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## Replication independent repair of DNA interstrand crosslinks mediated by XPF/SNM1A

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Interstrand crosslink (ICL) repair has been studied most extensively in the context of DNA replication. Yet crosslinks represent a barrier, both to replication and transcription, most cells in the body are not in cycle, and the genome is exposed to reactive agents independent of cell cycle phase. Classic models of replication independent ICL repair in *E. coli* propose dual incisions on one strand either side of the ICL, a process known as unhooking, and conceptually similar to the repair of single strand adducts by the Nucleotide Excision Repair (NER) system. Despite frequent reference, there has been no evidence for this pathway in mammalian cells. More recent models, based on *Xenopus* egg extracts and biochemistry data, involve a single incision followed by exonucleolytic degradation of one of the strands past the ICL and removal of the crosslink remnant by NER. We have designed an assay to follow replication independent ICL repair in G1 phase cells. We detect ssDNA generated as a repair intermediate in response to psoralen/UVA induced ICLs in nuclei of live cells. Kinetics analysis indicated it takes an hour for 50% of the cells to generate ssDNA at the laser localized ICLs. We found that XPC (NER helix-distortion-sensor) and the NER/Fanconi Anemia endonuclease XPF were required for efficient ssDNA display at the ICL stripe. In contrast, XPG was dispensable, arguing against an NER type double incision model for initial processing of the psoralen/UVA induced crosslink. The SNM1A exonuclease proved essential for generation of ssDNA at the targeted site. An adapted Proximity Ligation Assay (PLA) allowed us to show that SNM1A recruits in close proximity (30 nm) to the antigen-tagged ICL. Most importantly, by monitoring the persistence of an antigen-tagged ICL stripe in live cells, we showed that SNM1A is required for efficient removal of the crosslinks in G1 cells. This research was supported by the Intramural Research Program of the NIH, National Institute on Aging (Z01 AG000746-08) and the Fanconi Anemia Research Fund.

### Biography

Dr Bellani MA got her PhD in bacterial genetics from the University of Buenos Aires and conducted postdoctoral research on the role of SPO11 in meiotic recombination in mice at the NIDDK, NIH in Bethesda. She joined the NIA, NIH in Baltimore in 2010. The lab studies the cellular response to DNA damage and replicative stress. It has examined the dynamics of the replication apparatus in encounters with blocks to fork progression, developing a new technology for this inquiry. It discovered a novel and unexpected pathway, which proved to be the principle way through which replication forks respond to the severe impediment presented by ICLs, identifying some of the proteins required for the operation of the new scheme. It has also characterized the DDR to helix distorting DNA damage in the absence of replication and unveiled a pathway for replication independent repair of ICLs dependent on XPF and SNM1A.

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