THE EMBO JOURNAL

Manuscript EMBO-2016-95756

Cohesin acetylation and Wapl-Pds5 oppositely regulate translocation of cohesin along DNA

Mai Kanke, Pim Huis In't Veld, Eri Tahara and Tomoko Nishiyama

Corresponding author: Tomoko Nishiyama, Nagoya University

Review timeline:

Submission date:	26 September 2016
Editorial Decision:	14 October 2016
Revision received:	04 November 2016
Accepted:	07 November 2016

Editor: Hartmut Vodermaier

Transaction Report:

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

1st Editorial Decision 14 October 2016

Thank you again for submitting your manuscript on regulation of cohesin translocation to The EMBO Journal. It has now been evaluated by two expert reviewers. Both of them find your study potentially interesting and feel that your results significantly extends the earlier work of Stigler et al. We are therefore happy to consider the manuscript further for rapid publication in The EMBO Journal.

As you will see, the both referees are mainly concerned about how the data were analyzed and presented, which currently leaves it unclear whether the data really strongly support your conclusions. In principle, it appears that these issues might be realistically answered and addressed within a very short time frame. However, since there is also very limited time left for accepting manuscripts that could still be published in the current calendar year, it would be very important that you get back to me as early as possible with a proposal for addressing the referee comments and an estimate how long this would take.

REFEREE REPORTS

Referee #1:

In this work by Kanke et al. the authors use single-molecule imaging to investigate how cohesin translocation on DNA is regulated. This report goes beyond recent work by the Koshland and Greene labs reported in Cell Reports by using a full reconstitution of the cohesin complex and investigating cohesin localization in Xenopus extracts during active replication. However, I think

the paper would be strengthened if the authors more clearly articulated how their work differs from this study. In its current state, I do not believe this manuscript is ready for publication. I have significant technical concerns with how the data was analyzed and whether the data actually supports all the claims the authors make. If the authors are able to sufficiently address these points I believe the work will be impactful, appropriate for EMBO and will be of interest to the SMC field.

Major Concerns

1. The use of "...actively translocated on unreplicated DNA..." in the Abstract is misleading as it implies directed motion while the data are consistent with a passive diffusion process. The exception is during replication where the static cohesin complexes move unidirectionally. Presumably this is due to the replisome pushing the cohesin complex. Are the speeds of translocation in this case consistent with replication?

2. Figure S1B shows that a significant fraction of the Scc1-Halo spots presumably have multiple copies of Scc1-Halo. How was this treated in the data analysis? Did complexes with different composition have different diffusive properties?

3. The authors claim that acetylation of cohesin can increase translocation and cite Figure S2F which shows a clear difference between the two conditions. However, it seems that the same +/- acetylation data is plotted in Figure 2B and 2D (see cohesin alone in 2B and Ac-cohesin in 2D) and these plots up to the fitting window of 0.5 seconds are nearly indistinguishable. The conditions reported in the figure captions appear to be identical so it is unclear to me why there is such a dramatic difference between the SI and main text figures.

One possibility to explain this discrepancy is that the authors are plotting the MSD from a single particle, a likely possibility given how it is described in the figure caption. On their own single MSDs are meaningless. If this is the case the authors need to plot a histogram of the MSDs and show that the resulting distribution of diffusion coefficients are statistically distinguishable.

4. Figure 4A is very difficult to see. While it is apparent that MCM loading is severely attenuated in the presence of geminin, I still see a number of cohesin spots. It would be much more convincing if the authors showed a histogram of the number of cohesin and MCM spots per DNA in the presence and absence of geminin.

5. On page 12, it seems misleading to speak of chromatin structure when what is actually being done is loading nucleosomes onto doubly tethered DNA. Given the ~6-fold compaction of DNA upon forming a nucleosome how many nucleosomes are even being formed on the average substrate? Presumably any slack in the DNA substrate rapidly disappears and the resulting taut DNA substrate would inhibit any higher order nucleosome interactions.

