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Chromatin stiffening underlies enhanced locus mobility after DNA damage in budding yeast

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(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 14 November 2016

Thank you for submitting your manuscript on chromatin stiffening as the mode of enhanced locus mobility in damage budding yeast DNA. We have now received comments from three expert referees, which I am copying below for your information. As you will see, these referees are somewhat divided in their opinions: while referee 1 overall appreciates your results and their quality, referee 3 is not convinced that they provide a significant advance in our biological understanding beyond the current status of the literature. Finally, referee 2 acknowledges potential interest of your findings but raises substantive concerns with the decisiveness of the present data and their support for key conclusions of the study.

Faced with these mixed evaluations, I am afraid we have to conclude that the manuscript in its current form is not yet well-suited for publication in The EMBO Journal; but we also note that it may become a much more compelling candidate if substantiated and extended along the lines suggested in the first two reports. In particular, we feel it would be crucial to address point 2 of referee 2 regarding distance increase being possibly caused mainly by repair-associated processes (such as resection or nucleosome loss), and the related point 1 requesting some sort of alternative experimental support for increased chromatin rigidity. The other key conceptual issue that would need to be experimentally clarified is referee 2's point 3 regarding cis vs trans effects of H2A phosphorylation.

I realize that decisively addressing these points (as well as the various more specific/minor

concerns) and extending the study to provide deeper insight may require substantial further time and efforts; also, the uncertain results of such additional experimentation precludes me from predicting the outcome of an eventual re-evaluation by the referees and thus to make strong commitments regarding ultimate acceptance. Nevertheless, given the interest of the topic and the fact that the key concerns appear in principle addressable, I would still like to give you an opportunity to respond to the referees by way of a major revision of this work - if necessary within an extended revision period, during which publication of any competing work elsewhere would have as usual no negative impact on our final assessment of your own study. When revising the study, I would appreciate if you also improved its presentation through reorganizing the manuscript (currently only 4 main figures but 8 supplementary figures) by capitalizing on the extended format of an EMBO Journal article, which can easily accommodate 8 or more main figures and up to 5 Expanded View figures (see below for guidelines). Please also pay attention to the format and completeness of the reference list, and to provision of any referenced but not published articles (such as Arbona et al) for our review.

Should you have any questions or comments regarding the referee reports or this decision, or if you want to request an extended revision duration, please do not hesitate to get in touch with me already during the early stages of your revision work. Thank you again for the opportunity to consider this work for The EMBO Journal. I look forward to hearing from you in due time.

REFEREE REPORTS

Referee #1:

This study is very interesting and is carefully carried out. The paper is well written and provides new insights into an important topic. It should be published with minor additions. The authors confirm that double strand breaks result in enhanced chromatin subdiffusion and convincingly demonstrate that intrachromosomal distances increase with DNA damage across the entire chromosome 4R arm. The authors also show that a surprisingly minimalistic physical model of yeast chromosome fibers can predict dynamic features observed in the experiments. Specifically, they show that a chromosome fiber with enhanced rigidity can best explain the increased subdiffusion and increased intrachromosomal distances during DNA repair. These results point to a key role of altered physical chromatin properties in the response to DNA damage. Also the authors show that phosphorylation of H2A may contribute to the changes in physical properties of the chromatin.

A few comments:

1) It would be good if the authors could summarize all modeling parameters and provide a bit more details in the supp. materials. For example, it is not completely clear to me if parameters of the truncated LJ potential were modified when the compactation of the fiber is increased. It may be possible that the decrease in total number of beads was combined with an increased fiber diameter (i.e. bead volume) and subsequently to repulsive LJ interactions at larger distances in comparison to the control setting? In other words is the volume of the entire chromosome kept constant even though the length is reduced in the contracted state? On page 8, the authors state that the data agrees roughly with an ideal chain model of constant compactation and rigidity. Could they provide the parameters that agree best with the data? Are these the same parameters used for the reference state in Figure 3? It would also be good to know the rational in selecting the varied compactness and rigidity parameters in Figure 3.

2) For clarity and completeness it would also be good to provide more details on how the experimental MSDs are calculated across different cells.

3) It appears that the modeling is performed with an isolated chromosome 4 in a nuclear volume. Enhanced crowding in the nucleus due to the presence of all other chromosomes may have a pronounced influence on the location preference of the studied loci and presumably also on their dynamics. Could the authors comment if crowding effects could influence the outcome of their results?

4) Do the authors have an explanation why a decreased compactation decreases the loci mobility? This seems surprising to me.

5) The authors convincingly show that the measured MSD data are in rough agreement with an ideal chain model of constant compactation and rigidity and also that the model with increased rigidity best describes the changes in loci dynamics and distances when DSB are enforced. On the other hand it cannot be excluded that other settings may also agree with the data. I would expect local variations of compactness and rigidity in a biological fiber subject to binding of a variety of regulatory proteins, which eventually could still lead to a similar average compactness when comparing longer stretches of the chromosomes. Although this example is purely speculative.

Referee #2:

This manuscript by Zimmer and colleagues puts forward the interesting idea that DNA damage results in chromatin stiffening, which may, in turn, have significant implications for nuclear function. The authors use (live) imaging-based approaches to measure spatial distance between chromosome locations in cis, as well as chromatin mobility, both in the presence and absence of the DNA damaging agent zeocin. Combined with mathematical modeling, the authors conclude that DNA double-strand breaks (DSBs) induce chromatin stiffening, which appears to be at least in part dependent on histone H2A phosphorylation.

