

# Chapter 16 Translation

The translation of genetic information into amino acid sequences takes place on ribosomes and is mediated by special adaptor molecules known as tRNAs.

These tRNAs recognize groups of three consecutive nucleotides known as codons.

1 The Code Is Degenerate

2 How the code is cracked?

3 Three Rules Govern the Genetic Code

4 The Code Is Nearly Universal

5 Codon Usage Bias

# 1 The Code Is Degenerate

64 codons - 20 aa

Degeneracy (简并性):  
many amino acids are specified by more than one codon

18 {

1 codon: Met Trp

2 codons: Phe Tyr His Gln Asn Lys Asp Glu Cys

3 codons: Ile Stop

4 codons: Val Pro Thr Ala Gly

6 codons: Leu Ser Arg

Synonymous codons (同义密码子):  
Codons specifying the same amino acid

		second position				
		U	C	A	G	
first position (5' end)	U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA* stop UAG* stop	UGU Cys UGC UGA* stop UGG Trp	U C A G
	C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G
	A	AUU AUC Ile AUA AUG† Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
	G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G

# Codon in codon

Mutations in 1<sup>st</sup> position: similar (same) amino acid

Mutations in 2<sup>nd</sup> position: different amino acid

**NUN**: Hydrophobic aa

**NAN**: Hydrophilic aa

**NC/GN**: Neutral aa

Mutations in 3<sup>rd</sup> position: mostly same amino acid

**Codon in codn**: Nts in 2<sup>nd</sup> position play the most important role in determining the nature of aa.

**Minimize the deleterious effects of mutations.**

(1) $N_1N_2N_3$		(2) $N_1N_2N_3$	
GGN	疏	UGU/C	疏
CUN	↓	UUU/C	↓
AUA/C		AUA/C	
GUN		GUN	
GCU		CUN	
UUU/C		AUG	
UGU/G		UGG	
AUG	↓	CAU/C	↓
ACN	中	UAG/C	中
UCN		GCN	
UGG	↓	GGN	↓
UAU/C		ACN	
CAA/G		UCN	
AAA/G		CCN	
AAU/C		CGN	
GAA/G		AAU/C	
CAU/G	↓	CAA/G	↓
GAU/C	亲	GAA/G	亲
CGN		GAU/C	
		AAA/G	

# The genetic codon table

One tRNA for one codon?

Only 20 tRNAs!

The wobble hypothesis  
摇摆假说

		Second position					
		U	C	A	G		
First position (5'-end)	U	UUU	UUC	UUA	UUG	lys	U
		UUA	UUG	UUA	UUG	lys	U
		UUG	UUG	UUA	UUG	lys	U
		UUA	UUG	UUA	UUG	lys	U
	C	CUU	CUA	CUG	CUU	leu	C
		CUC	CUA	CUG	CUC	leu	C
		CUG	CUG	CUG	CUG	leu	C
		CUU	CUC	CUG	CUU	leu	C
	A	AUU	AUA	AUG	AUU	met	A
		AUA	AUA	AUG	AUA	met	A
		AUG	AUA	AUG	AUA	met	A
		AUU	AUA	AUG	AUU	met	A
	G	GUU	GUA	GUG	GUU	val	G
		GUC	GUA	GUG	GUC	val	G
		GUG	GUG	GUG	GUG	val	G
		GUU	GUC	GUG	GUU	val	G

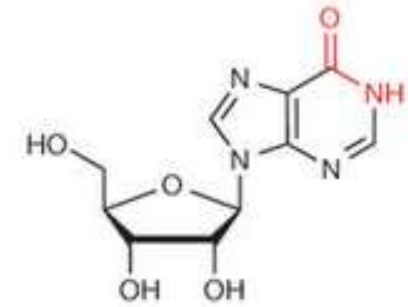
Francis Crick

# The wobble hypothesis

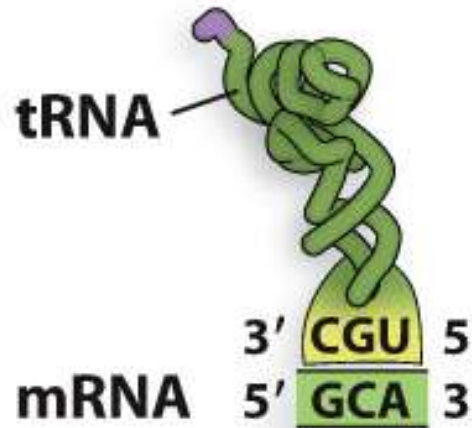
The first 2 bases of a codon must form Watson-Crick base pairs;

The last base of the codon can form wobble base pair. (**Wobble position**)

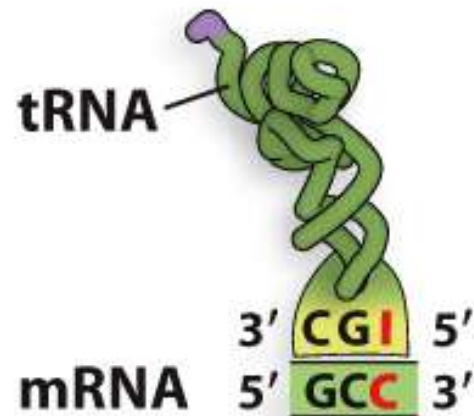
Inosine (I) 次黄嘌呤



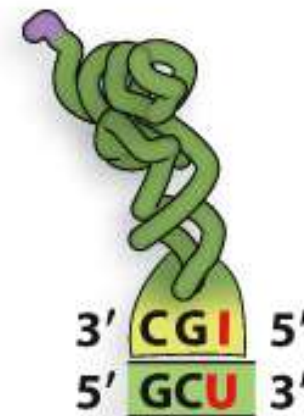
Normal A-U pair



I-C wobble pair



I-U wobble pair

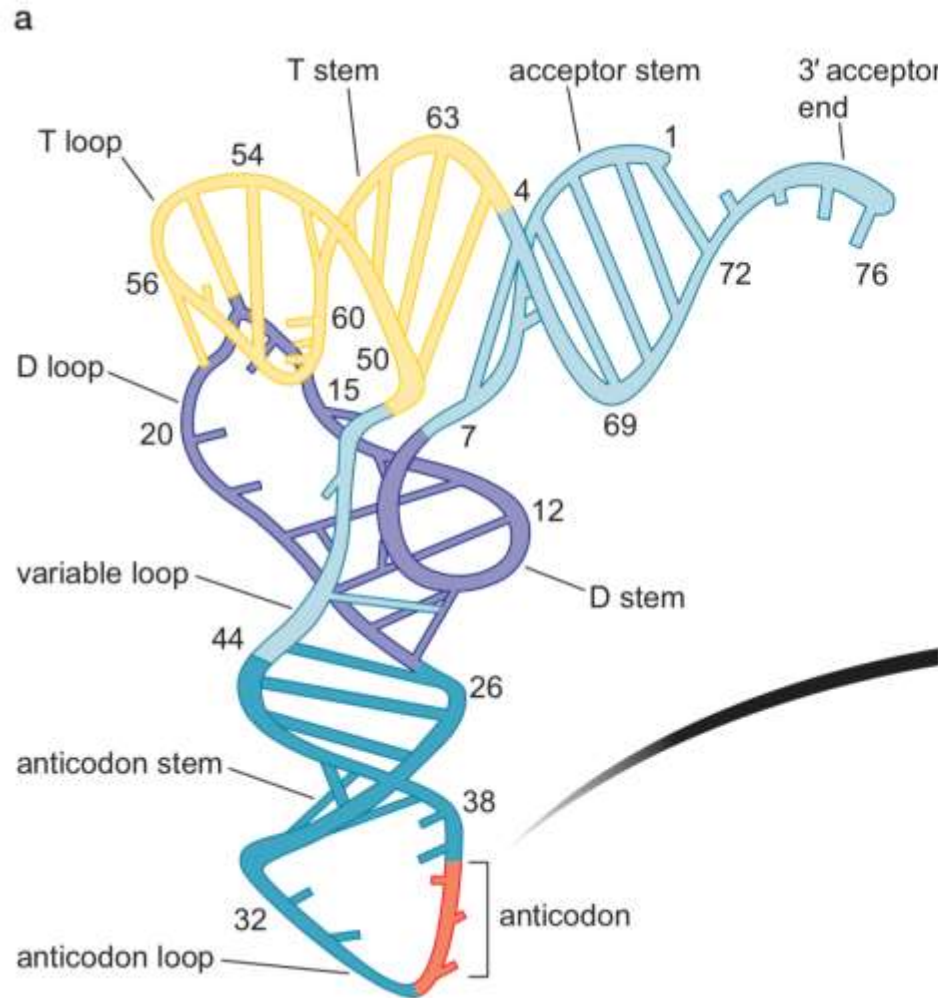


I-A wobble pair



**One tRNA recognize more than 1 codon.**

The base at the 5' end of the anticodon is easier to wobble than the fully stacked base at the 3' end of the anticodon



### 3.1 Codons are read in a 5' to 3' direction

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5' to 3' ✓

Met Val Arg Arg Val Asp Trp Arg Arg Lys Leu Val Arg Glu Tyr

AUG GUG CGU AGG GUC GAU UGG CGC AGA AAG UUA GUU AGA GAG UAC

Val Val Cys Gly Leu Stop Gly Arg Arg Glu Ile Leu Arg Glu His

3' to 5'



## 3.2 Codons are read with neither overlapping nor gaps

Nonoverlapping ✓

Wild type AUGGACGCU  
Met Asp Ala

Code without commas ✓

Reading frame 1: AUGCGAGCUUUUAGUCCUACGAGGUAAGUCCUCAUAGG GAGGUAAGUCCCUGA  
Met Arg Ala Phe Ser Ser Tyr Glu Val Gly Pro His Arg Glu Val Ser Pro Stop

G is deleted

Deletion: AUGCGACUUUUAGUCCUACGAGGUAA GUCCUCAUAGG GAGGUAAGUCCCUGA  
Met Arg Leu Leu Val Pro Thr Arg Stop

Incorrect amino acids



### 3. 3 Codons are read in a fixed reading frame

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Start

Start

Start

Start codon determines the RIGHT reading frame!

