

Ribosome Profiling

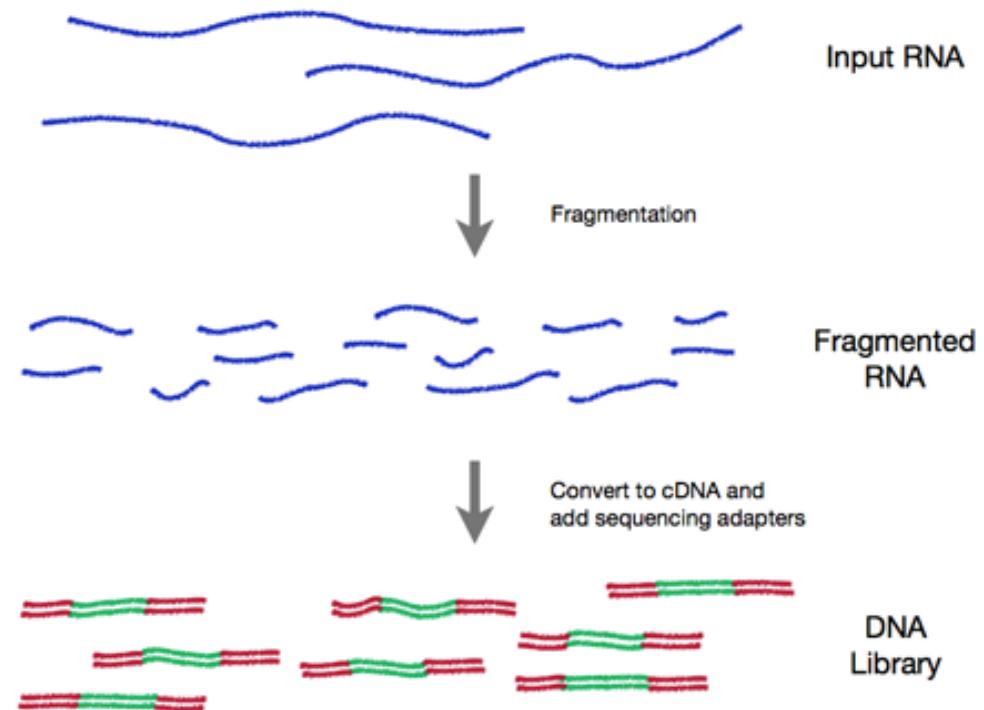


Dan Ofer
15.1.2015

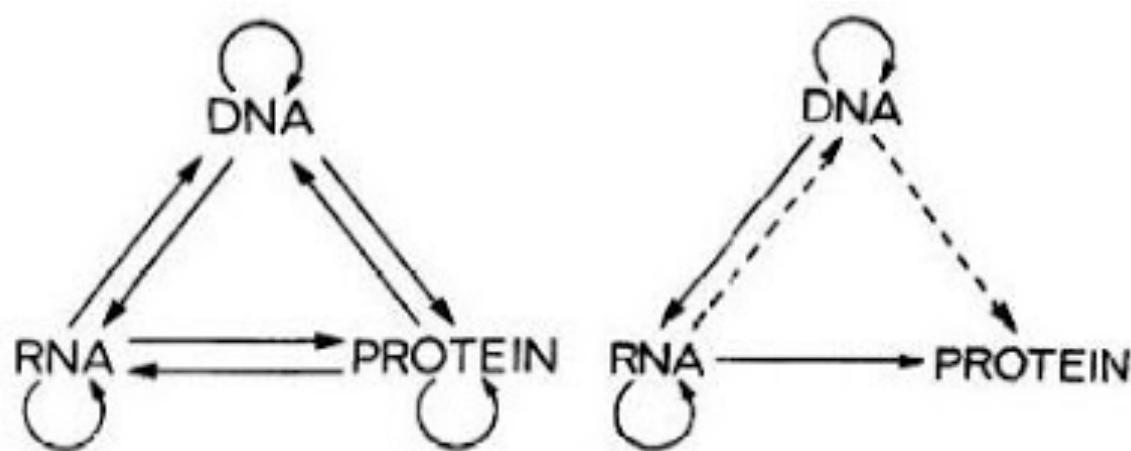
Mentors: Michal Linial, Nir Friedman

Functional Genomics :

Q: *Why* do we need Ribosome profiling? Isn't RNA-Seq enough?

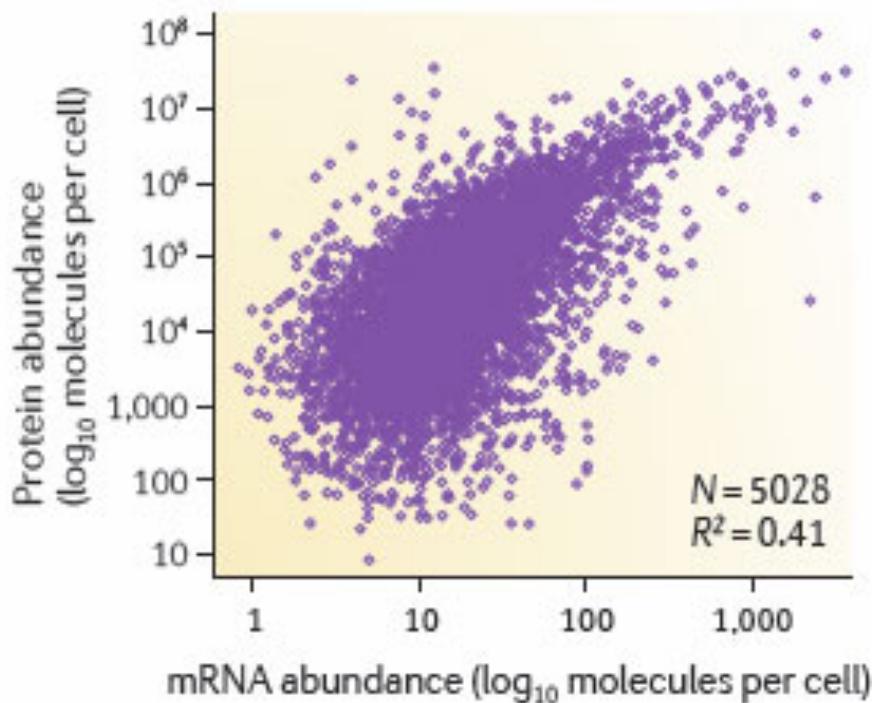


Protein Abundance: Not 1:1 with mRNA levels!

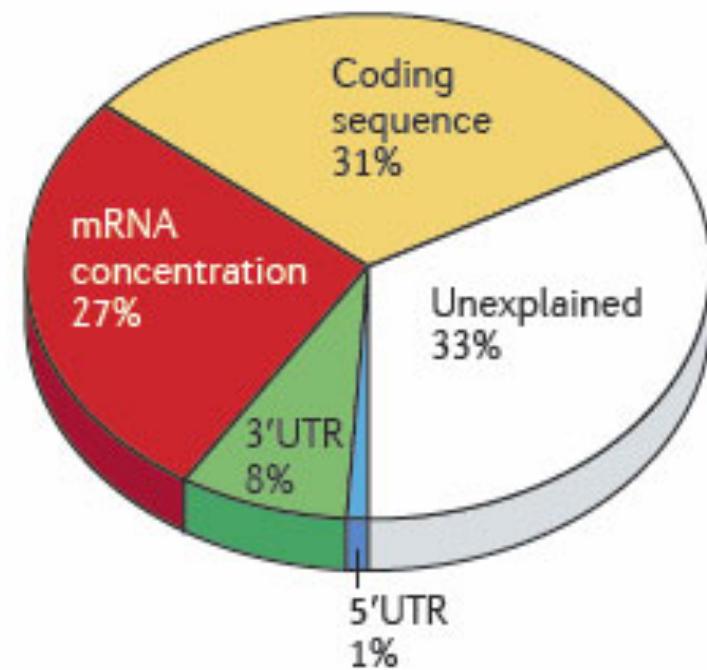


mRNA and protein levels abundance : Only ~34% Correlation!

a Mouse



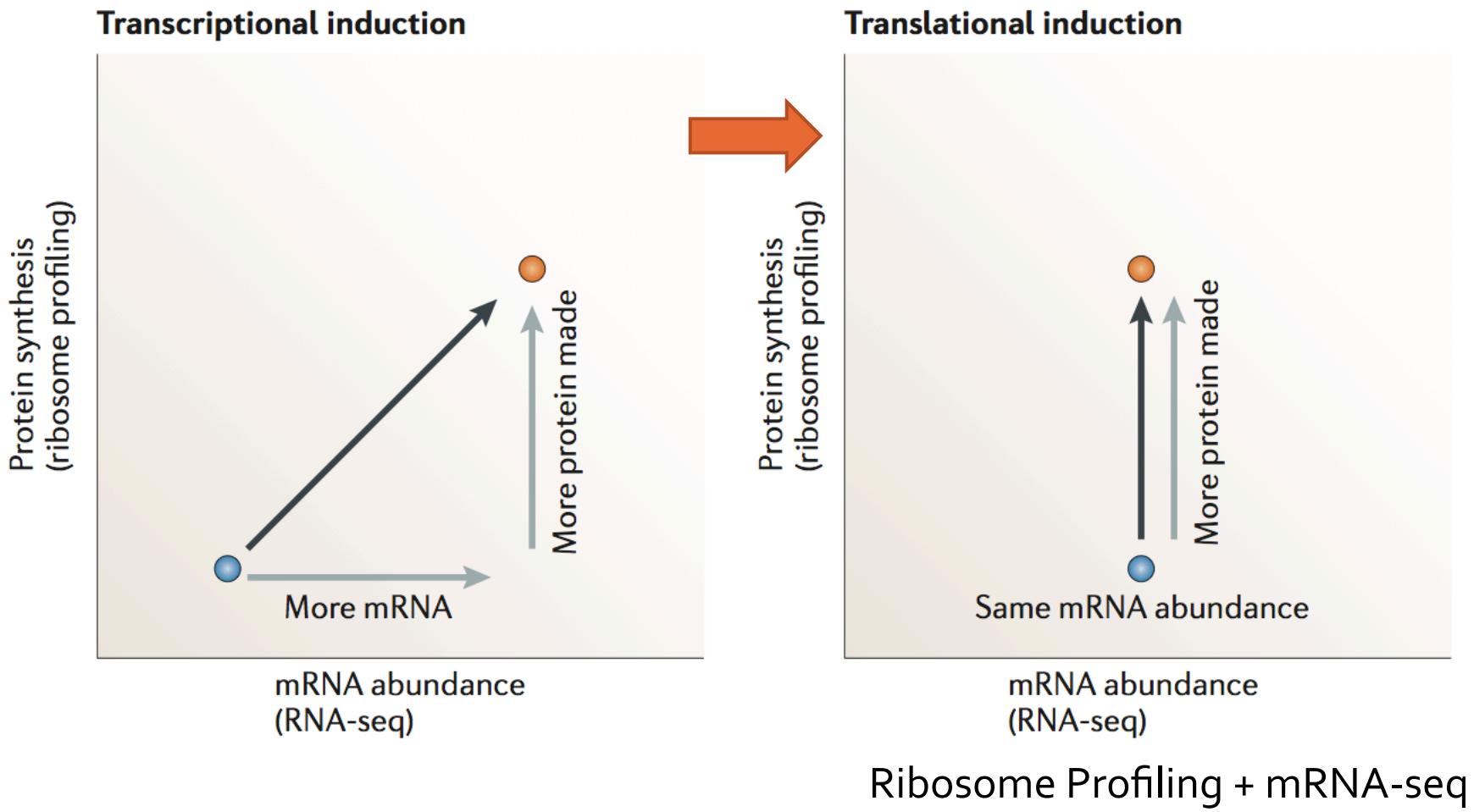
b Human



[Insights into the regulation of protein abundance from proteomic and transcriptomic analyses](#)

Christine Vogel & Edward M. Marcotte *Nature Reviews Genetics* 13, 227–232 (April 2012) doi:10.1038/nrg3185

Getting a *direct* picture of Translation levels



Follow the steps...

