Introduction
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 miRNAs, including circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and microRNA (miRNAs). In our laboratory we study the function of all these types of RNA- and protein regulators in tightly connected translation and mRNA decay and associated translation and decay function impact diseases like myotonic dystrophy (DM1), neurodegenerative disease and cancer.

Processing Body: mRNA-dependent aggregation of decapping enzyme (hDcp2) and its co-factors: hDcp1a, b, Edc3, Hedis, Lsm4, Rck/p54 among others. ARE-mRNAs, siRNA targets, miRNA targets, NMD substrates accumulate in PBs when decay factors are limiting.

Figure 1: Post-transcriptional regulation of gene expression. After a processed mRNA has been exported to the cell cytoplasm, it can become regulated at the level of localization, translation and decay. This is mediated by several regulatory factors including translation (e.g., 5'-terminal oligopyrimidine tract mRNAs (5'TOP-mRNAs)), decay factors (parame) 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.

Methods
Human cell culture
Stable inducible cell lines
RNA-Seq
miRNA-Seq
Immunofluorescence
Co-localization
Protein Immunoprecipitation
RNA immunoprecipitation
Translation analysis
Polyasomal fractionation
miRNA-Seq
Analysis
Subcellular fractionation
Protein-RNA binding
Recombinant protein expression
RNAi
Ribosome profiling

Figure 2: RNA Fluorescent In Situ Hybridization (RNA-FISH) for visualizing "stress granules" (SGs), non-localized, dead or mis-localized processing bodies (PBs) or redistributed nuclear mRNAs from patients with myotonic dystrophy (DM1).

Figure 3: Immunofluorescence revealing co-localization between two proteins in processing bodies (PBs).

Figure 4: co-immunoprecipitation

Figure 5: mRNA pulse-chase decay assay, Reporter mRNA expression is pulsed/chased.

Figure 6: “mRNA Immunoprecipitation” (RIP), Northern/RNA-seq.

Figure 7: Polysome profiling

Figure 8: De novo translation assay - BioMIST

Projects
1) Studying mechanisms regulating gene expression during cellular stress

Project 1: Identification and functional characterization of posttranscriptional modifications of AUBPs including LARP1, TIA-1 and TIAR proteins affecting functionality

Project 2: Identification of mRNA species that are regulated by LARP1/TIA-1/TIAR during cellular stress by RNA-seq.

Project 3: Functional analyses of mass-spec identified LARP1/TIA-1/TIAR interaction partners using global and biochemical approaches

2) Studying mechanisms of miRNA-mediated repression of local translation in polarized cells (epithelial and neuronal cells)

What is the spatial-temporal and functional interplay between miRNAs, mRNAs and RNA sponges in polarized cells?

Figure 9: Molecular mechanisms of circRNA-mediated regulation of translation and/or mRNA decay

Figure 10: DDX6 knockdown increases DMPK mRNA aggregation in patient cells.

Figure 11: Patient fibroblasts

Figure 12: Patient muscle

Figure 13: Mouse model

Figure 14: Molecular mechanism of circRNA-mediated repression of translation

Figure 15: Molecular mechanism of circRNA-mediated repression of mRNA decay

Figure 16: Schematic overview of regulated translation and decay

Figure 17: Visualizing mRNA processing events using cellular imaging and biochemical approaches.

Figure 18: DDX6 expression reveals nuclear foci formation and release splicing defect in patient cells.

Myotonic dystrophy type 1 - DDX6

Figure 6: mRNA pulse-chase decay assay, Reporter mRNA expression is pulsed/chased.