EMBO reports

Manuscript EMBOR-2012-35801

Pds5 promotes cohesin acetylation and stable cohesin-chromosome interaction

Sabine Vaur, Amelie Feytout, Stephanie Vazquez and Jean-Paul Javerzat

Corresponding author: Jean-Paul Javerzat, CNRS

Review timeline:

Submission date:	31 January 2012
Editorial Decision:	24 February 2012
Revision received:	06 April 2012
Accepted:	26 April 2012

Transaction Report:

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

1st Editorial Decision 24 February 2012

Thank you for your submission to EMBO reports. We have now received the enclosed reports from three referees that were asked to assess it. As you will see below, although all the referees are supportive of the study and its eventual publication here, they also raise a number of issues that need to be addressed experimentally and also request additional discussion of the data.

Given that all referees provide constructive suggestions on how to strengthen the work, I would like to give you the opportunity to revise your manuscript. Acceptable revision would entail addressing the following points experimentally:

- the possible effect of Pds5 on cohesin release from chromatin, its implication in generation and/or maintenance of the stable cohesin pool and whether it affects cohesin loading in G1 phase (main points of referee 1, referee 2 point 5 and referee 3 point 1)

- experimentally show (beyond the results in suppl figure 1) that "stably bound" cohesin is the one actually cohesive and Pds5 is localized there, as mentioned by referees 2 and 3

- determine Rad21 association with euchromatin after Pds5 deficiency, as requested by referees 2 and 3

In addition, it will be necessary to add further discussion at several points, for which I can increase

© European Molecular Biology Organization

our length limit to 29,000 characters (including spaces). If the referee concerns can be adequately addressed, we would be happy to consider your manuscript for publication here. However, please note that it is EMBO reports policy to undergo one round of revision only and thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

I have also noted several problems with the statistical analysis performed in several of the figures. Please note that it is incorrect to calculate error bars of replicate samples and sample size must be at least 3 independent experiments. In this regard, the data shown in figures 2D, 3B, 3D, 4D, 5B and 5D is incorrect, and I must ask you to repeat the experiments as needed, so that errors can be calculated from at least three independent experiments. For guidance, please refer to Cumming et al. JCB 2007. In addition, information on the number of samples analyzed is missing in the legends of figure 4A, 5A and SF1A.

I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, do not hesitate to get in touch with me if I can be of any assistance.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

Previous work has shown that the ability of cohesin to mediate sister chromatid cohesion depends on acetylation of the cohesin subunit Smc3 (Psm3 in fission yeast) by an acetyltransferase called Eco1 in budding yeast (Eso1 in fission yeast). Cohesin acetylation might antagonize the cohesin associated protein Wapl (Wpl1 in fission yeast) which has been proposed to either inhibit cohesion establishment, or to be able to release cohesin from DNA. Cohesion establishment also correlates with an increase in the residence time of cohesin complexes on DNA, and these stably bound complexes are thought to represent the cohesive form of cohesin. Wapl has a binding partner, called Pds5, whose role in sister chromatid cohesion is much less clear. In fission yeast, Pds5 is not essential for sister chromatid cohesion and cell viability, but is required for the maintenance of cohesion in cells that are artificially arrested in G2-phase for prolonged periods of time. In contrast, Eso1is required for cohesion establishment and viability, but this essential function can be bypassed by deletion of Pds5 (Tanaka et al., 2001).

In this study, Javerzat and colleagues report two interesting observations about Pds5 in fission yeast. First, the authors show, by using an antibody that recognizes an acetylated form of Psm3 in Western blots, that Pds5 is required for Psm3 acetylation. Based on this observation the authors speculate that Pds5 is required for recruitment of Eso1 to cohesin during DNA replication. Second, the authors address the role of Pds5 in stable binding of cohesin to chromatin by using a previously established assay in which they inactivate a cohesin loading factor in postreplicative cells and then measure the amount of cohesin on DNA by either immunofluorescence microscopy (IFM) or by chromatin immunoprecipitation (ChIP). The results obtained with this assay imply that Pds5 is not essential for the association of cohesin with chromatin (although cohesin levels on chromatin are reduced in the absence of Pds5) but that Pds5 is needed for the persistence of cohesin on DNA. As in their previous studies, the authors assume that this persistence is a reflection of the number of cohesin complexes that have been converted into a stably DNA bound cohesive state. Using this assay the Javerzat group has previously provided evidence that a role of Eso1 in cohesin stabilization on chromatin could be alleviated by deletion of Wpl1. Here, they report that a defect in cohesin stabilization that is caused by Pds5 deletion cannot be bypassed by deletion of Wpl1. This observation leads the authors to propose that Pds5 is needed for proper cohesin stabilization on DNA, and that the ability of Pds5 to execute this function is inhibited by Wpl1, until cohesin is acetylated by Eso1 during DNA replication.

