WHITE PAPER

Discover stories about translation that mRNA alone can't tell

Introduction

What happens when any of the rRNAs, 80 ribosomal proteins or about 150 non-ribosomal factors that are needed to bring ribosomes to life[1] go wrong? Ribosomal dysregulation has been implicated in multiple diseases including cancer, autism, cystic fibrosis, Alzheimer's disease and other neurodegenerative diseases[2].

Researchers continue to seek new technologies that will help them dig deeper into how cells orchestrate active translation and how dysregulation influences disease.

Isolated active ribosomes may reveal insights that might be obscured by the presence of inactive ribosomes. Ribosomes captured in action can provide access to underexplored translatome territory. With greater access, researchers may be able to uncover new insights about the factors that govern ribosomal function that cannot be identified from conventional mRNA analysis alone.

What's missing?

Differentiating effects of active vs. inactive ribosomes. Conventional ribosome profiling (e.g., with RIBO-seq) can provide positional information about ribosomal functional components and it can identify atypical ribosome behavior[3]. But RIBO-seq cannot distinguish between RNA that is within ribosomes during active translation vs. RNA that is bound to inactive ribosomes[2,3]. Conventional ribosome footprinting approaches can also face analytical challenges that may result in poor signal resolution[2].

Closer representation of active protein production. Using protein production as a proxy for pre-translation levels of mRNA does not account for downstream events that can impact protein quality or quantity. Mass spectrometry used to measure protein output directly is generally less sensitive than RNA-seq and may not be able to resolve nascent polypeptides[4–6]. Even used together, RNA-seq and mass spectrometry miss the most fundamental element of translation – the ribosome in action.

Capture ribosomes and RFPs in action

Immagina RiboLace technology is a new solution that captures ribosomes in action along with associated ribosome-protected fragments (RPFs). The fundamental component of RiboLace is a unique synthesized molecule (3P) comprised of a puromycin analog. To improve efficiency, 3P can be combined with cycloheximide to clamp active ribosomes onto translating mRNA fragments. The puromycin-trapped active ribosomes and RPFs are isolated from cell or tissue lysates by simple pull-down with magnetic beads, leaving the inactive ribosomes behind (Fig. 1).

Compared to total RNA sequencing (RNA-seq) and polysomal sequencing (POL-seq), capturing active ribosomes with RiboLace increases the concordance of transcriptomics data with the actual proteome (Fig. 2)[7].

Comparison of active ribosome profiling (RiboLace) and conventional ribosome profiling in HEK293 cells and mouse brain tissues also revealed significant differences in the translation rates of hundreds of individual genes (Fig. 3).

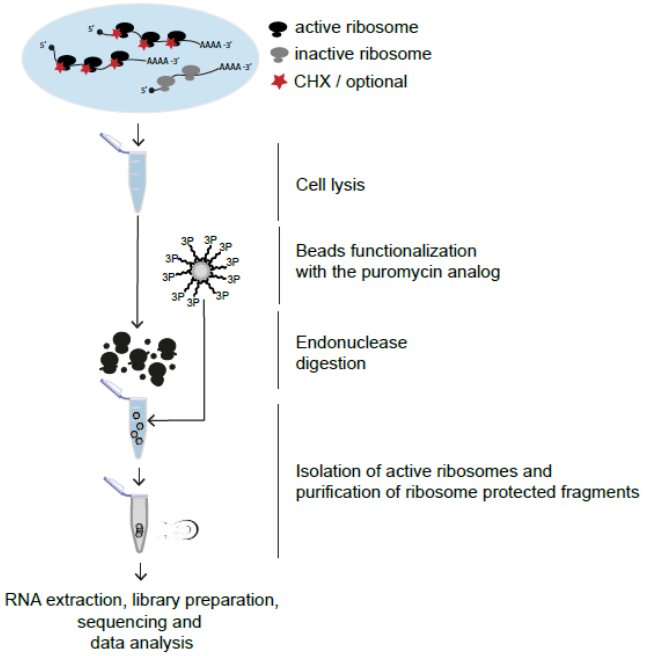

Figure 1: Schematic of the RiboLace protocol. Cells are pretreated with cycloheximide (CHX) to immobilize ribosomes. After cell lysis, the lysate is incubated with avidin-based magnetic beads functionalized with the puromycin analog. RiboLace beads are then washed and ribosomes isolated. Finally, ribosome-protected fragments are recovered for further library preparation, sequencing, and data analysis.

