

**CONJOINT 541**

**Translating a Transcriptome at  
Specific Times and Places**

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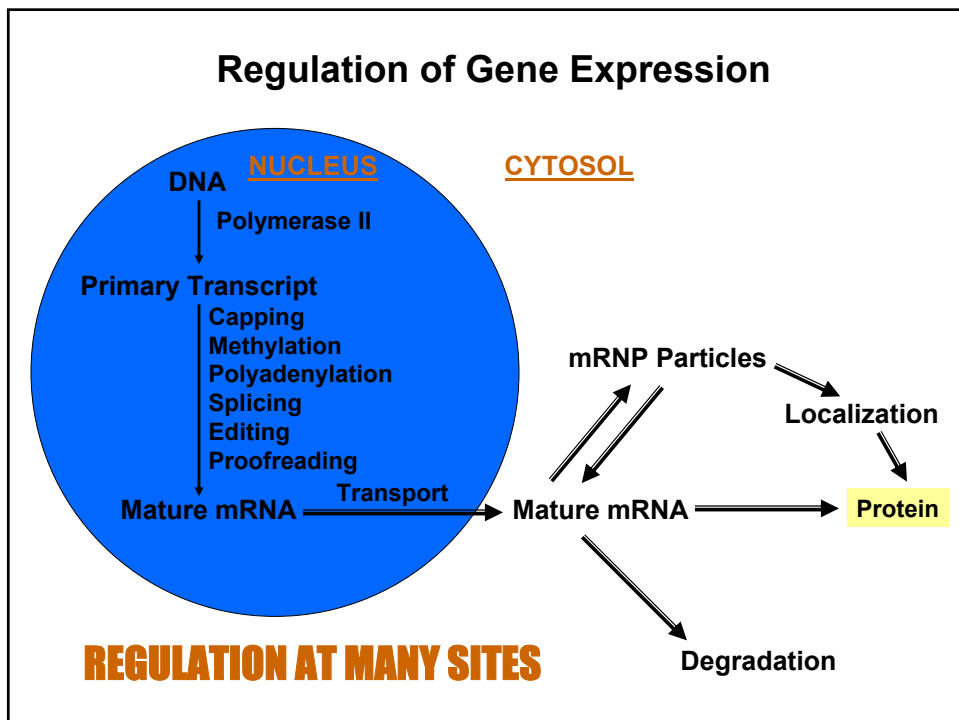
*<http://faculty.washington.edu/dmorris/>*