TABLE 16-6 Genetic Code of Mammalian Mitochondria

## 4 THE CODE IS NEARLY UNIVERSAL

Evolution

Genetic engineering

Exceptions:  
organelle  
prokaryote  
eukaryote

		second position				
		U	C	A	G	
first position (5' end)	U	UUU Phe UUC (GAA)† UUA Leu UUG (UAA)	UCU UCC Ser UCA (UGA) UCG	UAU Tyr UAC (GUA) UAA stop UAG stop	UGU Cys UGC (GCA) UGA Trp UGG (UCA)	U C A G
	C	CUU CUC Leu CUA (UAG) CUG	CCU CCC Pro CCA (UGG) CCG	CAU His CAC (GUG) CAA Gln CAG (UUG)	CGU CGC Arg CGA (UCG) CGG	U C A G
	A	AUU Ile AUC (GAU) AUA Met AUG (CAU)‡	ACU ACC Thr ACA (UGU) ACG	AAU Asn AAC (GUU) AAA Lys AAG (UUU)	AGU Ser AGC (GCU) AGA stop AGG stop	U C A G
	G	GUU GUC Val GUA (UAC) GUG	GCU GCC Ala GCA (UGC) GCG	GAU Asp GAC (GUC) GAA Glu GAG (UUC)	GGU GGC Gly GGA (UCC) GGG	U C A G

# 5 codon usage bias - 密码子使用偏好性

Synonymous codons are **not used randomly or equally** in almost all the genome

## *Neurospora crassa*



**C>G>A>U**

**Optimal codon**

**Non-optimal codon**

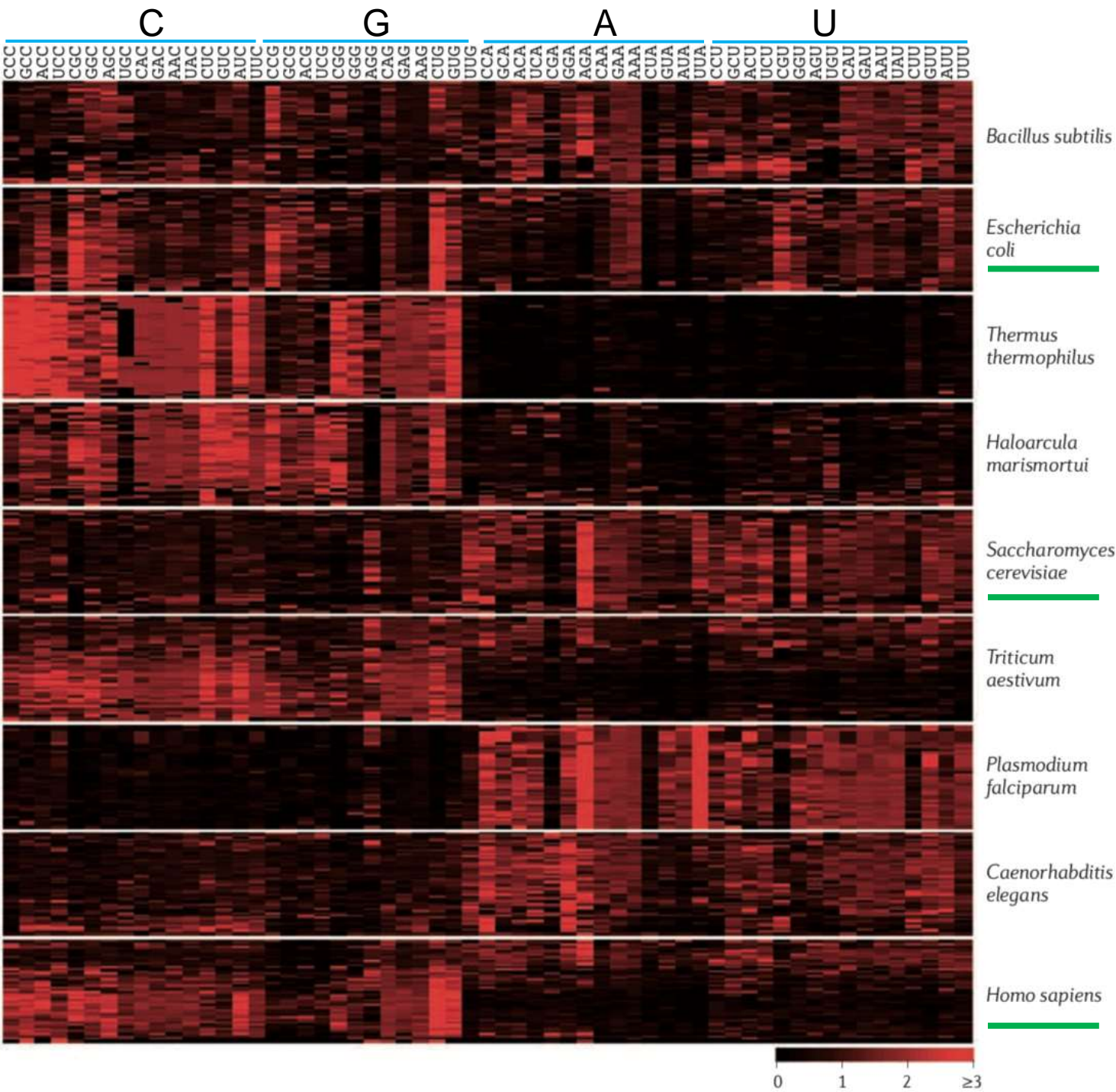
## *Neurospora crassa*

Phe	UUU 11.8	Ser	UCU 11.9	Tyr	UAU 8.5	Cys	UGU 3.4	
	UUC 22.1		UCC 20.0		UAC 17.5		UGC 7.7	
Leu	UUA 2.7		UCA 9.2	Stop codon	UAA 0.6	Stop codon	UGA 0.8	
	UUG 14.9		UCG 14.5		UAG 0.5	Trp	UGG 13.1	
	CUU 14.2		Pro	CCU 15.1	His	CAU 9.5	Arg	CGU 8.9
	CUC 26.8			CCC 22.4		CAC 14.8		CGC 17.6
	CUA 6.0	CCA 12.4		Gln	CAA 17.0	CGA 7.1		
	CUG 18.3	CCG 14.6			CAG 26.0	CGG 8.5		
Ile	AUU 14.0	Thr	ACU 11.2	Asn	AAU10.3	Ser	AGU 8.7	
	AUC 26.5		ACC 24.7		AAC 27.0		AGC 17.4	
	AUA 4.1		ACA 10.7	Lys	AAA11.7	Arg	AGA 7.9	
Met	AUG 21.8		ACG 13.5		AAG 40.4		AGG 11.8	
Val	GUU13.8	Ala	GCU 21.1	Asp	GAU 24.0	Gly	GGU 18.3	
	GUC 24.8		GCC 36.0		GAC 32.5		GGC 29.0	
	GUA 5.4		GCA 12.6	Glu	GAA 22.4		GGA 13.6	
	GUG 15.5		GCG 17.3		GAG 42.7		GGG 10.9	

Codon usage patterns across species

CG bias

AU bias





# How do we know the Codon Usage Bias of the organism of interest?

*Oryza sativa*— 水稻



Codon Usage Database

<https://www.kazusa.or.jp/codon/>

## Codon Usage Database

### Data source

[NCBI-GenBank](#) Flat File Release 160.0 [June 15 2007].

### Data amount

35,799 organisms

3,027,973 complete protein coding genes (CDS's)

### [Announcement](#)

### QUERY Box for search with Latin name of organism

Case: ☒ sensitive ☐ insensitive

# The study of codon usage from Dr. Yi Liu's Lab

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Yi Liu, Ph.D.

## Education

Undergraduate: Wuhan University (1989), Biology

Graduate School: Vanderbilt University (1995), Biology

## Research Interest

- New genetic codes, Mechanisms of circadian clocks, non-coding RNA
- Role of codon usage biases in regulating gene expression and protein structure
- Small non-coding RNAs and long non-coding RNAs

[UT Southwestern Profile](#)

Link to Yi Liu Lab: [Liu \(Yi\) Lab | UT Southwestern, Dallas, Texas](#)

# The effect of codon usage on the expression and function of genes

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[Published: 17 February 2013](#)

## Non-optimal codon usage affects expression, structure and function of clock protein FRQ

[Mian Zhou](#), [Jinhu Guo](#), [Joonseok Cha](#), [Michael Chae](#), [She Chen](#), [Jose M. Barral](#), [Matthew S. Sachs](#) & [Yi Liu](#) 

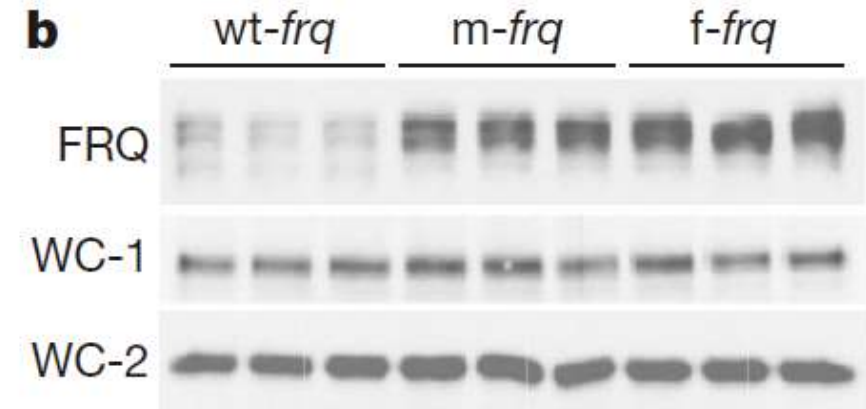
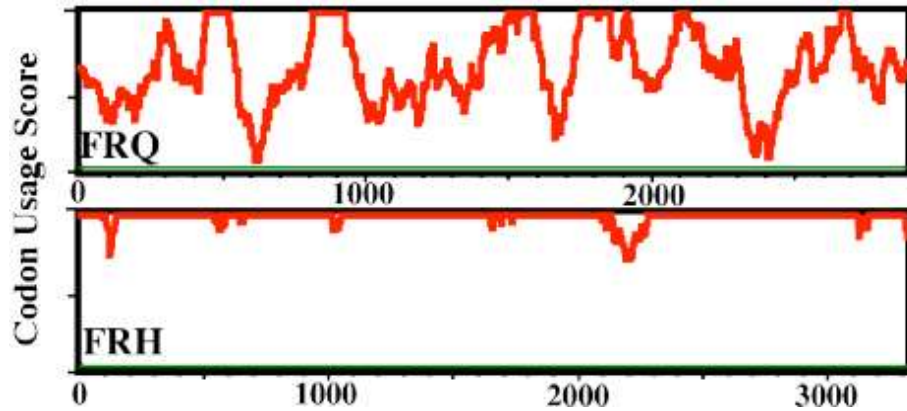
[Nature](#) **495**, 111–115 (2013) | [Cite this article](#)