The observations reported in this manuscript are interesting for the cohesion field, and I would

© European Molecular Biology Organization

therefore like to support their publication in EMBO Reports. However, I am not convinced that the authors' interpretation of their data is the only possible one. I would therefore recommend addressing alternative possibilities, both by additional experiments and by discussing these possibilities in the text, before the paper will be published.

The authors suggest that 'Wpl1 hinders Pds5's function in cohesin stabilization until counteracted by Eso1' (page 8) and make an even stronger claim in the Abstract '... showing that Pds5-mediated cohesin stabilization requires the neutralization of Wpl1 but not cohesin acetylation per se' (page 2; please note that this particular statement in the Abstract is very difficult to understand without having read the rest of the paper; it would be useful to improve the clarity here). This is an interesting idea, but I am not sure if this model is consistent with previous observations made about Pds5 and Eso1. If the key function of Eso1 was to inactivate Wpl1's inhibitory effect on Pds5, why would Eso1 be essential for cohesion establishment and cell viability, but Pds5 not? Would it not be more plausible to think that Eso1 inhibits Wpl1's ability to release cohesin from DNA, and that Pds5 has some independent function which is needed for proper binding of cohesin to DNA? Perhaps the absence of Pds5 would simply change the conformation of cohesin or weaken other protein-protein interactions in the complex, and as a result cohesin complexes lacking Pds5 might not be as stably bound to DNA as wild type complexes. Along these lines it would be very useful to know what happens to cohesin on chromatin in cells lacking Pds5 during G1 phase, either in the presence or absence of cohesin loading factors (similar to the experimental setup used in Bernard et al., 2008). If the authors were correct that 'Wpl1 hinders Pds5's function in cohesin stablilization until counteracted by Eso1' then Pds5 deletion should have no effect on the association dynamics of cohesin with DNA in G1 phase. If, however, Pds5 deletion also affected the amounts or dynamics of cohesin binding to DNA in G1, the authors' current interpretation would have to be changed very significantly.

Referee #2:

1. Do the contents of this manuscript report a single key finding? YES

2. Is the main message supported by compelling experimental evidence? YES

3. Have similar findings been reported elsewhere (e.g. on a closely related protein; in another organism or context)? NO

The role of Pds5 in sister chromatid cohesion, identified several years ago, has remained rather mysterious for long time and is nowadays gaining importance. An essential viability factor in budding yeast, but not so in fission yeast, its function appears to promote cohesion in some contexts but it has also been described as an anti-establishment factor, together with Wapl. Pds5 proteins have been studied also in human, mouse and Xenopus. The experiments presented in this manuscript contribute to our understanding of the role of Pds5 in cohesion, revealing its essential function to promote the stable association of cohesin with chromatin.

4. Is the main finding of general interest to molecular biologists? YES

As I mention in the previous section, the interest in understanding how Pds5, Wapl and Eco1 participate in cohesion establishment and maintenance by cohesin has produced excellent publications over the last years and it is a field of very active research in a number of organisms.

© European Molecular Biology Organization

5. After appropriate revision, would a resubmitted manuscript be most suited for publication:

in EMBO reports

6. Please add any further comments you consider relevant:

I have enjoyed reading the manuscript by Vaur et al. It is well written, the logic of the experiments is easy to follow, and the data support the conclusions of the authors. I also think that these conclusions contribute to further understand how Pds5 regulates cohesin´s function. Thus, I support the publication of this study in EMBO Reports. I only have a few suggestions, for the author´s consideration, in order to improve the manuscript:

1. In the Introduction, "Strains deleted for pds5 are viable showing that Pds5 is not required for the establishment of sister chromatid cohesion" is confusing when two sentences later, the authors claim the opposite, i.e., that "Pds5 is the cornerstone that couples replication with sister chromatid cohesion establishment and maintenance". Thus, It would be better to say "suggesting" instead of "showing".

