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A novel assay identifies transcript elongation roles for the Nup84 complex and RNA processing factors

Cristina Tous, Ana G. Rondón, María García-Rubio, Cristina González Aguilera, Rosa Luna and Andrés Aguilera

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 28 October 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has been now been evaluated by three referees and I enclose their reports below, as you will see they currently provide mixed recommendations regarding publication.

Overall the referees find that the development of the GLRO assay for transcription elongation will be of interest to the field and they appreciate that the study moves towards resolving the role of several factors whose role in elongation has been controversial. However, the referees do require further experimentation to make the study suitable for The EMBO Journal. There are three main points that need to be addressed, ChIP assays on the long reporter should be performed, natural genes should be examined for similar defects in elongation and finally that the analysis on at least one of the described factors should be extended, both referee #2 and #3 suggest experiments focusing on the role of Nup84. These three main issues are central to the conclusions proposed in the study and would very much strengthen it. Given the interest in the study should you be able to address these issues, we would be happy to consider a revised manuscript.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments. Please note that when preparing your letter of response to the referees' comments that this will form part of the Review Process File, and will therefore be available online to the community. For more

details on our Transparent Editorial Process initiative, please visit our website:
http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

In this manuscript, that authors present a new assay for transcription elongation in vivo, called GLRO. In this assay, they use a reporter where the level of RNA over the 5' region of the transcription unit can be distinguished from the level of RNA over the 3' region of the transcription unit. Using this assay, they test several mutants previously know or suspected to be defective in elongation and find that most of them look defective by this assay. The new assay will be of general interest to the transcription community.

1. From the gels shown, it is not possible to tell whether the two digestion products of 262 nt and 132 nt are the only two significant products. Please show gels with the entire length of the gel shown.

2. In a few cases, ChIP is used to support the findings of the GLRO assay. However, no ChIP is done on the actual long reporter, where defects are most strongly seen. For at least some cases this should be added. For example, include for a known elongation mutant, such as spt4 and then also include for some of the new ones.

3. The authors would like to conclude that they have demonstrated that these mutants are defective for transcription elongation. However, they have so far shown that they are only defective for elongation through lacZ, an E. coli gene. The generality of their findings would be much greater if they could identify some natural yeast genes that show a similar defect. It should be possible to identify coding regions that have a GC content similar to lacZ and then test one or a small number of those by a similar assay and/or ChIP.

4. page 7, bottom paragraph - This paragraph is confusing and needs revision so that it presents the data more clearly. What does each experiment test? What results are conflicting, if any? What is a summary of all fo these results? needs clarification/summary; emphasizes need for ChIP

5. page 8, bottom - Why conclude that sus1D has a minor role in elongation. Does it have any role in elongation?

6. page 9, top paragraph - For the sentence that begins, "Spt20 plays a ..." references should be cited.

7. The English needs some improvement throughout.

Referee #2 (Remarks to the Author):

In this paper the authors adopted a modification of the nuclear run-on (NRO) assay of transcription elongation developed by the Brow lab, using a reporter with two G-less cassettes, and modified it further by creating a second reporter with lacZ coding sequences inserted between the G-less cassettes. As transcription through lacZ has been shown previously to be impaired in various elongation mutants, notably those with a defective THO complex, this modified NRO assay system, which they dubbed GLRO, is well suited to analyze defects in elongation in vivo by a technique that

would likely be affected less than other techniques based on accumulated steady-state levels of long versus short transcripts. It also has advantages over ChIP analysis of Pol II density along a coding sequence, which does not provide information about the functional state of the Pol II molecules being detected; and over the in vitro transcription assay (developed by this group) involving two G-less cassettes and lacZ coding sequences, which would fail to reveal defects coupled to mRNP assembly or nuclear export that depend on intact nuclear function. By applying the GLRO assay to different mutants, the authors confirmed the involvement of the Paf1C complex, SAGA, THSC/TREX-2, an exosome subunit and two subunits of the termination complex CF1A in transcription elongation in vivo, and excluded the Sus1 component of SAGA and TREX-2. These results are valuable because in some cases, eg. Paf1C subunits, an important role in determining the efficiency of transcription elongation was controversial. They went on to demonstrate that mutations eliminating Nup84 or Nup133, two members of the same subcomplex of nuclear pore factors, also reduce the efficiency of elongation, at least for transcription through lacZ coding sequences.

