nature research

Peer Review Information

Journal: Nature Structural and Molecular Biology
Manuscript Title: Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II
Corresponding author name(s): Professor Patrick Cramer

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:

Our ref: NSMB-A46118

29th Apr 2022

Dear Patrick,

Thank you again for submitting your manuscript "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II". The reports of the referees are 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 the comments of the referees and our editorial requirements.

I hope that you will be pleased to see that both reviewers are positive about the work, and that Reviewer #2 offers a particularly complementary summary of the insights provided by the findings. Each reviewer queries aspects of the in vitro transcription assays presented in Figure 1 that can be addressed by text and figure modifications. Reviewer #1 requests an expanded discussion of why TFIIE/TFIIH does not increase promoter-specific transcription in these assays and requests that a quantitative comparison of specific and non-specific transcripts be provided. Reviewer #2 similarly requests that the low in vitro stimulatory activity of SNAPc be noted and addressed in the text. Reviewer #1 also suggests including a panel showing SNAPc:IIA/IIB interactions in context of the full PIC. Editorially, we agree that these suggestions would strengthen the presentation of the findings and ask that they be included in a revised manuscript.

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).*****

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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 #1 (Remarks to the Author):

Rengachari et al present several cryo-EM structures of SNAPc in complex with minimal Pol II preinitiation complex on U1 and U5 snRNA promoters. Using structural data complemented by in vitro transcription assays and EMSA the authors show that SNAPc in complex with minimal PIC allows for DNA melting in the absence of TFIIE and TFIIH. The authors also highlight the role of SNAPc extensions named wing-1 and wing-2 in binding TFIIA and TFIIB, respectively, and coordinating SNAPc-PIC-promoter interaction. The authors compare open and closed promoter complexes and propose a mechanism of Pol II DNA opening in the absence of TFIIH.

Comments:

TFIIE density is not seen in structures, and this is supported by IVT experiments. However, after sucrose gradient ultracentrifugation it seems that both TFIIE subunits are present in complex with PIC-SNAPc in stochiometric amounts (Fig 1 panel c). This needs more discussion.

Discuss why adding TFIIE/TFIIH does not increase transcription activity and leads to non-specific transcripts, even though their binding potentially is not restricted in the presented structures.

Extended Data Figure 5: typo, panel d) should be labeled as panel k)

Fig 6. Consider include a panel showing the interactions between SNAP and TFIIA/B in the context of the full complex.

Fig 1e. Show everything relative to PIC (w/o TFIIE/H). Quantitate the percentage of background products.

Ext Fig 6A. If you used a software to generate this figure please cite, otherwise disregard this comments.

In the methods please better define "scoop of DNase I"

Reviewer #2 (Remarks to the Author):

Rengachari et al have resolved cryo-EM structures of the SNAPc-containing Pol II preinitiation complex (PIC) assembled on U1 and U5 snRNA promoters. The structures show that the core of SNAPc binds two turns of DNA and recognizes the snRNA promoter-specific proximal sequence element (PSE) located upstream of the TATA box-binding protein TBP. Interestingly, SNAPc defines the TSS very precisely. The structures also show that two extensions of SNAPc called wing-1 and wing-2 bind TFIIA and TFIIB, respectively, explaining how SNAPc directs Pol II to snRNA promoters. Comparison of structures of closed and open promoter complexes indicate and explain that DNA opening is TFIIH-independent, unlike DNA opening for protein-coding gene initiation.
The results of this tour de force of structural and in vitro transcription analysis clearly provide the structural basis of Pol II initiation at PSE-containing promoters. As such, this is a timely and important piece of work.
I only have one quibble-in Figure 1d there is not a huge increase in transcription when SNAPc is added to the reaction, indicating that the in vitro conditions used are not great for PSE/SNAPc-dependent initiation. The authors should address why this might be.

Decision Letter, final checks:

Our ref: NSMB-A46118

16th May 2022

Dear Dr. Cramer,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Structural & Molecular Biology manuscript, "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II" (NSMB-A46118). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

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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 "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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If you have any further questions, please feel free to contact me.

