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The RSC chromatin remodeling enzyme plays a unique role in directing the accurate positioning of nucleosomes

Christian J. Wippo, Lars Israel, Shinya Watanabe, Andreas Hochheimer, Craig L. Peterson and Philipp Korber

Corresponding author: Philipp Korber, University of Munich

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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 17 September 2010

Thank you for submitting your manuscript for consideration at The EMBO Journal. I apologise that it has taken so long to evaluate your manuscript, but I understand that my colleague forwarded you the reports while I was away. Overall the three referees that evaluated your study find it to be interesting, however, there are a number of concerns and issues that need to be addressed. One of the major concerns is discrepancy between some of the in vitro and in vivo data, while referee #1 suggests that the in vivo data is removed, the other referees and myself believe it should remain part of the manuscript. Since the issues raised by the referees are all central to the manuscript, they should be experimentally addressed. Should you be able to address concerns we would be happy to consider a revised version of the 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):

This is useful, albeit disjointed, paper that is on the margin for publication in EMBO J; I lean slightly positive. The in vitro experiments are the strongest aspect of the paper. The authors show that RSC moves nucleosomes from their intrinsically preferred positions (nucleosome assembly via salt dialysis) to something resembling their in vivo positions. This is shown with purified RSC in the presence or absence of extract from rsc mutant cells. The authors do not perform detailed mapping of the position, which should be done to show that the positions are truly those in vivo. It is clear that RSC only does this at some genomic regions, and that other activities are required. An interesting result is that RSC is not equivalent to Isw2 or Swi/Snf, indicating that there is specificity to which remodeling complexes function at different regions. It could be argued that this is already known (by in vitro assays not being compared to in vivo positions), and that it is merely chance that RSC allows movement to in vivo positions at the relevant regions. On balance, however, this is a useful and novel contribution to the field.

The in vivo experiments involving Rsc mutants are less useful, and indeed these are not mentioned in the abstract. Such experiments have been done before, so the results here do not add much. Furthermore, there is no clear link to the in vitro experiments, which is a significant weakness. The results are poorly described; they are basically just listed with no coherent point being made. I think these should be deleted.

The experiments involving the RSC target site at -151 are useful and interesting. However, these are also not mentioned in the abstract, and they are not connected to the in vitro experiments. These could be retained, although a better effort to connect the results to the in vitro experiments would be nice.

In general, the manuscript is rather longwinded, especially in the description of results.

Referee #2 (Remarks to the Author):

The work described in this paper employed wild type and mutant yeast extracts to explore the molecular mechanisms of nucleosome positioning. The authors discovered a role for the RSC chromatin remodeling enzyme in generating in vivo-like nucleosome patterns at cloned S.cerevisiae promoters. RSC, but not equivalent remodeling units of SWI/SNF and ISW2, could rescue the mutant extract and restore in vivo-like nucleosome positioning. Given the widespread interest in nucleosome positioning, and the paucity of mechanistic studies, this work is interesting and important. Overall, the data are solid and credible. One weak point is the discrepancy between the in vitro and in vivo experiments in Figures 3 and 5. In vitro, it is shown that rsc3-ts extracts were unable to produce the NDRs of the CHA1 and SNT1 promoters (Fig. 3C, D). However, in vivo the same NDRs were present at the restrictive temperature in vivo (Fig. 5C, D). Additionally, in vivo, rsc3-ts at 37°C affected only NDR2 of the RIM9 promoter, but not NDR1 and NDR3 (Fig.5B). However, in vitro, the rsc3-ts extracts were unable to generate NDR2 and NDR3 (Fig. 3B). Although the authors explained this discrepancy by discussing the difference between generating (in vitro) and maintaining (in vivo) nucleosome positioning by RSC complex, there is still a possibility that this discrepancy may result from artifacts of the in vitro reconstitution system. A better experiment would be to isolate the plasmids containing the in vivo positioned nucleosomes and incubate them with the rsc3-ts extracts. A minor issue is the labeling of NDR1-3 should be consistent for the RIM9 promoter for example the labeling of Fig 2 and 5 is different than that of Fig 3 and 4. Additionally, the in vivo Rsc3 binding site study in Fig 6 should be recapitulated with the in

vitro system. In summary, this paper shows, for the first time, by in vitro reconstitution that the RSC nucleosome remodeling complex is required for proper nucleosome positioning at the yeast promoter NDRs. The authors' in vitro and in vivo data support the hypothesis that specific chromatin remodeling enzymes are required to generate proper positioned nucleosomes. The paper is clearly written and the data will be of general interest to the chromatin field.

