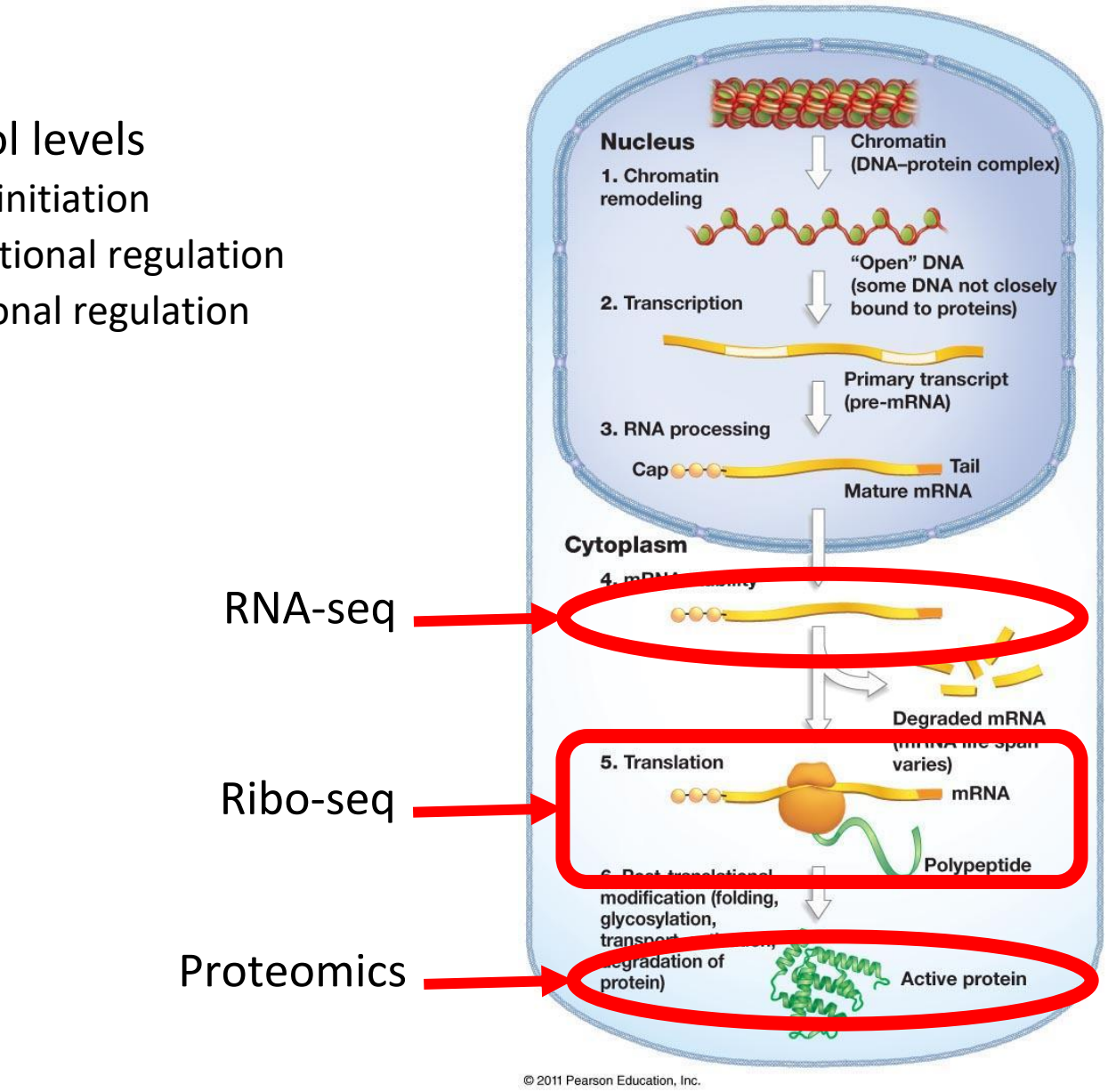
Traductomica como herramienta para comprender mecanismos regulatorios

Dr. José Sotelo

Gene expression

- Multiple control levels
  - Transcription initiation
  - Post-transcriptional regulation
  - Post-translational regulation



Gene expression

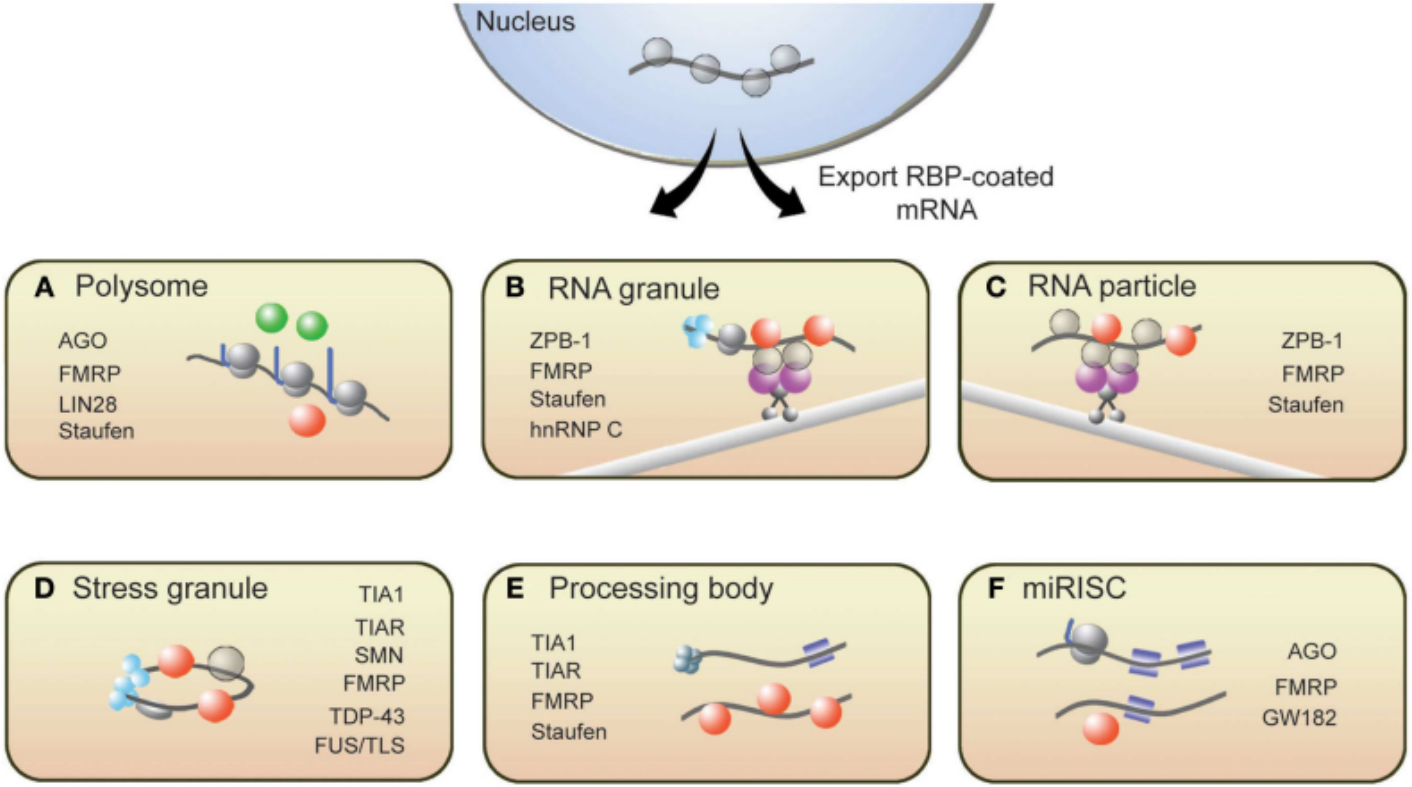
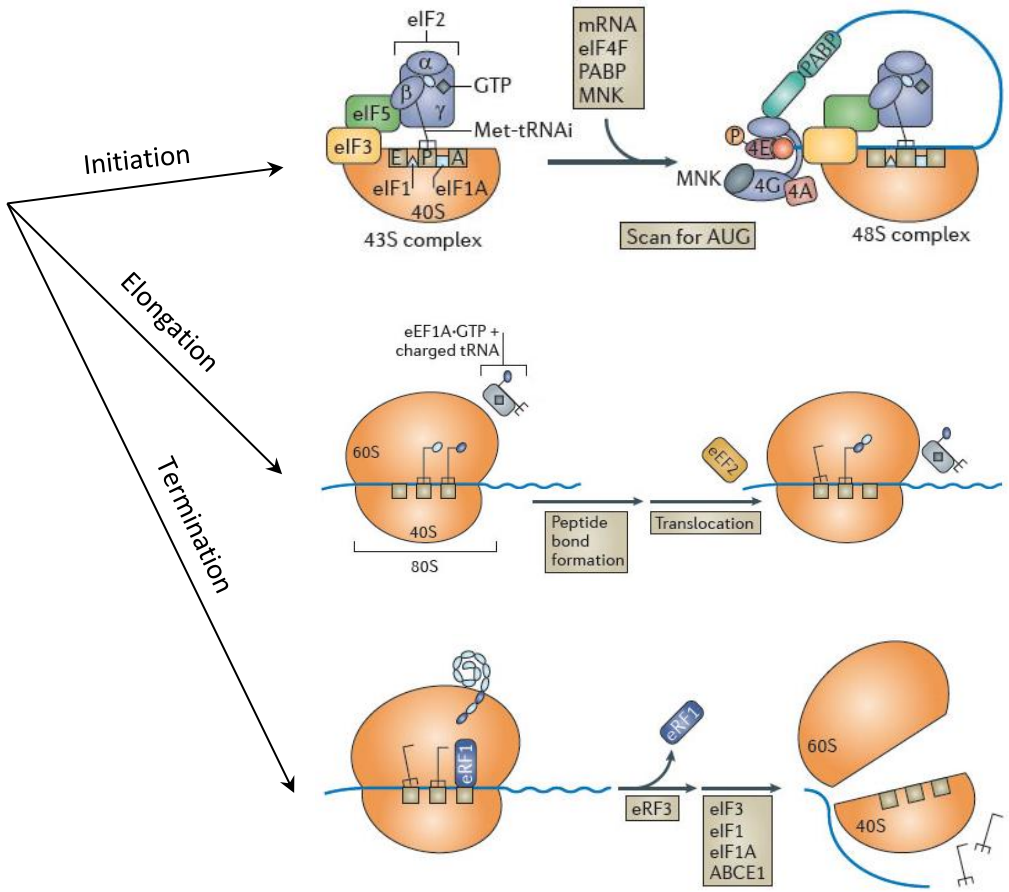


FIGURE 1 | Messenger RNAs associate with several RNP structures that influence their translational state. (A) Polysomes, sites of translation, contain RBPs that activate (green spheres) or repress (red spheres) translation. Following synthesis and processing, mRNA is exported from the nucleus and transported throughout the cell along microtubules via **(B)** RNA granules and **(C)** RNA particles. Repressor RBPs (red spheres) are present within RNA particles to ensure that mRNAs are not translated during transit. Messenger RNAs within RNA granules are associated with translation initiation machinery (light blue spheres) including ribosomes, suggesting that translation has commenced but is halted during transit. The translational fate of mRNA is dictated in part by the RBPs bound to them. If targeted by repressor RBPs or miRISCs (blue squares), mRNAs will associate with **(D)** stress granules, **(E)** processing bodies, or **(F)** miRISC structures resulting in either degradation or translational repression. Some RBPs present in neuronal RNP complexes are listed.

Kapeli; *Frontiers in Neuroscience* (2012)

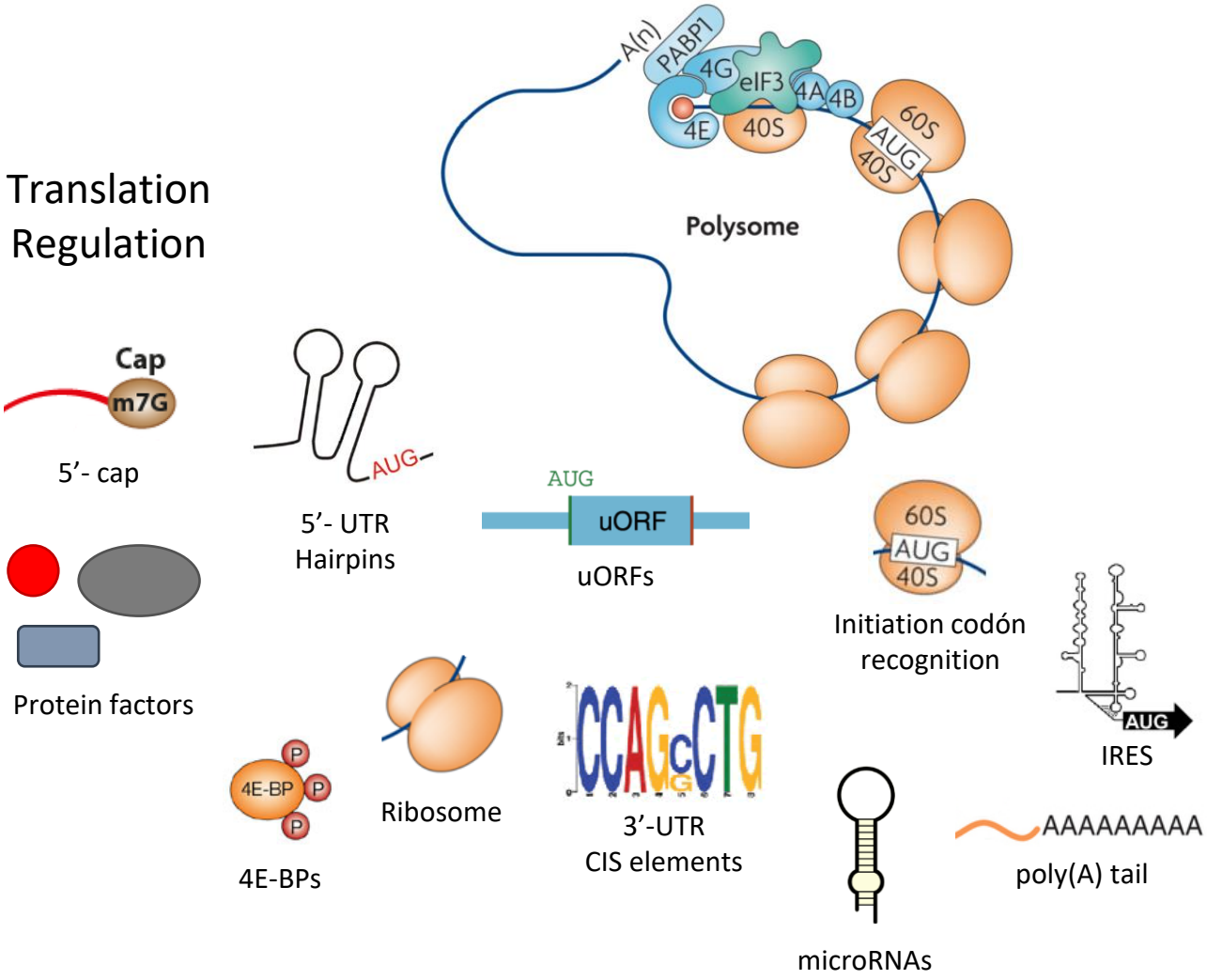
Protein Translation

Translation regulation



Walsh & Mohr; *Nature Reviews Microbiology* (2011)

