

Peer Review Information

Journal: Nature Structural and Molecular Biology

Manuscript Title: Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states

Corresponding author name(s): Christoph Muller

Reviewer Comments & Decisions:

Decision Letter, initial version:
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4th Sep 2020

Dear Christoph,

Thank you again for submitting your manuscript "Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states". I apologize for the delay while we awaited the comments (copied below) from the 2 reviewers who evaluated your paper. In light of those reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

I hope you will be pleased to see that both reviewers are quite positive about the interest of the findings, and that Reviewer #1, with expertise in pol III function, and finds the manuscript suitable for publication in its current form. Reviewer #2, our cryo-EM expert, offers suggestions for figure presentation and discussion to make aspects of the structural features more clear to readers; editorially, we agree these edits will improve the work and ask that they be incorporated in a revised manuscript.

Please be sure to address/respond to all concerns of the referee in full in a point-by-point response and highlight all changes in the revised manuscript text file. If you have comments that are intended for editors only, please include those in a separate cover letter.

When revising the manuscript, please bear in mind these guidelines for our Article format:

- Abstract should be under 150 words, with no references
- main text is typically between 3,000 and 4,000 words, and should be organized as introduction, results (with subheadings) and discussion.
- display items (figures and tables): typically between 6 and 8. Please note that the structural table

should be in main article.

- supplementary items: Supplementary Figures should be under 10; other supplementary items are Suppl Table, Note, Video, Data Set.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We expect to see your revised manuscript within 6 weeks. If you cannot send it within this time, please contact us to discuss an extension; we would still consider your revision, provided that no similar work has been accepted for publication at NSMB or published elsewhere.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Please follow the links below to download these files:

Reporting Summary:

<https://www.nature.com/documents/nr-reporting-summary.pdf>

Please note that the form is a dynamic 'smart pdf' and must therefore be downloaded and completed in Adobe Reader.

When submitting the revised version of your manuscript, please pay close attention to our [href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:](https://www.nature.com/nature-research/editorial-policies/image-integrity)

- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
- that control panels for gels and western blots are appropriately described as loading on sample processing controls
- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

If there are additional or modified structures presented in the final revision, please submit the corresponding PDB validation reports.

Data availability: this journal strongly supports public availability of data. All data used in accepted papers should be available via a public data repository, or alternatively, as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types,

deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:

<https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data>

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage.

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at

<http://www.nature.com/nsmb/authors/submit/index.html#costs>

Nature Structural & Molecular Biology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit www.springernature.com/orcid.

Please use the link below to submit your revised manuscript and related files:

[REDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

With kind regards,

Beth

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:

Girbig et al. have submitted a manuscript that describes structures of human RNA polymerase III in its free apo form and bound to DNA and RNA, mimicking the elongating state of the enzyme. The resolution of apo Pol III is 3.3 Å and elongating Pol III was resolved at 2.8 Å. Nucleic acid binding is mediated by the RPC1, RPC2 and RPABC1 subunits. The structures support an overall conservation of the yeast and human enzymes. However, important species-specific differences can be observed in the RPC3-RPC6-RPC7 heterotrimer and the RPC4-RPC5 heterodimer. The RPC4-RPC5 heterodimer cooperates with RPC10 in transcription termination by Pol III. The C-terminal zinc ribbon of RPC10 can adopt two conformations, one outside the polymerase funnel and one inside. Amino acids of the RPC1 linker in contact with RPC10 are not conserved in between yeast and human. Insertion of the zinc ribbon into the funnel coincides with open clamp formation which is important for RNA release and may favor reinitiation by facilitating the binding to the closed promoter. Promoter opening may then be enabled by the release of RPC10 from the active site. Structural differences of yeast and human RPC5 are mainly due to a 320 amino acid extension in the human protein, which fold into four winged helix domains, two of which are not visible in the structural reconstitution. WH1 and 2 can adopt two distinct conformations in the elongating enzyme, whereas WH3 and 4 seem to be mobile with possible roles in transcription initiation. The authors present the hypothesis that the WH domains co-evolved with BRF2 and SNAPc transcription factors.

In contrast to yeast RPC6, the human protein forms a 4Fe-4S cluster. Such a cluster was shown to be required for the interaction with TFE/IIEalpha transcription factors which show structural homologies to RPC3. Gribig et al. propose that an extension in yeast RPC3 may compensate for the 4Fe-4S cluster with respect to establishing a stable interaction of RPC3 and RPC6.