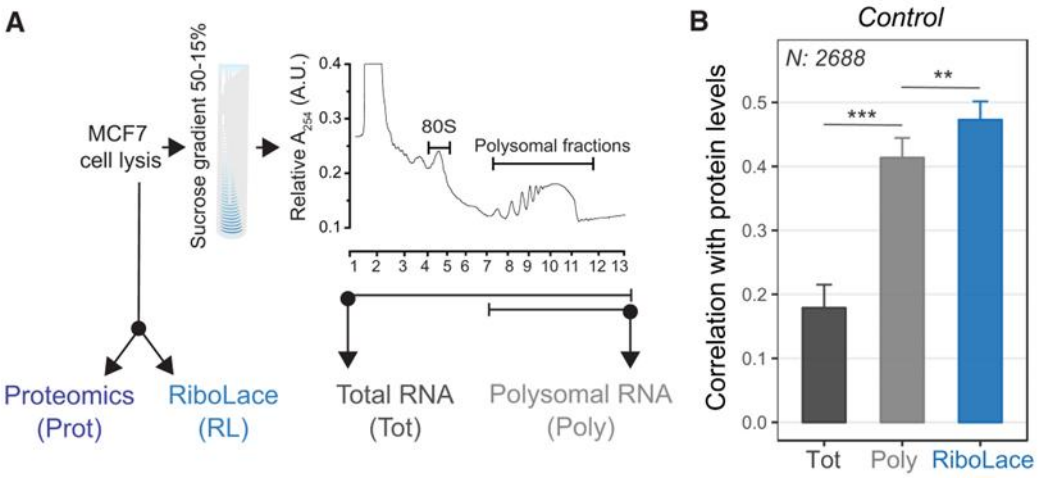

Figure 2 RiboLace yields better concordance of the transcriptome with the proteome. Pearson correlation analysis between MCF7 cell proteome and total RNA (correlation 0.18), polysomal RNA (correlation 0.41), and RiboLace RNA (correlation 0.48).