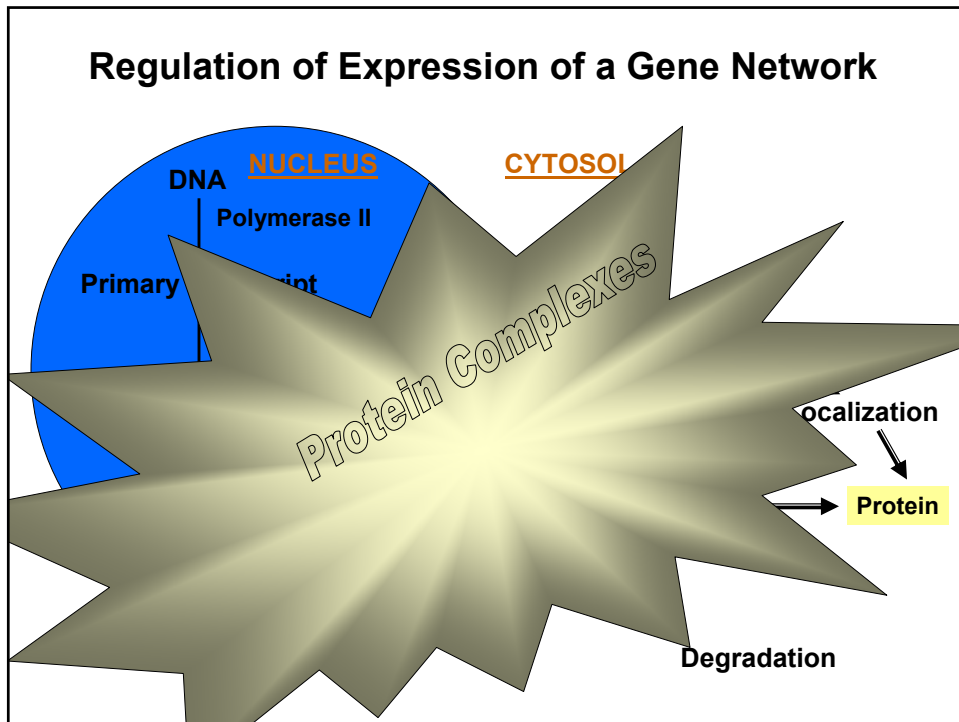
**Lecture 1**

*The Biology and Experimental  
Analysis of mRNA Translation*

# Lecture 1

- **Translational control – what and why?**
- **Genome-wide evaluation translation**
  - Global assessment of gene expression
  - Polyribosomes as a tool
  - Measurement of translational efficiency
  - Translation states across a transcriptome
- **Gene-specific translational control**
  - Sites of regulation of translation initiation
  - Cis*-acting elements in mRNAs

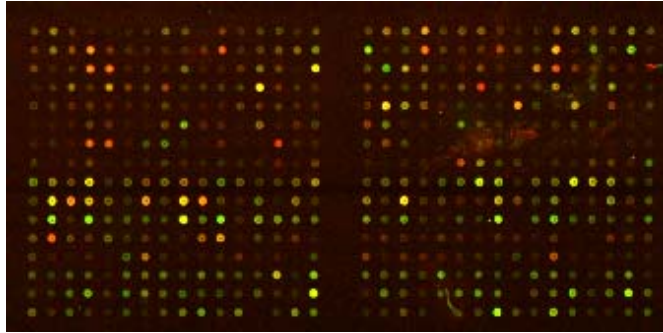




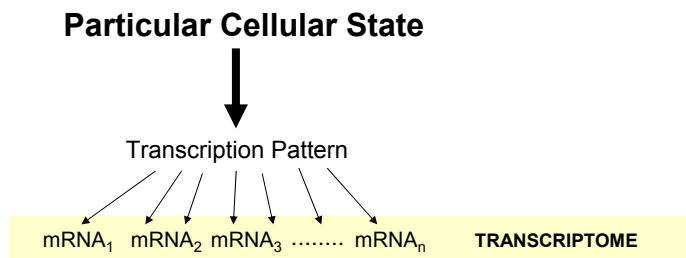
## Why regulate gene expression at the translational level?

- **Regulation of expression from stable mRNAs**
- **Rapid response**
  - ✓ Both on and off
  - ✓ Reversible
- **Fine tuning expression at levels beyond transcription**
- **Issues unique to big cells**
- **Localized expression in a cell**