2. The concept of cohesion erosion is very cryptic, and I think it merits some explanation since is a critical aspect of the Pds5 mutant phenotype in fission yeast. Since this is a G2 arrest, there is not, in principle, dissociation of cohesin (like the prophase pathway in vertebrates). In addition to cohesion mediated by cohesin, DNA catenation contributes to cohesion (even in yeast). One possible explanation for "cohesion erosion" is that during G2 arrest, more time is given for topoisomerase to perform decatenation. (This interpretation, of course, may not be shared by the authors!)

3. The authors equal the "stably bound" cohesin fraction with the cohesin actually engaging two sister chromatids, i.e., performing cohesion. Maybe this idea could be further supported by showing some GFP-dot assay, or at least include a few sentences describing their previous results in this regard (Feytout 2011). A recent paper by Guacci et al (MBC) shows that, in budding yeast, double depletion of eco1 and wpl1, rescues the lethality of eco1 mutants but that very little cohesion is actually established. This is a result distinct from the result presented in Feytout 2011 and in Figure 5A, in which there is stably-bound cohesin in the absence of eso1-wpl1 and the reasons for this difference could be discussed.

4. Regarding Figure 2D, it could be completed by showing how Pds5 deficiency affects the amount of Rad21 present at some euchromatic locations. From this figure also, it would be good to comment on the reasons for the more noticeably decrease in Rad21 binding at telomeres.

5. It would be interesting to try to see if there is any difference between a wpl1 mutant and a double Pds5-wpl1 mutant regarding the "kinetics" in the decrease of cohesin binding during G2 arrest in the absence of new loading. Shorter tiempoints between 0 and 60 min, when the most important decrease occurs, should be tested. In Figure 8 of Feytout 2011 it seems that the decrease in the absence of wpl1 is slower whereas there is not much difference between pds5 mutants and wildtype (in cdc25 mis4 background). I am trying to compare these results in fission yeast with results in Xenopus showing that in the absence of Pds5 dissociation during prophase is faulty. Is Pds5 required for Wpl1 function in releasing cohesin from chromatin?

Referee #3:

The investigation of Javerzat and colleagues provides novel information on the establishment and maintenance of sister chromatid cohesion. This should be valuable for the cohesin field in particular and the chromosome dynamics community in general. The authors focus on the cohesin-associated protein Pds5 which, together with Wpl1 and the acetyltransferase Eso1, is known to regulate

© European Molecular Biology Organization

establishment of sister chromatid cohesion during replication. Pds5 is also needed for cohesion maintenance in G2. In the manuscript it is convincingly shown that fission yeast Pds5 is required for Eso1-dependet acetylation of the cohesin subunit Smc3. Since this acetylation is crucial for establishment of cohesion the presence of Pds5 and Wpl1, it is an important observation. The authors also show that Pds5 is required for stable chromosomal association of cohesin in G2. As stated, it has already been established that Pds5 is needed for maintenance of cohesion, and budding yeast Pds5 maintains cohesin on chromosomes in G1 (see, for example, Panizza, 2000). The here presented observation that Pds5 protects and/or creates a fraction of stably bound cohesin in G2 takes the understanding of Pds5 and cohesion one step further. The authors also present evidence that the stabilization is executed independently of Eso1 and Wpl1, suggesting that this function of Pds5 is unrelated to its role during S-phase. So altogether, even though it remains unknown how Pds5 promotes Smc3 acetylation and stable chromosome binding of cohesin, I find that the presented observations could merit publication after the following issues have been addressed.

