Nuclear dynamics of mammalian DNA damage tolerance.

Parker L. Andersen, Fang Xu, Wei Xiao.

DNA damage tolerance is defined as a cellular process that balances cell survival and mutagenesis in the presence of replication blocking lesions, and plays a critical role in genomic instability. In budding yeast, this balance is maintained by sequential ubiquitination of PCNA that coordinates a mutagenic translesion synthesis (TLS) pathway mediated by Rev1 and polζ and an error-free lesion bypass pathway mediated by the ubiquitination complex Rad5-Ubc13-Mms2. In response to UV treatment, mammalian Y-family polymerases Polη and Rev1 co-localize with PCNA at stalled replication foci. However, previous conclusions are controversial and based on transfected cells, which often accumulate nuclear foci in the absence of DNA damage. Furthermore, there is no report on the in vivo characterization of the Rev3 catalytic subunit of the B-family TLS polymerase Polζ. We examined endogenous human Polη, Rev1 and Rev3 by immunocytochemistry using existing or newly created antibodies, as well as various means of inhibiting their expression, which allows us to determine the sequential assembly of TLS polymerases at the damaged site. We found that Rev1 and Polη are independently recruited to stalled replication foci, while Rev3 nuclear focus formation requires Rev1 but not Polη. In contrast, neither Rev1 nor Polη requires Rev3. These observations support and extend the current polymerase switch model. We also examined the assembly of mammalian Ubc13 and Mms2 to the damage site. Furthermore, we demonstrate that simultaneous suppression of Rev3 and Ubc13 results in a synergistic UV sensititvity, providing evidence for the mammalian two-branch DNA damage model.