The notion of a DSB-induced increase in chromatin rigidity is novel and may have important implications for the study and interpretation of DSB-associated chromatin reorganization. However, as it stands, several major issues would need to be addressed to support the model presented by the authors:

1. At this point, evidence for increased chromatin rigidity is circumstantial and largely based on a comparison of distance measurements and chromatin mobility with expectations based on mathematical modeling (Fig. 3). Experimental validation of increased chromatin rigidity would significantly improve the manuscript. Moreover, the predictions based on the model in Fig. 3 are somewhat counterintuitive, e.g. why is chromatin decondensation expected to result in decreased mean square displacement (chromatin mobility). This should be better explained.

2. Increased intra-chromosomal distances may be the result of a variety of known biological consequences of DSB formation, including nucleosome loss/histone variant exchange and increased DNA resection. The latter would further fit with the time dependence of the observed increase in square distance (Fig. 2). The authors need to address these possibilities using appropriate repair-defective yeast strains.

3. The authors estimate that the dose of zeocin used results in ~ 1 DSB per cell, suggesting that the increased chromatin rigidity observed on chromosome 4 may occur in trans to DSBs on other chromosomes. How does the latter fit with the dependence on H2A phosphorylation, which occurs in cis to DSBs? To distinguish between cis and trans effects, the authors should consider locally defined DSB induction, using CRISPR/Cas9 or endonuclease-based approaches. The latter would further provide independent validation for DSB dependence.

Minor comments:

1. Fig. 1 a should include a higher magnification of RAD52+ cells to get a sense of DSB frequency per cell.
2. The authors should discuss their work in the context of findings from the Gratton lab, which measured DSB-associated chromatin dynamics, e.g. PMID:24988341.
3. How does the proposed model fit with recent findings describing DSB-induced chromatin condensation? Are the observed effects dependent on continuous presence of DNA damage?

Referee #3:

Review of Herbert et al., Chromatin stiffening underlies enhanced locus mobility....

This manuscript delves to explain the mechanistic basis for increased chromatin dynamics following DNA damage observed in budding yeast. The paper describes a nice series of experiments using marked strains with fluorescent loci at different positions along the chromosome to quantitate packaging (compaction and rigidity) as a function of damage. The authors report no difference in compaction and rigidity following DNA damage. The data are well-presented, but it is unclear that there are any significant biological advances in this report.

Specific Comments:

The authors show the measured distance between a number of loci and conclude that the assumption of an ideal chain suffices to explain chromosome organization. Considering the state of the field and the numerous models using ideal chains, this conclusion is not surprising and in fact confirms findings in many prior reports. They provide additional data for intermediate distances that go beyond several prior reports, but nonetheless the findings are confirmatory and do not advance the field. The authors do find that the pericentromere region is stretched beyond the distance expected from an ideal chain. As the authors cite, this has been reported in mitosis to be a function of DNA loops functioning as a polymer brush(p. 9). This has been explicitly modeled in a recent report (Lawrimore et al., MBOC 2016).

In the presence of DNA damage, the intrachromosomal distances increase over the entire length of the chromosome. To understand the mechanistic basis for the increase the authors make a mutant in the CEP3 kinetochore protein (S575A). They do not replicate a previous report that concluded this change was instrumental in loss of tethering upon DNA damage.

The authors provide an alternative hypothesis that is based on chromatin rigidity. In the model, only chromatin rigidity was sufficient to account for the experimental results. This is an interesting hypothesis, but not evidence that rigidity is the key feature. In an effort to address the mechanism, the authors use the Histone 2A S129A mutant that is non-phosphorylatable. They find that in this mutant, the DNA damage response is largely abrogated (Fig. 4). This is an interesting finding and important for the field.

In summary, the authors address a previous publication that states centromeres are detached following DNA damage and provide good evidence that this is not the mechanism responsible for increased mobilization. In contrast, using a polymer model, chromatin rigidity can account for the observation. In an experiment with a non-phosphorylatable histone, they block the increase in DNA damage. This is an important first step toward understanding the chromatin modification in response to DNA damage.

1st Revision - authors' response 16 March 2017

We thank you for your editorial work on our paper (EMBOJ-2016-95842) and for having allowed for an extended revision period. We would also like to thank the referees, especially referees 1 and 2, for their insightful comments. As you will see, we have carefully taken into account each of the raised points and performed extensive new experiments and analyses to address them.

Briefly, the main additions to our paper are as follows:

- Using super-resolution (PALM/STORM) imaging of a repeated sequence array and a statistical analysis of the single molecule localizations, we provide independent new evidence in support of our proposed model of chromatin stiffening (new Fig 6 and Fig. S6)
- Using two repair-deficient mutants, we show that the reported increase in intrachromosomal distances des not result from Sae2 dependent resection or from Blm10 dependent histone degradation (new Fig. S4)
- Using inducible endonucleases, we confirm that targeting double strand breaks at a single locus is sufficient to increase chromatin mobility globally in the nucleus (new Figure S1)

We also provided answers to all other points raised by the referees, and adjusted our manuscript accordingly. In addition, we restructured the Supplementary Material as you requested and provide the cited Arbona et al. paper (now accepted for publication in Genome Biology).

