THE EMBO JOURNAL

Manuscript EMBO-2012-81557

# Myb-domain protein Teb1 controls histone levels and centromere assembly in fission yeast

Luis P. Valente, Pierre-Marie Dehé, Michael Klutstein, Sofia Aligianni, Stephen Watt, Jürg Bähler, Julia Promisel Cooper

*Corresponding author: Julia P. Cooper, Cancer Research UK - London Research Institute*

**Review timeline:**

| | |
|---|---|
| Submission date: | 28 March 2012 |
| Editorial Decision: | 16 May 2012 |
| Revision received: | 27 August 2012 |
| Editorial Decision: | 07 September 2012 |
| Revision received: | 27 November 2012 |
| Accepted: | 29 November 2012 |

## 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 16 May 2012

Thank you for submitting your manuscript on Teb1 and histone level control for our consideration. I am very sorry for the delay in getting back to you with a decision, owed to the fact that it took a while to assign a sufficient number of suitable referees, and that some of their reports were furthermore considerably delayed. We have now finally obtained all three sets of comments, which I am sending you enclosed in this email. As you will see, all three referees generally appreciate your study and its findings; nevertheless they also raise a number of concerns that would need to be addressed before publication. In this respect, the most important issue pointed out by all three referees is the currently somewhat limited insight into the mechanism linking Teb1 to histone expression and Cnp1 assembly, which should be extended by following up the referees' specific suggestions.

In light of these comments and recommendations, we shall be happy to consider a revised manuscript addressing the various concerns of the referees further for publication. I should remind you that it is our policy to allow only a single round of major revision, and that it will therefore be important to adequately respond to all the points raised at this stage in the process. In addition, there are also several editorial issues that I need to bring to your attention at this stage:

- please carefully revise the reference list - it is currently not only in a very heterogeneous format, but also lacks some references cited in the text (e.g. I noticed Maddox et al 2007, cited on page 17)
- we will need brief Conflict of Interest and Author Contribution statements at the end of the manuscript text (next to the Acknowledgements)
- finally, we noticed that the Western blot panels in Figure 6B currently show an assembly of

 

various individual lanes, thus not allowing a clear comparison and definitive assessment of these data. We would therefore need to ask you to kindly send us files containing the respective source images (scans) for the current figure accompanied by all necessary annotations and explanations; furthermore for eventual publication we would likely need a revised figure panel that allows more direct comparisons and assessment of the relations between the individual lanes and rows in these panels; this may require re-running these samples or experiments.

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

We generally allow three months as standard revision time, and it is our policy that competing manuscripts published during this period will have no negative impact on our final assessment of your revised study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider this work, and please do not hesitate to contact me in case you should have any additional question regarding this decision or the reports. I look forward to your revision.

With kind regards,
Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

This manuscript from the J. Cooper lab addresses the role of the telomere-like DNA -binding protein Teb1 in fission yeast. By combining Chip-Chip and transcriptome analysis, the authors provide compelling evidence that Teb1 binds the AACCCT box of the core histone gene promoters and regulates their transcription. They also show that Teb1 is required for a proper loading of CenpA at centromeres. Overall, the presented data are of high quality and are fully convincing.
Nevertheless, this reviewer regrets that the authors did not go deeper in the molecular mechanisms linking Teb1 to the GATA-like factor Ams2, previously shown to regulate histone genes in an AACCCT-dependent manner and Cenp-A deposition. The data suggest that Teb1 recruits Ams2 at histone promoter and maybe at centromere. This should be clarified and directly tested by in vitro and in vivo Teb1-Ams2 interaction assays. This would reinforce considerably the impact of this work for our understanding of histone regulation and centromere formation.

Referee #2 (Remarks to the Author):

Valente et al

It was earlier shown that S. pombe Ams2 is a GATA binding transcription factor, which binds centromeres (Chen et al 2003). Ams2 and H4 were isolated a multicopy-suppressors of a cnp1 (CENP-A) mutant. Ams2 in high dosage restores mutant Cnp1 localization at centromeres. An ams2 deletion strain has reduced Cnp1 levels at centromeres and defects in centromere function. It is also known that Ams2 binds to the gene promoters of histone genes and is required for cell cycle dependent activation of genes encoding H3, H4, H2A and H2B during S phase (Takayama and Takahashi 2007). The relative dosage of H3 and H4 is critical for assembly of Cnp1 nucleosomes at centromeres. If the H3 dosage is increased vs H4 (H3>H4) Cnp1 levels are reduced whereas H4>H3 leads to increased incorporation of Cnp1 (Castillo et al 2007).

