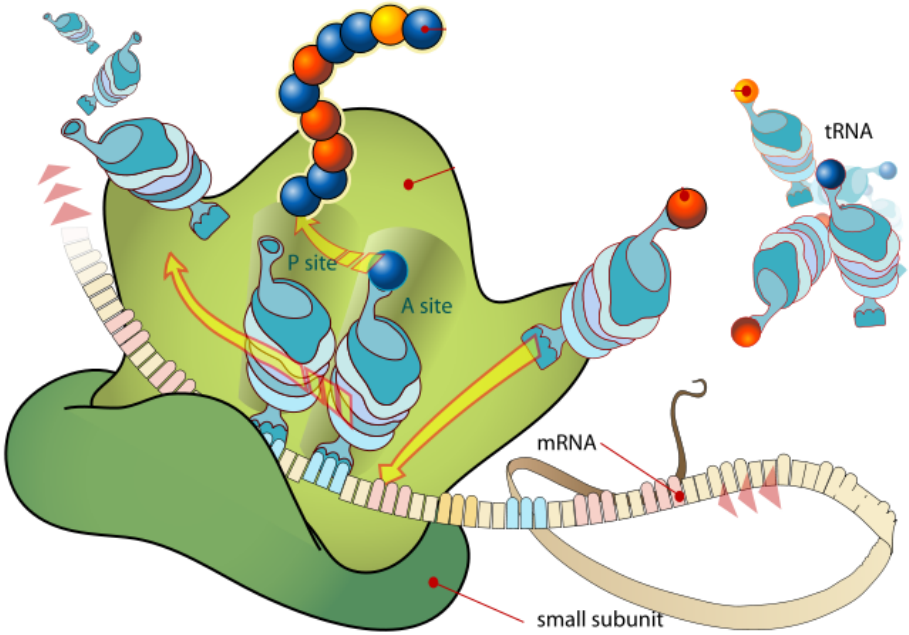
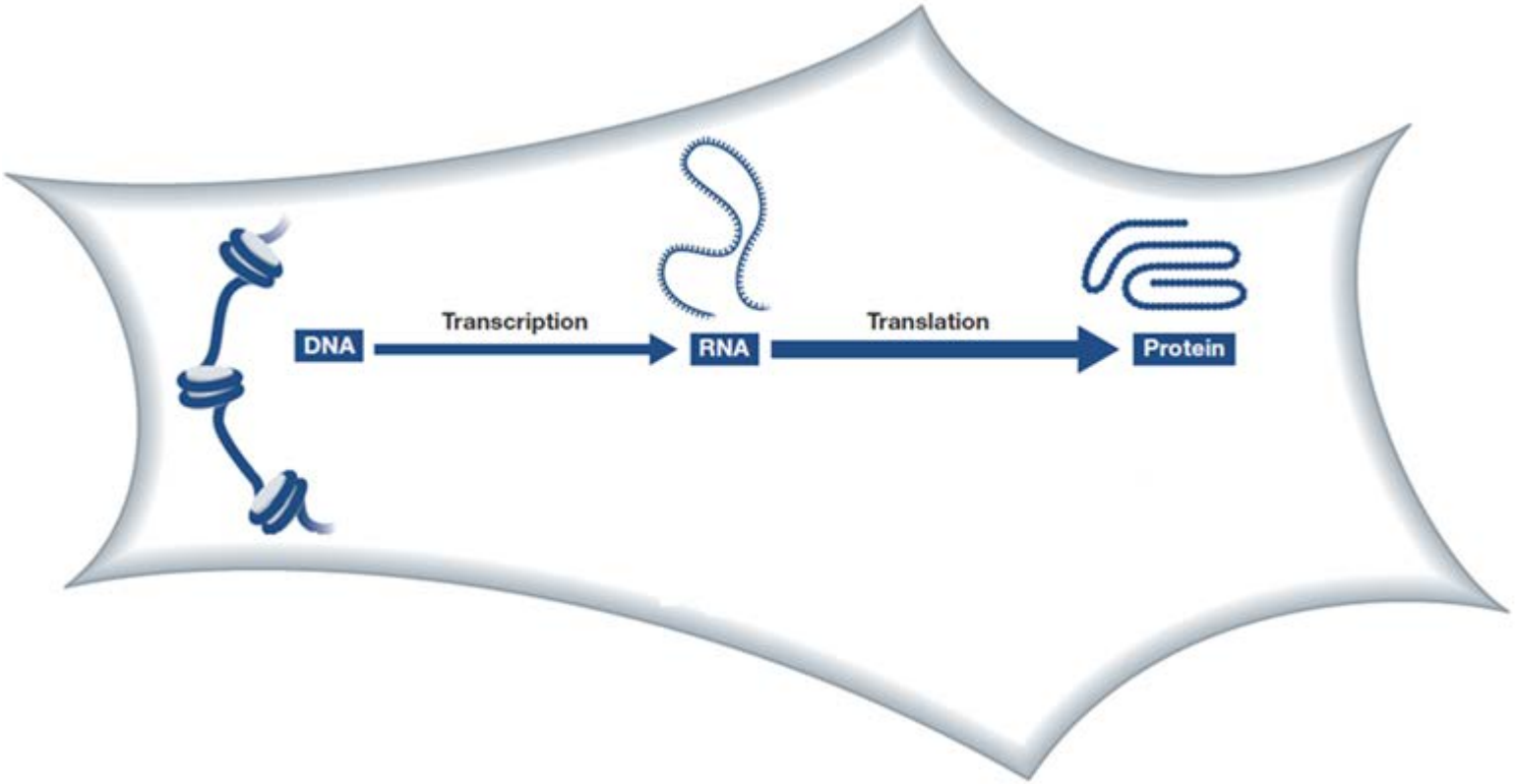
« Ribosome profiling strategy: exploring genome-wide landscape of translational control »

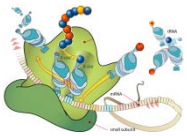


Parrinello Natalia
TJC
08.10.2013

Francis Crick's central dogma of molecular biology



Vogel C. Mol. Sys. Biol. (2011)



ARTICLE

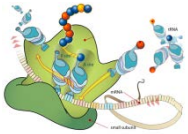
doi:10.1038/nature10098

Global quantification of mammalian gene expression control

Björn Schwanhäusser[1], Dorothea Busse[1], Na Li[1], Gunnar Dittmar[1], Johannes Schuchhardt[2], Jana Wolf[1], Wei Chen[1] & Matthias Selbach[1]

- 5000 mRNA and proteins analysed in mouse fibroblast (a quater of mouse genome)
- 40% of variation in protein levels is defined by mRNA levels (the results of transcription and mRNA degradation)
- 41–54% of the variation in concentration across proteins can be attributed to differences in translation rates (while the rates of degradation have surprisingly small roles)
- translation efficiency is the single best predictor of protein levels
- protein abundance seems to be predominantly regulated at the ribosome

the 'second half' of the central dogma of biology has a role much bigger than that has been recognized to date



Techniques for monitoring protein translation have lagged far behind methods for measuring mRNA levels

Microarray-based measurements of mRNA abundance have revolutionized the study of gene expression

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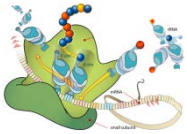
review

Exploring the new world of the genome with DNA microarrays

Patrick O. Brown[1,3] & David Botstein[2]

There is a critical need for techniques that directly monitor protein synthesis:

- mRNA levels are an imperfect proxy for protein measurement because mRNA translation is subject to extensive regulation
- Predicting the exact protein product from the transcript sequence is not possible because of effects such as internal ribosome entry sites, initiation at non-AUG codons, and nonsense read-through
- Programmed ribosomal pausing during protein synthesis is thought to aid the co-translational folding and secretion of some proteins

