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THE FACULTY OF NATURAL SCIENCE
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The summary of the doctoral thesis

Study of interactions of forkhead transcription factor FOXO4 with DNA and the 14-3-3 protein

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Abstract

This doctoral thesis deals with the interaction of human forkhead transcription factor FOXO4 with DNA and regulating 14-3-3 protein respectively. The main aim of this work was detailed characterization of interaction between DNA binding domain of protein FOXO4 with two canonical DNA sequences and further clarifying the role of the 14-3-3 protein in the regulation of activity of protein FOXO4.

FOXO transcription factors are potent activators of the transcription of genes, which affect a variety of cellular processes. FOXO4 protein belongs to the family of forkhead transcription factor, which is a group of several tens of proteins, whose common feature is a highly conserved DNA-binding domain. Summary of the DNA binding specificity of these proteins, namely what precisely determines the small differences in the binding properties of individual forkhead proteins, despite the large amount of available structural data remains still unclear. Therefore, detailed characterization of interactions between DNA binding domain of the protein FOXO4 and DNA using surface plasmon resonance (SPR) and time-resolved fluorescence spectroscopy was performed. The results of this study allowed to clarify the kinetic model of FOXO4 binding to DNA as well as characterize both the conformational change of FOXO4 upon its binding to the target DNA and the importance of particular amino acid residues on the stability of the complex between FOXO4 and DNA.

The transcriptional function of FOXO4 is regulated by phosphorylation and binding of the 14-3-3 protein, which is by its nature regulator of many cellular processes. The mechanism of this regulation is still unclear. The 14-3-3 protein affects FOXO transcription factors in two ways. The complex formation inhibits FOXO4 binding to the target DNA and masks nuclear localization sequence (NLS), which consequently blocks the transport of FOXO proteins into the nucleus. Methods of fluorescence spectroscopy were used to study the interaction of these two binding partners. Time-resolved fluorescence of intrinsic as well as extrinsic fluorophores was used to show that the binding of the 14-3-3 protein affects multiple regions of the DNA binding interface of FOXO4. The results suggest that observed changes are induced by direct protein-protein interactions of these two binding partners. In addition, to better describe the interaction between these two proteins, a realistic structural model of the FOXO4:14-3-3 complex was constructed using six intermolecular distances obtained from Förster resonance energy transfer measurements. This model shows clearly that FOXO4 is located deep inside the central channel of the dimer of the 14-3-3 protein and its DNA binding interface is blocked by this interaction.