This new version of the in vivo NRO assay incorporating lacZ sequences between G-less cassettes will undoubtedly be adopted by other labs studying elongation, as the authors' results show that it enabled detection of an elongation defect in vivo for various mutants, eg. thp1 and sac3 mutants, that did not score as defective using the original short reporter reporter developed by Brow et al. Their results also are valuable in providing strong evidence that the Paf1C complex contributes to the efficiency of elongation in vivo-a function long suspected, and demonstrable by certain assays, but challenged by a ChIP study of Pol II density published by the Struhl lab. The implication of Nup84/113 in elongation is a novel finding of general interest, although Nup proteins apparently were shown to localize with elongating Pol II on Drosophila polytene chromosomes and to affect the efficiency of gene expression in that organism. Moreover, certain other factors involved in nuclear export of mRNA have already been implicated in influencing elongation efficiency in yeast cells, such as TREX-2 subunits and the Mex67/Mtr2 mRNA export factor. It's not clear how the Nup proteins might affect elongation, and by analogy with the work in Drosophila, they might associate with elongating polymerase in the nucleoplasm rather than at nuclear pores. Despite the lack of mechanistic insight into how Nup84/113 impacts elongation, the work should garner attention both for its technological innovation and the new results implicating Paf1C subunits and nuclear pore factors in transcription elongation in vivo.

1. One issue that comes up repeatedly is why certain mutants register an elongation defect with both GLRO reporters while others only impair transcription through the long reporter containing lacZ coding sequences. In view of their previous conclusion that "elongation through a long gene is more demanding than through a short one (p. 12, lines 2-4), it's difficult to see why all mutations that impair elongation efficiency would not impact the long reporter more so than the short reporter. Most of the mutations studied here do so, but several, rpb9D, leo1D, and spt20D, seem to affect the two reporters about equally. The spt4D presents a third case in which only the long reporter is affected by a large amount and the short reporter is not affected at all, which seems to indicate that the long reporter differs importantly from the short one by a property other than length, such as sequence or chromatin structure. To make this understandable for a general audience, and also guide the interpretation of phenotypes obtained using the GLRO assay, the authors need to provide a detailed discussion and rationale for the different kinds of GLRO defects presented by the various mutants in this study.

2. There are two additional experiments that seem important to provide in order to bolster their interpretation of the elongation defect of nup mutants as resulting from a defect in coupling of elongation to mRNA export. First, they should analyze the nup mutants and a mex67 mutant in the in vitro transcription assay using G-less cassettes, as they would predict that no defect would be observed in this in vitro assay where nuclear structure is destroyed. This is important because one could imagine that these mutants affect elongation in vivo indirectly by affecting the expression of an elongation factor having a more direct role in transcription. In this event, one would observe the defect both in vitro and in vivo. Second, they should apply the new GLRO assay to a mex67 mutant to show that they can confirm an elongation defect associated with impairing this mRNA export factor using the new assay.

Referee #3 (Remarks to the Author):