Best regards,

Sophia Frank
Editorial Assistant
Nature Structural & Molecular Biology
nsmb@us.nature.com

On behalf of

Carolina Perdigoto, PhD
Chief Editor
Nature Structural & Molecular Biology
orcid.org/0000-0002-5783-7106

Reviewer #1:
Remarks to the Author:
Rengachari et al present several cryo-EM structures of SNAPc in complex with minimal Pol II preinitiation complex on U1 and U5 snRNA promoters. Using structural data complemented by in vitro transcription assays and EMSA the authors show that SNAPc in complex with minimal PIC allows for DNA melting in the absence of TFIIE and TFIIH. The authors also highlight the role of SNAPc extensions named wing-1 and wing-2 in binding TFIIA and TFIIB, respectively, and coordinating SNAPc-PIC-promoter interaction. The authors compare open and closed promoter complexes and propose a mechanism of Pol II DNA opening in the absence of TFIIH.

Comments:

TFIIE density is not seen in structures, and this is supported by IVT experiments. However, after sucrose gradient ultracentrifugation it seems that both TFIIE subunits are present in complex with PIC-SNAPc in stochiometric amounts (Fig 1 panel c). This needs more discussion.

Discuss why adding TFIIE/TFIIH does not increase transcription activity and leads to non-specific transcripts, even though their binding potentially is not restricted in the presented structures.

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Reviewer #2:
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Rengachari et al have resolved cryo-EM structures of the SNAPc-containing Pol II preinitiation complex (PIC) assembled on U1 and U5 snRNA promoters. The structures show that the core of SNAPc binds two turns of DNA and recognizes the snRNA promoter-specific proximal sequence element (PSE) located upstream of the TATA box-binding protein TBP. Interestingly, SNAPc defines the TSS very precisely. The structures also show that two extensions of SNAPc called wing-1 and wing-2 bind TFIIA and TFIIB, respectively, explaining how SNAPc directs Pol II to snRNA promoters. Comparison of

structures of closed and open promoter complexes indicate and explain that DNA opening is TFIIH-independent, unlike DNA opening for protein-coding gene initiation.
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I only have one quibble-in Figure 1d there is not a huge increase in transcription when SNAPc is added to the reaction, indicating that the in vitro conditions used are not great for PSE/SNAPc-dependent initiation. The authors should address why this might be.

Author Rebuttal, First Revision:

Detailed list of responses to reviewer comments

NSMB-A46118

Responses are in blue italics

Referees' comments:

Reviewer #1 (Remarks to the Author):

Rengachari et al present several cryo-EM structures of SNAPc in complex with minimal Pol II preinitiation complex on U1 and U5 snRNA promoters. Using structural data complemented by in vitro transcription assays and EMSA the authors show that SNAPc in complex with minimal PIC allows for DNA melting in the absence of TFIIE and TFIIH. The authors also highlight the role of SNAPc extensions named wing-1 and wing-2 in binding TFIIA and TFIIB, respectively, and coordinating SNAPc-PIC-promoter interaction. The authors compare open and closed promoter complexes and propose a mechanism of Pol II DNA opening in the absence of TFIIH.

We thank the reviewer for their work. We have addressed the minor issues as described below.

TFIIE density is not seen in structures, and this is supported by IVT experiments. However, after sucrose gradient ultracentrifugation it seems that both TFIIE subunits are present in complex with PIC-SNAPc in stochiometric amounts (Fig 1 panel c). This needs more discussion.

The approximate molecular weight of the Pol II PIC containing either SNAPc or TFIIE are similar. The resolution of the sucrose gradient is not high enough to distinguish between these two variants of the PIC and hence they co-migrate and are collected in the same gradient fractions. We observed this very early in our cryoEM data processing steps, where the PIC containing SNAPc or TFIIE separate as distinct sets of particles. The revised version of the manuscript includes a statement explaining this better.

Discuss why adding TFIIE/TFIIH does not increase transcription activity and leads to non-specific transcripts, even though their binding potentially is not restricted in the presented structures.