We believe that our changes considerably strengthen our study and hope that you will find the revised paper acceptable for publication in EMBO Journal.

POINT BY POINT RESPONSE

Referee #1:

This study is very interesting and is carefully carried out. The paper is well written and provides new insights into an important topic. It should be published with minor additions. The authors confirm that double strand breaks result in enhanced chromatin subdiffusion and convincingly demonstrate that intrachromosomal distances increase with DNA damage across the entire chromosome 4R arm. The authors also show that a surprisingly minimalistic physical model of yeast chromosome fibers can predict dynamic features observed in the experiments. Specifically, they show that a chromosome fiber with enhanced rigidity can best explain the increased subdiffusion and increased intrachromosomal distances during DNA repair. These results point to a key role of altered physical chromatin properties in the response to DNA damage. Also the authors show that phosphorylation of H2A may contribute to the changes in physical properties of the chromatin.

We thank the referee for appreciating the interest of our study.

A few comments:

1) It would be good if the authors could summarize all modeling parameters and provide a bit more details in the supp. materials. For example, it is not completely clear to me if parameters of the truncated LJ potential were modified when the compactation of the fiber is increased. It may be possible that the decrease in total number of beads was combined with an increased fiber diameter (i.e. bead volume) and subsequently to repulsive LJ interactions at larger distances in comparison to the control setting? In other words is the volume of the entire chromosome kept constant even though the length is reduced in the contracted state? On page 8, the authors state that the data agrees roughly with an ideal chain model of constant compactation and rigidity. Could they provide the parameters that agree best with the data? Are these the same parameters used for the reference state in Figure 3? It would also be good to know the rational in selecting the varied compactness and rigidity parameters in Figure 3.

We acknowledge that we provided little information about the modeling assumptions. Our basic modeling approach was first described in Wong et al. 2012 and the details of our new simulations are extensively described in the Arbona et al. paper cited (which was meanwhile accepted for publication in Genome Biology). We now added a few sentences in the main text (lines 279-285) and additional information in the Appendix Supplementary Methods about the main parameters.

To answer the referee's specific question: When changing the compaction of the fiber, we did not change the Lennard Jones potential or the volume of the beads, but only the number of beads (implying that the total volume of beads is different). We consider this approach to be conservative, since it is unclear how the effective volume of a chromatin fiber might change in response to decondensation or compaction. We made this explicit in the Appendix Supplementary Methods.

*Concerning page 8: for an ideal chain model, the mean squared intrachromosomal distances equals the genomic separation multiplied by 2*P/C, where P is the persistence length and C the compaction. From these data alone, we cannot determine P and C, but only the ratio P/C. From the fit to the data on loci R2, R3 and R4 in Figure 2c (dotted red line), we obtained a slope of 1,436 nm2/Kb, which would imply P/C = 0.718 nm2/bp.*

In our reference model of the old Figure 3 (now Figure 5), we used P=69 nm and C=50 p/nm, which implies P/C = 1.38 nm2/bp, approximately twice that value. These parameters are based on a systematic study of model parameters using hundreds of simulations and comparison to an extensive data set, containing many other imaging studies (although no dynamic data) and two Hi-C data sets, as described in Arbona et al. Unlike the ideal chain model, these simulations take into account nuclear confinement, centromeric tethering and crowding, and their predictions quantitatively differ from the ideal chain model. These simulations can also simultaneously explain the distances for the pericentromeric locus R1 and the internal loci R2-R4 with the above mentioned parameters, which the ideal chain model cannot.

In the present paper, however, we are not primarily concerned with estimating the precise parameters of chromatin, but in the qualitative changes of chromatin properties in response to DNA damage. Accordingly, we did not tune the compactness and rigidity parameters for Figure 3 (now Fig 5), but simply chose significantly different values from the reference model to facilitate the discussion of the effects of decondensation vs stiffening. We consider that a more quantitative assessment of the changes in chromatin structure as function of DNA damage lies outside of the scope of the present paper and should be addressed in subsequent work.

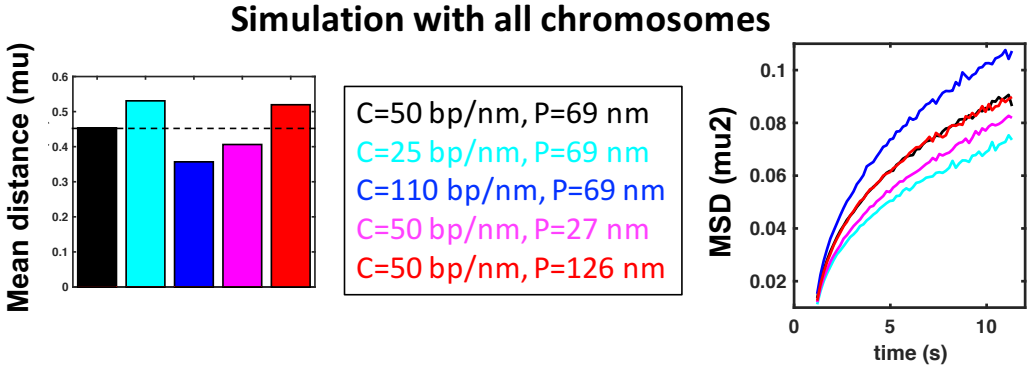
2) For clarity and completeness it would also be good to provide more details on how the experimental MSDs are calculated across different cells.