Recap:

- Functional Genomics need.



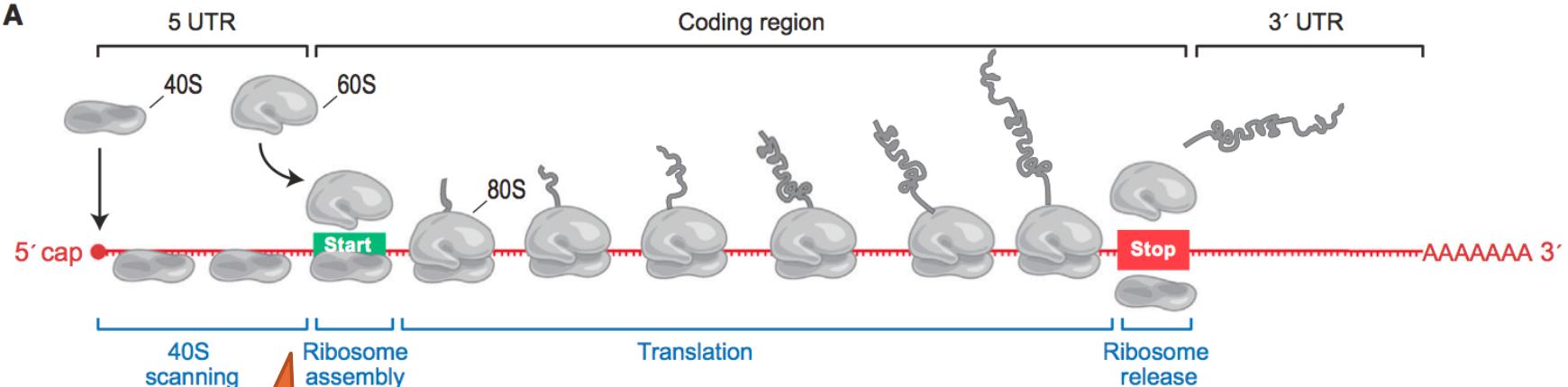
... To Ribosome Footprinting

Coming up:

- Ribosomal Profiling.
 - Translation 102.

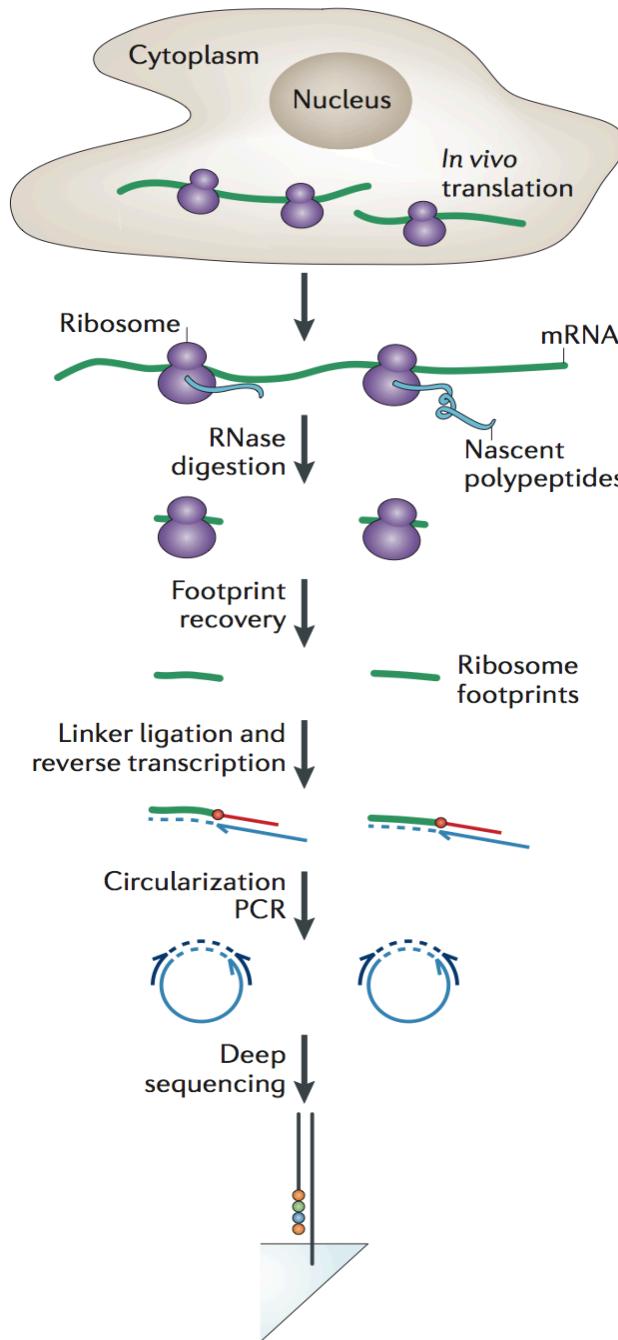
mRNA Translation

A



Note the initiation site,
scanning!

1. Initiation :
2. Elongation ---
3. Termination .



APPLICATIONS OF NEXT-GENERATION SEQUENCING — INNOVATION

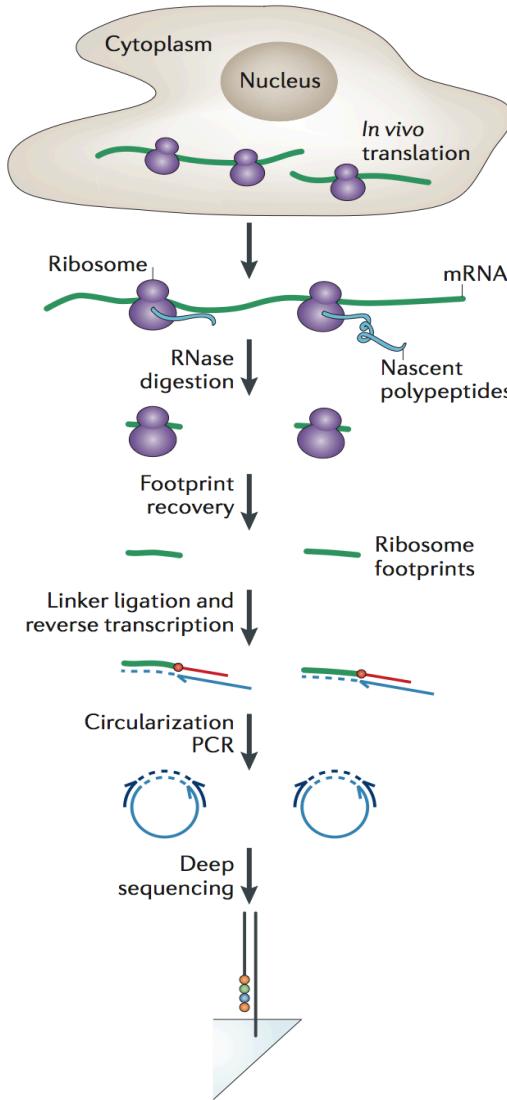
Ribosome profiling: new views of translation, from single codons to genome scale

Nicholas T. Ingolia

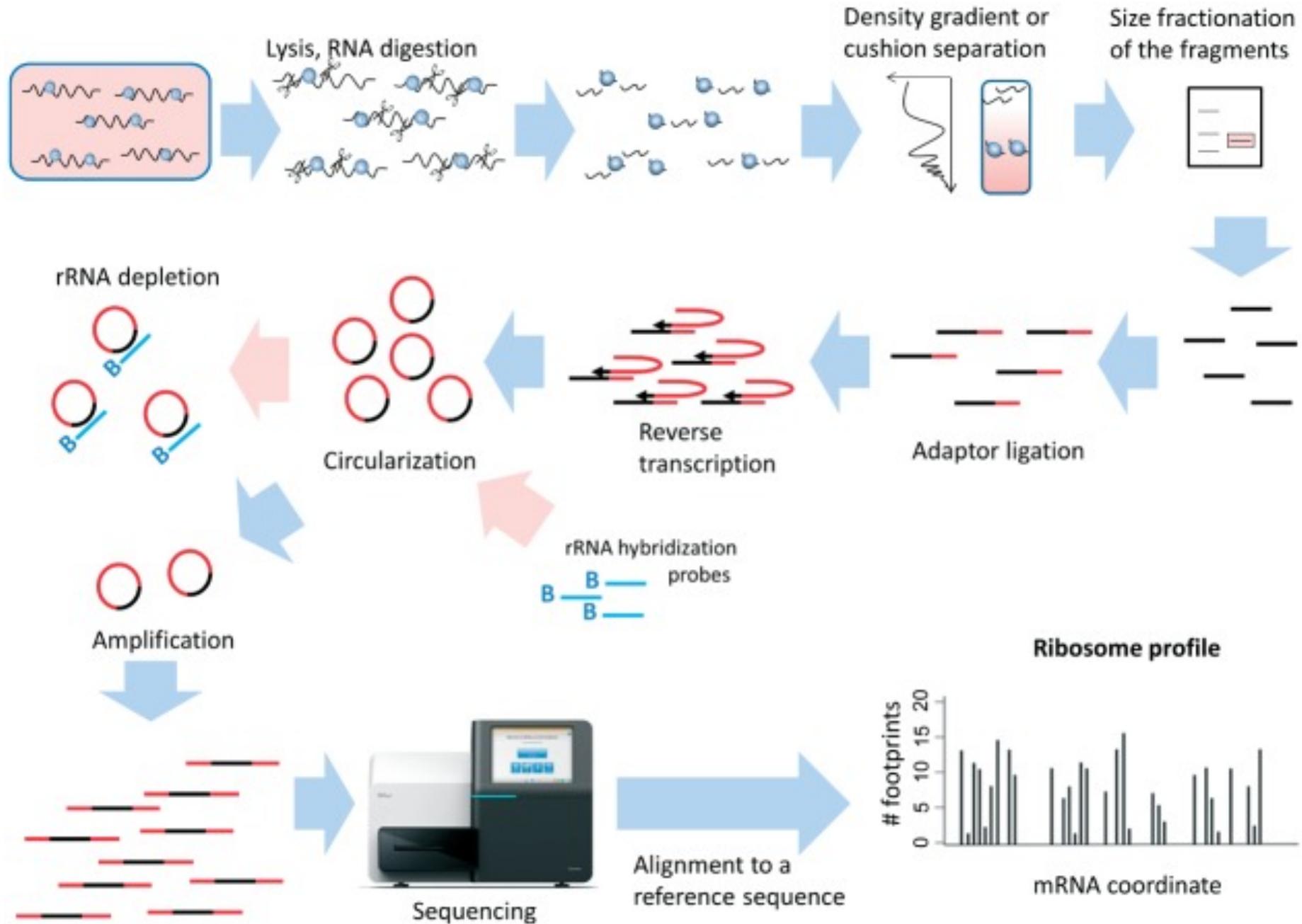
mRNA within a ribosome can be isolated using nucleases that degrade unprotected mRNA



Ribosomal profiling = Ribosome footprinting



- Get ribosome protected mRNA fragments.
- Convert these RNA footprints into a library of DNA for sequencing.
- Measure & Map the abundance of different footprints in this library.