Regulatory elements



Anderson & Kedersha, 2009 *Nature*

Study of polysomal RNA

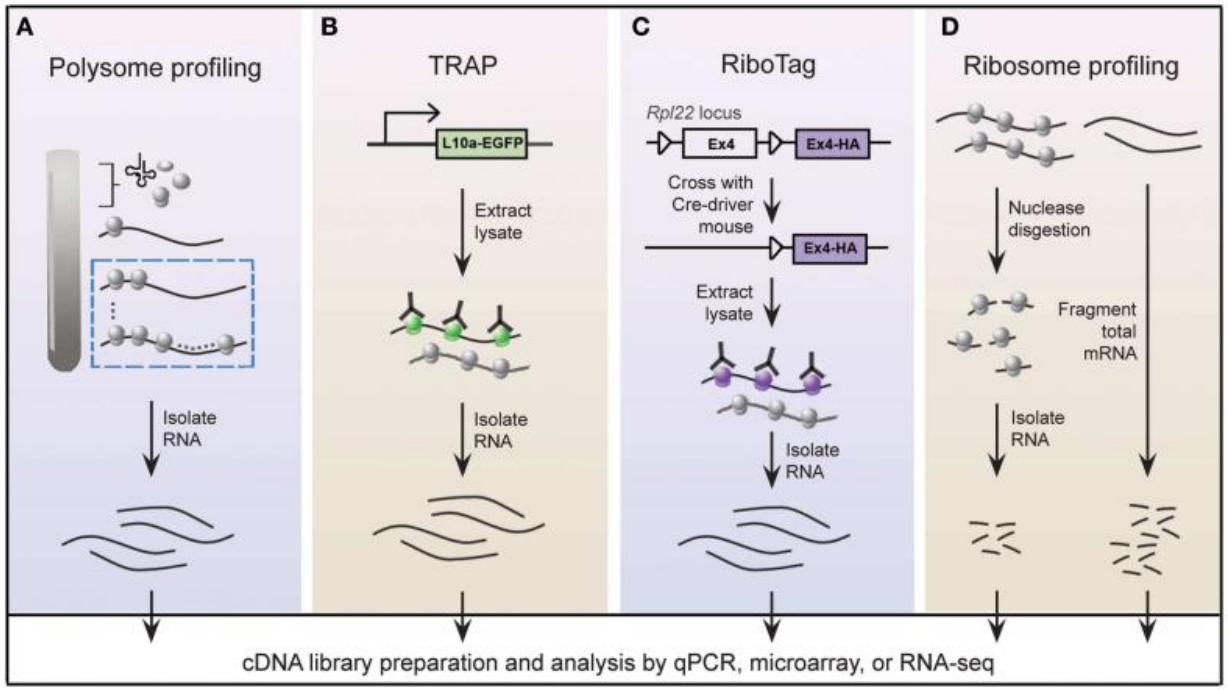


FIGURE 2 | Schematic of genome-wide methods to study polysome-associated mRNAs *in vitro* and *in vivo*. (A) With polysome profiling, cytoplasmic lysates from cells are layered onto a sucrose gradient and undergo centrifugation to separate tRNAs, 40S, 60S, and 80S ribosomes, and polysomes. Messenger RNAs from fractions corresponding to polysomes (dashed blue box) are isolated and identified by various approaches. **(B)** Engineered bacTRAP mice drive expression of EGFP-tagged L10a, a ribosomal protein found in polysomes (green ribosomes), from promoters that are activated in specific cells of the central nervous system. EGFP-L10a-mRNA complexes are immunopurified from brain tissue from bacTRAP mice, and associated mRNAs are identified by various techniques. **(C)** The RiboTag mouse carries an Rpl22 allele with a floxed wild-type C-terminal Exon4 followed by a HA-tagged Exon4. When the RiboTag mouse is crossed with a mouse expressing Cre-recombinase in a cell-type specific manner, Cre-recombinase activates expression of HA-tagged Rpl22, which incorporates into polysomes (purple ribosomes). Homogenized tissues from the offspring are subjected to co-immunoprecipitation using antibodies against HA, and associated mRNAs are identified by various techniques. **(D)** Using ribosome profiling to identify ribosome occupancy on mRNAs, cycloheximide-treated lysates from cultured cells are digested by micrococcal nucleases to remove mRNA sequences that are not bound by ribosomes (left). The resulting monosome complexes are purified by ultracentrifugation through a sucrose gradient or cushion. Ribosome-protected fragments are recovered and deep sequenced. In parallel, total mRNA from a similar preparation of cycloheximide-treated lysate is fragmented and deep sequenced (right), and serves as a normalizing control.

Kapeli; *Frontiers in Neuroscience* (2012)

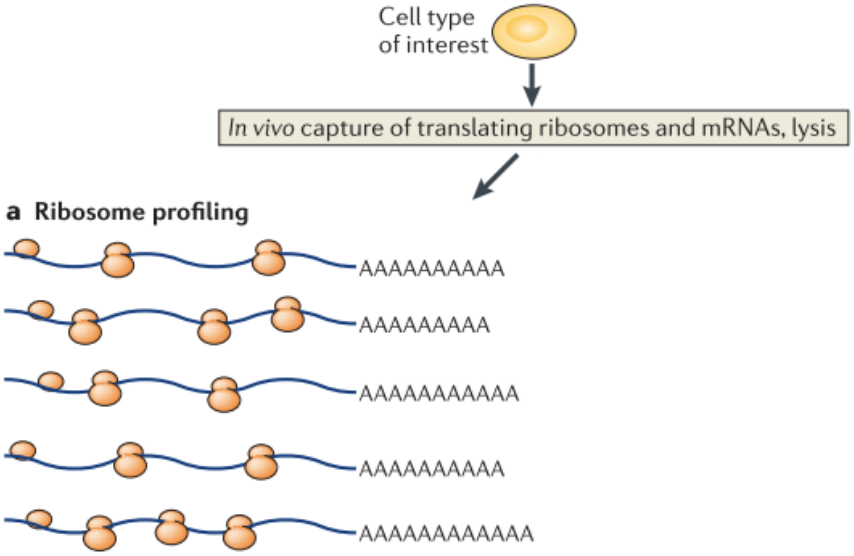
Ribosome profiling

Published in final edited form as:
Science. 2009 April 10; 324(5924): 218–223. doi:10.1126/science.1168978.

Genome-Wide Analysis in Vivo of Translation with Nucleotide Resolution Using Ribosome Profiling

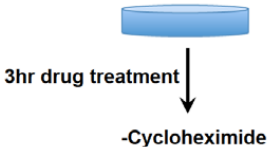
Nicholas T. Ingolia[*], **Sina Ghaemmaghami**[†], **John R. S. Newman**, and **Jonathan S. Weissman**
Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, University of California, San Francisco, and California Institute for Quantitative Biosciences, San Francisco, CA 94158, USA.

Ribosome profiling



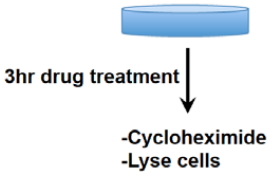
Ribosome profiling: Method

- Cell culture and ribosome stalling
  - *Cicloheximide*
  - Harringtonine, lactimidomycin
  - Rapid freeze



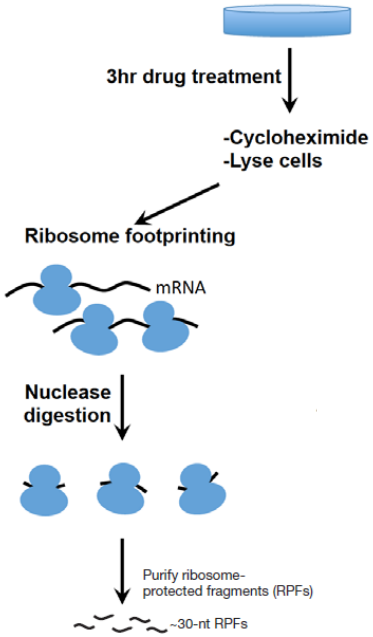
Ribosome profiling: Method

- Cell culture and ribosome stalling
  - *Cicloheximide*
  - Harringtonine, lactimidomycin
  - Rapid freeze
- Lysis



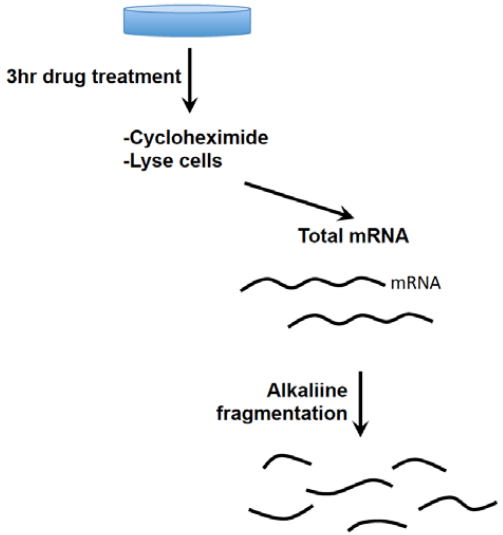
Ribosome profiling: Method

- Cell culture and ribosome stalling
  - *Cicloheximide*
  - Harringtonine, lactimidomycin
  - Rapid freeze
- Lysis
- *Ribosome Footprinting*
  - RNAse treatment
  - Sucrose gradient utracentrifugation
  - RNA extraction
  - Size selection (PAGE 15% + urea) 28-34nt



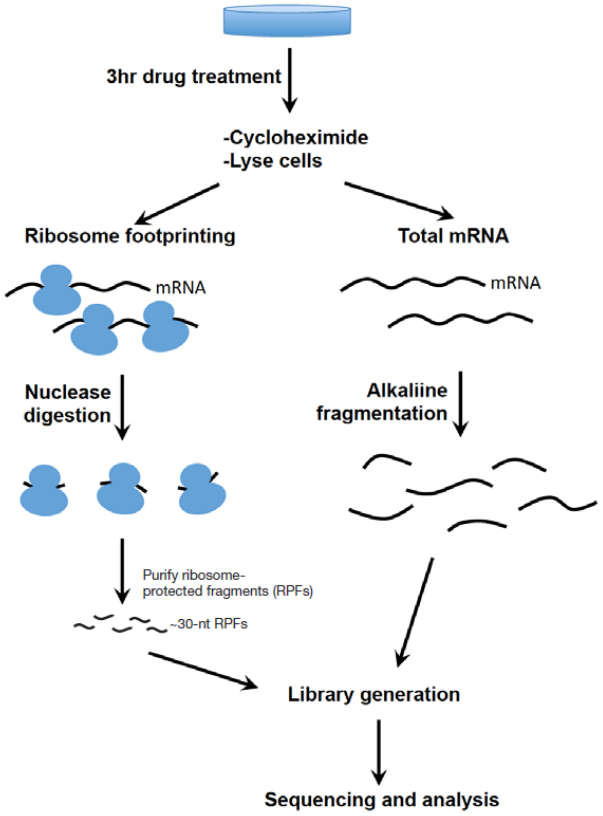
Ribosome profiling: Method

- *mRNA-Seq*
  - Total RNA extraction
  - poly(A) mRNA purification
  - Fragmentation
  - Size selection (PAGE) 50-80nt

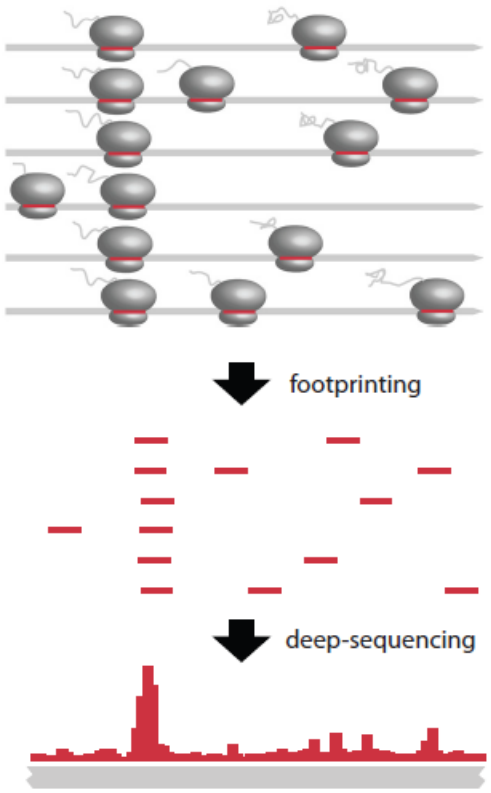


Ribosome profiling: Method

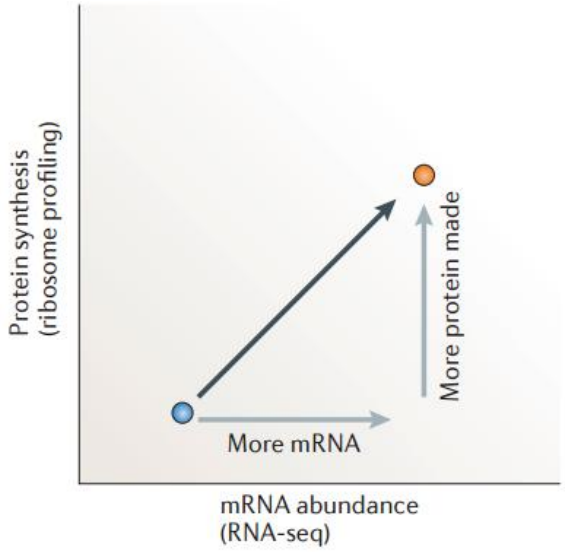
- *mRNA-Seq*
  - Total RNA extraction
  - poly(A) mRNA purification
  - Fragmentation
  - Size selection (PAGE) 50-80nt
- Library generation
  - T4 dephosphorilation
  - Adapter linking and purification
  - (for RFPs: rRNA depletion)
  - Reverse transcription
  - PCR amplification
- Sequencing and analysis