The RPC7 subunit assures the interaction of the RPC8-RPC9 stalk with the heterotrimer in apo Pol III and during transcription elongation. Interestingly, the N-terminal residues 10 to 33 interact with the RPC1 clamp coiled-coil domains overlapping with MAF1 binding sites. The authors propose that repression by MAF1 requires the replacement of RPC7alpha by RPC7beta.

Finally, Gribig et al. localize mutations in Pol III that cause neurodegenerative diseases and categorize them into four distinct types that affect the core of a subunit (type I), affect functional elements (type II), being located at the interface of subunits (type III) or at the surface with little contacts (type IV).

The manuscript provides a wealth of information that substantially contribute to a better understanding of human RNA polymerase III transcription. In particular, explanations of RPC4-RPC5, RPC10 and RPC7 functions by interpretation of structural data have been convincingly presented. In my opinion, the manuscript can be published as it is.

Reviewer #2:

Remarks to the Author:

The eukaryotic RNA Polymerase III transcribes tRNAs, some ribosomal and spliceosomal RNAs and is a central regulator of cell growth. The authors have provided structures of human RNA Polymerase III (Pol III) in apo and multiple elongation complex forms. These structures are informative with respect to the molecular mechanisms of Pol III transcription, and provides an important insight into Pol III's relationship to human disease by resolving the location of pathogenic mutations, and also by providing a testable hypothesis for tumour formation by RPC7alpha. The variations of the TFIIS-like subunit RPC10 are also interesting and have implications for RNA polymerases other than Pol III which utilise similar factors. This is an important advance in our understanding of RNA polymerase biology, and the work is clearly of high technical quality, hence it is suitable for publication in NSMB. I have these comments.

The multiple positions of RPC10 C-terminal domain are interesting but given their unusual positions (i.e. outside of the funnel, and within the funnel but not reaching the active site), they require further confidence in terms of depiction. Although Figs S5a,b show the density around the C-terminal domain in both positions, I found their fit fairly rough and their density possibly of lower resolution. I would ask the authors to show larger, isolated pictures of each domain position together with their densities. This is important, as these positions are unusual and require additional support, ideally by complementary methods but that is likely out of scope for this manuscript. Also, a comparison to C11 positions in yeast might also be worthwhile as I recall C11 C-terminal domain also adopting an unusual position outside of the funnel.

Given that the RPC10 acidic tip within the funnel doesn't quite reach the RNA, seeing its electron density, and its distance from the RNA should be shown in detail, as it could have implications beyond Pol III and be relevant for Pol I, Pol II and archaeal RNAPs. Similarly, was the proximity of the RPC10 acidic hairpin checked with backtracked RNA (which could be modelled from PolII-TFIIS, or bacterial RNAP structures)? I couldn't ascertain this from the inset figure panels of Fig S6b & d, which are far too small and indistinct for my eyes.

Does the acidic tip fail to reach the RNA because the entire domain is bound less deeply in the funnel or because of a local conformational change within the domain/hairpin? This is an important distinction as it informs about the plasticity of RPC10 binding and/or its conformation, and might be relevant for TFIIS and related factors in archaea. A figure comparing the position of the A12.2/TFIIS/RPC10 C-terminal domain after superposition of surrounding polymerase domains might be informative in this regard.

The proposition that RPC10 binding inside the funnel induces clamp opening and therefore assists with termination is compelling, but the nucleic acids are still present in their EC and needs reconciling with their theory. For example, does their EC represent the pre-termination complex? If so, does the DNA sequence contain a terminator? Similarly, the resolution of a 6bp hybrid is interesting, as most other RNAP structures from eukaryotes and bacteria resolve an 8-9bp hybrid which has often been thought to be the most stable length. Can the authors comment on why the visible hybrid is shorter?

Author Rebuttal to Initial comments

Point-by-Point Response

Reviewer #1 (Remarks to the Author):

The manuscript provides a wealth of information that substantially contribute to a better understanding of human RNA polymerase III transcription. In particular, explanations of RPC4-RPC5, RPC10 and RPC7 functions by interpretation of structural data have been convincingly presented. In my opinion, the manuscript can be published as it is.

Response: We acknowledge the positive feedback from Reviewer #1 and are very pleased that this referee recommends publication as it is.