## Transcriptome-Wide Evaluation of Translation

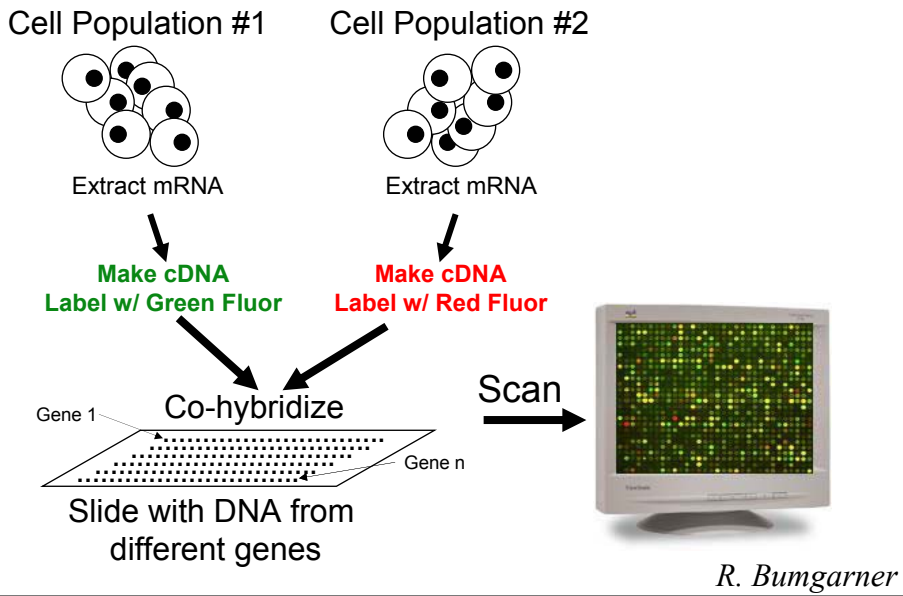


## Genetic Regulatory Networks



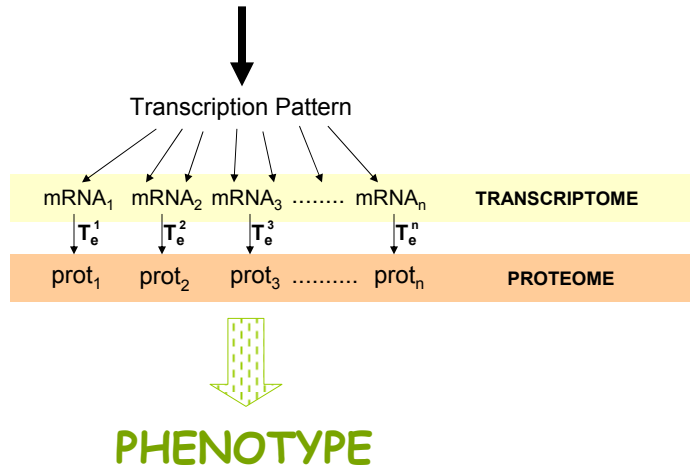
- ❖ Large-scale cDNA sequencing
- ❖ SAGE
- ❖ Microarray
  - Oligo
  - cDNA

# Classic Expression Array Experiment



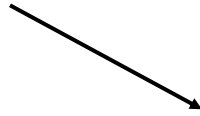
# Gene Expression Networks

Particular Cellular State



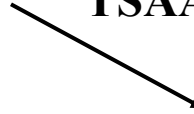
## Approaches to High-Throughput Analysis of Molecular Phenotype

**DNA**



**mRNA**

**TSAA**



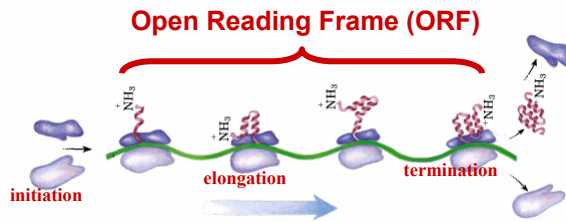
**Protein**

- ❖ Large-scale cDNA sequencing
- ❖ SAGE
- ❖ Microarray

- Proteome Analysis
- 2-D gels
  - Mass spect

# POLYRIBOSOME GYMNASTICS

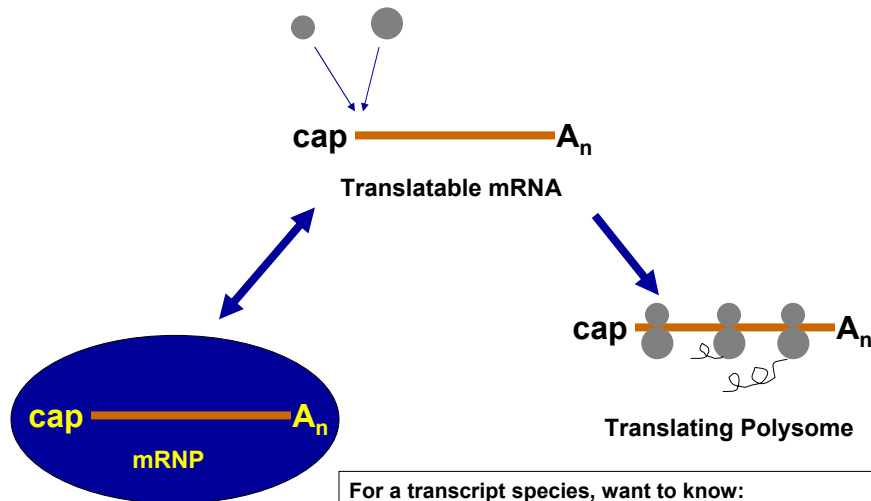
## mRNAs are Translated on Polyribosomes (Polysomes)