1. A key question is whether Pds5 makes and/or maintains the stably bound cohesin fraction. When reading the manuscript the impression is that the fraction of stably bound cohesin is the cohesive one, and responsible for cell survival. If this fraction is not made in pds5Δ cells it is difficult to understand how they survive during normal cell cycle progression (not held in a prolonged G2 arrest). Thus, their survival seems to argue that the stable fraction is made but not maintained in the pds5Δ mutant. Or, is it so that the "unstable" fraction of cohesin can provide enough cohesion to allow survival during normal cell cycle progression? The generation/ maintenance question could be further addressed by experiments where Pds5 is removed in S-phase or G2 only (in combination with different combination of eso1 and wpl1 mutations). This might be too much to ask at this stage, but in any case, the generation/maintenance issue should be more clearly discussed.
2. As the interplay anti-establishment/establishment/maintenance is rather complex, the manuscript would greatly benefit from a figure where a schematic model of these events is displayed, highlighting the new results.
3. As centromeres, rDNA and telomeres are specialized chromosomal domains it could be valuable to investigate Rad21 association at a non-repetitive cohesin binding site on a chromosome arm (i.e. one of the CAR sites investigated in a 2011 MBC publication from the same lab (Feytout et al.). This would confirm that the obtained data is valid for most types of cohesin binding regions.
4. The reason why the initial levels (before temperature increase) of Rad21 vary in pds5Δ should be discussed. Why is there more chromosome-bound Rad21 in pds5Δ than in wild type at the rDNA, less than in wild type at telomeres? (Fig. 2)
5. To be able to state that "cohesin co-localizes with the stable fraction on replicated chromosomes" a more detailed analysis (i.e. chip) than that shown in figure S1 is needed.

1st Revision - authors' response 06 April 2012

To the Referees,

We thank the Referees for supporting publication of our study and for their constructive comments and suggestions. Please find enclosed the revised manuscript in which we have addressed all questions, comments and suggestions. The point by point responses are listed below (bold letters are referees comments).

Referee #1:

The authors suggest that 'Wpl1 hinders Pds5's function in cohesin stabilization until counteracted by Eso1' (page 8) and make an even stronger claim in the Abstract '... showing that Pds5-mediated cohesin stabilization requires the neutralization of Wpl1 but not cohesin acetylation per se' (page 2; please note that this particular statement in the Abstract is very difficult to understand without having read the rest of the paper; it would be useful to improve the clarity here). This is an interesting idea, but I am not sure if this model is consistent with previous observations made about Pds5 and Eso1. If the key function of Eso1 was to inactivate

© European Molecular Biology Organization

Wpl1's inhibitory effect on Pds5, why would Eso1 be essential for cohesion establishment and cell viability, but Pds5 not? Would it not be more plausible to think that Eso1 inhibits Wpl1's ability to release cohesin from DNA, and that Pds5 has some independent function which is needed for proper binding of cohesin to DNA? Perhaps the absence of Pds5 would simply change the conformation of cohesin or weaken other protein-protein interactions in the complex, and as a result cohesin complexes lacking Pds5 might not be as stably bound to DNA as wild type complexes. Along these lines it would be very useful to know what happens to cohesin on chromatin in cells lacking Pds5 during G1 phase, either in the presence or absence of cohesin loading factors (similar to the experimental setup used in Bernard et al., 2008). If the authors were correct that 'Wpl1 hinders Pds5's function in cohesin stablilization until counteracted by Eso1' then Pds5 deletion should have no effect on the association dynamics of cohesin with DNA in G1 phase. If, however, Pds5 deletion also affected the amounts or dynamics of cohesin binding to DNA in G1, the authors' current interpretation would have to be changed very significantly.

In *eso1Δ wpl1Δ* cells, sister-chromatid cohesion is established and is properly maintained in a *pds5*-dependent manner. This shows that the sole but essential role of Eso1 is to neutralize the anti-establishment activity, a pre-requisite that authorizes cohesion establishment and maintenance. This also shows that Eso1 is not part of the mechanism that actually creates and maintains cohesion since cohesion establishment and maintenance do not require Eso1 when *wpl1* is experimentally neutralized. That's the reason why we wrote that "Wpl1 hinders Pds5's function in cohesion stabilization until counteracted by Eso1": as long as the anti-establishment is active, cohesion cannot be made and maintained. It does not mean that the key function of Eso1 is to inactivate Wpl1's inhibitory effect on Pds5. The key Eso1 function is to inactivate the anti-establishment as to unlock the door for cohesion establishment and maintenance. We agree that this sentence may be misleading and we've deleted it from the revised version. The abstract was also modified accordingly.
As suggested, we have now included G1 experiments (Figure S4) that address the effect of *pds5* and *wpl1* deletion on cohesin binding to chromatin in the presence (*mis4+*) and absence of cohesin loading (*mis4-367* 37°C) to assess the steady state amount of chromatin-bound cohesin and the kinetics of cohesin release. Pds5 again shows a dual behaviour: its deletion increased the steady state amount and Rad21 half-life of chromatin, but not to the same extent as *wpl1Δ*, and *pds5Δ* is epistatic to *wpl1Δ*. We conclude that Pds5 and Wpl1 act together to stimulate cohesin removal and the loss of Pds5 slightly alters the stability of cohesin on chromatin.
This experiment also shows that the deletion of *wpl1* or *pds5* does not abolish cohesin release. This was previously observed for the labile cohesin population in G2 cells (in *S. pombe* and mammals) and also in G1 cells in a previous report (EMBO 2008). Therefore, the sole neutralization of the anti-establishment (*wpl1Δ*) does not suffice to generate a fully stable cohesin interaction with chromosomes. Hence, we suggest that the stable cohesin fraction observed in G2 cells requires the neutralization of the anti-establishment, the Pds5 protein but also passage through S-phase (see new paragraph at the end of the result-discussion section, page 9).

