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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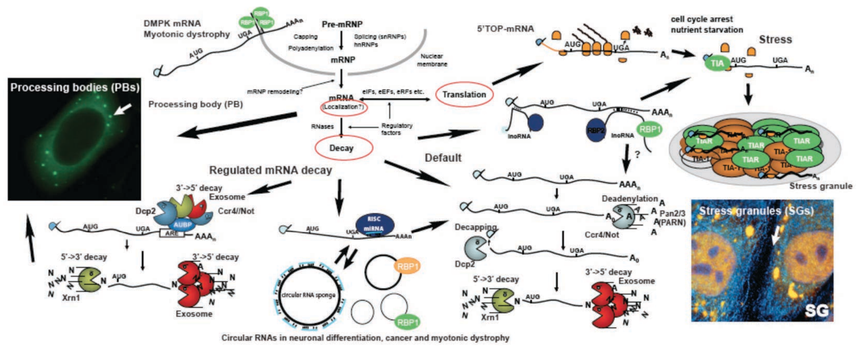
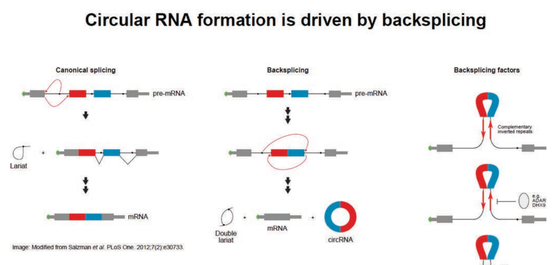
INTRODUCTION

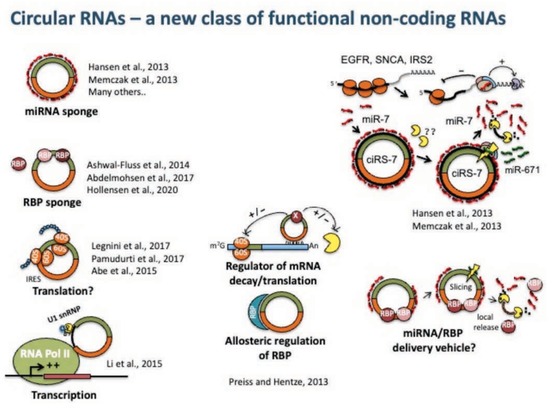


Circular RNAs in neuronal differentiation, cancer and myotonic dystrophy

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 cytoplasmic processes, including regulation of both global and local protein translation and mRNA stability are crucial modulatory instruments for 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 translation and mRNA decay and assess how their deregulated function impact diseases like myotonic dystrophy (DM1), Neurodegenerative disease and cancer. Recent efforts include the innovative use of circRNAs as therapeutic agents in amyothropic lateral sclerosis (ALS) and cancer.

Circular RNAs (circRNAs)





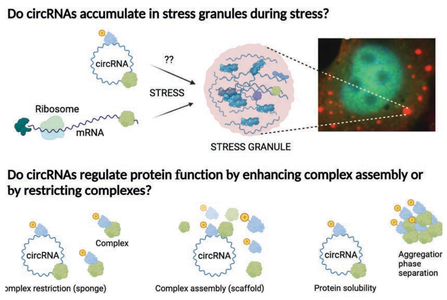
circRNAs are generated by the process of backsplicing, where a 5'ss engaged with an upstream 3'ss to create a circular RNA. Placing of splice sites into vicinity of each other can be facilitated by base pairingor by dimerization of RBP's binding in the surrounding introns.

circRNAs have been shown to "sponge" miRNAs and RBPs or to work 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.

METHODS

- Human cell culture - primary patient-derived cells, stem cells, and cell lines.
- CRISPR/Cas-manipulation of cells (gene knockout and knockin).
- Transfection and lentiviral transduction. Overexpression/knockdown
- Cell biological assays - Proliferation, migration, invasion, colony-formation, cell-cycle a.o.
- Imaging - Protein/RNA (immunoflourescence/RNA-FISH) and live-cell protein/RNA imaging (e.g. GFP-tagging/GFP-tetthering/SUNTAG).
- 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).
- Nanopore sequencing
- Flow cytometry
- In vitro RNA synthesis and design/selection of aptamers
- RNA therapeutics

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 or inhibit cells proliferation?