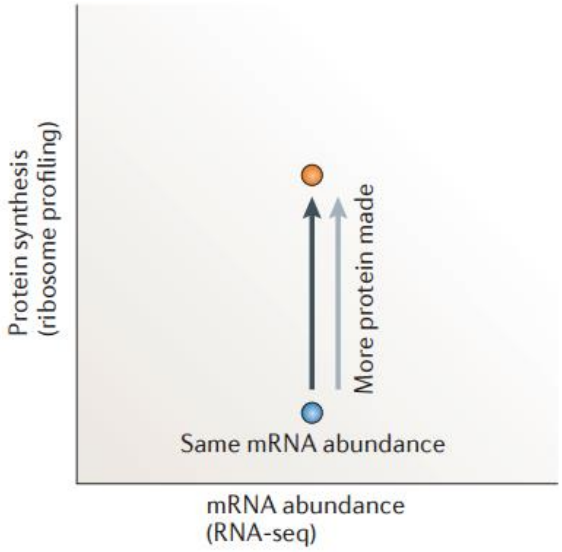


Ribosome profiling: Results



$$\text{Translation efficiency} = \frac{\text{Translation levels}}{\text{Steady state mRNA levels}}$$



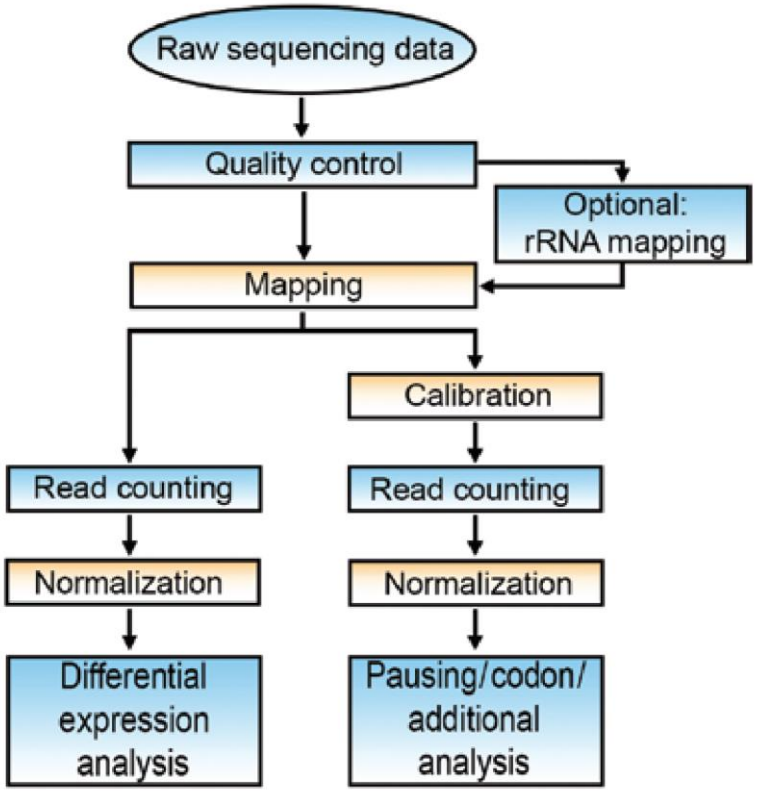


- Allows
  - Precise quantification of the proportion of mRNA actually bound to polysomes
  - The study of ribosome dynamics during translation

Ingolia, 2014 Nature Reviews

Analysis pipeline

- Similar to RNA-seq, so many standard tools can be used
- Additional information and issues
  - %rRNA removal
  - Footprint periodicity
  - Mapping coordinates
  - Size range



A. Bartholomäus, et al. 2015

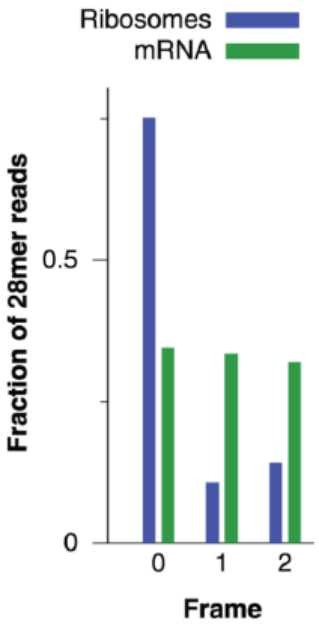
Specific software

Software available to analyze, interpret and visualize RP-derived data.
A list of some of the software used to analyze RP data is briefly described, indicating its main features and the adequate environment to use it.

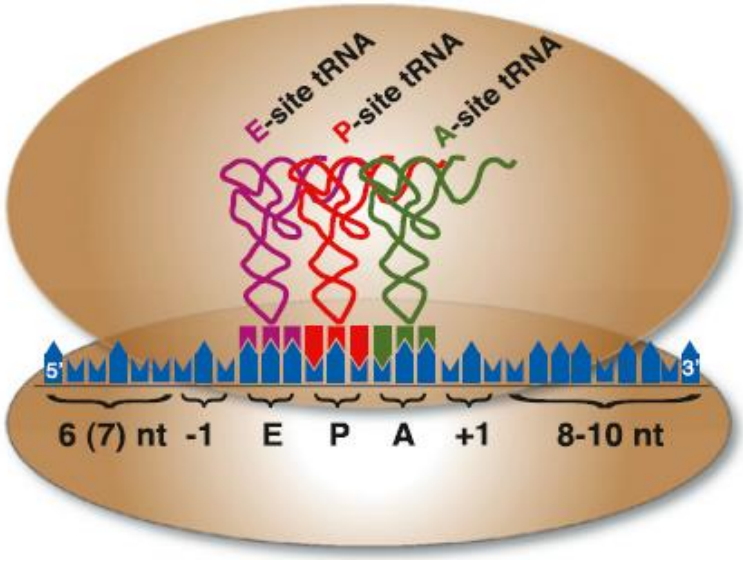
Name	Functions/description	Enviroment	Ref.
riboSeqR	Parsing data, align reads, plotting functions, frameshift detection and inferring alternative ORFs.	R	[101]
RiboProfiling	Quality assessment, read start position recalibration, counting of reads on CDS, 3′UTR, and 5′UTR, plotting of count data: pairs, log fold-change, codon frequency and coverage assessment, principal component analysis on codon coverage.	R	[102]
RiboGalaxy	On-line tools for the analysis and visualization of ribo-seq data (some of them use riboSeqR)	Galaxy webserver	[103]
Plastid	A handful of scripts for common high-throughput sequencing and ribosome profiling analyses, like: determining P-sites offsets	Python Library	[104]
Ribomap	Generates isoform-level ribosome profiles from ribosome profiling data	Unix	[105]
RiboTraper	Identifies translated regions	Unix	[106]
Rfoot	Identifies RNA regions protected by non-ribosomal protein complex present in Ribo-Seq data	Perl	[107]
anota	Analysis of differential translation and results visualization	R	[108]
RiboDiff	An statistical tool to detect changes in protein translation efficiency	Unix	[109]
Xtail	An analysis pipeline that identifies differentially translated genes in pairwise comparisons	R	[110]
RiboTools	Detection of translational ambiguities, stop codon readthrough events and codon occupancy. Provides plots for the visualization of these events.	Galaxy webserver	[111]
Proteoformer	Genome-wide visualization of ribosome occupancy and a translation initiation site calling algorithm. A protein database can be incorporated to increase protein identification	Galaxy webserver	[112]
ORFscore	Small ORF identification	In SPECTtre [106]; python	[75]
ORF-RATER	Coding sequence annotation	Python	[113]
FLOSS	A metric for distinguishing between 80S footprints and nonribosomal sources using footprint size distributions	In SPECTtre [106]; python	[61]
tRanslatome	Analysis of transcriptome, translatome and proteome data: Differentially expressed genes detection, gene ontology enrichment comparison and analysis of regulatory elements	R	[114]
TranslatomeDB	Differential gene expression, translation ratio, elongation velocity index and translational efficiency. Also comparision with other RP experiments can be done	Online	[115]
systemPipeR	Filter/trim sequences, quality control, alignments, counting, peak detection, differentially expressed genes detection, enrichment, classification, several reports and graphs	R	[116]