The MSD were first calculated for each cell based on the extracted trajectories, then averaged over all cells of the population. We added details in the "Image analysis and statistics" subsection of the Materials and Methods section (lines 520-525).

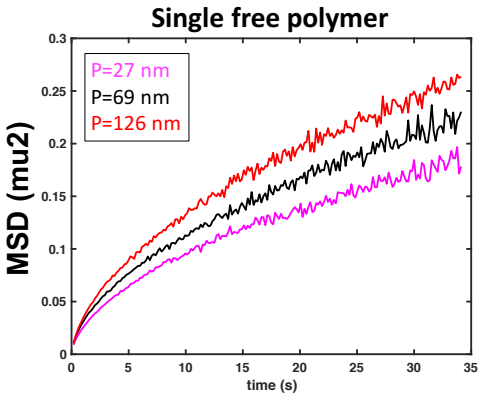
3) It appears that the modeling is performed with an isolated chromosome 4 in a nuclear volume. Enhanced crowding in the nucleus due to the presence of all other chromosomes may have a pronounced influence on the location preference of the studied loci and presumably also on their dynamics. Could the authors comment if crowding effects could influence the outcome of their results?

The reviewer is right; the presence of other chromosomes might influence positional preferences of loci and can in principle also affect their dynamics.

We have now run simulations with all other chromosomes present. These simulations are much more time consuming due to the large number of beads, and results somewhat noisy and less certain. Nevertheless, they do reproduce some of the main effects reported here as shown in the Figure below: increasing fiber rigidity from P=69 nm to P=126 nm increases mean intrachromosomal distance, and reducing it to P=27 nm decreases it (Figure below, left panel). Similarly, increasing compaction from C=50 bp/nm to C=110 bp/nm lowers the distance, while decreasing compaction to C=25 bp/nm increases it. Predicted MSDs are shown in the right panel. Compared to the reference, the simulation predicts that lower compaction reduces mobility and higher compaction increases mobility. Reducing rigidity P from 69 nm to 27 nm leads to reduced mobility, but increasing P to 180 nm did not clearly alter predicted MSDs, unlike for the single chromosome simulation shown in Fig. 5 (old Fig. 3).



However the predicted MSDs also likely depend strongly on the assumed size of the beads, W. This parameter could not be well determined from data analyzed in Arbona et al. (which contained no dynamic data). Here, we chose W=30 nm, but different values are in principle possible. We believe that this choice leads to excessive nuclear crowding. We tried to run simulations with W=15 nm beads, but unfortunately these simulations never reached an equilibrium, preventing us from fully addressing this point. Nevertheless, we believe that the increase of MSD due to stiffening holds true in presence of multiple chromosomes. Stiffening-induced enhancement mobility is robust to assumptions about tethering or nuclear confinement, as seen in the Figure below, which shows predicted MSD for a single untethered chromosome in absence of confinement.



Thus, our current model of all chromosomes does not fully account for the data, but since simpler models can robustly explain the observed effects, we believe that future simulations with a better choice of the parameter W will likely explain this effect quantitatively also in the context of nuclear crowding. We added a sentence in the relevant section to acknowledge the potential complications due to crowding (lines 321-324).

4) Do the authors have an explanation why a decreased compactation decreases the loci mobility? This seems surprising to me.

We acknowledge that this effect is not intuitive, but it can be understood using the Rouse model of polymer dynamics, which predicts that MSD(t) obeys three distinct regimes, each with distinct power law exponents (alpha): (i) free diffusion of the monomer at small time scales (alpha=1), (ii) the subdiffusive Rouse regime at intermediate time scales (alpha=0.5), where monomers are influenced by interactions with their neighbours, and (iii) free diffusion of the entire polymer chain (alpha=1). The time corresponding to the transition between the two latter regimes (the Rouse time) is proportional to the squared contour length of the polymer and therefore increases in our model of decreased compaction (which has more monomers with the same diffusion constant). This means that subdiffusive behavior extends to longer time scales. In reality, the real polymer behavior is characterized by smooth transitions between these regimes and slowly varying exponents alpha (eg between 0.5 and 1). Therefore, an increase in the Rouse time can lead to a reduction of alpha (and hence of MSD values) at time scales both below or above the Rouse time. In our simulations, the situation is complicated by additional factors, including tethering and confinement, but this effect is also predicted when we simulate polymers in free space (not shown). We added Appendix Figure S5 to explain this point about Rouse dynamics and mention it in our discussion of the simulation results (lines 302-305).

5) The authors convincingly show that the measured MSD data are in rough agreement with an ideal chain model of constant compactation and rigidity and also that the model with increased rigidity best describes the changes in loci dynamics and distances when DSB are enforced. On the other hand it cannot be excluded that other settings may also agree with the data. I would expect local variations of compactness and rigidity in a biological fiber subject to binding of a variety of regulatory proteins, which eventually could still lead to a similar average compactness when comparing longer stretches of the chromosomes. Although this example is purely speculative.

We agree with the reviewer. Although our model with increased global rigidity explains the observed changes in distances and loci dynamics, we cannot entirely rule out other scenario, such as local variations of compactness or rigidity. We did not investigate such potential local variations in the model because our data are not sufficient to address them directly. However, we now provide new experimental evidence that strengthens our proposed model of increased chromatin rigidity, as detailed in the response to Referee #2.