Tous et al. describe a new set of reporter plasmids for detecting transcription elongation defects in yeast cells. The reporters contain two G-less cassettes separated by either a long, GC-rich sequence (lacZ) or a short sequence that is not GC-rich. Previous work from the Aguilera lab demonstrated the utility of lacZ reporters for studying transcription elongation in vivo. The length and GC-rich nature of this bacterial gene provides a sensitive measure of elongation efficiency. Here, the authors use the G-less cassette reporters together with nuclear run on methods (i.e. the GLRO assay) to analyze potential elongation roles for several transcription (Spt4, Rpb9, PAF, SAGA), mRNA processing (Rna14, Rna15, Rrp6), and mRNA export factors (THSC and Nup84). The demonstration that this assay measures elongation defects is supported by "proof of principle" experiments on spt4 and rpb9 mutants. The authors also revisit elongation roles for the PAF, SAGA, THSC, CFIA (Rna14/15), and exosome (Rrp6) complexes. Previous in vitro and in vivo experiments, including RNA Pol II ChIP and lacZ northern assays, provided contradictory information on whether these complexes affected RNA Pol II elongation rate or processivity. Here, the authors demonstrate elongation defects for each of these complexes using the GRLO assay.

While the GLRO assay is a welcome addition to a rather small set of available transcription elongation assays, the development of this assay alone does not provide sufficient grounds for publication in EMBO J. The demonstration that PAF, SAGA, THSC, CFIA and exosome mutants have elongation defects with the reporters is important but mostly confirmatory of previous, though contradictory, studies. The demonstration of an elongation role for Nup84, which the authors identified in a transposon-based genetic screen, is interesting but is not carried far enough to reveal mechanism. This is also the case for the other proteins studied. For example, it would be interesting to know which aspects of the SAGA complex are required for efficient elongation. Are the histone modification activities of Gcn5 or Ubp8 involved? For the PAF complex, are the histone modifications important? Do nup84 mutants have any other phenotypes indicative of a transcription elongation defect, such as 6AU sensitivity or genetic interactions with TFIIS or Spt4? Are there any functional connections between Nup84 and THSC? Do mutations in these factors have combinatorial effects? In summary, while it provides a technical advance for the elongation field, this manuscript falls short of providing a mechanistic understanding of the results.

1st Revision - authors' response 26 January 2011

Referee #1 (Remarks to the Author):

In this manuscript, that authors present a new assay for transcription elongation in vivo, called GLRO. In this assay, they use a reporter where the level of RNA over the 5' region of the transcription unit can be distinguished from the level of RNA over the 3' region of the transcription unit. Using this assay, they test several mutants previously know or suspected to be defective in elongation and find that most of them look defective by this assay. The new assay will be of general interest to the transcription community.

1. From the gels shown, it is not possible to tell whether the two digestion products of 262 nt and 132 nt are the only two significant products. Please show gels with the entire length of the gel shown.

Changed as requested.
It can be appreciated that the two G-less cassettes correspond to the two predominant bands in each lane with the only exception of the undigested RNA that accumulates on top of the lane likely representing highly structured or protein-coated RNAs. Please, notice that if we removed the rest of the gel in the previous version it was because we followed exactly the same type of presentation published in the original paper by Steinmetz and Brow (MCB 2003) that focus in the G-less bands.

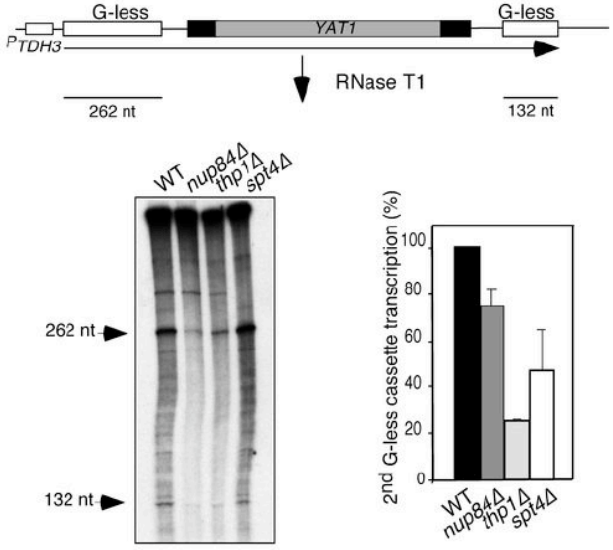
2. In a few cases, ChIP is used to support the findings of the GLRO assay. However, no ChIP is done on the actual long reporter, where defects are most strongly seen. For at least some cases this

should be added. For example, include for a known elongation mutant, such as spt4 and then also include for some of the new ones.