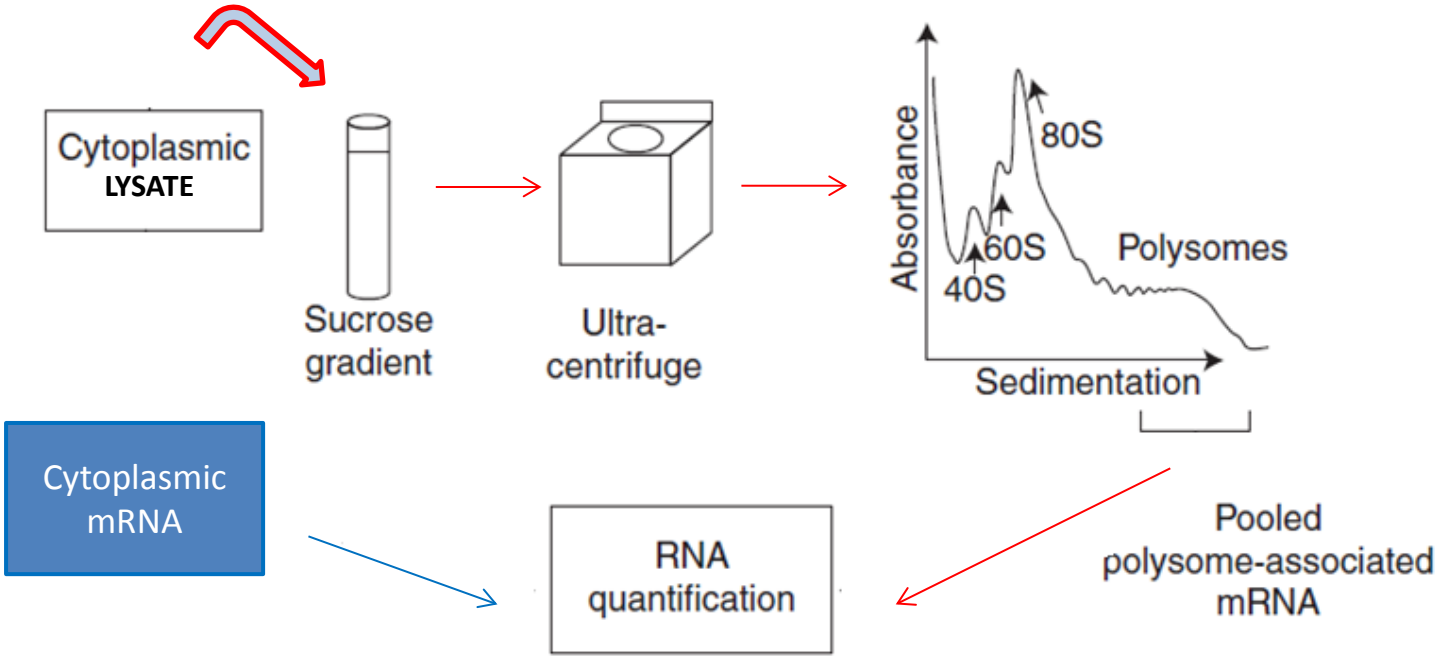


More direct evidence supporting the widespread role of translational control comes from studies of the global association between mRNAs and ribosomes.

Because mRNAs that have a higher translational activity are associated with more ribosomes, the polysome microarray technique has been used to study *genome-wide* mRNA translation.

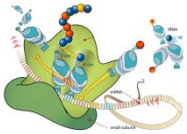
POLYSOME TECHNIQUE

POLYSOME: is a cluster of ribosomes, bound to mRNA molecule



O.Larsson et al. (2013)

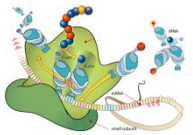
Fractionated mRNAs are then extracted and subjected to microarray analysis for identification and quantification



This approach suffers from limited resolution and accuracy.

Additionally, upstream open reading frames (uORFs)—short translated sequences found in the 5′ untranslated region (5′UTR) of many genes—result in mRNA that are bound to a ribosome and yet are not translated into a protein

Advances in quantitative proteomics circumvent some of these problems, but there currently are substantial limits on their ability to independently determine protein sequences and measure low-abundance proteins.

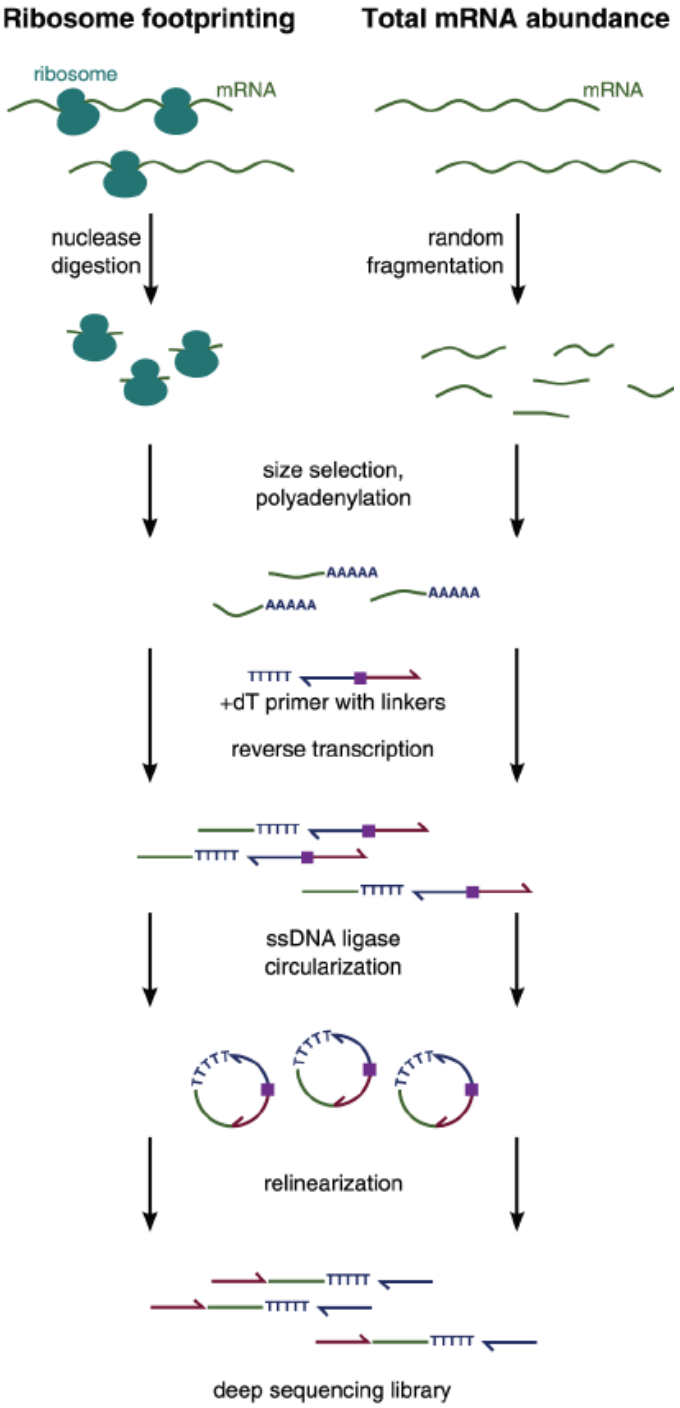


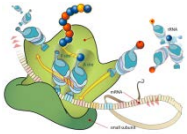
RESEARCH ARTICLE

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

Nicholas T. Ingolia,* Sina Ghaemmaghami,† John R. S. Newman, Jonathan S. Weissman