Referee #2:

1. In the Introduction, "Strains deleted for pds5 are viable showing that Pds5 is not required for the establishment of sister chromatid cohesion" is confusing when two sentences later, the authors claim the opposite, i.e., that "Pds5 is the cornerstone that couples replication with sister chromatid cohesion establishment and maintenance". Thus, It would be better to say "suggesting" instead of "showing".

Pds5 is indeed dispensable for cohesion establishment as previously shown by Tanaka and colleagues. Cells deleted for *pds5* are viable and do not show premature separation of sister-chromatids (cen2-GFP dots) in actively cycling cells (*cdc25-22 pds5Δ* cells before the shift to 37°C, Figure 3C of Tanaka et al. EMBO 2001). The sentence was modified to make this point clearer (page 4): "Strains deleted for *pds5* are viable and proficient for sister-chromatid cohesion in actively dividing cycling cells, showing that Pds5 is dispensable for the establishment of sister-chromatid

© European Molecular Biology Organization

cohesion [12, 18]. However, cohesion is progressively eroded during the G2 phase and is eventually lost when the duration of G2 is extended [12]."
We agree that the two sentences seemed to contradict each other. The second sentence was replaced by: "We show that Pds5 authorizes cohesion establishment by allowing Eso1-mediated neutralization of the anti-establishment. Pds5 is therefore required both for the anti-establishment activity and to bring about the mechanism that counteracts it." (page 4)

2. The concept of cohesion erosion is very cryptic, and I think it merits some explanation since is a critical aspect of the Pds5 mutant phenotype in fission yeast. Since this is a G2 arrest, there is not, in principle, dissociation of cohesin (like the prophase pathway in vertebrates). In addition to cohesion mediated by cohesin, DNA catenation contributes to cohesion (even in yeast). One possible explanation for "cohesion erosion" is that during G2 arrest, more time is given for topoisomerase to perform decatenation. (This interpretation, of course, may not be shared by the authors!)

In *S. pombe* and mammals there are two sub-populations of cohesin in G2 cells. One binds chromosome in a dynamic fashion (as in G1) while the other (~20% of total bound-cohesin) binds chromosomes in a very stable manner (no release can be observed during the course of the experiments, that is for up to 5 hours, see Feytout et al. 2011 and Gerlich et al. 2006 for mammalian cells). In the absence of *pds5*, we show that the whole population of cohesin binds G2 chromatin in the labile mode, ie the stable cohesin sub-population is not there. Pds5 is therefore required for the formation of the stable cohesin sub-population. As mentioned earlier, Pds5 is dispensable for cohesion establishment but essential for its maintenance. We therefore propose that cohesion is made in *pds5Δ* cells but these cohesive cohesin complexes are not stable enough on chromatin to ensure long term cohesion. Hence, cohesion is lost when this cohesive pool is exhausted. A sentence was added page 7 as to make this point clearer.
We agree that DNA intertwining may contribute to cohesion. A recent paper from the Nasmyth lab shows that cohesin by itself (without DNA intertwining) is sufficient for sister-chromatid cohesion but DNA intertwining can persist after S phase in a cohesin-dependent manner. Hence cohesin mediates cohesion directly but also indirectly by allowing the persistence of DNA intertwining (Farcas, Mol. Cell. 2011). In *pds5Δ*, cohesin interaction with G2 chromosomes is lost with time. The most straightforward explanation is that cohesion is lost because cohesin-mediated cohesion is lost and presumably, DNA intertwining is lost as well as it is dependent on cohesin.

