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Knowing the sources of spontaneous mutations is essential for understanding the origin of genetic variation that drives evolution in all organisms, and the mechanisms responsible for maintaining genome stability. This last issue is important for understanding the cancer etiology. Recently, it has been postulated that the lifetime risk of many types of cancer is correlated with the number of divisions (rounds of replications) of the normal cells maintaining tissue's homeostasis. Obtained results suggest that majority (70%) of cancers are due to the random, spontaneous mutations arising during DNA replication in normal cells.

It was shown that DNA replication errors are the major source of spontaneous mutations. DNA is a linear polymer consisting of deoxyribonucleotides. The fidelity of DNA replication is maintained primarily by selection of a correct deoxyribonucleotide by DNA polymerase. DNA polymerases preferentially insert deoxyribonucleotides into DNA rather than ribonucleotides, which is important for maintaining stable DNA-based genetic information as deoxyribonucleotides are more chemically stable. Ribonucleotides contain a reactive 2'-hydroxyl group on the ribose ring that makes them more sensitive to hydrolysis and, therefore, their presence in the DNA chain might make it more susceptible to breaks. Structure-function analyses of sugar discrimination revealed that the major barrier for ribonucleotides is confined to single residue in the DNA polymerase active site called the "steric-gate". The presence of "steric-gate" allows deoxyribonucleotide insertion into DNA but exclude incoming ribonucleotide. However, it has been demonstrated in vitro that ribonucleotides are incorporated into DNA by DNA polymerases with higher frequency (1/2500-1/5000 of replicated bases) than incorrect deoxyribonucleotide (1/10000 of replicated bases). Thus ribonucleotides are the most abundant non-canonical nucleotides inserted into DNA. Nonetheless, despite such high frequency of incorporation, ribonucleotides are not detected in the DNA. It suggests the existence of very efficient ribonucleotide repair mechanisms.

Due to their antiparallel nature, the two DNA strands are replicated in different fashion. One strand (the leading strand) is synthesized continuously, whereas the complementary (lagging) strand is synthesized discontinuously in short Okazaki fragments. The scientific goal of this project is to better understand the mechanisms of ribonucleotide incorporation and removal during chromosomal DNA replication. It is most interesting to find an answer to the question: is there a difference in the frequency and mechanisms of insertion and excision of ribonucleotides between the two DNA strands? We have developed a system that allows to assess mutation frequencies resulting from the leading and lagging strand replication. Previous results with this system revealed that in normal cells lagging strand replication is more accurate. This bias might be explained in terms of involvement of different DNA polymerases in DNA replication on each strand and/or by different efficiency of repair processes on leading and lagging strand. In the presented proposal we are going to use different Escherichia coli mutants in genes involved in ribonucleotide excision repair and mutants altering the "steric-gate" structure of different DNA polymerases. Results obtained in this study may have important implications for our understanding of genetic instability and sources of spontaneous mutagenesis.