosome-involved transcription initiation.

While the discussion section needs to be rewritten to better represent the actual finding of this paper. I also have a major concerns with the in vitro transcription assay results and the representation and description of the Complex C state in the results.

Concerns:

In Supplemental Figure 1 (the one that corresponds to Figure 1c) how is the length of the products determined? The gel does not appear to have a ladder. Additionally, FL product is presumably between 80-150bp and would mean that the TSS is locate within the Widom601 sequence. If this is the case, why then is the TSS the same for DNA and Nuc? Is it 60bp form the nucleosome dyad and when there is not nucleosome 60bp after the TATA? And these just happen to be the same place? What would occur with the complex C template? Previously results like from (Nagai et al. PNAS 2017: doi: 10.1073/pnas.1620312114) indicate that given the conditions of this experiment that there should be some product starting at ~30bp form the TATA.

In Supplemental Figure 1 (the one that corresponds to Extended Data Figure 1b) I have the same product size concern as the previous figure. The other concern I have is that the boxed lanes show a noticeable reduction in the band intensity for the right (Nuc) then any of the other bands. Presumably the other lanes are not just DNA and so which leads mean to wonder which are Nuc and why do they look no way near the intensity of the selected band. The in especially concerning given the supposed deviation of the band intensity is very low based on the circle values shown in Extended Data Figure 1b.

Comment:

A more detailed comparison is need for Complex B and C. Both in the text and in the Figure 4. In the text it is stated "overall orientation of the nucleosome with respect to the PIC in complex C resembled that observed for complex B." However this does not appear to be the case the nucleosome in the two complexes appear to engage TFIIH very differently. In complex B there appears to be a lot more contact then in complex C. If this is the case, then it would not support "similar PIC-nucleosome interaction can occur even with different initial PIC-nucleosome distances."

Author Rebuttal, first revision:

Responses to reviewer comments

manuscript NSMB-A46269-T

Structures of transcription preinitiation complex engaged with the +1 nucleosome

Wang, *et al.*

Responses are in italics.

Reviewer #1 (Remarks to the Author):

The revised paper from Wang et al. contains two additional structures that give more context to the structure (now Complex B) from the earlier version. This is helpful in that it shows the +1

nucleosome is not fixed relative to the PIC, validating the concern I raised in my first review. I have no technical issues with the work here, which lives up to the high standards of the Cramer lab. However, I still think the authors' interpretations go beyond what the data really show.

We thank the reviewer for the careful consideration. We have addressed the comments below.

They still claim they've shown "intimate interactions" (abstract) between TFIIH and the +1 nucleosome that "stabilize the PIC" (line 199). However, complexes A and B have completely different orientations and contact points (really just proximities, given the low resolutions). Complexes B and C only form after TFIIH is already incorporated into the PIC and beginning to translocate. What effect would any PIC stabilization have at this late point? If anything, the inhibition of transcription suggests collision with the nucleosome destabilizes the PIC to ITC conversion by impeding progression to an open complex. The +1 nucleosome might have a positive effect via TFIIID bromodomain interactions (which is the mechanism suggested in reference 11), but in the context shown here it clearly has a negative effect.

We observed intimate interactions between TFIIH and the +1 nucleosome in complex B. We agree with the reviewer that the +1 nucleosome may not stabilize the PIC directly and have removed this statement in discussion (line 199).

Multiple reviewers suggested that mutagenesis of the proposed TFIIH contact points could help test the authors' model, but the authors say those experiments aren't feasible. Without functional validation, the contact points could just be where TFIIH and the nucleosome collide as the translocase begins to pull in the DNA. In their reply, the authors seem to be moving in this direction ("we do not think that TFIIH specifically recognizes the nucleosome, rather, it serves as a docking site that restricts nucleosome orientation"), but this doesn't come across in the paper, which seems to suggest a stable interaction.