10 APRIL 2009 VOL 324 SCIENCE www.sciencemag.org

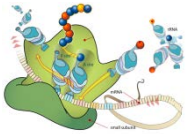




The technique involves two steps:

- isolation of mRNA fragments, obtained by RNAse treatment (or random RNA fragmentation);

- identification and quantification of these fragments by RNA-seq

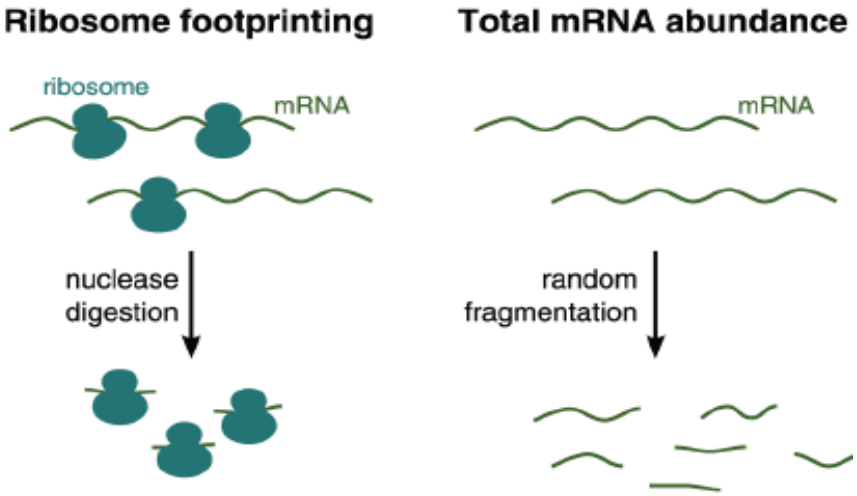


For each sample

Extract Preparation
(RNAseI)

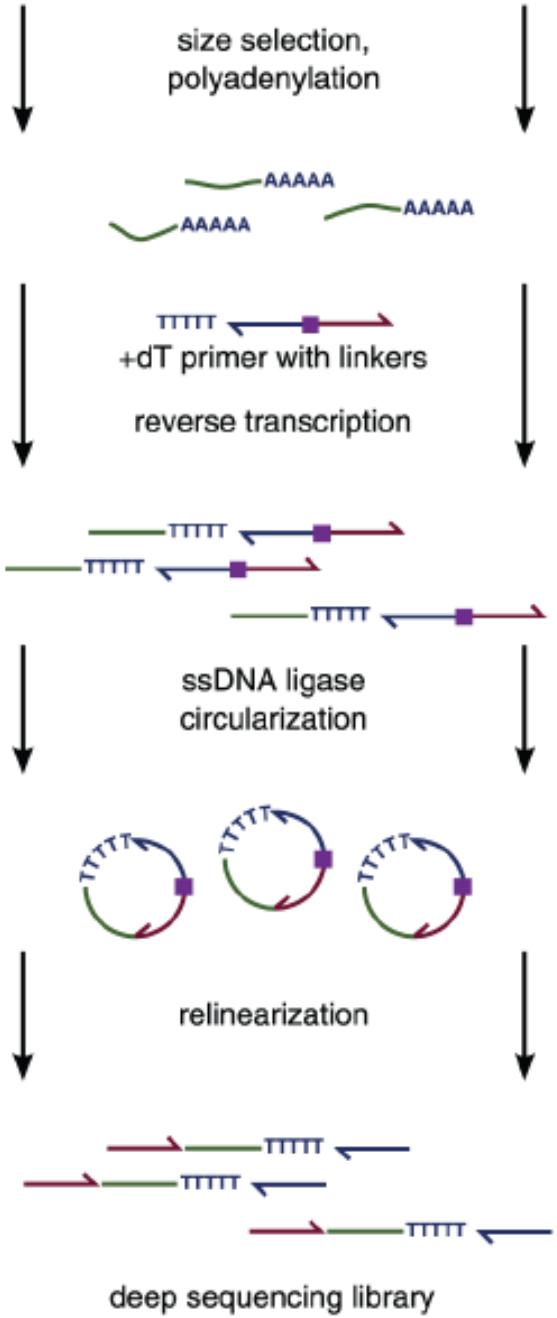
Total RNA Preparation
(alkaline fragmentation solution)

I STEP : *isolation of mRNA fragments, obtained by RNAse treatment or random RNA fragmentation*



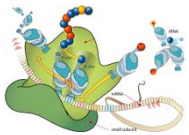
Ribosome footprint: portion of a mRNA template 30nt long that the ribosome protects from nuclease digestion

II STEP : *identification and quantification of these fragments by RNA-seq*



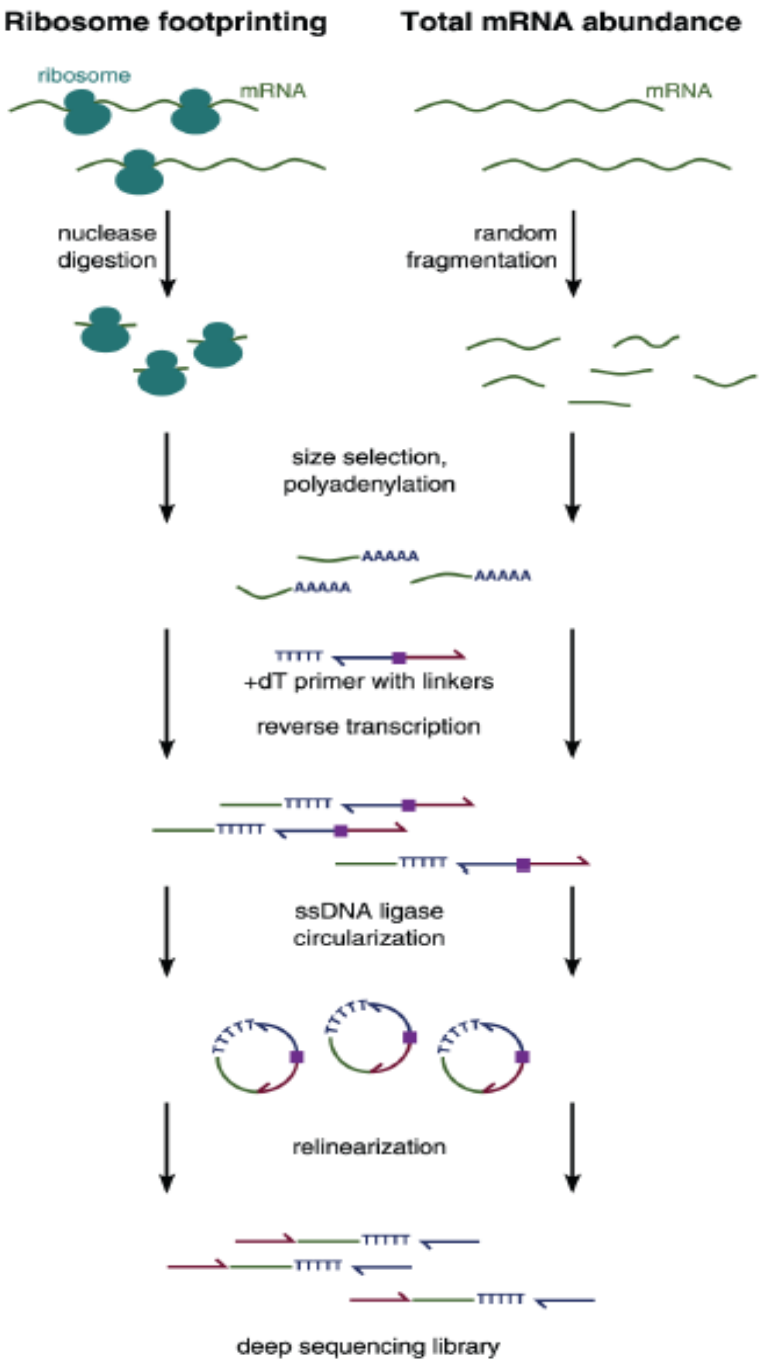
Depth (coverage) in DNA sequencing: refers to the number of times a nucleotide is read during the sequencing process.

