

The main goal of the project is to identify the dimer (functional complex composed with two proteins) assembly of yeast Rad50 protein, assess the molecular basis responsible for this assembly and determination of the impact of coiled-coil dynamics on Rad50 functioning. MRN(X) (Mre11:Rad50:Nbs1(Xrs2 in yeast)) is a protein complex responsible for sensing and repairing the double-stranded DNA breaks, which are caused by external factors, such as ionizing and UV radiation, as well as internal, physiological processes, namely cell division, meiosis and recombination in developing lymphocytes B. Rad50 is a protein of unique structural features. It is composed with globular (spherical) domain at one end, central Zn<sup>2+</sup>-binding motif at the other and a very long coiled-coil region that connects these two domains via central turn of the polypeptide chain. Due to significant flexibility of the coiled-coil region the MRN(X) complex can adopt many structural forms. However, recent structural studies show that only two Rad50 dimer assemblies are functional – the open form, determined years ago for Rad50 from hyperthermophilic archeon, and the closed form, newly discovered for human protein. We suspect that the dimer architecture of yeast Rad50 is analogous to human one, however, crystal structure of the yeast form is still beyond our reach and the amino acid sequence of both proteins differ quite significantly, which can indicate diverse spatial distribution of two chains in a dimer. Present data highlight a major role of coiled-coil segment regarding both dimer architecture and signal transmission between two apexes of the complex. We firmly believe that the mechanism of apex-to-apex communication lies in dynamic properties of the coiled-coil region. In order to evaluate aforementioned hypothesis we plan to conduct series of structural studies using different yeast Rad50 variants, namely spectrofluorimetric and mass spectroscopy with ion mobility separation measurements. Rad50 mutants will be diversified by means of coiled-coil and zinc-hook region separately, which will enable recognition of the molecular basis of dimerization related processes. Furthermore, the use of innovative coiled-coil constrained variants will grant a better understanding of the segment's dynamics and its role regarding Rad50 functioning in the MRN(X) complex.