Reviewer #2 (Remarks to the Author):

The eukaryotic RNA Polymerase III transcribes tRNAs, some ribosomal and spliceosomal RNAs and is a central regulator of cell growth. The authors have provided structures of human RNA Polymerase III (Pol III) in apo and multiple elongation complex forms. These structures are informative with respect to the molecular mechanisms of Pol III transcription, and provides an important insight into Pol III's relationship to human disease by resolving the location of pathogenic mutations, and also by providing a testable hypothesis for tumour formation by RPC7alpha. The variations of the TFIIIS-like subunit RPC10 are also interesting and have implications for RNA polymerases other than Pol III which utilise similar factors. This is an important advance in our understanding of RNA polymerase biology, and the work is clearly of high technical quality, hence it is suitable for publication in NSMB. I have these comments.

The multiple positions of RPC10 C-terminal domain are interesting but given their unusual positions (i.e. outside of the funnel, and within the funnel but not reaching the active site), they require further confidence in terms of depiction. Although Figs S5a,b show the density around the C-terminal domain in both positions, I found their fit fairly rough and their density possibly of lower resolution. I would ask the authors to show larger, isolated pictures of each domain position together with their densities.

Response: We are pleased to hear that the multiple positions of the RPC10 C-terminal are of interest to Reviewer #2. Following the request of this referee we have extensively revised Supplementary Fig. 6 that now shows larger pictures of the conformations of RPC10 (in two different orientations) and the corresponding cryo-EM density fits in **Supplementary Fig. 6b & g**.

This is important, as these positions are unusual and require additional support, ideally by complementary methods but that is likely out of scope for this manuscript. Also, a comparison to

C11 positions in yeast might also be worthwhile as I recall C11 C-terminal domain also adopting an unusual position outside of the funnel.

Response: The position of the C11 C-terminal domain is shown in **Supplementary Fig. 6c**.

Given that the RPC10 acidic tip within the funnel doesn't quite reach the RNA, seeing its electron density, and its distance from the RNA should be shown in detail, as it could have implications beyond Pol III and be relevant for Pol I, Pol II and archaeal RNAPs.

Response: The density of the acidic tip within the funnel is now shown in **Supplementary Fig. 6g**.

Similarly, was the proximity of the RPC10 acidic hairpin checked with backtracked RNA (which could be modelled from Pol II-TFIIS, or bacterial RNAP structures)? I couldn't ascertain this from the inset figure panels of Fig S6b & d, which are far too small and indistinct for my eyes.

Response: We acknowledge the feedback regarding the size of the inset figure panels. We, therefore, now show enlarged views of the RPC10/TFIIS inside the funnel in **Supplementary Fig. 6i & j**. The structure of yeast Pol II + TFIIS (reported by Cheung et al. 2011) is depicted in **Fig. 6j** and resembles a backtracked RNA polymerase in a 'reactivation intermediate' conformation, in which the +1 RNA base is modeled. In contrast, in the human Pol III 'inside funnel' conformation depicted in **Fig. 6i**, the 3' end of the RNA could be located to position -1, and thus, most likely, resembles an elongation complex. We hope that the differences between the two functional states become clearly apparent in **Supplementary Fig. 6i & j**.

Does the acidic tip fail to reach the RNA because the entire domain is bound less deeply in the funnel or because of a local conformational change within the domain/hairpin?

Response: As shown in **Supplementary Fig. 6i**, the acidic tip fails to reach the RNA tip for the following reasons: First, the entire domain inserts less deeply into the funnel. Second, the acidic hairpin adopts an inflected conformation, whereas the acidic tip in the yeast Pol II/TFIIS complex is extended. As also shown in **Supplementary Fig. 6i & j**, a key difference between the two structures is the different orientation of the gating tyrosines (also reported by reported by Cheung et al. 2011). In the human Pol III structure, Y684 points towards the acidic tip and essentially

blocks it and shields the 3' end of the RNA. Third, the distance between the RPC10-acidic tip and 3'-end of RNA is larger because it is located at position -1. In contrast, in the Pol II-TFIIS structure, the distance is shorter because a +1 RNA base is present. We have also included the description of these differences into page 5/6 of the main text.

This is an important distinction as it informs about the plasticity of RPC10 binding and/or its conformation, and might be relevant for TFIIS and related factors in archaea. A figure comparing the position of the A12.2/TFIIS/RPC10 C-terminal domain after superposition of surrounding polymerase domains might be informative in this regard.