 

In this manuscript Teb1 is identified as an important factor for Cnp1 assembly. Teb1 binds to promoters of many genes including the histone genes. A mutant teb1-1 shows reduced expression of all core histone mRNA except H2Aalpha. It is shown that Ams2 occupancy at the hht2+/hhf2+ gene promoter is reduced in teb1-1. teb1-1 cells have defects in centromeric silencing and show reduced Cnp1 localization. Furthermore H3 processing and other protein degradation in G1 arrested cells is defective in teb1-1 possibly due to reduced Isp6 expression (Isp6 is a vacuolar protease).

Points of concern

1. The graphical display of microarray ChIP-chip data in Figure 2A is difficult to interpret and needs to be improved. Genomic element such as centromeres subtelomeres and telomeres need to be annotated. Also chromosomes 2 and 3 should also be shown (as Suppl data).

2. The fold enrichment of Teb1 at histone gene promoters and histone variant gene promoters (Cnp1 and H2A.Z) and the effect of teb1-1 on gene expression from should be clearly shown. It is right now hidden in Supplementary Tables. I suggest including this in Figure 3 or a Table.

3. Interestingly H2Aalpha is not downregulated in teb1-1. Is the H2Aalpha vs H2Abeta ratio critical for Cnp1 assembly? Is Ams2 occupancy at the hta1+ locus affected in teb1-1 compared to hta2+?

4. In Figure 4 Ams2 occupancy is shown to be reduced at the hht2+ locus in teb1-1. The authors should also check if Ams2 occupancy at centromeres is affected.

5. Is there any chromatin/gene regulatory role of the dramatic H3 proteolysis during G1 arrest? Can the authors rule out that the clipping does not occur after lysis of cells due to leakage of enzymes from vacuoles etc. during the preparation? The details for how protein extractions are carried out need to be included.

6. The reduced histone dosage in teb1-1 could affect Cnp1 assembly but it is unclear how this fits with the earlier observations that altered H3:H4 ratio leads to reduced Cnp1. It would be interesting to test if increased dosage of any specific histone can suppress or enhance the Cnp1 assembly defect of teb1-1.

7. Finally it seems like Teb1 could affect Cnp1 assembly directly or indirectly (or both). Further evidence regarding the precise mechanism would considerably strengthen the manuscript. Can indirect effects of reduced expression of Cnp1 loading factors be ruled out by examining the teb1-1 gene expression data? See also point (4 above).

Referee #3 (Remarks to the Author):

This manuscript deals with the function of Teb1, a S. pombe Myb DNA binding protein. This protein has previously been shown to bind to vertebrate telomere repeat sequences in vitro. Despite this the authors demonstrate that Teb1 does not regulate telomere length but instead using ChIP - CHIP analysis they show that Teb1 associates with promoters of numerous genes including those of histone genes. Consistent with this finding, a point mutation in teb1 (teb1-1) significantly reduces histone gene mRNA levels and binding of the GATA activator Ams2. They also convincingly demonstrate that Teb1 is required for the proper levels of CENP-A loading and show that Teb1 is required for nitrogen-starvation induced histone H3 clipping through control of the expression of the vacuolar protease Isp6. Therefore this work identifies a specific protease responsible for histone clipping, which is important (although histone clipping has been shown in S. cerevisiae the protease responsible for this has not been identified -at least to my knowledge). This study also identifies Teb1 as a regulator of histone gene expression and centromere identity, which are both important findings. The slight weakness in the study is that evidence supporting the mechanism by which Teb1 influences CENP-A localization is limited. But this is likely to be a complex issue and is probably

 

beyond the scope of this study. Nevertheless, there are some points that should be addressed and these are outlined below.

1. A more detailed analysis of the Teb1-dependent genes identified by microarray analysis is warranted. For example the authors indicate that many genes are up regulated in a teb1-1 background. What are these genes? Do they fall into any specific ontology groups? Do they overlap with genes that are upregulated in other chromatin/transcription factor mutant backgrounds or under other conditions (meiosis, stress, DNA damage etc). How many were identified as being targets of Teb1 through ChIP analysis?