*Neurospora crassa*

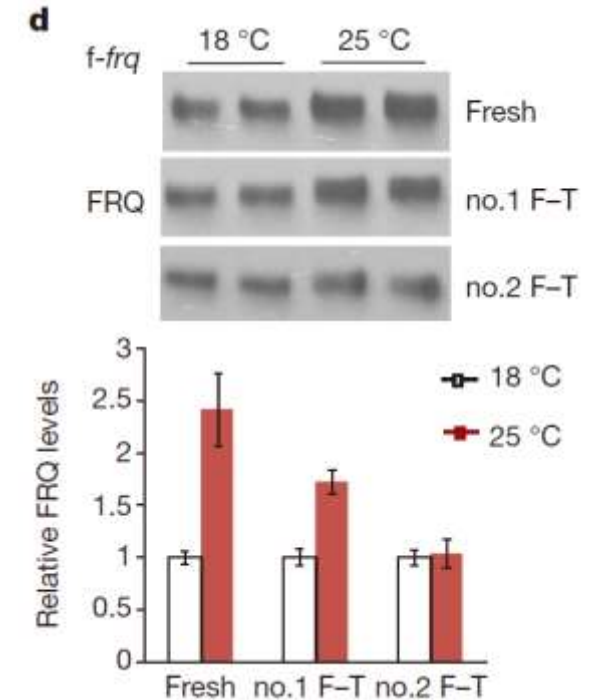
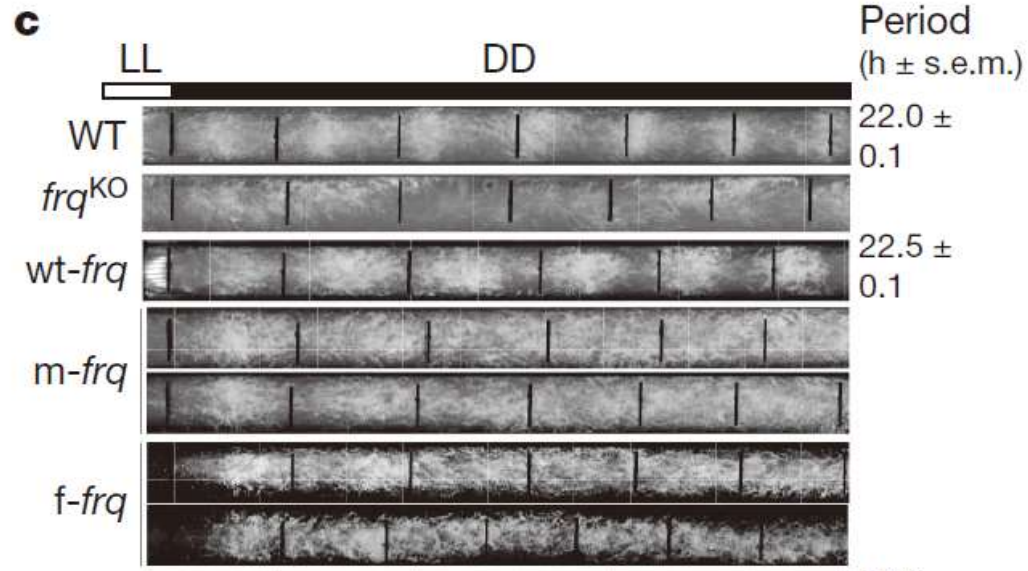




# The effect of codon usage in the fungus *Neurospora crassa*



**Q: How about animals?**

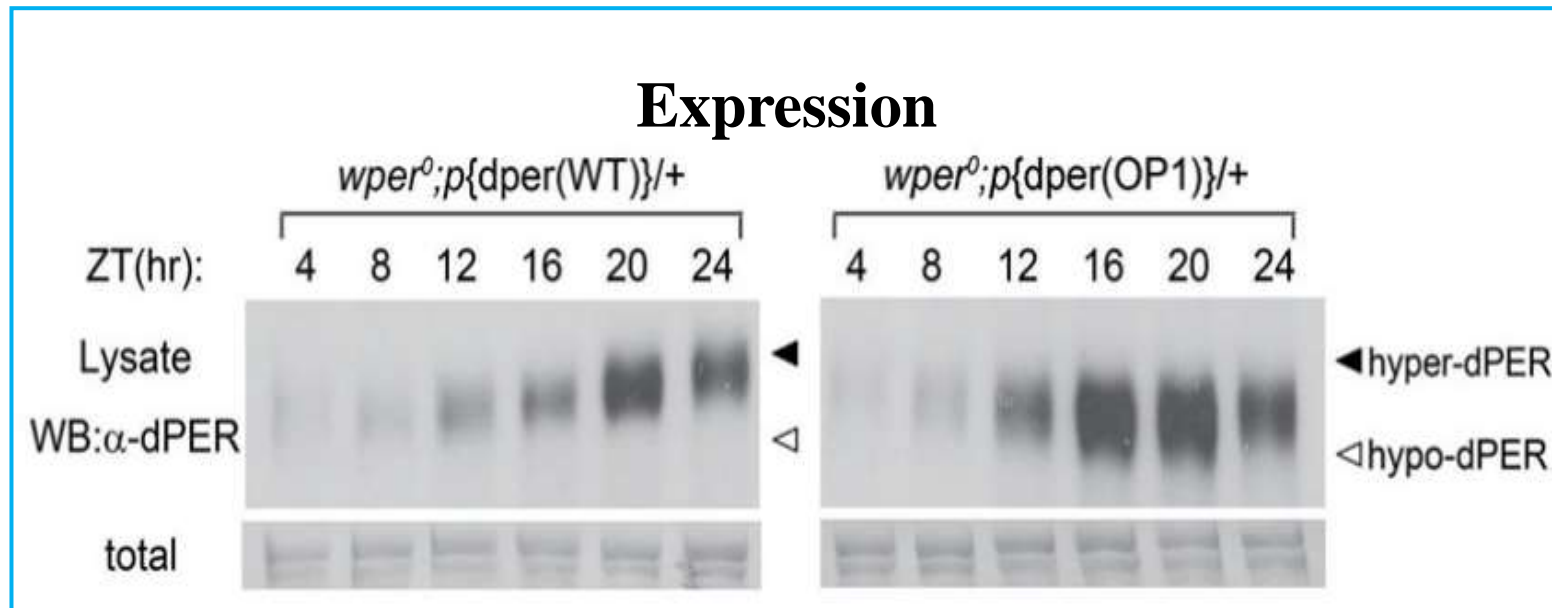


# Codon usage affects the expression of *Per* gene in *Drosophila*

## Q: How about human?

### Codon usage affects the structure and function of the *Drosophila* circadian clock protein PERIOD

Jingjing Fu<sup>1</sup>, Katherine A. Murphy<sup>2</sup>, Mian Zhou<sup>1,3</sup>, Ying H. Li<sup>2</sup>, Vu H. Lam<sup>2</sup>, Christine A. Tabuloc<sup>2</sup>, Joanna C. Chiu<sup>2</sup> and Yi Liu<sup>1</sup>



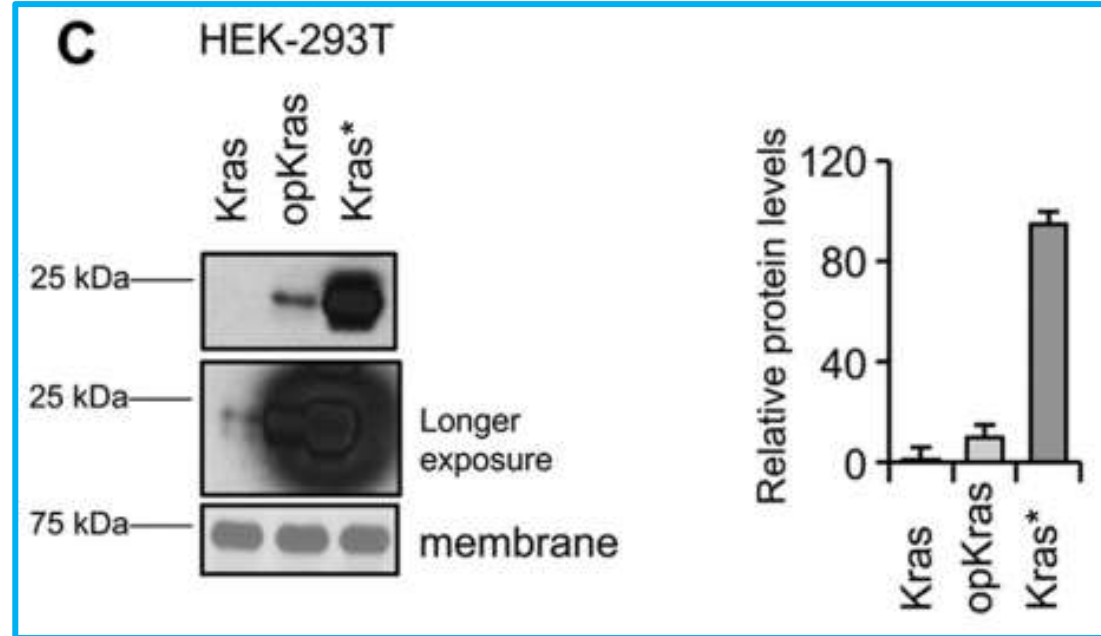
# Codon usage affects the expression of *KRAS* gene in Human

Volume 293, Issue 46, 16 November 2018, Pages 17929-17940

Gene Regulation

## Codon usage regulates human KRAS expression at both transcriptional and translational levels

Jingjing Fu <sup>‡</sup>, Yunkun Dang <sup>§</sup>, Christopher Counter <sup>¶</sup>, Yi Liu <sup>‡</sup>  

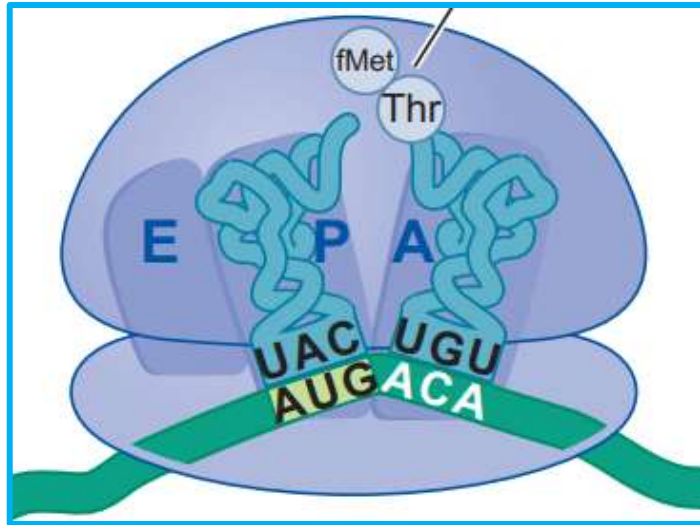


More than **100-fold increment** of the protein level of KRAS!