Coming up: What's been found? What can we find? What can we ask?

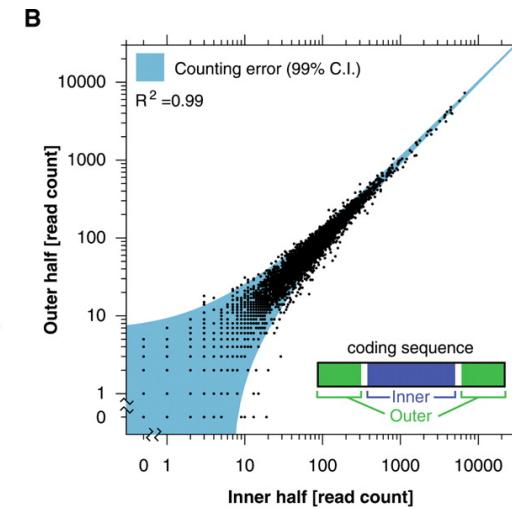
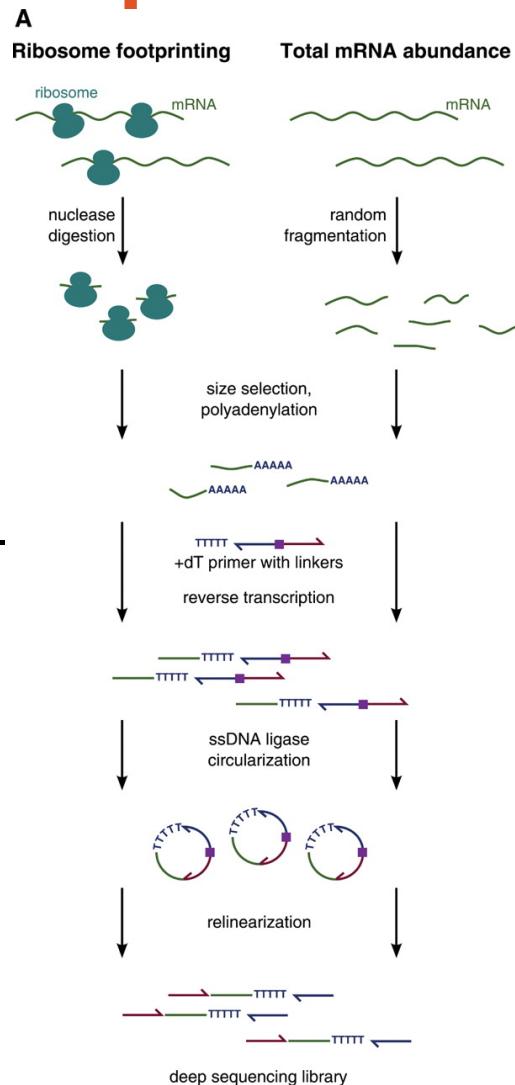


... To Ribosome Footprinting

- Functional Genomics: What's being Translated, how much, how fast...
- What's happening during Translation?
- Post-Transcriptional regulation of Translation
- מה מתרגם.
- מי מתרגם.
- איך מתרגם.
- איפה התרגום.

Ribosome footprint

- 42×10^6 protected fragments generated
- 7×10^6 (16%) aligned to CDSs (Coding DNA Sequences).
- Most of the remainder - rRNA



A – Process.
B – Checking that reads map correctly and equally to given CDSs.

Ribosome footprint



- Translation efficiency (TE):

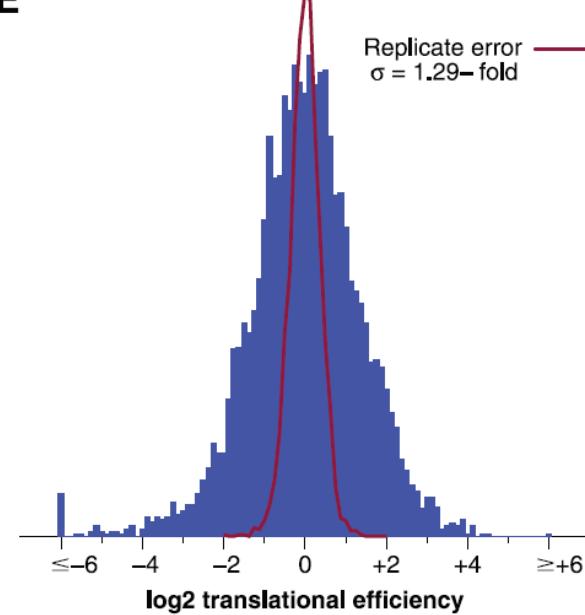
ribosome footprints

- 100-fold range between different yeast genes

- **Ribosome Density**
 - -> Less Ribosomes per unit length in (some) longer genes!

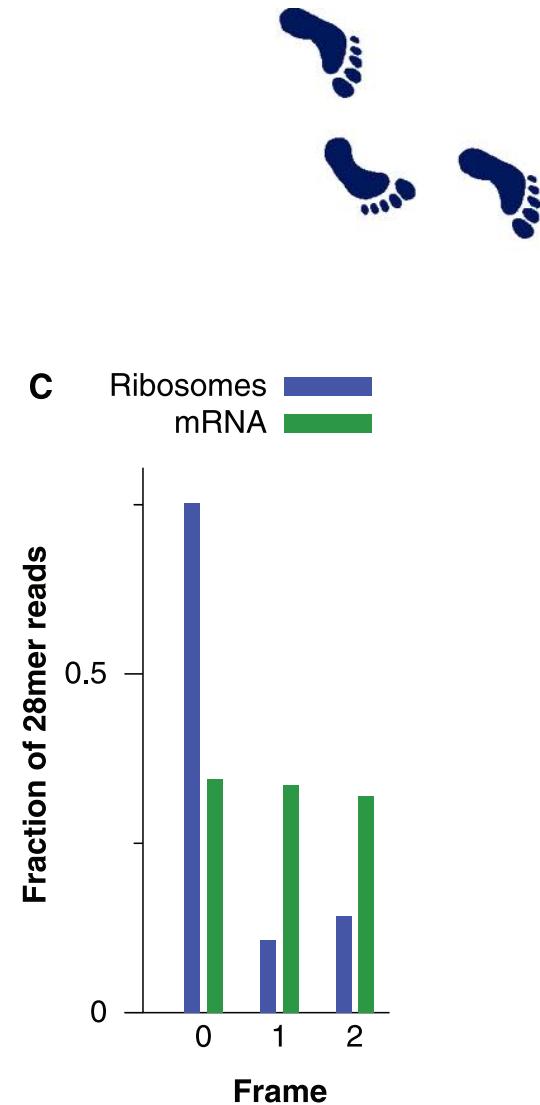
mRNA fragments

E



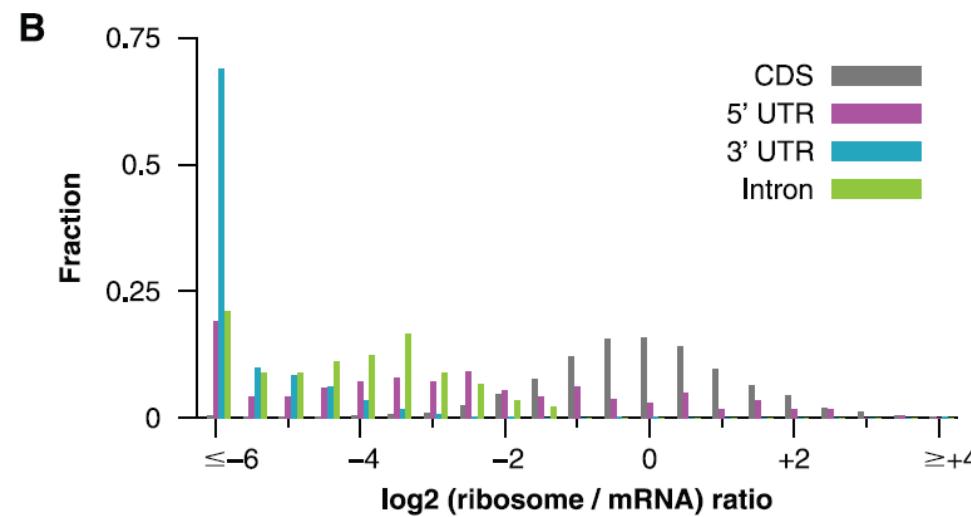
Ribosome footprint

- ~98.8% of fragments are from CDS.
- But, 56,105 fragments are not
 - Most from 5'UTR
- Strong 3-nt periodicity. (i.e ORFs maintained, codons..).
- 75% start on the first nucleotide of a codon.
- Enable studies of programmed frameshifting and stop codon skipping

