The Rna Code and Protein Synthesis

Ryan Collins, Gerissa Fowler, Sean Gamberg, Josselyn Hudasek, & Victoria Mackey

Timeline Leading up to Nirenberg's 1966 paper

1859:

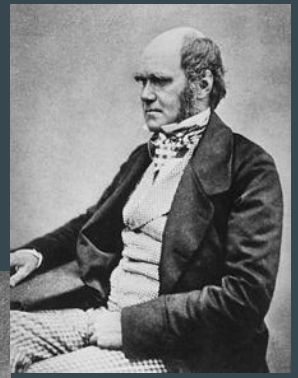
- Charles Darwin published his book “The Origin of Species”

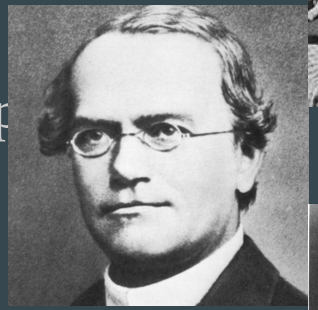
1866:

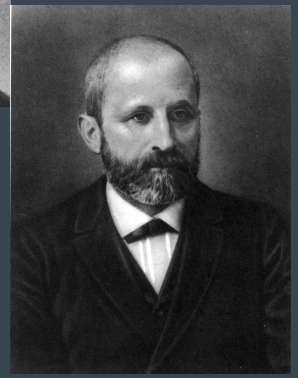
- Gregor Mendel completed his experiments on pea p
  thus marking the beginning of genetics as a science

1868:

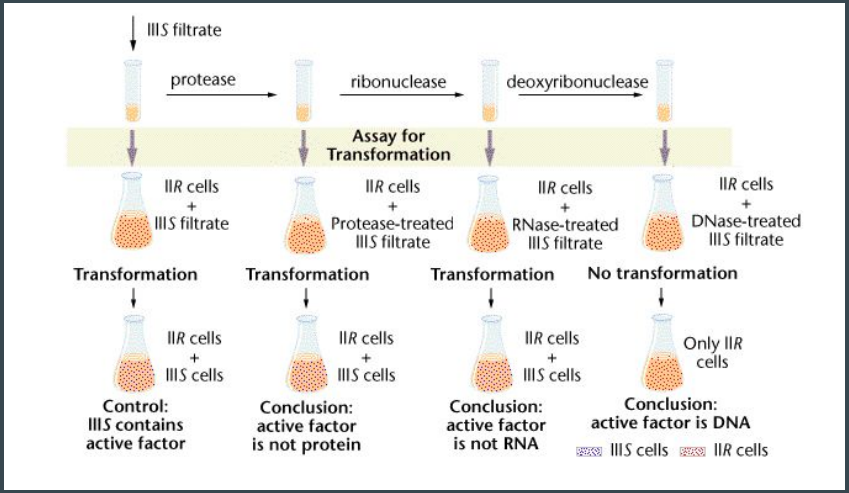
- Friedrich Miescher isolated nuclein from the cell nuclei

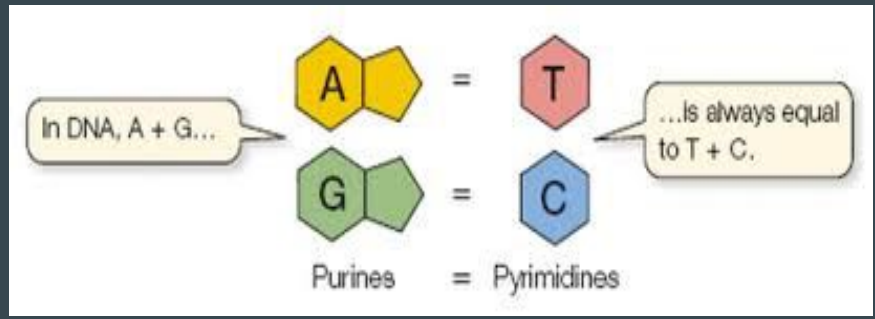






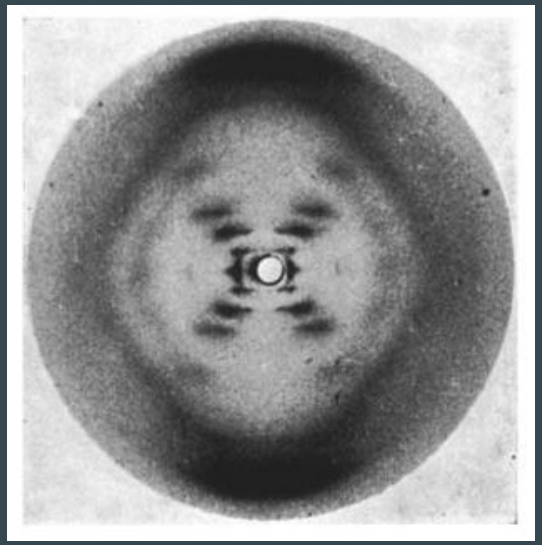
- 1944: Avery discovered DNA and suggested that it responsible for the transforming principle