6. On page 13 the authors argue that cohesin molecules reside in gaps between nucleosome free regions. They also argue earlier that cohesin is recruited to pre-RCs. Might the fact that cohesins prefer nucleosome -free regions just be a consequence that pre-RCs form preferentially in nucleosome-free regions?

7. The authors argue that the MCM helicase does not always colocalized with cohesin. To prove this point the authors show the distribution of Mcm2 and Smc3 on three different DNAs (Figure 5A). I just don't find these examples very compelling without quantification. The authors should easily be able to report the colocalized fraction.

Referee #2:

In this study, Kanke and colleagues use single molecule analyses based on flow-stretching techniques to follow the binding and movement of cohesin on DNA. They report that purified cohesin can bind and translocate along DNA in (ATPase)-dependent manner and that this movement is suppressed when Wapl-Pds5 and Sororin are added. Acetylation of Smc3, phosphorylation by

Plk1 or Aurora B rescues translocation. The authors also use chromatinized templates assembled in Xenopus egg extracts to show that also in this context cohesin is translocated on DNA in an Smc3 acetylation-dependent manner, and even that the movement becomes unidirectional during DNA replication.

This is a timely study that extends recent results from Stigler et al (2016) in which the movement of purified cohesin core complex over single DNA molecules was monitored. The study is of great interest and I am impressed by the state of the art technology employed. However, the paper is complicated and difficult to read, and even though the results show clear changes in cohesin's translocation ability in the presence of different regulators, additional data and further mechanistic insight would improve the relevance and impact of the study.

Main issues:

-In general, I would like to see an estimation of the cohesin molecules topologically entrapped per DNA molecule in each experiment. This is particularly important in the experiment in which Wapl-Pds5 heterodimer is added to DNA bound cohesin (Figure 2), since the effect of the heterodimer is to unload cohesin. It is unclear how the experiment is performed. In the main text page 9: "Even in the presence of Wapl-Pds5, a detectable amount of cohesin particles was bound to DNA if it was not treated with a high salt buffer. When those particles were analyzed in the presence of ATP, we found, unexpectedly, that Wapl-Pds5 significantly attenuated the translocation activity (Figure 2B)". I assume that a high salt wash was made after loading cohesin by Scc2-Scc4 in order to leave only topologically entrapped complexes before Wapl-Pds5 addition, but after incubation with Wapl-Pds5 they did not wash with high salt. If this second high-salt wash is important to remove complexes that have been "opened" by the action of Wapl (like in Figure 2A), why not perform it before measuring translocation? Otherwise, it is not clear how these molecules are binding to DNA. Are the cohesin molecules imaged in Figure 2B associated to Wapl-Pds5? They could be molecules "improperly" loaded (e.g., not loaded by Scc2-Scc4, similar to those observed in Figure S1I).

-The results with acetylated cohesin and sororin are intriguing. The authors write that "Although Sororin did not change the amount of cohesin bound to DNA (data not shown), Sororin further suppressed Wapl-Pds5-bound cohesin translocation activity (Figure 2D)." Since Sororin prevents Wapl unloading activity, I would expect that the number of cohesin complexes entrapping DNA would be higher in the condition +Sororin.

- In figure 3, showing how mitotic kinases affect translocation, the message is again confusing. Treatment with Plk1 affects Sororin binding and translocation without affecting Wapl whereas treatment with Aurora B, previously shown to promote Sororin dissociation, does not decrease Sororin intensity but does increase translocation. The authors should show whether Wapl is removed under this condition. They could also test the effect of the phosphorylation when only the core complex is bound.

- Does the presence of nucleosomes slow down translocation or promote dissociation of the complex?

- To explain the existence of two different behaviours of cohesin in HSS + AMP-PCP, the authors suggest that the addition of the AMP-PCP may affect the acetylation of Smc3. This could be tested by antibody staining of the single molecules.