2. The data relating to the control of histone gene expression by Teb1 could be strengthened. The authors demonstrate that Teb1 binds to histone gene promoters. Is this binding constitutive through the cell cycle or limited to S-phase? Do Teb1 levels fluctuate? Does Teb1 interact directly with Ams2? Does mutation of teb1 lead to loss of periodic histone gene expression or does expression remain periodic albeit at a lower level?

3. Nitrogen starvation leads to histone H3 clipping. Are the authors looking at soluble (unassembled) H3 in their western blots or does this clipping occur in the context of chromatin? The authors do not include their methodology for making protein extracts so this is difficult to judge.

Minor points
The first sentence of the abstract needs rewording. At present it suggests that heterochromatin function is linked to the promoters of many S. pombe genes.

Page 7 line 15 "(Fig 3; note that two of the histone gene promoters are divergent....." Surely this should be "four of the histone gene promoters are divergent" ?

Page 10 line 6: "(Figure4)" Insert space

1st Revision - authors' response 27 August 2012

 

August 22, 2012

I hereby submit a revised version of manuscript EMBOJ-2012-81557, entitled 'Myb-domain protein Teb1 controls histone levels and centromere assembly in fission yeast'. We are grateful to you and the reviewers for your positive assessment of the work and constructive suggestions on clarifying and improving it. Here we respond (in **bold**) to each of the reviewers' comments (*italic*) in turn:

*Referee #1 (Remarks to the Author):*

*This manuscript from the J. Cooper lab addresses the role of the telomere-like DNA - binding protein Teb1 in fission yeast. By combining Chip-Chip and transcriptome analysis, the authors provide compelling evidence that Teb1 binds the AACCCT box of the core histone gene promoters and regulates their transcription. They also show that Teb1 is required for a proper loading of CenpA at centromeres. Overall, the presented data are of high quality and are fully convincing.*

*Nevertheless, this reviewer regrets that the authors did not go deeper in the molecular mechanisms linking Teb1 to the GATA-like factor Ams2, previously shown to regulate histone genes in an AACCCT-dependent manner and Cenp-A deposition. The data suggest that Teb1 recruits Ams2 at histone promoter and maybe at centromere. This should be clarified and directly tested by in vitro and in vivo Teb1-Ams2 interaction assays. This would reinforce considerably the impact of this work for our understanding of histone regulation and centromere formation.*

**We appreciate the Reviewer's point that the paper raises questions of whether/where/how Teb1 and Ams2 interact without providing many answers. We have performed additional experiments to directly address this point. We had previously used ChIP to assess Ams2-Myc (endogenously tagged and fully functional) binding to the centromeric central core but had been unable to detect such binding, despite observing a robust Ams2 signal at the *hht2*$^+$*/hhf2*$^+$ promoter in the same ChIP samples. We have now taken this approach further by arresting cells in S-phase via HU treatment (new Figure 4). These HU-arrested cells showed a marked increase in Ams2-Myc levels relative to those in asynchronously growing cells, in line with previous observations that Ams2 is important for Cnp1 loading at the centromeric core specifically during S-phase. Nonetheless, we fail to detect Ams2-Myc binding to the centromeric central core in HU-arrested cells while detecting clear binding at both the *hht2*$^+$*/hhf2*$^+$ and *hta1*$^+$*/htb1*$^+$ promoters. This failure stands in contrast to the report by Chen et al (2003) in which ChIP using an anti-Ams2 antibody yielded evidence for centromeric binding. We have obtained an aliquot of a different batch of this anti-Ams2 antibody but could not detect centromeric binding by ChIP. These observations prompt us to favour a model in which Cnp1 loading is controlled by Ams2 via its role in regulating canonical histone gene expression, which is in turn dependent on Teb1 which perhaps serves as a DNA binding platform for Ams2. We feel that these observations augment our previous data significantly. Coimmunoprecipitation experiments have so far been inconclusive, since we obtain**

**Teb1 in anti-Ams2 IPs but also in negative control experiments – Teb1 seems to be rather 'sticky'; we feel that further optimization of this mode of assessment of how Ams2 and Teb1 interact is beyond the scope of the current report.**

