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# Nucleosome eviction in mitosis assists condensin loading and chromosome condensation

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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.)

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1st Editorial Decision                                                    30 September 2015

Thank you for submitting your manuscript on nucleosome eviction and condensin loading for consideration by The EMBO Journal. I apologize for the delay in getting back to you with an editorial decision, which was due both to a submission backlog and to absence from the office. We have in the meantime received the reports of three expert referees, which are copied below for your information. As you will see, the reviewers acknowledge the general interest of the topic as well as the potential importance of your findings. At the same time, all referees however raise major concerns regarding the decisiveness of the presented experimental evidence. Especially referees 2 and 3 remain unconvinced that some of the key conclusions are strongly supported by the current data. I am therefore afraid it would seem premature for us to commit to eventual future acceptance and publication in The EMBO Journal at this stage.

Given the potential interest of the study, we would nevertheless remain open to further considering a revised manuscript for publication, should further experimental efforts allow you to extend and solidify the evidence supporting the mechanistic conclusions. Key points in this respect would be to relieve the current over-reliance on strongly processed ChIP-PCR data partially lacking statistical analyses, and to complement them with ChIP-seq data for at least some of the key experiments. Likewise, it would be essential to directly analyze nucleosome eviction and to add histone profiling data.

Thank you again for the opportunity to consider this work for The EMBO Journal, and please do not hesitate to contact me should you have any comments or questions regarding the referee reports or this decision. I look forward to your eventual revision.

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REFEREE COMMENTS


Referee #1:

This manuscript uses genetics in the fission yeast to identify factors that contribute to condensin loading onto chromosomes. The authors identify the Gcn5 acetyl transferase of the SAGA complex and the RSC chromatin remodelling complex as factors that facilitate condensin binding to chromosomes. Together with recent results that the RSC complex facilitates cohesin loading onto chromosomes, this manuscript completes a picture in which nucleosome depleted regions are an integral part of loading all these SMC complexes onto chromosomes. This is an important insight that is worth publication in the EMBO Journal. Most of the authors' results and conclusions are thoroughly and convincingly documented, with a few exceptions noted below. A drawback that should also be corrected is the sketchy introduction. Several previous studies have characterised condensin loading factors and these should be mentioned and contrasted. Once these issues have been addressed, the authors' new results provide an important advance in our understanding of how condensin binds to chromosomes.

1. Introduction, page 3, "It is widely accepted that Topo II ensures decatenation between sister-chromatids and chromosomes". While Baxter et al. 2011 propose a model for how condensin action supports chromosome disentanglement, the demonstration that condensin actually promotes decatenation comes from Charbin et al. 2014, which should be mentioned.

2. page 3, "the mechanisms underlying the critical loading of condensins have remained elusive". This is not completely true, Johzuka and Horiuchi 2009 have characterised a condensin loading complex at the budding yeast rDNA, Hirano and Collas 2002 have described the human AKAP95 transcription factor as a condensin loading factor, while in fission yeast Schmidt et al. 2009 found that condensin loading coincides with TFIIIC and Fhl1 transcription factors (which in turn are linked to RSC). These studies form the relevant background for the present study and should be cited.

3. page 4, "condensins are enriched at centromeres, telomeres, and along chromosome arms nearby genes that are highly transcribed". In fission yeast, this was to my knowledge shown for the first time by Schmidt et al. 2009, while Sutani et al. 2015 and Nakazawa et al. 2015 have both characterised this in more detail. In particular, they noted that condensin loading takes place at highly transcribed genes, yet condensin appears to be excluded from the transcription unit. Again, these studies should be referred to.

4. page 4, "transcription by all three RNA Pols is shut down during mitosis", my understanding was that this is not the case in yeast, please clarify, as this is relevant to the authors' argument.

5. Figure 1B, a role of Gcn5 in chromosome condensation is not obvious from this figure. The difference between gcn5-47 and wild type is very small and is not specific to mitosis. It rather appears that chromosomes are overall larger in gcn5-47 cells, maybe as the consequence of enlarged cell size of the mutant? The authors could combine the gcn5-47 with a condensin mutation at an intermediate temperature, as in Figure 1A. This might demonstrate a role for Gcn5 in condensation more clearly.

6. Figure 2, a schematic of the SAGA complex with its three modules, indicating the respective subunit names, would be helpful for readers who are less familiar with the yeast gene nomenclature.

7. Figure 5, how were histone levels at promoters quantified? Shown is enrichment relative to wild type, which is a highly derived measure. It would be preferable to present less processed data, e.g. ChIP efficiency as % of input DNA. Greater ChIP efficiency in the Gcn5 mutant could reflect

greater histone occupancy or alternatively greater chromatin accessibility? Did the authors perform histone profiling to look at histone distribution at these promoters?

8. Throughout the study, it remains unclear whether condensin loading occurs at all transcribed genes, i.e. all H3K9/K18ac positive promoters, or at a specific subset of those? This is not conclusively answered by the genome wide correlation of condensin binding with NFRs reported in Figure 6. The correlation could arise from condensin binding to either a subset or to all NFRs. To fully understand the selection of condensin binding sites, it would be interesting to know the answer to this question.

9. Related to what is shown in Figure 6, there seem to be two possible explanations for why condensin binding at 3' ends of genes is affected by Gcn5 and RSC mutations. Either Gcn5 and RSC are present both at 5' and 3' ends of genes and affect condensin binding at both places. Alternatively, Gcn5 and RSC are concentrated at the promoters of active genes and load condensin there. Then condensin slides to 3' ends, as suggested for cohesin. Do the authors have an opinion on this?

Referee #2:

Toselli-Mollereau et al. describe in this manuscript how condensin is targeted to specific sites along the genome to promote chromosome condensation during mitosis. Their results show that the activity of the Gcn5 histone acetyltransferase is an important element in this process as supported by its genetic interaction with condensin and by the reduced binding of condensin to chromosomes in the absence of Gcn5 (Figures 1-3). In the second part of the study, the authors try to work out the underlying mechanism and suggest that histone acetylation by Gcn5 mediates nucleosome eviction at some promoters as a prerequisite for condensin uploading (Figures 4-6). Although this is a conceivable possibility, I think that results in this part are rather preliminary and, as suggested below, more work should be done to support their conclusions.

1. Figure 3A shows reduced binding of condensin to a selection of genes in Gcn5Δ and Gcn5Δ Mst2Δ double mutant in mitosis-arrested cells. Since several examples are shown, I would suggest to exclude from this analysis genes like 5S or tRNAS or, at least, to indicate that these genes are repeated and, therefore, the results represent the average of the multiple copies in the genome.

According to the model proposed by the authors, the seven single-copy genes bound by condensin in Fig. 3A, should belong to the limited number of regions that remain bound by Gcn5 during mitosis. Gcn5 binding is shown in Fig. 4B for three of the seven genes plus the ste11 gene. Although presented as an additional example, I am not sure that this gene is a good choice due to the small amount Gcn5 binding (Fig. 4B). In fact, binding to ste11 (described as positive) is lower than binding to the body of the prl53 gene (described as negative, page 9 line 12).

In addition to this, my main objection to this part of the work is that no example of negative controls are shown. To proof that condensin is targeted to specific genes in mitosis depending on Gcn5, the authors should select some genes with comparable levels of acetylation and Gcn5 binding in wild-type cycling cells (there must be plenty according to Fig. 4A). Then, they should show that only some of them (for example prl53, exg1, cdc22, and perhaps some other of the seven genes in Fig. 3A) retain this feature in mitosis while Gcn5 is lost from the remaining selected genes, which would act as negative controls. Then they should test whether condensin binds specifically to those that retained Gcn5 binding but not to those that failed to maintain Gcn5 binding and acetylation in mitosis relative to cycling cells.

2. The second part of the model proposes that nucleosomes are evicted from the Gcn5 acetylated promoters as a prerequisite for condensin binding. My second main objection is that no examples of nucleosome eviction are shown anywhere in the manuscript. The histone occupancy analyses in Fig. 5 do not have enough resolution to conclude that nucleosomes have been evicted from promoters as it is stated in many places of the manuscript and in its title. This is a key point of the model and the presence or absence of nucleosome-depleted regions (NDR) should be tested by micrococcal nuclease or DNAse I analysis and end-label hybridization (or, optionally, by genome-wide

nucleosome mapping) across the relevant regions to detect the presence/absence of NDRs at the 5' end of genes.