As requested, we now include a ChIP analysis of *spt4*Δ, *thp1*Δ and *nup84*Δ strains on the GLRO-long construct (new Figure 6C). As it can be seen these ChIPs reproduce the defect observed in the GLRO assay, with a reduction of RNAPII reaching the 3´end in the three mutants.

3. The authors would like to conclude that they have demonstrated that these mutants are defective for transcription elongation. However, they have so far shown that they are only defective for elongation through lacZ, an E. coli gene. The generality of their findings would be much greater if they could identify some natural yeast genes that show a similar defect. It should be possible to identify coding regions that have a GC content similar to lacZ and then test one or a small number of those by a similar assay and/or ChIP.

Following the reviewer suggestion we cloned the GC-rich yeast gene *YAT1* replacing *lacZ* in the GLRO-long system to generate a new GLRO reporter. Our previous studies on *spt4* mutants showed that transcription of *YAT1* gene was more sensitive to defects in elongation than other genes with a normal GC content (Rondon et a., 2003 EMBO J.). We found out that elongation through this new GLRO system is less efficient than through *lacZ*. Less 2nd G-less cassette transcript was observed in the WT. Thus, in mutants affected in elongation the 2nd G-less cassette is more difficult to see. Despite the difficulties, the YAT1 GLRO system reproduces the decrease in elongation observed in the GLRO-long system in the mutants we have analyzed: *nup84, thp1* and *spt4*. This clearly shows that our results are reproducible regardless of the DNA sequence used, whether from bacterial or yeast origin. However, we would prefer not to include this new system in the paper, because we do not think it is a reliable system to be made available. For this reason the Figure showing the results with the YAT1-GLRO system is included only for the reviewer view. In any case, we are open to include it as Suppl. data upon request.



Tous Fig. Yat1

In addition, following the reviewer suggestion, we have strengthened our conclusion by doing ChIP analysis in the endogenous *HSP104* gene (see new Figure 6B). As it can be seen the transcription elongation defect in these mutants is clearly appreciated, confirming that the GLRO-long data are reproducible by different assays regardless of the DNA sequence used.

4. page 7, bottom paragraph - This paragraph is confusing and needs revision so that it presents the data more clearly. What does each experiment test? What results are conflicting, if any? What is a summary of all fo these results? needs clarification/summary; emphasizes need for ChIP

We have re-phrased the paragraph to clearly specify the discrepancy in the Results section related to the PAF complex. In short we discuss three existing data bodies: Northern in different reporters under the same promoter, in vitro elongation assays on WCE and ChIPs. The Northern pointed to a defect in elongation occurring in any PAF mutant. The in vitro assay corroborated the defect in mutants of only two subunits (Paf1 and Cdc73). However, the ChIP analyses argued that none of PAF mutants altered elongation. We hope that the more detailed description included in this new version of the ms makes everything clearer.

5. page 8, bottom - Why conclude that sus1D has a minor role in elongation. Does it have any role in elongation?

Our GLRO and ChIP analyses of the *sus1*Δ mutant do not show a defect in elongation. However, Rodriguez-Navarro's lab showed that *sus1*Δ reduces RNAPII elongation by comparing transcription of a long reporter vs a short one and by RNAPII ChIP. We were not able to reproduce the small decrease in RNAPII occupancy at the 3´end region of YLR454w observed in Pascual-Garcia et al. (2008). As we stated in page 8 top paragraph, we think this is due to technical differences in the ChIP protocol. We prefer not to argue whether *sus1*Δ has an effect in elongation. If so, it is very minor and as we have proposed in a previous work (cited in text), probably mediated by THSC since *sus1*Δ behaves more similarly to THSC mutants than to SAGA mutants. We have changed this in Discussion (page 14). We hope that this is now satisfactory, but we are open to any additional suggestion on how to deliver this issue.