*Referee #2 (Remarks to the Author):*

*Valente et al*

*It was earlier shown that S. pombe Ams2 is a GATA binding transcription factor, which binds centromeres (Chen et al 2003). Ams2 and H4 were isolated a multicopy-suppressors of a cnp1 (CENP-A) mutant. Ams2 in high dosage restores mutant Cnp1 localization at centromeres. An ams2 deletion strain has reduced Cnp1 levels at centromeres and defects in centromere function. It is also known that Ams2 binds to the gene promoters of histone genes and is required for cell cycle dependent activation of genes encoding H3, H4, H2A and H2B during S phase (Takayama and Takahashi 2007). The relative dosage of H3 and H4 is critical for assembly of Cnp1 nucleosomes at centromeres. If the H3 dosage is increased vs H4 (H3>H4) Cnp1 levels are reduced whereas H4>H3 leads to increased incorporation of Cnp1 (Castillo et al 2007).*

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*Points of concern*

*1. The graphical display of microarray ChIP-chip data in Figure 2A is difficult to interpret and needs to be improved. Genomic element such as centromeres subtelomeres and telomeres need to be annotated. Also chromosomes 2 and 3 should also be shown (as Suppl data).*

**We thank the reviewer for these suggestions and have annotated the various chromosome regions as well as added Chr II and III (new Figure S1). Please note that telomere repeats are not present in the microarrays; the same is true for the repetitive rDNA regions.**

*2. The fold enrichment of Teb1 at histone gene promoters and histone variant gene promoters (Cnp1 and H2A.Z) and the effect of teb1-1 on gene expression from should be clearly shown. It is right now hidden in Supplementary Tables. I suggest including this in Figure 3 or a Table.*

**We have followed the Reviewer's suggestion and added information about Teb1 binding at histone variant gene promoters in the new Supplementary Figure II.**

*3. Interestingly H2Aalpha is not downregulated in teb1-1. Is the H2Aalpha vs H2Abeta ratio critical for Cnp1 assembly? Is Ams2 occupancy at the hta1+ locus affected in teb1-1 compared to hta2+?*

**These are interesting questions. We do not know of evidence that the H2Aalpha v beta ratio is critical for Cnp1 assembly. We find that Ams2 occupancy is in fact**

**regulated by Teb1 (see the new Figure 4B) but unlike at the Hht2/Hhf2 promoter, residual Ams2 binding at the Hta1/Htb1 promoter is still observed in the *teb1-1* background. This may explain the continued expression of H2Aalpha in *teb1-1* cells.**

*4. In Figure 4 Ams2 occupancy is shown to be reduced at the hht2+ locus in teb1-1. The authors should also check if Ams2 occupancy at centromeres is affected.*

**Please see our response to Reviewer 1 above and the new Figure 4B.**

*5. Is there any chromatin/gene regulatory role of the dramatic H3 proteolysis during G1 arrest? Can the authors rule out that the clipping does not occur after lysis of cells due to leakage of enzymes from vacuoles etc. during the preparation? The details for how protein extractions are carried out need to be included.*

**We have added details of protein extractions to the Methods section, and indeed we had the same concern about proteolysis in extracts of G1 cells and have endeavoured to avert this. Our observation that the clipping does not occur in G1-arrested *teb1-1* cells strongly argues against the possibility that the clipping is due to protein preparation, although we cannot rule out that something like vacuolar leakage is altered by the *teb1-1* mutation.**

*6. The reduced histone dosage in teb1-1 could affect Cnp1 assembly but it is unclear how this fits with the earlier observations that altered H3:H4 ratio leads to reduced Cnp1. It would be interesting to test if increased dosage of any specific histone can suppress or enhance the Cnp1 assembly defect of teb1-1.*

**We agree that the suggested experiments would be very interesting, especially in light of the fact that our Ams2 data suggests that Teb1's regulation of Cnp1 loading *via* Ams2 is due to altered histone levels. However, we feel that a proper analysis of histone levels and, for instance, whether manipulating histone levels ectopically can alter *teb1-1* phenotypes, are future experiments beyond the scope of this paper.**

*7. Finally it seems like Teb1 could affect Cnp1 assembly directly or indirectly (or both). Further evidence regarding the precise mechanism would considerably strengthen the manuscript. Can indirect effects of reduced expression of Cnp1 loading factors be ruled out by examining the teb1-1 gene expression data? See also point (4 above).*

**Please see again our response to Reviewer 1 above. Teb1 does not appear to regulate the expression of the Cnp1 loading factors Mis6, Ams2 or Scm3.**

