Uncovering PrimPol's roles in the maintenance of DNA replication & genome stability in vertebrate cells

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DNA damage and secondary structures act as potent obstacles to the cell's replication machinery and can induce replication stress (RS) in cells. Persistent RS leads to genomic instability and therefore numerous tolerance mechanisms exist to complete replication in the presence of such impediments. Eukaryotic cells contain a replicative enzyme called **Prim**ase-**Pol**ymerase (PrimPol) that is capable of repriming replication restart downstream of lesions / secondary structures. In this seminar, I will describe recent advances in our understanding of the 3Rs (roles, regulation and recruitment) of PrimPol during DNA replication processes in vertebrate cells and discuss potential links with human diseases, such as cancer.

To elucidate the cellular requirements for PrimPol, we generated PrimPol-deleted vertebrate cell lines (human & avian) and showed that it plays key roles in maintaining efficient replication, in both the nucleus and mitochondrion, even in the absence of exogenous damage. PrimPol-deficient cells are sensitive to genotoxins and exhibit delayed recovery after UV damage and increased mutation frequencies, micronuclei and sister chromatin exchanges, hallmarks of persistent RS. PrimPol is also required during mitochondrial replication, with null cells having increased mtDNA copy number but displaying a significant decrease in replication. Deletion of PrimPol in *XPV* (xeroderma pigmentosum variant) cells, lacking polymerase Eta that bypasses UV photo-lesions, causes an increase in damage sensitivity and pronounced fork stalling after UV treatment. We report that PrimPol is also important for allowing active replication to proceed in unperturbed cells, thus acting to prevent the accumulation of excessive fork stalling, genetic mutations and replication stress, even in the absence of exogenous damage.

We show that PrimPol's primase activity is requisite to restore wild-type replication fork rates in PrimPol$^{-/-}$ cells treated with genotoxins or encountering structures, establishing that repriming is a critical mechanism for replication restart in vertebrate cells. This capacity to reprime replication suggests that its deployment may be strictly regulated to prevent aberrant genome duplication. We demonstrate that PrimPol is recruited to stalled forks by the RPA complex to facilitate replication restart and identify that its RPA-binding motifs are critical for its recruitment to stalled forks *in vivo*. Together, these findings establish that PrimPol plays key roles in maintaining genome stability in vertebrate cells.