PP11: Alexander Hüttenhofer and Ronald Gstir

Identification of regulatory non-protein-coding RNAs in chronic CNS disorders by expression profiling employing a customized ncRNA microarray

In order to identify new regulatory ncRNAs in neural development and CNS disease, we performed deep sequencing analysis of cDNA libraries of ncRNAs derived from mouse embryonic stem (ES) cells, ES cells differentiated into neural progenitor (NP) and subsequently into a mixed population of neuronal-glial (NG) cells. The newly identified ncRNA candidates from the NP and NG cells showed 29 % overlap with ncRNA candidates derived from dorsal root ganglia (DRGs) and total mouse brain from our earlier studies (Skreka et al, unpublished; Rederstorff et al, Nucleic Acids Res., 2010).

Aim 1: Generation of a customized microarray chip for expression analysis of novel ncRNAs From the about 1000 most promising ncRNA candidates from above screens (i.e. differentiated ES cells, DRGs and total mouse brain) we have generated a customized micro-array chip. Thereby, the majority of these ncRNA candidates exhibited a size of 18 to 30 nucleotides in length while only a minor fraction showed sequences longer than 100 nt. The microarray platform employs a set of DNA oligonucleotide probes complementary to these ncRNA candidates, which enables differential expression analysis of complex RNA samples in a single experiment. With this approach, we aim to identify novel ncRNAs which are regulated in neurodegenerative diseases and might therefore contribute to signaling pathways involved in neurodegeneration. In addition to our customized ncRNA micro-array, we have established the LNA-miRCURY LNA microRNA array platform from EXIQON in the lab, to also provide expression profiling of known ncRNAs, i.e. miRNAs, to the SFB consortium.

Aim2: Identification of ncRNAs involved in CNS diseases Employing the customized neuro-ncRNA chip, we have performed expression analysis of ncRNAs from distinct brain regions of mouse models for Alzheimer's disease (Triple-transgenic mouse: PS1M146V, APPSwe, and tauP301L; collaboration with Ch. Humpel, P03), Multiple System Atrophy (MSA, transgenic mouse: □Synuclein overexpression; collaboration with G. Wenning, P02), and fear memory extinction (collaboration with N. Singewald, P06). Interestingly, cortex samples of the Alzheimer's disease model showed distinct expression differences of five ncRNA species in the disease model compared to wt mice. Their possible role in the etiology of Alzheimer's disease is currently further investigated. Up till now, by employing the customized ncRNA chip we could not detect significant expression changes of novel ncRNAs in a fear memory extinction mouse model (N. Singewald, P06), as well as in the MSA model (G. Wenning, P02). However, by employing the microRNA array platform from EXIQON distinct expression changes of four miRNAs could be detected in mice exhibiting impaired fear extinction compared to wt mice; thereby, their possible roles in fear memory extinction needs further investigation (performed in the Singewald lab; P06). In conclusion, we are now able to provide a comprehensive expression-profiling platform for complex ncRNA samples from mouse tissue for the SFB consortium.

Aim 3: Identification of biological functions of novel neuronal ncRNAsFor experimental analysis of biological functions, we are currently establishing three approaches: 1) In situ hybridization, to determine the subcellular localization of novel ncRNAs is currently underway. 2) By establishing a tag approach (RAT-Tag) we will isolate ncRNA-protein particles (RNPs) from cells and determine their protein composition by mass spectrometry. 3) By a systems biology approach, we are aiming to identify regulatory networks (e.g. mRNA targets) of novel ncRNAs as in case of miR-3096b involved in Alzheimers disease.

Papers published:

- *Skreka, Konstantinia, Simon Schafferer, Irina-Roxanna Nat, Marek Zywicki, Ahmad Salti, Galina Apostolova, Matthias Griehl, Mathieu Rederstorff, Georg Dechant, and Alexander Hüttenhofer. Identification of Differentially Expressed Non-Coding RNAs in Embryonic Stem Cell Neural Differentiation; Nucleic Acids Research (April 6, 2012). doi:10.1093/nar/gks311.*

Papers in preparation:

- *Gstir, R, Schafferer S. , Humpel, Ch, and Hüttenhofer A. Generation of a customized micro-array chip platform for expression analysis of novel ncRNAs involved in CNS diseases, ms in prep.*

Current SFB collaborations:

- *Ch. Humpel (PP05); G. Wenning (PP04); N. Singewald (PP10)*