**Q:** What are the underlying mechanisms?

# How does codon usage regulate gene expression?

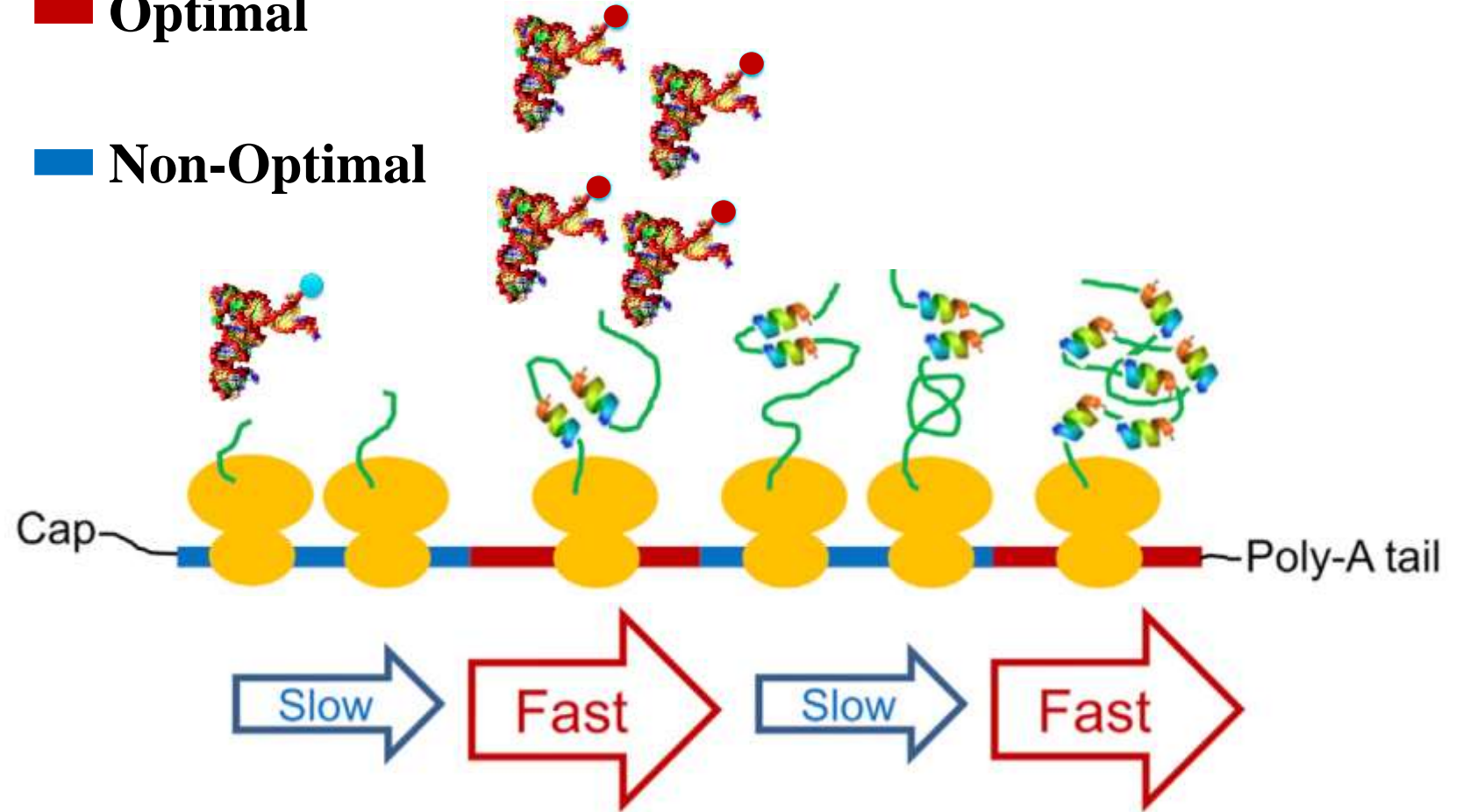
知识关联



Accepted theory

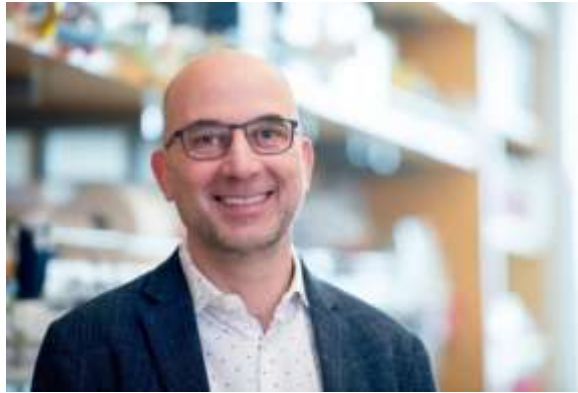
■ Optimal

■ Non-Optimal





# Does codon usage affect translation elongation speed?

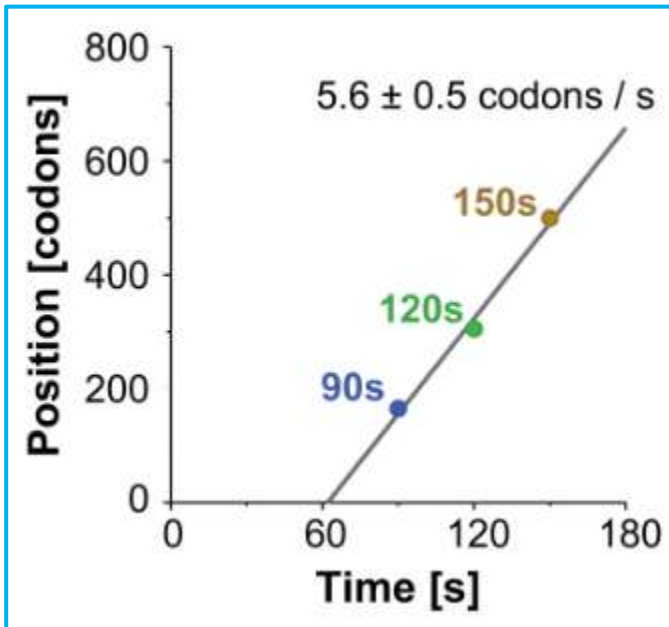


Jonathan S Weissman

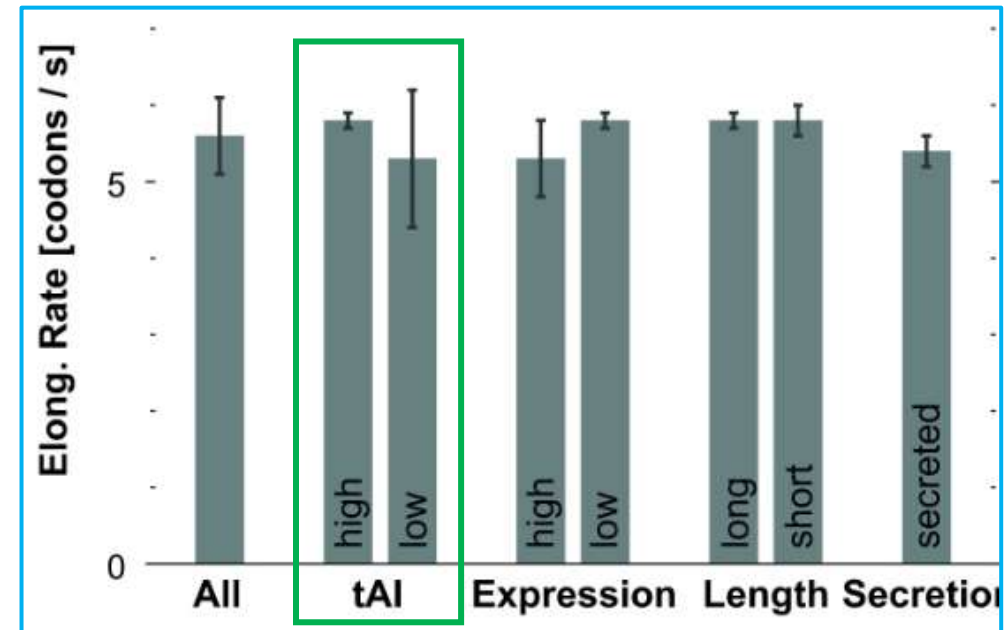
> Cell. 2011 Nov 11;147(4):789-802. doi: 10.1016/j.cell.2011.10.002. Epub 2011 Nov 3.

## Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes

Nicholas T Ingolia<sup>1</sup>, Liana F Lareau, Jonathan S Weissman



?

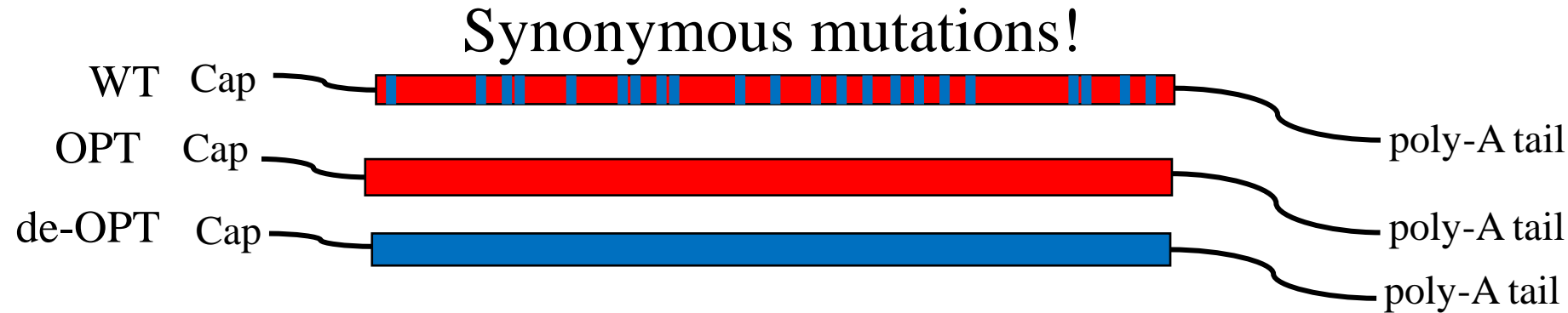


Translation speed is independent of codon usage.

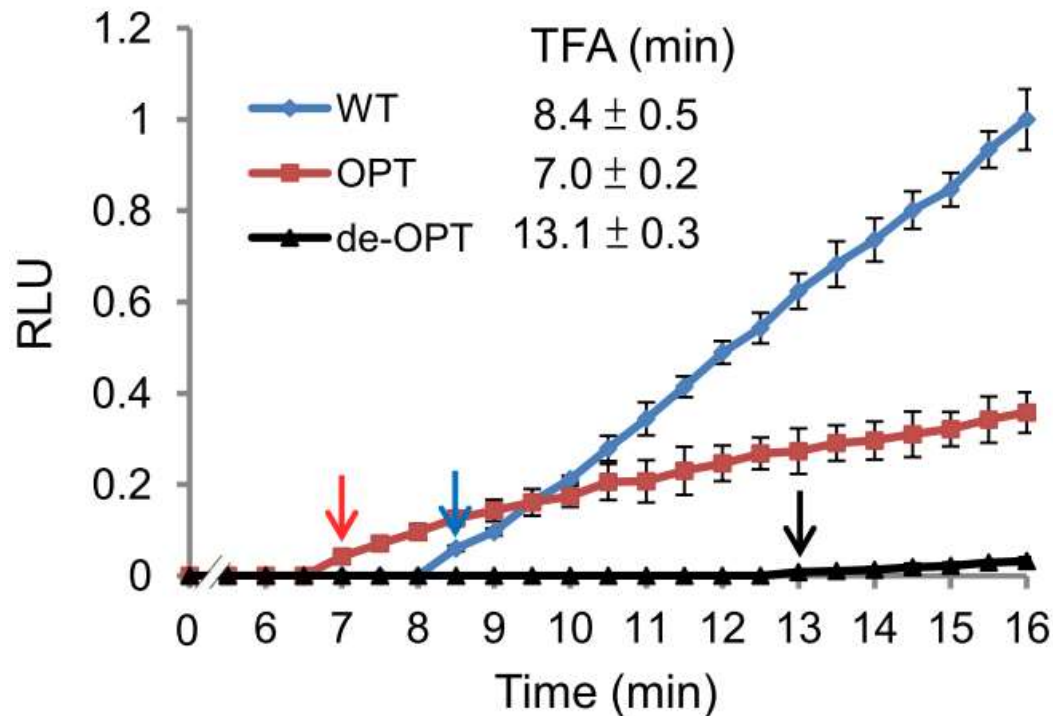
# Codon optimization enhances translation rate in *N. crassa*