Response: Given that the human Pol III 'inside funnel' conformation, most likely, still resembles an elongation complex, we propose that the acidic hairpin can transiently monitor the active site. In that regard, one could imagine that the RNA cleavage activity comes into action only, once backtracking occurs that allows the deeper insertion and conformational re-arrangement of the acidic tip. A similar working model could also be possible for related RNA-cleavage factors as suggested by Reviewer #2. We hope that the comparison between the RPC10 C-terminal domain and TFIIS depicted in **Supplementary Fig. 6i & j** is sufficient to outline the differences between the two functional states.

The proposition that RPC10 binding inside the funnel induces clamp opening and therefore assists with termination is compelling, but the nucleic acids are still present in their EC and needs reconciling with their theory. For example, does their EC represent the pre-termination complex? If so, does the DNA sequence contain a terminator?

Response: As now described in the results section on page 6, our structure does not resemble a pre-termination complex because the DNA does not contain a termination sequence. However, we propose that RPC10 can transiently monitor the active site. In that regard, RPC10 insertion and subsequent opening of the clamp would not affect an actively transcribing complex. On the contrary, once a meta-stable pre-termination complex forms upon recognition of the termination signal, RPC10-induced clamp opening could assist in destabilizing the complex further so that termination can take place.

Similarly, the resolution of a 6bp hybrid is interesting, as most other RNAP structures from eukaryotes and bacteria resolve an 8-9bp hybrid which has often been thought to be the most stable length. Can the authors comment on why the visible hybrid is shorter?

Response: We have included an interpretation of the shorter hybrid in the Discussion section on page 11/12. In brief, a shorter DNA-RNA hybrid is in line with the findings from our previous study (Hoffmann et al., 2015), in which we observed that yeast Pol III binds the hybrid less tightly than Pol II. Such loose hybrid binding is likely a Pol III-specific specialization in order to sense the poly-dT termination signal.

Decision Letter, first revision:

2nd Nov 2020

Dear Christoph,

Thank you again for submitting your manuscript "Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states". The referee's report is copied below, and based on these comments, we are happy to accept your paper, in principle, for publication as an Article in Nature Structural & Molecular Biology, on the condition that you revise your manuscript in response to our editorial requirements.

You will see that the referee is fully satisfied that the revisions have addressed prior concerns.

The text and figures require revisions. Note that, within a few days, we will send you detailed instructions for the final revision, along with information on editorial and formatting requirements. We recommend that you do not start revising the manuscript until you receive this additional information.

To facilitate our work at this stage, we would appreciate if you could send us the main text as a word file. Please make sure to copy the NSMB account (cc'ed above).

Data availability: this journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found below:

<https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data>

TRANSPARENT PEER REVIEW

Nature Structural & Molecular Biology offers a transparent peer review option for new original research manuscripts submitted from 1st December 2019. We encourage increased transparency in peer review by publishing the reviewer comments, author rebuttal letters and editorial decision letters if the authors agree. Such peer review material is made available as a supplementary peer review file.

Please state in the cover letter 'I wish to participate in transparent peer review' if you want to opt in, or 'I do not wish to participate in transparent peer review' if you don't. Failure to state your preference will result in delays in accepting your manuscript for publication.

Please note: we allow redactions to authors' rebuttal and reviewer comments in the interest of confidentiality. If you are concerned about the release of confidential data, please let us know specifically what information you would like to have removed. Please note that we cannot incorporate redactions for any other reasons. Reviewer names will be published in the peer review files if the reviewer signed the comments to authors, or if reviewers explicitly agree to release their name. For more information, please refer to our [FAQ page](https://www.nature.com/documents/nr-transparent-peer-review.pdf).

In recognition of the time and expertise our reviewers provide to Nature Structural & Molecular Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states". For those reviewers who give their assent, we will be publishing their names alongside the published article.

If you have any questions, please do not hesitate to contact me directly.

With kind regards,

Beth

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology

Reviewer #2 (Remarks to the Author):

These are comments for the revised version of the manuscript.

I thank the authors for the careful and detailed response to my review.

The altered and additional panels of supplementary figure 6 address my questions fully, and provides extra value with regard to the gating tyrosine.

If I've understood it correctly, the authors propose that Rpc10 is 'poised' to terminate Pol III transcription within the elongation complex; I found that the updated text clarifies this beyond what I had understood in the initial submission.

I recommend that this manuscript is ready for publication.

Author Rebuttal, first revision:

POLICY ISSUES:

1. Data availability: this journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as supplementary information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory.