We do not claim that TFIIH stably interacts with the nucleosome but suggest that it restricts the rotation of the nucleosome when TFIIH and the nucleosome collide. We have clear evidence for this and made this clear in the discussion part.

The observation that Complex C, with ten extra base pairs, shows a similar TFIIH-nucleosome orientation as Complex B could be significant, or it might just be because the non-native Widom 601 positioning sequence is only easily unraveled up to this point (as the authors suggest in the last sentences of the discussion). With only the current data, I don't think you can claim this is "a stable intermediate state that gets populated during the initiation phase". It could be one of many transient intermediate rotational positions of the nucleosome that we don't see,

artificially stabilized by the 601 sequence and crosslinking.

We believe that the TFIIDH-nucleosome orientation we observed in complexes B and C represent an intermediate state since they represent the conformation of the majority of the intact particles in our samples.

Line 201. I don't know of any data suggesting that TFIIDH activity is affected by histone modifications. Is there any basis for this speculation? Would this be a direct or indirect effect?

We have deleted this speculation.

Is the slight TFIIDH rotation in Complex B similar to that reported by Murakami in the PIC species leading to ITC (Yang et al., Mol Cell 2022)? If so, that would be worth noting.

This is an interesting point, but no, they are different. We think the slight rotation of TFIIDH observed here is caused by the collision with the +1 nucleosome.

In sum, I think the work here is technically impressive, but the authors are stuck on a model of a positive TFIIDH-nucleosome interaction. There's no functional data to support this positive model, and the in vitro transcription inhibition even argues against it. The structures are interesting and worth publishing if the authors were more careful in their interpretation.

We thank the reviewer for the help with improving our manuscript and supporting its timely publication.

Reviewer #2 (Remarks to the Author):

The authors addressed in a satisfactory way all my comments and suggestions. They provide additional structures that confirm their hypothesis and strengthen the manuscript. In my opinion this work is ready to be published in NSMB.

We thank the reviewer again for the suggestions and support.

Reviewer #3 (Remarks to the Author):

In this paper, Wang et al. present the structure of the PIC with an adjacent +1 nucleosome. They determined three structures that are potential intermediates in the collision of the PIC with the +1 nucleosome. The results are interesting and thought provoking, but the authors' many claims need further studies to determine. To dissect this, I will give comment on several of the major claims in the discussion of their paper first. Then go through some the major issues I see in the results.

We thank the reviewer for the insightful comments. We have addressed all the comments and rewritten the discussion.

We report here the first structures of Pol II PIC-nucleosome transcription complexes.
 - True, but this is only a partial PIC. Though the reason for this is provided by the author was that TFIID was too flexible in their attempts generate a complex. This could be interesting data to show in the paper.

We agree it would be very interesting to also have included TFIID but for unknown reasons this is technically not feasible in the yeast system so far, despite many years of trials in our (and likely other) laboratories. We note the PIC we have in our structures is sufficient to direct promoter-dependent transcription initiation in the absence of TFIID and thus our structure is functionally relevant.

The structures show that TFIH interacts with the nucleosome and that a preferred orientation of the nucleosome with respect to the PIC may exist that is characterized by multiple TFIH-nucleosome contacts.

- First the structure does appear to show multiple contact between TFIH and the nucleosome however it would seem that each structure appears to show different contacts and as such a "preferred orientation" would not appear to be true.
- Additionally, the contacts they do observe I question the importance of, they are not observed at high enough resolution to show any meaningful interaction (no specific interacting residue pairs can be seen).

We have rewritten this part to make it clearer. Briefly, TFIH restricts free nucleosome rotation and leads to preferred orientations of the nucleosome when the PIC and nucleosome are in contact distance.

These contacts suggest that the +1 nucleosome can help to stabilize the PIC via TFIH

interactions and may explain how PIC formation can be influenced by the +1 nucleosome.

- Is the nucleosome-PIC interaction stable? The three states show different interactions. It would seem that the template used for the assembly of these complexes are a bigger factor for how the nucleosome will interact with the PIC.

