One of the greatest breakthroughs in biology was the discovery of the structure of our genetic material - DNA. It consists of very long threads built by small blocks, called nucleotides, which form genes and various elements regulating genes' activity. Physical characteristics and the proper functioning of our organism depend on their linear sequence. Strikingly, in every human cell, DNA, which is around two meters long, is packed in a tiny nucleus with a diameter of only ten micrometers. In addition to the order of nucleotides in the genome, it has been recently discovered that the way in which DNA is folded in the cell nucleus also influences numerous cellular processes, as it determines the spatial interactions between the given elements of DNA. This means that cells performing distinct functions in the organism will differ in their three-dimensional DNA organization. Importantly, recent scientific findings indicate that disruption of the 3D interactions between specific DNA fragments can cause many human diseases, including cancer.

The three-dimensional DNA structure is, among other things, closely related to replication - a fundamental process through which a faithful copy of the whole genetic material of a cell (termed genome) is generated before each cell division. However, various internal and external factors can interfere with the replication process, eventually causing it to slow down. This situation is called replication stress, and it can lead to serious DNA damage, including chromosome breakage. Replication stress is one of the main causes of the phenomenon known as genetic instability, associated with increased frequency of mutations and other changes in the DNA. It is a characteristic feature of most cancers and has been found to contribute to the development of a number of other diseases such as neurodevelopmental disorders and neurodegenerative diseases.

The main aim of this project is to determine how the spatial organization of the genome changes upon replication stress and how these changes help to protect the cell from the harmful consequences of this stress. By using the most modern methods relying on next-generation sequencing, we will compare the three-dimensional structure of the genome in correctly replicating cells and those exposed to replication stress. DNA regions that are particularly susceptible to stress-related damage will also be identified allowing to generate a comprehensive, genome-wide map of fragile sites in human cells. It will enable us to determine the characteristic features of those sites and subsequently to get novel insights into the causes of their breakage. In addition, we will investigate the consequences of permanent 3D changes emerging in cells due to replication stress in terms of their potential contribution to the development of cancer. Based on the obtained results, a computational model, determining genomic sites, where the damage may initiate or stimulate the development of cancer, will be created. Additionally, we will develop new tools enabling modeling of structural elements of DNA such as single DNA loops and dynamics of their formation. The execution of the project will significantly broaden our understanding of the mutual relations between replication stress, the spatial structure of the genome, and genetic instability. In addition, the obtained results can potentially be used in the diagnosis and therapy of diseases associated with chromosome fragility, such as cancer, autism, or mental developmental disorders.