

Processing of DNA Interstrand Cross-links by XPF-ERCC1 is Required for the Efficient Localization of FANCD2 to Chromatin

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Objective: A number of laboratories, including our own, have previously demonstrated that the XPF-ERCC1 structure-specific nuclease plays a critical role in the repair of DNA interstrand cross-links (ICLs). Interestingly, other factors essential for the mammalian nucleotide excision repair (NER) pathway are much less important during the processing of ICLs. Cellular and biochemical analysis suggests that XPF-ERCC1 might be required for the initial incisions that flank the ICLs, releasing the cross-linked strands and permitting downstream processing by translesion synthesis and/or recombination. Although both XPF-ERCC1-deficient cells and cells defective in Fanconi anemia proteins show hypersensitivity to interstrand cross-linking agents, it is not known if these proteins participate in the same repair mechanism(s).

Results and Conclusions: Here, we determined the kinetics of FANCD2 monoubiquitination and its chromatin recruitment as markers of activation of the Fanconi anemia pathway in cells lacking XPF, ERCC1 or other NER factors (including XPG and XPA). Monoubiquitination of FANCD2 (to FANCD2-L) was readily detected in whole cell extracts from all cell lines following treatment with the cross-linking drugs nitrogen mustard (HN2) and mitomycin C (MMC). FANCD2 monoubiquitination persisted longer in XPF-ERCC1-deficient cells than wild type cells. However, the levels of FANCD2-L associated with chromatin after crosslink damage were significantly reduced in XPF-ERCC1 mutated cells compared to both wild-type and XPA or XPG deficient cells. In contrast, when chromatin-bound FANCD2 was analysed following 10 Gy of ionising radiation, the levels of FANCD2-L in wild-type and XPF-ERCC1 cells were similar. Based on these data we conclude that XPF-ERCC1 is not required for initial activation of the Fanconi anemia pathway, but it is essential for the efficient recruitment and/or retention of monoubiquitinated FANCD2 to chromatin.

Translational Applicability: Fanconi anemia patients are defective in ICL repair, as are the XPF and ERCC1 subgroup of *Xeroderma pigmentosum* individuals. Our evidence that the XPF-ERCC1 must be active for the efficient chromatin recruitment of FANCD2 has significant consequences for our understanding of the early events required to engage the FA pathway. Patients suffering from FA and XP could both ultimately benefit from this knowledge. The discovery that XPF-ERCC1 is required for full engagement in ICL repair by the Fanconi pathway reveals a possibility that mutations in Xpf or Ercc1 could account for some FA patients that do not fit into classical complementation groups.