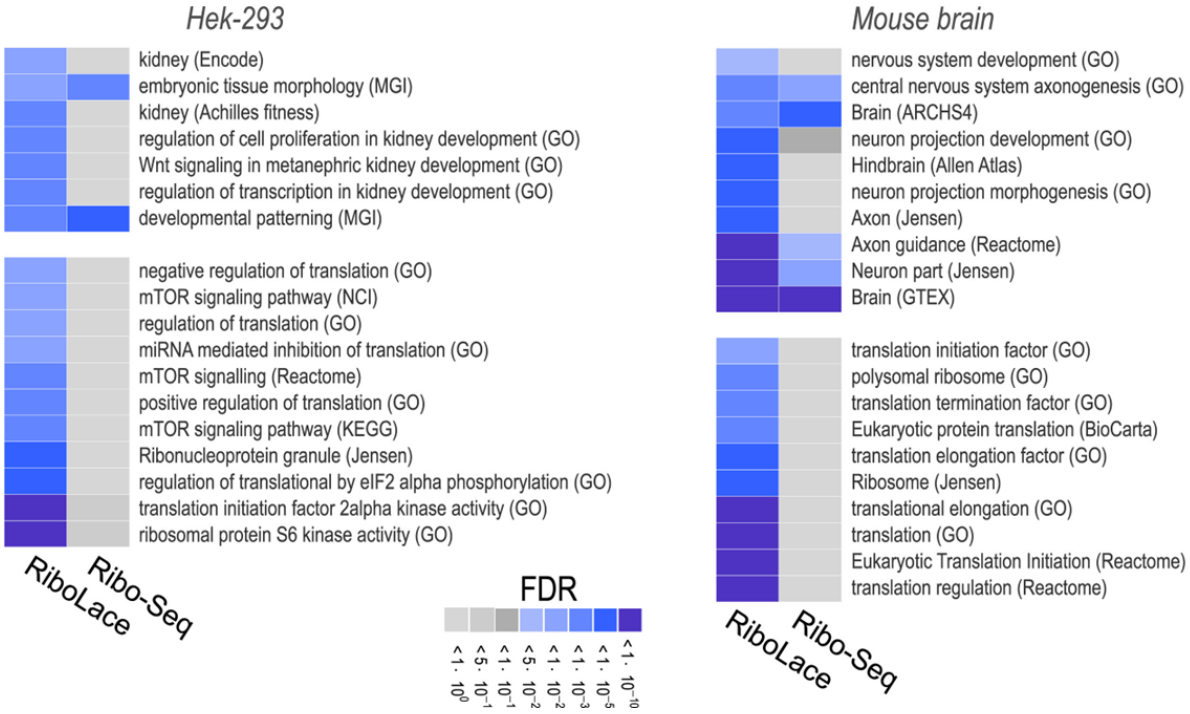

Fig. 3 Ribolace reveals significant differences in translation rates of individual genes: Comparisons of transcripts enriched in RiboLace and Ribo-seq in HEK293 cells and mouse brains. Heat map colors correspond to the degree of enrichment.

Simplicity and sensitivity, even with precious samples

With RiboLace, no antibodies or tags are required. Bead-based pull-down speeds and simplifies isolation and purification of active ribosomes. Capturing active ribosomes with RiboLace greatly improves data-to-noise ratios compared to RNA-seq and POL-seq. In addition, **RiboLace requires significantly less starting material** than conventional sucrose centrifugation or size-exclusion chromatography methods. A recent publication has used RiboLace to profile active ribosomes from 100 to 250 mouse oocytes or embryos[8].

Disome-seq possible with RiboLace

Since most mRNAs are associated with multiple ribosomes[9], and the speed of translational elongation depends on factors such as tRNA availability, codon usage, positively charged nascent peptides, and mRNA secondary structures, a slowdown of the leading ribosome might generate a collision between two active ribosomes close to each other (disome), which therefore results in a single RNase I resistant fragment with about twice the footprint length of a single ribosome.

The only current method to detect disomes is a long, error-prone and tedious procedure consisting of a sucrose gradient centrifugation [26] followed by disomes isolation and RNA extraction. Due to this technical limitation the genomic landscape and the sequence determinants of endogenous ribosome collisions are only partially known[10,11].

We developed a **gel-free RiboLace variant that coupled with a library preparation developed in-house (available upon request as a service) allows to profile disomes together with standard monosomes** without laborious additional experimental work. As expected, disome footprints (~68 nt) captured by RiboLace are double the size of the monosome-protected fragments (~34 nt) (Fig. 4A). It is known that disomes are quite widespread (about 5 % of ribosomes are trapped in disomes in fast-proliferating CHO cells[10]). Accordingly, our data showed that 80% of the transcripts that were detectable at the monosome footprint level showed robust, albeit lower disome coverage (data not shown). Both monosome and disome-protected fragments show an enrichment of P-sites in the first frame on the coding sequence but not in the UTRs (Fig. 4B) and both have a strong 3-nt periodicity on the protein coding sequences (Fig. 4C). Within the disomes, the P-site was computed for the 5' translating ribosomes. As a consequence, in the disome metaprofile (Fig 4C), the last peak appeared around 34 nt (~1 ribosome length) upstream the translation termination site, consistent with a disome formed by a translating ribosome colliding with a terminating ribosome

Ribosome profiling for the future

The capabilities of RiboLace to isolate and capture active ribosomes, RAPs, RPFs and nascent peptides are enabling new approaches to exploring translational control and ribosomal dysregulation. It's time for deeper exploration of the components of translation in action to reveal vital information about the influence of translational control on human disease.