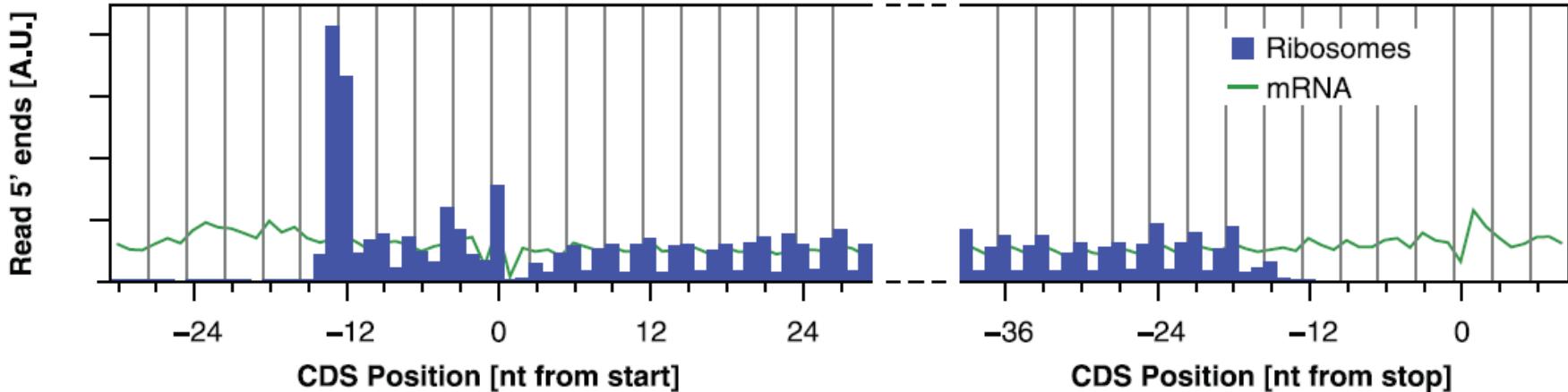


(C) - Position of ribosome footprints relative to the reading frame

Ribosome footprint



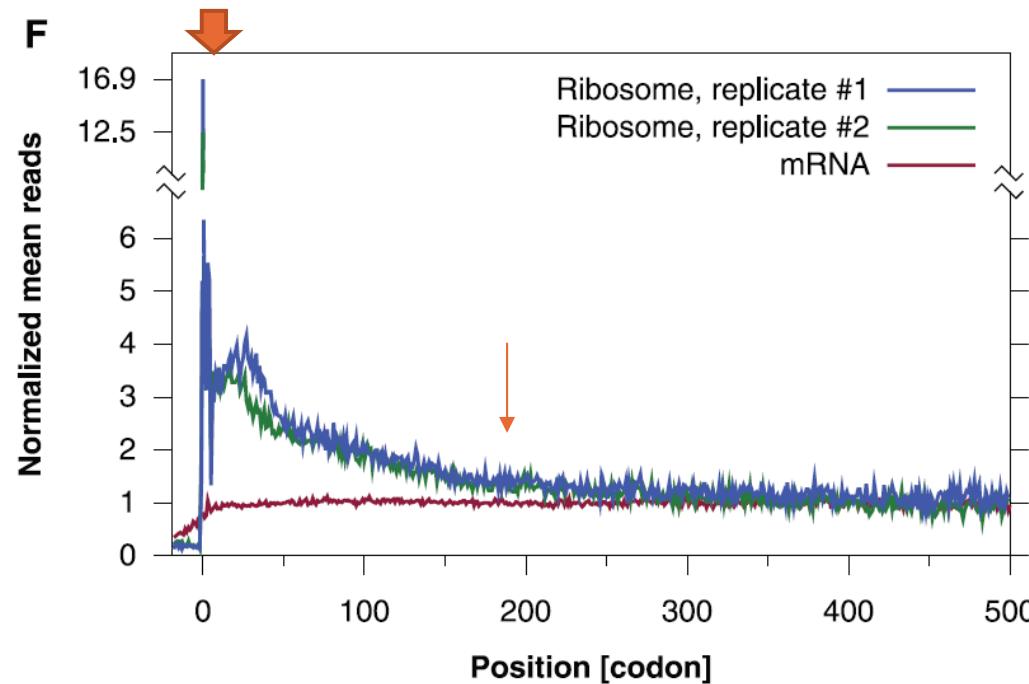
A



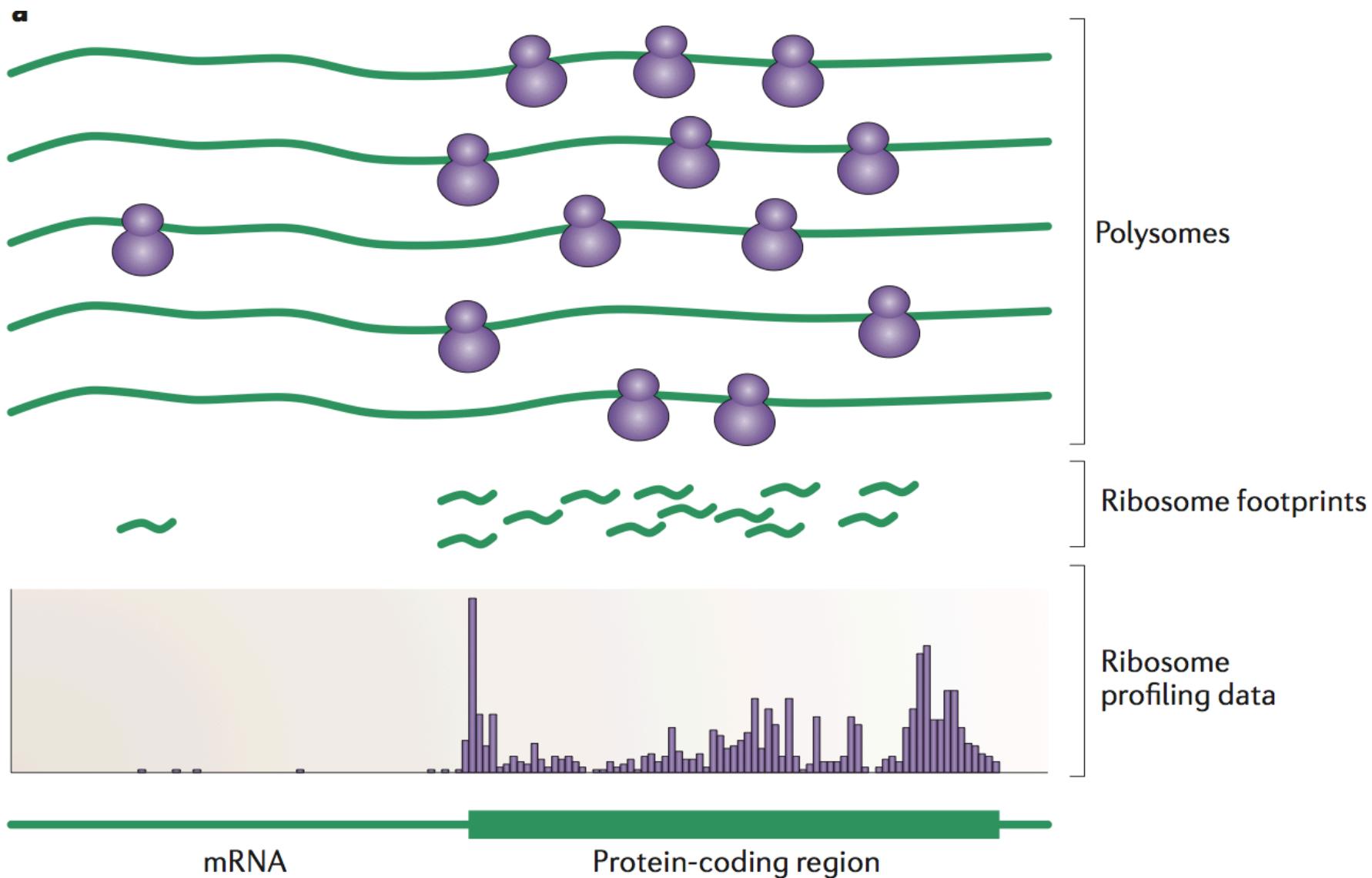
Ribosome footprints



- Ribosome density is greater (~3-fold) for the first 30 – 40 codons.
- After 100 – 200 codons: converges to a uniform density till translation termination



Ribosome occupancy analysis

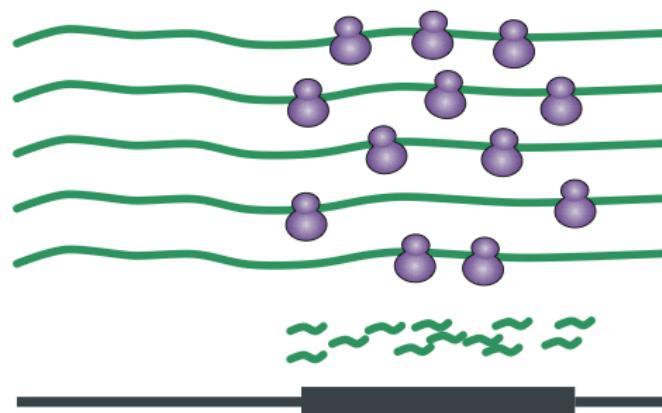


[Ribosome profiling: new views of translation, from single codons to genome scale](#)

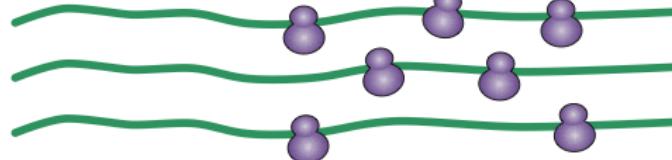
• Nicholas T. Ingolia *Nature Reviews Genetics* (2014). doi:10.1038/nrg3645

+ Ribosome found Translating = More Translation!

