EMBO reports

hnRNP R negatively regulates transcription by modulating the association of P-TEFb with 7SK and BRD4

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Transaction Report: This manuscript was transferred to EMBO reports following peer review at The EMBO Journal.

Dear Dr. Briese,

Thank you for the transfer of your revised manuscript to our editorial offices. I have now received the reports from two of the referees that have evaluated your study at The EMBO journal and were asked to look into the revision, you will find below. Original referee #3 was unresponsive to this request. However, going through your point-by-point-response, I consider his/her concerns as adequately addressed. As you will see, the other two referees now fully support publication of your study in EMBO reports. Referee #1 (original referee #2) has a final suggestion to improve the manuscript I ask you to address in a final revised manuscript.

Moreover, the manuscript needs formatting according to our journal style. Please carefully review the instructions that follow below.

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Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
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12) Please provide the abstract written in present tense.

In addition, I would need from you:
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- three to four short one sentence bullet points highlighting the key findings of your study.
- a schematic summary figure (synopsis image) in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels that can be used as a visual synopsis on our website.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

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Best,

Achim Breiling
Senior Editor
EMBO Reports

Referee #1:

In this revised manuscript, the authors adequately addressed the points I raised in the previous review. In fact, newly added data strengthen the authors' conclusion very well. Particularly, since CycT1 dissociated from CDK9 is rapidly degraded, the data showing that a proteasome inhibitor rescued CycT1 expression and that different cyclin (CycK) was increased when CycT1 level is low, present a fascinating model of switching CDK9's cyclin partners in particular condition (hnRNP R depletion in this case).

The only concern I have is that Cyclin K is now considered to be the cyclin partner of CDK12/13 rather than CDK9 (https://www.uniprot.org/uniprot/O75909). Also, authors dismissed cyclin T2a/b because mRNA levels are low. However, since CycT protein levels are not very well correlated with the mRNA levels (because of post-transcriptional regulations such as proteolysis), I would still think it is worth looking at T2a/b, or at least including these points in the Discussion.

Other than that, I recommend the manuscript to be published in EMBO Rep.

Referee #2:

This is a thoroughly revised manuscript and the revision addresses all main points raised from the first review process by additional data, discussion, and extended data figures. I recommend to the journal editors to publish this study in EMBO Reports.

Referee #1:

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Other than that, I recommend the manuscript to be published in EMBO Rep.

Author's response: According to the referee's suggestion we extended the second paragraph of the Discussion as following:

We observed that CDK9 levels were increased in hnRNP R knockout cells whereas Cyclin T1 levels were reduced due to proteasomal degradation. Since CDK9 requires a Cyclin to become activated, depletion of Cyclin T1 would be predicted to reduce functional P-TEFb complexes. However, we found that, even though total levels of Cyclin T1 were reduced, the levels of Cyclin T1 in the chromatin fraction remained stable in HNRNPR-/- cells. Thus, we hypothesize that the increased amounts of CDK9 present in the nuclear soluble fraction of HNRNPR-/- cells can associate with BRD4, which then assembles fully functional P-TEFb on chromatin. Indeed, a recent report has shown that P-TEFb dissociates into monomers upon release from 7SK and requires BRD4 to re-assemble (Zhou et al, 2022). Additionally, we observed that CDK9 association with Cyclin K is enhanced in hnRNP R-deficient cells, indicating that increased levels of CDK9/Cyclin K complexes contribute to the enhanced RNA pol II phosphorylation in HNRNPR-/- cells. While our transcriptomics data indicate that Cyclin K is expressed at higher levels than Cyclin T2a/b, we cannot rule out the possibility that Cyclin T2a/b is stabilized through post-translational mechanisms in HNRNPR-/- cells and thus might form additional complexes with CDK9 that can further phosphorylate RNA pol II. Either way, our results indicate that loss of an RNA-binding protein can induce a shift in CDK9 binding to different Cyclins, thereby adjusting its activity.

Referee #2:

This is a thoroughly revised manuscript and the revision addresses all main points raised from the first review process by additional data, discussion, and extended data figures. I recommend to the journal editors to publish this study in EMBO Reports.

Dear Dr. Briese

Thank you for the submission of your revised manuscript files. A few things still need to be corrected:

The number of EV figures needs to be reduced to 5. Please chose the 5 most important figures as EV figures and add the remaining figures to the Appendix file. Alternatively, you can also add all supplementary figures to the Appendix file. Please also correct all callouts in the manuscript text accordingly.

Fig 4 panels are not called out alphabetically, and the EV figure panels are not called out. Please correct.

The title and short summary are a little vague. Terms such as "regulate" and "modulate" should be avoided where possible, and more specific terms used instead. Please re-write where possible. E.g. the title could be:

hnRNP R negatively regulates transcription by .....

I look forward to seeing a final version of your manuscript as soon as possible. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

The authors have addressed all minor editorial requests.

Dr. Michael Briese
Universitätsklinikum Würzburg
Institute of Clinical Neurobiology
Versbacherstr. 5
Würzburg 97080
Germany

Dear Dr. Briese,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

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Esther Schnapp, PhD
Senior Editor
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- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

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Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
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- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
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  - are tests one-sided or two-sided?
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Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	7SK-/- RNA-seq replicate 1 was identified as outlier and removed from the analysis as reported in the Materials and Methods section
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Statistical tests are described in the Figure legends

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