G. Eastman, et al. 2018

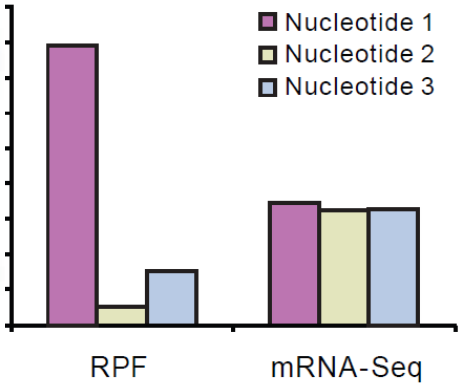
Footprint periodicity



Yeast

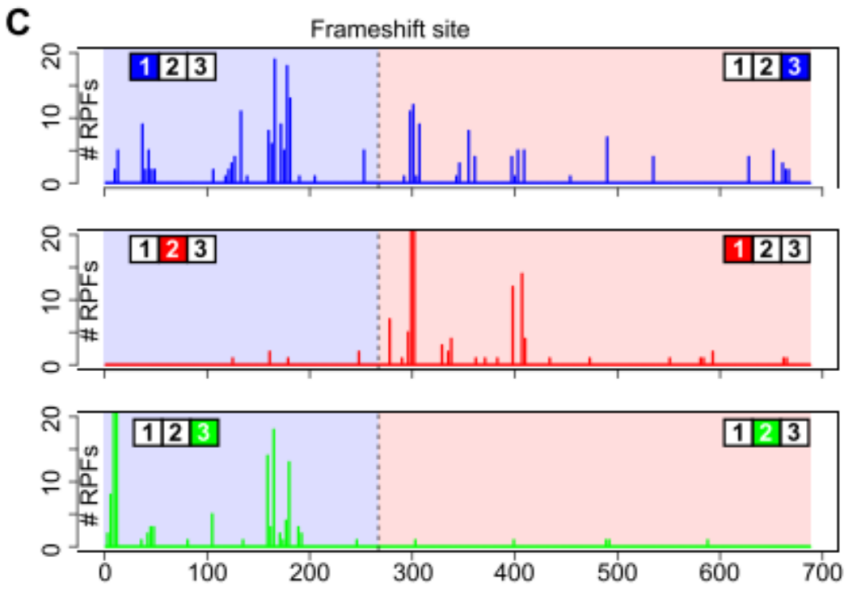


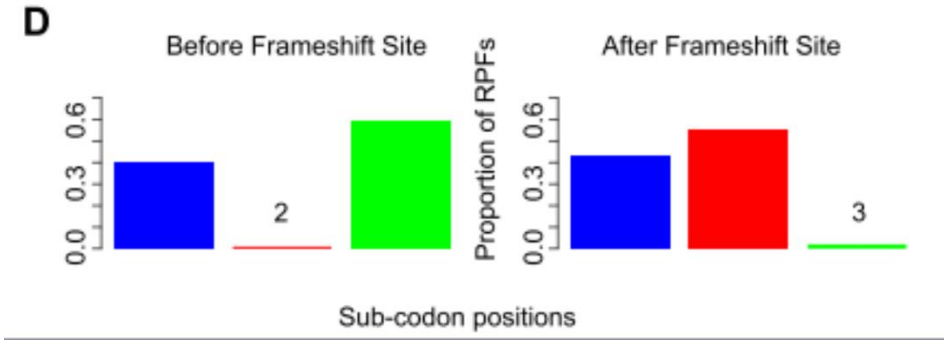
HeLa



Mice

Footprint periodicity

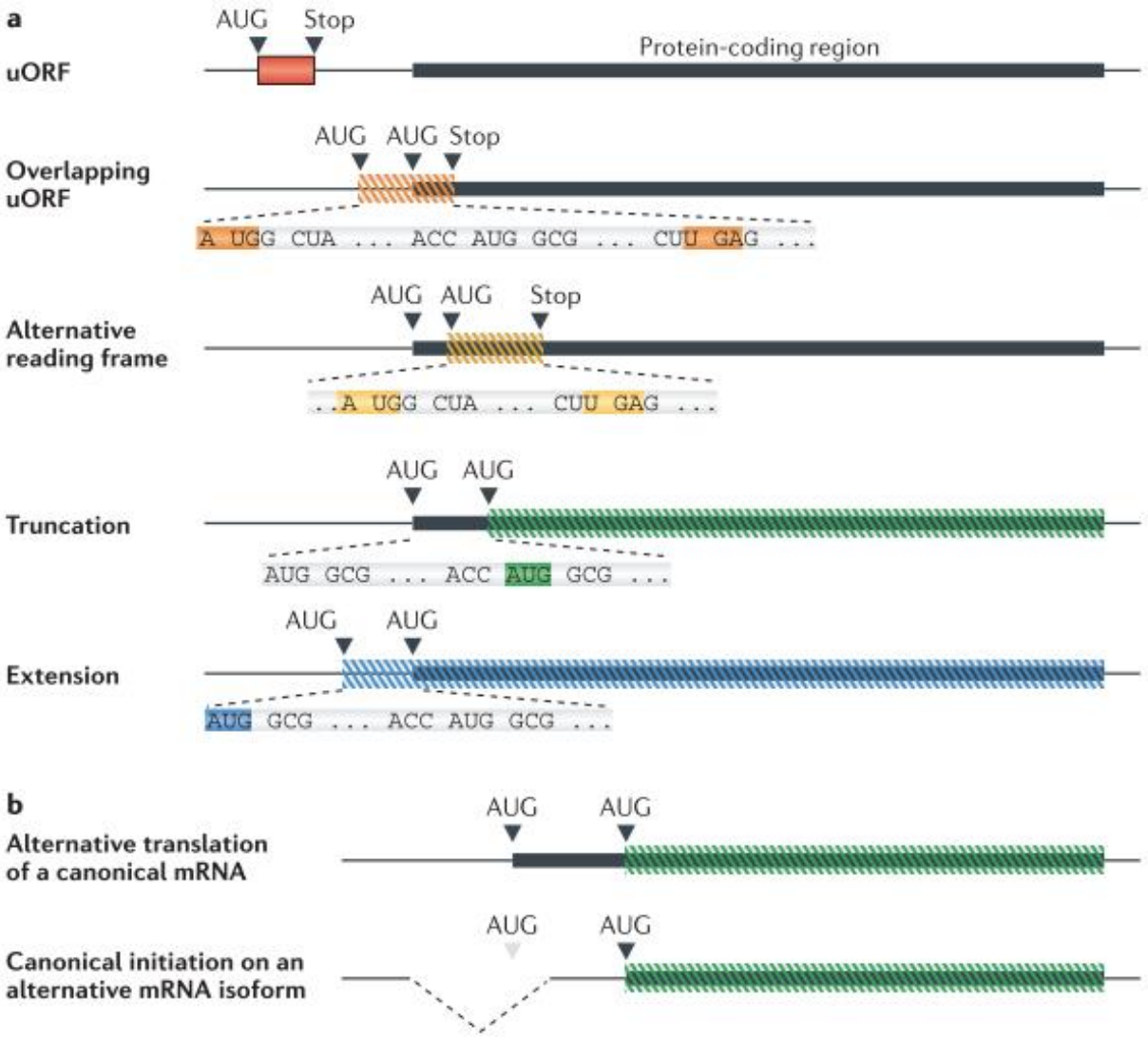




Frameshift detection

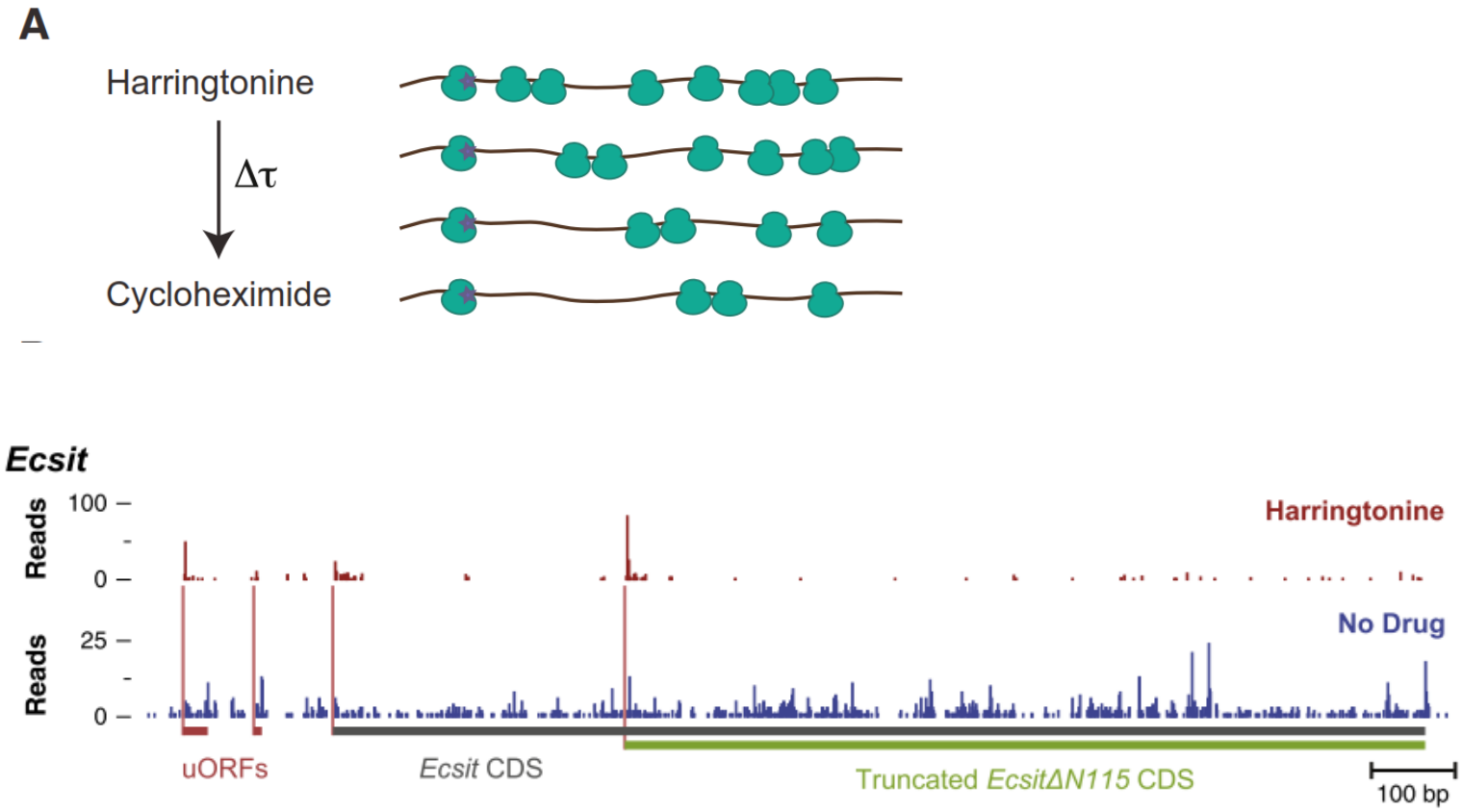
Michel, *et al.* 2012 Genome Research

Alternative Reading frames



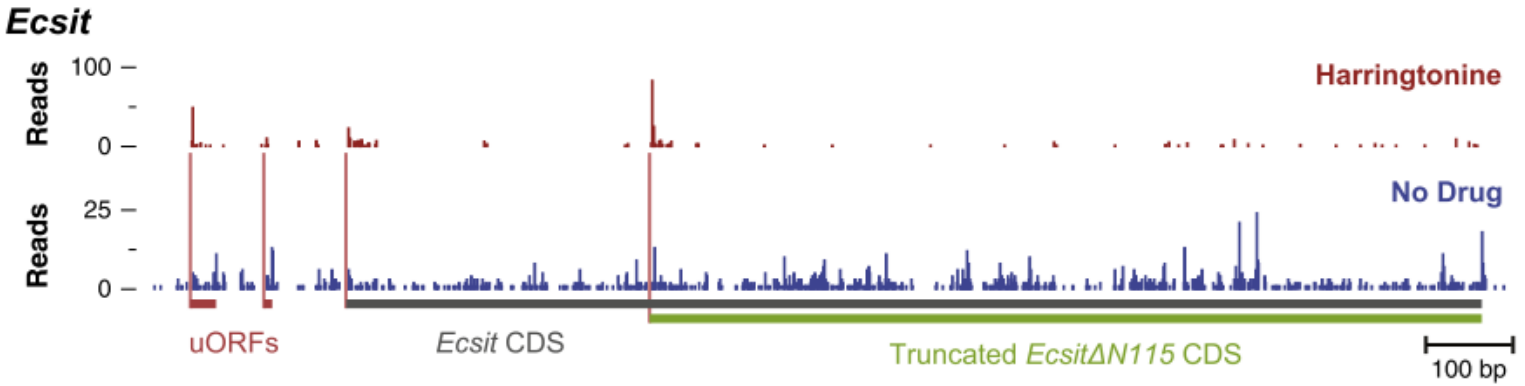
Ingolia, 2014 Nature Reviews Genetics

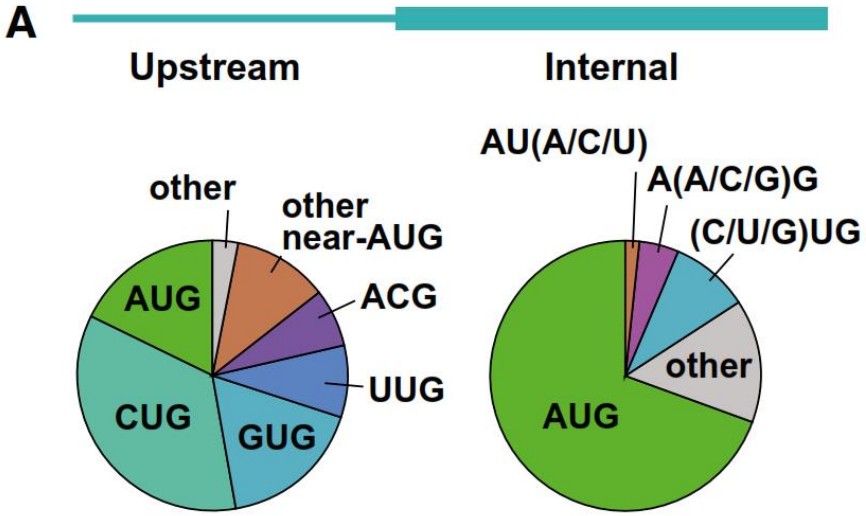
Initiation site determination and uORFs detection



Ingolia, Cell 2011

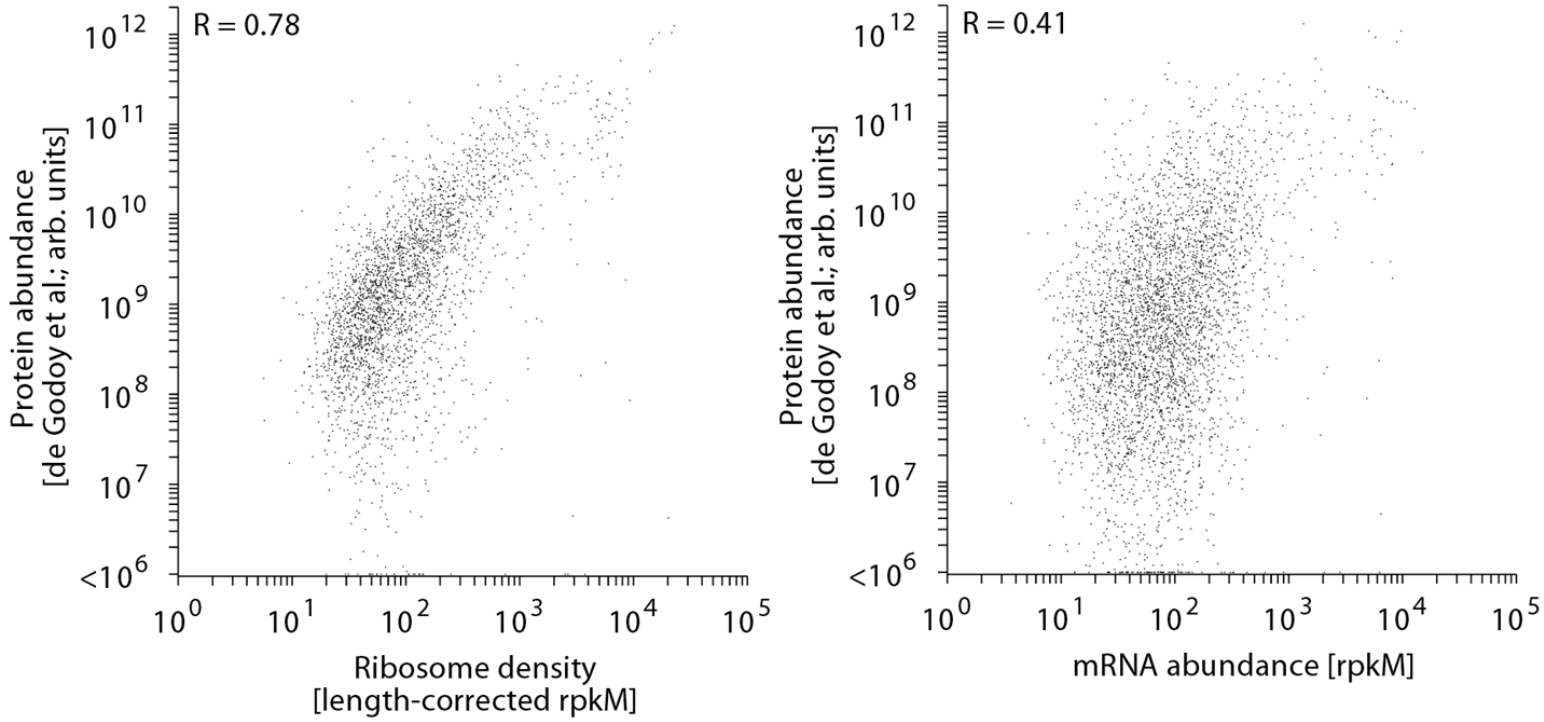
Initiation site determination and uORFs detection