b

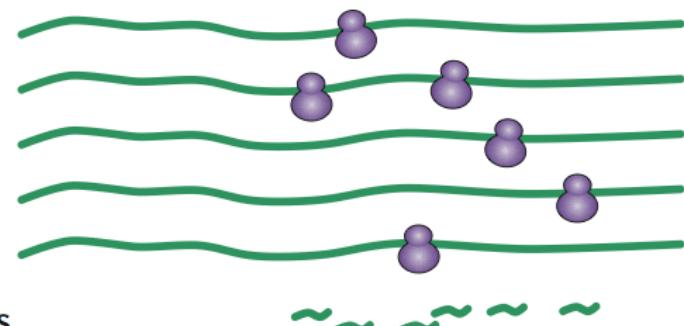


Fewer mRNAs



Fewer ribosomes per mRNA

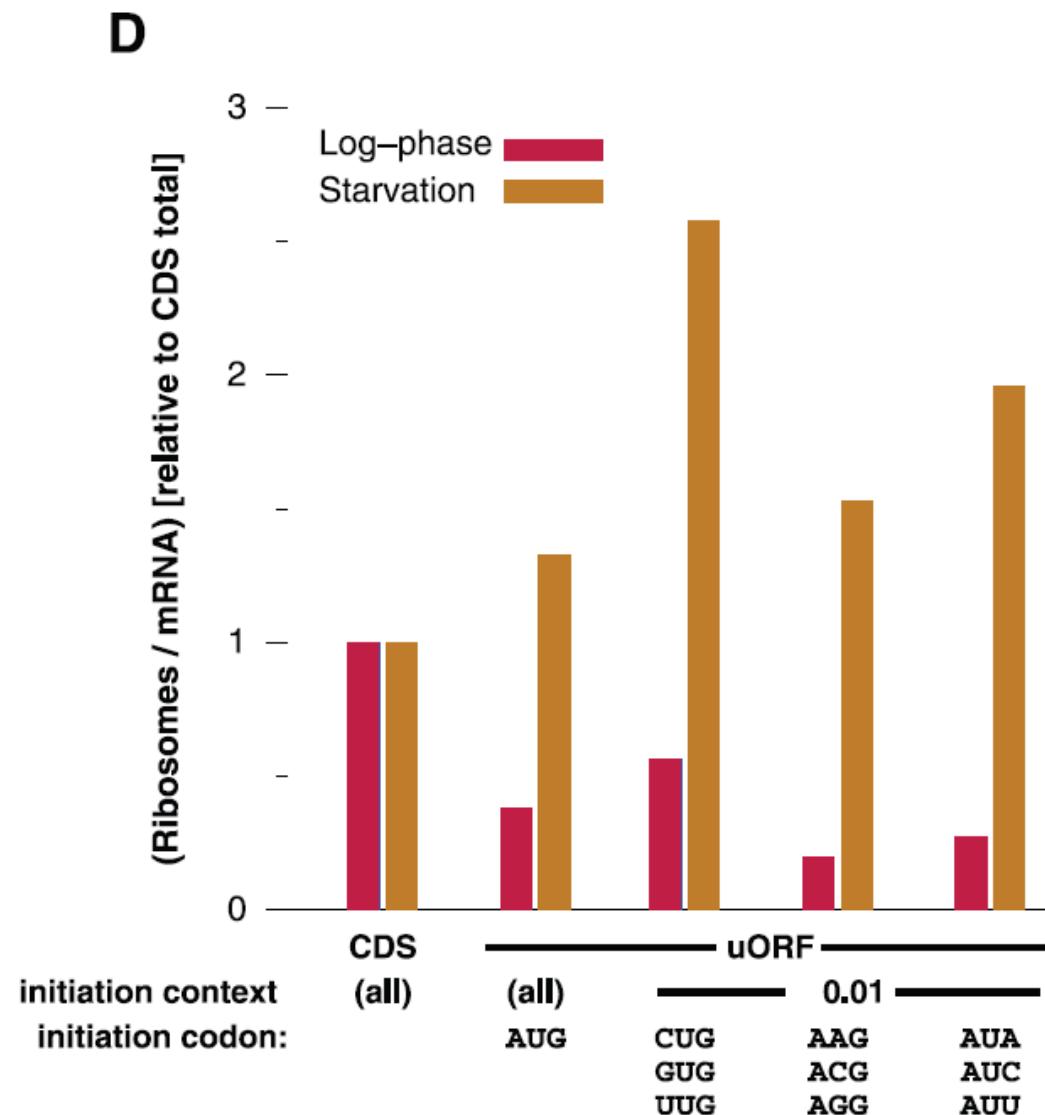
Or

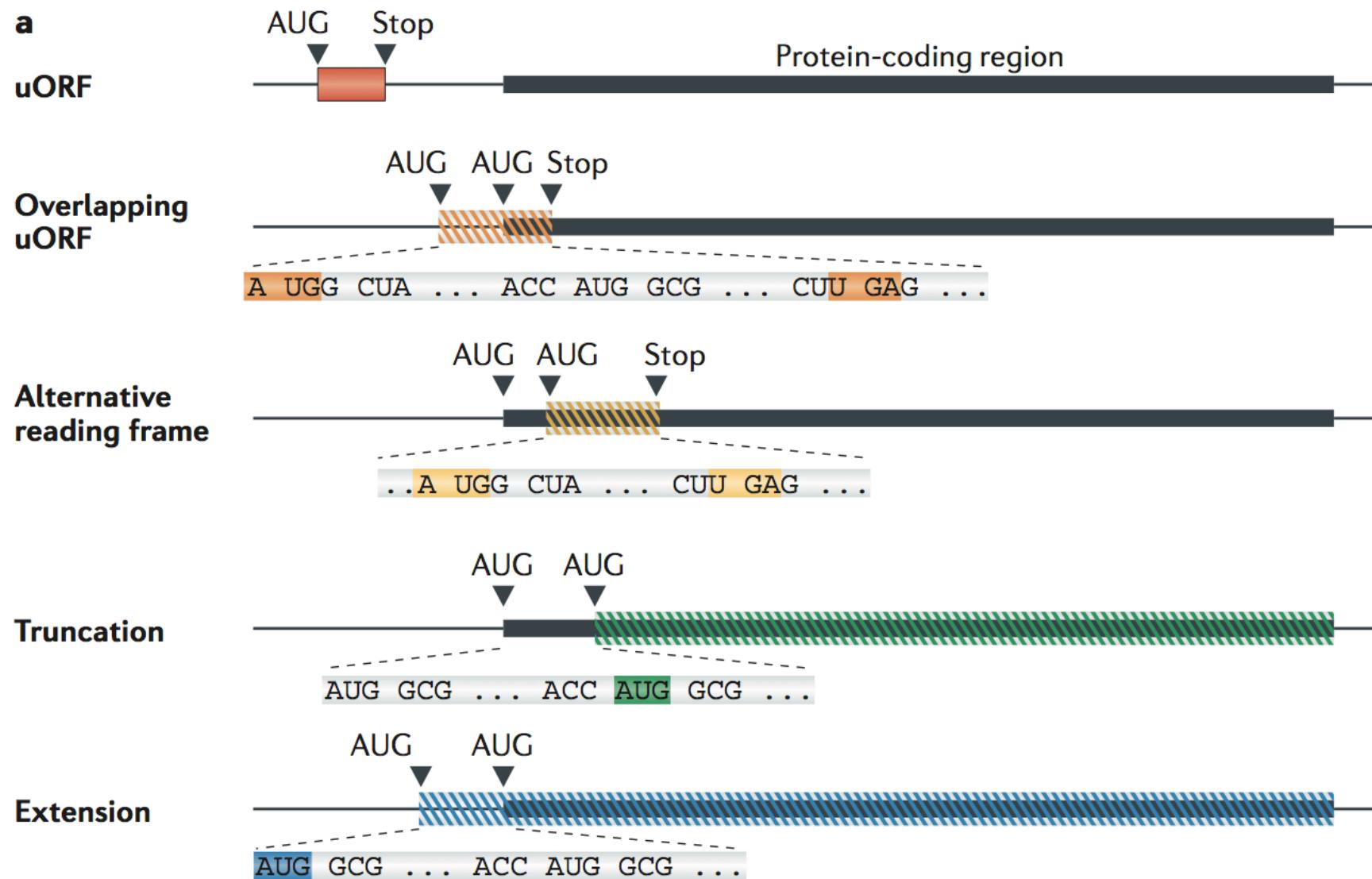


Fewer ribosome footprints

Non-AUG uORFs

uORF = Unknown
Reading Frames

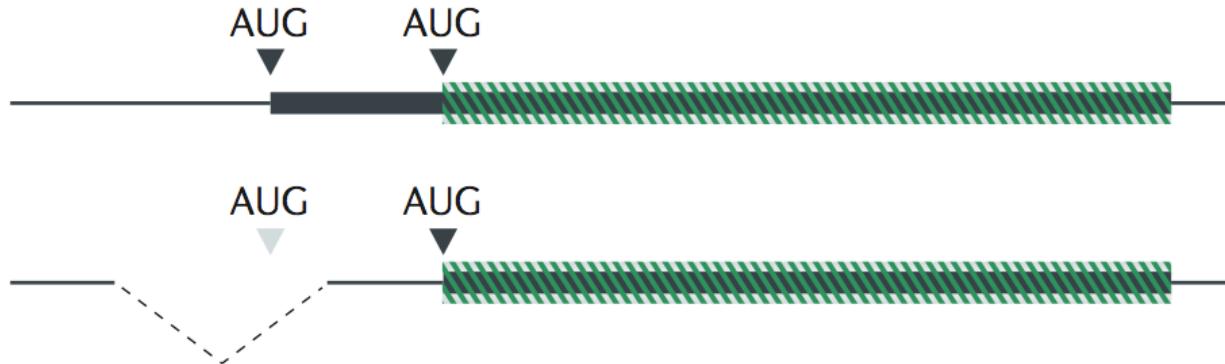


a

Alternative Translation Start Sites.

b

Alternative translation
of a canonical mRNA

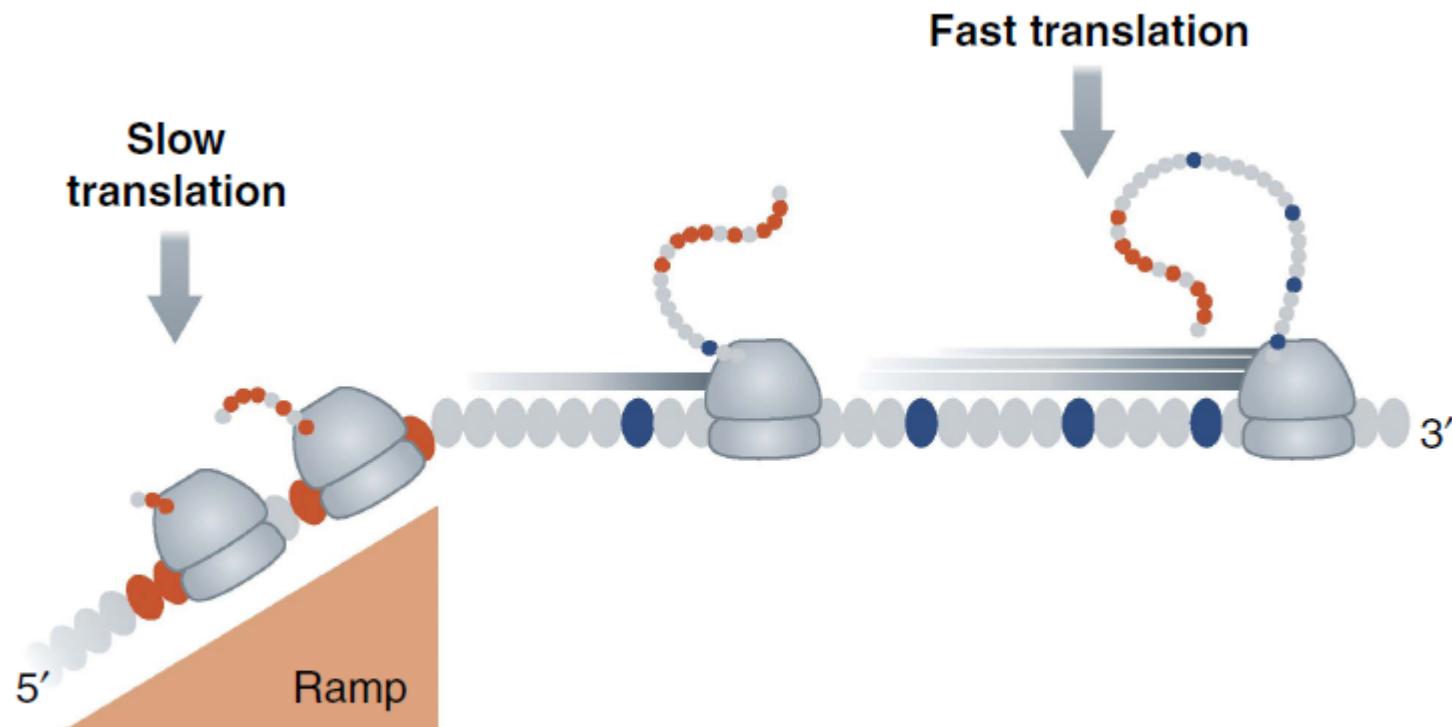


- Alternate Start codons (UUG..)
-> N-Terminal Extended isoforms.
-> Truncated isoforms.
- Alt. ORFs/ reading frames