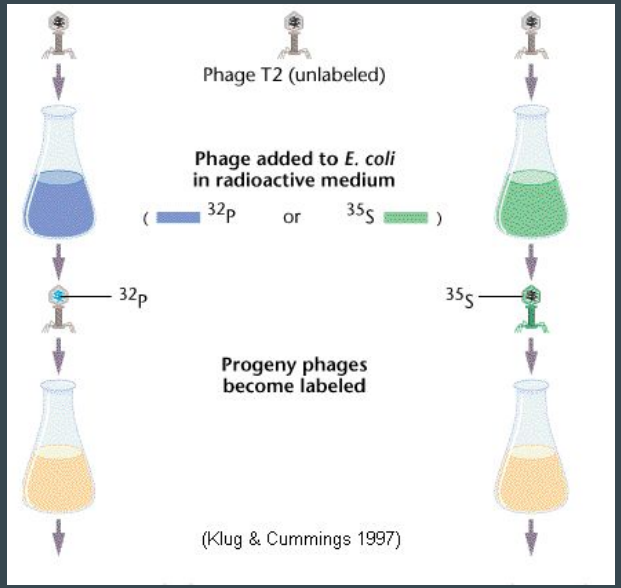


- 1950: Chargaff's rules

- 1952: Photo 51 by Franklin and Gosling

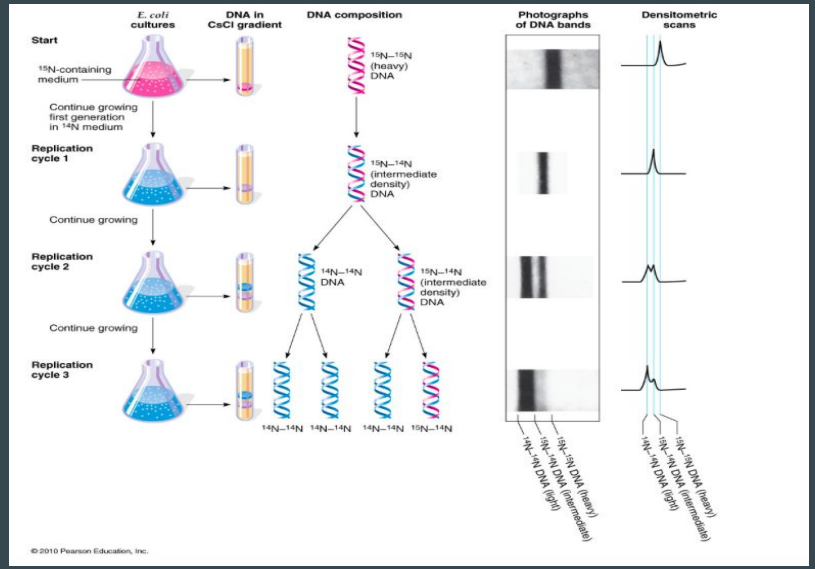


- 1952: Hershey & Chase blender experiment



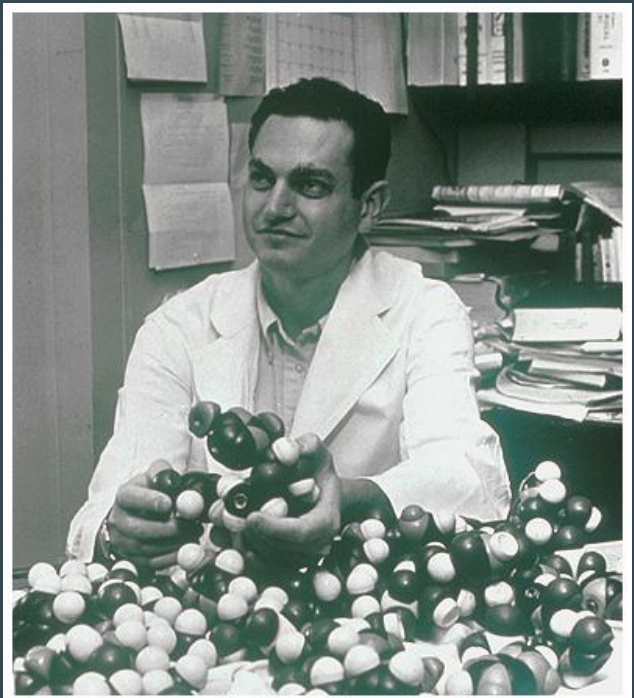
- 1953: Watson & Crick's DNA model

- 1958: DNA is Semiconservative



- 1961:
  - Brenner, Jacod, Crick & Monod discovers mRNA
  - Gamow suggests triplet code
  - Nirenberg and Matthaei identify the amino acid for poly-U

