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## SUMOylation and PARylation cooperate to recruit and stabilize SLX4 at DNA damage sites

Roman Gonzalez-Prieto, Sabine A.G.Cuijpers, Martijn S. Luijsterburg, Haico van Attikum and Alfred C.O. Vertegaal

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### Review timeline:

|                     |                  |
|---------------------|------------------|
| Submission date:    | 17 December 2014 |
| Editorial Decision: | 12 January 2015  |
| Revision received:  | 03 February 2015 |
| Editorial Decision: | 05 February 2015 |
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### Transaction Report:

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

Editor: Nonia Pariente

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1st Editorial Decision

12 January 2015

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Thank you for your patience during the peer-review of your study at EMBO reports. I have now heard back from the three referees that were asked to assess it. As you will see, all find the topic of interest and referees 1 and 2 are largely supportive of the study, although referee 3 raises a number of issues.

Both referees 1 and 3 are concerned regarding the MMC rescue data, which they find unconvincing and in contrast to the recent studies on the topic. Upon further discussion with the referees, we think this issue should be addressed experimentally before publication by testing whether the deltaSIM mutant is more sensitive to other types of DNA damage. In addition, please provide a quantification of the results of PML nuclear body localization and a more detailed description of the mutants used, as referee 3 requests. Please note that we do not agree with taking the PARylation data out, as/he suggests.

If these issues can be adequately addressed, we would be happy to accept your manuscript for publication. As I mentioned in our earlier correspondence, and given the time sensitivity in this case, we ask that you return your revised version within 3-4 weeks.

From a purely formal point of view, data regarding the type of error bars used seems to be missing from the legend to figure 4E. In addition, the mass spectrometry data needs to be deposited in an appropriate, publicly available database and the accession number provided in the manuscript.

I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, please contact me if I can be of any assistance.

#### REFEREE REPORTS:

##### Referee #1:

This manuscript reports that SLX4 makes multiple non-covalent contacts with SUMO2, leading to SLX4 sumoylation and participating in the recruitment of SLX4 to DNA damage sites (and PML bodies).

Overall, the experiments are well carried out and make an important connection between protein sumoylation and the SLX4 scaffold even though the experiments are largely circumscribed to the localization of SLX4.

The only issue I have with the manuscript is the partial rescue of MMC sensitivity by the SLX4 deltaSIM mutant. The results seem at odds with equivalent analyses described in the recent Gaillard and Zou papers and thus the authors may want to explore if the SIM-less mutant is sensitive to agents other than MMC or at the very least contrast and compare their results with the recent published work.

##### Referee #2:

The authors show that SLX4 has three SIMs that interact with poly-SUMO and that are required for SLX4 SUMOylation. Mutating the SIMs causes a slight defect in ICL repair, and has a marked effect on localization of SLX4 in PML bodies and at DNA damage sites.

The paper is short and succinct but the findings are novel and interesting. The paper is sound technically. Given the other papers on the topic recent, it would seem sensible to publish this paper as soon as possible. Further experiments are not required to validate the conclusions reached by the authors.

##### Referee #3:

SLX4 is a key genome stability factor and tumor suppressor that acts as a scaffold for DNA repair endonucleases such as XPF-ERCC1, MUS81-EME1 and SLX1. Because of this hub function, SLX4 promotes the repair of multiple DNA lesions including interstrand crosslinks (ICLs), and is also required for common fragile site (CFS) cleavage under conditions of DNA replication stress. Therefore, understanding the regulation of SLX4's diverse roles is of major interest to a broad spectrum of life scientists.

In this study, Gonzalez-Prieto et al document the role of SUMOylation and PARylation in the regulation of SLX4 subnuclear localization. They find that SLX4 contains 3 SUMO interacting motifs (SIMs) that are required for both its non-covalent interaction with SUMO-2/3 and its covalent modification by SUMO. Deletion of its SIMs (SLX4  $\Delta$ SIM) reduces SLX4 recruitment to and stabilization at laser induced DNA damage stripes. In addition, the presence of SLX4 at PML nuclear bodies appears to be abolished by SLX4  $\Delta$ SIM (although no quantification is shown). These localization data agree with 2 recently published Mol. Cell papers from the Zou and Gaillard labs.