Following the same logic as in point 1, the authors should show that NDRs appear specifically (or are maintained) in promoters that retain Gcn5 binding during mitosis in wild type cells and that NDRs are lost (or were never present) in genes bound by Gcn5 in cycling cells that do not retain it during mitosis (negative controls cited in point 1). If this were the case, the model predicts that these mitosis-specific and Gcn5-dependent NDRs should be totally or partially lost in Gcn5Δ mutants. This prediction should also be tested by nuclease analysis.

3. As regards the ChIP analysis to measure the level of H3K9ac, H3K18ac and H3K14ac (Fig 4E and 4F), it is not clear how the ratio of the three froms of acetylated H3 relative to total H3 can be higher than 1. The most extreme case is the 5' IGR of cdc22, which seems to have more than five times H3K18ac than total H3 (Fig. 4F).

4. Figure 6A shows that 50% of NDRs map between 1 and 5 kb away from cohesing binding sites (median above 2 kb). In fact, condensin accumulates towards the 3' end of genes on average (Fig 6B). These data by themselves, do not argue in favour (or against of the proposed model. Also, data in Fig. 6 C-D are somehow redundant with those in Fig. 3A because they show that condensing binding is reduced at the 3' end of three genes in the Gcn5Δ and Gcn5Δ Mst2Δ double mutant as it was shown in the body of the same genes in Fig. 3A.

In summary, I think that the model proposing that Gcn5 acetylates some promoters during mitosis followed by nucleosome eviction as a prerequisite for condensin uploading, although plausible, is not sufficiently supported by the evidence presented and the predictions it makes should be tested more rigorously.

Referee #3:

Considerable progress has been made in a number of organisms showing where condensin binds but how and what features of DNA facilitates this process are still unclear. This paper makes a number of important findings and sheds new insight on a difficult problem. The study finds that nucleosome-depleted regions formed at highly expressed genes forms an entry point to condensin binding. Through a synthetic lethal screen they show the histone acetyltransferase Gcn5 mediates condensin binding and demonstrate a relationship between Gcn5,acetylated H3 and condensin binding during mitosis. They further show Gcn5 and another HAT Mst2 collaborate strongly together to remove promoter nucleosomes to assist condensin binding and demonstrate the parallel activity and principle for RSC. Some very nice data on Gcn5 and condensation is also provided early in the manuscript. The data is generally very tight and the findings significant. The frustrating aspect was the over reliance of ChIP-PCR to confirm many findings which somewhat limits the breadth of the conclusions.

Comments:

1) A better explanation of the synthetic lethal screen with cut-477 is needed. As I understand, cut3-477 is lethal at 36 degrees and is defective but not lethal at 32 degrees. Therefore the synthetic lethality is at 32 degrees and should be stated clearly in the results section. Some indication on the panel (Figure 2A) as to which module (HAT, Spt, deubiquitylase) each subunit belongs would also assist the reader.

2) Although the ChIP-PCR confirms the observations throughout, a more informative assay would be ChIP-seq that would also take away any notion of bias. For instance, in Figure 3,10 genes occupied by condensin are chosen for ChIP analyses to illustrate the relationship between Cdn2-GFP and Gcn5. However, the rationale for selecting these is not entirely clear other than they were common to 3 genome wide condensin maps. A more definitive experiment would be a ChIP-seq comparison of cnd2, gcn5, gcn5 Δcnd2, and perhaps acetylated H3, including alignment of peaks for select genes. Although it is too much to ask ChIP-seq for all the histone marks and ChIP analyses, a limited number of ChIP-seq on the key findings would significantly broaden the conclusions.

3) Statistics are provided for ChIP-PCR in Figure 3A but not for the remaining ChIPs in the main and expanded view figures. Some statistics should be provided where any significant differences are stated.

4) The fluorescence panel of Figure 3C shows No tag and cut3-HA but not cut3-HA Δgcn5 or cut3-HA Δgcn5 Δmst2 which is what is being quantitated and therefore should be shown as a representative image. For completeness, cut3-HAΔgcn5 should be added to the immunoblot (Fig 3D).

5) Figure 4A has no representative images for Gcn5-myc and Cut14-HA immunofluorescence on chromosomes to accompany the quantification. These should be added.

6) For Figure 4B,E,F ste11 is added to a subset of the 10 condensin enriched genes used for Figure 3, but it not clear why this extra gene has been added.

7) The discussion seems to concentrate on histone eviction and condensin binding at highly expressed genes, but does not mention if this mechanism might be relevant to other condensin enriched sites such as centromeres and telomeres.

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Additional correspondence (author)                                    07 October 2015

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Thank you for offering us the opportunity to submit a revised manuscript for publication. My colleagues and I are willing to address the concerns raised by the three referees. However, before committing into time consuming and costly experiments, we would like to have your opinion on our plan to succeed at final assessment of our revised study.

Main points raised by reviewer#1:

(a) Perform histone profiling at promoters adjoining condensin binding sites to rule out the possibility that greater ChIP efficiency for histones H3 and H4 in the gcn5 mutant reflects greater chromatin accessibilityrather than increased histone occupancy per se.

(b) Determine whether condensin associates with a subset or all nucleosome depleted regions (NDRs).

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Main points raised by reviewer#2:

(c) Provide example of negative controls, by showing that genes that do not retain Gcn5 during mitosis do not strongly recruit condensin.

(d) Provide unambiguous example of NDRs adjoining condensin binding sites by MNase or DNase I assay.

(e) Correlates the presence of NDR in mitosis with the presence of Gcn5.

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Main point raised by reviewer#3:

(f) Request a ChIP-seq comparison of Cnd2-GFP (the Cnd2 sub-unit of condensin, tagged with GFP) in wt and gcn5 mutant to relieve over-reliance on ChIP-qPCR and take away any notion of bias.

*To provide robust evidence for nucleosome eviction and add histone profiling data, *we will determine at the genome wide scale the

positioning of nucleosomes, during mitosis, in wt cells and /gcn5Δ
mst2Δ/ double mutant cells, by performing MNase-seq experiments.

*To relieve over-reliance on ChIP-qPCR data and complement them with
ChIP-seq data, *we will assess by ChIP-seq the chromosomal association of Cnd2-GFP in wt and
/gcn5Δ mst2Δ/ double mutant cells arrested in mitosis.

These experiments will allow us to:

(1) Define the nucleosome landscape (histone profiling) at all condensin binding sites in wt cells and
in cells lacking Gcn5 and Mst2 (points a and e).

(2) Assess at the genome-wide scale whether a nucleosome depleted region (NDR) is present in the
vicinity of major condensin binding sites (point d), and whether condensin binds to a subset or all
NDRs (point b).

(3) Determine, with high resolution, whether Gcn5 and Mst2 evict
nucleosomes from promoters adjoining condensin binding sites, i.e.
whether nucleosome free regions adjoining condensin binding sites are
filled-in by one or more nucleosomes when Gcn5 and Mst2 are lacking
(points a, d, e).

(4) Identify at the genome-wide scale the chromosomal sites where Gcn5 and Mst2 assist condensin
binding (point f)

(5) Draw a robust correlation between nucleosome eviction at gene
promoters by Gcn5 and Mst2 and condensin loading (point e)

To provide negative controls (point c), we plan to combine MNase-seq and ChIP-qPCR against
Gcn5-myc. We anticipate two scenarios depending on whether condensin binds to a subset or all
NDRs in mitosis. If condensin binds only a subset of NDRs, then comparing NDRs locations
(MNase-seq) and condensin peaks (Cnd2-GFP ChIP-seq) will identify NDRs that do not recruit
condensin. We will test by ChIP-qPCR against Gcn5-myc whether or not Gcn5 is retained at these
negative controls. If condensin accumulates at all NDRs present on mitotic chromosomes, then
searching for NDRs present on chromosomes in interphase but absent in mitosis might identify
appropriate negative controls. The positioning of nucleosomes has been determined in interphase in
fission yeast cells by MNase-seq (Soriano /et al/, 2013), but not during mitosis. By comparing
NDRs locations in mitosis and interphase, we will determine whether some interphase NDRs are
absent from mitotic chromosomes and assess Gcn5 binding at these locations by ChIP-qPCR.