- What can be the reason for the variability observed in the mock depleted extracts shown in Figure 5E? Does the graph corresponding to Esco2 depletion represent a single experiment? More importantly, can addition of acetylated cohesin rescue the translocation in the Esco2-depleted Xenopus egg extracts (Figure 5D)?

-The result in Figure 5F is potentially important but as it is presented is difficult to understand. The kymograph represents the movement of a particle over 15-20 min, i.e., much longer than in previous experiments. In the HSS condition (left) the authors should indicate which of the dots correspond to the same particle. In the NPE condition, I assume that the image of the DNA corresponds to the last timepoint in the kymograph. Most important: How representative are the images shown?

Minor issues:

-The authors claim that cohesin acetylation does not affect the ATPase activity and must affect instead the conformation of the complex. They cite a study by Ladurner et al (2014) in which ATPase assays with acetylation mutants were performed in vitro, without DNA involved, and ATPase activity was not changed. More recent experiments by Murayama and Uhlmann (2015) have instead suggested that the ATPase activity of cohesin is stimulated by DNA sensing by the unacetylated Lysines in Smc3.

- In Discussion, the authors say that "cohesin acetylation reverts the translocation activity of Wapl-Pds5-bound cohesin" or that "acetylation of cohesin facilitates its active translocation in the presence of Wapl-Pds5". However, they also show that cohesin acetylation increases translocation in the absence of Wapl-Pds5 (Figure 2B-D). This may be important since it is not clear what proportion of complexes present in the cell are associated with Wapl-Pds5.

Additional correspondence - author 14 October 2016

Thanks for your positive reply. Now I looked through the reviewers' comments. Although all comments could be technically answered, we still need several experiments and analyses for satisfactory explanation. So, I would expect that we need 2 weeks for the revision.

Additional correspondence - editor 14 October 2016

Thank you for your reassuring response - I am glad to hear that you are confident about addressing the comments (but still, do contact me if unsure about anything). So I could actually offer you November 7th as very latest date for resubmission of a revised manuscript. Should you be finished earlier, that would of course be fine, but I hope these ~3 weeks give you a bit more freedom to address the points with all necessary diligence and carefulness.

It will however be important that the manuscript is already in very much the correct format for EMBO Journal at the time of resubmission, so please carefully double-check adherence to our author guidelines.

1st Revision - authors' response 04 November 2016

We would like to thank the Referees for their positive comments on our manuscript and for providing many helpful suggestions in a short period of time. We would like to answer the referee's comments point by point.

Referee #1:

1. The referee felt that the wording of "actively translocated" in the text is misleading, since all the cohesin translocations are based on passive diffusion process.

Because translocation ability of cohesin is inhibited by AMP-PCP or AMP-PNP, we called it "active translocation." However, we agree that cohesin translocation is based on diffusion. Therefore, we changed "active translocation" to "translocation" in the abstract and main text and misleading words like "translocation activity" were also changed. As the referee also pointed out, we think that cohesin translocation observed during DNA replication depends on replisome progression as cohesin translocation and expansion of replication bubble are synchronized. Now we show the new replication results in new Fig 6, where we monitored DNA replication by incorporation of xFen1-PAGFP and observed replication and cohesin movement at the same time. Fig 6B (group 1) clearly shows that the expansion of replication bubble and cohesin translocation is synchronized, although those population was only ~15% in our system. We think that the group3 (~32%), which is incorporated into replicating DNA, would be more physiologically important to make cohesive cohesin.

2. The referee pointed out that significant population of Scc1-Halo forms multimer (Appendix Fig S1B) and asked if those multimers had the same diffusive property as the single copy of cohesin complex.

As the referee pointed out, we observed multimers of cohesin-Halo complexes on DNA. However, most of those multimers are immobile on DNA, presumably because they are just aggregated. In all tracking experiments in our manuscript, we chose only 1) the mobile particles and 2) the particles, which intensities were corresponding to those of single molecules. Therefore we expect that our MSD plots and the diffusion coefficients would not consider the multimer complexes. We added the new Appendix Fig S1G to show that multimer complexes are immobile.