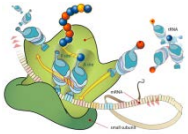
Deep sequencing indicates that the depth of the process is many times larger than the length of the sequence under study.



RIBOSOME PROFILING STRATEGY

- Quantifying mRNA abundance by deep sequencing
- Monitoring ribosome position with single codon resolution by deep sequencing
- Genome-wide measurements of translation
- Genome-wide investigation with subcodon resolution





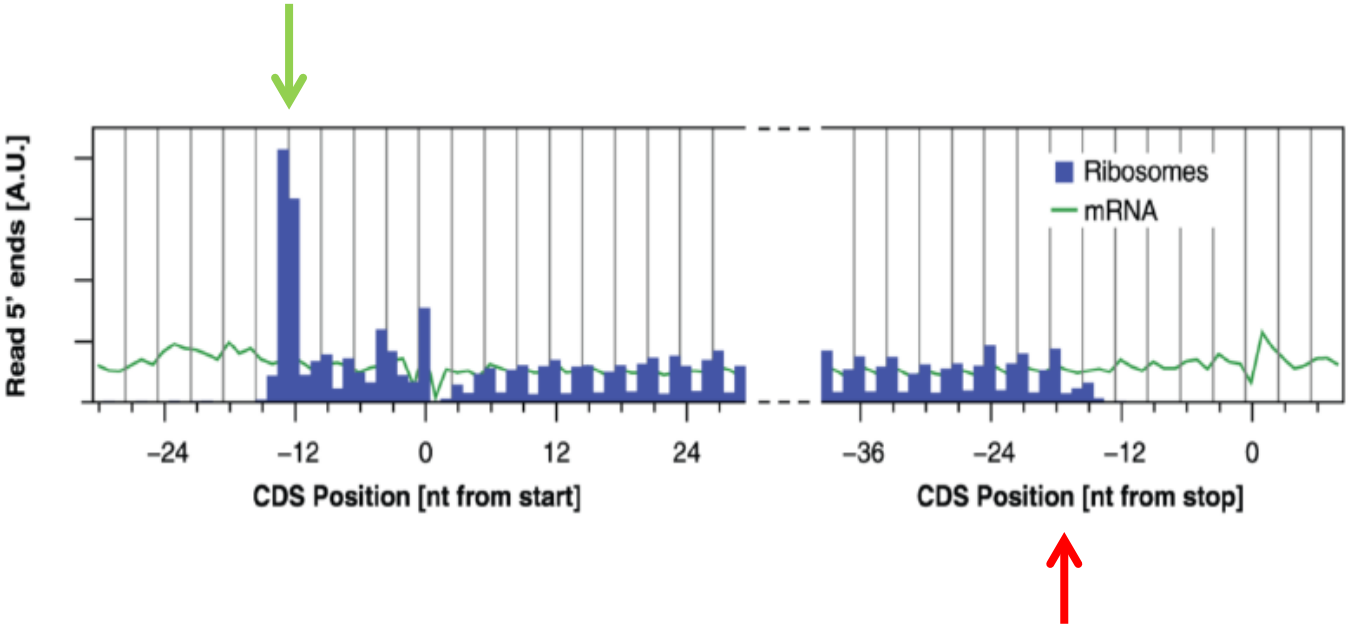
Quantifying RNA by deep sequencing

To establish ribosome profiling as a quantitative tool for monitoring translation, they implemented three steps:

- robustly generate ribosome-protected mRNA fragments ("footprints") whose sequences indicate the position of active ribosomes;
- convert these RNA footprints into a library of DNA molecules in a form that is suitable for deep sequencing;
- measure the abundance of different footprints in this library by means of deep sequencing

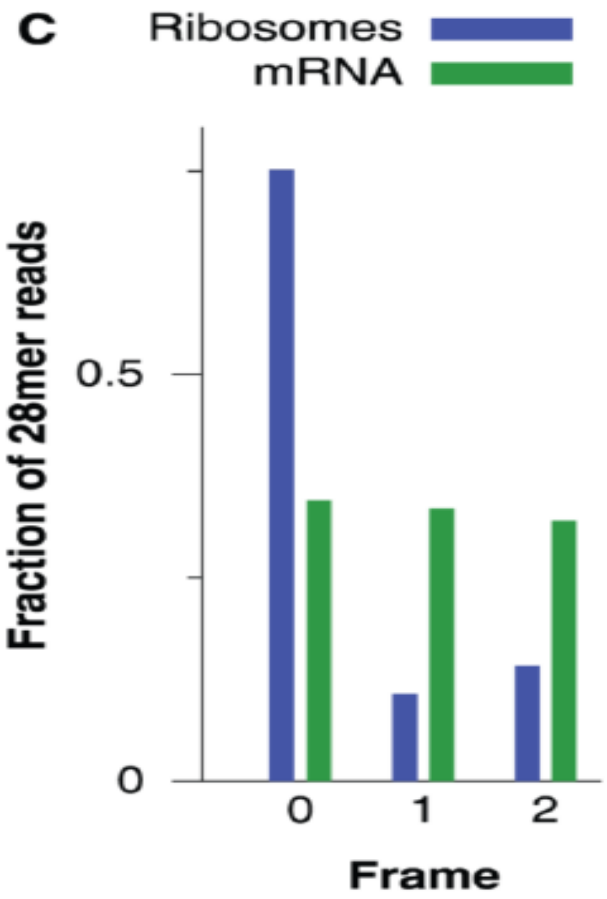
- **deep sequencing performed on 5295 genes**

Monitoring ribosome position with single codon resolution by deep sequencing



sequencing 42 million fragments generated by ribosome protection assay (on *Saccharomyces cerevisiae*)