Do circRNAs impact subcellular localization and/or functions of RNA binding proteins? Are circRNAs primarily RBP sponges (inhibition) or RBP scaffolds that assemble protein complexes (positive regulation)?

Could circRNAs affect phase separated RBPs/RNAs accumulate in large granules (stress granules)? Do cells even localize circRNAs in granules?

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).

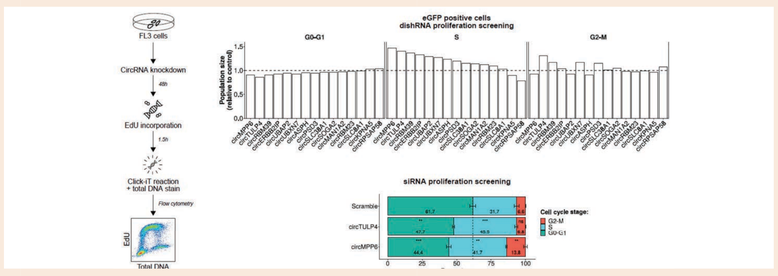
PROJECTS (EXAMPLES)

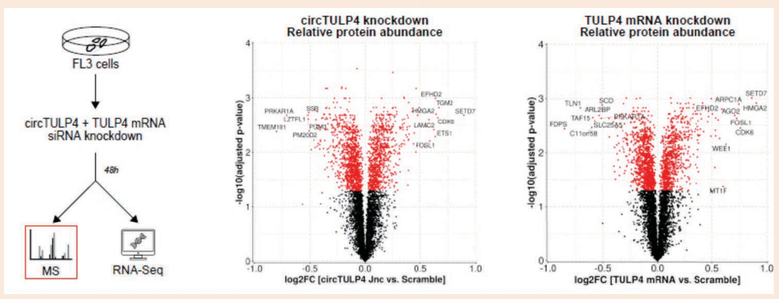
Project - Bladder cancer: Study of circRNA impact on bladder cancer cell proliferation, viability and survival.

Some circRNAs affect cell cycle progression in bladder cancer.
Upper figure shows distribution of cells in G1, S or G2/M phases by EdU incorporation followed by flow cytometry. Note that circTULP4 and circMPP6 knockdown pushes cells beyond G1/S transition and increases DNA replication. Lower figure shows that circTULP4 regulates the expression of many proteins (and RNAs - not shown here).

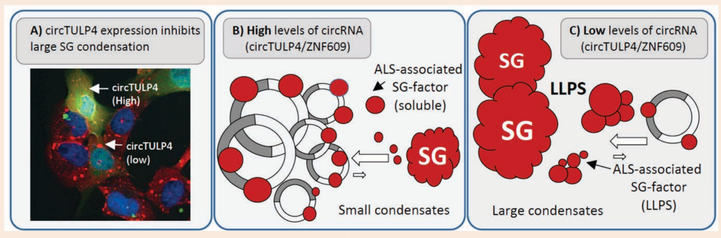
How are these effects achieved? What are the molecular mechanisms?

Identify circRNA protein interaction partners and perform manipulations with circRNA/proteins to measure impact on gene-expression, in cell biological assays or on subcellular localization. Are these circRNAs funcitioning as miRNA, protein sponges or scaffolds for protein complexes? Are circRNAs necessary for the formation of protein complexes?
Perform co-immunoprecipitations after knockdown or overexpression of circRNA. Imaging: Combined RNA-FISH and immuno-fluorescence to test whether proteins/RNAs co-localize.