6. page 9, top paragraph - For the sentence that begins, "Spt20 plays a ..." references should be cited.

Thank you. This has been amended.

7. The English needs some improvement throughout.

We have thoroughly revised the text and corrected the mistakes detected. I must add that for some reasons grammatical errors that we had annotated to be changed in the original version were not appropriately corrected. We apologize for this oversight.

Referee #2 (Remarks to the Author):

…This new version of the in vivo NRO assay incorporating lacZ sequences between G-less cassettes will undoubtedly be adopted by other labs studying elongation, as the authors' results show that it enabled detection of an elongation defect in vivo for various mutants, eg. thp1 and sac3 mutants, that did not score as defective using the original short reporter reporter developed by Brow et al. Their results also are valuable in providing strong evidence that the Paf1C complex contributes to the efficiency of elongation in vivo-a function long suspected, and demonstrable by certain assays, but challenged by a ChIP study of Pol II density published by the Struhl lab. The implication of Nup84/113 in elongation is a novel finding of general interest, although Nup proteins apparently were shown to localize with elongating Pol II on Drosophila polytene chromosomes and to affect the efficiency of gene expression in that organism. Moreover, certain other factors involved in nuclear export of mRNA have already been implicated in influencing elongation efficiency in yeast cells, such as TREX-2 subunits and the Mex67/Mtr2 mRNA export factor. It's not clear how the Nup proteins might affect elongation, and by analogy with the work in Drosophila, they might associate with elongating polymerase in the nucleoplasm rather than at nuclear pores. Despite the lack of mechanistic insight into how Nup84/113 impacts elongation, the work should garner attention both for its technological innovation and the new results implicating Paf1C subunits and nuclear pore factors in transcription elongation in vivo.

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coding sequences. In view of their previous conclusion that "elongation through a long gene is more demanding than through a short one (p. 12, lines 2-4), it's difficult to see why all mutations that impair elongation efficiency would not impact the long reporter more so than the short reporter. Most of the mutations studied here do so, but several, rpb9D, leo1D, and spt20D, seem to affect the two reporters about equally. The spt4D presents a third case in which only the long reporter is affected by a large amount and the short reporter is not affected at all, which seems to indicate that the long reporter differs importantly from the short one by a property other than length, such as sequence or chromatin structure. To make this understandable for a general audience, and also guide the interpretation of phenotypes obtained using the GLRO assay, the authors need to provide a detailed discussion and rationale for the different kinds of GLRO defects presented by the various mutants in this study.

We have modified the Discussion to attend the reviewer suggestion (see paragraphs 2 and 3). The results obtained with the GLRO assay suggest that at least three classes of elongators exist: those that work since an early elongation phase (like Rpb9 and Spt20), those acting exclusively at a late elongation phase (whose effect is only appreciated when transcribing long genes: Spt4, THSC, CFIA, Rrp6 and Nup84) and an intermediate class whose effect is mainly observed at long genes (Paf1, Rtf1, Cdc73, Sgf73 and Gcn5). We agree that the GLRO-long construct differs from the GLRO-short in other features apart from the length. These features are GC content, sequence and chromatin structure. We now discuss how the different parameters could affect elongation (Discussion, paragraph 3). Having said this, we prefer not to speculate beyond what we believe it is reasonable with the available data.

2. There are two additional experiments that seem important to provide in order to bolster their interpretation of the elongation defect of nup mutants as resulting from a defect in coupling of elongation to mRNA export. First, they should analyze the nup mutants and a mex67 mutant in the in vitro transcription assay using G-less cassettes, as they would predict that no defect would be observed in this in vitro assay where nuclear structure is destroyed. This is important because one could imagine that these mutants affect elongation in vivo indirectly by affecting the expression of an elongation factor having a more direct role in transcription. In this event, one would observe the defect both in vitro and in vivo. Second, they should apply the new GLRO assay to a mex67 mutant to show that they can confirm an elongation defect associated with impairing this mRNA export factor using the new assay.