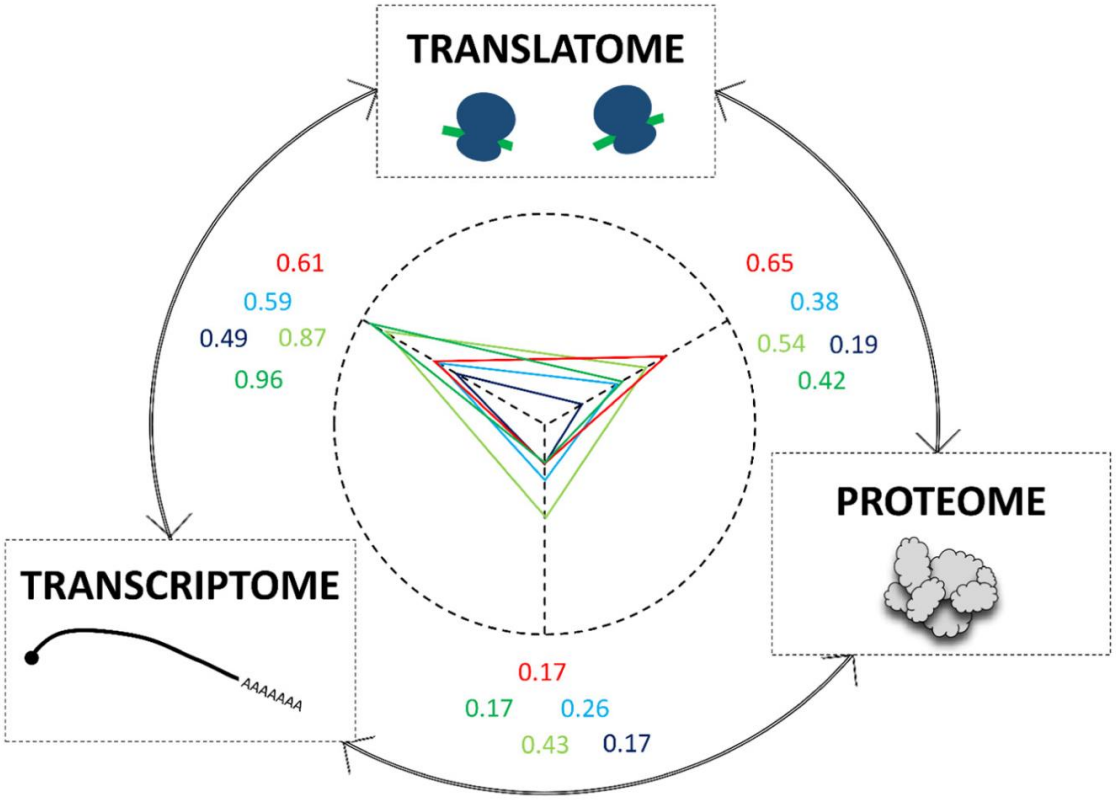


Ingolia, Cell 2011

Correlations with proteome



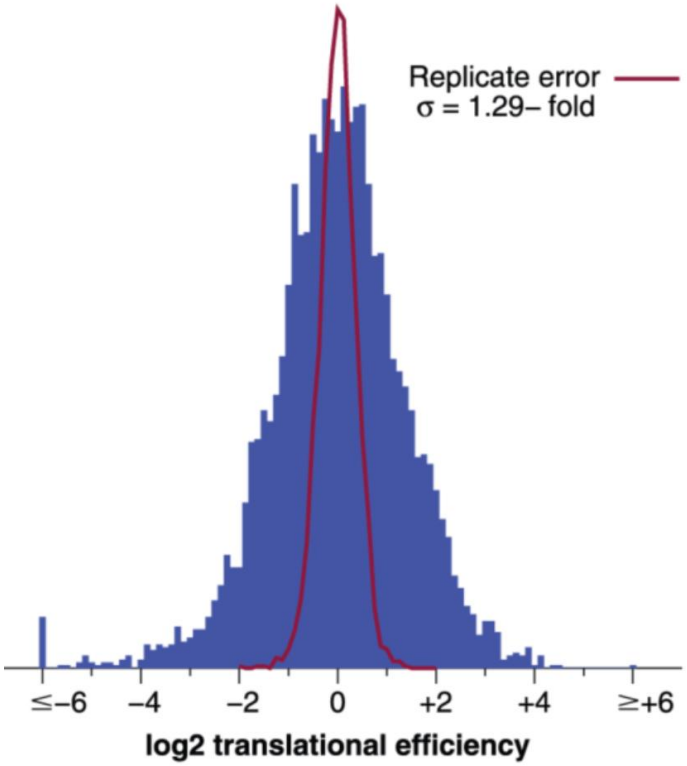
Correlations with proteome

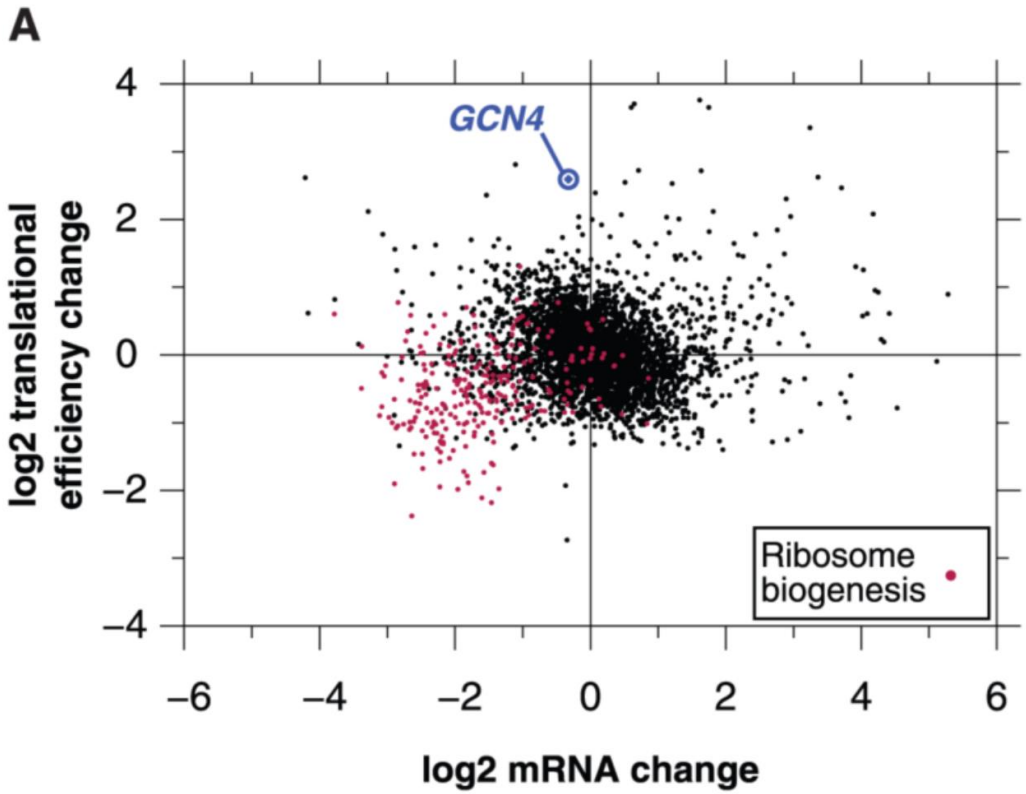


Authors	*Journal*	*Year*	*Organism*	*Correlation score*
Ingolia	Science	2009	Yeast	R^2
Smircich	BMC Genomics	2015	Trypanosoma cruzi	Pearson R^2
Cenik	Genome Research	2015	Lymphoblastoid cell lines	Spearman ρ
Wang & Sun	Genome Biol Evol	2015	Yeast	Pearson R^2
Miranda-CasoLuengo & Staunton	BMC Genomics	2016	Mycobacterium abscessus	Pearson R^2

Eastman, *et al.* 2018

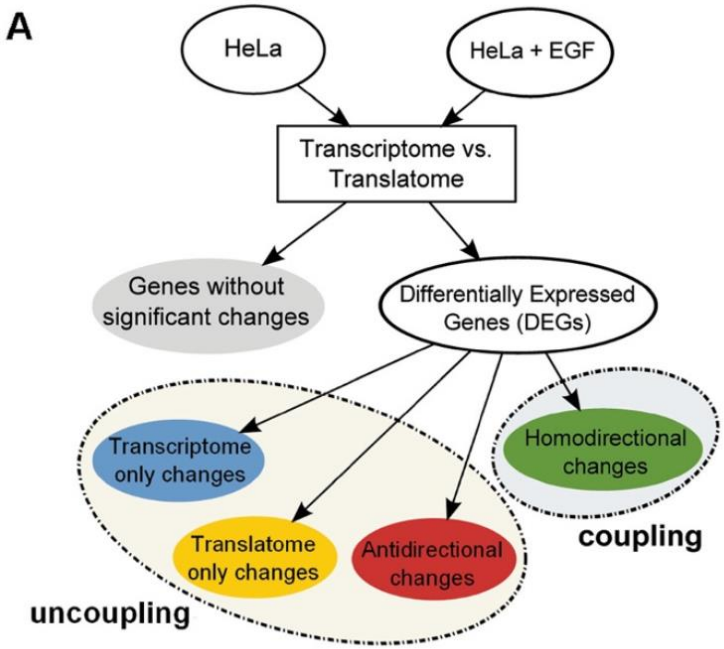
Translation regulation

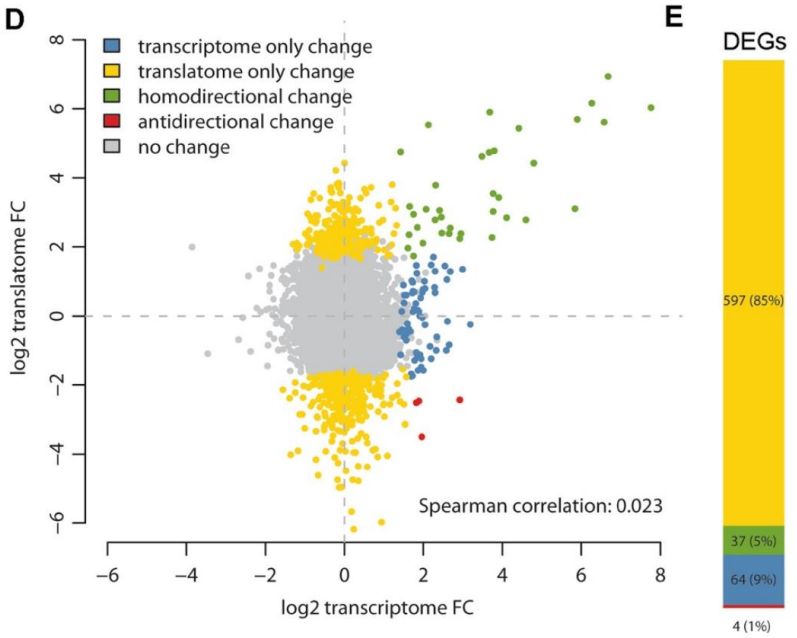




Ingolia, Science 2009

Translation regulation



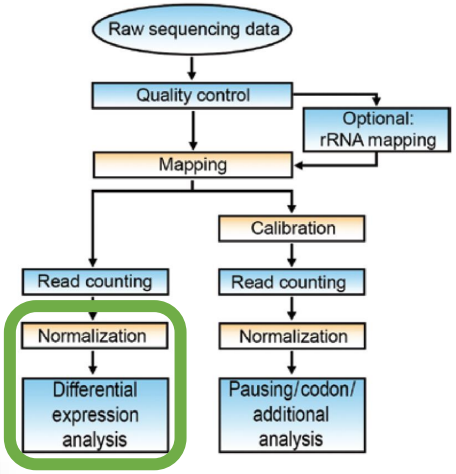


- Translation regulation is a major contributor of gene expression levels
  - Most important for rapid response to environmental signals

Tebaldi BMC Genomics 2012

Translation regulation: RiboDiff and Riborex

They take into account the dependence of translatome leveles on transcriptome levels



RiboDiff | Detecting Changes of mRNA Translation Efficiency from Ribosome Footprints

Riborex: fast and flexible identification of differential translation from Ribo-seq data

Wenzheng Li[1], Weili Wang[1], Philip J. Uren[1], Luiz O. F. Penalva[2,3] and Andrew D. Smith[1,*]