Referee #2:

This manuscript by Zimmer and colleagues puts forward the interesting idea that DNA damage results in chromatin stiffening, which may, in turn, have significant implications for nuclear function. The authors use (live) imaging-based approaches to measure spatial distance between chromosome locations in cis, as well as chromatin mobility, both in the presence and absence of the DNA damaging agent zeocin. Combined with mathematical modeling, the authors conclude that DNA double-strand breaks (DSBs) induce chromatin stiffening, which appears to be at least in part dependent on histone H2A phosphorylation.

The notion of a DSB-induced increase in chromatin rigidity is novel and may have important implications for the study and interpretation of DSB-associated chromatin reorganization. However, as it stands, several major issues would need to be addressed to support the model presented by the authors:

1. At this point, evidence for increased chromatin rigidity is circumstantial and largely based on a comparison of distance measurements and chromatin mobility with expectations based on mathematical modeling (Fig. 3). Experimental validation of increased chromatin rigidity would significantly improve the manuscript. Moreover, the predictions based on the model in Fig. 3 are somewhat counterintuitive, e.g. why is chromatin decondensation expected to result in decreased mean square displacement (chromatin mobility). This should be better explained.

We accept the referee's criticism that our evidence for increased chromatin rigidity was circumstantial and based largely on comparison of our data (distances and chromatin mobility) to computational modeling. We therefore decided to perform new experiments to assay potential changes in chromatin properties by independent means. We performed PALM/STORM super-resolution imaging of a ~7 Kb long Lac operator sequence targeted by nanobodies against lacI-GFP (new Fig. 6). We analyzed the first and second axes of variance of the resulting localization clouds. Decondensation of the chromatin fiber should lead to an increase of both these variances, while an increase of the rigidity can lower the second variance if the chromatin region's contour length is on the order of the persistence length (see simulation results in Fig. EV5). We imaged more than 100 cells in absence or presence of Zeocin and observe a small, but significant, reduction of the second variance in cells treated by Zeocine (Fig. 6d). This is consistent with a rigidification, but not a decondensation of the chromatin fiber. These data therefore provide new evidence for our proposed model of global chromatin stiffening. We describe these results in a new section "PALM imaging of Lac operator array is consistent with chromatin stiffening" (lines 328-356).

We acknowledge that the reduction of mean square displacement in response to chromatin decondensation is not intuitive and therefore added an explanation based on the Rouse model of polymer dynamics in Appendix Figure S5 (see response to point 4 of referee #1).

2. Increased intra-chromosomal distances may be the result of a variety of known biological consequences of DSB formation, including nucleosome loss/histone variant exchange and increased DNA resection. The latter would further fit with the time dependence of the observed increase in square distance (Fig. 2). The authors need to address these possibilities using appropriate repair-defective yeast strains.

We agree that multiple processes can potentially cause the increase in intrachromosomal distances, notably histone degradation and DNA resection. We therefore followed the referee's suggestion and analyzed repair defective mutants, for which we measured intrachromosomal distances in response to Zeocin treatment.

Blm10 is an activator of the 20S proteasome and is important for degradation of acetylated histones. Core histone acetylation is a consequence of DNA damage (eg. Qian et al., Cell 2013). Our Δblm10 mutant shows a growth defect as previously published (Doherty et al., G3 2012). However, the intrachromosomal distances in the mutant increased identically to the wild type cells: the distributions of distances were similar (no statistical difference), both in untreated cells and after 6h of Zeocin exposure. The distance increase in the mutant cells was very significant and undistinguishable from the increase in the WT. This implies that acetylated histone loss via the 20S proteasome is not involved in the DNA damage dependent increase of intrachromosomal distances. We acknowledge, however, that we cannot rule out the implication of other histone degradation pathways.

In order to analyze the involvement of DNA resection, we wished to delete Mre11, Exo1 or Sae2. Together with Rad50 and Xrs2, Mre11 forms the MRX complex that is recruited after DSB to hold the two broken extremities together (Lobachev et al., 2004; Kaye et al., 2004). An initial resection is performed by MRX together with Sae2. Exo1 is an excision machine that degrades DNA at a rate of 4kb/hour (Mehta and Haber 2017).
We did not manage to delete either Mre11 or Exo1 in our strain background (BY). However, we succeeded in constructing the Sae2 deletion mutant. Sae2 is essential for recombination (Sarangi et al., Plos Genetics 2015; Baroni et Mol Cell Biol 2004) and Sae2 deletion slows down resection of an HO-induced DSB (Clerici et al., 2005). As for Δblm10, the intrachromosomal distances in the Δsae2 mutant had the same distribution as the WT cells in untreated cells, and increased similarly upon Zeocin exposure. This indicates that resection initiated by Sae2 and association of the broken ends does not significantly contribute to the observed increase in intrachromosomal distances. However we cannot presently exclude the involvement of other repair factors.

We have included these new data in the new Fig. EV4 and added a paragraph at the end of the section "DNA damage increases intrachromosomal distances" (lines 228-244).

3. The authors estimate that the dose of zeocin used results in ~ 1 DSB per cell, suggesting that the increased chromatin rigidity observed on chromosome 4 may occur in trans to DSBs on other chromosomes. How does the latter fit with the dependence on H2A phosphorylation, which occurs in cis to DSBs? To distinguish between cis and trans effects, the authors should consider locally defined DSB induction, using CRISPR/Cas9 or endonuclease-based approaches. The latter would further provide independent validation for DSB dependence.