## ➤ Cell-free Translation

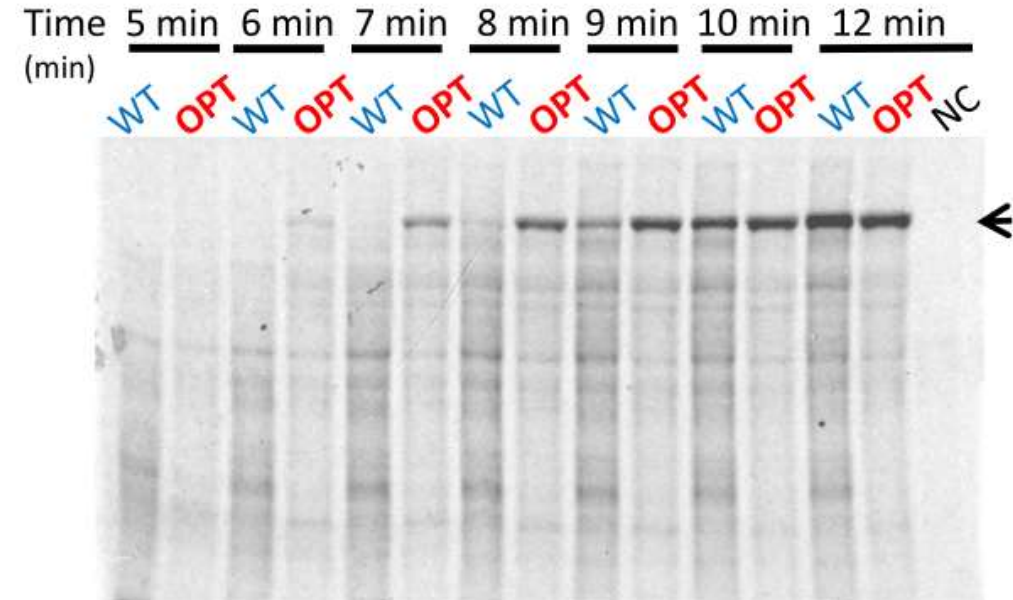
## ➤ Luciferase



**Luciferase activity**



**Full length luciferase protein**

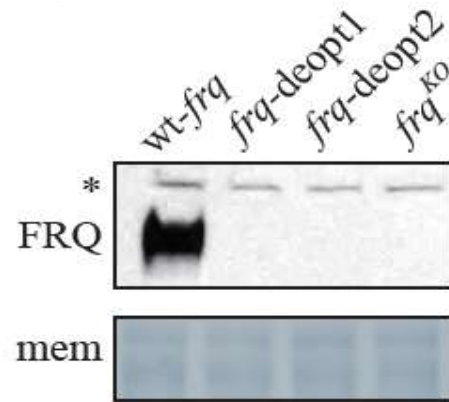
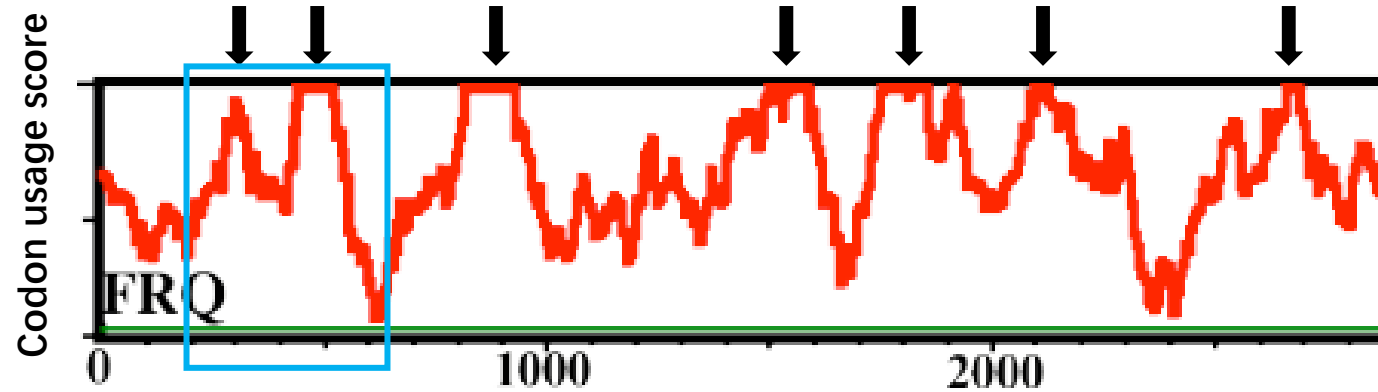


**Q: Other mechanisms?**

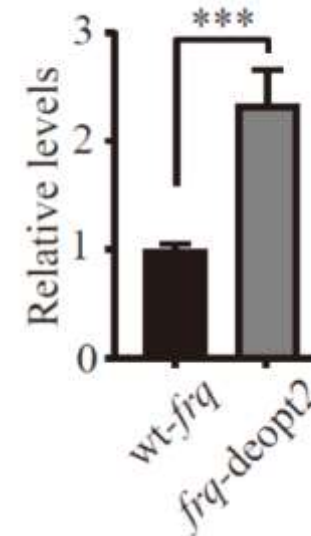
PMID: 26321254

# Codon usage affects the cleavage and polyadenylation of mRNA

What if we de-optimize the codon usage?



*frq* pre-mRNA levels

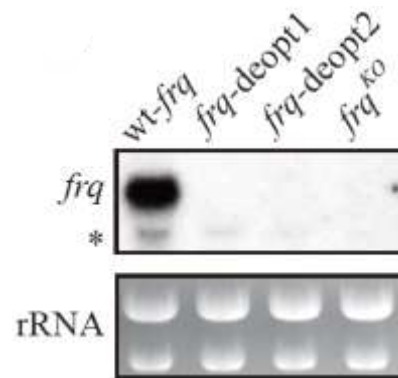




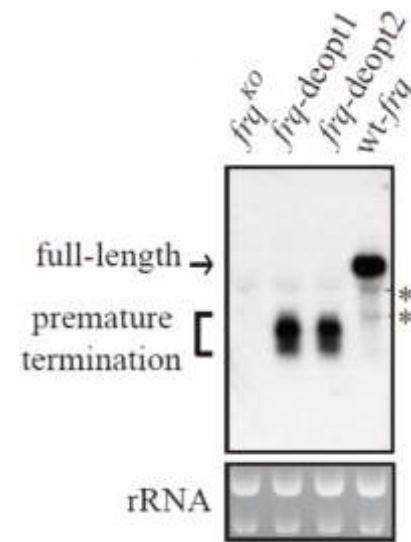
# Codon usage affects the cleavage and polyadenylation of mRNA



3' end probe

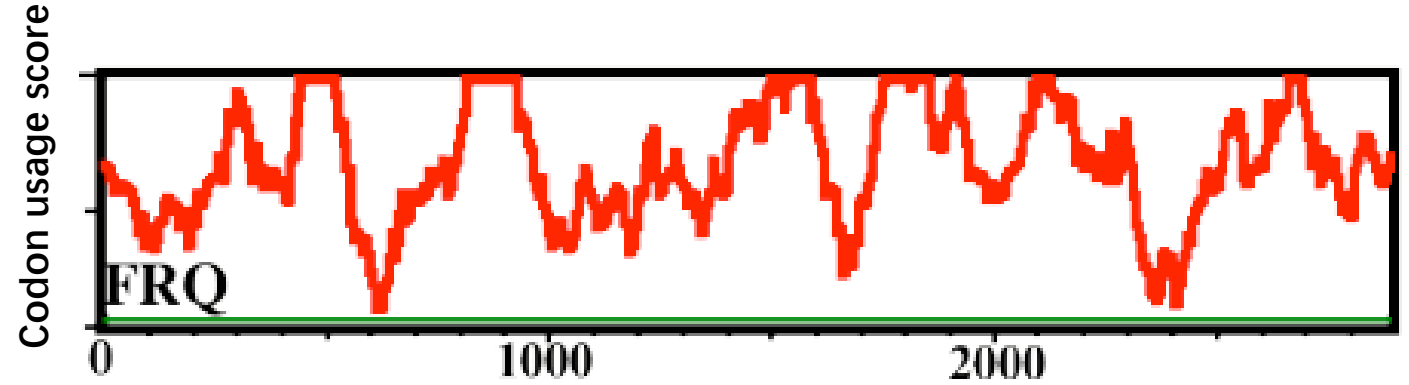
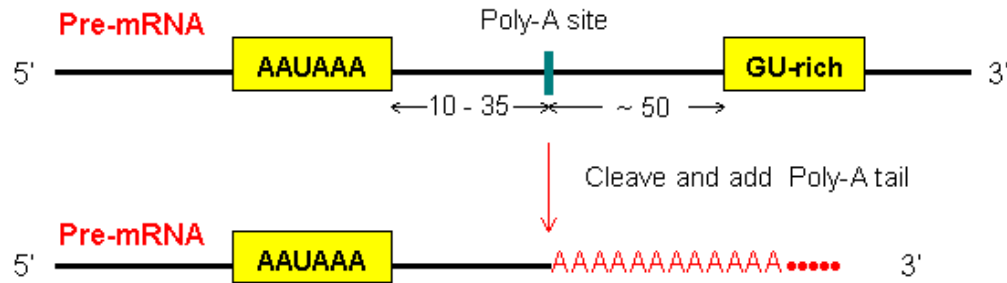


5' end probe



# Codon usage affects the cleavage and polyadenylation of mRNA

Poly(A) signal = AAUAAA



Codon de-optimization



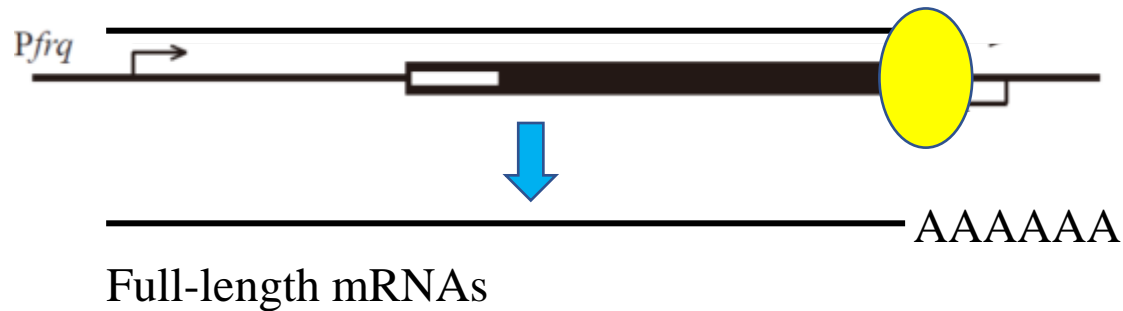
AU↑



Poly(A) signal

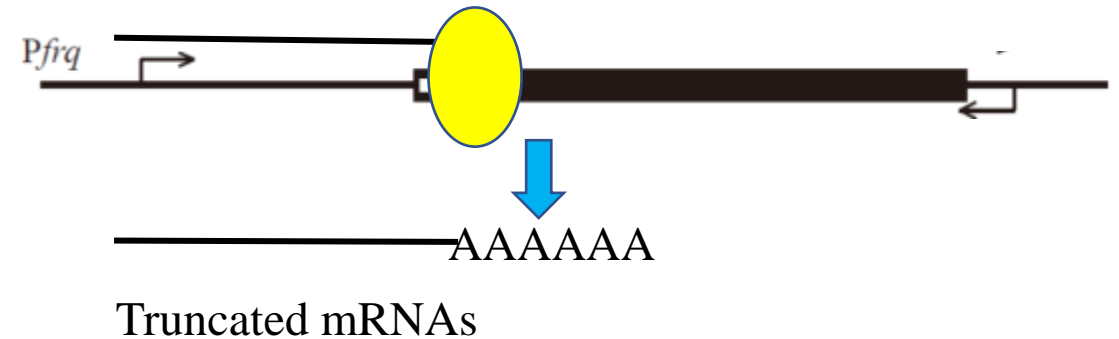
WT

Without Poly(A) signal



de-OPT

With Poly(A) signal



# What can you learn?

## ➤ Codon Usage Bias

Synonymous

but

NOT Always Silent!