Referee #3 (Remarks to the Author):

Review of EMBOJ-2010-75466, by Wippo et al. (P. Korber lab)

The manuscript by Wippo et al. explores the relationship between chromatin remodelers and nucleosome positioning. Unlike previous reports that looked strictly at in vivo nucleosome positions, or strictly in vitro assemblies of chromatin, these authors assembled chromatin templates in the presence of nuclear extracts in an attempt to identify responsible factors that generated chromatin templates that matched the in vivo pattern.

The paper is well designed, executed and written. The quality of the data is acceptable, but one would have more confidence if clearer. Still, it is acceptable. Partial purification revealed components of the RSC chromatin remodeler as involved in nucleosome positioning in vitro at certain promoters. This result is interesting in relation to the more well-established role of RSC in altering nucleosome positioning rather than the assembly of chromatin. However, purified RSC was not competent in reproducing the in vivo pattern. This might be considered a serious limitation for the paper, however RSC itself could complement RSC-deficient cell extracts, defining a role for RSC in vitro, and suggesting additional components are required. Importantly, other remodelers were tested and were found insufficient in reproducing the in vivo pattern. Another strong section is the examination of the potential Rsc3 binding sites in the formation of this structure and the basal transcription state. As mentioned above, these results could describe a new role for RSC in establishing a preset nucleosome pattern.

I have three issues with the paper that need to be addressed. First, the authors elect to incubate the strains overnight at the restrictive temperature. This greatly increases the possibility that the affects seen are indirect and due to a cell viability issue. The authors need to verify the full viability of the strains by plating for single colonies following the Ts shift. This may help reconcile the authors' work with previous work where the shifts were for shorter periods, and viability was tested.

Second, more often than not, the observations in vitro and in vitro do not give the same results. The authors offer ad hoc reasons that might underlie this, but these inconsistencies remain a weakness. I agree that it is too high a bar to expect a series of promoters to all behave the same in vitro as in vivo. It remains for future work to make these connections, and I have an overall favorable view of this paper in the hope that those connections are made, but it remains entirely possible that the in vitro work will not connect, or that the in vivo work suffers from the long incubation times at the restrictive temperature.

Third, the authors need to show in supplemental data and discuss more thoroughly the impact of the Ts shift in the genomics work of others (Madhani, Hughes, and Ptashne). Here, it is not clear the extent to which the prior whole-genome work supports or does not support the authors' changes at the loci tested.

One more relevant issue is testing whether the omission of the Rsc3 site at -151 affects the presence of RSC at that location by ChIP.

Overall, this paper has several good elements and a new twist on RSC function, and with moderate improvement could be acceptable for the EMBO Journal.

1st Revision - authors' response 16 December 2010

Referee #1 (Remarks to the Author):

This is useful, albeit disjointed, paper that is on the margin for publication in EMBO J; I lean slightly positive. The in vitro experiments are the strongest aspect of the paper. The authors show that RSC moves nucleosomes from their intrinsically preferred positions (nucleosome assembly via salt dialysis) to something resembling their in vivo positions. This is shown with purified RSC in the presence or absence of extract from rsc mutant cells. The authors do not perform detailed mapping of the position, which should be done to show that the positions are truly those in vivo.

> This is a valid comment and we wholly agree that the direct comparison of in vivo and in vitro nucleosome patterns is crucial to our study. We point out now explicitly in our paper that all samples were analysed side-by-side by the same technique to allow such direct comparison (p. 6, lines 8 to 6 from bottom). With regard to the degree of detail, this study defines in vivo positioning at the resolution of indirect endlabeling, a technique that monitors the midpoint of the Gaussian distribution of nucleosome positions, which ranges in 10 bp increments for at least 30 bp even for highly positioned nucleosomes (Jiang and Pugh, 2009, Nat Rev Genet). In this analysis, it is very significant that we can reconstitute this Gaussian distribution in vitro. We would like to carry out base pair resolution mapping at a latter date, which will reveal if some positions of this distribution are more heavily occupied in vitro than in vivo. However, this would not affect the main conclusion of our present manuscript (see also Tanaka S, Livingstone-Zatchej M, Thoma F, JMB, 1996, especially last sentence of abstract).