3. The referee pointed out that MSD vs time plots of cohesin alone in the previous Fig 2B and Ac-cohesin in the previous Fig 2D were nearly indistinguishable, although their diffusion

coefficient are significantly different. And the referee further suggested plotting of diffusion coefficient to avoid the discrepancy.

First, we would like to thank the reviewer for pointing out this and apologize that we have shown wrong value of diffusion coefficient for Ac-cohesin in the previous Fig 2D and S2F, although the MSD plots and line regressions were correct. We corrected the diffusion coefficient in new Fig 2D and EV2C. As the referee pointed out, plots for "cohesin alone" ($D = 0.19\ \mu m^2/s$) in Fig 2B and "Ac-cohesin" ($D = 0.228\ \mu m^2/s$) in Fig 2D and EV2C are almost indistinguishable. From our experiences, cohesin mobility can be slightly variable depending on the room temperature, the purification lot, and their freshness after the purification. In every MSD plot, we used the cohesin purified in the same day to directly compare their diffusion coefficients. However, we cannot directly compare the MSD between the different panels, since their freshness and the room temperatures, which we could not tightly regulate, could be different. Nevertheless, we would like to show another example of comparison between Ac-cohesin and unacetylated cohesin, both of which were purified in the same day but a different lot from what is shown in new Fig EV2C (For referees Figure (1)). In this case, their diffusion coefficients are lower than the case shown in the Fig EV2C. Nonetheless, acetylation of cohesin facilitates the translocation of cohesin on DNA.

Regarding the diffusion coefficient (D), we estimated D from the initial slope of the MSD vs. Δt curve. MSD was calculated as indicated in supplementary methods. Every MSD (N, n) was obtained after normalization of ≥ 279 data from single particle and the MSD vs. Δt curve was drawn as a mean of ≥ 45 particles. Therefore we believe that we could obtain reliable results.

4. The referee suggested showing a histogram of the number of cohesin and Mcm spots per DNA in the presence or absence of geminin to make the previous Figure 4A more visible.

We added the new Fig 4A (magnified representative pictures) and 4B, where the numbers of DNA-bound Smc3 and Mcm2 particles were counted. There are still some residual Smc3 spots after geminin treatment. On untreated DNA, the numbers of Mcm2 particles were lower than that of Smc3 (shown in Figs 4B) presumably because 1) Mcm complex would be less stable than cohesin or 2) a few number of preRC contributes to load many cohesin molecules.

5. The referee pointed out that it would be misleading to speak of chromatin structure in our experimental system where nucleosomes formed on doubly tethered DNA and the formation of nucleosomes should be restricted.

We agree with that it would be misleading to mention about chromatin structure. We changed the wording in the main text from “chromatin structure” to “nucleosome density”.

6. The referee asked if preference of cohesin for nucleosome-free region is a consequence of that pre-RCs formed preferentially in nucleosome-free regions.

To test this, we have performed immunofluorescence microscopy for nucleosome (H3) and pre-RC (Mcm2). Colocalization analysis revealed that there is no preference of pre-RC formation for nucleosome-poor region, suggesting that cohesin rather than pre-RC is preferentially localized to nucleosome-poor regions. We mentioned this in the main text and added new Fig 4F.

7. The referee suggested quantifying the colocalization of Smc3 and Mcm2 in the previous Figure 5A.

We quantified the number of DNA-bound Smc3 and Mcm2 IF signals and the result was shown in new Fig5A. More than 90 % of Smc3 signals (particles) were not colocalized with Mcm2 (green), whereas ~ 50% of Mcm2 signals (particles) were colocalized with Smc3 (magenta).

Referee #2:

1. The referee suggested performing the second high salt wash after Wapl-Pds5 addition to remove cohesin complexes that have been opened by the action of Wapl.