Ribosome profiling: new views of translation, from single codons to genome scale

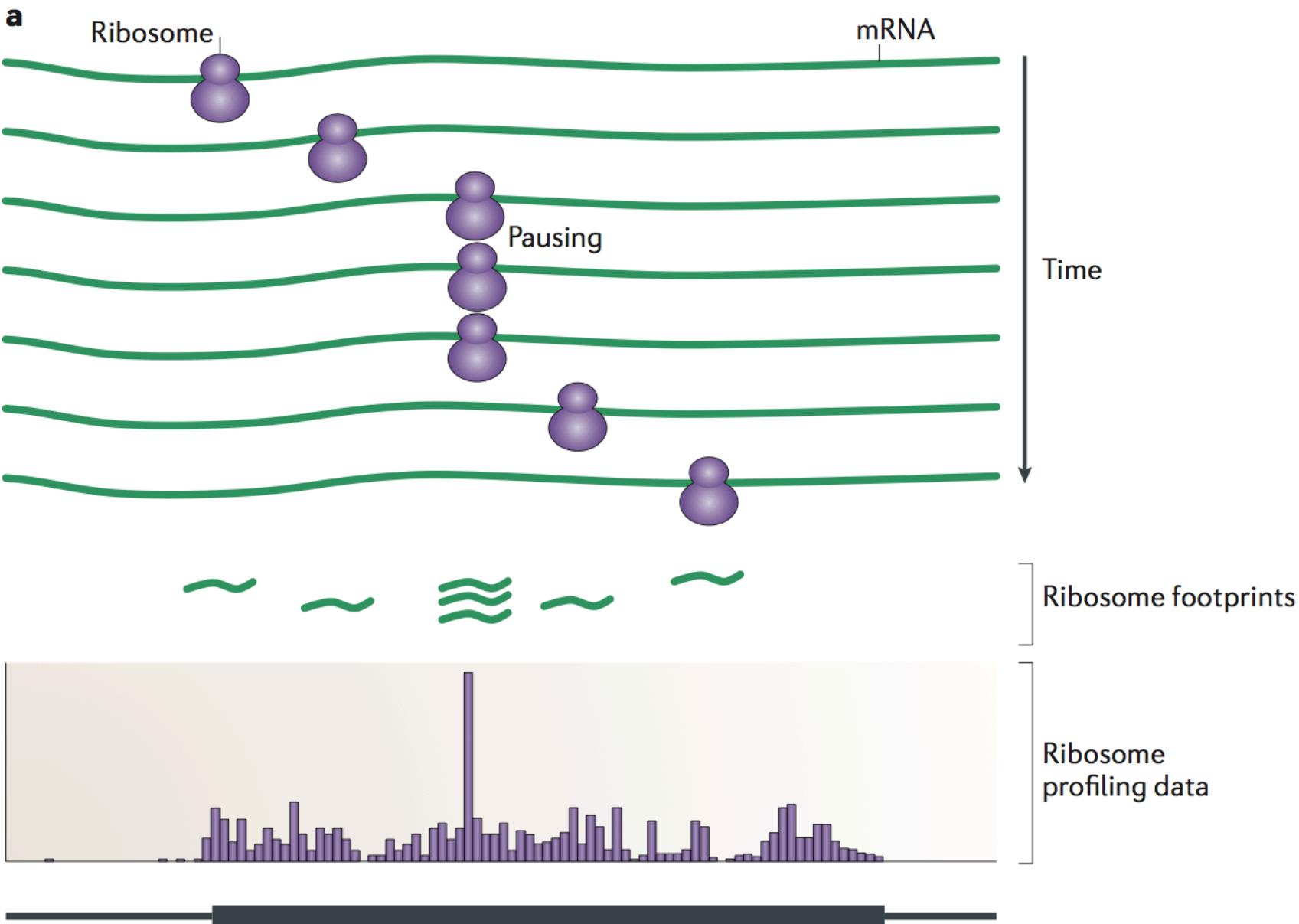
• Nicholas T. Ingolia *Nature Reviews Genetics* (2014). doi:10.1038/nrg3645

Flux of ribosomes:



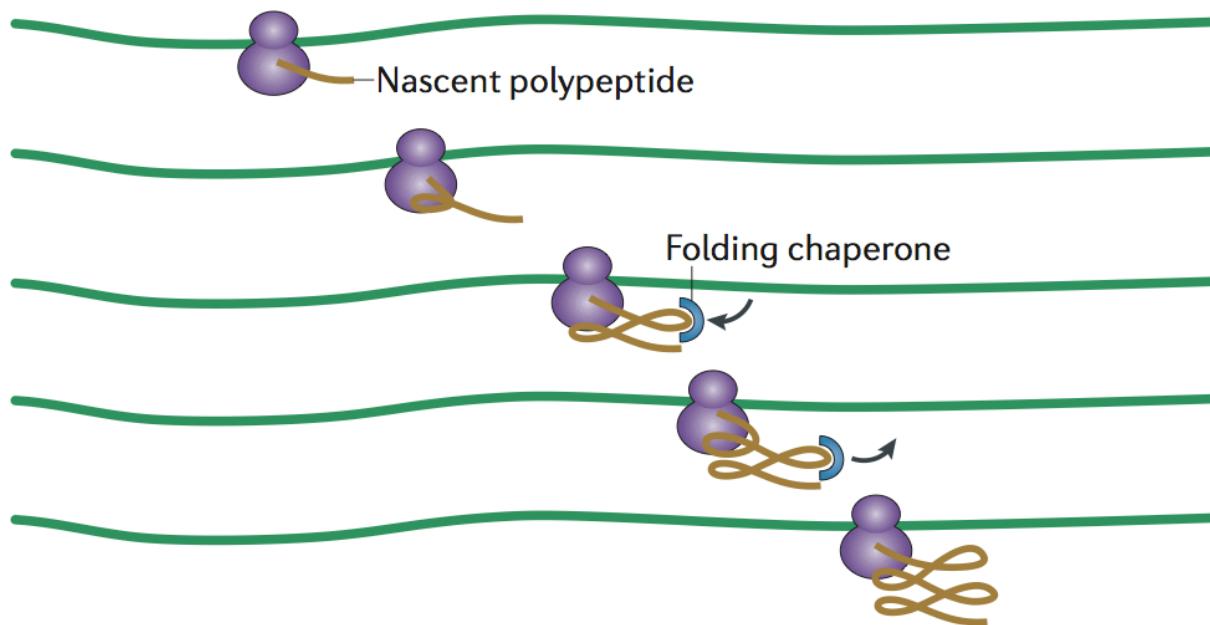
- Remember Ribosome density near the start site?

Flux, Speed, kinetics, Traffic jams, Speed bumps:

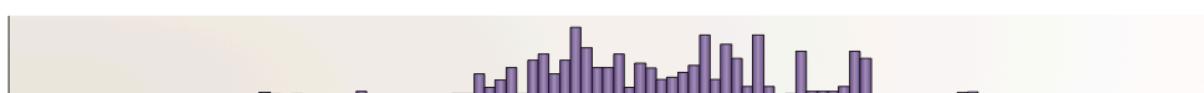


Co-Translational Folding and slowdowns

b



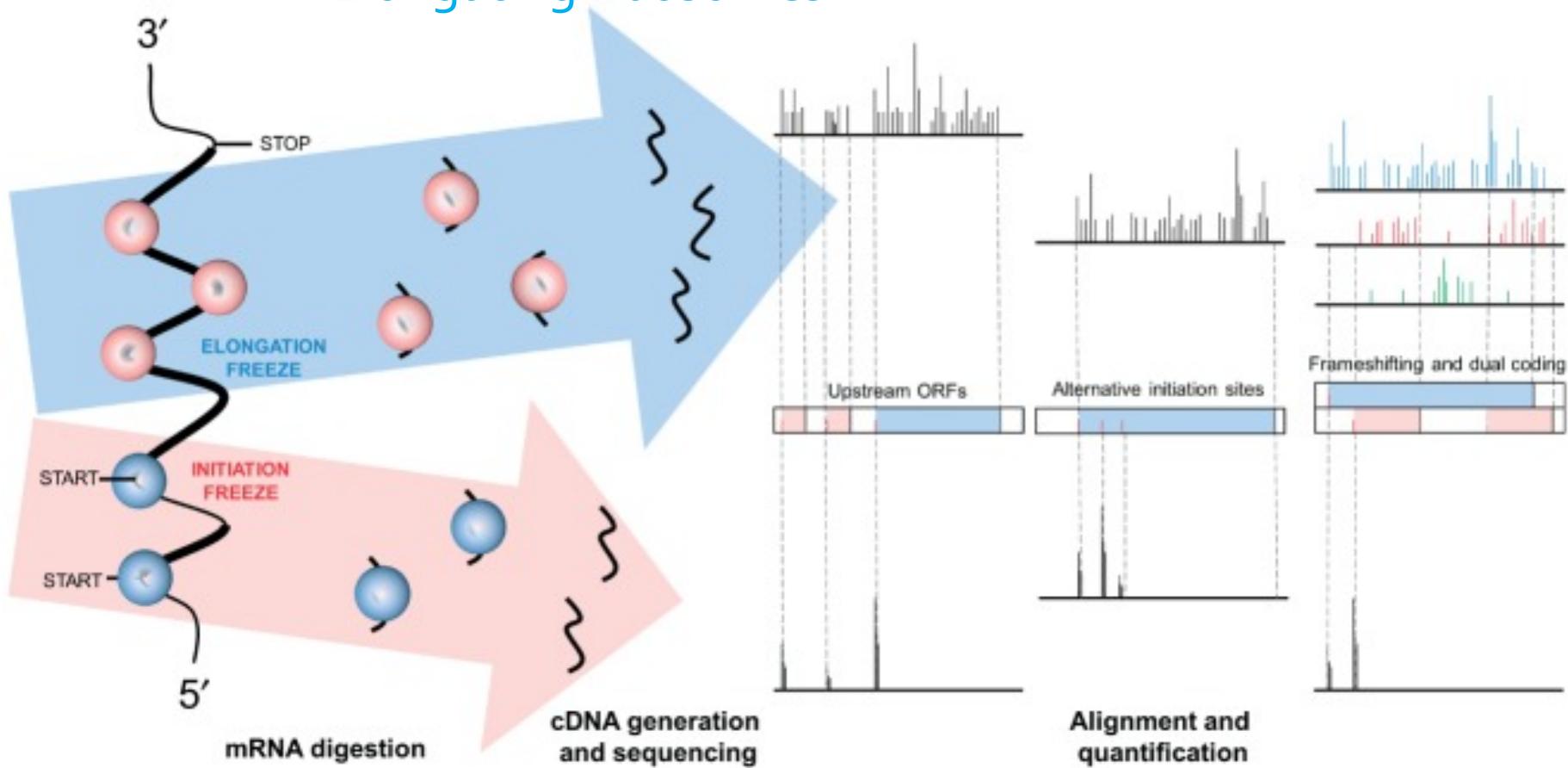
Total footprints
Pulldown footprints



Total ribosome profiling data
Pulldown ribosome profiling data



Elongating ribosomes

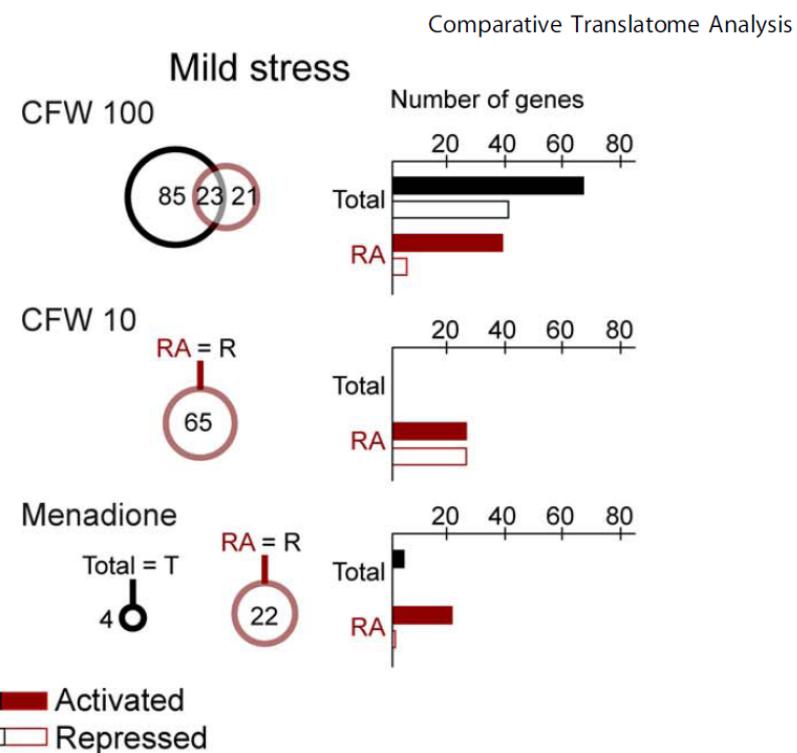
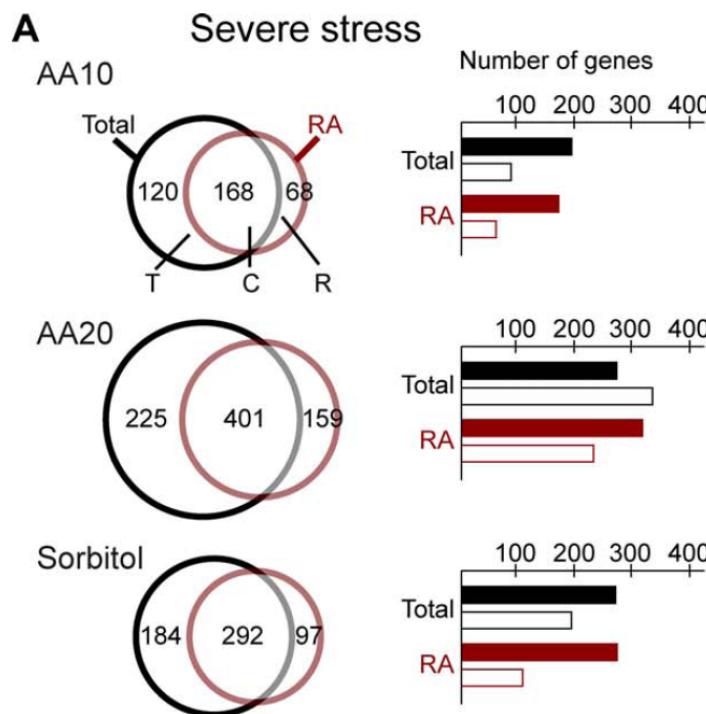