3. **The authors equal the "stably bound" cohesin fraction with the cohesin actually engaging two sister chromatids, i.e., performing cohesion. Maybe this idea could be further supported by showing some GFP-dot assay, or at least include a few sentences describing their previous results in this regard (Feytout 2011). A recent paper by Guacci et al (MBC) shows that, in budding yeast, double depletion of eco1 and wpl1, rescues the lethality of eco1 mutants but that very little cohesion is actually established. This is a result distinct from the result presented in Feytout 2011 and in Figure 5A, in which there is stably-bound cohesin in the absence of eso1-wpl1 and the reasons for this difference could be discussed.**

As suggested, we now show (by FISH using 6 cosmid probes on chromosomes 1 and 2) that sister-chromatid cohesion is indeed intact in *mis4-367* cells arrested for 3 hours in G2 by the use of *cdc25-22* (Fig. S1), further supporting the notion that stably bound cohesin is indeed cohesive.
We do not know why cohesion establishment and maintenance are intact in *S. pombe* cells deleted for both *eso1* and *wpl1* whereas this is not the case in budding yeast. The mechanism of cohesion establishment may be different between the two (very) distantly related yeast species. Alternatively, budding yeast Eco1 and Wpl1 may have additional functions which when altered, have indirect effects on sister-chromatid cohesion (for instance, an effect on transcription may alter the expression of genes in the cohesion pathway). I assume new data from budding yeast will shed light on this particular issue. We agree this point deserves discussion but it may be better suited for a review article rather than in a short format research paper.

4. Regarding Figure 2D, it could be completed by showing how Pds5 deficiency affects the amount of Rad21 present at some euchromatic locations. From this figure also, it would be good to comment on the reasons for the more noticeably decrease in Rad21 binding at telomeres.

© European Molecular Biology Organization

As suggested, we have now included 2 euchromatic sites in all ChiP assays.
We do not have any data that would explain why the amount of Rad21 binding at telomeres (and centromeres) is reduced in cdc25-22 *pds5Δ* arrested cells (Fig. 2D). It is an interesting issue but the main point in this study was to point out that *pds5Δ* cells lose cohesion after such a prolonged G2 arrest although there are cohesin complexes bound to chromosomes, suggesting that they do not ensure functional cohesion and indeed, Fig. 3 shows that those cohesin complexes are not stably bound.

5. It would be interesting to try to see if there is any difference between a wpl1 mutant and a double pds5-wpl1 mutant regarding the "kinetics" in the decrease of cohesin binding during G2 arrest in the absence of new loading. Shorter timepoints between 0 and 60 min, when the most important decrease occurs, should be tested. In Figure 8 of Feytout 2011 it seems that the decrease in the absence of wpl1 is slower whereas there is not much difference between pds5 mutants and wildtype (in cdc25 mis4 background). I am trying to compare these results in fission yeast with results in Xenopus showing that in the absence of Pds5 dissociation during prophase is faulty. Is Pds5 required for Wpl1 function in releasing cohesin from chromatin?

The experiment in Figure 4A compares the kinetics of Rad21 removal from chromatin in *pds5Δ* and *pds5Δ wpl1Δ* cells and indeed, they are very similar. This shows that Wpl1 is not responsible for cohesin removal in *pds5Δ*. We agree that the experiment also suggests that Wpl1 may not be able to remove cohesin in the absence of Pds5. It is however difficult to draw conclusions from these G2 experiments since they display 2 different cohesin sub-populations in wild-type and that the stable sub-population is lost in *pds5Δ* but not in *wpl1Δ*.
We therefore looked at G1 cells (Figure S4) to address the effect of *pds5* and *wpl1* deletion on cohesin binding to chromatin in the presence (*mis4+*) and absence of cohesin loading (*mis4-367* 37°C) to estimate the steady state amount of chromatin-bound cohesin (in a *mis4+* background) and the kinetics of cohesin release (upon inactivation of Mis4-367). Indeed, the deletion of *pds5* increased the steady state amount and Rad21 half-life of chromatin, consistent with Pds5 being required for cohesin removal. Still the effect is not to the same extent as in *wpl1Δ*, and *pds5Δ* is epistatic to *wpl1Δ*. We conclude that Pds5 and Wpl1 are both required and act in the same pathway that stimulates cohesin removal but in the mean time loss of Pds5 slightly alters the stability of cohesin on chromatin.