Galaxy / Rätsch Lab

RStudio

- edgeR
- DESeq2
- Voom

Y. Zhong, et al. 2017

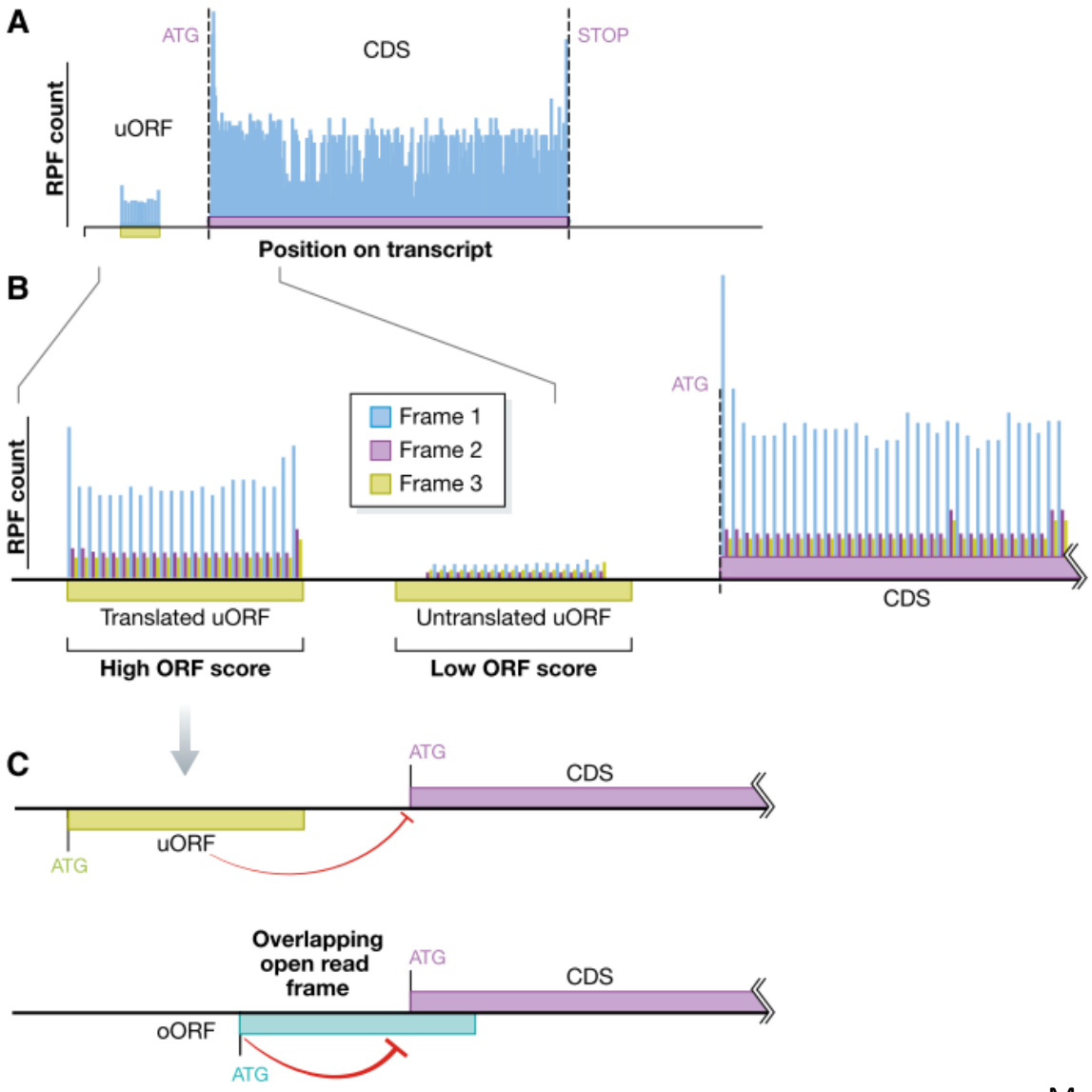
W. Li, et al. 2017

A. Bartholomäus, et al. 2015

https://public.bmi.inf.ethz.ch/user/zhongy/RiboDiff/

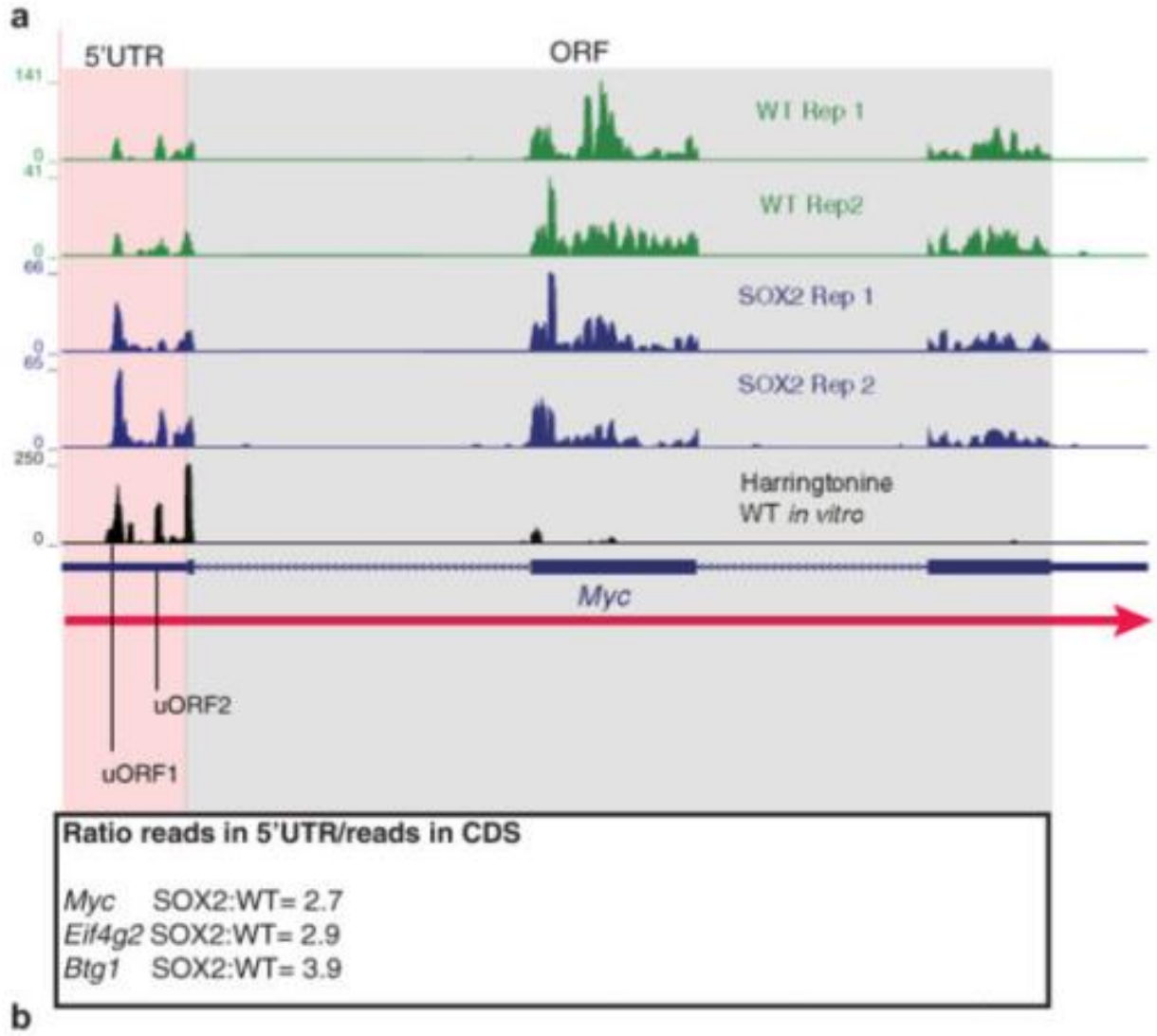
https://github.com/smithlabcode/riborex/blob/master/DESCRIPTION

Translation regulation: uORFs



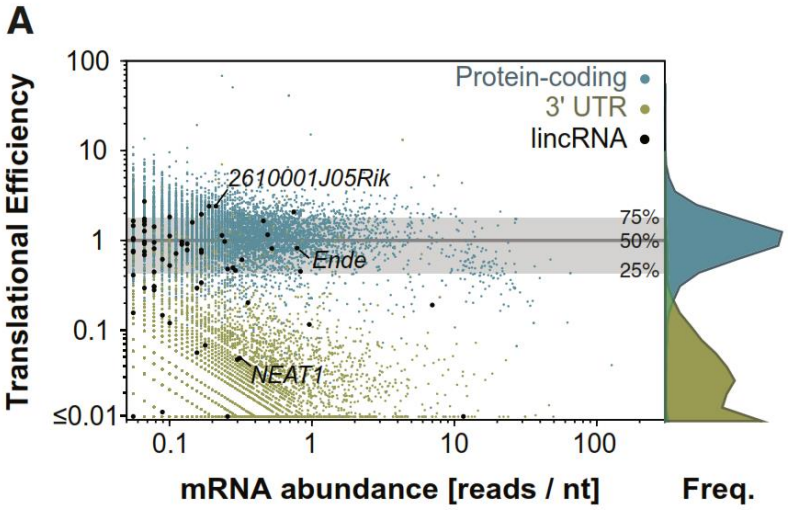
McGeachy, EMBO J 2016

Translation regulation: uORFs



Sendoel, Nature 2017

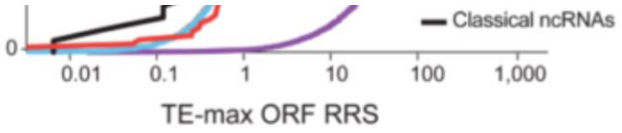
Non-coding RNA tranlslation

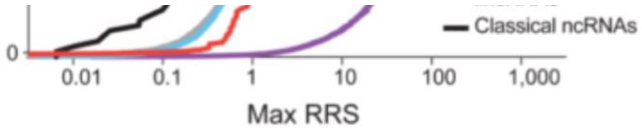


- lincRNAs have ribosome occupancies similar to CDS
- Other specific characteristics of translation are not present
  - Some exceptions

Cell Reports
Article

Ribosome Profiling Reveals Pervasive Translation Outside of Annotated Protein-Coding Genes





Ingolia Cell 2011
Guttman Cell 2013
Ingolia Cell 2014

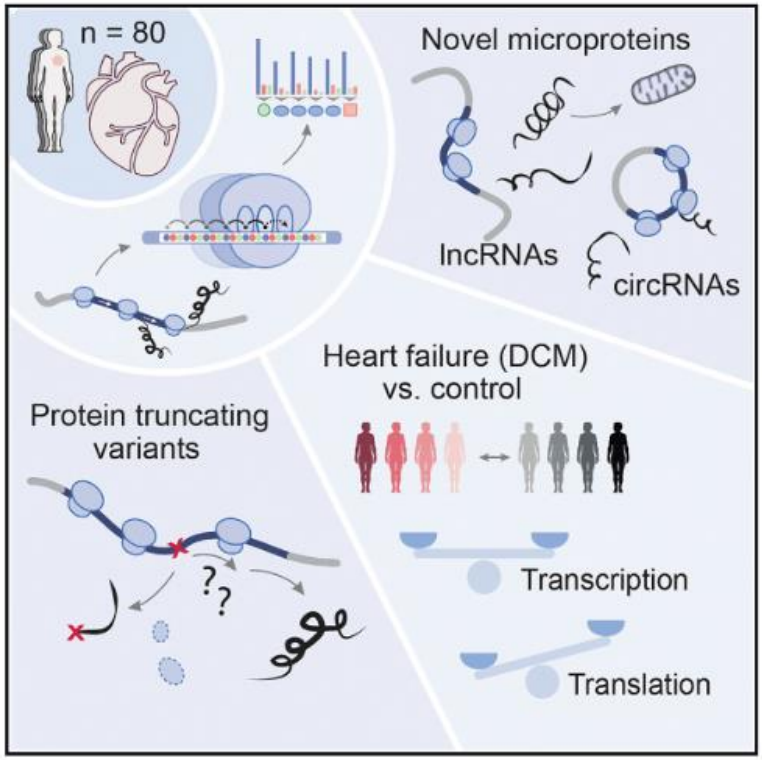
Non-coding RNA tranlslation

Resource

Cell

The Translational Landscape of the Human Heart

Graphical Abstract



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Correspondence

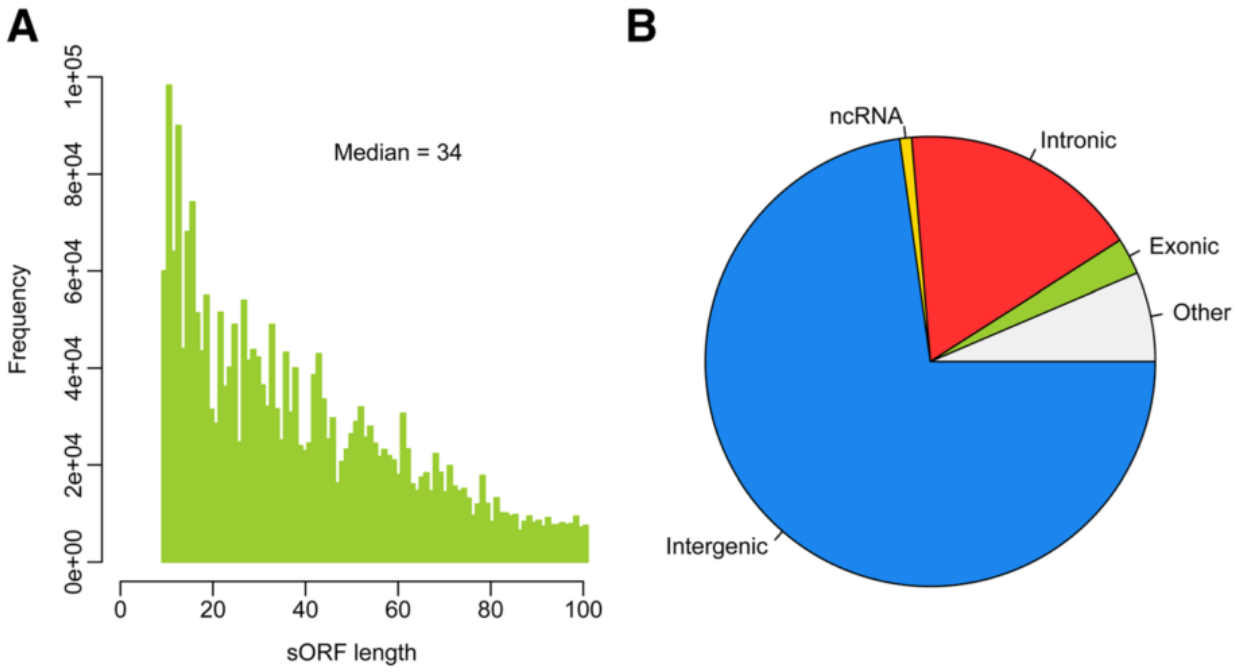
sebastiaanvanheesch@gmail.com (S.v.H.), nhuebner@mdc-berlin.de (N.H.)

In Brief

Translational profiling in a primary human tissue reveals frequent translation downstream of predicted disease-causing variants as well as translation of hundreds of microproteins from long noncoding RNAs and circular RNAs.

Heesch, Cell 2019

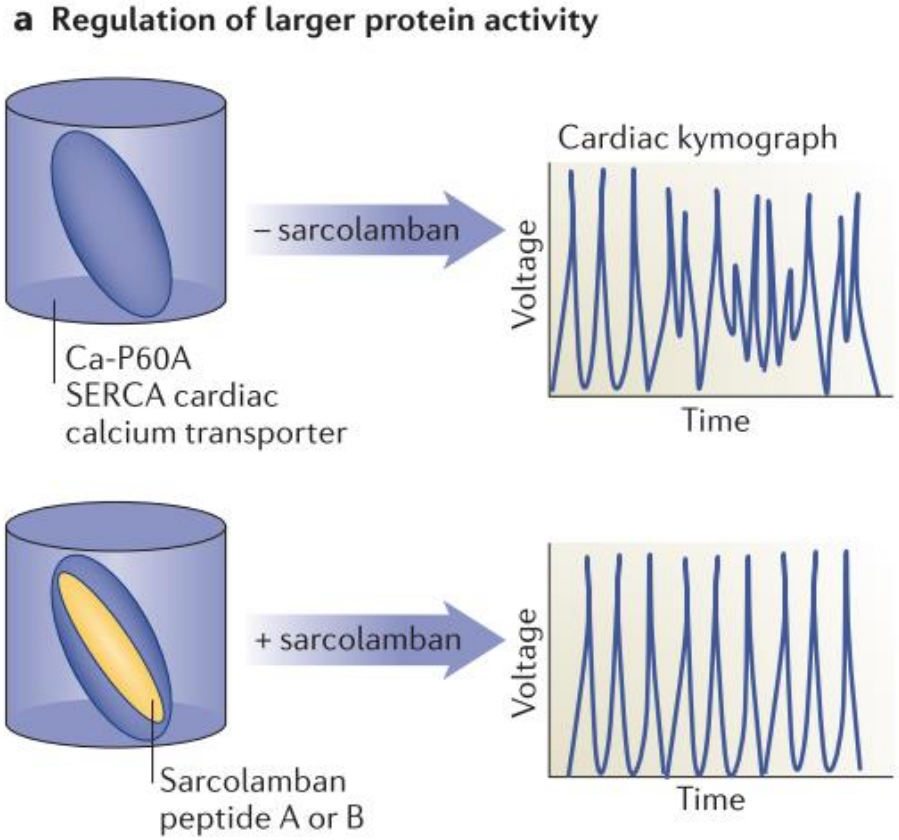
Translation of small ORFs (micropeptides)

