ABSTRACT

Cells in our body are challenged every day by DNA damage arising as a result of both endogenous and exogenous insults. The ability of cells to repair DNA lesions is essential for correct propagation of genetic information. The most cytotoxic DNA lesion is DNA double-strand break (DSB) while oxidative DNA damage is one of the most frequent lesions. The aim of this thesis was to improve current the knowledge of the molecular mechanisms underlying the repair of DSBs and oxidative DNA damage.

The major source of oxidative damage in cells are reactive oxygen species that are constantly generated as by-products of cell metabolism. One of the most frequent lesions is 7,8-dihydro-8-oxo-guanine (8-oxo-G) that gives rise to 8-oxo-G:A mispairs during DNA replication and if left unrepaired, results in accumulation of DNA mutations. We found that Werner helicase (WRN) physically interacts with DNA polymerase λ (Polλ) and stimulates DNA gap-filling by Polλ opposite to 8-oxo-G followed by strand displacement synthesis in MutY DNA glycosylase homolog (MUTYH) initiated base excision repair (BER) of 8-oxo-G:A mispairs.

There are two major pathways involved in repair of DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ). NHEJ is highly error-prone while HR is error-free. There are two subpathways of HR, namely synthesis-dependent strand annealing (SDSA) and canonical double strand break repair (DSBR). DSBR results in both crossovers (CO) and non-crossover (NCO) products while SDSA yields only NCOs. Undesired CO formation may lead to chromosomal rearrangements and loss of heterozygosity. Thus, in mitotic cells, majority of HR events proceed via SDSA to avoid crossing-over.

The molecular mechanism underlying promotion of SDSA is not well studied in human cells. RECQ5 and FBH1 have been suggested to be functional orthologs of Srs2 helicase that promotes SDSA in yeast cells. We have demonstrated that RECQ5 can prevent illegitimate RAD51 nucleofilament formation during post-synaptic phase of SDSA to promote formation of NCO products. Thus we propose that RECQ5 is the functional ortholog of Srs2 in human cells.

In yeast, Exonuclease 1 (Exo1) and Dna2 in conjunction with Sgs1 represent two separate pathways of long-range DNA end resection. In human cells, Bloom helicase (BLM) has been suggested to cooperate with DNA2 to mediate long-range resection. We were able to show that

DNA2 cooperates with either BLM or WRN in human cells to mediate this process. In addition, our experiments suggest that BLM promotes DNA end resection in complex with TopIIIα-RMI1-RMI2.

Activation of *Ataxia telangiectasia* and Rad3 related (ATR) kinase upon DSB induction depends on DNA end resection. We have identified the mismatch recognition complex MSH2-MSH3 as a component of the ATR signalling pathway. We have shown that MSH2-MSH3 is recruited to sites of DSBs in a DNA end resection dependent manner and promotes DSB repair by HR. Moreover, our results suggest that the MSH2-MSH3 complex binds to DNA hairpin structures in replication protein A-coated ssDNA and recruits the ATR-ATRIP complex hence stimulating ATR activation and DNA repair.