As the referee assumed, we washed the flow cell in high salt buffer only after Scc2-4-dependent loading to remove improperly loaded cohesin complex. In this study, we aim to understand the effect of Wapl-Pds5 as well as other binding proteins or modifications on cohesin’s translocation ability. As the major role of Wapl-Pds5 is to unload cohesin from DNA, we are not able to observe cohesin after the second high salt wash in the presence of Wapl-Pds5. Indeed, in the presence of Wapl-Pds5, both cohesin and Wapl-Pds5 are dissociated from DNA after high salt wash (For referees Figure (2)). In order to evaluate the translocation activity of cohesin bound to Wapl-Pds5, we need to avoid dissociation of Wapl-Pds5-bound cohesin. This is the reason why we did not perform the second high salt wash in the presence of Wapl-Pds5. Because 1) topological loading of cohesin is achieved by Scc2-4 (Fig 1B), 2) non-topological cohesins should be washed away by high-salt buffer (Fig EV1B), and 3) most of high-salt-treated cohesin are

colocalized with Wapl (Appendix Fig S2B), we believe that we could properly evaluate the Wapl-Pds5-bound topological cohesins.

The referee also suggested showing estimation of cohesin molecules topologically entrapped per DNA molecule in each experiment. From the reason mentioned above, we do not have the estimation of the number of cohesin molecule topologically entrapping DNA in each experiment. Nevertheless, we can roughly estimate it from the high-salt washing experiments. In the high-salt washing experiment shown in Fig EV1B (previous Figure S1E), we roughly estimate that 16.2±4.4 cohesin molecules are topologically loaded on a 50 kbp DNA in the absence of Wapl-Pds5 (not mentioned in the manuscript). Around 90% of these topological cohesins were removed from DNA in the presence of Wapl-Psd5, whereas it is ~50% in the presence of Sororin in addition to Wapl-Pds5. We added this result in new Appendix Fig S2G.

2. In the original manuscript, we have mentioned that Sororin did not change the amount of cohesin bound to DNA. However, since Sororin is known to have an inhibitory activity against Wapl-Pds5, the referee asked if Sororin increased the number of cohesin entrapping DNA.

To answer this question, we have performed high-salt wash experiment in the presence of Wapl-Pds5 and Sororin. As shown in new Appendix Fig S2G, Sororin indeed suppressed Wapl-Pds5 activity to remove cohesin from DNA. However, again because of the reason mentioned in the previous point, we did not perform the second high salt wash. Since the cohesin loading and dissociation is highly dependent on salt concentration (Murayama and Uhlmann 2014, 2015, Appendix Fig S1E), we could not observe Sororin-dependent increase of cohesin amount in our tracking assay condition without high-salt washing. In any case, as this sentence about Sororin amount is confusing, we replaced the sentence to explain the new Appendix Fig S2G.

3. The referee pointed out that the result in the previous Figure 3, where we showed that Aurora B did not affect Sororin amount on cohesin, is inconsistent with our previous observation (Nishiyama *et al*., 2013 *PNAS*) and also suggested testing if Wapl is removed from cohesin in the presence of Aurora B.

As the referee pointed out, we have previously shown that Aurora B is sufficient for the dissociation of Sororin from Wapl-Pds5, whereas Plk1 does not affect Sororin binding to Pds5 *in vitro*. Therefore, it was unexpected for us too that Plk1 and Cdk1 but not Aurora B dissociated Sororin from cohesin complex (Fig 3B). We assume that this discrepancy is due to the difference of *in vitro* system. Our previous *in vitro* experiments were binding assay using affinity beads, where we can wash the Pds5-bound beads with a buffer containing detergent. On the other hand, in this study, we can wash the reaction only with a mild salt condition without any detergents,

