Molecular mechanisms in human health and disease
- circular RNAs and RNA-binding proteins -
- NEW! RNA therapeutics to target cancer and ALS -
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The eukaryotic cell possesses numerous gene-regulatory mechanisms to control cell function according to given conditions and environmental cues. These include rapid changes in gene expression elicited at almost every thinkable level inside the cell - events often deregulated in disease. Historically much attention has been given to the regulation of transcription and mRNA processing events, which in turn produce a tremendous diversity from metazoan genes. These important regulated events aside, there is now increasing evidence that post-translational processes, including regulation of both global and local protein translation and mRNA stability are crucial regulatory tools in the cell during development, cell growth and as primary responses to environmental changes. These processes are governed by both RNA-binding proteins (RBPs) and large classes of ncRNAs, including circular RNAs (circRNAs), long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). In my laboratory we study the function of all these types of RNA- and protein regulators in tightly controlled mRNA translation and mRNA decay and assess how their deregulated function impact diseases like myotonic dystrophy (DMD), Neurodegenerative diseases and cancer. Recent efforts include the innovative use of circRNAs as therapeutic agents in amyotrophic lateral sclerosis (ALS) and cancer.

INTRODUCTION
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METHODS
- Human cell culture - primary patient-derived cells, stem cells, and cell lines.
- CRISPR/Cas-manipulation of cells (gene knockout and knockin).
- Cell-cycle checkpoint analysis
- Cell biological assays - proliferation, migration, invasion, colony formation, cell-cycle a.o.
- Imaging - Protein/RNA (immunofluorescence/RNA-FISH) and live cell protein/RNA imaging (e.g. GFP-tagging/GFP-extraction/SLIMAR)
- Gene expression analyses - Western blotting, RT-qPCR, RNA-seq and mass spectrometry.
- Polysome profiling
- Protein and RNA immunoprecipitation (followed by RT-qPCR, RNAseq, Western or mass spectrometry).
- Flow cytometry
- RNA localization and targeting
- RNA therapeutics and design/selection of aptamers

HYPOTHESES/QUESTIONS
Many circRNAs and RNA binding proteins (RBPs) are dysregulated in disease (e.g. cancer, ALS, Myotonic dystrophy). Could dysregulated circRNAs be biomarkers in cancer; have driver functions in ribbped cells proliferate?

- Do circRNAs impact subcellular localization and/or functions of RNA binding proteins? Are circRNAs primary RBPs (activation) (inhibition) or RBPs scaffolds that assemble protein complexes (regulatory)?

- Could circRNAs affect phase separated RBPs/RNA-accumulation in large granules (stress granules)? Do cells even localize circRNAs in the cytoplasm?

- circRNAs could work as negative or positive regulators of weak protein-protein interactions. circRNAs could affect the solubility of proteins containing intrinsically disordered regions (IDR).

PROJECTS (EXAMPLES)

**Project: Bladder cancer: Study of circRNA impact on cancer cell proliferation, viability and morphology**

Some circRNAs affect cell cycle progression in bladder cancer. DNA-methylation analysis shows similar DNA-methylation profiles of circRNAs, however, the impact of circRNA expression on cell cycle progression is not well understood. Method: Upper: EdU incorporation assay followed by flow cytometry to screen impact of circRNA knockdown. Middle: Identification of circRNA binding proteins (DNA-chip/mass spec assay). Lower: Knockdown of circRNA or mRNA followed by mass spectrometry.

**Project: Study of ALS-associated granules and stress granules (SG) forming circRNA.**

Why are some circRNAs overrepresented in RNA granules? Do they play structural roles here? High levels of certain circRNAs are found in ALS patient-specific granules and SGs. What is the basis for this and are pathological inclusions found in ALS regulated by circRNAs?

**Upper: ESU incorporation assay followed by flow cytometry to screen impact of circRNA knockdown. Middle: Identification of circRNA binding proteins (DNA-chip/mass spec assay). Lower: Knockdown of circRNA or mRNA followed by mass spectrometry.**

**Project: Can small circRNAs be used to target and harness disease-causing protein and RNA-binding partners?**

- circRNAs are generated by the process of backsplicing, where a 5’ss engaged with an upstream 3’ss to create a circular RNA. Placing of splice sites in vicinity of each other can be facilitated by base-pairing or dimerization of RBP’s binding in the surrounding introns.

- circRNAs have been shown to ‘sponge’ miRNAs and RBPs or as scaffolds for protein complex assembly. Many circRNAs are deregulated in disease and may play a direct role in the disease etiology by skewing gene-regulatory mechanisms.

**Top: Upper: RNA localization by tyramide amplification of DIG-probe signal in RNA-FISH.**

**Middle: Identification of circRNA binding proteins (DNA-chip/mass spec assay).**

**Lower: Knockdown of circRNA or mRNA followed by mass spectrometry.**