*Referee #3 (Remarks to the Author):*

*This manuscript deals with the function of Teb1, a S. pombe Myb DNA binding protein. This protein has previously been shown to bind to vertebrate telomere repeat sequences in vitro. Despite this the authors demonstrate that Teb1 does not regulate telomere length but instead using ChIP -CHIP analysis they show that Teb1 associates with promoters of numerous genes including those of histone genes. Consistent with this finding, a point mutation in teb1 (teb1-1) significantly reduces histone gene mRNA levels and binding of the GATA activator Ams2. They also convincingly demonstrate that Teb1 is required for the proper levels of CENP-A loading and show that Teb1 is required for nitrogen-starvation induced histone H3 clipping through control of the expression of the vacuolar protease Isp6. Therefore this work identifies a specific*

*protease responsible for histone clipping, which is important (although histone clipping has been shown in S. cerevisiae the protease responsible for this has not been identified -at least to my knowledge). This study also identifies Teb1 as a regulator of histone gene expression and centromere identity, which are both important findings. The slight weakness in the study is that evidence supporting the mechanism by which Teb1 influences CENP-A localization is limited. But this is likely to be a complex issue and is probably beyond the scope of this study. Nevertheless, there are some points that should be addressed and these are outlined below.*

*1. A more detailed analysis of the Teb1-dependent genes identified by microarray analysis is warranted. For example the authors indicate that many genes are up regulated in a teb1-1 background. What are these genes? Do they fall into any specific ontology groups? Do they overlap with genes that are upregulated in other chromatin/transcription factor mutant backgrounds or under other conditions (meiosis, stress, DNA damage etc). How many were identified as being targets of Teb1 through ChIP analysis?*

**We do include Tables summarizing the number of genes up- or down-regulated in a *teb1-1* background along with a summary of its effects on histone gene expression and the iron regulatory genes. We hope that investigators interested in taking these analyses beyond the scope of our work will use the datasets we have submitted to explore the relationship between Teb1 and other chromatin/transcription factors.**

*2. The data relating to the control of histone gene expression by Teb1 could be strengthened. The authors demonstrate that Teb1 binds to histone gene promoters. Is this binding constitutive through the cell cycle or limited to S-phase? Do Teb1 levels fluctuate? Does Teb1 interact directly with Ams2? Does mutation of teb1 lead to loss of periodic histone gene expression or does expression remain periodic albeit at a lower level?*

**Please see our response to Reviewer 1 above with respect to Teb1-Ams2 interactions. The reason we noticed histone clipping, and the role of Teb1 therein, in G1 arrested cells is that we were attempting to synchronize cultures in order to determine whether the periodicity of gene expression was lost in *teb1-1* cells. However, we felt that the *teb1-1*-induced alterations we observed in the G1 arrest response would complicate interpretation of such experiments. We now show in the new Figure 4A that Teb1 levels increase during an HU-induced arrest; the enrichment of histone promoter sequences in Ams2 ChIP also increases during HU arrest and this is largely dependent on wild type Teb1.**

*3. Nitrogen starvation leads to histone H3 clipping. Are the authors looking at soluble (unassembled) H3 in their western blots or does this clipping occur in the context of chromatin? The authors do not include their methodology for making protein extracts so this is difficult to judge.*

**Please see our response to Reviewer 2, point 5 above. We are looking at soluble H3.**

*Minor points*

*The first sentence of the abstract needs rewording. At present it suggests that heterochromatin function is linked to the promoters of many S. pombe genes.*

**Thank you – we have corrected this.**

*Page 7 line 15 "(Fig 3; note that two of the histone gene promoters are divergent....." Surely this should be "four of the histone gene promoters are divergent" ?*

**Again thanks - we have changed this statement to say 'two pairs of histone genes share divergent promoters'.**

*Page 10 line 6: "(Figure4)" Insert space*

**Done.**

Again we thank the reviewers for their time and trouble in reading and making suggestions on our manuscript. We hope that you will now find the paper suitable for publication in the *EMBO J,* and we look forward to hearing from you.

2nd Editorial Decision 07 September 2012

Thank you for submitting your revised manuscript for our consideration. Two of the original reviewers have now assessed it once more (see comments below). I am pleased to inform you that they both of them consider the manuscript significantly improved and now in principle suitable for publication. However, one of the originally raised issues remains in the eyes of 2 still insufficiently addressed, regarding the results on histone H3 clipping. Since these concerns would potentially affect the interpretation and conclusions of some aspects of this work, I feel it would be important to experimentally clarify them as requested, and I am therefore returning the manuscript to you for one final round of modification, to allow you to address this concern.