The data availability statement is included in the main text.

2. DATA DEPOSITION: We require deposition of coordinates (and structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). EM maps must be deposited in the EMDB. Accession codes must be provided in your final submission for acceptance, and entries must be accessible or HPUB at the galley proof stage.

Coordinates and EM maps were submitted to the Protein Data Bank and EMDB, respectively for immediate release upon publication (HPUB). Accession codes are listed in Table 1.

3. Nature Research is taking an active approach to improving our transparency standards and increasing the reproducibility of all of our published results. Detailed information on experimental design and reagents is now collected on our Life Sciences Reporting Summary, which will be published alongside your paper. Please provide an updated version of the Reporting Summary (which will be published with the paper) with your final files.

<https://www.nature.com/authors/policies/ReportingSummary.pdf>

The updated Life Sciences Reporting Summary has been provided as requested.

Please also upload a revised Editorial policy checklist. <https://www.nature.com/authors/policies/Policy.pdf>

We have also uploaded a revised Editorial policy checklist.

GENERAL FORMATTING:

4. The manuscript is currently 4065 words (main text; Introduction, 402 words; Results: 3323 words; Discussion: 340 words). [OK]

The manuscript has been edited to address Point 9. The word count has thus been changed to: Introduction, 402 words; Results: 3388 words; Discussion: 340 words.

5. Current title: Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states (**13 words, 86 characters**, spaces included). [OK]

The title has not been changed.

6. Your abstract is currently 150 words. It must remain max 150 words and should not include citations.

The abstract has not been changed.

7. References: the current manuscript has 52 references in main text and 39 in methods. Up to 60 references are allowed in the main text; additional 20 references can be included in the online Methods. Please make sure all references are cited in numerical order and place Methods-only references after the Methods section, following the numbering of the main reference list (i.e. do not start at 1).

We have transferred the section "Cryo-EM data collection and processing and structural model building" to the Supplementary Information (Supplementary Note 1). The main text in the revised version contains 52 references and the Methods section now contain 12 references.

The reference list should contain papers that have been published or accepted by a named publication or recognized preprint server. Published conference abstracts, numbered patents and research datasets that have been assigned a digital object identifier may also be included in the reference list.

FIGURES AND TABLES:

8. There are currently 5 Figures and 1 Table in main article. [OK]. Tables {in this case, the cryoEM data collection table} should be pasted into Word files as editable tables, not as images.

No changes have been made to the number of Figures and Tables in the revised manuscript.

9. Please make sure all figures and tables, including Extended Data Figures, are cited in the text in numerical order. **Please correct the following: Supplementary Figure 4 is cited before Supplementary Figure 3, and Supplementary Figure 6 is cited before Supplementary Figure 5. Supplementary Table 4 is cited before Supplementary Table 3.**

We have changed the main text accordingly so that all figures and tables are now cited in numerical order.

10. Cropping of gel and/or blot images: gel pieces should be separated with white space (do not add borders). When cropped gels or blots are shown in the main figures, all key data should be presented in uncropped form with molecular weight markers, as Source Data, as instructed below. These data can be displayed in a relatively informal style, but must refer back to the relevant figures; figure legend text should refer to the uncropped image and cite the Source Data (e.g., Uncropped blot/gel images are shown in the Source Data".

We provided the non-processed source data for Extended Data Fig.1 and Extended Data Fig. 6 in the following files: Human_Pol_III_2020_source_data_SI1.pdf and Human_Pol_III_2020_source_data_SI6.pdf.

11. *When submitting the revised version of your manuscript, please pay close attention to our [href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines](https://www.nature.com/nature-research/editorial-policies/image-integrity). and to the following points below:*

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.

-- that control panels for gels and western blots are appropriately described as loading or sample processing controls

-- that all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the production process or after publication if any issues arise.

Figures were prepared to match Digital Image Integrity Guidelines.

SUPPLEMENTARY INFORMATION

All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

1. **EXTENDED DATA FIGURES:** Extended Data Figures are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data Figures, and each must be referred to in the main text. Each Extended Data Figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.

All Extended Data Figure must be called out in order as Extended Data Fig. 1, Extended Data Fig 2, etc.

We have changed the caption titles of the Extended Data Figures accordingly. References in the figure legends can be found in the Supplementary Information file.

2. **SUPPLEMENTARY INFORMATION:** Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, large figures, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Supplementary Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be

presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

Supplementary items (such as Supplementary Tables, Videos, Notes, and additional Supplementary Figures if permitted), should be numbered and called out in main article, as Supplementary Figure 1 (not SI1) and so on.