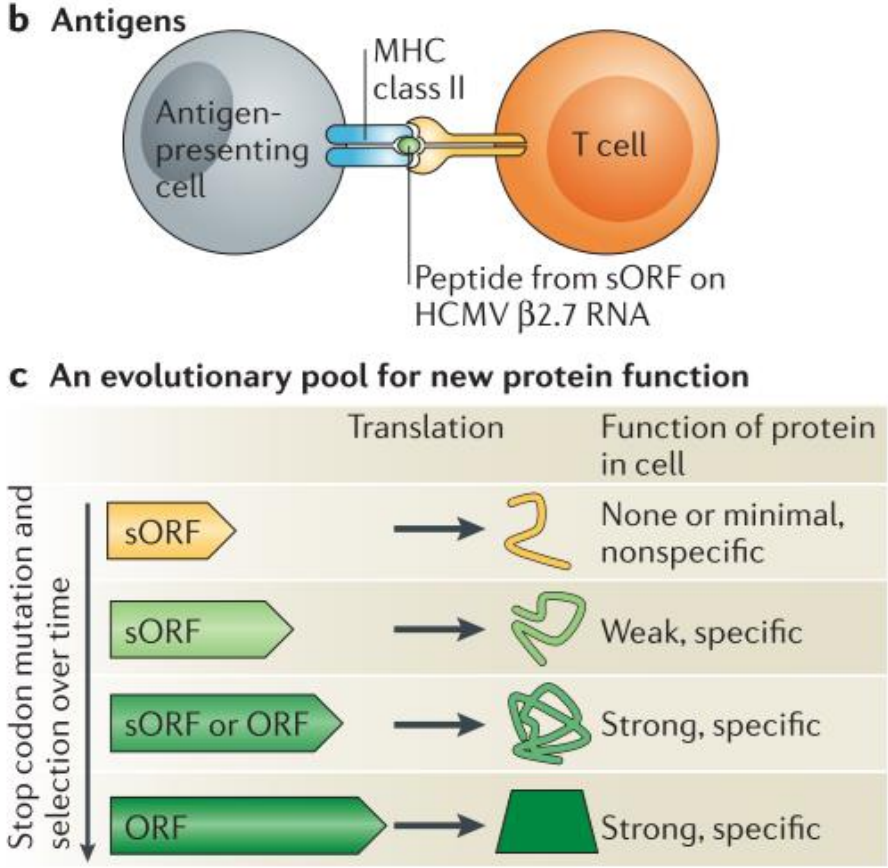


- Less than 100AA
- Translated in the cytoplasm (non membrane bound ribosomes)
- Evolutionary conserved

Crappe, BMC Genomics 2013

Translation of small ORFs (micropeptides)





Translation of small ORFs (micropeptides)

RESEARCH

MOLECULAR BIOLOGY

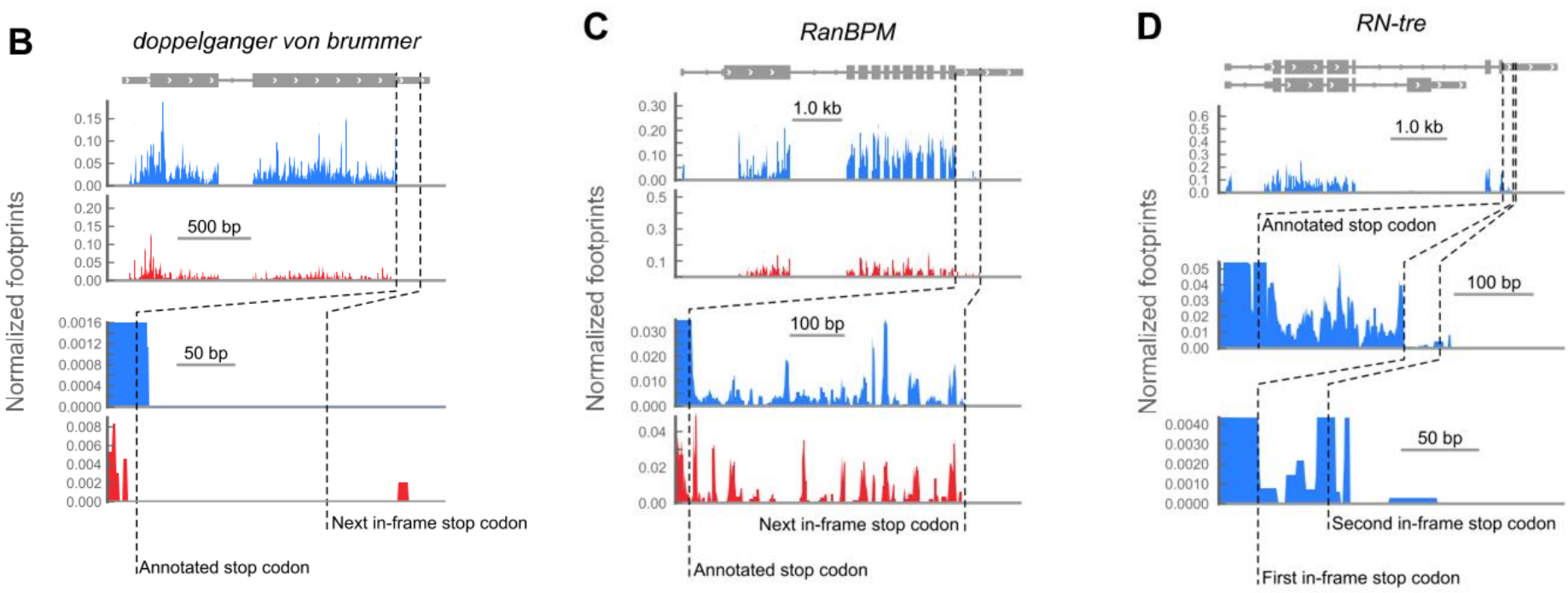
Pervasive functional translation of noncanonical human open reading frames

Jin Chen[1,2], Andreas-David Brunner[3], J. Zachery Cogan[1,2], James K. Nuñez[1,2], Alexander P. Fields[1,2]*, Britt Adamson[1,2]†, Daniel N. Itzhak[4], Jason Y. Li[4], Matthias Mann[3,5], Manuel D. Leonetti[4], Jonathan S. Weissman[1,2]‡

Ribosome profiling has revealed pervasive but largely uncharacterized translation outside of canonical coding sequences (CDSs). Here, we exploit a systematic CRISPR-based screening strategy to identify hundreds of non-canonical CDSs that are essential for cellular growth and whose disruption elicit specific, robust transcriptomic and phenotypic changes in human cells. Functional characterization of the encoded microproteins reveals distinct cellular localizations, specific protein binding partners, and hundreds that are presented by the HLA system. Interestingly, we find multiple microproteins encoded in upstream open reading frames, which form stable complexes with the main, canonical protein encoded on the same mRNA, thus revealing the diverse use of functional bicistronic operons in mammals. Together, our results

Chen, Science 2020

Stop codon read-through



- Several hundred Drosophila genes express C terminal extensions

Dunn eLife 2013

Stop codon read-through

PLOS | GENETICS

RESEARCH ARTICLE

Stop-codon read-through arises largely from molecular errors and is generally nonadaptive

Chuan Li[¤], **Jianzhi Zhang***

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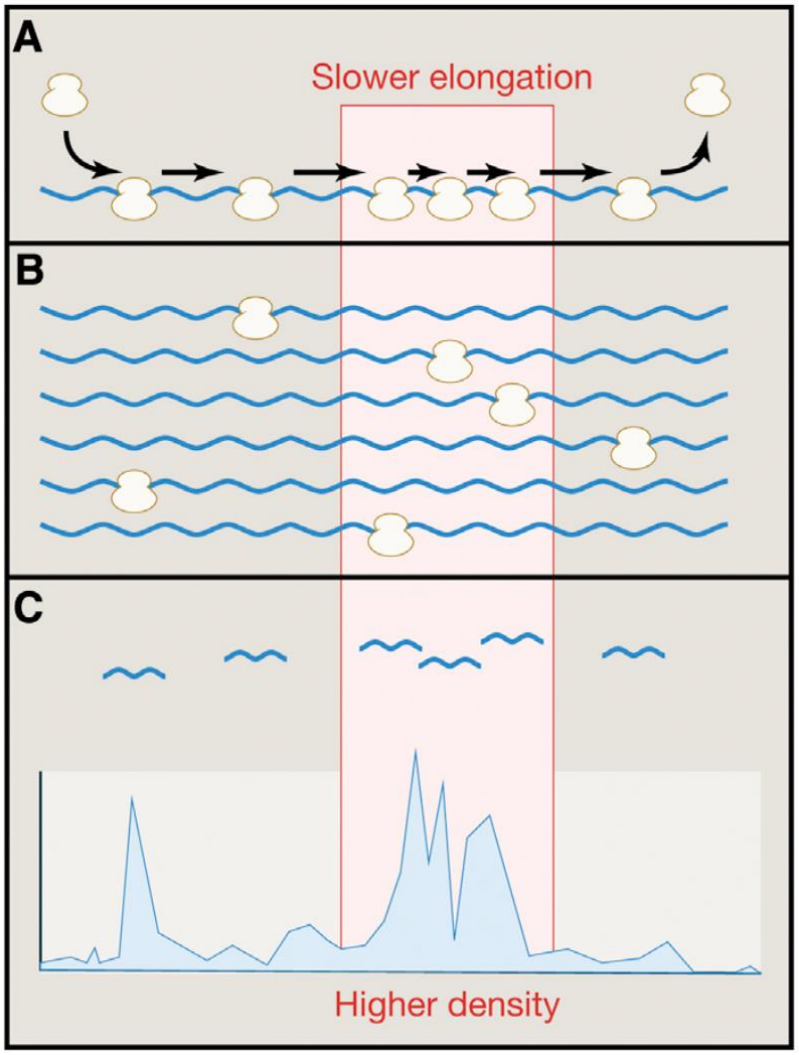
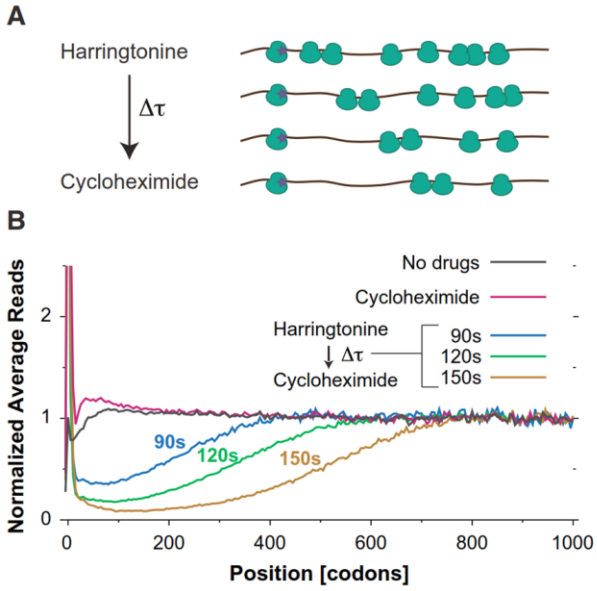
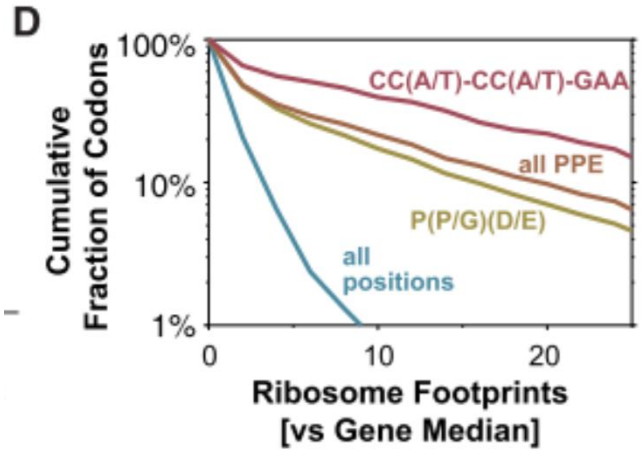
sity. Here we propose and test a competing hypothesis that stop-codon read-through arises mostly from molecular errors and is largely nonadaptive. The error hypothesis makes distinct predictions about the probability of read-through, frequency of sequence motifs for read-through, and conservation of the read-through region, each of which is supported by genome-scale data from yeasts and fruit flies. Thus, except for the few cases with demonstrated functions, stop-codon read-through is generally nonadaptive. This finding, along with other molecular errors recently quantified, reveals a much less precise or orderly cellular life than is commonly thought.