which might not be sufficient for removing Sororin from Wapl-Pds5 if the effect is relatively weak. Similarly, the addition of Wapl-Pds5 without any high-salt wash in our current condition is not sufficient to remove cohesin from DNA as mentioned in main text (p9), whereas washing the flow cell in a high salt buffer could remove cohesin (Appendix Fig S2C and S2G). This may be a reason why Aurora B treatment was not sufficient to dissociate Sororin from Wapl-Pds5. We also confirmed that amount of Wapl bound to cohesin was not changed by Aurora B (Appendix Fig S3D). So far, we do not have any answers to explain how Aurora B facilitates cohesin translocation without affecting Sororin amount on cohesin. As mentioned in main text, we assume that phosphorylation-dependent conformational changes or negative charge may increase the translocation ability.

Regarding the effect of Plk1, this was also unexpected that Plk1 was sufficient to remove Sororin from Wapl-Pds5-bound cohesin. Because, in our current flow cell system, Plk1 is expected to phosphorylate not only Sororin but also whole cohesin-Wapl-Pds5 complex (Appendix Fig S3B), whereas only Sororin was phosphorylated by Plk1 in a previous report (Nishiyama et al., 2013 *PNAS*), this could explain why Plk1 was sufficient for the Sororin dissociation in this study. Presumably, the Plk1-dependent phosphorylation of cohesin and/or Wapl-Pds5 could facilitate Sororin dissociation from Pds5.

The referee also suggested testing the effect of phosphorylation on cohesin core complex itself. We could have only tested Plk1 and we found that cohesin translocation was "suppressed" by Plk1 treatment and it was reverted by the inhibitor (BI4834) treatment (new Appendix Fig S3F). It is quite interesting that the phenotype is opposite to that observed in the presence of Wapl-Pds5-Sororin, although we do not know the reason so far. We assume that Wapl-Pds5 would be an important regulator of cohesin translocation. Although we could not test the effect of Aurora B and Cdk1 on cohesin core complex due to the limitation of time, we added the new Appendix Fig S3F showing the effect of Plk1 and mentioned in the text.

4. The referee asked if the presence of nucleosomes slows down translocation or promote dissociation of the complex.

We now have observed the cohesin translocation in HSS followed by immunostaining for histone H3 to identify the position of nucleosomes. So far it is technically difficult to observe fluorescently labeled nucleosomes with cohesin at the same time in HSS, we first performed time-lapse imaging of cohesin and, immediately after the imaging, histone H3 was immunostained. As shown in new Fig 5C, cohesin particles were lagging around nucleosomes but were able to passing through them, which was similar to the *in vitro* observations (Stigler et al., 2016 Cell Rep.; Davidson et al., 2016 EMBO J). This result indicates that nucleosomes act as semipenetrable

barriers also on chromatin in *Xenopus* egg extracts. Fig 5C also suggests that nucleosomes do not necessarily promote cohesin dissociation from DNA. Although we could frequently observe the cohesin dissociation from DNA in HSS, so far we do not have any evidence showing that nucleosomes facilitate the cohesin dissociation.

5. The referee asked if acetylation of cohesin is affected in HSS in the presence of AMP-PNP/PCP.

We compared the cohesin acetylation in HSS with ATP or AMP-PNP by immunostaining of Smc3-ac. In the presence of ATP, ~27% of cohesin was acetylated on HSS chromatin, whereas it was decreased to 21% in the presence of AMP-PNP. Although we did not detect significant decrease of acetylation, we cannot rule out the possibility that we underestimated the acetylation because cohesin acetylation may less stable (or dynamic) on *Xenopus* chromatin due to it is translocated from the loading sites (pre-RC), where xEco2 is present. We assume that AMP-PNP suppresses the cohesin translocation at least partially because of reduced acetylation but mainly because of the forced closure of cohesin ring as seen in *in vitro* using AMP-PCP (Fig 1F). We added this result as a new Appendix Fig S5B and mention this in main text.