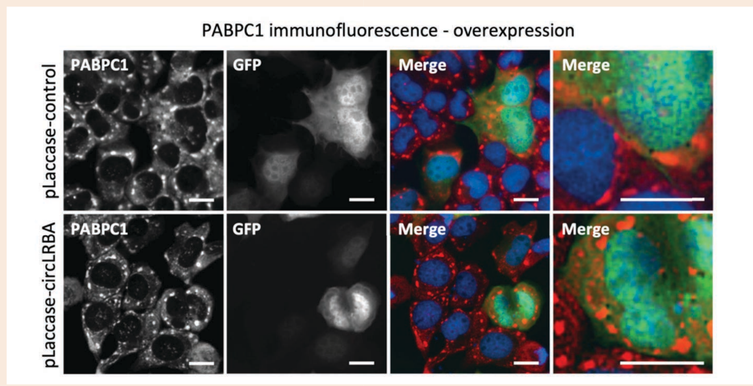




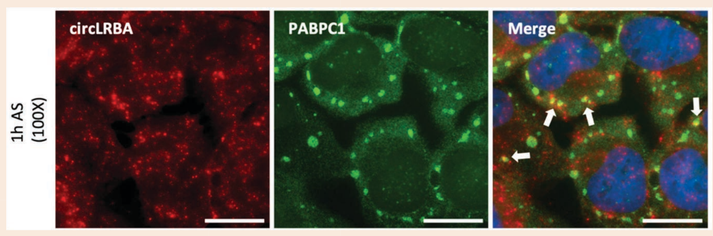
Upper: EdU incorporation assay followed by flow cytometry to screen impact of circRNA knockdown. Middle: Identification of circRNA binding proteins. circRNA pulldown- mass spec assay. Lower: Knockdown of circRNA or mRNA followed by mass spectrometry.



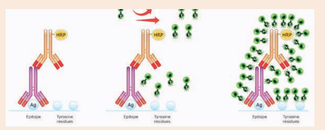
Overexpression of certain cirRNAs inhibit RNA granule formation, potentially by inhibition of weak IDR interactions and phase separation.



RNA granule visualization by immunofluorescence.



circRNA localization by tyramide amplication of DIG-probe signal in RNA-FISH



Principle for tyramide-mediated amplication of target signal. In circRNA-FISH a single backsplicing junction probe is used (DIG or biotin labeled). Signal is amplified by fluorophore conjugation by antibody-HRP activity.

Project: Study of ALS-associated granules and stress granule (SG) homing circRNAs.

Why are some circRNAs overrepresented in RNA granules? Do they play structural role here? High levels of certain circRNAs can inhibit SG-formation. What is the basis for this and are pathological inclusions found in ALS regulated by circRNAs?

Test dependence of RBP phase separation on circRNA. Perform knockdown of circRNAs or overexpression and probe ALS-specific RBPs to test if they localize differently (granules smaller/bigger, higher/lower numbers, more/less intense, faster/slower dynamics). Test post translational modifications (using phosphospecific antibodies, biochemistry or introductions of mutations) and signalling pathways during stress (using drugs, knockdown or inactivating/activating mutations).

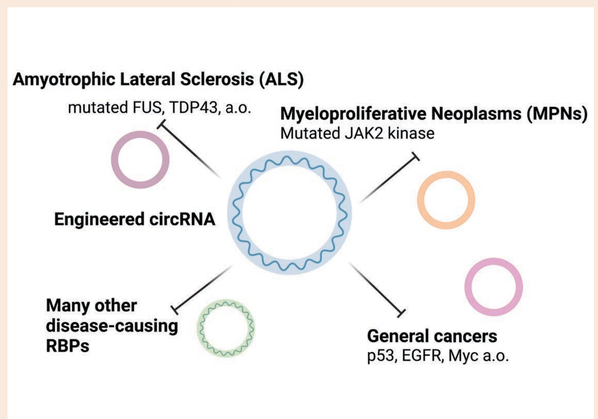
Project: Can small circRNAs be used to target and harness disease-causing protein and RNA-binding proteins?

- circRNAs are incredibly stable in cells.

- circRNAs can be engineered to express proteins or short peptides.

- circRNAs can function as scaffolds or sponges for RNA binding proteins.

We have developed strategies for targeting specific oncogenes in specific cancers and neurodegenerative disease. These include Amyotrophic Lateral Sclerosis (ALS) and Myeloproliferative Neoplasms (MPNs). However, our strategies also allow us to target general cancer related genes including p53 and EGFR, that are defective or dysregulated in many cancers.



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