We have removed this statement since there is no evidence to support that the +1 nucleosome can directly help to stabilize the PIC. Compare also the response to reviewer #1.

The location of TFIID in the vicinity of histone tails might allow the regulation of TFIID activity by histone modifications.

- Is this a known phenomenon? Please give reference. I think this is the first time in the paper this is mentioned.

We have removed this speculative comment. Compare also the response to reviewer #1.

Although the nucleosome may initially be found at different rotational states with respect to the PIC, it is likely that action of the Ssl2 translocase leads to a preferred nucleosome orientation that allows for multiple TFIID contacts as observed here.

- Would it not be possible that if a differently spaced template was used that TFIID could also stabilize this interaction? Complex C used a 10 bp extended template that would give the nucleosome the same rotational state as complex B. Even still it appears that TFIID interact with the nucleosome in a different manner.

This is true. In both complexes B and C, the captured states of the TFIID-nucleosome were after the action of the Ssl2 translocase. Even though that TFIID interacts with the nucleosome in a slightly different manner, the common feature is that TFIID uses the same region to contact the nucleosome, creating a barrier for further rotation of the +1 nucleosome.

The observation that TFIID stops unravelling nucleosomal DNA at SHL -5 indicates that the translocase activity of TFIID is not enough to enable PIC to pass through the strong histone-DNA interacting sites (such as SHL -5 and -1) within nucleosome.

- Is this true in the both the A/B and C template containing structures? The comparison of Complex B and C needs to be expanded in the text and figures. It appears in Figure 5 that the TFIID-nucleosome interactions are different.

Passage of PIC through the +1 nucleosome may require the help from chromatin remodelers and chromatin modifying complexes.

- While not a major point are the authors stating that a chromatin modifier directly helps the passage of the PIC through the +1 or that having a modified nucleosome would help?

- Secondly, would not the acetylation (if this is the type of modification the authors are thinking about) of the +1 typically precede the assembly of the PIC? This would lead to the bigger question as to whether a modified nucleosome would stall the PIC, given that acetylated nucleosome is thought to be slightly less stable than unmodified ones.

As shown in the structures of both complexes B and C (in the presence of NTP), TFIID alone does not allow efficient passage through the +1 nucleosome. We agree with the reviewer that it will be interesting to test whether an acetylated nucleosome will help the passage of PIC but we have no means to test this in a timely manner.

Together with modeling, the structures also provide an explanation for why the transcription start site (TSS) is often located ~60 bp upstream of the dyad of the +1 nucleosome in yeast.

- This is the first sentence of the second and last paragraph of the discussion. This claim is meant to be supported by the rest of this paragraph. I do not see how this claim is at all supported by data.

To initiate RNA chain synthesis, the yeast PIC scans downstream DNA for the TSS.

- While this may seem obvious, the how has never been determined. No one has yet shown the nature of this scanning and how the PIC can start at the TATA box and make its way to the TSS.

- What the authors capture is the beginning of this scanning, and to explain “why the transcription start site (TSS) is often located ~60 bp upstream of the dyad of the +1 nucleosome in yeast” they would need to show the TSS in the active site of the polymerase.

We have tried to explain things better so there is no misunderstandings. The issue is that the barrier to Pol II caused by the nucleosome is well-characterized over the years and in the context of our structures may explain how Pol II progression during scanning is counteracted, and this may lead to usage of a TSS. We think the proposed mechanism is a reasonable model that is consistent with our structures we present. Elucidating the very complicated mechanism of promoter scanning is clearly beyond the scope of this work. But it would be a pity if we are not allowed to speculate on how the nucleosome may influence scanning and the selection of the start site.

Modeling based on complex B indicates that scanning requires further progression of the PIC into the +1 nucleosome and detachment of three additional turns of DNA (Extended Data Fig. 10). This may be achieved by the ATP-dependent Ssl2 translocase that is required for scanning.