Rates of elongation and termination are constant for most transcripts, therefore rate of synthesis of the encoded protein is proportional to ribosome spacing

$$[\text{ribosome spacing}] \times k = \text{rate of synthesis}$$

## mRNA Translation in Eukaryotes

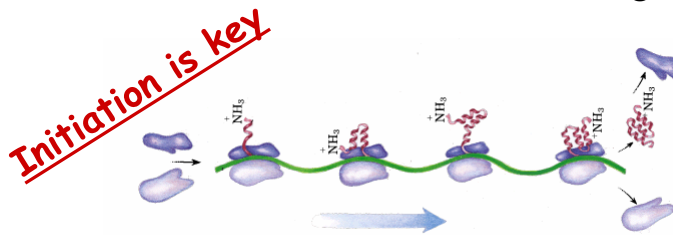


For a transcript species, want to know:

- > fraction of mRNA associated with ribosomes
- > average polysome size (# ribosomes)

## Two parameters dictate number of ribosomes loaded on an mRNA:

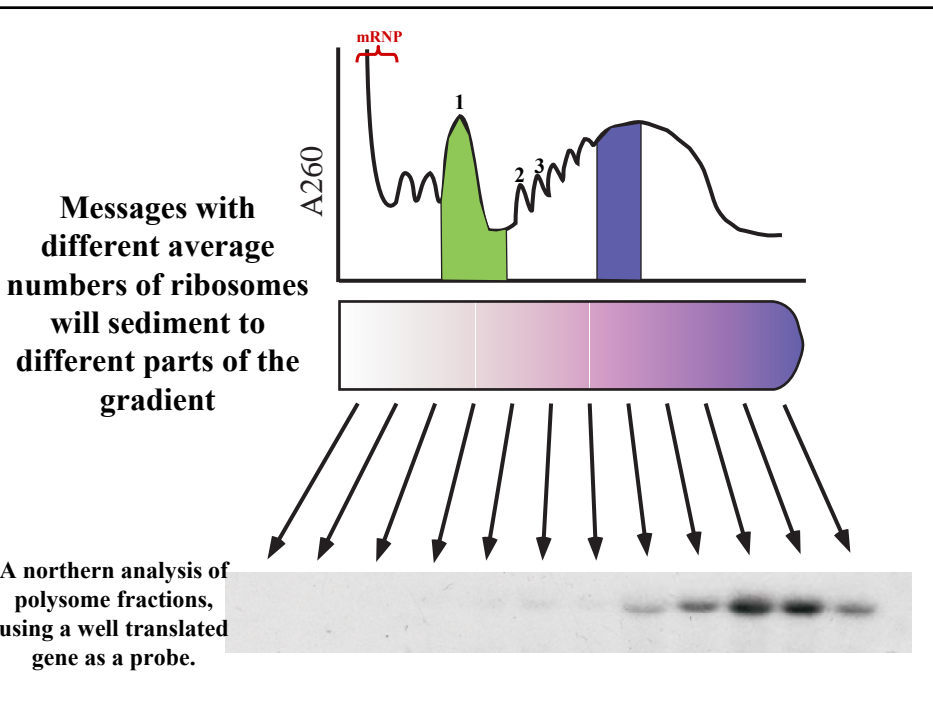
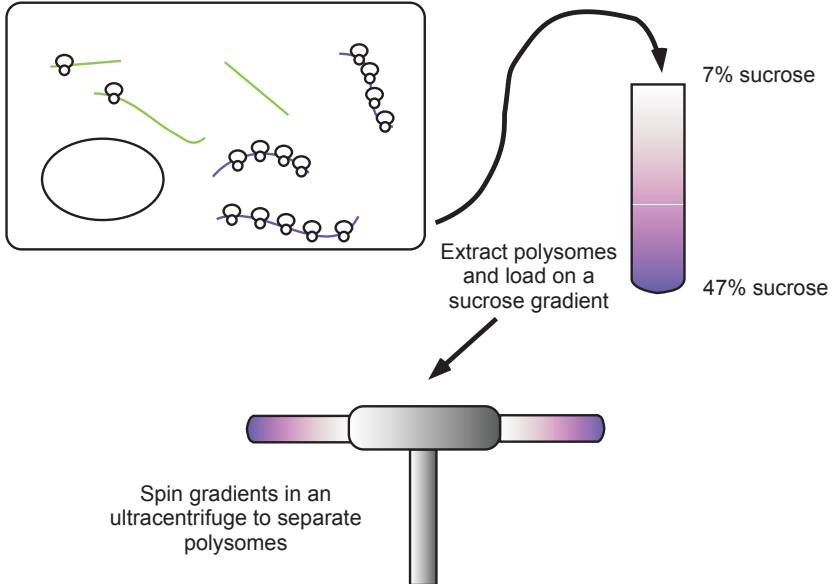
- Size of the ORF
- Rate of ribosome loading