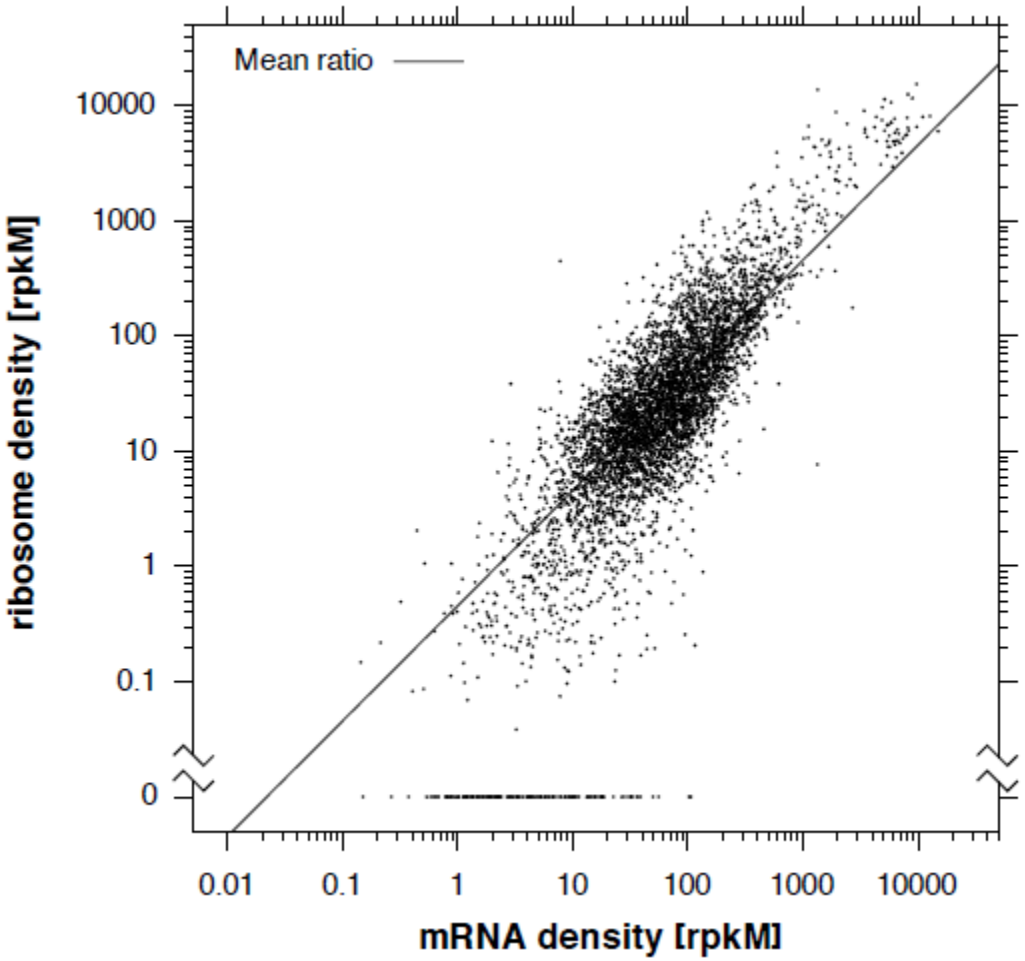
Monitoring ribosome position with single codon resolution with deep sequencing



75% of the 28-nt
oligomer ribosome-protected fragments started on the first nucleotide of a codon

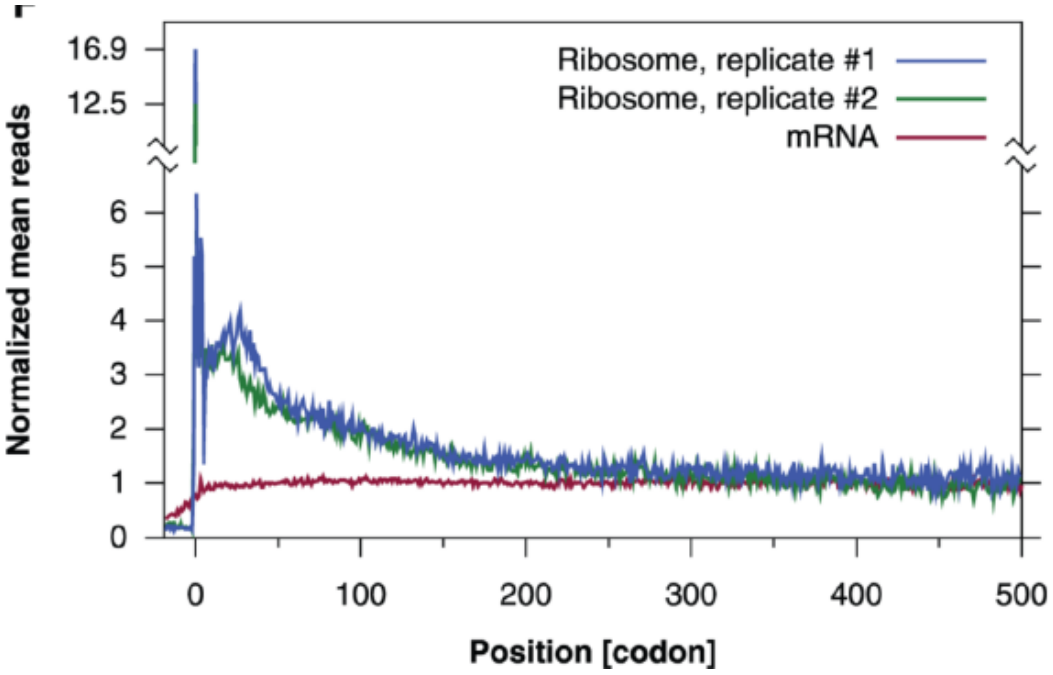
Genome-wide measurements of translation

From 7 million footprint sequences, they measured the translation of 4648 of 5295 genes



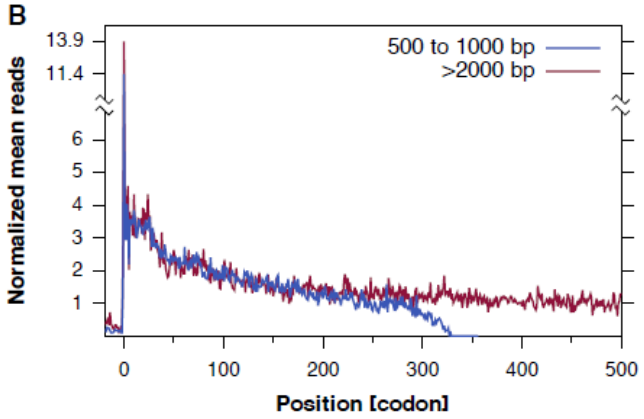
Genome-wide investigation with sub-codon resolution

Ribosome profiling reveals different phases of translation

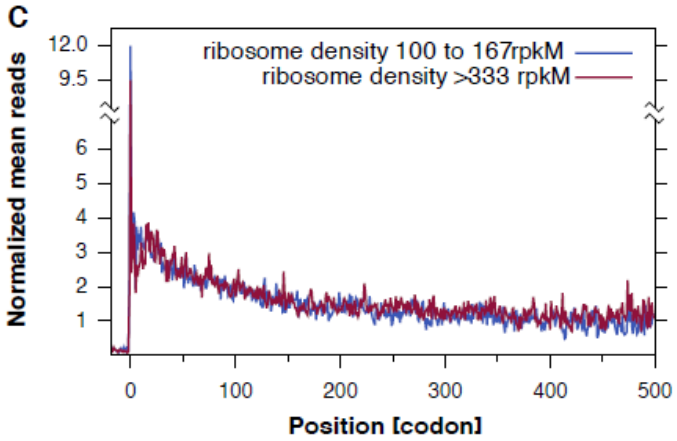


over hundreds of
well-translated genes

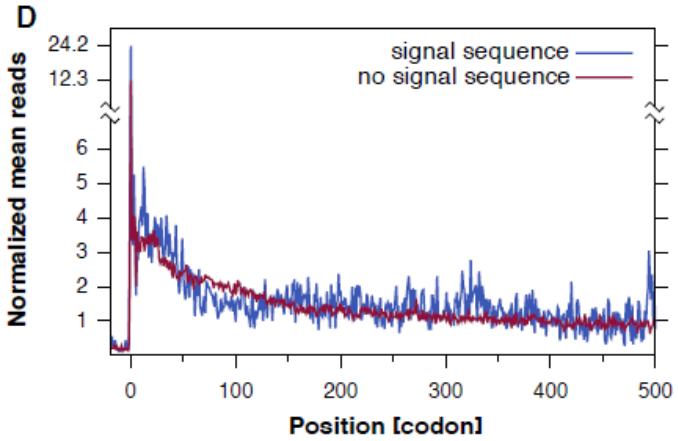
Ribosome profiling reveals different phases of translation



