Structural characterization of interaction between transcription factors and DNA

Mgr. Růžena Filandrová

Abstract

Transcription factors are proteins that mediate gene expression regulation through interactions with DNA and other factors. They allow a cell to respond to various stimuli and play a crucial role in many biological processes such as control of cell cycle progression, differentiation of cells during development or immune response. To understand these processes, the knowledge of the transcription factors 3D structure together with the mechanism of their interaction with DNA is essential. However, some of the typical features of transcription factors, such as is for example the presence of intrinsically unstructured regions, make the 3D structure determination by the commonly used high resolution methods challenging. Therefore, utilization of complementary methods like structural mass spectrometry (MS), which was used in this thesis, might prove to be beneficial to explore the structural basis of the transcription factor-DNA interaction.

In first part of this work, a set of structural mass spectrometry methods with the main focus on hydrogen/deuterium exchange mass spectrometry (HDX-MS) was optimized and tested on two transcription factor-DNA complexes and their DNA binding motifs and proved to be able to provide structural information about regions of transcription factors inaccessible by the classical high-resolution methods as well as about structural dynamics of the transcription factor-DNA complex.

In the other part of the thesis, the structural mass spectrometry methods were, together with other techniques, such as smFRET, gel shift or fluorescence anisotropy, used to investigate whether and how the sequential context of the M-CAT motif and its orientation affects its interaction with the DNA binding domain of transcription factor TEAD1 (TEAD1-DBD). The obtained results have shown that the sequences of the DNA regions flanking the M-CAT motif affect its binding affinity to TEAD1-DBD and moreover, the transcription factor was found to be able to bind also to the inverted 5'-CCTTA-3' M-CAT motif, albeit with lower affinity. This low affinity interaction was then structurally characterized and might present a potential way of regulation of the transcription factor activity. Finally, the binding cooperativity of FOXO4 and TEAD1 transcription factors were studied utilizing oligonucleotides with adjacent response motifs.