In our submitted paper, we mentioned previous studies according to which 1h of Zeocin treatment is expected to lead to "at least one DSB, on average, per cell". We now removed this statement, as we do not believe that our Zeocin treatment causes only 1 DSB per cell (see below).

The question raised by the referee about cis vs trans effects is of course an important one. However, it is difficult to estimate the actual number of DSB per cell. The number of Rad52 foci is not a good proxy, since multiple DSB can colocalize into a single Rad52 focus (Lisby et al 2004) and some DSB might not lead to detectable Rad52 foci.

To directly address the question of the referee about trans effects, we therefore performed additional experiments using HO endonucleases to target DSB to a single locus on chromosome 3 and examine chromatin mobility on chromosome 15. Our results (Fig. EV1) indeed show increased mobility upon induction of DSB, confirming earlier studies, and supporting a model whereby DNA damage leads to of global increase in chromatin dynamics. The increase in MSD observed with HO is somewhat smaller than for Zeocin treatment, suggesting that Zeocin induces more than one DSB, on average, per cell.

These new results are described in lines 140-149.

Minor comments:

1. Fig. 1a: should include a higher magnification of RAD52+ cells to get a sense of DSB frequency per cell.

We added magnified views of the RAD52 labeled cells in Fig. 1a as requested.

2. The authors should discuss their work in the context of findings from the Gratton lab, which measured DSB-associated chromatin dynamics, e.g. PMID:24988341.

We thank the reviewer for pointing out this study by Hinde et al. Although this paper assesses chromatin compaction rather than rigidity, it shows that local DNA damage can increase chromatin compaction at distances of up to ~ 3 mu around the damage site (where they observe decreased chromatin compaction). Since a distance of 3 mu is sufficient to cover the entire yeast nucleus (diameter ~2 mu), the same mechanism could potentially explain changes in chromatin structure in mammalian cells and throughout the yeast nucleus. We added a few lines and incorporated a reference to this paper in the Discussion (397-401).

3. How does the proposed model fit with recent findings describing DSB-induced chromatin condensation? Are the observed effects dependent on continuous presence of DNA damage?

We thank the reviewer for pointing out these findings. Khurana et al. 2014 reported an immediate chromatin expansion at the damaged site, followed by an unexpected chromatin re-condensation at later times. We have updated our Discussion section accordingly (line 394-397).

Unfortunately, our present experiments cannot fully address this time-course and would require a better temporal control of DNA damage induction and we added a sentence to acknowledge this limitation (lines 402-405). We likewise cannot presently say whether our effects depend on continuous DNA damage.

Referee #3:

Review of Herbert et al., Chromatin stiffening underlies enhanced locus mobility ....

This manuscript delves to explain the mechanistic basis for increased chromatin dynamics following DNA damage observed in budding yeast. The paper describes a nice series of experiments using marked strains with fluorescent loci at different positions along the chromosome to quantitate packaging (compaction and rigidity) as a function of damage. The authors report no difference in compaction and rigidity following DNA damage. The data are well-presented, but it is unclear that there are any significant biological advances in this report.

In fact, our paper does report differences in chromatin rigidity following DNA damage. This part of the referee's report may be based on an earlier version of our paper from 2015 (which was then under review in a different journal), in which we had used short (1 hour) Zeocine exposure times and had failed to detect significant changes in intrachromosomal distances (see Appendix Figure S4). With the longer exposure times used here, we do in fact detect a significant change, as shown in Fig. 3 and discussed in the section "DNA damage increases intrachromosomal distances". This increase in distances (together with our data on chromatin dynamics) is a central result of our study, as also reflected by the title of the paper.

Specific Comments:

The authors show the measured distance between a number of loci and conclude that the assumption of an ideal chain suffices to explain chromosome organization. Considering the state of the field and the numerous models using ideal chains, this conclusion is not surprising and in fact confirms findings in many prior reports. They provide additional data for intermediate distances that go beyond several prior reports, but nonetheless the findings are confirmatory and do not advance the field. The authors do find that the pericentromere region is stretched beyond the distance expected from an ideal chain. As the authors cite, this has been reported in mitosis to be a function of DNA loops functioning as a polymer brush(p. 9). This has been explicitly modeled in a recent report (Lawrimore et al., MBOC 2016).

Unlike the increase in intrachromosomal distances mentioned above, our observation that measured distances between loci are in agreement with ideal chains is not a central result of this paper. We

mentioned it in a single sentence (lines 170-173) and showed it in a Supplementary Figure (former Fig. S3). We nonetheless believe that this observation is interesting and worth mentioning, even if ideal chains have been successfully used before in models of yeast chromosomes. Our statement about the ideal chain behavior is based on an analysis of the entire distribution of distances, not just mean distances. To our knowledge this is novel and provides much stronger evidence for Gaussian chain behavior than studies based only on average distances.

As to the pericentromeric stretching: our explanation for this observation is very different from that of Lawrimore et al., since our model does not require any DNA loops and applies to interphase chromatin, not mitosis. As described in more detail in Arbona et al., our model assumes homogeneous chromatin properties (constant compaction and rigidity) all along the chromosomes. The stretching predicted by our simulation results from interactions between generic polymers as in a polymer brush and does not require assuming any specific structure for the peri-centromeric region. We added a sentence in lines 202-204 to mention this.