It is clear that RSC only does this at some genomic regions, and that other activities are required. An interesting result is that RSC is not equivalent to Isw2 or Swi/Snf, indicating that there is specificity to which remodeling complexes function at different regions. It could be argued that this is already known (by in vitro assays not being compared to in vivo positions), and that it is merely chance that RSC allows movement to in vivo positions at the relevant regions. On balance, however, this is a useful and novel contribution to the field.

The in vivo experiments involving Rsc mutants are less useful, and indeed these are not mentioned in the abstract. Such experiments have been done before, so the results here do not add much. Furthermore, there is no clear link to the in vitro experiments, which is a significant weakness. The results are poorly described; they are basically just listed with no coherent point being made. I think these should be deleted.

> We agree with the other two Reviewers and with the Editor who all expressed that the in vivo results should remain part of the manuscript. The Reviewer is correct that similar experiments have been done before. Nonetheless, it is usually advisable to confirm genome-wide data by locus-specific techniques for regions of interest. In addition, no one checked so far if the chromatin changes at the PHO8 promoter were Pho4-dependent (Suppl. Fig. S5; p. 13, line 6 from bottom to p. 14, line 6). Importantly, our in vivo analysis was done by the exact same method as the in vitro experiments thus allowing a more direct comparison. We make this link between the in vitro and the in vivo data now clearly in the manuscript (p. 12, last paragraph to p.13, line 5) and mention the in vivo data now in the abstract.

The experiments involving the RSC target site at -151 are useful and interesting. However, these are also not mentioned in the abstract, and they are not connected to the in vitro experiments. These could be retained, although a better effort to connect the results to the in vitro experiments would be nice.

> We appreciate the recognition of the Rsc3 binding site experiments as useful and interesting, but we also see that they are not essential for the main focus of our study, i.e. the in vitro experiments. As we could not observe an effect on RSC recruitment by ChIP (see reply to Reviewer 3 below), we decided to clarify the cause of the Rsc3 binding site effects later and to delete this part from the manuscript.

In general, the manuscript is rather longwinded, especially in the description of results.

> We apologize for seeming longwinded. We went through the whole manuscript again with special focus on concise writing. Especially by deleting the part relating to the Rsc3 sites, we shortened the manuscript substantially.

Referee #2 (Remarks to the Author):

The work described in this paper employed wild type and mutant yeast extracts to explore the molecular mechanisms of nucleosome positioning. The authors discovered a role for the RSC chromatin remodeling enzyme in generating in vivo-like nucleosome patterns at cloned S.cerevisiae promoters. RSC, but not equivalent remodeling units of SWI/SNF and ISW2, could rescue the mutant extract and restore in vivo-like nucleosome positioning. Given the widespread interest in nucleosome positioning, and the paucity of mechanistic studies, this work is interesting and important. Overall, the data are solid and credible. One weak point is the discrepancy between the in vitro and in vivo experiments in Figures 3 and 5. In vitro, it is shown that rsc3-ts extracts were unable to produce the NDRs of the CHA1 and SNT1 promoters (Fig. 3C, D). However, in vivo the same NDRs were present at the restrictive temperature in vivo (Fig. 5C, D). Additionally, in vivo, rsc3-ts at 37°C affected only NDR2 of the RIM9 promoter, but not NDR1 and NDR3 (Fig.5B). However, in vitro, the rsc3-ts extracts were unable to generate NDR2 and NDR3 (Fig. 3B).

> We agree with the Reviewer, and already pointed out in the first version, that there are discrepancies between the in vitro and in vivo results. Particularly, already the wt extract fails to generate the in vivo patterns at some loci (p. 8, lines 11 to 5 from bottom).
However, of all the examples (CHA1, SNT1 and RIM9) pointed out by the Reviewer, only the NDR at SNT1 remains a true discrepancy. Our new data on CHA1 (new Fig. 5C, data of old Fig. 5C now in new Suppl. Fig. S7C, see also p. 14, last paragraph) do show an effect of RSC ablation also in vivo, consistent with our in vitro results and with in vivo data of others (new Suppl. Fig. S4C, new Suppl. Table S3). Further, NDR3 at RIM9 (note that this is now NDR1 in the new version due to consistent re-numbering, see below) is one example that could not be generated by the wt extract (see above). So the in vitro rsc3-ts 37°C effect at RIM9 (Fig. 3B) relates only to NDR2, just as in vivo.

