

Abstract

Multifunctional adapter protein and histone chaperone Daxx has been described in numerous cellular processes, including the regulation of apoptotic and stress signalling, antiviral response and processes connected to chromatin (e. g. transcription). Its influence on chromatin-related processes is mainly carried out by several associated enzymes, such as DNA-methyltransferase-1, histone deacetylases and chromatin-remodelling ATPase ATRX. In the complex with ATRX Daxx functions as a chaperone of histone-3.3, maintaining the constitutive heterochromatin e. g. at centromeric and telomeric regions.

The main aim of this Thesis was a better understanding of the Daxx cellular functions through identification and functional characterization of its novel interacting proteins. Using the yeast two-hybrid screen, several such new Daxx-interacting proteins were identified. These proteins were mainly nuclear, connected to the regulation of chromatin-related processes. More detailed analysis focused on the interaction of Daxx with chromatin-remodelling ATPase Brg1. This interaction was confirmed both *in vitro* and in the cells, where Daxx and Brg1 associated mainly in high molecular weight protein complexes. These likely chromatin-remodelling complexes contain, in addition to Brg1, several other Brg1-associated factors (BAFs). The co-localization of ectopically expressed Daxx and Brg1 was also observed in PML nuclear bodies. The mapping of the sites that mediate the Daxx-Brg1 interaction showed that Daxx binds not only the Brg1 region between its N-terminal QLQ and HSA domains but also some unidentified region(s) in the C-terminal half of Brg1. Consistently with this finding, Brg1 associates not only with the N-terminal third of Daxx but also with its central region. The Daxx-Brg1 interaction is thus probably mediated by at least two parts of both proteins. Subsequent functional analysis of Daxx-Brg1 interaction revealed that whereas Daxx participates in repression of several Brg1-activated genes in SW13 cell line, both proteins are necessary for the expression of SNCAIP gene in MCF10a cells. It thus seems that Daxx could act both as co-repressor and a co-activator of Brg1-driven gene expression. Daxx/Brg1 complex could also regulate other chromatin-related processes, not connected with gene expression.