Spontaneous mutations in DNA play fundamental role in evolution, but also in aging, carcinogenesis and genetic diseases. Therefore, the knowledge about the mechanisms responsible for maintaining a low level of spontaneous mutagenesis is of great importance in various aspects of medicine. The specific aim of the proposed study is to recognize the mechanisms involved in newly discovered process leading to an increase of spontaneous mutagenesis due to augmented cellular level of Mms2. It has been previously shown that cellular abundance of Mms2 increases in response to DNA damage. Additionally, it has been revealed for number of cancer cell lines that the level of mRNA information encoding human homolog of Mms2 is increased in comparison to control cell lines. Proposed research will employ *S. cerevisiae* model organism, in which Mms2 protein was first identified and for which it was first shown that this protein plays structural role in poliubiquitnation processes in complex with ubiquitin conjugating enzyme, Ubc13. The basic mechanisms engaging this complex in yeast function in similar manner in higher eukaryotes confirming usefulness of *S. cerevisiae* as a research model.

Mms2 protein does not possess enzymatic activity by itself, therefore increase of spontaneous mutations frequency due to increase of Mms2 cellular level is intriguing. Despite high similarity to ubiquitin conjugating enzymes, Mms2 lacks the canonical aminoacid residue in potential active site what makes Mms2 unable to function as ubiquitin conjugating enzyme. Therefore, Mms2 is a member of proteins named ubiquitin-enzyme-variant (UEV). Activity of UEVs is dependent on its interaction with canonical ubiquitin conjugase and so far only Ubc13 was identified as a conjugase interacting with Mms2. Protein complex of Ubc13-Mms2 takes part in error-free DNA damage tolerance pathway that allows continuous replication of DNA despite the presence of DNA lesions blocking replicating DNA polymerases. Activity of this engaging Mms2 pathway prevents genetic information against mutations. In contrary, investigated by me effect connects Mms2 activity with mutator effect. Additionally, according to my results the promutagenic function of Mms2 does not depend on Ubc13. Instead, it requires ubiquitin conjugating enzyme Ubc4 and ligase Rad5. I have also determined that mutagenesis in Mms2 overproducing cells is entirely dependent on Pol zeta which is specialized DNA polymerase able to replicate DNA through damaged template. Obtained results suggest an existence of so far unknown ubiquitinating pathway, engaging Ubc4 and Mms2 activities in activation of error-prone function of Pol zeta.

In the presented project I plan to characterize the mechanisms associated with a new, promutagenic function of Mms2 by further corroborating the activities of proteins, which I have already shown to be involved in examined effect and identify new enzymes engaged in this process. Both Ubc4 and Rad5 possess several domains which are associated with distinct activities. To asses which domains of Ubc4 and Rad5 take part in the Mms2 mediated process leading increased mutagenesis I am going to employ site directed mutagenesis technique to introduce specific mutations in specific domains of those proteins. Additionally I will evaluate contribution of other potential Mms2-interacting proteins in this mechanism. I will also determine the genetic requirements of Pol zeta activity in mutagenesis associated with *MMS2* overexpression. Additionally, by use of genetic and immunochemistry techniques I will examine the role of PCNA modifications in promutagenic function of Mms2.

Taking into consideration that Mms2 is ubiquitous protein in higher eukaryotes, including human cells, the results obtained for yeast Mms2 may potentially suggest its functions in other organisms. Similarly, Pol zeta is ubiquitous protein with conserved activity. Importantly, activity of this polymerase was shown to counteract effective anticarcinogenic treatment based on generating DNA damage in proliferating cells of cancer. Because of that, identification and characterization of mechanism involved in Pol zeta regulation could be crucial not only for the basic research area of interest but also in designing new anticancer therapies.