Adenoviruses are large, non envelopped viruses with double stranded DNA genome. Human adenoviruses cause usually mild infections but in young, elderly and immunocompromised individuals the infection can have serious effects. Mutated adenoviruses that are not able to replicate in normal human cells and are tested as a tools to deliver to the cells DNA encoding proteins that could have therapeutic potential as vaccines or in an anticancer therapy. The detailed knowledge of the process of adenovirus replication may lead to the development of better gene-delivery tools and better antiviral therapeutic strategies.

Virus replication requires synthesis of large quantities of the relatively few types of proteins. Viral proteins are often synthesized as a large precursors that undergo complex process of specific proteolysis and folding that results in the mature proteins formation. Moreover, proteins that are components of the viral capsid often have natural tendency to form aggregates. Therefore, many viral proteins depend on the assistance from the host cell proteins called chaperones. Chaperones help other proteins to acquire proper three-dimensional structure during the maturation process. One of the chaperone proteins, called Hsp90, present in the cytoplasm of every cell proved to be essential for the replication of many viruses. We decided to test whether Hsp90 chaperone is also necessary for the replication of human adenovirus.

Results of our experiments proved that inhibition of Hsp90 activity in cells infected with Adenovirus 5 abolished replication of the virus and strongly inhibited replication of the viral genomic DNA. In the proposed project we will identify proteins of adenovirus that depend on Hsp90 chaperone activity. We will also investigate which processes during virus replication are affected by the inhibition of Hsp90. The effects of the Hsp90 inhibition on the process of the synthesis viral proteins synthesis will be studied using antibodies that bind specifically to the viral proteins. These proteins can be used to detect quantity and localization of the viral proteins in the cells. Assembling of the viral particles will be studied using electron microscopy. We will also isolate Hsp90 proteins from the cells infected with adenovirus to analyze which viral proteins interact directly with Hsp90. We will also analyze Hsp90 interaction with the viral proteins using “two-hybrid assay”. This assay rely on the detection of an easy to quantify enzyme expressed from the ‘reporter’ gene that is activated when Hsp90 and the viral protein bind together in a stable complex.

Human cells express two forms of Hsp90, named Hsp90α and Hsp90β. We noticed that only Hsp90β is necessary for the adenovirus replication. We will identify structural features of Hsp90β that are responsible for its activity in this process by study chaperoning activity of the hybrid proteins composed of Hsp90α and β fragments.

Hsp90 activity is necessary for replication of all viruses, but the specific proteins and processes chaperoned by Hsp90 are different for each virus. Identification of the adenoviral proteins chaperoned by Hsp90 will increase our knowledge of this subject. Many studies overlooked the existence of the two types of Hsp90 in the cytoplasm of mammalian cells and both proteins are treated as one ‘Hsp90’. Our results demonstrating that only Hsp90β is able to chaperone adenovirus proteins will prove for the first time that each form of Hsp90 may have unique role in the process of viral infection. Hsp90 inhibitors are intensely studied as potential anticancer drugs. The results of our studies will provide an evidence that Hsp90 is also an attractive therapeutic target in treatment of viral infections. Despite numerous studies on Hsp90 chaperone mechanism and identification of several hundred proteins interacting with Hsp90 little is known about the protein-Hsp90 interactions at the level of the protein structure. Identification of the fragments of Hsp90 structure necessary for the interaction with the specific adenoviral proteins will increase our understanding of the mechanism of Hsp90 chaperoning activity.