DNA Damage Tolerance in Mammalian Cells

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ABSTRACT

DNA is susceptible to both exogenous and endogenous damaging agents. Damage is constantly reversed by a wide range of DNA repair pathways. Lesions which escape such repair may cause nucleotide mis-pairing and stalled replication, resulting in mutagenesis and cell death, respectively if left unresolved. Stalled replication is particularly dangerous because replication fork collapse can lead to double-strand breaks (DSBs) and chromosome rearrangement, a hallmark of cancer. DNA damage tolerance (DDT) is defined as a mechanism that allows DNA synthesis to occur in the presence of replication-blocking lesions.

DDT, also known as post-replication repair (PRR) in yeast, has been well characterized in the lower eukaryotic model *Saccharomyces cerevisiae* to consist of error-free and error-prone (mutagenic) pathways. Mono-ubiquitination of proliferating cell nuclear antigen (PCNA) by the Rad6-Rad18 complex promotes mutagenesis by recruiting low fidelity translesion synthesis (TLS) polymerases, while continual Lys63-linked poly-ubiquitination of PCNA by the Mms2-Ubc13-Rad5 complex promotes error-free lesion bypass. Since most of the genes involved in DNA metabolism are conserved within eukaryotes, from yeast to human, I tested the hypothesis that mammalian cells also possess two-pathway DDT in response to DNA damage. Namely, the error-free pathway is dependent on the Ubc13-Mms2 complex, while the error-prone pathway utilizes the TLS polymerases, such as Rev3.

By utilizing cultured mammalian cells and producing antibodies against human Ubc13, Mms2 and Rev3, I was able to show that all three proteins associate with PCNA in S-phase cells, and that this association is enhanced following DNA damage. Ubc13-Mms2 association with PCNA was enhanced in response to DSBs. Furthermore, suppression of Ubc13 or Mms2 using interfering RNA technology resulted in increased spontaneous DSBs. In response to UV exposure, Rev3 co-localized with PCNA and two other TLS polymerases, Rev1 and Pol-η, at the damage site. UV-induced Rev3 nuclear focus formation was dependent on Rev1 but

independent of Pol-η. Surprisingly, over-expression of Pol-η was sufficient to induce spontaneous Rev3 nuclear foci. It was further demonstrated that Rev1 and Pol-η were independently recruited to the damage site and did not require Rev3. These observations support and extend the polymerase switch model which regulates the activity of the replicative and TLS polymerases. Finally, simultaneous suppression of Rev3 along with Ubc13 or Mms2 resulted in a synergistic sensitivity to UV, whereas simultaneous suppression of Ubc13 and Pol-η resulted in an additive effect. These results are consistent with those in yeast cells, implying a comparable mammalian two-pathway DDT model.

Additional interesting observations were made. Firstly, Ubc13 interacts with Uev1A, a close homolog of Mms2, which is involved in the NF-κB signaling pathway independent of DNA damage. Secondly, Rev3 appears to be excluded from the nucleus in a fraction of low passage normal non-S-phase cells, whereas in tumor derived cell lines, Rev3 is consistently enriched in the nucleus independent of cell cycle stage. Finally, Rev3 is elevated during mitosis and associates with condensed chromosomes, suggesting a possible novel role in mitosis. Consistent with this notion, chronic ablation of Rev3 resulted in cell death with inappropriate chromosome segregations. The above preliminary observations require further investigation.