PARylation is also suggested to modulate SLX4 dynamics at DNA lesions. A direct role in SLX4 recruitment i.e. the presence of a PAR-binding domain is not determined, but an SLX4-PARP1 (PARylation enzyme) interaction is detected. Again, whether this is a direct interaction is not tested,

but PARylation appears to support SLX4 recruitment to DNA lesions (laser-induced) in parallel to SUMO binding. However, as stated by the Authors, these data are uncertain due to the mixed nature of DNA lesions induced by lasers e.g. single strand DNA "nicks" and DNA double strand breaks. Whilst potentially interesting, this aspect of the paper is not well integrated and adds little at this stage, as it is underdeveloped. As indicated below, this section could be removed and replaced with data that better support an important role for the SLX4-SUMO interaction in DNA repair.

In an attempt to find a functional output for SUMO-dependent recruitment of SLX4 to DNA lesions, the Authors tested the effect of SLX4  $\Delta$ SIM on cellular resistance to the DNA crosslinking agent MMC. They suggest that there is a minor defect in MMC resistance in cells expressing SLX4  $\Delta$ SIM versus wild-type. This result is not very convincing and contrasts with the findings of the above-mentioned Mol. Cell papers. In these papers, convincing effects of the SLX4-SUMO interaction on the repair of topoisomerase I-dependent lesions (camptothecin resistance) and CFS instability were revealed, but no significant defect in ICL repair was detected. The reason for this difference is not clear, but in the current manuscript, the Authors say they deleted the SIM sequences. No other information was given in the paper, so perhaps they did not make the standard hydrophobic to alanine substitutions in the SIM sequence, and instead deleted whole sections of protein that could adversely affect SLX4 function beyond SUMO interaction. This should be described in the Materials and Methods/figure legend if deletions were not made, or repeated with more conservative substitutions if they were.

Overall, the paper convincingly defines a novel role (Mol. Cell papers aside) for an SLX4-SUMO interaction in governing the subnuclear localization of SLX4 e.g. at DNA breaks or PML nuclear bodies. However, what is lacking is functional data that clearly demonstrates the importance of the SLX4-SUMO interaction in DNA repair. Therefore, in my opinion, as a self-standing study it currently falls short of an advance significant enough to warrant publication in EMBO Reports.

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1st Revision - authors' response

03 February 2015

Gonzalez-Prieto et al. Reply to referee reports.

Referee #1:

*This manuscript reports that SLX4 makes multiple non-covalent contacts with SUMO2, leading to SLX4 sumoylation and participating in the recruitment of SLX4 to DNA damage sites (and PML bodies).*

*Overall, the experiments are well carried out and make an important connection between protein sumoylation and the SLX4 scaffold even though the experiments are largely circumscribed to the localization of SLX4.*

In reply: We are pleased with the support of the referee for our project.

*The only issue I have with the manuscript is the partial rescue of MMC sensitivity by the SLX4  $\Delta$ deltaSIM mutant. The results seem at odds with equivalent analyses described in the recent Gaillard and Zou papers and thus the authors may want to explore if the SIM-less mutant is sensitive to agents other than MMC or at the very least contrast and compare their results with the recent published work.*

In reply: in contrast to the Gaillard and Zou papers, we observe that cells expressing the SLX4 SIM-less mutant are more sensitive to MMC compared to cells expressing wild-type SLX4. We have carefully checked the expression levels of wild-type SLX4 and the SIM-less mutant in our experiments and found that they are expressed at equal levels. Moreover, we have carried out this experiment four times and found this result to be highly reproducible. In addition to MMC, we have now also tested for camptothecin sensitivity of these cells since it was published that SLX4  $-/-$  MEFs are sensitive to MMC and camptothecin, but not to UV or hydroxyurea (Castor et al. 2013 Molecular Cell Figure. S3.). In agreement with the published data, we confirmed the sensitivity of SLX4  $-/-$  MEFs for camptothecin, but we found that wild-type SLX4 and the SIM-less mutant rescued equally efficiently. Due to time constraints, we have performed this experiment only twice in contrast to the MMC sensitivity experiments and therefore have added the camptothecin sensitivity assay to the expanded view part of our manuscript instead of in the main manuscript. We would like to point out a few differences between our experiments and the experiments published in the Gaillard and Zou papers that could potentially explain the observed differences:

- 1-Unequal expression levels for SLX4 w.t. and SIM-mutant in both other studies. In the case of the Gaillard group, the expression levels between SLX4 w.t. and SIM-mutant are estimated to be two-fold different (Guervilly et al. Figure 5B). In the case of the Zou group, the expression levels between W.T. and SIM-mutant are estimated to be at least three-fold different (Ouyang. et al. Figure S3). Obviously, if the expression levels of the SLX4 SIM-mutant are already lower compared to SLX4 w.t., the rescue by the SLX4 SIM-mutant might also be lower compared to SLX4 w.t. as observed for camptothecin. In our case, we have carefully analyzed the expression levels of SLX4-w.t. and SIM-mutant for all experiments and found them to be virtually equal (Figure 2D).
- 2-A variation in MMC survival rates for the positive control groups in the case of the Gaillard group estimated to be in between 65% and 45% (Guervilly et al. Figure 5C). This might be the reason why all the different mutants are not shown together in one graph, but instead are each shown in a separate panel. Since these differences in the control group are already significant, it is unclear whether the differences in survival observed for the different mutants is meaningful.
- 3-Differences in assay set up. The camptothecin experiments carried out by the Zou group are different from our experiments. The camptothecin doses used by the Zou group are high, but treatment time is relatively short (4 hours). The lowest survival rates observed in response to camptothecin is still around 20% (Ouyang. et al. Figure 3B), in contrast to our survival assays, where the lowest survival rates are about 1%.
- 4-We are using a log-scale, which is standard to do for survival assays and which for the eye might underestimate differences. Nevertheless, the difference that we observe between w.t. SLX4 and the SIM-mutant is about 50%. In contrast, the difference in the survival observed between w.t. SLX4 and the SIM-mutant in the camptothecin experiment performed by the Zou group is at the highest dose in the assay (3 $\mu$ M) only about 5%.
- 5-We are using wild-type MEFs as a positive control for the rescue of the SLX4  $-/-$  MEFs by w.t. SLX4. In both other studies, such a proper positive control is missing and therefore, it is unclear

whether the rescues obtained by w.t. SLX4 are as efficient as the rescue that we have observed for w.t. SLX4.

6-Both other groups are focusing on human SLX4. In contrast, we are working with mouse SLX4. Differences observed could be due to the differences between mouse and human SLX4. Some differences between mouse and human SLX4 have already been observed in the past, whereas human SLX4 partially localizes at telomeres, mouse SLX4 was not found at telomeres (Wilson et al. 2013 Cell Reports 4:853-860).

7-For our survival assays, we prefer the system developed by Prof. J. Rouse (Dundee, U.K.) which is based on a specific deletion of the SLX4-gene only, compared to knockdowns that are not 100% efficient.

Referee #2:

*The authors show that SLX4 has three SIMs that interact with poly-SUMO and that are required for SLX4 SUMOylation. Mutating the SIMs causes a slight defect in ICL repair, and has a marked effect on localization of SLX4 in PML bodies and at DNA damage sites.*

*The paper is short and succinct but the findings are novel and interesting. The paper is sound technically. Given the other papers on the topic recent, it would seem sensible to publish this paper as soon as possible. Further experiments are not required to validate the conclusions reached by the authors.*

In reply: We are pleased with the support of the referee for our project.

Referee #3:

*SLX4 is a key genome stability factor and tumor suppressor that acts as a scaffold for DNA repair endonucleases such as XPF-ERCC1, MUS81-EME1 and SLX1. Because of this hub function, SLX4 promotes the repair of multiple DNA lesions including interstrand crosslinks (ICLs), and is also required for common fragile site (CFS) cleavage under conditions of DNA replication stress. Therefore, understanding the regulation of SLX4's diverse roles is of major interest to a broad spectrum of life scientists.*

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In reply: the editor has informed us that the section about PARylation should not be removed from the manuscript.