Initiating ribosomes

Stress dependent regulation

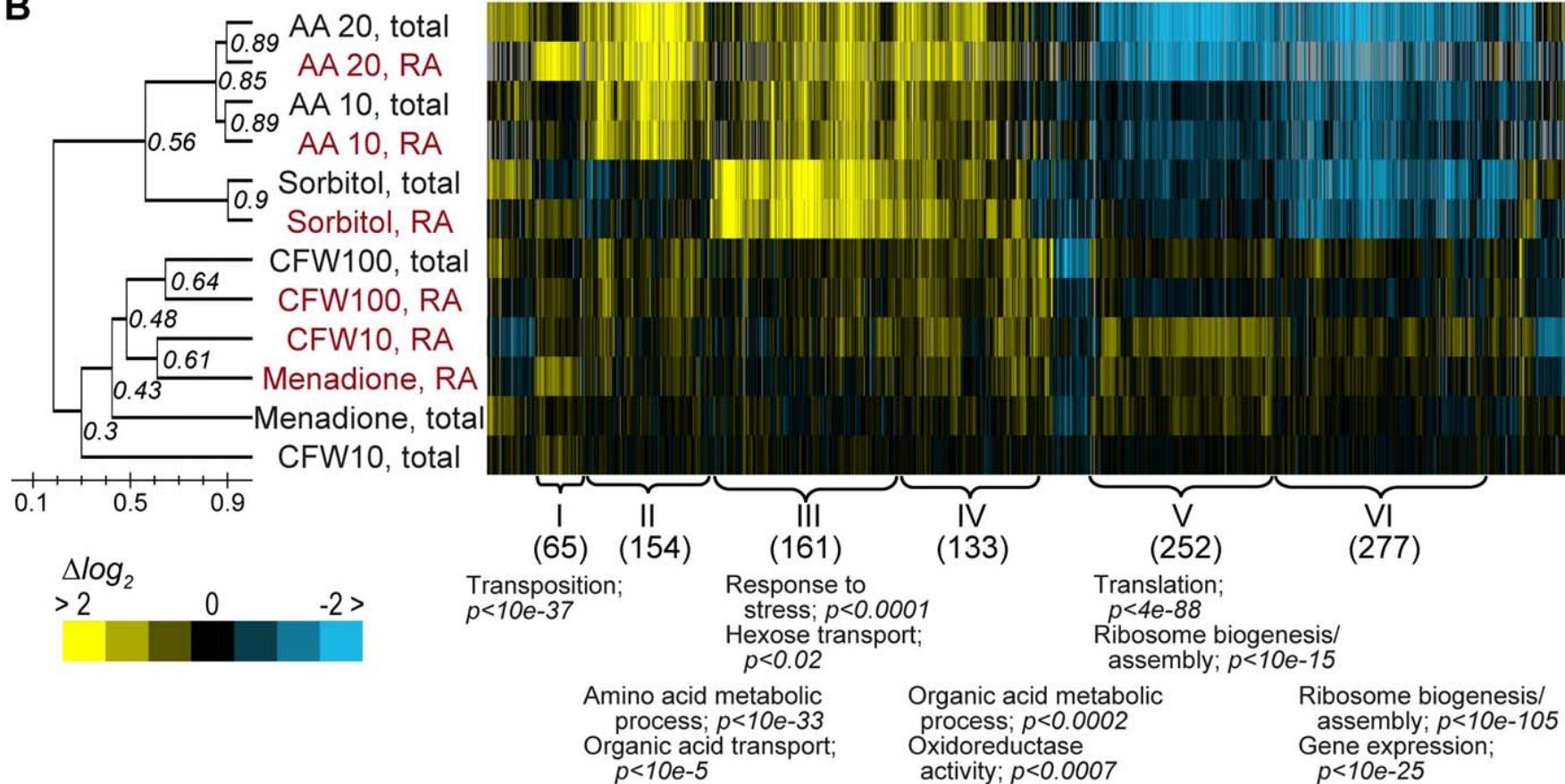
- Severe stress – Environmental Stress Response (ESR)
 - A.A starvation, osmotic shock
- mild oxidative stress
- First: Effects on Translation!

Stress...

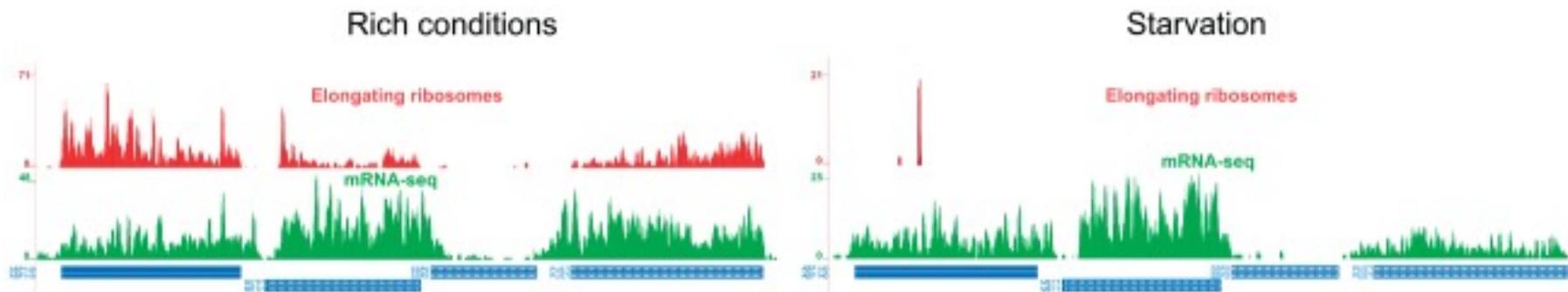


RA – Ribosome Associated mRNA.

Total - Total mRNA levels

B

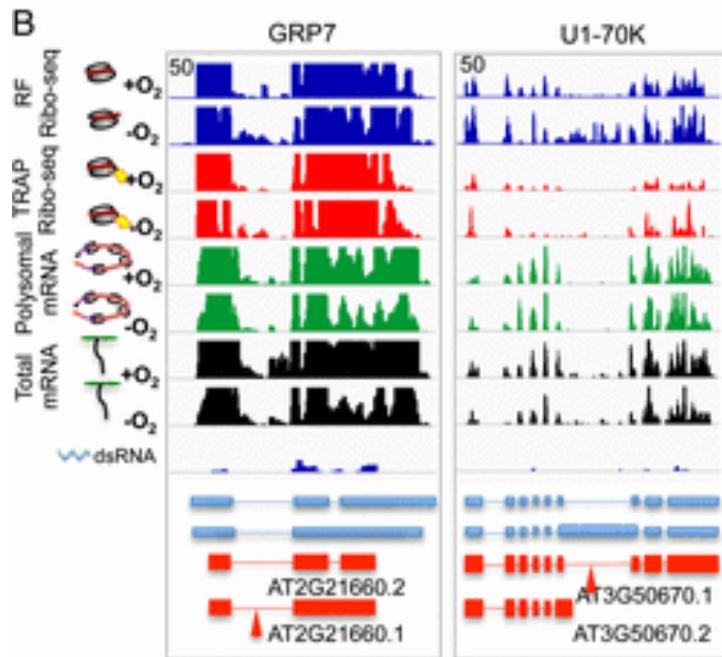
Stress Alters Translational profiles



Ribo-seq (red) and mRNA-seq (green) coverage plots for the *S. cerevisiae* genome. Under starvation conditions (right), *ABP140*, *MET7* and *PUS7* are transcribed, but not translated.

Stress Alters Translational profiles

- Different Initiation sites.
- + Stalling at start.
- Alternative ORFs.
- Seen in multicellular eukaryotes, not just yeast!

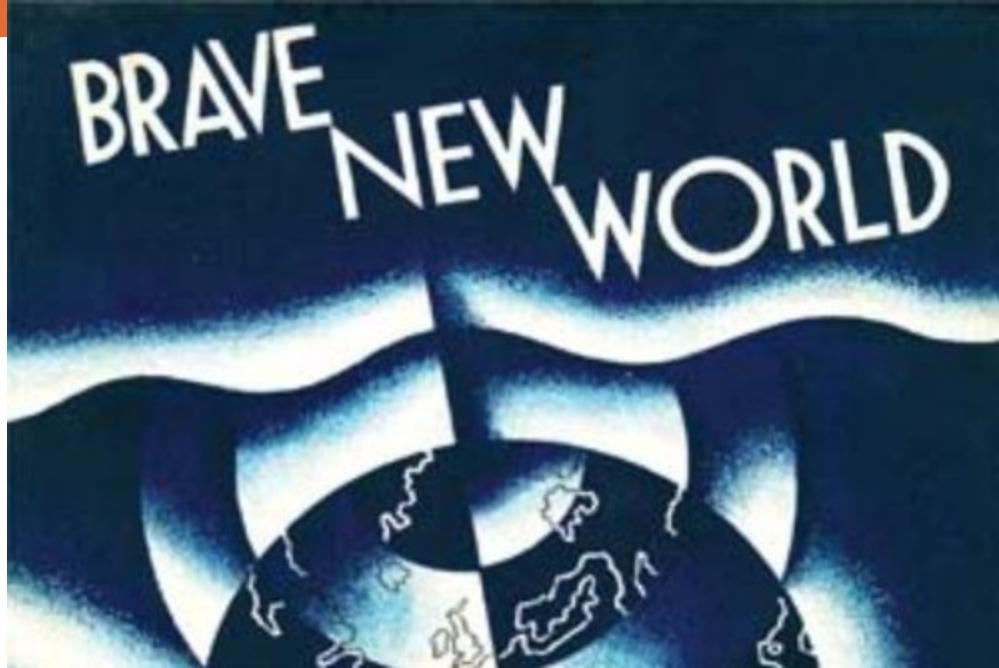


Conditional alternative
splicing in *Arabidopsis*

A Whole New World!



What have we seen? A Whole New World!