We believe that these MNase-seq and ChIP-seq experiments will extend and solidify our previous
data and significantly strengthen the
conclusionthat nucleosome eviction assists condensin binding in mitosis. However, performing
these experiments is not trivial, and their realisation will constitute an important commitment for my
lab, in both terms of financial and human resources. Therefore, since EMBO J allows only a single
round of major revision, I would really appreciate having your opinion on this revision strategy, and
notably whether you believe that, if successful, the proposed experiments could guarantee us a
publication in EMBO J.

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Additional correspondence (editor)                                      12 October 2015

Thank you for contacting me with your detailed revision proposal. I have now carefully looked
through it, and further discussed it with one of the original reviewers, whose feedback is copied
below for your information. In conclusion, we agree that the planned experiments should be able to
address what we think are the key issues, and thus in principle warrant publication in The EMBO
Journal, provided that they turn out sufficiently conclusive. Please let me know in case you should
need an extension beyond the standard three months revision time frame to carry out this revision
work.

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REFEREE COMMENTS


Referee 2:
The proposed plan of action is reasonable, represents a significant amount of work and it will address my two main concerns about the manuscript. I would suggest that they try micrococcal nuclease instead of DNAse I for the mapping of the NDRs. This is the most widely used method and it will also give information on the positioning of nucleosomes flanking the NDR.

Even better, they intend to profile nucleosomes genome-wide. This will certainly be the best option because it will provide many examples of genes with differential NDRs to correlate with the presence/absence of Gcn5 and condensin in mitosis versus G2 (if this turns out to be the case).


1st Revision - authors' response                                                29 March 2016


*Point by point response to reviewers*

Referee #1

*This manuscript uses genetics in the fission yeast to identify factors that contribute to condensin loading onto chromosomes. The authors identify the Gcn5 acetyl transferase of the SAGA complex and the RSC chromatin remodelling complex as factors that facilitate condensin binding to chromosomes. Together with recent results that the RSC complex facilitates cohesin loading onto chromosomes, this manuscript completes a picture in which nucleosome depleted regions are an integral part of loading all these SMC complexes onto chromosomes. This is an important insight that is worth publication in the EMBO Journal. Most of the authors' results and conclusions are thoroughly and convincingly documented, with a few exceptions noted below. A drawback that should also be corrected is the sketchy introduction. Several previous studies have characterised condensin loading factors and these should be mentioned and contrasted. Once these issues have been addressed, the authors' new results provide an important advance in our understanding of how condensin binds to chromosomes.*
*1. Introduction, page 3, "It is widely accepted that Topo II ensures decatenation between sister-chromatids and chromosomes". While Baxter et al. 2011 propose a model for how condensin action supports chromosome disentanglement, the demonstration that condensin actually promotes decatenation comes from Charbin et al. 2014, which should be mentioned.*

The reference is now mentioned page 3, line 42, as requested.  Condensin aids sister chromatid decatenation by topoisomerase II. Adrian Charbin, Céline Bouchoux, Frank Uhlmann. Nucleic Acids Res. 2014 January 1; 42(1): 340–348

*2. page 3, "the mechanisms underlying the critical loading of condensins have remained elusive". This is not completely true, Johzuka and Horiuchi 2009 have characterised a condensin loading complex at the budding yeast rDNA, Hirano and Collas 2002 have described the human AKAP95 transcription factor as a condensin loading factor, while in fission yeast Schmidt et al. 2009 found that condensin loading coincides with TFIIIC and Fhl1 transcription factors (which in turn are linked to RSC). These studies form the relevant background for the present study and should be cited.*

The introduction has been reshaped to include these references and others. Please, see page 3-4, lines 61 to 86.

*3. page 4, "condensins are enriched at centromeres, telomeres, and along chromosome arms nearby genes that are highly transcribed". In fission yeast, this was to my knowledge shown for the first time by Schmidt et al. 2009, while Sutani et al. 2015 and Nakazawa et al. 2015 have both characterised this in more detail. In particular, they noted that condensin loading takes place at highly transcribed genes, yet condensin appears to be excluded from the transcription unit. Again, these studies should be referred to.*

The paper entitled "Conserved features of cohesin binding along fission yeast chromosomes." (Schmidt CK, Brookes N, Uhlmann F. Genome Biol. 2009;10(5):R52) is now cited page 4, line 70.

*4. page 4, "transcription by all three RNA Pols is shut down during mitosis", my understanding was that this is not the case in yeast, please clarify, as this is relevant to the authors' argument.*

Luis Aragon's lab has shown that, in budding yeast, transcription by RNA Pol I and RNA Pol II is repressed by Cdc14 during anaphase, and that this repression is necessary for condensin binding to DNA repeats (Clemente-Blanco *et al*, 2009, 2011). Thus, the repression of transcription during mitosis observed in higher eukaryotes might be conserved, at least partly, in yeasts. These two papers are cited in the manuscript at page 4, lines 97-98.

*5. Figure 1B, a role of Gcn5 in chromosome condensation is not obvious from this figure. The difference between gcn5-47 and wild type is very small and is not specific to mitosis. It rather appears that chromosomes are overall larger in gcn5-47 cells, maybe as the consequence of enlarged cell size of the mutant? The authors could combine the gcn5-47 with a condensin mutation at an intermediate temperature, as in Figure 1A. This might demonstrate a role for Gcn5 in condensation more clearly.*

We did not expect a severe condensation defect in *gcn5* mutant cells since condensin binding is reduced but not abolished. Some condensation activity is expected to persist along chromosome

arms in *gcn5* mutant cells. This might explain why the difference between gcn5-47 and wild type is rather small in the condensation assay. Regarding the fact that chromosomes appear less condensed throughout the cell cycle, we cannot rule out the possibility that chromatin structure might be relaxed during interphase because of a reduced acetylation in the absence of Gcn5. Alternatively, or additionally, lack of Gcn5 might affect the functioning of a minute fraction of condensin that operates on chromosomes in interphase. We now clearly mention these possibilities in the manuscript at page 6, lines-148-152:

"The distances between the fluorescently labelled loci were also slightly enlarged during interphase in gcn5-47 cells (Fig. 1C). Reduced acetylation of nucleosomes might relax chromatin fibers during interphase. Alternatively, or additionally, lack of Gcn5 might impair condensin-mediated chromosome shaping throughout the cell cycle. »

Importantly, we reinforce the idea of a condensation defect by two additional phenotypes. We show (1) the presence of chromatin bridges during anaphase in *gcn5* mutant cells (see Fig.1 D-E), and (2) that the arms of chromosome III remain untangled during anaphase when Gcn5 is impaired (Fig. EV1). These two phenotypes are typical of a chromosome condensation defect, and are also displayed by the condensin mutant *cut3-477*, used as control.

Page 6, lines 153-157, we write: "Consistent with impaired condensation, gcn5-47 mutant cells exhibited frequent chromatin bridges or chromatin trailing in anaphase (Fig. 1D) and failed to efficiently disentangle the rDNA repeats located on the arms of chromosome III (Fig. EV1). These phenotypes are frequently observed as a consequence of defects in mitotic chromosome condensation (Tada et al, 2011), for example in the cut3-477 condensin mutant (Fig. 1D and EV1A)."

Note that, to put more emphasis on the presence of chromatin bridges during anaphase, we moved this result from Fig1 EV1 in the submitted version of the manuscript to Fig. 1 D-E in the revised version.

We believe that, together, the chromosome condensation assay and the chromosome segregation defects provide convincing evidence for a partially defective condensation of chromosome arms in mitosis.


*6. Figure 2, a schematic of the SAGA complex with its three modules, indicating the respective subunit names, would be helpful for readers who are less familiar with the yeast gene nomenclature.*

This has been done. See Fig. 2A. We thank the reviewer for this suggestion.


*7. Figure 5, how were histone levels at promoters quantified? Shown is enrichment relative to wild type, which is a highly derived measure. It would be preferable to present less processed data, e.g.*

*ChIP efficiency as % of input DNA. Greater ChIP efficiency in the Gcn5 mutant could reflect greater histone occupancy or alternatively greater chromatin accessibility? Did the authors perform histone profiling to look at histone distribution at these promoters?*

Point 1. ChIP enrichments.  Enrichment initially shown in Fig. 5 corresponded to the % of Input DNA measured in mutant cells divided by the % of Input DNA measured in wt controls. The objective of this rather conventional normalization was to ease comparison. Nevertheless, we now provide graphs with non-normalized % IP in Fig. 5F, along with MNase-seq analyses (see below). Results remain unchanged.