*In an attempt to find a functional output for SUMO-dependent recruitment of SLX4 to DNA lesions, the Authors tested the effect of SLX4  $\Delta$ SIM on cellular resistance to the DNA crosslinking agent MMC. They suggest that there is a minor defect in MMC resistance in cells expressing SLX4  $\Delta$ SIM versus wild-type. This result is not very convincing and contrasts with the findings of the above-mentioned Mol. Cell papers. In these papers, convincing effects of the SLX4-SUMO interaction on the repair of topoisomerase I-dependent lesions (camptothecin resistance) and CFS instability were revealed, but no significant defect in ICL repair was detected. The reason for this difference is not clear.*

In reply: See referee #1. In contrast to the Gaillard and Zou papers, we observe that cells expressing the SLX4 SIM-less mutant are more sensitive to MMC compared to cells expressing wild-type SLX4. We have carefully checked the expression levels of wild-type SLX4 and the SIM-less mutant in our experiments and found that they are expressed at equal levels. Moreover, we have carried out this experiment four times and found this result to be highly reproducible. In addition to MMC, we have now also tested for camptothecin sensitivity of these cells since it was published that SLX4  $-/-$  MEFs are sensitive to MMC and camptothecin, but not to UV or hydroxyurea (Castor et al. 2013 Molecular Cell Figure. S3.). In agreement with the published data, we confirmed the sensitivity of SLX4  $-/-$  MEFs for camptothecin, but we found that wild-type SLX4 and the SIM-less mutant rescued equally efficiently. Due to time constraints, we have performed this experiment only twice in contrast to the MMC sensitivity experiments and therefore have added the camptothecin sensitivity assay to the expanded view part of our manuscript instead of in the main manuscript. We would like to point out a few differences between our experiments and the experiments published in the Gaillard and Zou papers that could potentially explain the observed differences as detailed also for reviewer 1:

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*Methods/figure legend if deletions were not made, or repeated with more conservative substitutions if they were.*

In reply: SIMs in SLX4 were mutated by replacing large hydrophobic residues for alanines by PCR mediated mutagenesis in line with common practice in the field. This was mentioned in the expanded view part of our manuscript, but appears to have been missed by the referee. Therefore, these important details have now been included in the main body of the manuscript too.

*Overall, the paper convincingly defines a novel role (Mol. Cell papers aside) for an SLX4-SUMO interaction in governing the subnuclear localization of SLX4 e.g. at DNA breaks or PML nuclear bodies. However, what is lacking is functional data that clearly demonstrates the importance of the SLX4-SUMO interaction in DNA repair. Therefore, in my opinion, as a self-standing study it currently falls short of an advance significant enough to warrant publication in EMBO Reports.*

In reply: See our previous comments on the MMC - and camptothecin sensitivity experiments.

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2nd Editorial Decision

05 February 2015

Thank you for the submission of your revised study. I have now carefully gone through your file once more and discussed it with an editorial advisor, to avoid unnecessary loss of time. I am happy to say that we are both positive about the publication of your study in EMBO reports. I am therefore writing with an 'accept in principle' decision, which means that we will accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

- We strongly feel that the results of the camptothecin experiment should be included in figure 2. I understand that this experiment was performed only twice and must therefore ask you to remove the error bars and plot both data measurements for each point.

- Some discussion of the discrepancies between your results and those of the Gaillard and Zou labs needs to be included in the main text, along the lines of what you discuss for the referees, although less detailed. We very much support the log-scale reporting of the results, which is the gold standard. Many readers will have the same issues as referees 1 and 3 did, and therefore it is important to address these discrepancies in the text.

After all remaining corrections have been attended to, you will receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

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2nd Revision - authors' response

06 February 2015

We are pleased to read your decision and would like to thank you for handling our manuscript very efficiently.

We have uploaded the final files according to your instructions.

3rd Editorial Decision

09 February 2015

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I am 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.