In the presence of DNA damage, the intrachromosomal distances increase over the entire length of the chromosome. To understand the mechanistic basis for the increase the authors make a mutant in the CEP3 kinetochore protein (S575A). They do not replicate a previous report that concluded this change was instrumental in loss of tethering upon DNA damage.
The authors provide an alternative hypothesis that is based on chromatin rigidity. In the model, only chromatin rigidity was sufficient to account for the experimental results. This is an interesting hypothesis, but not evidence that rigidity is the key feature. In an effort to address the mechanism, the authors use the Histone 2A S129A mutant that is non-phosphorylatable. They find that in this mutant, the DNA damage response is largely abrogated (Fig. 4). This is an interesting finding and important for the field.

We appreciate the referee's acknowledgement of the importance of this finding. As to the importance of rigidity, we have performed new experiments using super-resolution microscopy that provide independent evidence for an increase of rigidity (new Figure 6), as explained in response to referee 2.

In summary, the authors address a previous publication that states centromeres are detached following DNA damage and provide good evidence that this is not the mechanism responsible for increased mobilization. In contrast, using a polymer model, chromatin rigidity can account for the observation. In an experiment with a non-phosphorylatable histone, they block the increase in DNA damage. This is an important first step toward understanding the chromatin modification in response to DNA damage.

We indeed propose that a change in chromatin rigidity is responsible for increased chromatin dynamics after DNA damage rather than loosened centromeric tethering. We thank the referee for acknowledging the importance of this new mechanism.

2nd Editorial Decision 06 April 2017

Thank you for submitting your revised manuscript, which has now been assessed once more by referee 2. I am pleased to inform you that this reviewer, who originally raised the most serious but also constructive criticisms, now considers the study substantially improved, however s/he still retains some well-taken concerns that would need to be experimentally addressed before the manuscript would be ready for EMBO Journal publication. Given the significant improvements on a majority of points at this stage, we shall be happy to give you the opportunity to address these few remaining specific points in an exceptional additional round of revision.

REFEREE REPORTS

Referee #2:

This revised manuscript is significantly improved. However, the question of cis versus trans effects requires further clarification. The authors now demonstrate that HO-mediated DSBs can affect chromatin mobility in trans at a sub-telomeric locus. Given that centromeres and telomeres may be

susceptible to tethering effects, the authors should include a more distal probe, such as R2 or R3. More importantly, the model proposed by the authors is largely focused on cis effects, in part based on the observations with the H2A mutant. It will be important to determine if the mobility changes in trans are also influenced by H2A phosphorylation/the DNA damage response. If so, a local "change of charge model" due to H2A phosphorylation is not sufficient to explain the observed global chromatin stiffening.

An extension of HO analyses is particularly important as the prolongued zeocin treatment is likely to alter cell function and may cause cell cycle arrest in S/G2 (the authors comment on increased cell size), which complicates a comparison of untreated and 6 h zeocin treated cells.

Minor comment:

The magnified lacR images in Figure 6 should be on the same scale. How do the auhors explain that, despite the reduction in lambda2, no increase in lambda1 is observed. Wouldn't that suggest an overall compaction rather than a stiffening of the LacR fiber?

2nd Revision - authors' response 15 May 2017

We are now submitting a newly revised manuscript. The revised paper incorporates the changes outlined in this letter, as well as a few additional improvements. We provide a point-by-point response to the last review separately. We hope that with these changes you will find the paper acceptable for publication in the EMBO Journal. We look forward to hearing back from you.

POINT BY POINT RESPONSE

We thank the reviewer for the insightful comments, which led us to further improve our manuscript. We provide a detailed response to each comment below. We also performed a few relatively minor changes to the manuscript, including:

- An additional paragraph about cis vs trans effects on chromatin mobility in the Discussion
- A more complete discussion of measurement errors in the Supplementary Methods (first section and Fig. S3)

This revised manuscript is significantly improved. However, the question of cis versus trans effects requires further clarification. The authors now demonstrate that HO-mediated DSBs can affect chromatin mobility in trans at a sub-telomeric locus. Given that centromeres and telomeres may be susceptible to tethering effects, the authors should include a more distal probe, such as R2 or R3.

We acknowledge the importance of cis versus trans effects, as well as that of potential tethering effects. However, several independent studies have already shown that a targeted DSB can induce increased chromatin mobility in trans, including on loci that are less susceptible to tethering. For example, MinÈ-Hattab and Rothstein (Nat Cell Biol 2012, Fig. 5) showed that induction of a DSB on chromosome III increased the mobility of locus URA3 on chromosome V. This locus is located at 36kb from the centromere and 116kb from the telomere, and its 3D position is close to the center of the nucleus (Berger, Cabal et al. Nat Meth 2012, Fig. 1). More recently, Durocher and collaborators (Strecker et al., Nat Cell Biol 2016, Fig. 5) showed that a DSB induced in the internal region of chromosome IV (at 101 Kb from the centromere and 981 Kb from the telomere) led to increased mobility of a locus in the middle of the left arm of chromosome V (locus MAK10 locus, at 98kb from the centromere and 52kb from the telomere).

Therefore, increased mobility in trans is a robust effect and not restricted to loci in proximity of centromere and telomeres. We added relevant sentences with these references towards the end of the section "DNA damage by prolonged Zeocin exposure increases chromatin subdiffusion."