The exacerbated turnover of non-specific products evident for the PIC assembly containing both TFIIE and TFIIH are also mildly observed across the entire IVT experiment. This suggests the presence of a few putative non-specific binding events and start sites for Pol II PIC in the IVT template. In the absence of TFIIE and TFIIH, promoter opening and snRNA transcription occurs preferentially from the original PSE-directed start site. Once TFIIE and TFIIH are present, they appear to over-ride this start site preference because the sophisticated translocase machinery of TFIIH can enable opening of all the ungainly start sites. To a lesser extent, TFIIE alone is able to generate a similar effect, as has been observed in the yeast system (Plaschka, Hantse et al 2016, Dienemann et al 2019), on promoters with high meltability. A statement addressing this has been included in the updated version of the manuscript. With respect to the second point, TFIIE and TFIIH are not occluded sterically.

Extended Data Figure 5: typo, panel d) should be labeled as panel k)

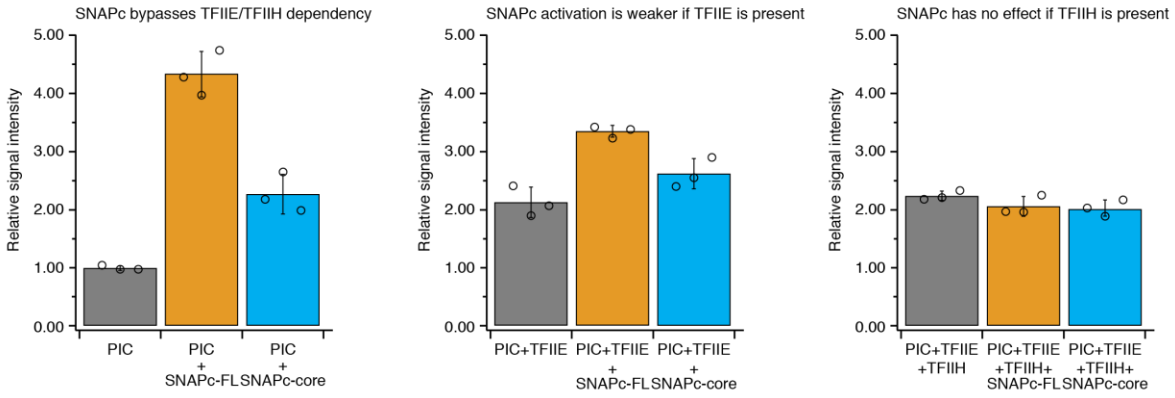
The updated Extended Data Figure 5 has the suggested change incorporated.

Fig 6. Consider include a panel showing the interactions between SNAP and TFIIA/B in the context of the full complex.

This fine suggestion can help the reader with the bigger picture. SNAPC-TFIIA/TFIIB interaction belongs to Figure 5 and it has been updated accordingly.

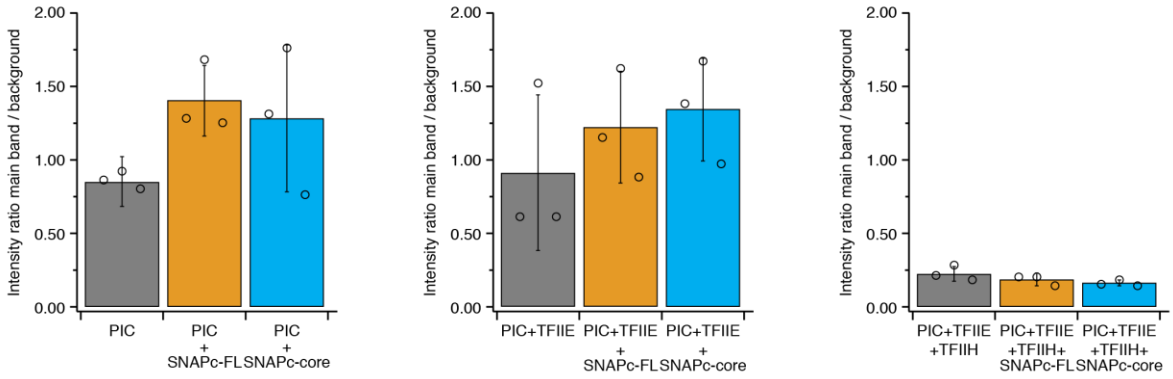
Fig 1e. Show everything relative to PIC (w/o TFIIE/H). Quantitate the percentage of background products.

