Exploring molecular mechanisms in human disease

- Using CRISPR/Cas9 knock-out and knock-in technologies to screen and study gene function -
- Functions of circular RNAs and their protein binding partners (RBPs) -

Damgaard lab
1 technician, 2 post doc, 3 PhD students, on average 2 MSc and 2 BSc

Introduction

The eukaryotic cell possesses numerous gene-regulatory mechanisms to control cell function according to gene expression and environmental cues. This includes rapid changes in gene expression elicited at almost every transcribable level inside the cell - events often dysregulated in disease. Historically, much attention has been given to the regulation of transcription and mRNA processing events, a fact that has produced a tremendous diversity from within genes. These important regulatory events aside, there is now increasing evidence that cytoplasmic processes, including regulation of both global and local protein translation and mRNA stability, are crucial mediators of cell function during development, cell growth and in particular, stress-resistant changes. These processes are governed by both RNA-binding proteins (RBPs) and large classes of non-coding RNAs (ncRNAs), exosome-consisting RNA (exRNA) and microRNAs (miRNAs). In our laboratory, we study the function of all these types of RNA and protein regulators in tightly controlled translation and mRNA decay and assess how their dysregulated function impact disease-like conditions in DMD (Duchenne muscular dystrophy) and cancer.

Hypotheses/questions

- Do circRNAs and RBPs generally misregulate in disease?
- How are circRNAs degraded?
- Protection by RBPs?
- Which RBPs interact with which circRNAs and what is the consequence at the cellular level, disease level?

Methods

- Mammary cell culture
- CRISPR/Cas9 knockdown
- CRISPR/Cas9 knock-in
- Stable inducible cell lines
- GFP/TCF
- Northern blotting
- RIP-seq
- RNA-Seq for RNA-seq
- Flow Cytometry
- Nucleofection
- Immunofluorescence
- Western blotting
- Fluorescence
- Protein/RNA co-localization
- Protein/RNA immunoprecipitation
- Polyribosome fractionation
- Ribotoxic RIP
- 45S/5S RNA Decay
- Subcellular fractionation
- Protein-RNA interactions
- Recombinant protein expression

Figure 1: Posttranscriptional regulation of gene expression. After a processed mRNA has been exported to the cytoplasm it can become regulated by RNA binding proteins (RBPs) at the level of localization, translation and decay. This is mediated by several regulatory factors including translation (e.g. green/orange TIA-proteins), decay factors (pacmen) and RNA binding proteins (e.g. “AU-rich binding proteins” (AUBPs)). Current work in the lab is concerned with the regulation of mRNA localization, translation and decay. Many regulated mRNAs accumulate in cytoplasmic foci termed processing bodies (PBs) and stress induce accumulation of certain mRNAs in stress granules (SGs). Both granules contain repressed mRNAs but PB tethered mRNAs are thought to undergo rapid decay.

Figure 2: Examples - RNA Fluorescent In Situ Hybridization (RNA-FISH) for visualizing “stress granules” (SGs), “processing bodies” (PBs) or sequestered nuclear mRNPs from patients with myotonic dystrophy (DMD).

Figure 3: mRNA pulse-chase decay assay. Reporter mRNA expression is pulse-chased.

Figure 4: RNA-sequencing

Figure 5: CRISPR/Cas9 (or CRISPR/Cas12a) used to generate knockouts or knock in cell lines.

Figure 6: Immunofluorescence revealing co-localization between two proteins reading in processing bodies (PBs).

Figure 7: “mRNA immunoprecipitation” (RIP). Northern/GFP/TCF/RNA-seq.

Bachelor projects

- Using CRISPR/Cas9 to create knockout cell lines and subsequent functional characterization in cancer or myotonic dystrophy.

Examples:
Knockout in cancer cells of various important RNA-binding protein genes, followed by translation assays, proliferation assay (longer project: also transcriptome by RNAseq). cirRNAs abundance?

- Using CRISPR/Cas9 to create knock-in cell lines and subsequent functional characterization.

Examples:
Generation of GFP-tagged, immune-tagged (e.g. FLAG or HA) or degradation tag-encoded RNA-binding proteins. We can then localize and follow proteins in live-cells. Immunoprecipitation to look for interaction partners or bound RNAs. Degradation induced to remove degradation tag-encoded endogenous protein within 3-5 hours and the immediate impact on cells can be tested (e.g. cancer cell proliferation, migration or invasion) potential.

Knockdown of specific RBPs or cirRNAs and assay for importance in different established cellular processes. Do cirRNAs affect fundamental processes in the cell and are given RBPs important for these?