We directly address the remaining discrepancy regarding the NDR at SNT1 (p. 20, lines 3 to 18). The redundancy between Abf1 and Reb1 in keeping the SNT1 NDR open (new Suppl. Fig. S4D and Hartley PD and Madhani HD, 2009, Cell), makes it very likely, that these factors could maintain the NDR even after RSC ablation, while the in vitro system could not set it up de novo. In addition, the residual RSC activity under restrictive conditions in vivo may be still sufficient at SNT1 whereas the in vitro rsc3-ts 37°C extract apparently contained even less RSC activity.

Although the authors explained this discrepancy by discussing the difference between generating (in vitro) and maintaining (in vivo) nucleosome positioning by RSC complex, there is still a possibility that this discrepancy may result from artifacts of the in vitro reconstitution system. A better experiment would be to isolate the plasmids containing the in vivo positioned nucleosomes and incubate them with the rsc3-ts extracts.

> We agree that this would be a better experiment. However, in our hands we were not able to find conditions that remove remodeling activities from pre-assembled templates without disturbing nucleosome positioning. For example, treatment with sarkosyl or high salt may remove RSC, which is a prerequisite of the suggested experiment, but concomittantly leads to repositioning of nucleosomes (Korber P and Hörz W, JBC, 2004, Fig. 3 and the respective text on p. 35116-7, and Varga-Weisz PD et al., Nature, 1997, Figure 1).

In order to address the Reviewer's concern, we tried an alternative experiment. We wished to use the rsc3-ts 25°C extract for proper nucleosome positioning (see Fig. 3, lanes 12-13) and then incubate this chromatin at 37°C in the hope to inactivate RSC in vitro. This would amount to the establishment of proper positioning in the presence of RSC and then to testing the maintenance of positioning in the absence of RSC as suggested by the Reviewer. However, incubation at 37°C

failed to inactivate the rsc3-ts 25°C extract in vitro (data not shown). So this alternative experiment was not technically feasible.

A minor issue is the labeling of NDR1-3 should be consistent for the RIM9 promoter for example the labeling of Fig 2 and 5 is different than that of Fig 3 and 4.

> The labeling is now consistent.

Additionally, the in vivo Rsc3 binding site study in Fig 6 should be recapitulated with the in vitro system.

> As the in vivo effect of deleting Rsc3 binding sites turned out not to affect RSC recruitment in vivo (see reply to Reviewer 3 below), we decided to follow up this issue in a future study and deleted this part from the current manuscript.

In summary, this paper shows, for the first time, by in vitro reconstitution that the RSC nucleosome remodeling complex is required for proper nucleosome positioning at the yeast promoter NDRs. The authors' in vitro and in vivo data support the hypothesis that specific chromatin remodeling enzymes are required to generate proper positioned nucleosomes. The paper is clearly written and the data will be of general interest to the chromatin field.

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I have three issues with the paper that need to be addressed. First, the authors elect to incubate the strains overnight at the restrictive temperature. This greatly increases the possibility that the affects seen are indirect and due to a cell viability issue. The authors need to verify the full viability of the strains by plating for single colonies following the Ts shift. This may help reconcile the authors' work with previous work where the shifts were for shorter periods, and viability was tested.

> We entirely agree with the Reviewer's concern. Indeed, it is one of the main points of our in vitro data that we can show a direct, necessary and specific role for RSC in nucleosome positioning without the unavoidable uncertainties of conditional mutant experiments in vivo.
As the rsc3-ts data by Badis et al are the most comprehensive and our main reference point, we analysed again all loci where we saw rsc3-ts effects (PHO8, RIM9, CHA1, RIO1, GAL10) after 6.5 h at 37°C, which are the same conditions as used by Badis et al. The data are now shown in new Suppl. Fig. S7 and reassured us that both conditions produced identical results.