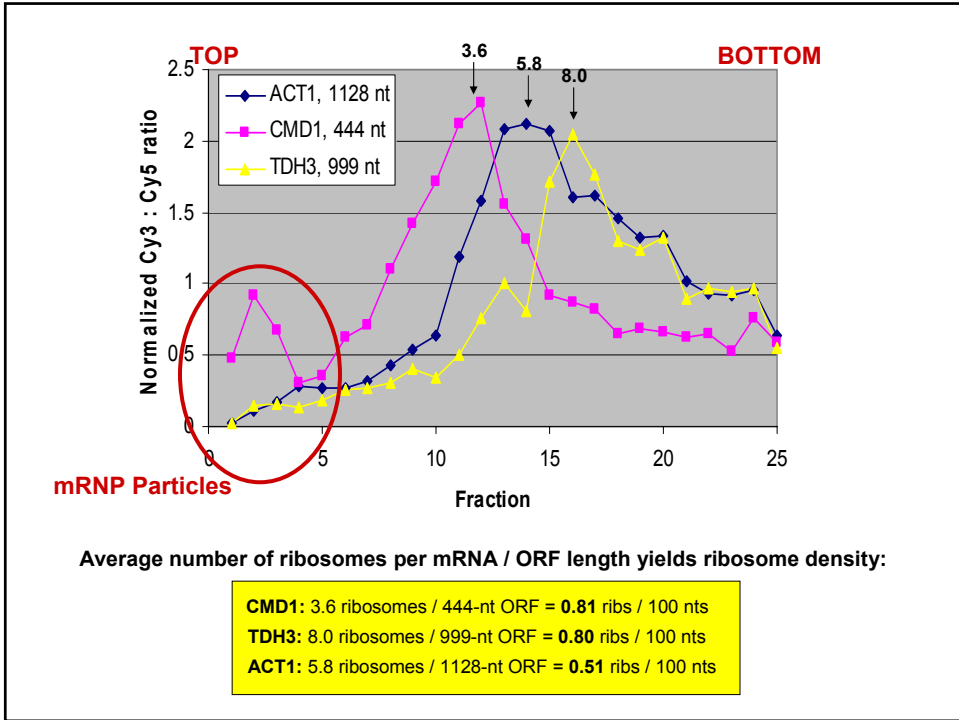


How to measure number of ribosomes on a transcript?