Point2. Nucleosome accessibility vs occupancy.  We totally agree with the reviewer that greater ChIP efficiency in the gcn5 mutant could possibly reflect increased chromatin accessibility rather than increased occupancy. This criticism has been thoroughly addressed by performing MNase-seq experiments (see Fig. 5) and MNase-qPCR (nucleosome scanning assay) at a candidate promoter (see Fig. EV6C). Briefly, wt, *gcn5Δ* and *gcn5Δ mst2Δ* mutant cells were arrested in mitosis, chromatin was digested by MNase to produce mononucleosomes (Fig. EV6A), and mononucleosomal DNA fragments have been sequenced by massive parallel sequencing, or quantified by qPCR. As shown in Fig. 5 and Fig. EV6C, these experiments have revealed (1) that condensin tends to accumulate at or in the vicinity of nucleosome-depleted regions (NDRs), and (2) that Gcn5 and Mst2 evict nucleosome for NDRs occupied by condensin and/or adjoining condensin binding sites.

These new data are described in the manuscript pages 10-11, line 284-315.

The results of these direct analyses of nucleosome occupancy corroborate our previous conclusion that Gcn5 and Mst2 evicts nucleosome from condensin binding sites in mitosis.

*8. Throughout the study, it remains unclear whether condensin loading occurs at all transcribed genes, i.e. all H3K9/K18ac positive promoters, or at a specific subset of those? This is not conclusively answered by the genome wide correlation of condensin binding with NFRs reported in Figure 6. The correlation could arise from condensin binding to either a subset or to all NFRs. To fully understand the selection of condensin binding sites, it would be interesting to know the answer to this question.*

We addressed this point by comparing the results of our MNase-seq experiment with those of the condensin ChIP-seq experiment performed by Sutani et al (*Sutani et al. 2015*). Note that both experiment have been done on cells arrested at the same stage of the cell cycle, ie in pro/metaphase. Our results suggest that condensin binds only a subset of NFRs, and, therefore, that NFRs are necessary but not sufficient for creating a condensin binding site. This is mentioned in the manuscript page 14, lines 401-407.

"In addition, it should be noted that nucleosome depletion is unlikely to drive condensin binding by itself. We identified ~7000 NDRs in mitotic chromosomes in cells arrested in pro/metaphase, but solely ~400 condensin peaks (48 high and 340 low-occupancy) have been identified by ChIP-seq at a similar cell cycle stage (Sutani et al, 2015). This suggests the existence of NDRs devoid of condensin during mitosis. The corollary, therefore, is that nucleosome eviction is necessary but not sufficient for condensin binding. Hence, additional features/activities must attract condensin."

*9. Related to what is shown in Figure 6, there seem to be two possible explanations for why condensin binding at 3' ends of genes is affected by Gcn5 and RSC mutations. Either Gcn5 and RSC are present both at 5' and 3' ends of genes and affect condensin binding at both places. Alternatively, Gcn5 and RSC are concentrated at the promoters of active genes and load condensin there. Then condensin slides to 3' ends, as suggested for cohesin. Do the authors have an opinion on this?*

We assessed Gcn5 binding by ChIP at the 3' ends of genes highly occupied by condensin, but failed to detect Gcn5 at these locations (see Fig. EV5A). Regarding the sliding of condensin from promoters towards 3' ends, we address this point in the Discussion, page 13, lines 385-393.

"Like Gcn5, RSC is present at promoters adjoining condensin binding sites during mitosis and is necessary for condensin binding at the 3' end of genes. However, RSC deficiency increases nucleosome occupancy strongly at gene promoters but only moderately at the 3' end of genes (see Fig. 6B and EV7). This suggests that nucleosome eviction at gene promoters plays a crucial role in the binding of condensin at the 3' end of genes. Thus, given the enrichment of Gcn5 at gene promoters, and the physical and functional interactions between condensin and the TATA Binding Protein 1 (Iwasaki et al, 2015), it is tempting to speculate that condensin rings first associate with chromosomes at promoter NDRs and subsequently translocate towards the 3' end of genes, as proposed for the related cohesin complex (Lengronne et al, 2004)."

Referee #2:

*Toselli-Mollereau et al. describe in this manuscript how condensin is targeted to specific sites along the genome to promote chromosome condensation during mitosis. Their results show that the activity of the Gcn5 histone acetyltransferase is an important element in this process as supported by its genetic interaction with condensin and by the reduced binding of condensin to chromosomes in the absence of Gcn5 (Figures 1-3). In the second part of the study, the authors try to work out the underlying mechanism and suggest that histone acetylation by Gcn5 mediates nucleosome eviction at some promoters as a prerequisite for condensin uploading (Figures 4-6). Although this is a*

*conceivable possibility, I think that results in this part are rather preliminary and, as suggested below, more work should be done to support their conclusions.*

*1. Figure 3A shows reduced binding of condensin to a selection of genes in Gcn5Δ and Gcn5Δ Mst2Δ double mutant in mitosis-arrested cells. Since several examples are shown, I would suggest to exclude from this analysis genes like 5S or tRNAS or, at least, to indicate that these genes are repeated and, therefore, the results represent the average of the multiple copies in the genome.*

Regarding the 5S rRNA and tRNA genes, we used primers located in unique sequences located within their upstream 5'InterGenicRegions. This is mentioned is the legend of Fig. 3A page 20, line 602:"For repeated genes 5S and gly05, qPCR primers were designed within adjacent, unique 5' intergenic sequences. »

Moreover, we kept these two chromosomal sites as they served as negative controls for the persistence of Gcn5 during mitosis (see below).

*According to the model proposed by the authors, the seven single-copy genes bound by condensin in Fig. 3A, should belong to the limited number of regions that remain bound by Gcn5 during mitosis. Gcn5 binding is shown in Fig. 4B for three of the seven genes plus the ste11 gene. Although presented as an additional example, I am not sure that this gene is a good choice due to the small amount Gcn5 binding (Fig. 4B). In fact, binding to ste11 (described as positive) is lower than binding to the body of the prl53 gene (described as negative, page 9 line 12).*

The *ste11* gene. As suggested by the reviewer #2 and #3 we removed *ste11* in the revised version of our manuscript. However, to avoid any confusion and disbelief, we would like to emphasize the presence of a small, overlapping gene, called *SPAC27E2.11c,* within the body of *prl53*. The presence of this overlapping gene, and its promoter, within the body of *prl53* most likely explains why Gcn5 ChIP signals within the body of *prl53* appeared stronger than Gcn5 ChIP signal measured at the promoter of *ste11* in cycling cells. We probably not mentioned clearly enough the presence of this overlapping gene in the previous manuscript. For simplicity, the ste11 gene has been removed.

*In addition to this, my main objection to this part of the work is that no example of negative controls are shown. To proof that condensin is targeted to specific genes in mitosis depending on Gcn5, the authors should select some genes with comparable levels of acetylation and Gcn5 binding in wild-type cycling cells (there must be plenty according to Fig. 4A). Then, they should show that only some of them (for example prl53, exg1, cdc22, and perhaps some other of the seven genes in Fig. 3A) retain this feature in mitosis while Gcn5 is lost from the remaining selected genes, which would act as negative controls. Then they should test whether condensin binds specifically to those that retained Gcn5 binding but not to those that failed to maintain Gcn5 binding and acetylation in mitosis relative to cycling cells.*

Negative controls. We agree with reviewer 2 on the importance of providing examples of negative controls and addressed this criticism. See page 9, lines 248 -263.

As a preamble, we would like to mention that the condensin binding profile along chromosome arms in mitosis consists of low-occupancy binding sites and hot spots of association (high-occupancy binding sites), which correlate with highly expressed genes (D'Ambrosio *et al*, 2008; Schmidt *et al*, 2009; Nakazawa *et al*, 2015; Sutani *et al*, 2015). We used low-occupancy condensin binding sites as negative controls.

In Fig. 3A, we analyse condensin binding by ChIP at 9 high occupancy binding sites and at 5 low occupancy binding sites. We show that condensin binding is reduced at high-occupancy binding sites in cells lacking Gcn5, but remains unchanged at low occupancy-binding sites analyzed. Thus Gcn5 is required for condensin binding at high occupancy binding sites.