Further, even though viability under our conditions is indeed compromised as determined by single colony plating (47 +/- 2 % for arp9-ts and < 5% for rsc3-ts and sth1-td; p. 15, lines 7 to 8) our data are generally in excellent agreement with published data or show even fewer effects, for example at CHA1 or GAL10 in the sth1-td strain (see new Suppl. Table S3 and new Suppl. Figure S4 and our reply below). So there is no indication that our overnight incubation conditions artifactually exacerbated or generated new effects at the tested loci. The only exception is the changed chromatin pattern that we observed at the PHO8 promoter in the sth1-td mutant after overnight incubation, whereas Parnell et al did not see changes in this strain after 2h at 37°C. However, as this changed PHO8 pattern was the same as for the other two mutants (Figure 5A) and also the same in the rsc3-ts mutant after shorter incubation times (6.5 h at 37°C, new Suppl. Fig. S7A), we are very confident that it reflects the true effect at PHO8 upon ablation of RSC activity.

We discuss the issue of cell viability and also the comparison of our data to published data (see Reviewer's point below) now extensively in the new version (p. 15, lines 5 to 22).

Second, more often than not, the observations in vitro and in vitro do not give the same results. The authors offer ad hoc reasons that might underlie this, but these inconsistencies remain a weakness. I agree that it is too high a bar to expect a series of promoters to all behave the same in vitro as in vivo. It remains for future work to make these connections, and I have an overall favorable view of this paper in the hope that those connections are made, but it remains entirely possible that the in vitro work will not connect, or that the in vivo work suffers from the long incubation times at the restrictive temperature.

> We agree with the Reviewer that it is "too high a bar" to expect perfect correspondence between in vitro and in vivo results at all these loci. Nonetheless, at the vast majority of loci, i.e., at PHO8, CHA1, SNT1 (all in Fig. 2), PHO5 (Hertel CB et al., 2005, MCB) and PHO84 (Suppl. Fig. S2 and Wippo CJ et al., 2009, MCB) the extract system reconstitutes very much in vivo-like chromatin patterns. It is really mainly the RIM9 locus (Fig. 2B) and some other NDRs (see p. 8, lines 11 to 5 from bottom) where the in vitro system could not recapitulate well the in vivo pattern. It will be a goal of future studies to find out what is missing in the extract to achieve proper positioning at these loci. Nonetheless, we wish to underscore that our in vitro assembly system generates the best correspondence technically possible at the moment. We are not aware of any other approach that is able to generate more in vivo-like nucleosome positioning in vitro. Despite its limitations we show here that our approach is valid as it identified the same factor, RSC, as was previously found, and confirmed here, by in vivo studies.

As argued above, it is unlikely that the long incubation times of the in vivo experiments are the reason for any discrepancies as the same effects were seen at shorter incubation times and as we usually see less effects in vivo (e.g. at SNT1 or CHA1) than in vitro.

Third, the authors need to show in supplemental data and discuss more thoroughly the impact of the Ts shift in the genomics work of others (Madhani, Hughes, and Ptashne). Here, it is not clear the extent to which the prior whole-genome work supports or does not support the authors' changes at the loci tested.

> We show and discuss now all available data from other groups regarding RSC effects and RSC binding at our test loci (new Suppl. Fig. S4, new Suppl. Tables S3 and S4, p. 13 lines 12 to 18; p. 14, line 7 to p. 15, second line from bottom; p. 17, lines 7 to 3 from bottom). The overview in new Supplementary Table S3 shows that we observe the same or sometimes a bit fewer effects as others. It also shows discrepancies within the literature, both for nucleosome occupancy changes upon ablation of different RSC subunits (at PHO8, CHA1 and RIO1) and even more with regard to RSC binding data (at all loci besides RIM9 and RIO1).

One more relevant issue is testing whether the omission of the Rsc3 site at -151 affects the presence of RSC at that location by ChIP.

> We are especially grateful for this suggestion. We did this ChIP experiment, observed clear RSC

recruitment at the PHO8 locus relative to control loci, but were surprised that deletion of the Rsc3 sites did not affect RSC recruitment to PHO8 in vivo. This leaves the role of the Rsc3 binding sites unresolved and requires a more detailed future study. As it is not essential for the main conclusion of our manuscript, we decided to delete this part.

