The Siz1-dependent sumoylation of PCNA down-regulates spontaneous translocations

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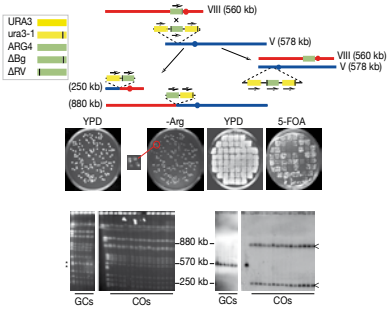
Summary

Unrepaired DNA damage is a threat to cell survival, particularly during DNA replication. To cope with replicative damage, the cells have evolved a mechanism capable of setting up a selective recruitment of different sets of proteins by triggering distinct post translational modifications of the processivity factor PCNA. For instance, the RAD6/RAD18 dependent ubiquitination of PCNA lysine residue 164 (K164) triggers the recruitment of mutagenic translesion synthesis polymerases, while the further polyubiquitination depending on MMS2/UBC13/RAD5 promotes a more error-free template switch repair. The same lysine residue can also become sumoylated by the E3 ligase Siz1, a modification that stimulates the recruitment of the Srs2 helicase to prevent the formation of recombination prone Rad51-covered DNA single-strands.

We have previously established that absence of either the Srs2 helicase or the replicative checkpoint protein Mrc1 dramatically elevate the spontaneous rates of crossover-associated events. In addition, a genetic background in which PCNA cannot be posttranslationally modified on lysine residues 127 and 164 also yields elevated levels of translocations.

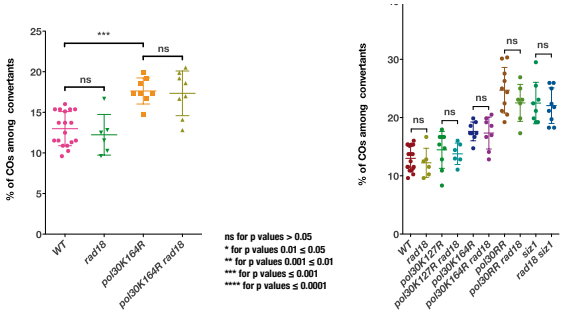
In the present study, we have investigated the post-translational modifications of PCNA required for spontaneous crossover control in a yeast haploid cell and found that, unlike ubiquitination, sumoylation of PCNA is required for crossover control. Interestingly, both lysine residues K127 and K164 must be mutated to observe crossover levels of a siz1Δ mutant indicating that both residues are indeed a substrate for the E3 ligase and can compensate for one another. Unexpectedly, although the absence of RAD18 has no incidence on spontaneous crossovers, we found that RAD5 and MMS2 are involved in crossover control in the absence of PCNA sumoylation.

Assay to measure spontaneous CO events

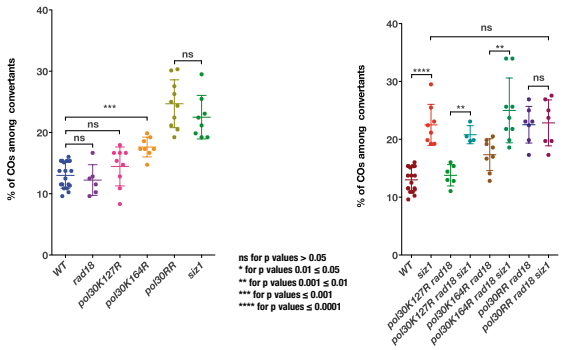


Robert, T., Dervins, D., Fabre, F. & Gangloff, S. (2006) EMBO J. 25, 2837-2846.

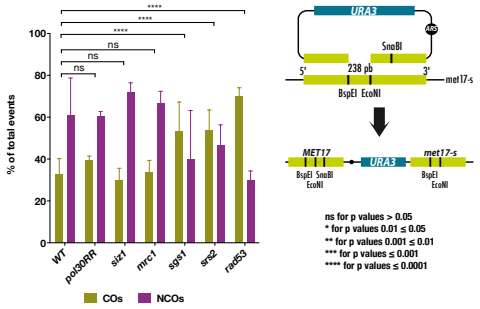
Ubiquitination does not affect genome stability



PCNA sumoylation affects genome stability



COs following a DSB do not depend on Mrc1, Siz1 or PCNA posttranslational modifications



Bartsch, S., Kang, L. E., and Symington, L. S. (2000). Mol. Cell. Biol. 20, 1194-1205.

Conclusions

- The Siz1-dependent sumoylation of PCNA is required to down-regulate spontaneous COs.
- Siz1-dependent sumoylation of K127 can complement the absence of a K164 residue that cannot be sumoylated.
- PCNA ubiquitination has no effect on unchallenged genome stability.
- The crossover levels observed in Srs2R1 mutants lacking the SIM domain correspond to the fraction triggered by the interaction with sumoylated PCNA, indicating that unchallenged cells use the Siz1-dependent sumoylation of PCNA to regulate genome rearrangements by concentrating Srs2 at the replication fork.
- The absence of proteins traveling with the replication fork (PCNA, Mrc1) does not noticeably bias the resolution of a DSB-induced recombination intermediate, whereas it greatly affects the resolution of spontaneous events. The same is true for the E3 ligase Siz1 responsible for the sumoylation of PCNA. This suggests that PCNA sumoylation reduces the production of COs during DNA replication. Conversely, checkpoint proteins and the Srs2 and Sgs1 helicases prevent CO formation independently of the initiating event and the position in the cell cycle.

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