When re-submitting your final version, please also take care of the following editorial points:

- please upload the main article (excluding figures) in a text file format (e.g. .doc, .rtf...), this is needed for production purposes

- please revise Figure 4A regarding contrast/brightness adjustments, since the adjustments in the current version have led to loss of background signals

- please revise Figure 6B: according to the source data you kindly provided, essentially all lanes were originally adjacent to each other, except for the removal of one irrelevant lane per panel. Presentation of these data would therefore be much more straightforward and natural if you kept all adjacent lanes together, separately adding only the one/two lanes before the 'M' lane with a visual separation line (and a figure legend pointing out the removal of an irrelevant lane as well as mentioning the availability of the full blot in the source data). The current version of the figure, where each lane is separately shown, is unnecessarily confusing.

Please let me know should you need any further clarification regarding this additional round of revision. I look forward to receiving your final version in due course.

With best regards,
Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #2 (Remarks to the Author):

Valente et al revised version

This manuscript has been significantly improved and most of the points of concern by the three reviewers have been addressed. The only remaining issue, which needs to be dealt with is the H3 clipping data and its interpretation.

This was pointed out before as major concerns both by reviewer 2 (point 5) and reviewer 3 (point 3).

The authors have not addressed this issue in the revised version, although they have provided their protocol for histone preparations and western blotting. The authors admit in response to reviewer 2 (point 5) that vacuolar leakage can occur during preparation but they need to experimentally address this. Otherwise they cannot conclude that the clipping occurs in cells (page 12).
One way of preventing proteolysis during preparations is to resuspend the cells in 7M GuHCl buffer containing Tris-Cl pH 7.5 and 1 mM DTT before lysis, and to carry out the lysis in the same buffer. This would inhibit the activity of vacuolar proteases. In the current protocol (described on page 23) the cells are resuspended in 20% TCA and then washed in Tris base prior to lysis, i.e. the lysis takes

 

place in Tris base buffer. I am worried that this procedure is not sufficient to prevent a putative artefact of H3 clipping during or after lysis. The counterargument in response to reviewer 2 (point 5) that teb1-1 mutations abolishes clipping is not valid since the vacuolar protease in question (Isp6) is downregulated in this situation.

Referee #3 (Remarks to the Author):

The authors have dealt with the majority of the issues associated with the original manuscript. Importantly, they have strengthened the data relating to the control of histone gene expression by Teb1. Although they have not been able to resolve the role of Teb1 in periodic histone gene expression or to confirm a direct interaction between Teb1 and Ams2, for technical reasons they have demonstrated that Teb1 levels accumulate in HU arrested cells and that the enrichment of Ams2 at histone gene promoters is largely dependent upon Teb1 function. The other points that were raised have been addressed.

2nd Revision - authors' response 27 November 2012

I hereby submit our revised manuscript. We have performed the final experiment requested by Reviewer 2, who asked us to resuspend and lyse cells in 7M GuHCl to inhibit vacuolar proteases during extraction of G1-arrested cells. As the reviewer suspected, extracts made with this protocol fail to yield evidence of protein clipping. Therefore, Teb1 regulates the G1-specific vacuolar protease Isp6, which appears to act during extraction rather than in vivo to clip histone H3. Conceivably this activity of Isp6 is relevant to cell physiology, if for instance H3 is shuttled through the vacuole in G1-arrested cells; however, such trafficking and protein handling would be subjects far beyond the scope of our paper, so we have moved this data to the Supplementary information (adding the GuHCl results) and altered our text accordingly. We have also acknowledged this anonymous reviewer for suggesting the GuHCl protocol, which may be useful for others examining protein clipping in quiescent cells.

The main points of the paper, that Teb1 is a newly recognized master regulator of histone gene expression and CenpA loading, acting through its Myb-domain-mediated ability to bind TTAGGG repeats in histone promoters and regulate transcription, are unaltered. We hope you will now find the paper suitable for publication in EMBO J and look forward to hearing from you.

Acceptance letter 29 November 2012

Thank you for submitting your re-revised study for our consideration. I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal!

I have to admit that I had some qualms regarding the histone clipping data, since this was initially a quite prominent claim (2/7 figures) and appreciated by the referees. But I realize that the main aspects of the paper remain unaffected, and therefore decided that we will proceed with publication also without this additional aspect of the conclusions. And I agree that the altered Isp6-dependent proteolytic activity, even if only in lysosomes or during extraction, remains an interesting observation worth including in the supplementary information.

With best regards,
Editor
The EMBO Journal

 