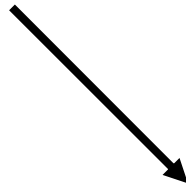


## Sucrose Gradient Centrifugation



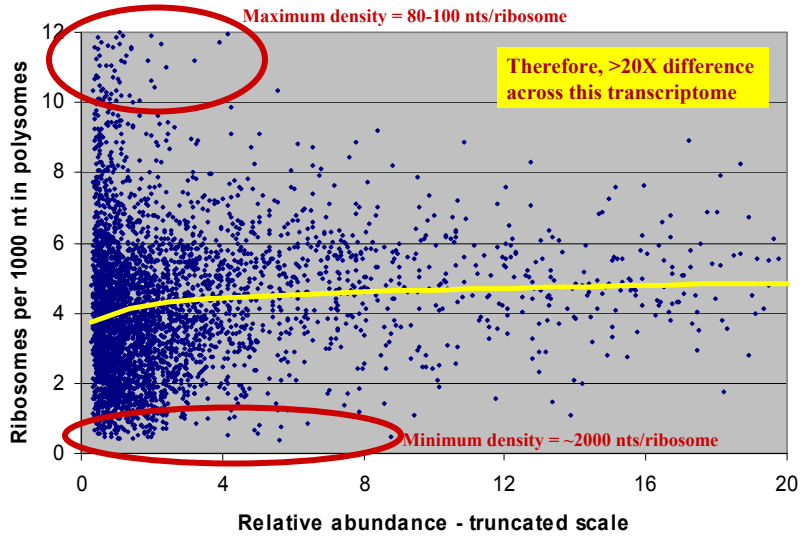


## High throughput analysis



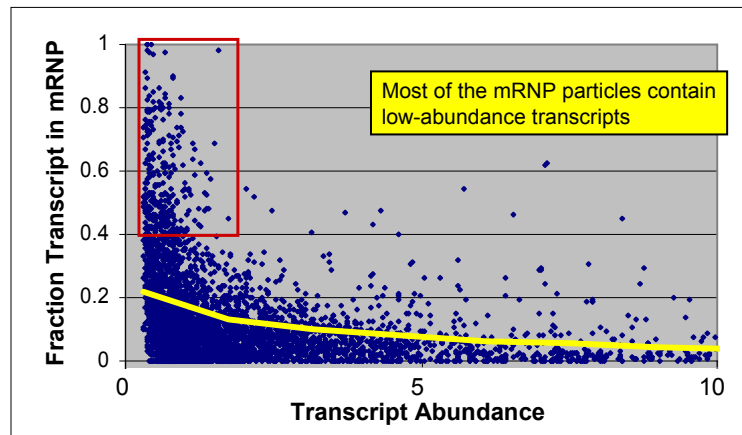
**TSAA**

## Ribosome Density Across a Transcriptome



MacKay et al., 2004

## Occurrence of Transcripts in mRNP Particles



MacKay et al., 2004

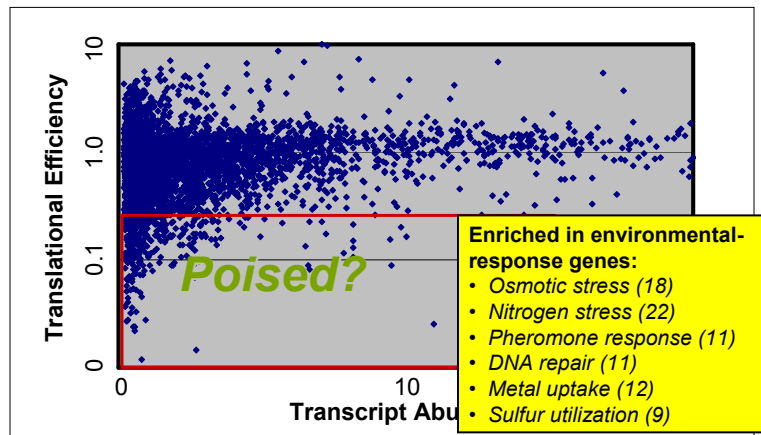
## Synthetic Rates of Individual Proteins

For protein “x”