Q: What else?

Phe	UUU 11.8	Ser	UCU 11.9	Tyr	UAU 8.5	Cys	UGU 3.4
	UUC 22.1		UCC 20.0		UAC 17.5		UGC 7.7
Leu	UUA 2.7		UCA 9.2	Stop codon	UAA 0.6	Stop codon	UGA 0.8
	UUG 14.9		UCG 14.5		UAG 0.5	Trp	UGG 13.1
	CUU 14.2	Pro	CCU 15.1	His	CAU 9.5	Arg	CGU 8.9
	CUC 26.8		CCC 22.4		CAC 14.8		CGC 17.6
	CUA 6.0		CCA 12.4	Gln	CAA 17.0		CGA 7.1
	CUG 18.3		CCG 14.6		CAG 26.0		CGG 8.5
Ile	AUU 14.0	Thr	ACU 11.2	Asn	AAU 10.3	Ser	AGU 8.7
	AUC 26.5		ACC 24.7		AAC 27.0		AGC 17.4
	AUA 4.1		ACA 10.7	Lys	AAA 11.7	Arg	AGA 7.9
Met	AUG 21.8		ACG 13.5		AAG 40.4		AGG 11.8
Val	GUU 13.8	Ala	GCU 21.1	Asp	GAU 24.0	Gly	GGU 18.3
	GUC 24.8		GCC 36.0		GAC 32.5		GGC 29.0
	GUA 5.4		GCA 12.6	Glu	GAA 22.4		GGA 13.6
	GUG 15.5		GCG 17.3		GAG 42.7		GGG 10.9

# The study of codon usage from Dr. Yi Liu's lab



Yi Liu codon usage



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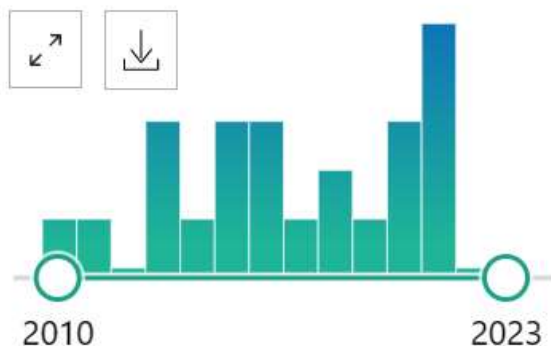
Display options ⚙

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21 results

Page 1 of 3

RESULTS BY YEAR



1

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**Codon usage** and protein length-dependent feedback from translation elongation regulates translation initiation and elongation speed.

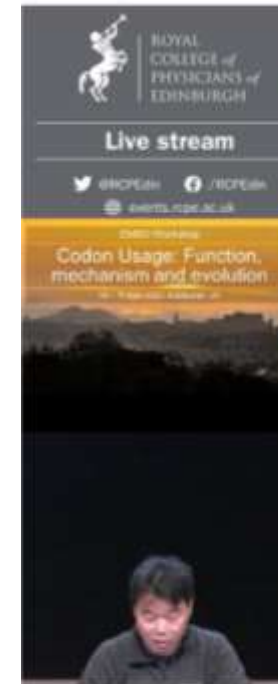
Lyu X, Yang Q, Zhao F, **Liu Y**.

Nucleic Acids Res. 2021 Sep 20;49(16):9404-9423. doi: 10.1093/nar/gkab729.

PMID: 34417614 [Free PMC article](#).

Here, we discovered that there is a conserved but **codon usage**-dependent genome-wide negative correlation between protein abundance and CDS length. The **codon usage** effects on protein expression and ribosome flux on mRNAs are influenced by CDS length; op ...

# The EMBO Workshop - Codon Usage



Decoding the codon usage codes in protein folding and gene expression

Yi Liu

University of Texas Southwestern Medical Center

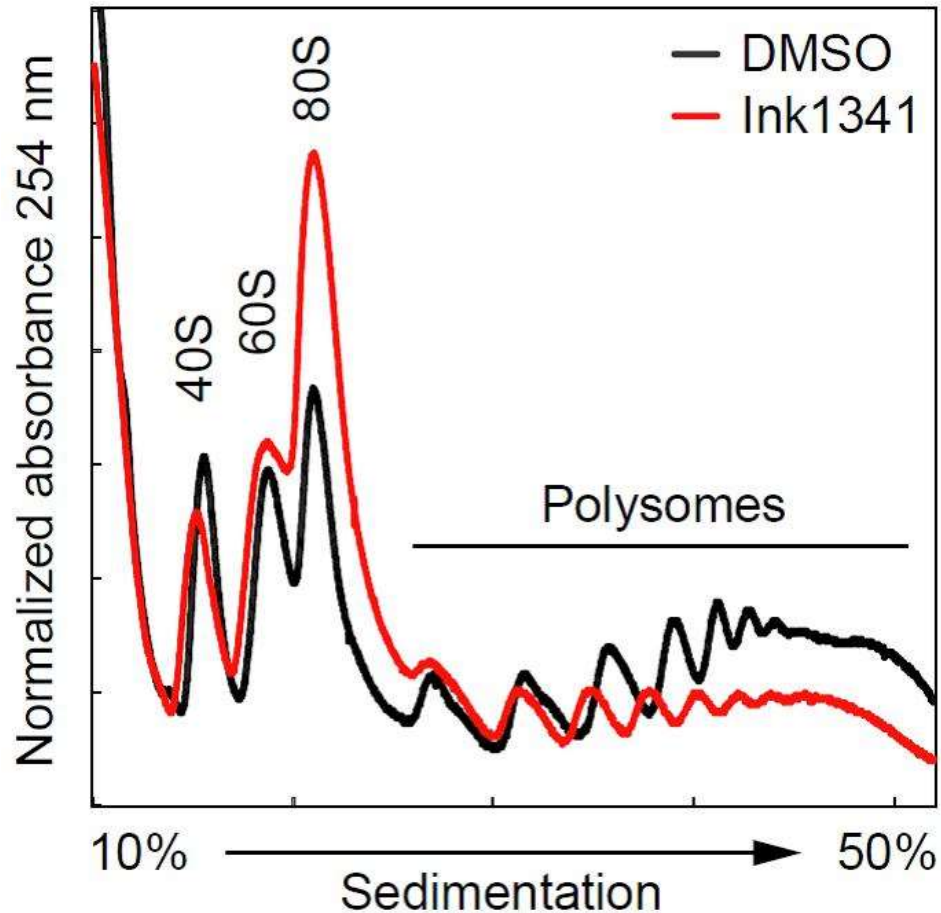


EMBO workshop 9th April 2022 (Morning)

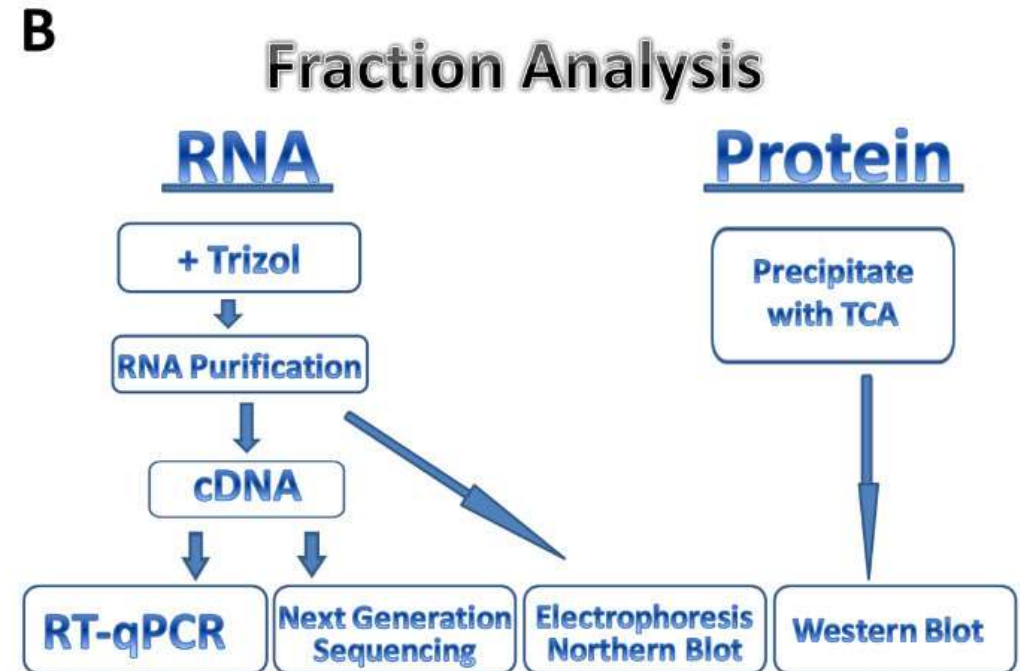
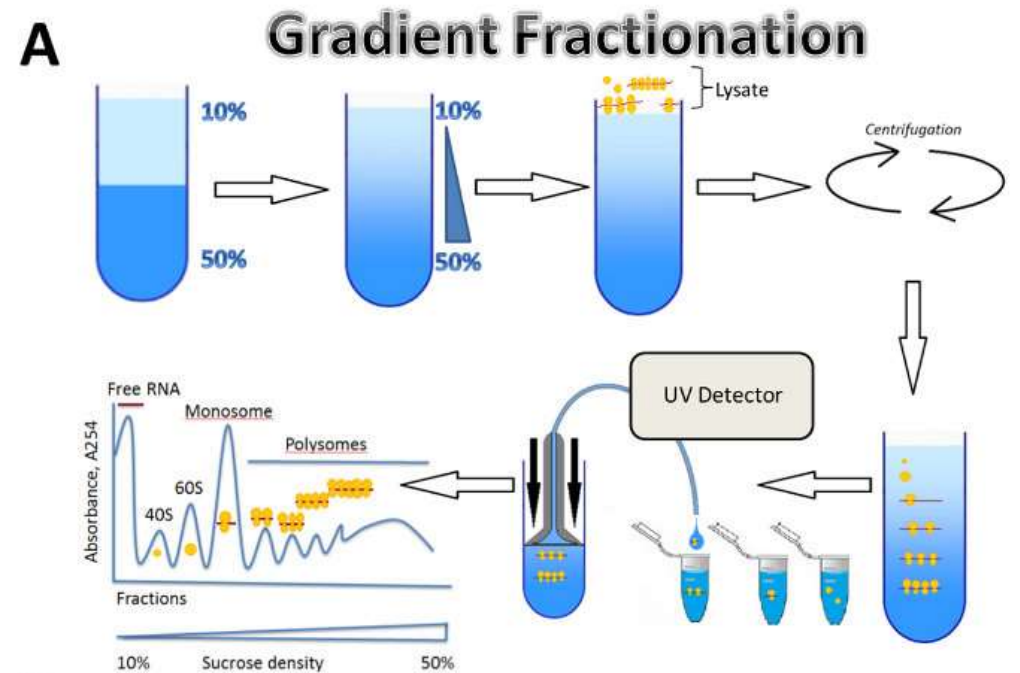
♥ 0 ▶ 171 ◻ 0



# Polysome Profiling



**P/M = Polysome/Monosome**



# How to do Polysome Profiling?





# The translating ribosome affinity purification (TRAP) strategy

Cell type-specific?