In Fig. 4B, we assess using ChIP against Gcn5-myc the levels of Gcn5 at promoters upstream of condensin binding sites, in interphase versus mitosis. We show that Gcn5 is bound to all promoters during interphase. During mitosis, however, Gcn5 becomes enriched at promoters of high-occupancy condensin binding sites, whilst it is reduced at low-occupancy condensin binding sites. This indicates that whether Gcn5 occupancy increases or drops at promoters during mitosis is linked to condensin occupancy. Importantly, the levels of Gcn5 are similar during interphase at the *slp1* high-occupancy condensin binding site and at low-occupancy binding sites. Thus, whether Gcn5 occupancy increases or drops at promoters during mitosis is unrelated to its absolute binding levels in interphase.

Low occupancy binding sites constitute the negative control requested by reviewer #2.

Together, our data indicate that Gcn5 is specifically retained or even enriched during mitosis at promoters adjoining high-occupancy condensin association sites, where condensin binding in return relies upon Gcn5.


*2. The second part of the model proposes that nucleosomes are evicted from the Gcn5 acetylated promoters as a prerequisite for condensin binding. My second main objection is that no examples of nucleosome eviction are shown anywhere in the manuscript. The histone occupancy analyses in Fig. 5 do not have enough resolution to conclude that nucleosomes have been evicted from promoters as it is stated in many places of the manuscript and in its title. This is a key point of the model and the presence or absence of nucleosome-depleted regions (NDR) should be tested by micrococcal nuclease or DNAse I analysis and end-label hybridization (or, optionally, by genome-wide nucleosome mapping) across the relevant regions to detect the presence/absence of NDRs at the 5' end of genes.*

Nucleosome occupancy. We agree with reviewer#2 that ChIP against H3 was insufficient to precisely measure nucleosome eviction at condensin binding sites. We addressed this point by

performing MNase-seq experiments. Results are shown in Fig. 5 and EV6, and described in the manuscript page 10-11, line 284-315.

Our MNase-seq experiments provide direct evidence that (1) condensin accumulate over NDRs at the 3' ends of genes, and (2) that Gcn5 and Mst2 are necessary for nucleosome eviction at or in the immediate vicinity of high-occupancy condensin binding sites during mitosis, where condensin binding relies upon Gcn5 and Mst2.

We confirmed this conclusion by performing MNase-qPCR (see Fig. EV6C).

*Following the same logic as in point 1, the authors should show that NDRs appear specifically (or are maintained) in promoters that retain Gcn5 binding during mitosis in wild type cells and that NDRs are lost (or were never present) in genes bound by Gcn5 in cycling cells that do not retain it during mitosis (negative controls cited in point 1). If this were the case, the model predicts that these mitosis-specific and Gcn5-dependent NDRs should be totally or partially lost in Gcn5Δ mutants. This prediction should also be tested by nuclease analysis.*

These prediction have been tested and verified. We compared condensin ChIP-seq data (Sutani et al. 2015) with our MNase-seq data, and provide evidence that the vast majority of condensin binding sites overlap with NDR during mitosis (see Fig. EV6B). We also show that Gcn5 and Mst2 are necessary for nucleosome eviction at high occupancy-binding sites (Fig. 5), where Gcn5 is enriched during mitosis (Fig. 4B), and where Gcn5 is, in return, required for condensin binding (Fig. 3A). Reciprocally, we show that Gcn5 and Mst2 are dispensable for nucleosome residence at the low occupancy binding site 5S (Fig. 5F and EV6D), where Gcn5 levels decreases in mitosis (Fig. 4B) and where condensin binding is independent of Gcn5 and Mst2 (Fig. 3A).

These data are described in details pages 10-11, lines 284-315 of the manuscript.

*3. As regards the ChIP analysis to measure the level of H3K9ac, H3K18ac and H3K14ac (Fig 4E and 4F), it is not clear how the ratio of the three froms of acetylated H3 relative to total H3 can be higher than 1. The most extreme case is the 5' IGR of cdc22, which seems to have more than five times H3K18ac than total H3 (Fig. 4F).*

Measuring histone H3 acetylation by ChIP necessitate to take into account the total amount of H3 bound to the chromosomal sites that are studied. Thus, for any acetyl-lysine assessed within histone H3, acetylation was calculated as the ratio H3ac/H3-Total. This ratio is determined, in part, by the respective affinities of the antibodies used for ChIP. When the anti H3-acetyl antibody has a higher affinity than the anti-H3 CTerm, this leads to H3-ac/H3 ratio superior to one.

*4. Figure 6A shows that 50% of NDRs map between 1 and 5 kb away from cohesing binding sites (median above 2 kb). In fact, condensin accumulates towards the 3' end of genes on average (Fig 6B). These data by themselves, do not argue in favour (or against of the proposed model. Also, data in Fig. 6 C-D are somehow redundant with those in Fig. 3A because they show that condensing binding is reduced at the 3' end of three genes in the Gcn5Δ and Gcn5Δ Mst2Δ double mutant as it was shown in the body of the same genes in Fig. 3A.*

Former Figure 6 has been removed since these previous analyses were performed using interphase NDRs from Soriano et al. (Soriano *et al*, 2013), and since we have now mapped NDRs on mitotic chromosomes by MNase-seq.

By comparing our MNase-seq data with condensin-ChIP-seq data acquired at a same stage of the cell cycle, we provide robust evidence that most condensin binding sites overlaps with an NDR at the 3' end of genes and/or resides in the immediate vicinity of an NDR at TSS (See Fig. 5B-C, and Fig. EV6B).

These data by themselves argue that nucleosomes constitute an obstacle for condensin localization, which is consistent with the proposed model.

*In summary, I think that the model proposing that Gcn5 acetylates some promoters during mitosis followed by nucleosome eviction as a prerequisite for condensin uploading, although plausible, is not sufficiently supported by the evidence presented and the predictions it makes should be tested more rigorously.*

We believe that the new data provided fully respond to the concerns of reviewer #2 and strongly support our previous model.

Referee #3:

*Considerable progress has been made in a number of organisms showing where condensin binds but how and what features of DNA facilitates this process are still unclear. This paper makes a number of important findings and sheds new insight on a difficult problem. The study finds that nucleosome-depleted regions formed at highly expressed genes forms an entry point to condensin binding. Through a synthetic lethal screen they show the histone acetyltransferase Gcn5 mediates condensin binding and demonstrate a relationship between Gcn5,acetylated H3 and condensin binding during mitosis. They further show Gcn5 and another HAT Mst2 collaborate strongly together to remove promoter nucleosomes to assist condensin binding and demonstrate the parallel activity and principle for RSC. Some very nice data on Gcn5 and condensation is also provided early in the manuscript. The data is generally very tight and the findings significant. The frustrating*

*aspect was the over reliance of ChIP-PCR to confirm many findings which somewhat limits the breadth of the conclusions.*

*Comments:*

*1) A better explanation of the synthetic lethal screen with cut-477 is needed. As I understand, cut3-477 is lethal at 36 degrees and is defective but not lethal at 32 degrees. Therefore the synthetic lethality is at 32 degrees and should be stated clearly in the results section. Some indication on the panel (Figure 2A) as to which module (HAT, Spt, deubiquitylase) each subunit belongs would also assist the reader.*

1a) A better explanation of the synthetic lethal screen. The synthetic lethality is now better explained. See page 6, line 129:

*"Fission yeast cells carrying the thermo-sensitive cut3-477 mutation in the Smc4 condensin subunit cease to divide at 36°C, but continue to proliferate at the semi permissive temperature of 32°C, even though condensin binding to chromosomes is reduced and mitotic chromosome condensation is partly impaired (Saka et al, 1994; Tada et al, 2011; Robellet et al, 2014). To identify factors that collaborate with condensin, we screened for mutations synthetically lethal with cut3-477 at 32°C (Robellet et al, 2014). »*

1b) A scheme of SAGA. A scheme of the SAGA complex has been added in Figure 2A to indicate the connection between subunits and modules. We thank the reviewer for this suggestion.

