Mammalian cells for proper functioning require instructions and nutrients from the outside environment. One of the processes that enable them to internalize signaling molecules and nutrients is clathrin-mediated endocytosis (CME). This process consists of several subsequent steps: (1) selection and grouping of cargo, (2) formation of initial cell membrane invagination, (3) formation of clathrin coated pit, (4) separation of the pit from cell membrane and formation of the endocytic vesicle and (5) transport of the vesicle away from the cell periphery. Proper progression of CME requires dozens if not hundreds different proteins but AP2 adaptor complex, which forms a bridge between the clathrin molecules, cargo and the cell membrane, plays a key role in this process. AP2 consists of four subunits: α2, β2, μ2 and σ2. Individual subunits can undergo post-translational modifications, which significantly affect their functions and thus regulate the entire AP2 adaptor complex. One of these modifications is phosphorylation of threonine 156 of μ2 subunit, which has been suggested to affect the activation of the AP2 complex. However, preliminary studies conducted in our laboratory have shown that there are other phosphorylation sites: serine 45 and serine 309 of μ2. Our results indicate that phosphorylation of these amino acids occurs in a p70S6 kinase dependent-manner. Furthermore, we have shown that phosphorylation of serine 45 affects the efficacy of the clathrin mediated endocytosis. However, the role of these post-translational modifications in the regulation of the AP2 adaptor complex has not yet been revealed, and it is not known how exactly they affect the process of clathrin-dependent endocytosis.

The purpose of this project is to test the hypothesis that the phosphorylation of serine 45 and serine 309 regulates the conformational changes of the AP2 complex and thus contributes to the modulation of clathrin-dependent endocytosis. At the same time we plan to test the role of p70S6K in regulating AP2 functions through these phosphorylations.

The proposed research will be conducted on two levels of complexity. At first we will examine the role of phosphorylation of serine 45 and serine 309 at the molecular level and then we will look at the physiological functions at the cellular level.

As a result of this project, we expect to primarily describe the new regulatory mechanism for AP2 complex and clathrin-dependent endocytosis by phosphorylation of serines 45 and 309 of the μ2 protein.