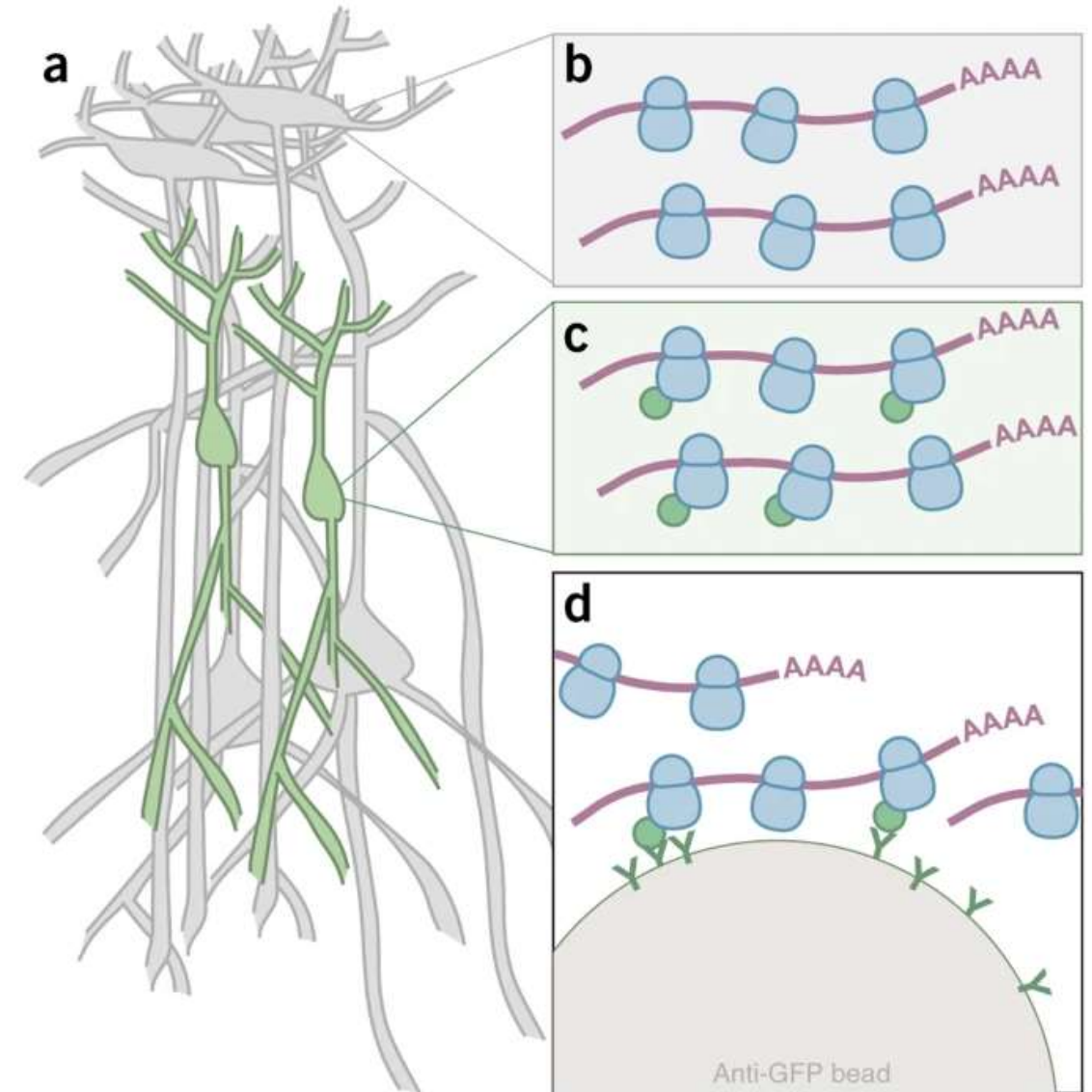
Published: 08 May 2014

## Cell type-specific mRNA purification by translating ribosome affinity purification (TRAP)

Myriam Heiman , Ruth Kulicke, Robert J Fenster, Paul Greengard & Nathaniel Heintz

*Nature Protocols* **9**, 1282–1291(2014) | [Cite this article](#)

**7025** Accesses | **167** Citations | **29** Altmetric | [Metrics](#)



# Ribosome Profiling or Ribo-seq

Genome-wide analysis in vivo of translation with ...

<https://pubmed.ncbi.nlm.nih.gov/19213877>

Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Techniques for systematically monitoring protein translation have lagged far behind methods for...

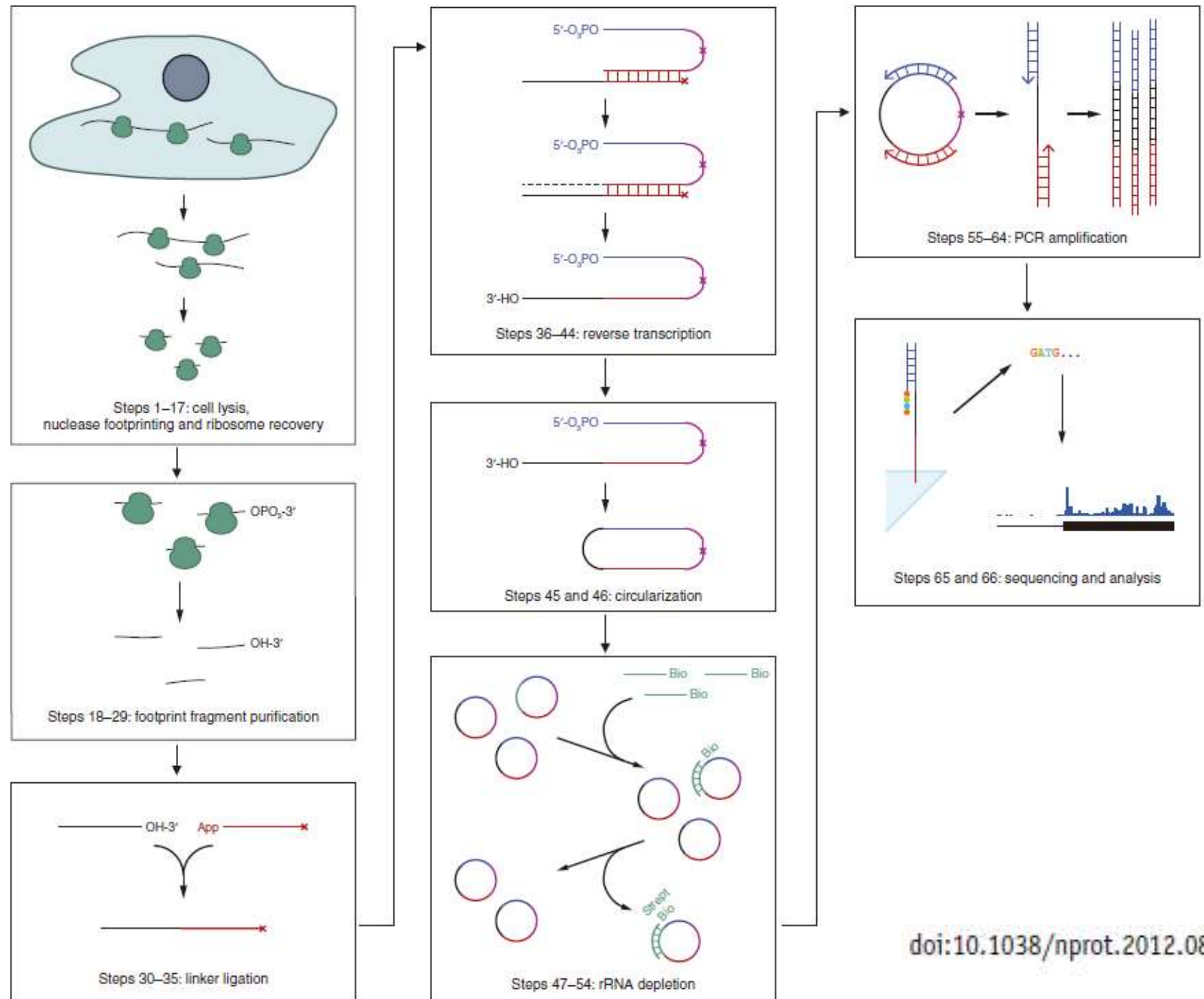
Cited by: 2967

Author: Nicholas T. Ingolia, Sina Ghaemmaghami, J...