*2)   . For instance, in Figure 3, 10 genes occupied by condensin are chosen for ChIP analyses to illustrate the relationship between Cdn2-GFP and Gcn5. However, the rationale for selecting these is not entirely clear other than they were common to 3 genome wide condensin maps. A more definitive experiment would be a ChIP-seq comparison of cnd2, gcn5, gcn5 Δcnd2, and perhaps acetylated H3, including alignment of peaks for select genes. Although it is too much to ask ChIP-seq for all the histone marks and ChIP analyses, a limited number of ChIP-seq on the key findings would significantly broaden the conclusions.*

The rationale for choosin condensin binding sites. The condensin binding profile in mitosis consist of low-occupancy binding sites punctuated by high-occupancy binding sites which correspond to genes highly transcribed by RNA Pol II. Genes selected to analyse condensin binding by ChIP in Fig.3 where chosen to represent both high-occupancy and low-occupancy binding sites.

ChIP-seq. We totally agree we reviewer #3 that a genome-wide comparison of condensin binding in wt versus mutants would significantly broaden/strengthen our conclusions. However, we would like to emphasize that conventional ChIP-seq is not quantitative (see Hu *et al*, 2015; Bonhoure *et al*, 2014; Orlando *et al*, 2014), and, therefore, cannot be used to compare condensin binding in wt and mutant cells. To circumvent this problem, we tried during the revision period to apply a quantitative,

calibrated ChIP-seq method, called chromodynamics, which allows quantifying ChIP-seq profiles (Hu *et al*, 2015). The principle is to mix defined number of *S. cerevisiae* cells with *S. pombe* cells, both expressing GFP-tagged condensin, to ChIP condensin from the mixture and to assess occupancies onto both genomes. *S. cerevisiae* cells provides the calibrating genome (the internal standard) that enables to compare occupancy ratios between wt and mutant *S. pombe* cells. Unfortunately, we failed to obtain experimental conditions where both *S. cerevisiae* and *S. pombe* chromatin are simultaneously and efficiently ChIPed.

Thus, to try to broaden our conclusions, we increased the number of arm condensin binding sites analysed to 14: with 9 high-occupancy binding sites and 5 low occupancy. Results remain unchanged.

Note that we broaden our conclusion on the importance of Gcn5 and Mst2 on chromatin structure at condensin binding sites by performing MNase-seq (see Fig. 5).

Cited References for ChIP-seq: Bonhoure N, et al. (2014) Quantifying ChIP-seq data: a spiking method providing an internal reference for sample-to-sample normalization. Genome Res. 24: 1157–1168.   Hu B, et al. (2015) Biological chromodynamics: a general method for measuring protein occupancy across the genome by calibrating ChIP-seq. Nucleic Acids Res. 43: e132.  Orlando DA, et al. (2014) Quantitative ChIP-Seq normalization reveals global modulation of the epigenome. Cell Rep. 9: 1163–1170


*3) Statistics are provided for ChIP-PCR in Figure 3A but not for the remaining ChIPs in the main and expanded view figures. Some statistics should be provided where any significant differences are stated.*

Performing Wilcoxon Mann Whitney-tests necessitates at least 4 measurements per condition. We now provide statistics anywhere it is possible, ie when more than 3 measurements per condition are available.


*4) The fluorescence panel of Figure 3C shows No tag and cut3-HA but not cut3-HA Δgcn5 or cut3-HA Δgcn5 Δmst2 which is what is being quantitated and therefore should be shown as a representative image. For completeness, cut3-HAΔgcn5 should be added to the immunoblot (Fig 3D).*

Representative images of chromosome spreads and surface plots are shown in Fig. 3C. Immunoblots of cut3-HA gcn5Δ and cut3-HA gcn5Δ mst2Δ are now shown in Fig. 3D.


*5) Figure 4A has no representative images for Gcn5-myc and Cut14-HA immunofluorescence on chromosomes to accompany the quantification. These should be added.*

Representative images have been provided. See Figure 4A.

I must mention here that while looking for those images, I found that an error occurred during the first chromosome spreading experiment in the acquisition of Gcn5-myc signals. Images of Gcn5-myc immunofluorescence acquired with a short acquisition times were mistakenly mixed with the set of images acquired with a normal (longer) exposure time. This mistake led to the erroneous conclusion that the bulk of Gcn5 is detached from chromatin throughout mitosis. Having identified this mistake, we reproduced the chromosome spreading experiments three times (by two independent investigators). Our results shown in Fig. 4 confirm that the bulk of Gcn5 dissociates from chromosomes during prophase. However the chromosomal amount of Gcn5 in prometaphase/metaphase chromosomes increases and is not different from interphase chromosomes. These results imply that Gcn5 might only temporarily dissociate from chromosomes upon entry into mitosis (prophase) but then reassociate at a time when condensin levels further increase (prometaphase/metaphase).

We deeply apologize for this mistake. Please, note that ChIP experiments regarding the association of Gcn5-myc with chromosomes remain correct. They confirm that Gcn5 increased during mitosis at high occupancy condensin binding sites whilst it decreased at low-occupancy condensin binding sites.

Importantly, this rather minor correction has no impact on the main conclusion of the manuscript.


*6) For Figure 4B,E,F ste11 is added to a subset of the 10 condensin enriched genes used for Figure 3, but it not clear why this extra gene has been added.*

Ste11 was initially used as a positive control for Gcn5 binding in interphase and as a negative control for Gcn5 maintenance in mitosis. The *ste11* gene has been replaced by more appropriate negative controls, which correspond to low-occupancy condensin binding sites.


*7) The discussion seems to concentrate on histone eviction and condensin binding at highly expressed genes, but does not mention if this mechanism might be relevant to other condensin enriched sites such as centromeres and telomeres.*

The role of Gcn5 in condensin binding at telomeres is unknown. Regarding centromeres, the importance of nucleosome eviction for condensin binding is now discussed page 13, line 393.

"The central domain of centromeres (cnt1) is transcribed by RNA Pol II and is a site of high nucleosome turn-over (Choi et al, 2011; Sadeghi et al, 2014). The reduced association of condensin at cnt1 in the absence of Gcn5 (Fig. 3A) might therefore indicate that nucleosome eviction and/or dynamics contribute at to the association of condensin, along with Monopollin (Tada et al, 2011), at centromeres."

2nd Editorial Decision                                                                                       25 April 2016

Thank you for submitting your revised manuscript for our consideration. It has now been assessed once more by the three original referees, all of whom consider the study significantly improved and now in principle suited for publication. Referees 2 and 3 still raise a limited number of minor issues (mostly arising from the newly added data), which I would kindly invite you to respond to and address in one additional, final round of revision. As you will see from the comments below, these points refer mainly to analysis, presentation and discussion of the available data but should in my view probably not require generation of any additional data sets. When uploading the modified final manuscript, please also provide again a brief point-by-point response to the referees' remaining comments; and please make sure to incorporate the respective ArrayExpress (and possible other) accession numbers for submitted data in the final version of the text file as well.

I hope you will be able to make these necessary additional revisions as early as possible, and look forward to receiving the final version of your manuscript.

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REFEREE COMMENTS

Referee #1:

I have read the revised manuscript. With additional experiments, the authors have strengthened their conclusions. Changes to the text have further improved the manuscript that I can now recommend for publication.

Referee #2:

Toselli-Mollereau et al. have made a significant effort to address the points I raised in my review. They have incorporated new data in the revised manuscript that lend stronger support to their conclusions. As regards my first main point, concerning the need of showing negative controls, the authors have included new positive and negative examples in Figs. 3 and 4 and have reorganized the presentation of the data in a much clearer format. A minor suggestion is that the use of two different scales in the %IP in the two panels in Fig. 3A should be indicated in the legend.

As regards my second main point, concerning the need of higher resolution analysis of NDR occupancy, they have generated genome-wide MNase maps of nucleosome occupancy (Fig. 5B and EV6D) and have tested the dynamics of the prl53 NDR by MNase Q-PCR (Fig. EV6C). My comments on these new results are below. They can be addressed using the MNase-seq datasets already generated by the authors.

1. The sequencing protocol used (single-read or paired-end) should be indicated in Materials and Methods.

2. Table S2 should have a legend since the content of some of the columns is not clear.

3. Do the authors have any explanation for the very different height of the peaks in each map in Figs 5B and EV6? Can they also explain why peaks do not show the periodicity and the approximate expected internucleosomal distance if nucleosomes were positioned along these regions?

4. Given the subtle differences in the level of occupancy of NDRs in gcn5D and gcn5D mst2D relative to WT cells, it would be useful to show in a new Suppl. Figure the same regions shown in Fig. 5B in the three biological replicates to support further the observed differences.