Following the reviewer suggestion we performed the in vitro elongation assay on *nup84*Δ WCE (see new Figure 6C). As previously observed for the *thp1* mutant, ablation of Nup84 does not interfere with RNAPII elongation *in vitro*. Therefore, we confirmed that the Nup84 role in elongation is coupled to mRNA export. We attempted to assay the *mex67-5* ts mutant (Mex67 is essential) in vivo as suggested by the reviewer, with no success. We believe that this is due to the fact that we have to shift the cells to 37ºC to do this experiment, and we do not have reliable conditions in which this system works in a repetitive manner at temperatures above 30ºC. We think *mex67-5* cells shifted to 37ºC are not able to resist the sarkosyl treatment used to permeabilize the cell, the first step in the GLRO protocol. Having said this and having performed the requested experiments, we are not sure that Mex67 is the best control. It is known from the work of F. Stutz and others that Mex67 is recruited to RNAPII at the promoter also, and physically interacts with proteins with a clear effect in elongation both *in vivo* and *in vitro.* Therefore it is not clear to us that *mex67* mutants should behave as proposed by the reviewer. However, Nup84, as expected, behaves exactly as the control the reviewer suggested. For this reason with think that Nup84 data fulfill the control requested.

Referee #3 (Remarks to the Author):

Tous et al. describe a new set of reporter plasmids for detecting transcription elongation defects in yeast cells. The reporters contain two G-less cassettes separated by either a long, GC-rich sequence (lacZ) or a short sequence that is not GC-rich. Previous work from the Aguilera lab demonstrated the utility of lacZ reporters for studying transcription elongation in vivo. The length and GC-rich nature of this bacterial gene provides a sensitive measure of elongation efficiency. Here, the authors use the G-less cassette reporters together with nuclear run on methods (i.e. the GLRO assay) to analyze potential elongation roles for several transcription (Spt4, Rpb9, PAF, SAGA), mRNA processing (Rna14, Rna15, Rrp6), and mRNA export factors (THSC and Nup84).

The demonstration that this assay measures elongation defects is supported by "proof of principle" experiments on spt4 and rpb9 mutants. The authors also revisit elongation roles for the PAF, SAGA, THSC, CFIA (Rna14/15), and exosome (Rrp6) complexes. Previous in vitro and in vivo experiments, including RNA Pol II ChIP and lacZ northern assays, provided contradictory information on whether these complexes affected RNA Pol II elongation rate or processivity. Here, the authors demonstrate elongation defects for each of these complexes using the GRLO assay.

While the GLRO assay is a welcome addition to a rather small set of available transcription elongation assays, the development of this assay alone does not provide sufficient grounds for publication in EMBO J. The demonstration that PAF, SAGA, THSC, CFIA and exosome mutants have elongation defects with the reporters is important but mostly confirmatory of previous, though contradictory, studies. The demonstration of an elongation role for Nup84, which the authors identified in a transposon-based genetic screen, is interesting but is not carried far enough to reveal mechanism. This is also the case for the other proteins studied. For example, it would be interesting to know which aspects of the SAGA complex are required for efficient elongation. Are the histone modification activities of Gcn5 or Ubp8 involved? For the PAF complex, are the histone modifications important? Do nup84 mutants have any other phenotypes indicative of a transcription elongation defect, such as 6AU sensitivity or genetic interactions with TFIIS or Spt4? Are there any functional connections between Nup84 and THSC? Do mutations in these factors have combinatorial effects? In summary, while it provides a technical advance for the elongation field, this manuscript falls short of providing a mechanistic understanding of the results.