Publish Year: 2009

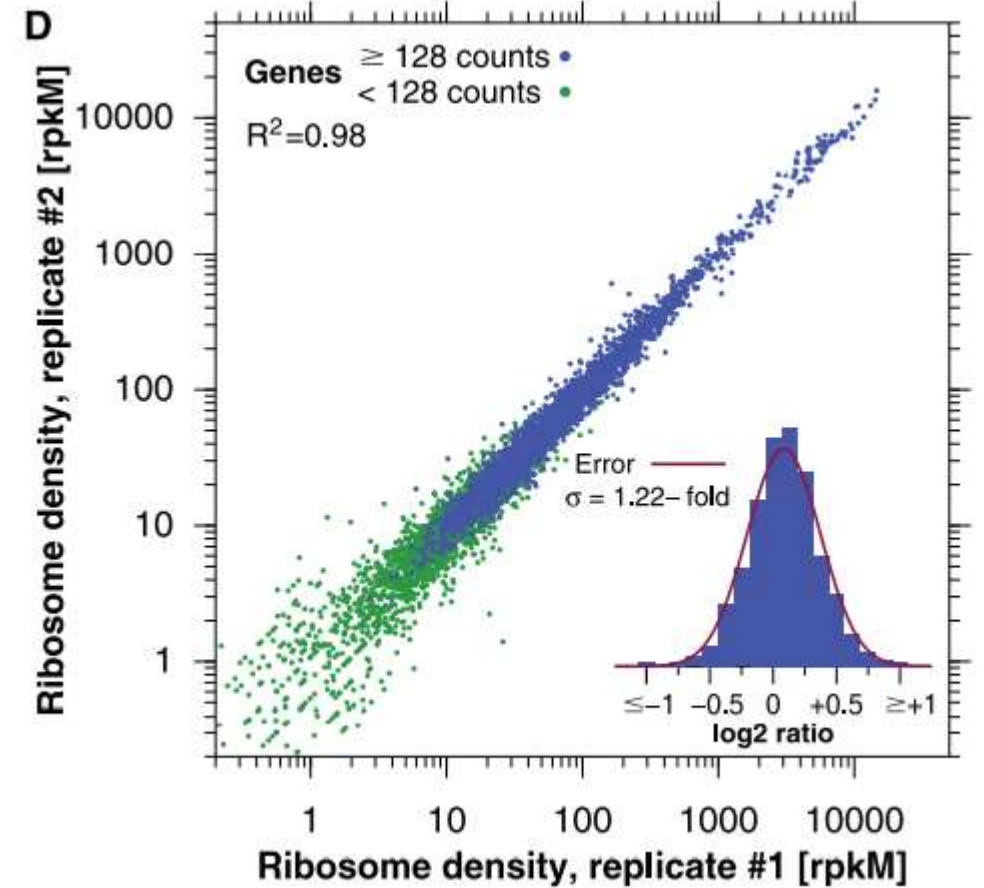
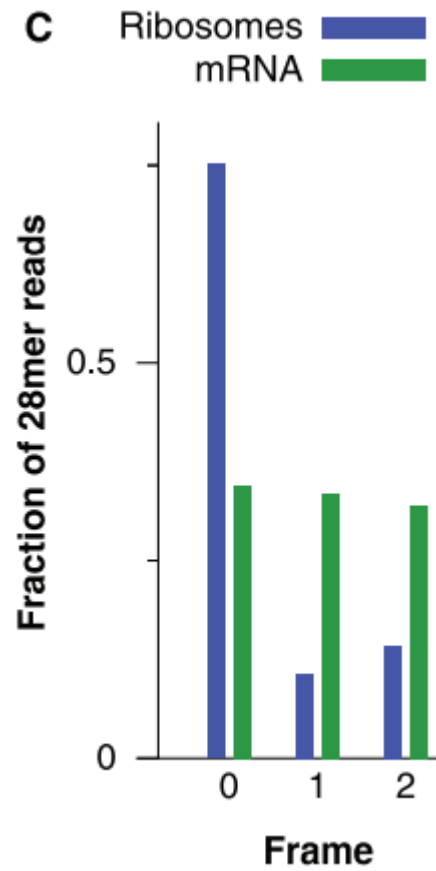
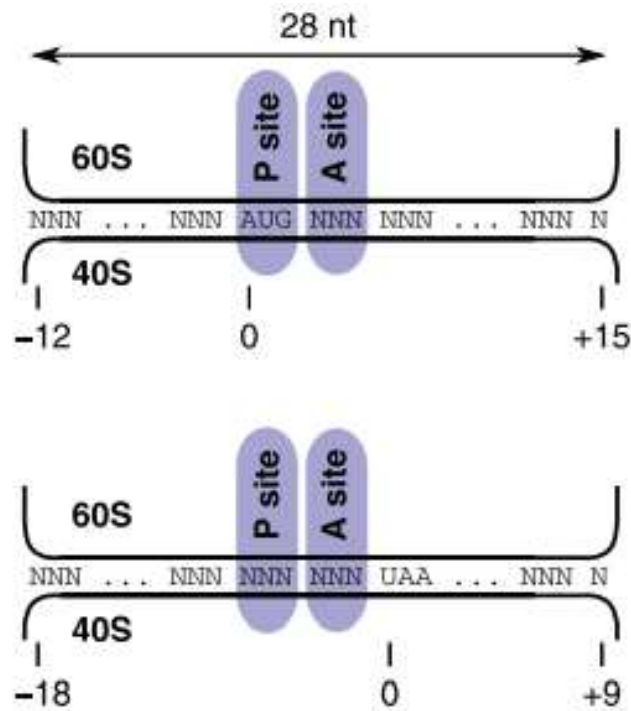
# Overview of the ribosome profiling protocol

**Ribosome protected fragments = RPFs**



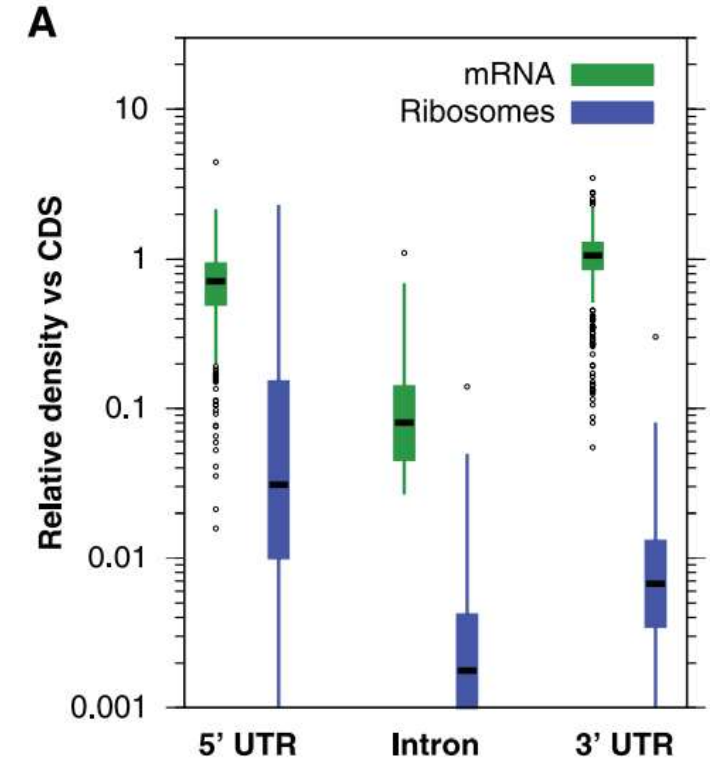
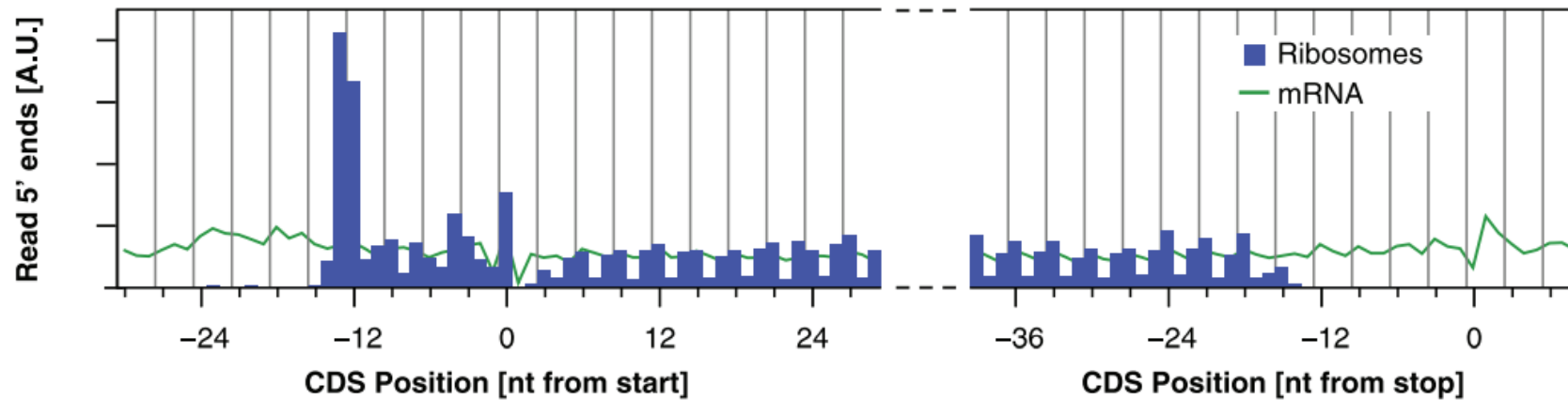
doi:10.1038/nprot.2012.086

# How evaluate the quality of a Ribo-seq?



DOI: 10.1126/science.1168978

# How evaluate the quality of a Ribo-seq?

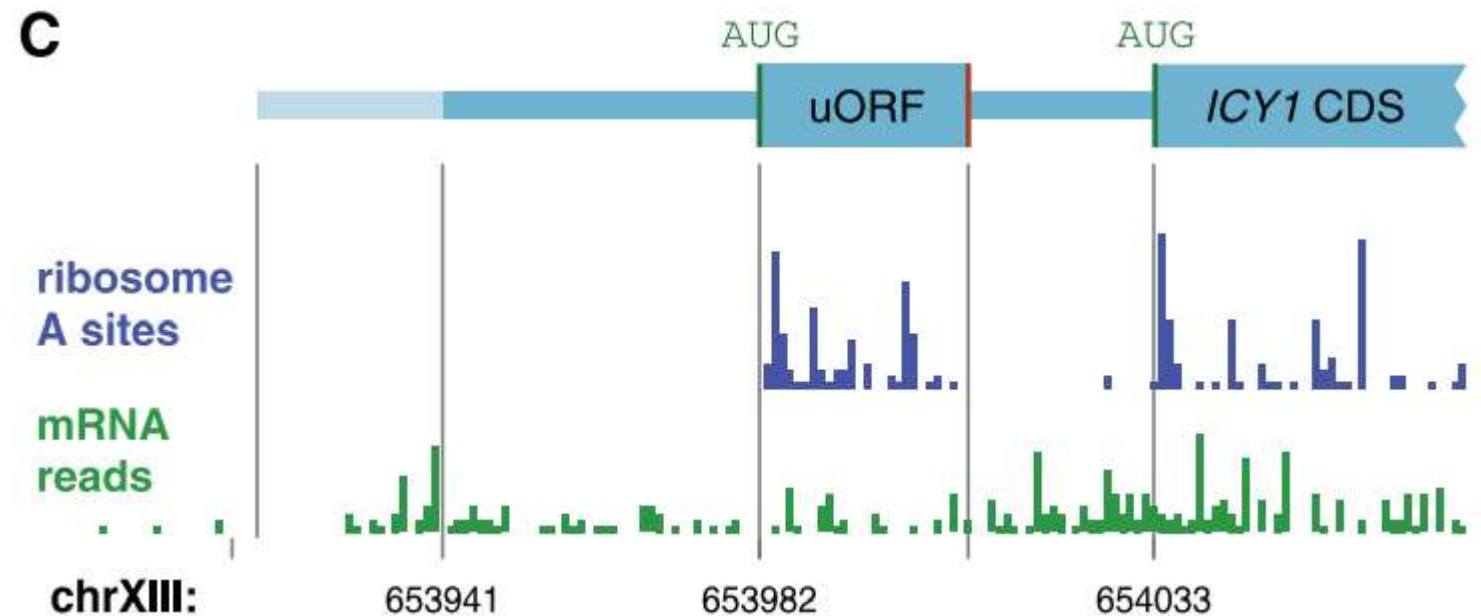


DOI: 10.1126/science.1168978



# What can we learn from Ribo-seq?

- Yes or no?
- Where?
- Which?
- How?



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# Key Points of Chapter 15

- The major challenge of translation compared with transcription.
- The components of translational machinery.
- Three steps of translation in prok and euk.
- Translational regulation in prok and euk.



[Published: 17 May 2017](#)

## Global translational reprogramming is a fundamental layer of immune regulation in plants

[Guoyong Xu](#), [George H. Greene](#), [Heejin Yoo](#), [Lijing Liu](#), [Jorge Marqués](#), [Jonathan Motley](#) & [Xinnian Dong](#) 

[Nature](#) **545**, 487–490 (2017) | [Cite this article](#)

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## Plant HEM1 specifies a condensation domain to control immune gene translation

[Yulu Zhou](#), [Ruixia Niu](#), [Zhijuan Tang](#), [Rui Mou](#), [Zhao Wang](#), [Sitao Zhu](#), [Hongchun Yang](#), [Pingtao Ding](#) & [Guoyong Xu](#) 

[Nature Plants](#) **9**, 289–301 (2023) | [Cite this article](#)

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# Homework

What is the difference between prokaryotic and eukaryotic mRNAs, and how does it affect translation initiation?