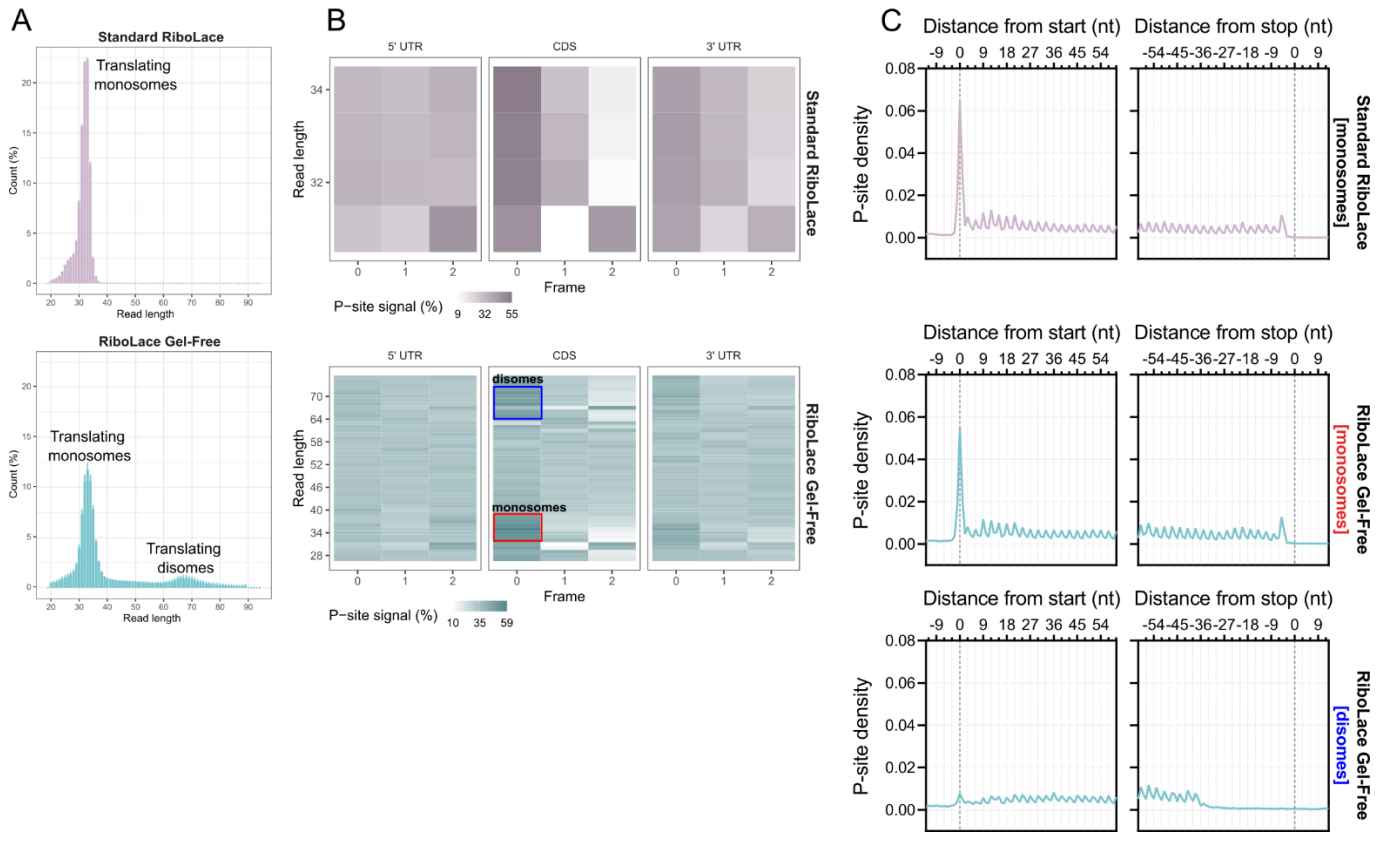

Fig. 4. Gel-free Ribolace captures both monosomes and disomes. Ribosome footprint data analysis. (A) Length distribution of ribosomes captured by standard RiboLace (pink) and RiboLace Gel-Free (green). (B) Percentage of P-sites corresponding to the three possible reading frames along the 5' UTR, CDS, and 3' UTR, stratified for read length. (C) Metaprofiles showing the density of P-sites around the translation initiation and termination sites for monosomes captured by standard RiboLace (pink) and for monosomes and disomes captured by RiboLace Gel-Free (green).

CASE STUDY RiboLace identifies a key spinal muscular atrophy protein as a ribosome-associated protein (RAP) with a role in spinal muscular atrophy pathogenesis[12]

Spinal muscular atrophy (SMA) is a neuromuscular disease that is caused by loss of survival motor neuron (SMN) protein, resulting in muscle atrophy and wasting. SMN protein is known to be involved in ribonucleoparticle biogenesis, but it may also play a significant role in translational control. In recent studies, **Lauria *et al.* used RiboLace to investigate the significance of SMN in translation dysregulation and ribosome-associated defects in SMA disease.** Initial RiboLace experiments revealed three key findings: 1) SMN is a ribosome-associated protein; 2) SMN positively regulates translation by binding to actively translating ribosomes; and 3) active ribosomes bound with SMN ("SMN-primed") predominantly occupy the first five codons of the transcripts.

