Project Title:

High-Throughput profiling of Purkinje neuron dendrites during memory formation and neuronal stress

Name: ○Anton Kratz

Laboratory at RIKEN: Center for Life Science Technologies, Division of Genomic Technologies

Background and purpose of the project

The cerebellum is a highly organized brain structure, playing a critical role in motor learning and movement coordination. Motor learning is associated with long-term changes of the synaptic contacts on Purkinje neurons, the sole output of the cerebellar cortex. As in several other neuronal networks, protein synthesis in neuronal cells is known to be necessary for the formation of long-term synaptic plasticity in Purkinje neurons. In neurons, translation occurs not only in the soma but also within dendrites. There is mounting evidence that this local protein synthesis beneath or within dendritic spines responds to neural stimulation by altering spine morphology and molecular signal processing, thus resulting in synaptic plasticity. We conduced an analysis of the transcriptome before and after induction of synaptic plasticity to obtain novel insights into the molecular basis of long-term memory formation. In addition, there has been increasing speculation about the involvement of non-coding RNA in regulating synaptic plasticity, but so far only very few actual findings on this phenomenon have been reported.

We used a hybrid Adeno-Associated virus to specifically infect Purkinje neurons in the cerebellum and express a ribosome-binding fusion protein (L10a-EYFP). Immunoprecipitation of the EYFP-tagged polysomes allowed us to specifically harvest the polysome-associated transcriptome of Purkinje cells, at different time points, in different cellular fractions of Purkinje neurons: cytoplasmic and associated to nucleus and rough endoplasmatic reticulum (ER). Deep-sequencing combined with the nanoCAGE and CAGEscan technologies which we have developed allowed us to quantitatively identify the 5' ends of RNA transcripts, which comprises both mRNA and non-coding RNA.

Combining analysis of differential digital gene expression (DGE), Gene Ontology and KEGG-pathway analysis, transcript assembly, structural comparison and detection of known and putative non-coding RNAs, we identified the RNAs specifically expressed in the different cellular compartments, with a view to elucidating the mechanisms controlling synaptic plasticity, including the interaction between non-coding and translated RNA.

Usage of RICC

RICC has not been used for computation in fiscal year 2013 (but the attached D2S mass storage was of critical importance to back up experimental data). There was no reason to do so / the amount of experimental data currently present could be dealt with on the local cluster. I would like to keep my account on RICC though with a view to future work in FY 2014.

Fiscal Year 2013 List of Publications from the Use of RICC

[Publication]

We have one publication currently under review at Genome Research, under the title "Digital expression profiling of the compartmentalized translatome of Purkinje neurons".

We did not use results computed on RICC in FY 2013 in the actual paper, but RICC has been evaluated during the research and D2S has been of critical importance to make backups of the large amount of data generated; to acknowledge this, we wrote that "We thank […] RIKEN, Japan, for an allocation of computing resources on the RIKEN Integrated Cluster of Clusters (RICC) system […]" in the paper.

[Proceedings, etc.]

n/a

[Oral presentation at an international symposium]

n/a

[Others]

n/a