- Not just a more direct view of transcriptomics...
- Ribosome and translation behavior.
- Novel translatory behavior. (uORFs, targets..)
- Ribosome “Driving behavior” . (Speed, spacing, density).
- Superb resolution. High Thoroughput.
- Caveats...
 - Translation levels, not abundance...

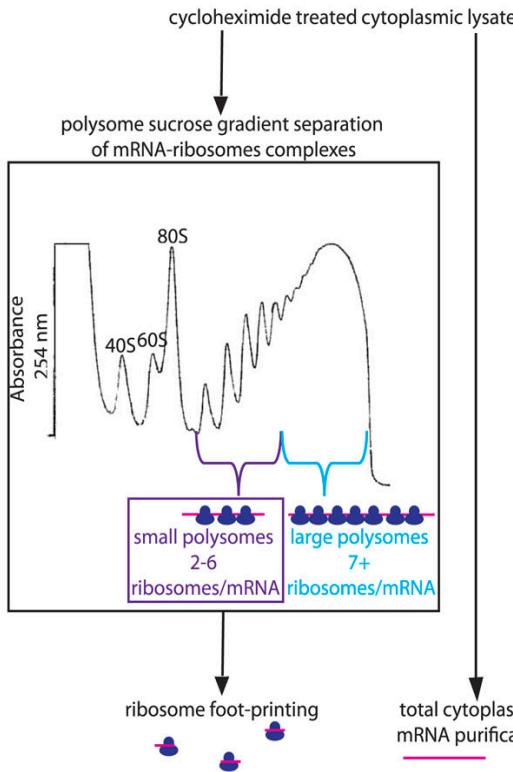
Additional Methods

Poly-Ribo-Seq

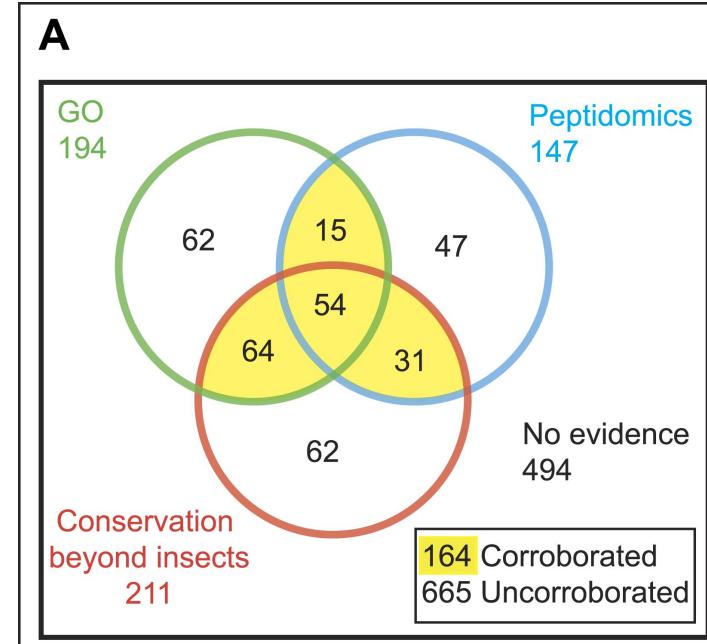
Q: How to find small Open Reading Frames (smORFs), micro-peptides (<100 aa), with less false positives?

A: Look for multiple Ribosomes! (Polysomes).

B



A



Extensive translation of small Open Reading Frames revealed by Poly-Ribo-Seq. Elife. 2014. doi: 10.7554/eLife.03528. . Aspden, Couso JP et al

smORF discovery

Hundreds of smORFs found to be translated.

- ~80aa smORFs. (Peptides).
 - Structural role? Many transmembrane domains.
 - Conserved. Well expressed.
- ~20aa dwarf smORFs
 - Less conserved.
 - Unknown functions

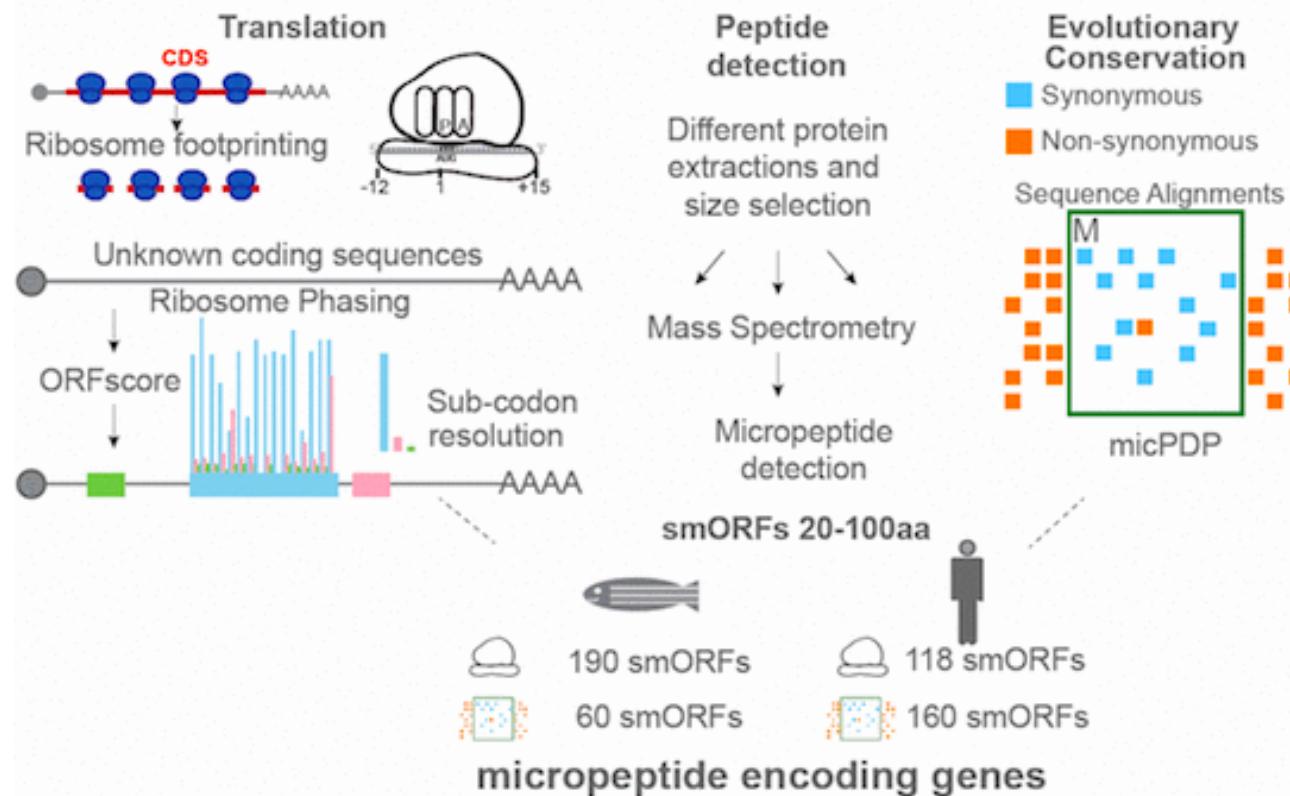
Annotated smORFs in different organisms

DOI: <http://dx.doi.org/10.7554/eLife.03528.003>

	smORFs	ORFs	% smORFs
Drosophila	829	21,870	3.8
Zebrafish	854	43,148	2.0
Mouse	1131	51,745	2.2
Human	1938	104,109	1.9

Extensive translation of small Open Reading Frames revealed by Poly-Ribo-Seq. Elife. 2014. doi: 10.7554/eLife.03528. . Aspden, Couso JP et al

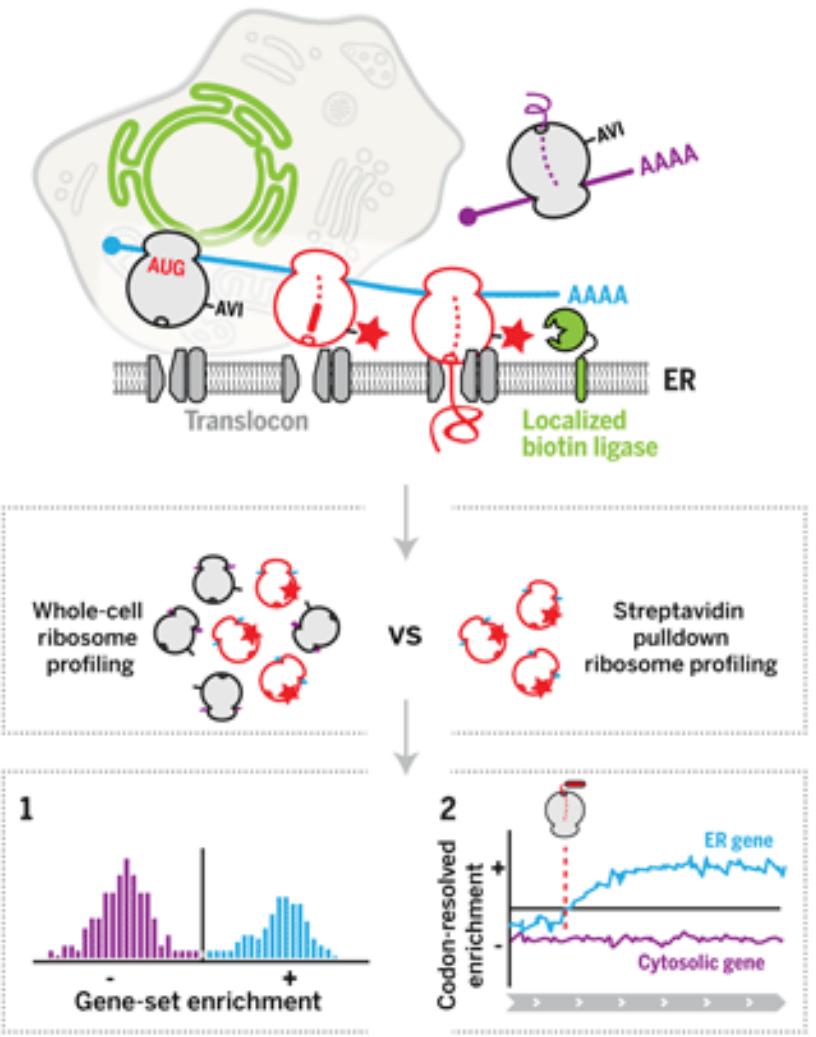
Validation of Detected Micropeptides, smORFs : MS



Additional Methods

Proximity-specific ribosome profiling

- Biotin ligase is expressed as a **fusion** to a protein that localizes to a specific compartment.
- This fusion protein is coexpressed with ribosomes that contain a biotin acceptor peptide.
- Ribosomes that come in close contact with the biotin ligase are biotinylated and then subject to streptavidin pulldown and ribosome profiling.



Principles of ER cotranslational translocation revealed by proximity-specific ribosome profiling . Weissman[†] et al. Science 7 November 2014:

Additional Methods

Proximity-specific ribosome profiling

Some findings:

- Most secretory proteins: targeted to the ER cotranslationally.
 - Functional diversity of signal sequences length, SRPs, translocons...
- 30% mitochondrial proteins are cotranslationally translocated.
- Some proteins can be targeted to both the ER and mitochondria.
 - E.g: Osm1 - Has 2 Translational start sites, and 2 isoforms.

GAME OVER

Thank you for playing