Overall, this paper has several good elements and a new twist on RSC function, and with moderate improvement could be acceptable for the EMBO Journal.

2nd Editorial Decision 04 January 2011

Your revised manuscript has been reviewed by one of the original referees who finds that you have addressed most of the concerns and recommends publication of the study in The EMBO Journal, once the additional viability data are included. Pending this minor revision, we would be happy to publish your manuscript in the EMBO Journal.

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

Referee #3 (Remarks to the Author):

Review of revised EMBOJ-2010-75466R (Wippo et al., P. Korber lab)

The revised version is moderately improved, as the authors made efforts to address my concerns. On the major issue regarding viability I am both quite disappointed in the measured viability (quite low in some cases), but also pleased that the authors repeated the experiment at an earlier time point (6.5 hrs) with similar results. However, the authors still do not report the viability at this time point in their hands. I request that the authors please add the information regarding viability at 6.5 hrs to the manuscript so that this data can be properly evaluated by the reader. It remains possible that cell viability is also low at 6.5 hrs. The fact that the author's time point is the same as another investigator does not prove that the cells are viable in their system - it must be tested and reported.

On the second issue, no experiment was needed - I was looking whether the authors' had more information or work that might help the in vitro and in vivo results become more congruent - but we will have to wait for this.

One the third issue, the author's offer additional data analysis in the form of a Supplemental Table, and a bit of discussion. It appears that there is variability among the groups, which makes things hard to reconcile - though reconciliation of the field is not the charge of this paper. The current data, as now presented, gives the reader the current the lay of the land.

I'm very glad the authors did the ChIP experiment in the -151 mutant, which shows a lack of need for this site. This takes away a section of the paper, but not one critical for acceptance. It is fortunate that this experiment was done, otherwise the reader might have assumed that this cis site was a major player for recruitment.

Overall, the paper is a solid advance with some new evidence that RSC might help place nucleosomes in particular positions in vivo, and that there might be an in vitro system in hand to reveal additional players. The variability in the assay and the need for other factors suggest that we are only part way with this story, but it is a significant step in an interesting direction, and the role of remodelers in positioning in vivo is a difficult and important area.

2nd Revision - authors' response 21 January 2011

Referee #3 (Remarks to the Author):

Review of revised EMBOJ-2010-75466R (Wippo et al., P. Korber lab)

The revised version is moderately improved, as the authors made efforts to address my concerns. On the major issue regarding viability I am both quite disappointed in the measured viability (quite low in some cases), but also pleased that the authors repeated the experiment at an earlier time point (6.5 hrs) with similar results. However, the authors still do not report the viability at this time point in their hands. I request that the authors please add the information regarding viability at 6.5 hrs to the manuscript so that this data can be properly evaluated by the reader. It remains possible that cell viability is also low at 6.5 hrs. The fact that the author's time point is the same as another investigator does not prove that the cells are viable in their system - it must be tested and reported.

> We agree with the Referee that this is an important point. We measured the viability for the rsc3-ts mutant after 6.5 hours at the restrictive temperature and report it now (p. 15, lines 13 to 14) as 31 ± 3%, which is substantially higher than after overnight incubation (< 5%). So together with the other arguments outlined on p. 15 we are confident that our in vivo data do not just reflect exaggerated or artifactual effects due to severely compromised cell viability under restrictive conditions. Instead, these data confirm, in line with data from other groups, that RSC has a role in nucleosome positioning also in vivo as strongly suggested by our in vitro data.

On the second issue, no experiment was needed - I was looking whether the authors' had more information or work that might help the in vitro and in vivo results become more congruent - but we will have to wait for this.

One the third issue, the author's offer additional data analysis in the form of a Supplemental Table, and a bit of discussion. It appears that there is variability among the groups, which makes things hard to reconcile - though reconciliation of the field is not the charge of this paper. The current data, as now presented, gives the reader the current the lay of the land.

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Overall, the paper is a solid advance with some new evidence that RSC might help place nucleosomes in particular positions in vivo, and that there might be an in vitro system in hand to reveal additional players. The variability in the assay and the need for other factors suggest that we are only part way with this story, but it is a significant step in an interesting direction, and the role of remodelers in positioning in vivo is a difficult and important area.

3rd Editorial Decision 25 January 2011

I have looked through your revised manuscript and I am happy to accept it 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

