Regulation of Translesion Synthesis by the Fanconi Anemia Core Complex

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Objective: Fanconi anemia (FA) is a rare autosomal and X-linked disorder, characterized by cancer susceptibility, bone marrow failure and multiple congenital abnormalities. FA is caused by recessive mutations in genes encoding proteins that function coordinately in a DNA repair pathway involved in replication-dependent removal of interstrand crosslinks (ICLs) and other genotoxic stresses. Most of the FA proteins described to date form a nuclear complex (known as the core complex) with ubiquitin ligase activity. Activation of the pathway results in the modification with a single ubiquitin moiety of FANCD2 and of the newly-identified FANCI proteins by the core complex. Ubiquitinated FANCD2-I heterodimers form DNA repair foci on chromatin, where they co-localize with proteins involved in homologous recombination (HR) repair. Besides HR, the FA pathway appears to also exploit other repair mechanism, in order to bypass ICLs. Thus, it was reported that FA cells show reduced levels of mutagenesis. This suggests that translesion synthesis (TLS), a damage-tolerance mechanism that employs specialized, mutagenic DNA polymerases to replicate through DNA lesions that would normally block the progression of high fidelity replicative polymerases, is involved in FA-dependent DNA repair. We aim to identify the missing link between FA and TLS, in order to unravel how FA activates mutagenic DNA repair.

Methods: Using the SupF mutagenesis assay, that allows for accurate quantification of mutation efficiency, we were able to genetically dissect the FA pathway for its role in mutagenesis. Moreover, fluorescence microscopy experiments were employed to study the requirement of FA proteins for the correct localization of TLS polymerases.

Results: We have demonstrated that cells deficient in the FA core complex display a reduced frequency of mutations. Interestingly, this is not the case of FANCD2 or FANCI-depleted cells, suggesting that the core complex controls mutagenesis independent of FANCD2 and FANCI ubiquitylation. We also identified Rev1, a deoxycytidil transferase frequently used for TLS, as a protein essentially required for repair of interstrand crosslinks, as cells treated with siRNA targeting Rev1 displayed hypersensitivity to mytomicin C. Importantly, using a GFP-tagged variant of Rev1, we showed that its localization to DNA damage-induced chromatin foci requires an intact FA core complex, arguing that this complex is involved in recruiting TLS components to their sites of action. Biochemical experiments and siRNA-based screens are underway to elucidate the molecular mechanisms involved and identify factors regulating them.

Conclusions and Translational Applicability: Our studies suggest that the FA pathway modulates mutagenesis by directly controlling the localization of TLS polymerases. Understanding how FA cooperates with TLS is expected to reveal important insights into the cellular mechanisms employed for maintenance of genomic integrity, and how their deregulation leads to cellular transformation and other FA-associated pathologies, thus opening the way for identification of novel therapeutic approaches.