PP08: Alexandra Lusser

"Chromatin remodeling in neurogenesis and neurological disease"

The energy-dependent modulation of histone-DNA contacts that is carried out by chromatin remodeling factors (CRFs) is one of the key strategies of the cell besides posttranslational histone modifications, DNA methylation and incorporation of histone variants to exert epigenetic changes. Several CRFs have been shown to play important parts during development and have been linked to a wide variety of diseases. Comparably few studies have been conducted to assess the roles of CRFs in development and function of the nervous system as well as their involvement in neurological disease. In the current project we strive to increase our knowledge about CRFs in the brain by pursuing the following Aims:

Aim 1: Characterize expression and complex composition of various CRFs in healthy and diseased brain.

a) To gain an overview about the presence and distribution of CRF complexes in the brain, we have, in a first step, analyzed the expression of 24 CRFs in seven regions of mouse brain and in embryonic stem cells (ESC) by RT-qPCR. We found that while some CRFs exhibit rather broad expression throughout the brain, others are specifically present in certain areas (e.g CHD5, CHD7). In addition, some CRFs show particularly high expression in the brain compared to ESCs (e.g. CHD3, Brm), while others seem to be excluded from the brain (e.g. HARP, LSH). We have then used a biochemical approach to fractionate different CRF- as well as HDAC-(complexes) by chromatographic methods. These experiments revealed characteristic elution patterns of the factors on an anion exchange column, in some cases pointing towards their presence in multiple distinct protein complexes (e.g. HDACs 1, 2 and 3; CHD5). Currently, we are trying to further separate and to determine the sizes of these complexes. In a next step, these experiments will be repeated with brain extracts from MSA mice (collaboration with Wenning/Stefanova) to investigate potential disease-related differences as well as with extracts from mouse liver to determine brain specificity of any of these complexes.

b) In collaboration with the Singewald group, we have analyzed the potential regulation of various CRFs and HDACs in the context of fear memory formation/extinction. To this end, we have compared protein amounts of chromatin modifying factors in subregions of the brain (ventral and dorsal hippocampus, amygdala, prefrontal cortex) of S1 mice fed with regular or zinc-restricted diet (because Zn2+ had been shown to improve fear extinction). The experiments revealed that CRF levels were predominantly altered in a Zn2+-dependent manner in dorsal hippocampus of mice tested for both expression of fear as well as fear extinction. In contrast, mostly class-I HDACs showed Zn2+-dependent changes in the ventral hippocampus and prefrontal cortex. The fewest changes were observed in the amygdala which is consistent with its role in the acute fear learning process.

c) The goal of this subaim is to investigate chromatin-related differences in the brains of MSA and control mice. In collaboration with the Wenning/Stefanova group we have extracted histones from 4 brain regions (forebrain, midbrain, brain stem, cerebellum) and examined their acetylation and methylation levels. Our preliminary results suggest altered patterns of acetylation of H3 and H3 lysine 9 as well as of H4, while no changes were detected for H3 lysine 9 trimethylation. In addition, we have examined the expression of several genes that have potential roles in disease development by RT-qPCR. Among those, the signaling factor Rab5a was upregulated in midbrain of MSA mice and the transcription factor Sox17 exhibited moderate downregulation in the brain stem. In ongoing and future experiments we will attempt to identify additional misregulated genes and use ChIP assays to determine, if the misregulation is accompanied or caused by chromatin changes at the gene loci.

d) In collaboration with the Flucher/Obermaier group we have been analyzing a potential link between the Ca2+-channel subunit □4b and chromatin modifying factors. To this end, we have first analyzed nuclear extracts from cerebella of wild-type and lethargic mice by western analysis and were able to detect at least 3 closely spaced bands with an antibody against □4. Most likely, these bands correspond to the different splice forms of the □4 subunit. In addition, we have established an immunoprecipitation protocol using the □4 antibody in order to be able to test for interaction partners by co-immunoprecipitation. Thus far, we have performed activity assays with immunoprecipitates to identify potentially interacting histone acetyltransferases, HDACs and histone methyltransferases. However, these experiments did not reveal stable interactions of soluble □4 with any of these enzymatic activities.

Aim 2: Study the role of the chromatin remodeling factor CHD1 in neurogenesis

The chromatin remodeling factor CHD1 has been implicated in the control of all stages of transcription (initiation, elongation, termination). Downregulation of CHD1 in ESCs led to a loss of pluripotency and preferential differentiation into the neural lineage. In this project we are studying the biological role of CHD1 in ESCs and in the mouse. Specifically, we have been addressing two subaims:

a) To generate and analyze a conditional CHD1 knock-out mouse: Although we faced many setbacks in the work on this subaim before, during the past year we have been able to make significant progress. After several unsuccessful attempts, we have now obtained mice with germline transmission of the floxed CHD1 allele. We have been breeding these mice to a mouse strain ubiquitously expressing Cre recombinase (Ubi-Cre), and we have obtained the first litters with heterozygous CHD1 knock-out mice. So far, we have not detected any obvious phenotypes except for a slight reduction in overall size. We have also started to breed the floxed mice to an inducible Nestin-Cre mouse line in order to assess the effects of CHD1 mutation on brain development. These mice were provided by the Aigner/Couillard-Despres group.

b) Study pluripotency, neural differentiation capabilities and chromatin structure in Chd1 knock-out ESCs: To this end, we have generated an ESC line in which both Chd1 alleles are targeted at exon 2 (which harbors the translation start codon). We have expressed Cre recombinase in these cells and purified several clones containing a homozygous deletion of exon 2. Unexpectedly, deletion of the translation start codon appears to result in the selection of another down-stream AUG (probably the 4th or 5th) and the production of an N-terminally truncated Chd1 protein. When we analyzed these ESCs, we detected no effects on self renewal and proliferation abilities but profound defects in the differentiation potential of the ESCs. Using ESC differentiation assays, immunostaining and RT-qPCR for different developmental marker genes we determined that the presence of the mutated protein led to an inability of the ESCs to properly form mesodermal and ectodermal cell populations and resulted in preferential, albeit aberrant, differentiation into the neural lineage. Our results suggest that the N-terminus of Chd1, which harbors several phosphorylation sites, is essential for the functions of Chd1 in ESC pluripotency. A manuscript describing this work is currently in preparation.