length of the CDS

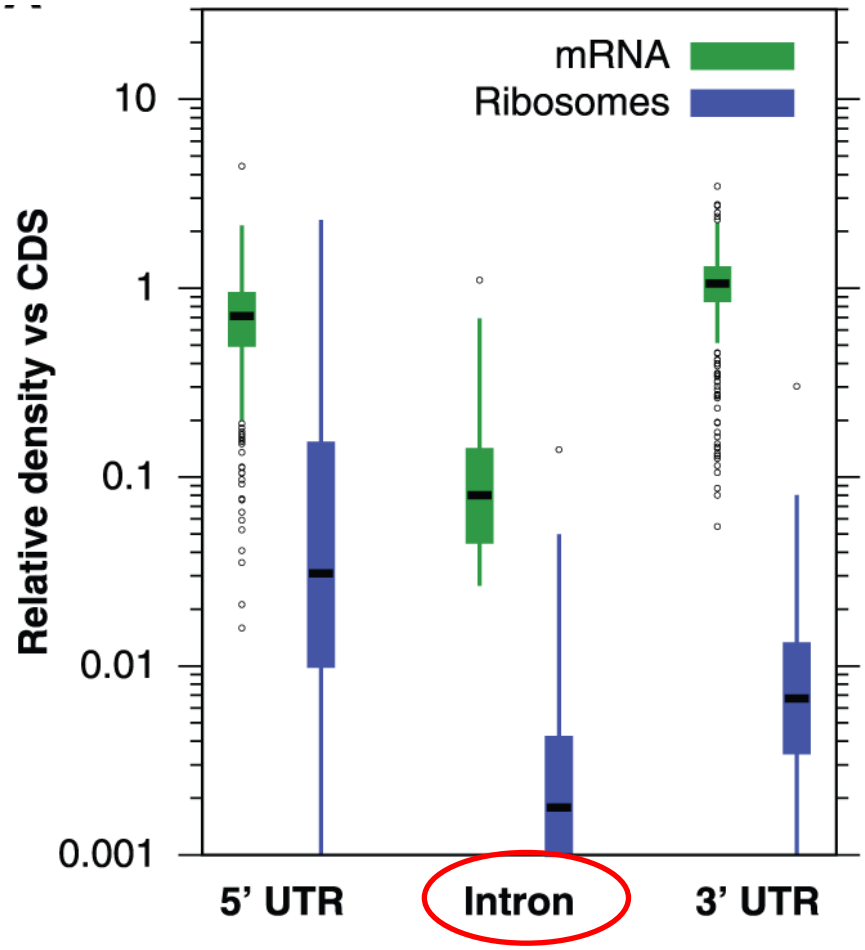


translation level



presence of an N-terminal signal sequence

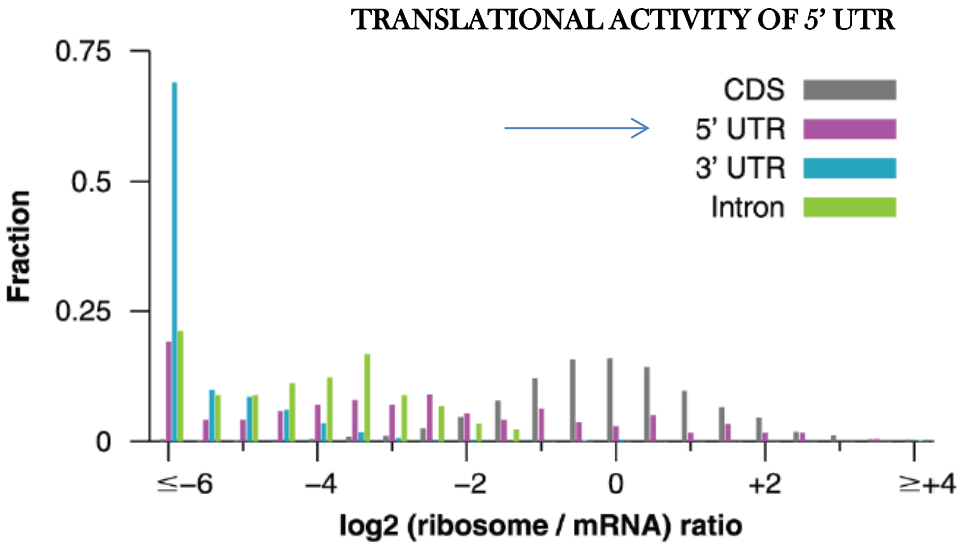
Codon-specific measurements of ribosome positions



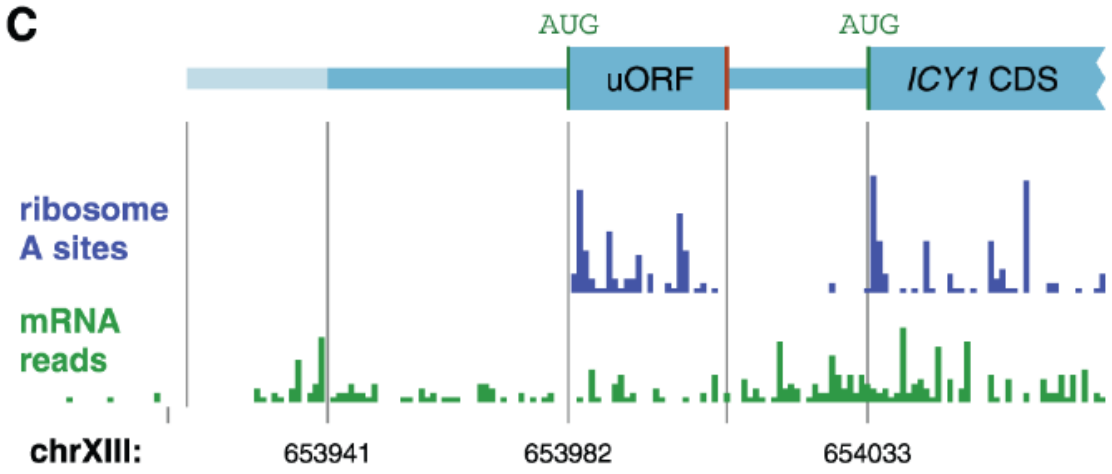
RIBOSOME OCCUPANCY

98.8% of ribosome footprints--protein-coding regions

56,105 unexplained footprints



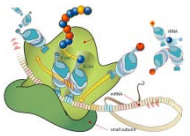
Codon-specific measurements of ribosome positions