- Clearly this is not enough, or else you would see the promoter open.

We speculate that scanning may be impaired at the major nucleosomal barrier just upstream of

the nucleosome dyad, which then may trigger TSS usage and RNA chain initiation as suggested.

- What do the authors mean by “major nucleosomal barrier”? The nucleosome itself or a specific DNA-octamer interaction (e.g. SHL -1)?

This model of nucleosome defined TSS usage may explain why TSSs in yeast are generally located at a distance of ~60 bp from the dyad of the +1 nucleosome, whereas the distance from the TSS to the TATA box varies in the range of ~40-120 bp.

- Yes, the model laid out can explain “why TSSs in yeast are generally located at a distance of ~60 bp from the dyad of the +1 nucleosome,” but it does not explain why “the TSS to the TATA box varies in the range of ~40-120 bp.” Like I stated before the “No one has yet shown the nature of this scanning and how the PIC can start at the TATA box and make its way to the TSS”

In our experimental system, the high stability of the nucleosome on the Widom-601 positioning sequence may have prevented scanning and led to a stable intermediate amenable to structure determination.

- Possibly, and could also have prevented the authors from explaining “why the transcription start site (TSS) is often located ~60 bp upstream of the dyad of the +1 nucleosome in yeast” Future studies should focus on the roles of Mediator and TFIID in nucleosome-involved transcription initiation.

It is indeed possible that some not yet determined factors may be required to help the PIC to scan the promoter for a TSS. We have clarified in the text the use of the term “major nucleosomal barrier” to refer to specific DNA-octamer interactions within the nucleosome (e.g. SHL -1).

While the discussion section needs to be rewritten to better represent the actual finding of this paper. I also have a major concerns with the in vitro transcription assay results and the representation and description of the Complex C state in the results.

Concerns:

In Supplemental Figure 1 (the one that corresponds to Figure 1c) how is the length of the products determined? The gel does not appear to have a ladder. Additionally, FL product is presumably between 80-150bp and would mean that the TSS is locate within the Widom601 sequence. If this is the case, why then is the TSS the same for DNA and Nuc? Is it 60bp form the nucleosome dyad and when there is not nucleosome 60bp after the TATA? And these just happen to be the same place? What would occur with the complex C template? Previously results like from (Nagai et al. PNAS 2017: doi: 10.1073/pnas.1620312114) indicate that given the conditions of this experiment that there should be some product starting at ~30bp form the TATA.

In Supplemental Figure 1 (the one that corresponds to Extended Data Figure 1b) I have the same product size concern as the previous figure. The other concern I have is that the boxed

lanes show a noticeable reduction in the band intensity for the right (Nuc) then any of the other bands. Presumably the other lanes are not just DNA and so which leads mean to wonder which are Nuc and why do they look no way near the intensity of the selected band. The in especially concerning given the supposed deviation of the band intensity is very low based on the circle values shown in Extended Data Figure 1b.

We have made multiple edits to the discussion and hope the reviewer is satisfied. The length of the product was determined based on an RNA ladder on the other gel run under the same conditions. As shown in Fig. 1a, the TSS locates within the Widom 601 sequence. The same sequence was used in in-vitro transcription experiments for naked DNA (DNA) or nucleosome-containing (Nuc) template. Even though there is a potential TSS ~30 bp from the TATA in our template, we didn't observe a clear product starting from this site. A potential explanation for this observation could be that the promoters used in our experiment and their paper are different (His4 vs. Pho5). On the gel that corresponds to Extended Data Figure 1b, the boxed lanes are one of three replicate experiments we carried out on different dates. The other lanes were the same templates tested with other variations (the four lanes to the left) or just DNA (the two lanes to the right). The deviation of the band intensity was calculated between replicates done on different dates. We made sure these things are described correctly in the legend.