5. What is the situation of NDRs of genes with low levels of Gcn5 and low-occupancy of condensin like uge1, cnd1 and 5S (Fig. 4B)? I would suggest that the nucleosome map of uge1 and cnd1 regions should also be shown along with 5S in Fig. EV6D. If these two genes have NDRs at their 5'

or 3' ends, do they remain unchanged in gcn5D and gcn5D mst2D relative to WT cells as predicted by the model?

Referee #3:

The revised version is much improved, in response to a number of reviewer comments. My main gripe being the key experiment I requested could unfortunately not be done, due to technical problems. This aside, the manuscript is still an important contribution in what is currently a very hot topic.

1-My main query (point 2) on the original submission was the absence of any ChIP-seq data to support findings over locus specific ChIP-PCR that was used throughout the manuscript. Unfortunately the revised manuscript was not able to produce this data. Given the Cnd2 ChIP works well, I would have thought at least some ChIP-seq would be a logical extension in the mutant background especially considering the length of revision time. My point was to broaden the depth of the analyses through some ChIP-seq and give the reader confidence the findings apply genome wide (not for absolute quantification as the authors responded). I am not clear why the authors chose to use chromodynamics to address my point over the more conventional ChIP-seq. There are good examples of comparative condensin ChIP-seq in S. pombe to draw on (i.e. Nakazawa N et al, Genes to Cells, 2015; Sutani T et al, Nature Comm 2015). However, as the chromodynamics technique uses ChIP-seq, can any of the data be included? It is not clear what step didn't work and what experiments were actually performed in this technique. Even a more simple comparison of Cnd2 ChIP-seq analysis between WT and Gcn5Δ strains would certainly build a much stronger base for all downstream arguments. That said, I do believe the ChIP-PCR in the manuscript is still very solid and the loci chosen appropriate and supportive of the paper's conclusions, and at least some extra condensin sites were added in the revision.

2-Regarding my above point, a paper from the Hinnebusch lab (Qui H., et al. Genome Research, 2016) recently appeared and seems to have significant overlap especially in regard to histone eviction at promoters and Gcn5. The Qui paper that is in yeast should be discussed with regard to how it impacts or supports the author's conclusions.

3-The response to my point 5 is somewhat perplexing. The authors did not include a representative image for Figure 4A in the first submission, but upon looking for this found the original figure used for quantification was not correct and have completely changed their finding. I do appreciate the honesty here and I am glad this has been changed and the erroneous data removed, but saying originally that bulk of Gcn5 is not associated with chromatin during mitosis and changing to Gcn5 associates transiently during prophase and then increases during prometaphase/metaphase represents quite a change in findings.

4-Line 70. Papers from Kim JH., et al. (Nature Communications, 2013), and Sutani T., at al. (Nature Communications, 2015) are relevant when discussing condensin binding at promoters of highly expressed genes and should be added here.

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2nd Revision - authors' response                                                           03 May 2016

Point by point response to reviewers

Referee #1:

*I have read the revised manuscript. With additional experiments, the authors have strengthened their conclusions. Changes to the text have further improved the manuscript that I can now recommend for publication.*

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*Toselli-Mollereau et al. have made a significant effort to address the points I raised in my review. They have incorporated new data in the revised manuscript that lend stronger support to their conclusions. As regards my first main point, concerning the need of showing negative controls, the authors have included new positive and negative examples in Figs. 3 and 4 and have reorganized the presentation of the data in a much clearer format. A minor suggestion is that the use of two different scales in the %IP in the two panels in Fig. 3A should be indicated in the legend.*
This is now mentioned in the legend of Figure 3, at lines 820-821, with the sentence *"Note the use of different scales in the arm: high-occupancy and arm: low-occupancy panels."*

*As regards my second main point, concerning the need of higher resolution analysis of NDR occupancy, they have generated genome-wide MNase maps of nucleosome occupancy (Fig. 5B and EV6D) and have tested the dynamics of the prl53 NDR by MNase Q-PCR (Fig. EV6C). My comments on these new results are below. They can be addressed using the MNase-seq datasets already generated by the authors.*

*1. The sequencing protocol used (single-read or paired-end) should be indicated in Materials and Methods.*
Single-end reads is now indicated in Material and Methods, line 517, with the sentence *"Between 46,818,494 and 62,258,601 single-end reads of 75 bp in length were obtained per sample and aligned to the S. pombe genome (Ensembl ASM294v2, May 2009)."*

*2. Table S2 should have a legend since the content of some of the columns is not clear.*
A legend has been added, as requested.

*3. Do the authors have any explanation for the very different height of the peaks in each map in Figs 5B and EV6? Can they also explain why peaks do not show the periodicity and the approximate expected internucleosomal distance if nucleosomes were positioned along these regions?*
Height of peaks: The amplitude of the MNase-seq peaks reflects the frequency at which a given bp is protected from MNase digestion in the cell population, sequenced and aligned to the reference genome. High and thin peaks reveal the recurrent presence of well-positioned nucleosomes. Small or large peaks indicate less frequent and/or less tightly positioned nucleosomes (i.e. nucleosomes that exhibit more lateral flexibility in their positioning). For more details, please see the refs Pugh BF (2010) A preoccupied position on nucleosomes. *Nat. Struct. Mol. Biol.* 17: 923, and Struhl K & Segal E (2013) Determinants of nucleosome positioning. *Nat. Struct. Mol. Biol.* 20: 267–273.
We do not have a definitive explanation for the differences in the amplitude of the peaks in Figs. 5B and EV6. These differences might be due, in part, to different transcription rates of underlying genes. Highly expressed genes exhibit low nucleosome occupancy (Lanterman et al. 2009, Soriano et al. 2013), because chromatin is constantly disrupted by travelling polymerases. We cannot rule out that differences in amplitudes might be due also to differences in mappability (i.e. the ability to sequence a DNA fragment or to align reads) and/or to mitotic chromosome condensation *per se*. Regarding chromosome condensation, however, Soriano et al. similarly reported differences in the amplitude of MNase-seq peaks when they assessed nucleosome occupancy during interphase in fission yeast cells (see Soriano et al. 2013, Fig. 2, 3 and 5). Thus, differences in the height of MNase-seq peaks is not a feature specific to mitosis. Also, Sutani et al have reported that the nucleosome pattern, assessed by ChIP-seq against H3 at the top 10% of condensin binding sites, remains unchanged in a condensin *cut3-477* mutant (Sutani et al. 2015, see Supp. Fig 5), suggesting that small MNase-seq peaks are not due to the local binding of condensin. Thus, we tend to believe that the differences in height of the peaks are largely due to differences in transcription rates.

Periodicity and internucleosomal distance: The internucleosomal distance (or nucleosome repeat length) is short in *S. pombe*, with ~ 152 bp (Lanterman et al. 2009, Soriano et al. 2013). So there is very little linker DNA between two adjacent nucleosomes. Some regularly spaced nucleosome occupancy peaks are visible in Figs. 5 and EV6, and in the new Fig. EV7. For instance, see on the left side of *ecm33* and on the right side of *cdc22* in Figs. 5 and EV7, or in the *hba1* gene in Fig. EV6. However, we agree that the spacing appears more irregular than previously observed in vegetative cells by Soriano et al. 2013. This difference might be experimental and/or due to the fact that we used cells arrested in early mitosis, with highly condensed mitotic chromosomes, and not

vegetative cells. Internucleosomal spacing is expected to be inversely proportional to nucleosome density. Further dedicated work is necessary to thoroughly compare the nucleosome landscapes in interphase and mitosis.

*4. Given the subtle differences in the level of occupancy of NDRs in gcn5D and gcn5D mst2D relative to WT cells, it would be useful to show in a new Suppl. Figure the same regions shown in Fig. 5B in the three biological replicates to support further the observed differences.*
MNase-seq nucleosome patterns observed at the *snoU14*, *ecm33* and *cdc22* genes in the three wt, *gcn5Δ* or *gcn5Δ mst2Δ* biological replicates are now shown in a new Fig. EV7. Also, Fig. EV7 is now cited in the text at lines 299, 301, 311.