In addition, to assess the potential effect of telomeric tethering in our experiment of HO-mediated DSB, we now estimated the distance of this locus to the nuclear periphery both in absence or presence of HO induction (new panel Fig EV1 e) and found no evidence for untethering, as shown in the new panel Fig. EV1 f. This further indicates that tethering effects alone cannot explain increased mobility in our experiment. We added a relevant sentence mentioning Fig. EV1e,f at the end of this same section.

We acknowledge that the amplitude of the mobility increase may depend on the genomic location of the tracked locus along the chromosome arm, and also on the genomic location of the induced DSB. A full quantitative characterization of this dependency would, however, require systematically varying the positions of the DSB and the tracked locus and is beyond the scope of our paper.

More importantly, the model proposed by the authors is largely focused on cis effects, in part based on the observations with the H2A mutant. It will be important to determine if the mobility changes in trans are also influenced by H2A phosphorylation/the DNA damage response. If so, a local "change of charge model" due to H2A phosphorylation is not sufficient to explain the- observed global chromatin stiffening.

We agree that in addition to our Zeocin experiments it would be interesting to analyze the effect of H2A phosphorylation using the HO system, which allows to specifically monitor mobility in trans. In principle, this might be possible using a mutant of H2AñS129A that also permits targeted DSB induction and has a fluorescently tagged locus in trans. However, this would require rebuilding the yeast strains from scratch and imply considerable additional experimental work. We therefore consider that these experiments should be done in a distinct follow-up paper.

Furthermore, although we acknowledge that H2A phosphorylation in cis might be sufficient to explain increased mobility after Zeocin exposure (provided that random DBSs occur sufficiently frequently on the same chromosome as the tagged locus), our data are entirely consistent with a model where H2A phosphorylation occurs throughout the genome, i.e. also in trans. We indeed favor this model of global (cis + trans) H2A phosphorylation because it can explain our data without having to assume a high frequency of DSB. We admit that we currently did not address the mechanism by which H2A phosphorylation propagates from single DSBs throughout the genome. Investigating such mechanisms will be the subject of future studies.

An extension of HO analyses is particularly important as the prolongued zeocin treatment is likely to alter cell function and may cause cell cycle arrest in S/G2 (the authors comment on increased cell size), which complicates a comparison of untreated and 6 h zeocin treated cells.

The referee is correct that DNA damage by Zeocin leads to cell cycle arrest, and the size of nuclei indeed increases for longer Zeocin exposure (Fig. EV3 inset). However, HO induced DNA damage is not fundamentally different: Keogh et al. (Nature, 2005) showed that a single DSB induced by HO also leads to transient cell cycle arrest. Therefore, we do not see HO experiments as a means to decouple the effect of cell cycle arrest from DNA damage. It might be possible to decouple these effects using mutants of the checkpoint pathway. However, this would require time-consuming construction of new cell strains and additional experiments that we consider to be outside the scope of the present study.

Nonetheless, the effect of cell cycle arrest can be reduced with shorter Zeocin exposure, as Fig. EV3 (inset) shows. When we use shorter exposure times of 2 or 4 h, we still observe an increase of intrachromosomal distances (albeit weaker), as shown in Fig. 3 and EV3. In addition, we also observe an increase in chromatin mobility for these shorter exposure times, and longer exposure leads to higher mobility increase. This is now shown in the new Figure S3 of the Appendix for three chromatin loci. Therefore, the main effects reported in our study are not restricted to DNA damage levels that lead to high levels of cell cycle arrest.

Minor comment:

The magnified lacR images in Figure 6 should be on the same scale. How do the auhors explain that, despite the reduction in lambda2, no increase in lambda1 is observed. Wouldn't that suggest an overall compaction rather than a stiffening of the LacR fiber?

We now adjusted the magnified views in Fig 6 to have the same scale.

As to our interpretation of the change in eigenvalues lambda1 and lambda2: We indeed observe a slight reduction in lambda2 but no significant change in lambda1. Under the assumptions of our paper, theory predicts a slight increase of lambda1 and a slight reduction of lambda2 upon stiffening, as shown in the updated Figure EV5 (in this new version of the Figure we added simulations for a softer and a more compact chromatin chain, and the chains were simulated in 3D). By contrast, decondensation predicts a strong increase of both lambda1 and lambda2, while an increase in compaction (condensation) leads to a strong decrease of both lambda1 and lambda2. The observed reduction in lambda2 might therefore point to either stiffening or compaction. However, compaction is not consistent with the observed increase in intrachromosomal distances (Fig. 3). Thus, the only scenario that can explain both the intrachromosomal distance data and the super-resolution imaging results is chromatin stiffening.

The fact that in our experimental data lambda1 does not significantly change, unlike in the simulations, may be due to multiple factors, including insufficient statistics (although we analyzed roughly 100 cells per condition), unspecific labeling by nanobodies, or larger random localization errors than in the simulations. Future super-resolution imaging studies in 3D with more specific labeling and/or even higher resolution might yield significant difference in both eigenvalues, but such experiments are beyond the scope of the present paper.

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Corresponding Author Name:	EMMANUELLE FABRE AND CHRISTOPHE ZIMMER
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The data shown in figures should satisfy the following conditions:

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
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- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
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- a statement of how many times the experiment shown was independently replicated in the laboratory.
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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.

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