Comment:

A more detailed comparison is need for Complexes B and C. Both in the text and in the Figure 4. In the text it is stated "overall orientation of the nucleosome with respect to the PIC in complex C resembled that observed for complex B." However this does not appear to be the case the nucleosome in the two complexes appear to engage TFIIH very differently. In complex B there appears to be a lot more contact then in complex C. If this is the case, then it would not support "similar PIC-nucleosome interaction can occur even with different initial PIC-nucleosome distances."

We have added a more detailed comparison between Complexes B and C in text and Fig. 5. From the new Fig. 5b, it is clear to see a similar orientation between TFIIH and nucleosome in both complex B and C even though the detailed PIC-nucleosome interaction is partially different.

Final Decision Letter:

Message 7th Oct 2022

:

Dear Dr. Cramer,

We are now happy to accept your revised paper "Structures of transcription preinitiation complex engaged with the +1 nucleosome" for publication as a Article in Nature Structural

& Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Structural & Molecular Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately.

You will not receive your proofs until the publishing agreement has been received through our system.

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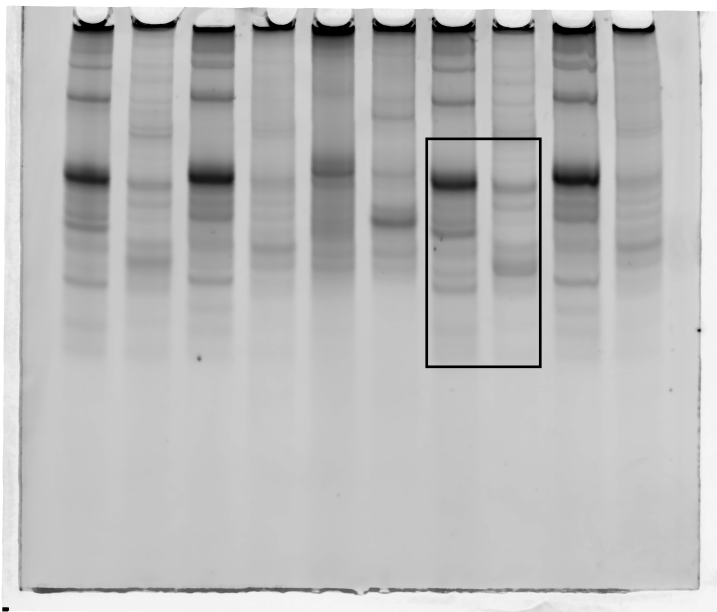
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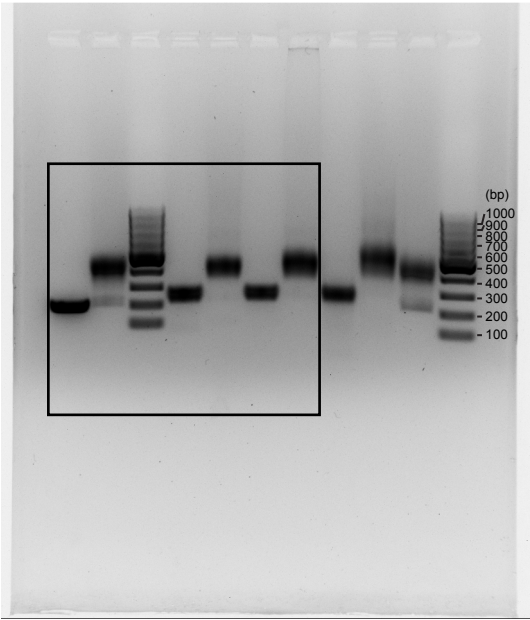
Kind regards,
Florian

Dr Florian Ullrich
Associate Editor, Nature
Consulting Editor, Nature Structural & Molecular Biology
ORCID 0000-0002-1153-2040

Uncropped scanned gel (cf. Figure 1c).



Uncropped scanned gel (cf. Extended Data Figure 1a).



Uncropped scanned gel (cf. Extended Data Figure 1b).

