Duplication (replication) of genetic information is essential to the life cycle of cells. Information is carried by deoxyribonucleic acid (DNA), which acts as a template for protein synthesis. The base sequence of DNA determines the order of amino acids, and thus the structure and properties of proteins. Prior to cell division that information must be copied, so that each daugher cell gets one set of DNA molecules (chromosomes). DNA replication must must be strictly controled. If daughter cell gets incomplete or flawed genetic information, such a cell will not be able to function properly, which generally leads to her death. Sometimes, as a result of receiving wrong information, cell undergoes oncogenic transformation. Therefore, an accurate understanding of the DNA replication process is very important in the context of developing effective methods of prevention and treatment of cancer.

Replication consists of: changing the spatial organization of DNA, separating the double strand DNA, synthesis of the initial section (primer), synthesis of complementary strand of DNA, and the termination of the process. DNA fragment, where seperation occurs between complementary DNA strands and where replication machinery functions, is called replication fork. It should be noted, that, in the nucleus, new replication forks are activated in the vicinity of the forks that are already active. Created in this way are replication factories, structures built with a network of protein complexes and nucleic acids that are visible in light microscope. It is believed that their formation and dissociation are influenced by local structure of DNA and associated structural proteins (chromatin) in the nucleus. It has been shown that each cell follows a pattern, whereby different regions of the nucleus are assigned a specific time at which their replication begins.

Ezymes that contribute to the replication process usualy belong to one of the following groups: topoisomerases, helicases, DNA polymerases, ligases or proteins supporting replication. Among the latter an important role is played by PCNA (proliferating cell nuclear antigen), which is composed of three subunits connected in a ring shaped trimer. This structure allows movement of PCNA along the DNA strand which passes through the center of ring trimer. PCNA serves as a docking platform for proteins that catalyze the replication of DNA and, in particular, polymerases ε and δ which adds nucleotides to a new DNA strand (based on the existing strand). It should be noted that because polymerase only works in one direction, DNA synthesis takes place continuously along one of the sperated strands (leading strand), and along the other(delayed strand) polymerase creates fragments (Okazaki fragments), which are linked afterwards. Because of the lagging strand synthesis structure one replication fork can contain multiple molecules of PCNA.

Positioning of PCNA on the DNA strand is a task performed by protein called replication factor C(RFC). RFC catalyses: PCNA ring opening, provision of link between PCNA and DNA, and the ring closing. Replication, particulary PCNA-RFC intearaction, has been investigated in the past mainly by biochemical methods. However, there are not many research that compare in vitro results to the conditions of living cells. In particular, it is not clear whether after loading of PCNA onto the matrix RFC dissociates, or is still associated with the replication machinery and participates in the next stages of replication.

The aim of the project is therefore to investigate the dynamics of RFC, with particular emphasis on its interaction with PCNA. Obtained data will be interpreted in the context of biochemical models of this interaction. During the tests, fluorescent biosensors will be introduced into the HeLa model cells, and that will allow determination of the level and distribution of RFC and PCNA protein in cell nuclei during replication. By using advanced microscopic techniques we will test the exact concentration of proteins and how it changes over time, how fast single molecule is moving and whether the two proteins travel the nuclei together. By using genetic engineering techniques we will make the protein RFC lose some of its function, then check to see whether there will be a difference in behavior of PCNA in the cell.