6. The referee suggested performing add-back experiment in XEco2-depletion assay and also asked the reason of variability of MSD in the previous Figure 5E.

We have now performed add-back experiments of XEco2 depletion (new Fig 5D-F). After depletion of XEco2 from HSS, purified human Esco1 protein was added to the depleted HSS and confirmed that the cohesin acetylation was fully restored on chromatin (Fig 5D). In this condition, we observed that cohesin translocation was restored (Fig 5E and 5F). Thus, we conclude that XEco2 is required for the cohesin translocation ability in *Xenopus* egg extracts. Regarding the variability of MSD, it was because the each MSD plot was corresponding to each single particle as the referee concerned. We have now analyzed 15 particles in each condition and obtained plots as mean ± s.e.m (Fig 5F).

7. The referee suggested to indicating the same particles in HSS in the previous Figure 5F. The referee also asked how to select the representative image.

Since the previous kymograph in Figure 5F showing 1-min-interval of HSS cohesin does not provide any meaningful information, we deleted the figure. The cohesin mobility in HSS

is already clearly shown in Fig 5 in much shorter interval. Instead, we have now performed new replication assay to monitor DNA replication and cohesin at the same time. We observed 34 particles and their motions were categorized into 5 groups and each percentage and their kymographs were shown in new Fig 6B. The result indicates that ~15% of cohesin particles exhibited "unidirectional translocation" presumably by pushing force of replisome, whereas ~32% of particles became immobile and were incorporated into replicating DNA. These mobile and immobile particles would be what we observed in the previous manuscript. We added this result in Fig 6B and also described and discussed in the text.

Minor issues:

8. The referee pointed out that it has been suggested by Murayama and Uhlmann (2015) that the ATPase activity of cohesin is stimulated by DNA sensing by the unacetylated Lysines in Smc3.

We considered this point and the sentences in the result and discussion were changed based on their report.

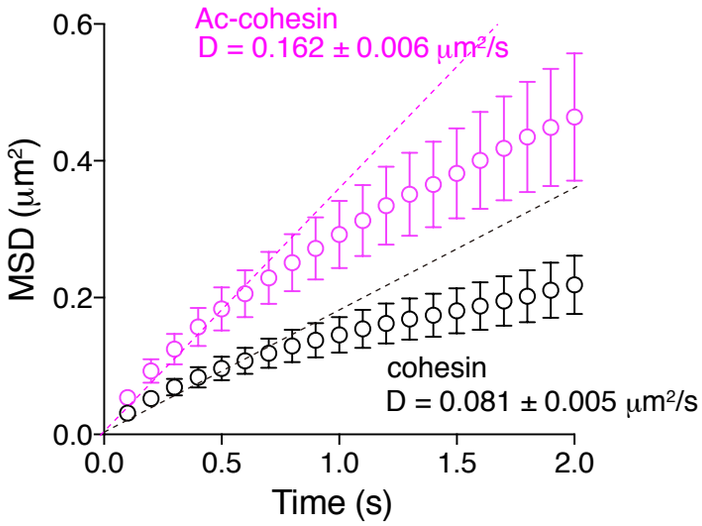
9. The referee pointed out that it would be important to consider that acetylation could directly facilitate cohesin translocation even in the absence of Wapl-Pds5, because it is not clear what proportion of complexes present in the cell are associated with Wapl-Pds5.

According to the suggestion, we changed the sentences in result and discussion sections.

For referee #1

(1) MSD comparison between unacetylated and acetylated cohesin of another purification lot.