Li Plos Genetics 2019

Translation dynamics

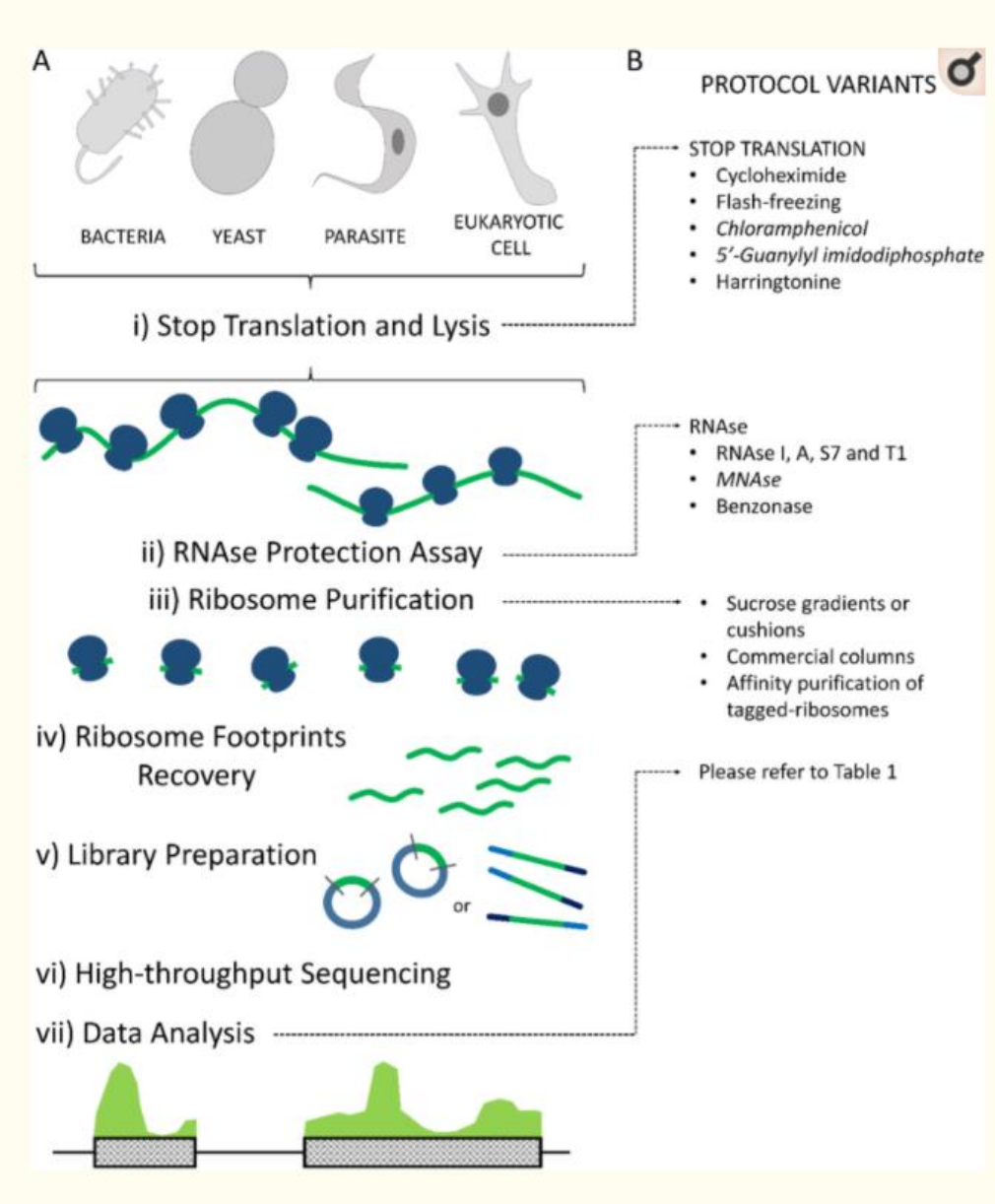






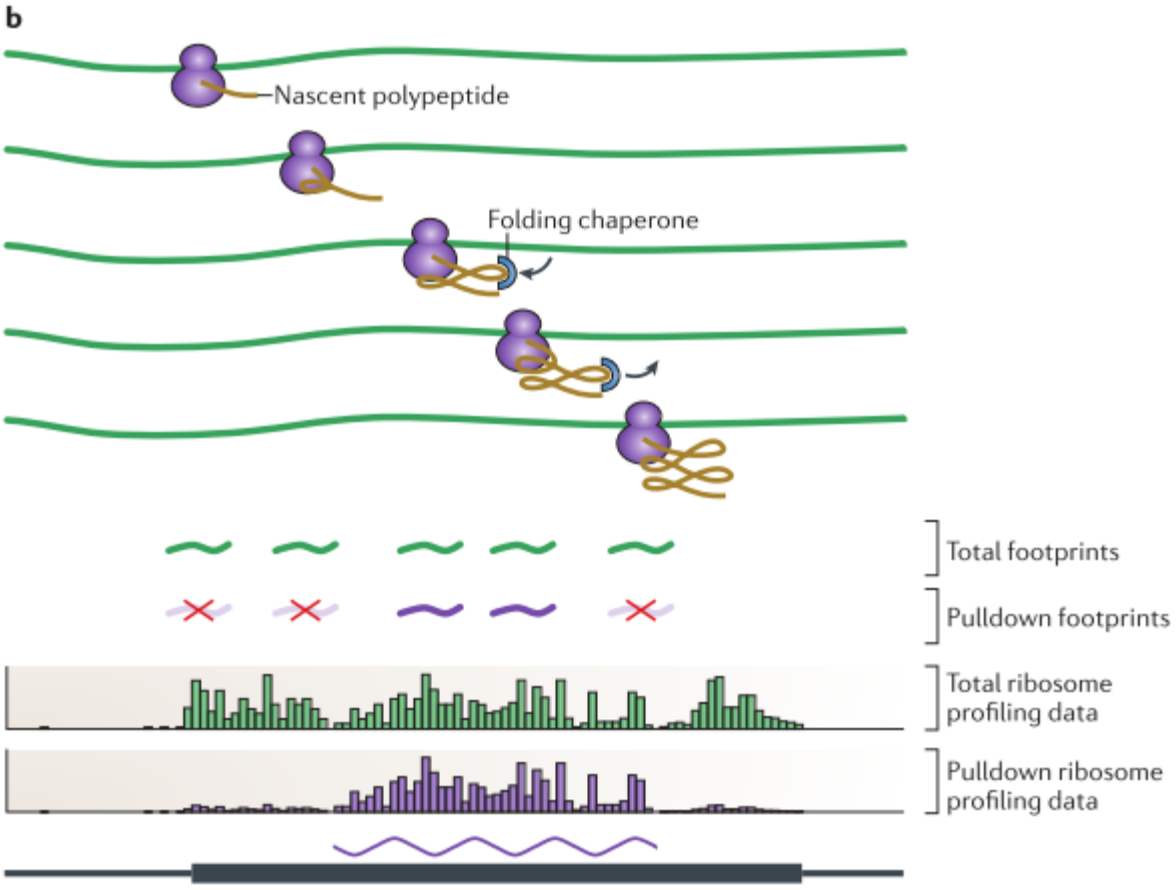
Ingolia Cell 2011

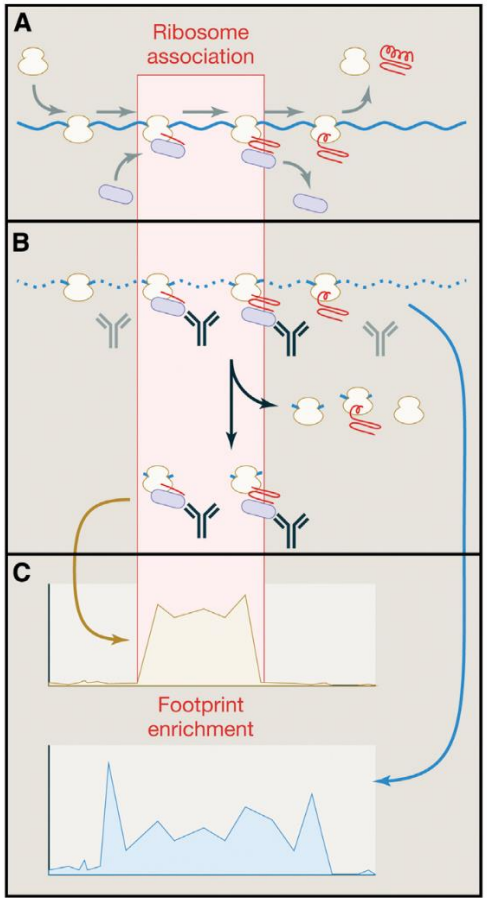
Ribo-seq variations



Eastman, *et al*. Comput Struct Biotechnol J. 2018

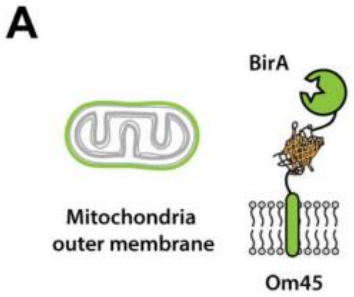
Ribo-seq variations



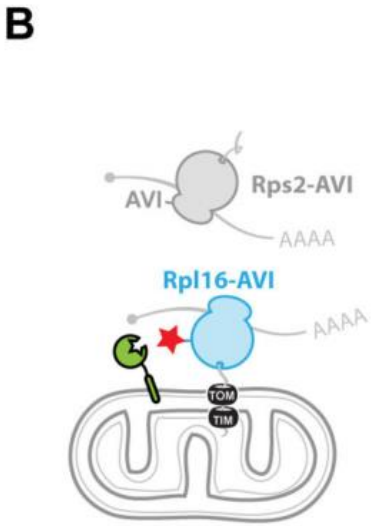


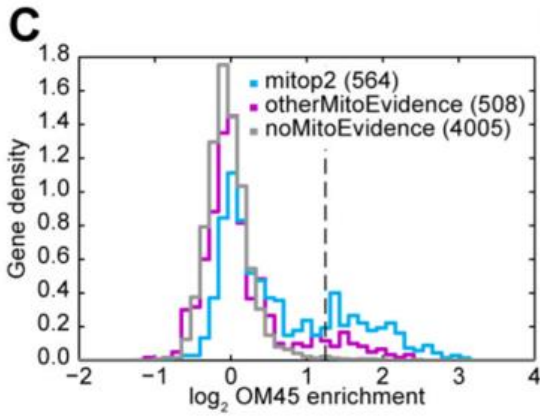
Ingolia, Nature Reviews Genetics 2014
Ingolia, Cell 2016

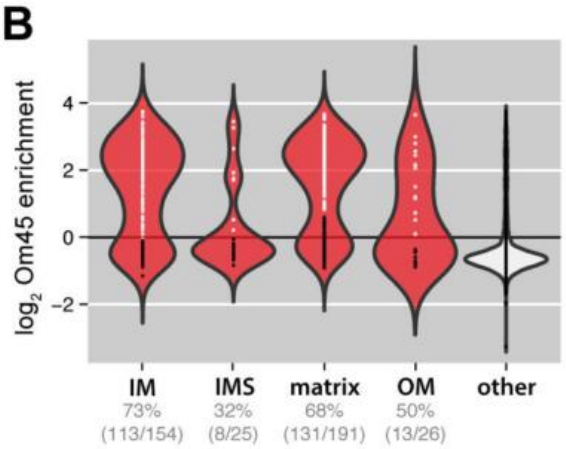
Proximity-specific ribosome profiling



Detects translation occurring close to the mitochondrial membrane







Williams, Science 2014

Translatomics goes single cell…

nature methods

ARTICLES

https://doi.org/10.1038/s41592-021-01128-0

Check for updates

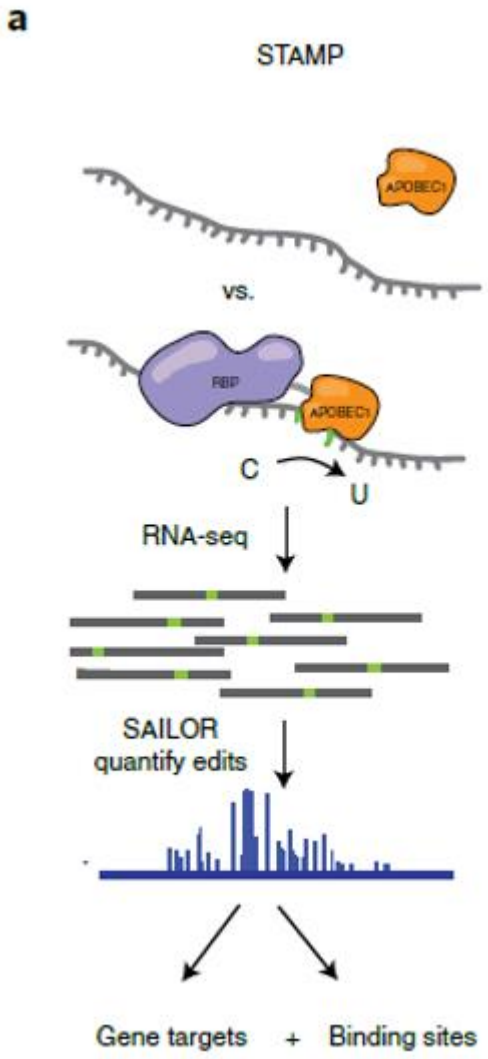
Robust single-cell discovery of RNA targets of RNA-binding proteins and ribosomes

Kristopher W. Brannan[1,2,3], Isaac A. Chaim[1,2,3], Ryan J. Marina[1,2,3], Brian A. Yee[1,2,3], Eric R. Kofman[1,2,3], Daniel A. Lorenz[1,2,3], Pratibha Jagannatha[1,2,3], Kevin D. Dong[1,2,3], Assael A. Madrigal[1,2,3], Jason G. Underwood[4] and Gene W. Yeo[1,2,3] ✉

Translatomics goes single cell…

Results

STAMP identifies RBP binding sites without immunoprecipitation. Our strategy for IP-free detection of RBP targets involves fusing full-length RBPs of interest to the cytidine deaminase enzyme APOBEC1, which is known to catalyze C-to-U editing on single-stranded RNA targets (Fig. 1a). Upon expression of an RBP–APOBEC1 fusion protein (RBP–STAMP), RBPs direct the



Translatomics goes single cell…

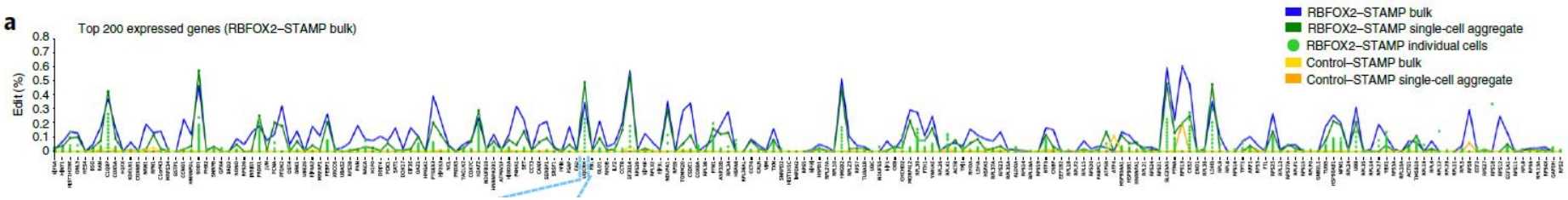
Ribosome-subunit STAMP edits are enriched in highly translated coding sequences and are responsive to mTOR inhibition. Since ribosomes have extensive association with mRNAs during translation, we reasoned that ribosomal subunits fused to APOBEC1 (Ribo–STAMP) have the potential to edit mRNAs in a manner that reflects ribosome association. We generated independent HEK293T cell lines expressing APOBEC1 fusions to ribosomal subunits RPS2 and RPS3. For RPS2–STAMP and RPS3–STAMP, we observed that edits were enriched relative to control–STAMP on exons of protein-coding genes that are highly translated in HEK293T cells, such as *ATP5PB*[36], coincident with RPS3 eCLIP signal enrichment over size-matched input control (Fig. 2a). In comparison, RPS2–STAMP and RPS3–STAMP signals were minimally detected on highly expressed noncoding genes such as the long noncoding RNA *MALAT1*, which is localized to the cytoplasm in mitotic cell lines[37] (Fig. 2b). We performed replicate RPS2–STAMP

Translatomics goes single cell…

10x Genomics standard single-cell RNA-seq

844 RBFOX2–STAMP cells and 5,242 control–STAMP cells.

Comparison of bulk and single-cell edit fractions for control–STAMP and RBFOX2–STAMP experiments across the top 200 expressed genes (ranked by transcripts per million (TPM) from bulk RBFOX2–STAMP RNA-seq) revealed nearly identical edit enrichment profiles of RBFOX2 samples above controls and further uncovered a spectrum of editing frequencies across individual cells (Fig. 4a). To illustrate, we next ranked individual control–STAMP



Translatomics goes single cell…

Ribo–STAMP reveals translational landscapes at single-cell resolution

We performed stable 72-h high-induction control–STAMP and RPS2–STAMP and conducted scRNA-seq