To address the reviewer's concern, we have prepared a version of Fig. 1e in which all bars are shown relative to PIC (w/o TFIIE/H) (see below). However, we refrain from the use of this version as it may confuse the reader and distract from the original message of the figure, which is to allude to the stimulatory effect of SNAPc on transcription.



We have quantified the background products and present the ratio between the main transcript and the background products in another separate figure (see below). There are two major observations. First, background transcription (all transcription that does not result in production of the main transcript) is increased by 4-7-fold in the presence of TFIIH. This points to the effectiveness of TFIIH in inducing DNA opening and transcription initiation also at such promoter sites as discussed above. Second, for the samples without TFIIH, background transcription is decreased for SNAPc-containing samples. This reflects

the ability of SNAPc to direct the Pol II machinery to the preferred TSS within the promoter sequence and assist in DNA opening at that position as discussed in the manuscript.



However, we would like to note that due to the nature of the experiment, which relies on quantification of bands from different urea-PAGE gels, the assignment of a defined baseline can vary between gels. Whereas this has no significant effect on quantification of a defined single band in the gel (such as the main transcript), this effect is much stronger for quantification of the background signal, which is the sum of all additional signal from a respective lane in the gel. This inconsistency is reflected in larger error bars. In order not to confuse the reader we would suggest not to include the background quantification figure in the manuscript. We also believe that it does not add any additional insights that are not obvious from the gel image itself. To this end, we have included a statement mentioning the 4-7-fold increased background signal in the presence of TFIIH in the legend for Figure 1e.

Ext Fig 6A. If you used a software to generate this figure please cite, otherwise disregard this comments.

We have cited all the software used for the generation of Ext Fig 6 in the methods section.

In the methods please better define "scoop of DNase I"

We have changed the text in the methods.

Reviewer #2 (Remarks to the Author):

Rengachari et al have resolved cryo-EM structures of the SNAPc-containing Pol II preinitiation complex (PIC) assembled on U1 and U5 snRNA promoters. The structures show that the core of SNAPc binds two turns of DNA and recognizes the snRNA promoter-specific proximal sequence element (PSE) located upstream of the TATA box-binding protein TBP. Interestingly, SNAPc defines the TSS very precisely. The structures also show that two extensions of SNAPc called wing-1 and wing-2 bind TFIIA and TFIIB, respectively, explaining how SNAPc directs Pol II to snRNA promoters. Comparison of structures of closed and open promoter complexes indicate and explain that DNA opening is TFIIH-independent, unlike DNA opening for protein-coding gene initiation.

The results of this tour de force of structural and in vitro transcription analysis clearly provide the structural basis of Pol II initiation at PSE-containing promoters. As such, this is a timely and important piece of work.

We thank the reviewer for a very positive assessment of our work.

I only have one quibble-in Figure 1d there is not a huge increase in transcription when SNAPc is added to the reaction, indicating that the in vitro conditions used are not great for PSE/SNAPc-dependent initiation. The authors should address why this might be.

Although SNAPc does not induce transcription in the presence of TFIIE and TFIIH, there is a pronounced increase in transcript formation in the absence of these two factors, especially for SNAPc-FL (Figure 1d, 1e). The effect observed in the presence of TFIIE and TFIIH could be the result of non-specific initiation (please see our response to reviewer #1). The effect of TFIIE alone increasing transcription of PIC has been well documented also in Pol II mRNA transcription on promoters with high meltability (Plaschka, Hantse et al 2016, Dienemann et al 2019). However, the lack of an additive effect of TFIIE and SNAPc together and the absence of TFIIE in the cryoEM structures, supports the non-cooperative role of TFIIE in Pol II snRNA transcription.

Final Decision Letter:

Dear Dr. Cramer,

We are now happy to accept your revised paper "Structural basis of SNAPc-dependent snRNA transcription initiation by RNA polymerase II" 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.

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Carolina Perdigoto, PhD
Chief Editor
Nature Structural & Molecular Biology
orcid.org/0000-0002-5783-7106

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