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Master level degree thesis project available at Dept. of Cell & Molecular Biology, Uppsala University

Title

Role of Poly(A)-specific ribonuclease (PARN) in telomere biology disorders

Background

PARN is a eukaryotic processive poly(A) degrading exoribonuclease. It degrades poly(A) tails present on a subset of mRNAs and non-coding RNAs, including among others certain snoRNAs, miRNAs and precursor rRNAs. We and others have recently found that human patients with genetic lesions in PARN suffer from a spectrum of syndromes called telomere biology disorders (TBD). All TBDs are associated with short telomeres. This unexpected discovery demonstrated that RNA poly(A) tail metabolism is intimately linked to growth control, ribosome biogenesis and telomere biology. To further investigate the effects of PARN deficiency, we have generated stable PARN KO zebrafish using CRISPR/cas9 technology. We have so far identified three candidate PARN mutant zebrafish with bi-allelic mutations and also generated a significant number of mono-allelic mutants by out-crossing with wild type zebrafish. Thus, different mutant zebrafish models were generated to titrate the PARN deficiency. We have also observed that some fish have developmental and growth defects. These models can be used to study the variations in PARN penetrance during the development of the diseased state in the whole life span and over several generations of the zebrafish.

Specific goals

- To identify the genotypes of fish to check for the correlation with the phenotype.
- Measure the average and chromosome specific telomere length of wt vs parn deficient fish.
- Characterize the snoRNAs and ribosome profile of the parn deficient fish

Work plan

Isolate genomic DNA from the offspring of homo/heterozygous zebrafish (fins or larvae) having mutations in *parn* gene and genotype them with specific primers that can distinguish wt and mutant alleles by PCR/Sequencing the PCR products. Fish will be sorted out according to their genotypes and the average and chromosome specific telomere lengths will be measured using TRF and STELA methodology and check the correlation between the genotype and phenotypes. Analyze the gene expression of mature and precursor snoRNAs and ribosome profile in wt vs mutant fish lines using qPCR/Northern blotting.

Methods

During the project several important techniques will be utilized, for eg., DNA/RNA isolation, Genotyping, Telomere length measurement assays, qPCR assay, Northern blotting

Contact

Students who are interested to join us to perform their degree project at Anders Virtanen's lab, can contact anders.virtanen@icm.uu.se or sethu.gunja@icm.uu.se