Summary

Nuclear proteins containing polyglutamic stretches: The role of parathymosin in the structure and function of chromatin

Doctorate thesis

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Parathymosin (ParaT) is a small (101 aa), ubiquitously expressed nuclear protein. It contains a central acidic amino acid stretch and a bipartite nuclear localisation signal. The biological role of ParaT remains controversial. Initially, it was thought that ParaT has immunomodulating activity. Subsequent studies revealed a putative role of this polypeptide in regulating the glycolytic pathway. Latest work proposed a role of this protein in early replication.

Other proteins containing long acidic stretches in their amino acid sequence have well-characterised functions related to the organisation of chromatin. Previous studies identified linker histone H1 as a specific interacting partner of ParaT. Histone H1 is the major component that organises chromatin fibers into higher order structures. In addition, there is accumulating evidence that H1 stoichiometry plays a crucial role in many chromatin dependent processes. Therefore, the elucidation of the physiological significance and the functional consequence of the interaction of ParaT with H1 may provide additional information on the function of this acidic polypeptide in chromatin related processes and its biological role in the cell.

In this study and on the basis of previous interaction studies, we present first evidence on the effect of the interaction of ParaT with linker histone H1 on chromatin structure. We investigated the changes in nucleosome repeat length (NRL) of chromatin

assembled with H1 and ParaT *in vitro*. Furthermore, using a physiological chromatin reconstitution system we assessed the effect of ParaT on histone H1 binding to chromatin. Our studies indicate that ParaT binds to linker histone H1 and that this interaction leads to a decrease in the NRL and an inhibition of H1 binding to chromatin. The data suggest that upon interaction of H1 with ParaT the linker histone alters its mode of interaction with the chromatin template.

We performed biophysical experiments to assess a possible conformational change of H1 upon binding to ParaT. Circular Dichroism (CD) and fluorescence emission spectra studies indicate that H1 undergoes a conformational change when binding to ParaT occurs. A quantitative fluorescence-based binding assay revealed a K_d value of 19.6±11.3 μM for the H1-ParaT interaction.

Since linker histone H1 is the major factor responsible for the organisation of higher order chromatin structure, the obtained data indicate a putative involvement of ParaT in creating a relaxed chromatin structure through an interaction with histone H1. In a subsequent step we used *in vitro* and *in vivo* chromatin decondensation assays to study this putative functional consequence of an interaction of ParaT with H1. In both systems, ParaT induced a significant increase in nuclear surface indicating chromatin decondensation activity. In addition, fractionated chromatin of control GFP- and GFP-ParaT over-expressing HeLa cells was subjected to micrococcal nuclease digestion to visualise a change in chromatin accessibility. In the ParaT over-expressing HeLa cells the fraction containing chromatin remodeling complexes and actively transcribed genes showed a different, more 'relaxed' DNA profile when compared to control chromatin. Furthermore, indirect immunofluorescence and confocal scanning microscopy indicate that upon overproduction of ParaT the nuclear envelope shows gaps in the lamin B staining and ParaT content is released in the cytoplasm. These observations suggest that overproduction of ParaT leads to an increased pressure in the nucleus generated by chromatin, most probably due to the decondensation of chromatin by ParaT.

All collected data point to a function of the protein in chromatin decondensation through an interaction with linker histone H1. It is well established that chromatin relaxation is obligatory for nuclear factors to access DNA during gene-expression and the findings indicate a possible biological role of ParaT during this process. In an attempt to

elucidate this hypothesis, we performed immunohistochemistry studies with tissues of the lymphoid system to study ParaT expression in different cell-types in an organised environment. This system is well studied and it is becoming evident that chromatin remodeling factors play a crucial role during lymphocyte differentiation and T-cell function. Sections obtained from lymph node, spleen and thymus were incubated with antibodies against ParaT and with known cell specific markers of the lymphoid lineage (CD3, CD4, CD8 and CD20). Our studies revealed a cell-type specific protein profile of ParaT in T lymphocytes. Moreover, our data indicate that ParaT expression might be restricted to CD4 helper and absent in CD8 cytotoxic T cells.

In conclusion, this study extends our previous knowledge about the interaction of ParaT with linker histone H1 and indicates a possible mode of action and consequence of this acidic polypeptide on H1 binding to chromatin. Most specifically, our work imply for the first time the involvement of ParaT in thymocyte development and T cell function and point to an important biological function of this nuclear protein.

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Parathymosin affects the binding of linker histone H1 to nucleosomes and remodels chromatin structure.

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Linker histone H1 is the major factor that stabilizes higher order chromatin structure and modulates the action of chromatin-remodeling enzymes. We have previously shown that parathymosin, an acidic, nuclear protein binds to histone H1 in vitro and in vivo. Confocal laser scanning microscopy reveals a nuclear punctuate staining of the endogenous protein in interphase cells, which is excluded from dense heterochromatic regions. Using an in vitro chromatin reconstitution system under physiological conditions, we show here that parathymosin (ParaT) inhibits the binding of H1 to chromatin in a dose-dependent manner. Consistent with these findings, H1-containing chromatin assembled in the presence of ParaT has reduced nucleosome spacing. These observations suggest that interaction of the two proteins might result in a conformational change of H1. Fluorescence spectroscopy and circular dichroism-based measurements on mixtures of H1 and ParaT confirm this hypothesis. Human sperm nuclei challenged with ParaT become highly decondensed, whereas overexpression of green fluorescent protein- or FLAG-tagged protein in HeLa cells induces global chromatin decondensation and increases the accessibility of chromatin to micrococcal nuclease digestion. Our data suggest a role of parathymosin in the remodeling of higher order chromatin structure through modulation of H1 interaction with nucleosomes and point to its involvement in chromatin-dependent functions.

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The histone chaperone SET/TAF-Ibeta interacts functionally with the CREB-binding protein.

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The oncoprotein SET/TAF-Ibeta is a histone chaperone which is involved in cell-cycle control and chromatin remodeling. Confocal laser scanning microscopy reveals that SET is localized in distinct foci of variable size throughout the nucleoplasm of interphase cells. We report here that SET interacts directly with the acetyltransferase CREB-binding protein (CBP) and enhances the transactivation potential of the transcription coactivator. Our data suggest that the histone chaperone SET regulates the CBP-mediated transcription and may indicate a general principle by which transcriptional regulators cooperate with histone chaperones for gene activation.

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Prothymosin alpha associates with the oncoprotein SET and is involved in chromatin decondensation.

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Prothymosin alpha (ProTalpha) is a histone H1-binding protein that interacts with the transcription coactivator CREB-binding protein and potentiates transcription. Based on coimmunoprecipitation and mammalian two-hybrid assays, we show here that ProTalpha forms a complex with the oncoprotein SET. ProTalpha efficiently decondenses human sperm chromatin, while overexpression of GFP-ProTalpha in mammalian cells results in global chromatin decondensation. These results indicate that decondensation of compacted chromatin fibers is an important step in the mechanism of ProTalpha function.

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