To study the role that loss of SMN protein plays in causing defects in translation, the team analyzed positioning of the SMN-primed ribosome translation initiation site (P-site) on SMN-specific transcripts. In healthy control mice, SMN-specific transcripts that were isolated from only active ribosomes (using RiboLace) revealed predominant binding of P-sites within the first five codons of the transcripts (as previously observed). In comparison, isolation of both active and inactive ribosomes using RiboSeq showed lower resolution. In early-stage SMA mice, initiation sites of active ribosomes were tightly constrained to very early codons. The authors conclude that SMN-specific transcripts from early-stage SMA mice are defective in initiating translation (Fig. 5).

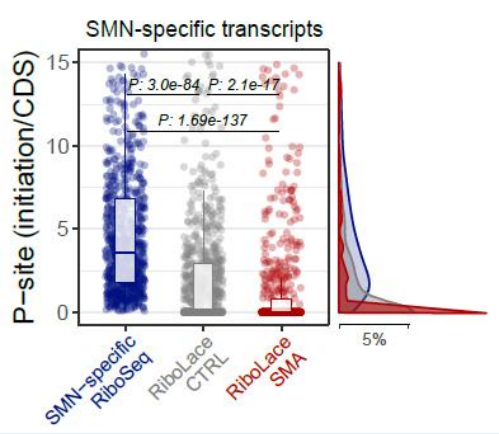

Fig 5: RiboLace reveals defects in positioning of active ribosomes on the first five codons in transcripts from early-stage SMA mice Comparison of P-site binding positions on SMN-primed ribosomes on SMN-specific transcripts. Blue: Signal from transcripts (n=874) isolated from control mice using classical RiboSeq (i.e., both active and inactive ribosomes). Grey: Transcipts (n=859) isolated from control mice using RiboLace (i.e. active ribosomes only). Red: Transcipts (n=774) isolated from early-stage SMA mice using RiboLace

The team then investigated the impact of SMN loss on specific transcripts that were grouped according to different functional roles associated with SMN and specific defects in SMA disease. **They used RiboLace to isolate active ribosomes from mice with early symptoms of SMA and from healthy control mice.** The results revealed that in early-SMA mice, binding of SMN-primed ribosomes to SMN-specific mRNAs is significantly diminished across all functional groupings compared to the control mice (Fig. 6). These studies reveal that SMN is a master modulator of ribosomal flow on a subset of specific mRNAs that are relevant to SMA disease. The authors conclude that RAPs have an important role in regulating disease pathogenesis in SMA and related conditions.

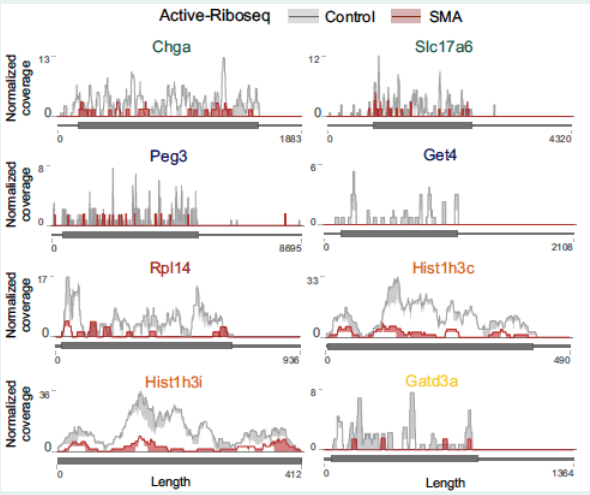

Fig. 6: RiboLace reveals diminished ribosome binding in SMA mice Tracks show coverage of active SMN-primed ribosomes on eight selected SMN-specific transcripts representing different functional roles associated with SMA. Active SMA-primed ribosomes were isolated using RiboLace. Red: early-SMA mice. Grey: control mice. Grey bar below each profile shows the boundaries of the coding sequence and 5'UTR regions.

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Ordering information

Product	Quantity	Cat. no
RiboLace Mod. 1	9 rxns	RL001_Mod1
RiboLace XL	9 rxns	RL001_XL
RiboLace Gel-Free	*Available as a service*	-
Ribosome Profiling All-In-One Set	9 rxns	RS-001s
PAGExt	18 rxns	KGE-002
LACEseq	9 rxns	LS001
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