Microscopic identification of proteins involved in DNA repair of CAG repeats regions

Repeated sequences, which occur very commonly in the human genome, are unstable genetic elements, which in some cases, may be a causative factor in genetic diseases. One of the examples is **CAG repeats**, which expansion is responsible for many **neurodegenerative disorders**, called poliglutamine diseases (poliQ), such as **Huntngton's disease** or spinocerebellar ataxias. Number of repeats in poliQ diseases is positively correlated with earlier onset and severity of the disorders. Novel therapies for poliQ diseases aim is to decrease the expression of the defected genes, and recently attempts to cut out or shorten the CAG tract in the genome have been made. Progress in molecular biology and genetic engineering allows precisely editing the chosen sequence in the genome for therapeutic purposes. **CRISPR-Cas9 technology** is now the most versatile tool for genome editing, which can be used for CAG repeats contractions. In our research, we have shortened the CAG tract in the *HTT* gene by induction of the double strand break (DSB) in the region with repeats, using nuclease Cas9. One hypothesis explaining these shortenings is the involvement of DNA repairing pathways utilizing microhomology regions to rejoin two ends of cleaved DNA: microhomology-mediated end joining/ alternative end joining (MMEJ/alt-EJ) or single strand annealing (SSA), which employ different proteins. This hypothesis has been justified by the results of our recent experiments. However, dependency between choosing the repairing pathway and the type and site of DSB induction still needs to be settled. Another interesting issue is the CAG tract contractions in the effect of usage of D10A nickase (Cas9n), which is able to cleave only one DNA strand (single-strand break, **SSB**).

The project aims to directly (microscopically) determine which mechanism is engaged in DSB/SSB repair in the CAG repeats regions and responsible for the contractions of this type of repeats. The direct determination of which proteins are involved in this process would create opportunities to develop new poliQ diseases therapy.

In this project, the cutting-edge CRISPR-Cas9 genome editing technology will be used in two aspects: to induce DSB/SSB within the CAG tract and also to visualize sequences that flank the edited region. For the second purpose, nucleotically inactive dCas9 and MS2-MSP tagging system will be utilized. Proteins of interest will be detected in the repaired locus using the **immunofluorescence** method and **confocal microscopy**. This experiment will unequivocally determines the *HTT* CAG tract shortening mechanism after DSB/SSB induction.