*5. What is the situation of NDRs of genes with low levels of Gcn5 and low-occupancy of condensin like uge1, cnd1 and 5S (Fig. 4B)? I would suggest that the nucleosome map of uge1 and cnd1 regions should also be shown along with 5S in Fig. EV6D. If these two genes have NDRs at their 5' or 3' ends, do they remain unchanged in gcn5D and gcn5D mst2D relative to WT cells as predicted by the model?*
The *cnd1* and *uge1* genes have a NDR at their 5' ends, and these NDRs remain unchanged in the gcn5 and gcn5 mst2 mutants, as expected. This result is now shown along with the 5S gene in Fig. EV6D. These results are mentioned in the text, lines 313-315 with the sentence: *"Note that nucleosome occupancy appeared unchanged at the 5S rRNA, cnd1 and uge1 genes in cells lacking Gcn5 or both Gcn5 and Mst2 (Fig. EV6D and Fig. 5F), where the binding of condensin remained unchanged (Fig. 3A)."*

Referee #3:

*The revised version is much improved, in response to a number of reviewer comments. My main gripe being the key experiment I requested could unfortunately not be done, due to technical problems. This aside, the manuscript is still an important contribution in what is currently a very hot topic.*

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*It is not clear what step didn't work and what experiments were actually performed in this technique. Even a more simple comparison of Cnd2 ChIP-seq analysis between WT and Gcn5Δ strains would certainly build a much stronger base for all downstream arguments. That said, I do believe the ChIP-PCR in the manuscript is still very solid and the loci chosen appropriate and supportive of the paper's conclusions, and at least some extra condensin sites were added in the revision.*

The length of revision time was caused both by the MNase-seq experiment and our unsuccessful attempts to perform quantitative ChIP-seq. Regarding ChIP-seq, to the best of our knowledge, Nakazawa et al. (Genes to Cells 2015) normalized to 10 millions the read numbers in IP and WCE fractions in each sample before calculating IP/WCE ratios. Since the amount of DNA recovered by ChIP is necessarily different between wt and mutant samples, we were concerned that such a total read normalization might not be totally adequate for comparing wild-type and gcn5 mutants. Moreover, such a normalization would leave undetected a global reduction in condensin binding in a mutant background.
To allow sample-to-sample comparison of ChIP-seq data, Sutani et al. normalized their ChIP-seq raw data by applying a correction factor calculated from ChIP-qPCR at few loci. We see three limitations to this method. (1) There is no robust internal control to check that the quality of IP are

similar between samples and between ChIPs. (2) The correction factor is calculated from the rather small number of replicates generally used in ChIP-seq, and, as such, might be inaccurate. (3) To apply this type of correction factor, we must assume that the ratio mutant/wild-type remains constant throughout the genome. However, if you look at our data in Fig. 3A, you will see that this is not the case. For instance, lack of Gcn5 reduces condensin binding at the *snoU14* and *gas1* genes, but the reduction is clearly stronger at *snoU14*.

It is for all these limitations and/or concerns that we decided to embark on chromodynamics, even though this technique was clearly more complicated and more risky than conventional ChIP-seq.

The step that didn't work was to efficiently IP condensin from both *S. cerevisiae* and *S. pombe* cells. Fixed *S. cerevisiae* cells and *S. pombe* cells were mixed at a ratio of 1:3, chromatin prepared for ChIP, and we tried to simultaneously IP the kleisin subunit of condensin from *S. cerevisiae* (Brn1-GFP) and *S. pombe* (Cnd2-GFP). To do this, we scaled up the conventional Cnd2-GFP ChIP, and set up new sonication conditions. We assessed the simultaneous IP of Cnd2-GFP and Brn1-GFP by qPCR. We managed to ChIP Cnd2-GFP but, unfortunately, failed to efficiently IP Brn1-GFP under these conditions. We did not send DNA samples to sequencing and, after unsuccessful repeated trials, decided to focus on the MNase-seq experiment. We are still working on condensin chromodynamics and believe that the initial cell ratio (3:1) was inadequate.

*2-Regarding my above point, a paper from the Hinnebusch lab (Qui H., et al. Genome Research, 2016) recently appeared and seems to have significant overlap especially in regard to histone eviction at promoters and Gcn5. The Qui paper that is in yeast should be discussed with regard to how it impacts or supports the author's conclusions.*
The paper from Qui H. et al. published in Genome research in 2016 (entitled "Genome-wide cooperation by HAT Gcn5, remodeler SWI/SNF, and chaperone Ydj1 in promoter nucleosome eviction and transcriptional activation") supports our conclusion.
This paper is now cited in the discussion at lines 407-412, with the following sentences: "The fact that nucleosome residence increases at high-occupancy condensin binding sites in the absence of Gcn5 and Mst2, despite the presence of RSC, implies that Gcn5 and Mst2 promote condensin binding at these sites by recruiting additional chromatin remodellers, which are at least partly redundant with RSC. The recent finding that budding yeast Gcn5 acts cooperatively, and often redundantly, with the Swi/Snf nucleosome remodelling enzyme to evict promoter nucleosomes (Qiu et al, 2016), supports our conclusion.

*3-The response to my point 5 is somewhat perplexing. The authors did not include a representative image for Figure 4A in the first submission, but upon looking for this found the original figure used for quantification was not correct and have completely changed their finding. I do appreciate the honesty here and I am glad this has been changed and the erroneous data removed, but saying originally that bulk of Gcn5 is not associated with chromatin during mitosis and changing to Gcn5 associates transiently during prophase and then increases during prometaphase/metaphase represents quite a change in findings.*
We totally agree, and can only further thank the reviewer for avoiding us presenting erroneous data. Having said that, we would like to emphasize that our finding that Gcn5 persists at high-occupancy condensin binding sites during mitosis, whilst it dissociates from low condensin binding sites, was correct from the very beginning.

*4-Line 70. Papers from Kim JH., et al. (Nature Communications, 2013), and Sutani T., at al. (Nature Communications, 2015) are relevant when discussing condensin binding at promoters of highly expressed genes and should be added here.*
These two reference have been added. See lines 70-71.

Accepted                                                                                              06 May 2016

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

## EMBO PRESS

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### Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

### A- Figures
### 1. Data
**The data shown in figures should satisfy the following conditions:**

➜ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
➜ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
➜ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
➜ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
➜ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

### 2. Captions

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

➜ a specification of the experimental system investigated (eg cell line, species name).
➜ the assay(s) and method(s) used to carry out the reported observations and measurements
➜ an explicit mention of the biological and chemical entity(ies) that are being measured.
➜ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

➜ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
➜ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
➜ a statement of how many times the experiment shown was independently replicated in the laboratory.
➜ definitions of statistical methods and measures:
  • common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  • are tests one-sided or two-sided?
  • are there adjustments for multiple comparisons?
  • exact statistical test results, e.g., P values = x but not P values < x;
  • definition of 'center values' as median or average;
  • definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

**Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.**

### B- Statistics and general methods

| Question | Answer |
|---|---|
| 1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? | Sample size was > 6 in order to assess statistical validiy by Wilcoxon and Mann-Withney test. See page 18 "Statistical Methods" |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | NA |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | NA |
| 3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe. | NA |
| For animal studies, include a statement about randomization even if no randomization was used. | NA |
| 4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe. | NA |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | NA |
| 5. For every figure, are statistical tests justified as appropriate? | Yes |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | The Mann-Whitney U-test does not require the assumption of normal distributions |
| Is there an estimate of variation within each group of data? | YES, standard deviation is provided |
| Is the variance similar between the groups that are being statistically compared? | YES as judged by similar standard deviations |

### C- Reagents

| 6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right). | Catalog numbers for antibodies are provided in Appendix Table S5 |
|---|---|
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | Strain list is provided in Table S3 |

\* for all hyperlinks, please see the table at the top right of the document

## D- Animal Models

| 8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals. | NA |
|---|---|
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. | NA |
| 10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance. | NA |

## E- Human Subjects

| 11. Identify the committee(s) approving the study protocol. | NA |
|---|---|
| 12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. | NA |
| 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. | NA |
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## F- Data Accessibility

| 18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>e. Proteomics and molecular interactions | GEO accession number requested for Mnase-seq data (see page 18) |
|---|---|
| 19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right). | NA |
| 20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right). | NA |
| 21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section.<br><br>Examples:<br>**Primary Data**<br>Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462<br>**Referenced Data**<br>Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26<br>AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208 | NA |
| 22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information. | NA |

## G- Dual use research of concern

| 23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could. | NA |
|---|---|