Biological significance of DNA replication-dependent changes in local higher-order chromatin structure.

Human genome consists of 2-meter-long DNA separated into 46 differently-sized chromosomes which must fit into the cell nucleus with diameter of 10 μM - approximately 200 thousand times smaller. This massive disproportion causes that genome in the interphase nucleus needs to be compacted in very organized way. DNA, besides being highly packed, needs to be at the same time easily accessible for the enzymatic complexes participating in reading and copying of the genetic information. The intriguing research question - how the cells manage to satisfy these two contradictory requirements – has been since a long time the subject of intensive investigations. Improper genome organization can lead to wrong gene expression, DNA replication and further to serious developmental abnormalities and diseases including cancer.

Copying of DNA is a highly complex process during which due to coordinated activity of multiple protein complexes DNA double helix is being unwound and complementary strand is being synthesized on both maternal strands. DNA replication machinery needs access to the DNA – therefore local chromatin structure needs to significantly be re-modelled. Interestingly, replication is localized in in limited number of replication centers called “replication factories”. It is thought that majority of the factories contains several replication complexes and copies ~ 1 million base pairs of DNA. Exact structure and biological significance of those structures is not known.

My project aims to describe replication factories and their importance for genome organization and also their role in cancerogenesis. I am planning to achieve this in the comprehensive way combining genome-wide methods using next generation sequencing, bioinformatics and 3D microscopy.

The levels of DNA organization between nucleosomes and arranging chromatin into chromosome territories had been largely unknown until recently. Thanks to the new method: Hi-C there has been unprecedented progress in the past several years. Hi-C enables genome-wide insight into 3D chromatin organization of all levels by mapping the interactions between distinct fragments of chromatin. It lead to the discovery of the compartmentalization of the genome into reproducible, 1 Mbp sized domains of increased chromatin interactions. Further computer analysis of those domains showed that they could be divided into two types: A and B and also that domains of the same type interact preferentially with each other not only within one chromosome but also genome-wide.

Progress in understanding of the three-dimensional genome structure as well as my interest in the spatio-temporal organization of its replication (replication factories) lead me to perform preliminary research aiming to investigate influence of the DNA replication on the chromatin structure and conformation. It is well known that mistakes in replication can cause cancer eg. due to chromosomal translocations. Therefore, I have decided to study also how local 3D chromatin structure contributes to translocations characteristics to known cancer cells.

To address these questions, I have developed a modified version of Hi-C and related methods which selectively measures the interaction frequencies of chromatin replicating in the factories. I have called these methodologies: RepliC (genome-wide version), Repli3C and RepliCaptureC (for the high-resolution analysis of local interactions). Repli3C allows detection of DNA-replication specific interactions by relatively simple and inexpensive protocol based on qPCR, hence was ideal for proof of concept experiment. Based on the comparative bioinformatic analysis of 4C experiments made in proliferating and replicationally inactive lymphocytes I have chosen the regions with differences in local chromatin conformation and I tested them with Repli3C. **It turned out that Repli3C in clear way confirmed bioinformatic analysis.**

This encouraged me to apply for funding necessary to conduct further investigation of the importance of replication factories on the chromatin structure and the risk of cancerous translocations.