Referee #3:

1.A key question is whether Pds5 makes and/or maintains the stably bound cohesin fraction. When reading the manuscript the impression is that the fraction of stably bound cohesin is the cohesive one, and responsible for cell survival. If this fraction is not made in pds5D; cells it is difficult to understand how they survive during normal cell cycle progression (not held in a prolonged G2 arrest). Thus, their survival seems to argue that the stable fraction is made but not maintained in the pds5D; mutant. Or, is it so that the "unstable" fraction of cohesin can provide enough cohesion to allow survival during normal cell cycle progression? The generation/ maintenance question could be further addressed by experiments where Pds5 is removed in S-phase or G2 only (in combination with different combination of eso1 and wpl1 mutations). This might be too much to ask at this stage, but in any case, the generation/maintenance issue should be more clearly discussed.

As shown earlier by Tanaka and colleagues, Pds5 is dispensable for cohesion establishment. Cells deleted for *pds5* are viable and do not show premature separation of sister-chromatids (cen2-GFP dots) in actively dividing cells (*cdc25-22 pds5Δ* cells before the shift to 37°C, Figure 3C of Tanaka et al. EMBO 2001). However, cohesion is progressively lost during the G2 arrest. We therefore propose that cohesion is made in *pds5Δ* cells but these cohesive cohesin complexes are not stable enough on chromatin to ensure long term cohesion and cohesion is lost when this unstable cohesive pool is exhausted. We added a sentence to make this point clearer (page 7).
We agree it would be interesting to know whether Pds5 is continuously required during G2 for the maintenance of cohesion. We tried to do this experiment by fusing a thermosensitive N-degron to Pds5-GFP but this experimental set up was not satisfactory. When *cdc25-22 Ndeg-Pds5-GFP* cells

© European Molecular Biology Organization

were shifted to 37°C, the amount of total Ndeg-Pds5-GFP was reduced but unfortunately the chromatin-bound fraction remained intact, suggesting that chromatin-bound Pds5 was sheltered from the degradation machinery. Other strategies may be envisaged but at the present time we cannot address this question.

2. As the interplay anti-establishment/establishment/maintenance is rather complex, the manuscript would greatly benefit from a figure where a schematic model of these events is displayed, highlighting the new results.

Considering the short format of EMBO Reports, we cannot afford an additional figure. However, the new results have been discussed in more detail in the last paragraph of the result-discussion section.

3. As centromeres, rDNA and telomeres are specialized chromosomal domains it could be valuable to investigate Rad21 association at a non-repetitive cohesin binding site on a chromosome arm (i.e. one of the CAR sites investigated in a 2011 MBC publication from the same lab (Feytout et al.). This would confirm that the obtained data is valid for most types of cohesin binding regions.

As suggested, we have now included 2 euchromatic sites in all ChiP assays.

4. The reason why the initial levels (before temperature increase) of Rad21 vary in pds5Δ; should be discussed. Why is there more chromosome-bound Rad21 in pds5Δ; than in wild type at the rDNA, less than in wild type at telomeres? (Fig. 2)

We do not have a clear explanation for this. As suggested by the G1 experiment (Fig. S4), Pds5 may act together with Wpl1 to stimulate cohesin removal but in the mean time, Pds5 stabilizes the interaction of cohesin with chromatin. Some unknown locus-specific factors may fine tune the balance between the two, resulting in the observed steady-state amounts of cohesin? We think however that this is out of the scope of the present study and too speculative at this stage to deserve discussion.

5. To be able to state that "cohesin co-localizes with the stable fraction on replicated chromosomes" a more detailed analysis (i.e. chip) than that shown in figure S1 is needed.

As suggested, we have now included a ChiP assay showing that the pattern of Pds5 closely matches that of stably-bound Rad21 (now Figure S2).

2nd Editorial Decision 26 April 2012

Thank you for your patience while your revised version has been under peer-review. It was seen by referees 1 and 2, who support its publication with no further comments. I am thus very pleased to accept your manuscript for publication in the next available issue of EMBO reports.

Thank you for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Editor
EMBO Reports

© European Molecular Biology Organization