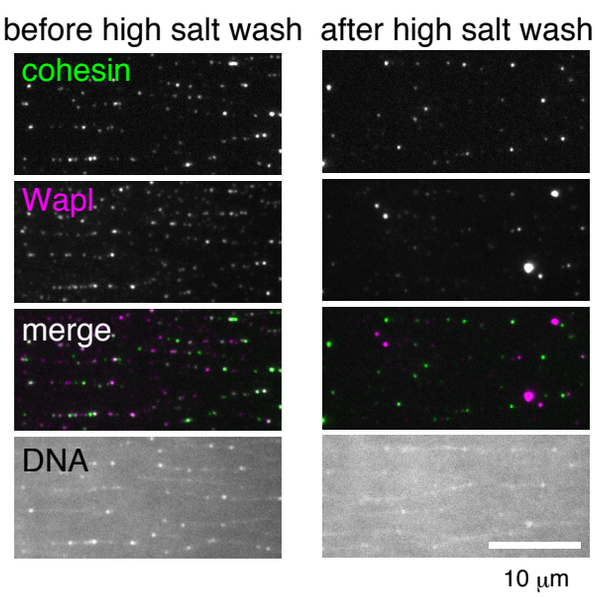
In this purification lot, the diffusion coefficients of both Ac-cohesin and un-acetylated cohesin are lower than that shown in Fig EV2C. Nevertheless, cohesin acetylation accelerates the translocation.

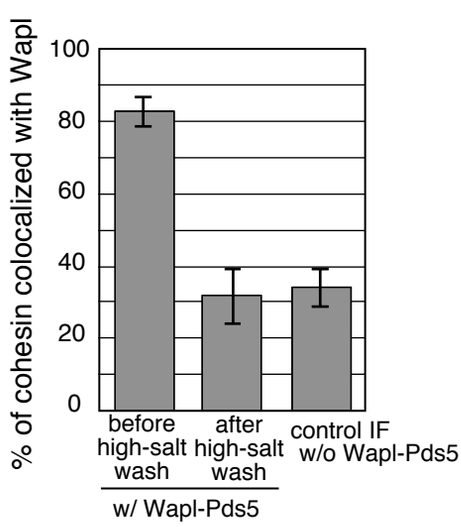


For referee #2

(2) Wapl-Pds5 is dissociated from DNA together with cohesin after high-salt treatment.

Wapl-Pds5-bound cohesin is washed in high-salt buffer and subjected to immunofluorescence microscopy. Very few cohesin could be detected on DNA after high-salt washing and those cohesins were not bound to Wapl-Pds5 (left picture). Note that control IF using Wapl antibody in the absence of Wapl-Pds5 exhibited the ~30% of cohesin colocalized with Wapl (right panel), indicating that the Wapl IF signals after high salt wash could be nonspecific signals.





2nd Editorial Decision 07 November 2016

Thank you for submitting your final revised manuscript for our consideration. We have now gone through your response letter as well as the new version, and found the original comments satisfactorily addressed. I am therefore pleased to inform you that we have now accepted your study for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name:	Tomoko Nishiyama
Journal Submitted to:	EMBO Journal
Manuscript Number:	EMBOJ-2016-95756

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 $\chi 2$ 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.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For every MSD, it was calculated as indicated in supplementary methods. Every MSD (N, n) was obtained after normalization of ≥ 279 data from single particle and the MSD vs. Δt curve was drawn as a mean of ≥ 45 particles. For intensity data, we measured fluorescence intensities on n ≥ 98 DNAs, and for particle colocalization data, ≥ 50 particles were counted. All these data were taken from at least three independent experiments.
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?	Samples were not excluded from analysis, except in special cases e.g. bad image quality.
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?	Statistical tests were justified as appropriate and clearly understandable.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normality was tested by D'Agostino & Pearson omnibus normality test and if it meets normal distribution, the significance was tested by unpaired t-test. Whereas, if it does not meet normal distribution, the significance was tested by Mann Whiteny U test.
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	In Figure 4E, it is similar. For other statistical analyses were performed by Mann Whiteny U test, which does not assume the equal variance of the two populations.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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).	Described in the materials and methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* 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
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
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).	We included all dataset in Extended View 1-2, Appendix Figure S1-6
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. Examples: **Primary Data** 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 **Referenced Data** 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 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	This section was not included in the manuscript.
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.	No.