POPULAR SCIENCE ABSTRACT

DNA replication is a process during which genetic material of the cell (genome) becomes duplicated, in preparation to become divided into two daughter cells, allowing for multiplication of the organism or its development from the embryonic stage. From the organism's health point of view, it is imperative for the genome to remain in a damage-free form, and for genome's replication to be performed with the highest fidelity. DNA is a long double-stranded molecule made of deoxyribonucleotides, which are composed of, among others, sugar (deoxyribose) and nitrogenous base (adenine, guanine, thymine, or cytosine). The nitrogenous base sequence in the DNA chain is the true information hidden in DNA. DNA is transcribed into RNA, which contains a different sugar (ribose). RNA is translated into proteins, and their structure is determined by deoxyribonucleotide sequence of DNA. As a consequence, DNA determines height, hair colour, and many other important human characteristics, including health.

DNA replication is carried by specialised proteins, DNA polymerases. During replication, DNA polymerases add deoxyribonucleotides to the terminus of a nascent DNA chain, using parental strand as a template (two DNA polymerases simultaneously replicate DNA using two parental strands, producing two double-stranded DNA molecules). DNA polymerases are required to choose correct deoxyribonucleotides so as to ensure proper pairing of the nitrogenous bases (adenine "fits" only to thymine, whereas cytosine to guanine). Replication errors (for example, errant adenine-guanine basepairing) can lead to formation of genetic mutations, which, in most cases, have deleterious effects for health. Fortunately, such mismatches are not occurring frequently – DNA polymerases are much more accurate than any human-made device (1 error per 10 million basepairs).

Throughout the past decade, it has been shown that DNA polymerases insert ribonucleotides (RNA building blocks) into the genome with much higher (even 1000-fold) frequency than mismatched deoxyribonucleotides. Ribonucleotides are the most commonly occurring DNA lesions, with 1 million incorporated into human genome during every cycle of replication. As has been concluded from numerous studies, ribonucleotides in DNA, if left unrepaired, may cause extensive damage to DNA (including genome breaks), negatively affect various cellular processes, and lead to the development of genetic disorders, cancer, and embryonic lethality. As a consequence, living cells evolved specialised systems capable of recognition and removal of ribonucleotides from DNA.

It is known that bacteria are more resistant to the negative effects of ribonucleotide presence in DNA. This creates an opportunity to utilise them as a tool in a thorough examination of the mechanistic basis underlying the incorporation of ribonucleotides into DNA, as well as cellular pathways that remove them. Studies employing *Escherichia coli* as a model organism have revealed that both DNA strands are replicated with differential fidelity; in other words, the frequency of genetic mutations occurring on two DNA strands is unequal. Therefore, the aim of this project is to check:

- whether ribonucleotide incorporation into DNA affects the fidelity of replication of both DNA strands;
- whether the number of ribonucleotides inserted to both DNA strand is equal;
- what cellular pathways are involved in the removal of genomic ribonucleotides and whether their efficiency differs between two DNA strands;
- what can be the consequences for the cellular processes of the failure to remove ribonucleotides from DNA.

As a main research tool, a mutated variant of a DNA polymerase, characterised by increased ribonucleotide incorporation frequency, will be employed. During research project realization, the following studies are planned:

- the analysis of genetic mutation spectra on both DNA strand, in order to confirm the observed strand specificity of ribonucleotide removal systems;
- direct estimation of the amount of ribonucleotides in DNA when different ribonucleotide removal systems are inactive (a significant increase in the number of genomic ribonucleotides is expected);
- the pursuit, using genetic and biochemical methods, of new systems that may be involved in ribonucleotide repair (preliminary data suggest the participation of the proofreading activity of the main replicase responsible for replication of both DNA strands);
- the measurement of the amount of single-stranded DNA, which would indicate the existence of problems with maintaining the processivity of replication (a high level of single-stranded DNA is expected when ribonucleotide repair systems are inactive, in agreement with preliminary results suggesting replication disturbances).

The results obtained will significantly broaden our knowledge regarding ribonucleotide removal pathways, their evolutionary significance and possible consequences of their impairment.