Abstract

Genetic mechanisms of regulation of gene expression form the basis for proper development, function of organisms and their responses to variable life conditions. However, they are relatively slow. Life processes that require a fast response to the changing environmental and metabolic conditions are mostly executed on the level of proteins especially their posttranslational modifications and protein-protein interactions.The goal of the experimental work that led to the presented thesis consisted in exploitation of the model organism *Caenorhabditis elegans* for analysis of regulation of gene expression by transcription factors from the protein family of nuclear receptors. The model system *C. elegans* enables very efficient experimental procedures in the field of genetics, genomics and functional analysis of phenotypes. In the experimental work connected with this thesis, I studied the regulation of gene expression under specific experimental conditions from the perspective of advanced functional proteomics and I focused on the employment of separation methods and methods of advanced proteomics, especially by mass spectrometry.In the first part of the work, I characterized the nuclear receptor NHR-60 on the protein level. This nuclear receptor is expressed as two protein forms with a mass of 50 kDa and 64 kDa that are detectable in variable intensities in materials originating from individual cultures of *C. elegans*, despite that *nhr-60* is expressed as a single form of mRNA corresponding to the protein with the molecular mass of 50 kDa. The goal was to characterize NHR-60 (64 kDa) at the molecular level and its possible functions. The detection of NHR-60 using the specific antibody was then used for characterization of the power and limitations of two-dimensional chromatography. The main research goal was the development of methodology for comparative two-dimensional chromatography for the analysis of proteomes from high volume *C. elegans* cultures affected by specific experimental conditions. In the second part of the work, I focused on the identification of protein interactions of the Mediator subunit MDT-28. The results confirmed that MDT-28 shows interactions with subunits comprising the Head module of the Mediator complex of *C. elegans*. The experimental work confirmed the classification of MDT-28 as the orthologue of MED28 and revealed additional proteins that display an affinity to MDT-28. The experimental work connected with this thesis is in line with the very fundamental contribution of proteomic methods for studies of mechanisms of gene expression.