Supplementary Information contains: Supplementary Note 1, Supplementary Table 1-4, Supplementary References.

3. **SOURCE DATA:** We encourage you to provide source data for your figures. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels with the linked figure noted directly in the file. Statistical source data (i.e., data behind graphs) should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. For imaging source data, we encourage deposition to a relevant repository, such as figshare (<https://figshare.com/>) or the Image Data Resource (<https://idr.openmicroscopy.org>).

Source data should be cited in the legend text (e.g., "Uncropped images for panels a-c are available as source data" or "Data for graphs in d-f are available as source data").

We provide the non-processed source data for Extended Data Fig.1 and Extended Data Fig. 6 in the following files: [Human_Pol_III_2020_source_data_SI1.pdf](#) and [Human_Pol_III_2020_source_data_SI6.pdf](#).

STATISTICS and REPRODUCIBILITY

12. **Cell lines:** the Methods should include a section with cell lines used, origin, whether they were tested for mycoplasma and, where relevant, whether they were authenticated or not.

This information is included in the Methods section under the heading "Generation of a cell line with endogenously tagged Pol III".

13. **Competing interests statement:** Please include a competing interests statement as a separate section after the Author Contributions, under the heading "competing interests", and enumerate any such circumstances there, or read: The authors declare no competing interests.

A competing interests statement has been included under the heading "competing interests".

14. **Reporting Summary statement:** This should be placed after Online Methods section and read: Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

A Reporting Summary statement has been included.

15. **Data Availability statement:** This should be placed after Reporting Summary statement (before Methods-only references). We suggest that you list in this order:

- data deposited in public repositories, with accession codes or DOIs.
- data available as Source Data (e.g. "Source data are available with the paper online.")
- if any data can only be shared upon request, please specify what those data are and explain why.

More information and examples can be found at

<http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf>

A data availability statement has been included that contains the PDB and EMDB entry codes.

TRANSPARENT PEER REVIEW

16. Nature Structural & Molecular Biology offers a transparent peer review option for new original research manuscripts submitted from 1st December 2019. We encourage increased transparency in peer review by publishing the reviewer comments, author rebuttal letters and editorial decision letters if the authors agree. Such peer review material is made available as a supplementary peer review file. **Please state in the cover letter 'I wish to participate in transparent peer review' if you want to opt in, or 'I do not wish to participate in transparent peer review' if you don't.** Failure to state your preference will result in delays in accepting your manuscript for publication.

Please note: we allow redactions to authors' rebuttal and reviewer comments in the interest of confidentiality. If you are concerned about the release of confidential data, please let us know specifically what information you would like to have removed. Please note that we cannot incorporate redactions for any other reasons. Reviewer names will be published in the peer review files if the reviewer signed the comments to authors, or if reviewers explicitly agree to release their name. For more information, please refer to our [FAQ page](https://www.nature.com/documents/nr-transparent-peer-review.pdf).

AUTHORSHIP AND OTHER REQUIREMENTS

We wish to participate in transparent peer review. This is also stated in the cover letter.

Ensure that all **required forms** found in the Policy Worksheet are uploaded to our Journal Processing system as "Supplementary Materials".

All required forms found in the Policy Worksheet

Final Decision Letter:

21st Dec 2020

Dear Christoph,

We are now happy to accept your revised paper "Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states" 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.

Before the manuscript is sent to the printers, we shall make any detailed changes in the text that may be necessary either to make it conform with house style or to make it intelligible to a wider readership. If the changes are extensive, we will ask for your approval before the manuscript is laid out for production. Once your manuscript is typeset you will receive a link to your electronic proof via email within 20 working days, with a request to make any corrections within 48 hours. Please read proofs with great care to make sure that the sense has not been altered. If you have queries at any point during the production process then please contact the production team at rjsproduction@springernature.com. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

Please note that due to tight production schedules, proofs should be returned as quickly as possible to avoid delaying publication. If you anticipate any limitations to your availability over the next 2-4 weeks (such as vacation or traveling to conferences, etc.), please e-mail rjsproduction@springernature.com as soon as possible. Please provide specific dates that you will be unavailable and provide detailed contact information for an alternate corresponding author if necessary.

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My kind regards,

Beth

Beth Moorefield, Ph.D.
Senior Editor
Nature Structural & Molecular Biology