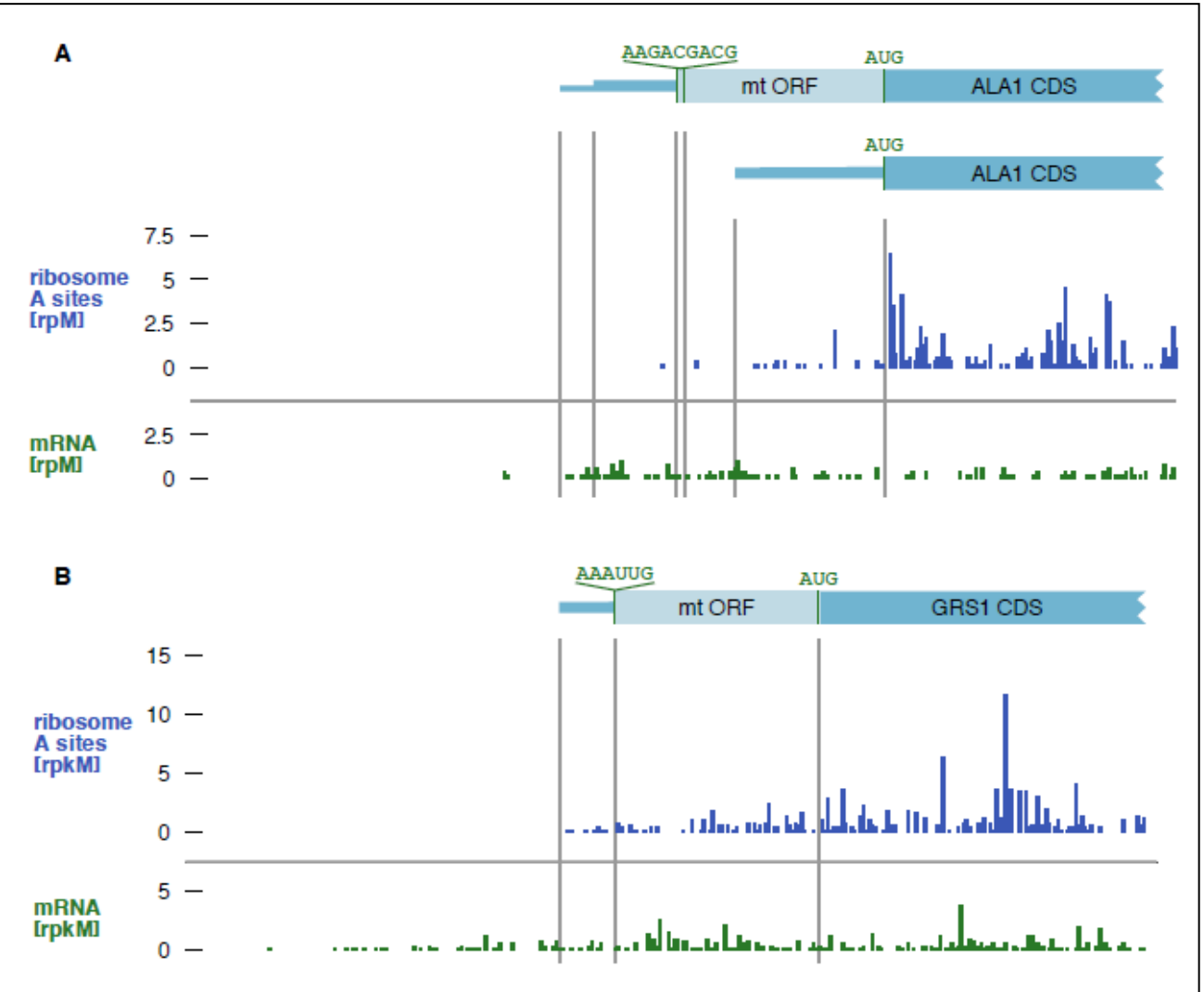
1048 candidate uORFs in the yeast genome (presence of an upstream AUG codon)

Only 20 of the uORF were well traslated like ICY1

Codon-specific measurements of ribosome positions

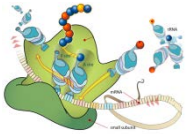


mtORF: protein with a mitochondrial signal sequence



143 non-AUG uORFs with evidence of translation

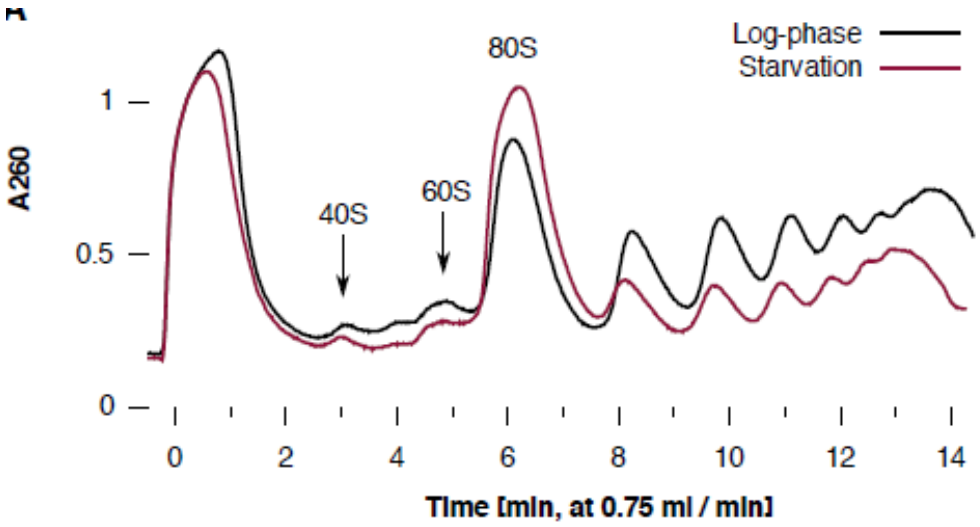
Strategy Application



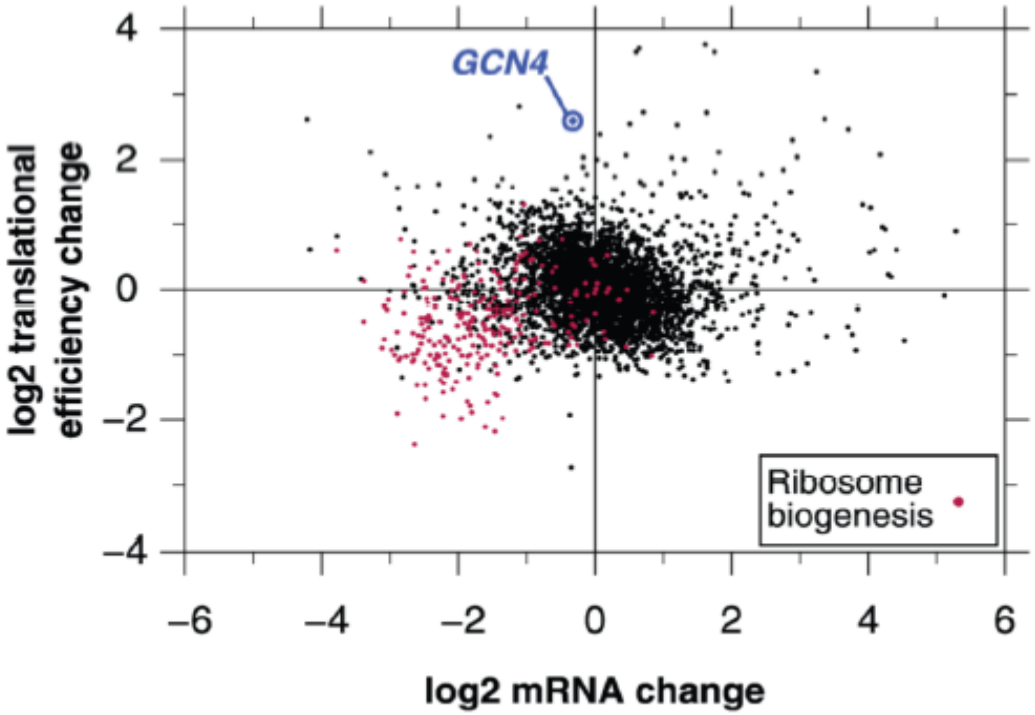
Translational responses to starvation

- 20 min of amino acid deprivation and made ribosome-footprint and mRNA-abundance measurements