$$\frac{dP^x}{dt} = T_e^x \cdot [mRNA_x]$$

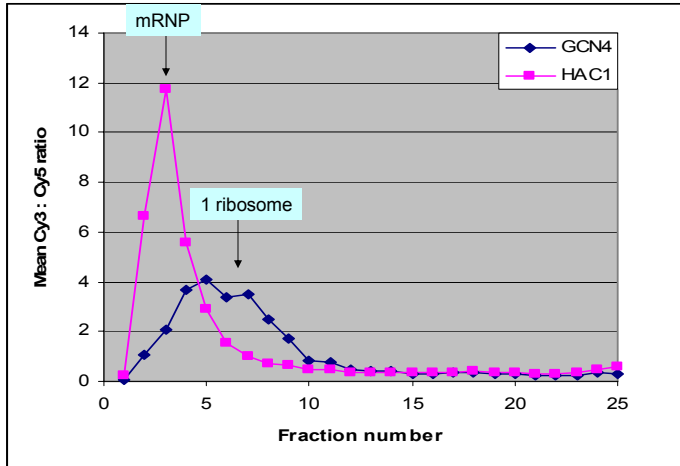
$T_e = (\text{polypeptide chains completed}) \cdot \text{mRNA}^{-1} \cdot \text{min}^{-1}$

## *Translation of the transcriptome of growing yeast*



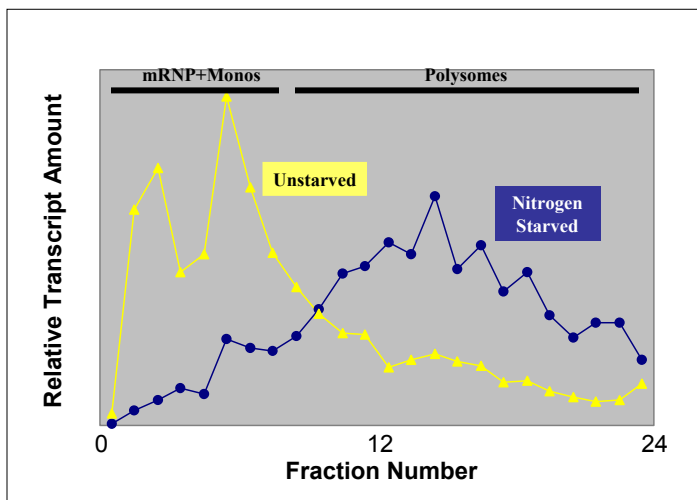
*Law et al., 2005*

## Two Poorly Translated mRNAs: *GCN4* and *HAC1*



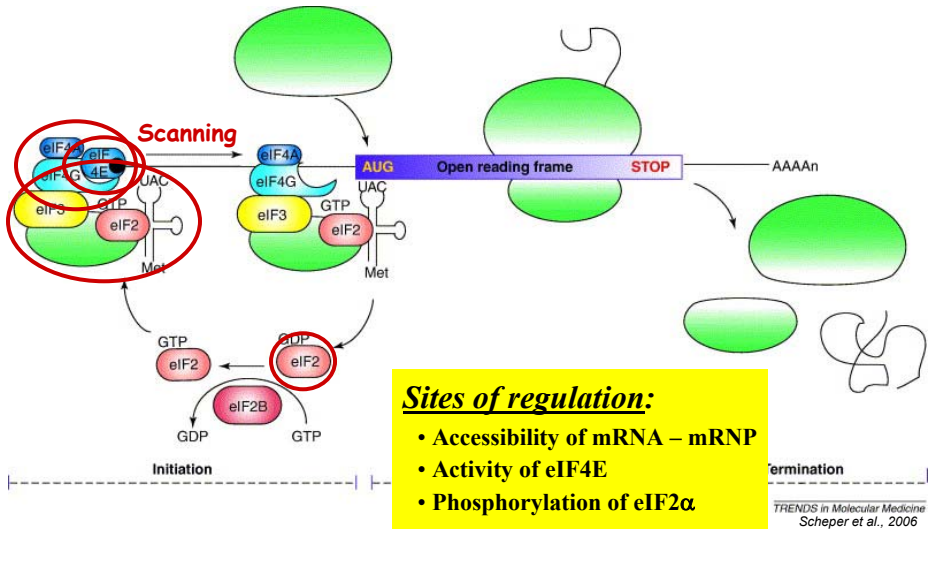
*MacKay et al., 2004*

## Sucrose gradient analysis of the response of *YSP3* transcripts to nitrogen starvation



*Lynn Law*

## Translation is generally regulated at initiation



## MECHANISMS

- What are the cis-acting elements that confer unique translational properties to individual mRNAs?
- How are these regulated?

## mRNA *cis* Elements Located in Non-Protein-Coding Regions