We have focused this work in the analysis of the role of the Nup84 complex, as the three reviewers indeed has nicely suggested. First, we have determined the genetic interactions of *nup84*Δ with the *spt4*Δ and *dst1*Δ mutations of two well-known elongation factors. We show that the double mutants *spt4*Δ *nup84*Δ and *dst1*Δ *nup84*Δ are more sensitive to MPA, a drug that depletes the nucleotide pool hindering elongation (new Figure 5). Moreover, we show that the double mutant *nup84*Δ *sac3*Δ is lethal indicating they have a combinatorial effect in mRNA export. Next, we assessed the effect of *nup84*Δ on transcription elongation *in vitro*. As stated above (see reviewer 2 answers) Nup84 relies on an intact nuclear envelope to influence elongation, and an elongation defect is only seen *in vivo*, as it was also the case of the THSC/TREX2 complex. Thus we conclude the role of Nup84 complex in elongation is mediated by its function in mRNA export. We consider that these new results substantially add to the ms. Thank you. We appreciate the reviewer suggestion about dissecting PAF and SAGA role in elongation. However, we consider that this is beyond the goal of this ms. We would like to add that there are other groups that are dedicating a lot of efforts to these complexes and we are happy to provide them with this system.

We do not agree that this manuscript it is just about one system for measuring elongation. The important point is that using this new assay, we are able to solve the inconsistencies about the role in transcription elongation of a number of proteins that existed for years. We feel that this is an important contribution to the field. More importantly, our work shows for the first time a new role for the Nup84 complex on transcription elongation, which together with the analysis on mRNA processing factors reveals new data on the connection between RNA export and transcription elongation.

2nd Editorial Decision 02 March 2011

Your revised manuscript has now been reviewed once more by the three original referees who find the study to be significantly strengthened and recommend publication in the EMBO Journal pending some minor revisions.

When you send us your revision, please include a cover letter with an itemised list of all changes made, or your rebuttal, in response to comments from review. When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our

Transparent Editorial Process initiative, please visit our website:
http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to reading the revised manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

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The revised manuscript has adequately addressed my previous comments. The new assay, coupled with the findings about Nup84, will make this paper of general interest to the transcription elongation field.

Referee #2 (Remarks to the Author):

I am fully satisfied with the new data and revisions to the text.

Referee #3 (Remarks to the Author):

In this revised manuscript, the authors adequately address the previous reviewer comments and provide additional experimental results to substantiate the view that Nup84 positively regulates transcription elongation. These results include the (1) demonstration of genetic interactions between a nup84 deletion mutation and deletion mutations of SPT4 and DST1, (2) additional ChIP assays that reveal decreased Pol II occupancy at the 3' ends of three genes in nup84 mutants, and (3) an in vitro transcription elongation assay which did not reveal a significant elongation defect with the nup84 mutant extract. The latter result bolsters the view that the newly developed GLRO assay will identify elongation defects that are only apparent in vivo, such as those involving nuclear pore structure. Prior to publication, the authors are asked to address the following minor points:

1. Based on phenotypic scoring, the identities of the nup84 dst1 and nup84 spt4 double mutants should be known. The double mutants should be indicated, as was done for the nup84 sac3 double mutants.

2. Page 8, first complete paragraph, second sentence-sfg73 should be sgf73.

3. Page 11, Discussion, paragraph 1, last sentence--- this sentence is overly strong considering the fact that only one nucleoporin outside of the Nup84 complex was examined.

4. Page 19, Figure 6C legend--- "Idem" needs to be changed.

2nd Revision - authors' response 08 March 2011

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4. Page 19, Figure 6C legend--- "Idem" needs to be changed.

We have attended the 4 minor changes suggested by reviewer 3. The typos of pages 8 and 19 have been corrected, the double mutants nup84 dst1 and nup84 spt4 have been indicated in the Figure, and the sentence of Discussion in the first paragraph of page 11 has been deleted.

3rd Editorial Decision 09 March 2011

I have looked through the revised version of your manuscript and find that you have addressed all the remaining concerns. I am happy to accept the manuscript for publication in The EMBO Journal. You will receive the official acceptance letter in the next day or so.

Yours sincerely,

Editor
The EMBO Journal

