DNA Interstrand Crosslinks Repair in Mammalian Cells

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We studied the formation of double strand breaks (DSBs) as intermediates in the repair of DNA interstrand crosslinks (ICLs) by homologous recombination (HR). The plasmid EGFP-N1 was crosslinked with trioxsalen to give one ICL per plasmid on average. HeLa cells were transfected with the crosslinked plasmids and the ICL repair was monitored by following the restoration of the GFP expression. It was accompanied by γ-H2AX foci formation suggesting that DSBs were formed during the process. However, the same amount of γ-H2AX foci was observed when cells were transfected with native plasmid, which indicated that γ-H2AX foci appearance could not be used to determine the amount of DSBs connected with the ICL repair in this system. For this reason we further monitored the DSB formation by determining the amount of linearized plasmids, since having one crosslink per plasmid on average, any ICL-driven DSB formation would lead to plasmid linearization. Native and crosslinked plasmids were incubated in repair-competent cell-free extracts from G1 and S phase HeLa cells. Although a considerable part of the ICLs was repaired, no linearization of the plasmids was observed in the extracts, which was interpreted that DSBs were not formed as intermediates in the process of ICL repair. In another set of experiments HR-proficient HeLa and HR-deficient irs3 cells were transfected with native and crosslinked plasmids, and 6 h and 12 h later the plasmid DNA was isolated and analyzed by electrophoresis. The same amount of linear plasmid molecules was observed in both cell lines, regardless of whether they were transfected with native or crosslinked pEGFP-N1, which further confirmed that DSB formation was not an obligatory step in the process of ICL repair by HR.

Key words: DNA Interstrand Crosslinks, Homologous Recombination, DNA Repair