- comparison starvation and log-phase growth measurements for the 3769 genes

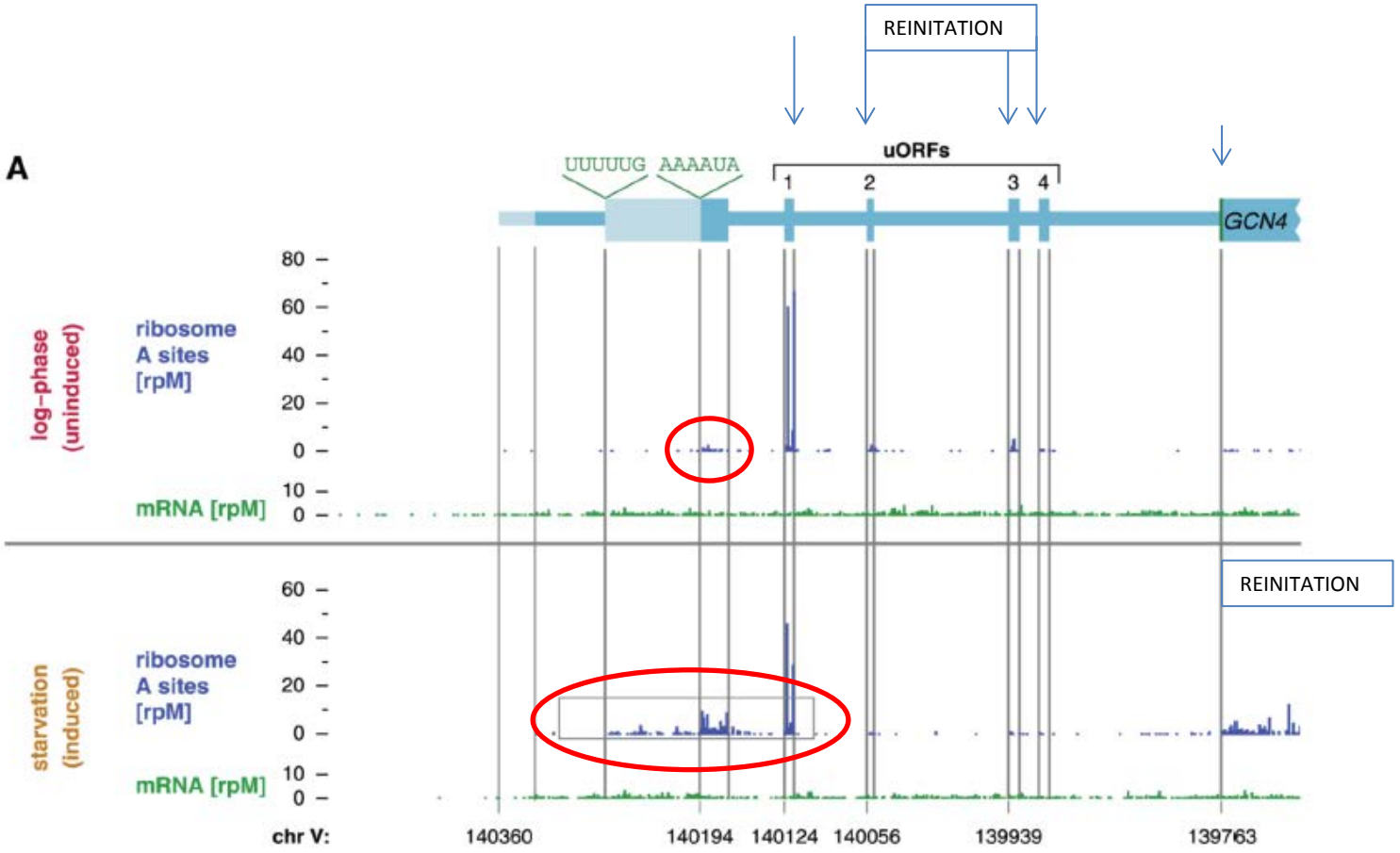


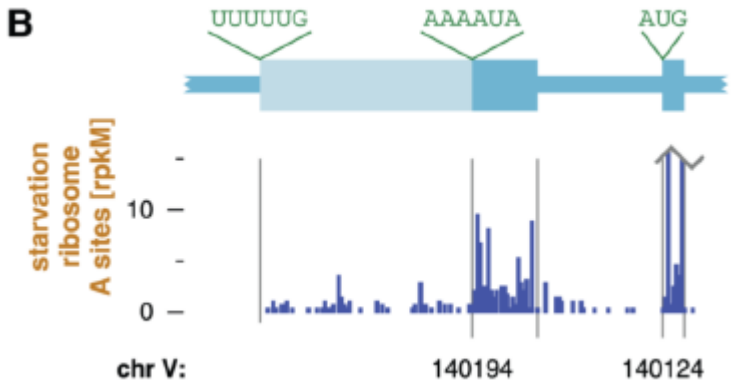
changes in mRNA abundance and translational efficiency changes in response to the starvation

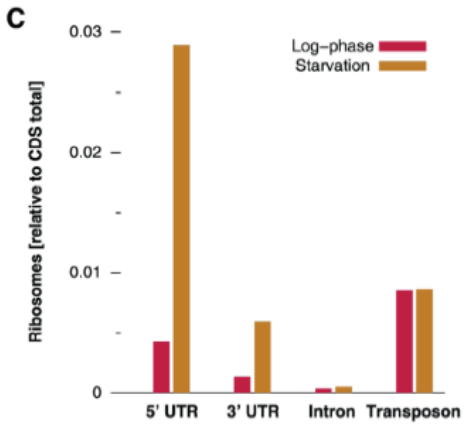


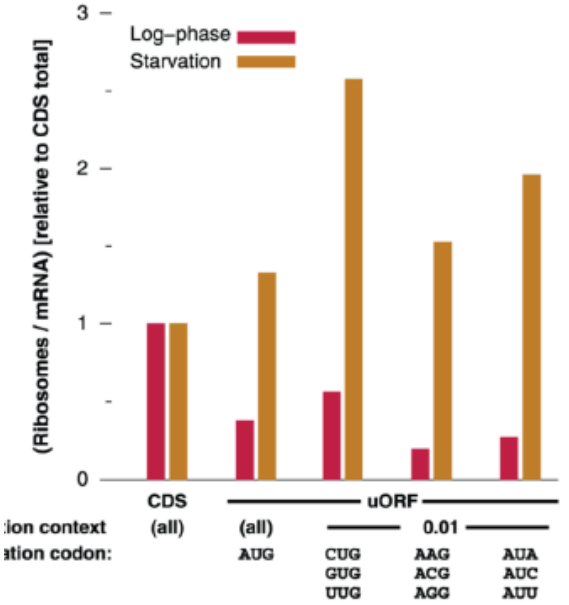
Changes in 5' UTR translation during starvation

Ribosome and mRNA densities in the GCN4 5′UTR in repressive and inducing conditions. The four known uORFs are indicated along with the proposed initiation sites for upstream translation.





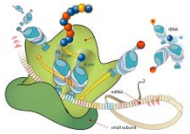




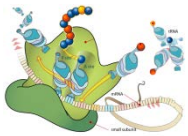
increase in the fraction of ribosome footprints derived from 5′UTRs but little change in introns

The non-AUG uORFs showed a particularly dramatic increase in ribosome occupancy during starvation, apparently exceeding the translation not only of canonical AUG uORFs but of the CDSs themselves

PERSPECTIVE



- Ribosome profiling greatly increases the ability to quantitatively monitor protein production
- It should become a central tool in the repertoire available for studying the internal state of cells
- The basic strategy is readily adaptable to other organisms, including mammals
- Could be use for studies of the translational control of gene expression and molecular characterization of disease states such as cancer, in which associated cellular stress will probably directly affect translation
- Measurements of the effects of starvation on translational activity also revealed widespread and regulated initiation at non-AUG codons
- The high-resolution gene-specific ribosome density profiles will enable efforts to explore how variations in the rate of translation, as well as effects such as ribosomal pausing, modulate protein synthesis and folding.



THANKS FOR YOUR ATTENTION!