Dr. Marshall Nirenberg (1927-2010)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- Born in NY city and grew up in Florida
  - Interest in bird-watching
- University of Florida
  - B.Sc. and master's
- University of Michigan
  - Ph.D.
- National Institute of Health
  - Interested in fundamentality of life

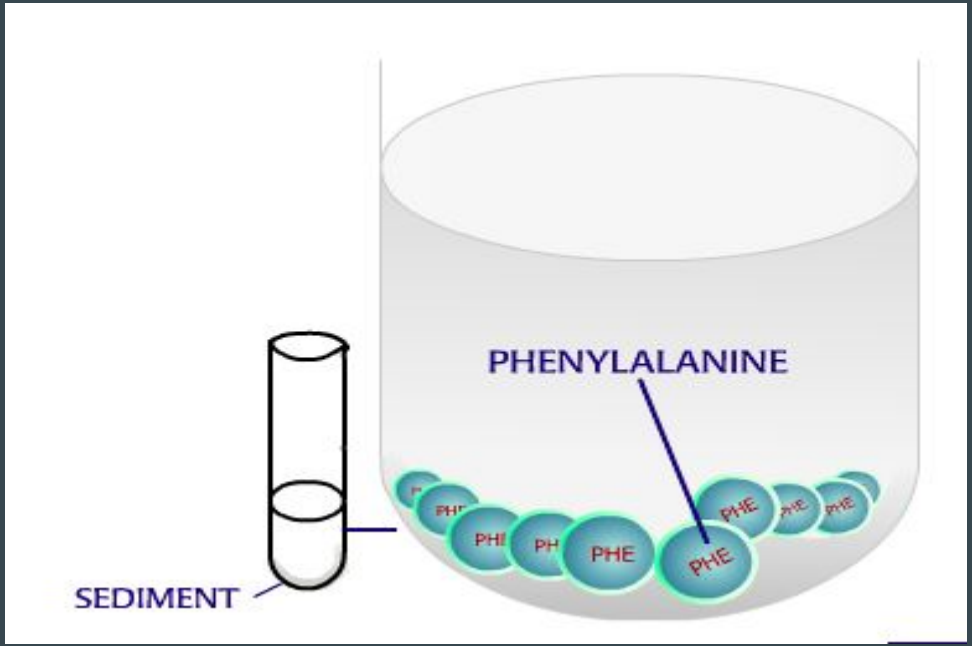
Poly-U Experiment

- E. Coli bacteria is ground up to produce a cell-free system
  - Treated with DNase

- 20 test tubes were used, one radioactively labeled, containing:
  - E. Coli extract
  - Synthetic RNA made of uracil
  - Amino acids

Results

- When radiolabeled Phenylalanine was added to the test tube with synthetic RNA composed of only uracil they found polypeptides made of only Phenylalanine
- The code can be broken!!



1963 Cold Spring Harbor Meeting

- Central Dogma and properties of the RNA code

- Questions raised about the fine structure of RNA

Formation of codon-ribosome-AA-sRNA complexes

Base sequence assay requires the following:

i. trinucleotides are able to serve as templates for AA-sRNA-ribosome binding

ii. codon-ribosome-AA-sRNA complexes can be retained by cellulose nitrate filters

TABLE 1. CHARACTERISTICS OF AA-sRNA BINDING TO RIBOSOMES

Modifications	C^{14}-Phe-sRNA bound to ribosomes ($\mu\mu$mole)
Complete	5.99
– Poly U	0.12
– Ribosomes	0.00
– Mg^{++}	0.09
+ deacylated sRNA at 50 min	
0.50 A^{260} units	5.69
2.50 A^{260} units	5.39
+ deacylated sRNA at zero time	
0.50 A^{260} units	4.49
2.50 A^{260} units	2.08

Formation of codon-ribosome-AA-sRNA complexes

Poly U: codon

Ribosome: translational apparatus. Sourced from *E. coli*

Mg++: Critical for Aminoacyl tRNA synthetase action

deacylated sRNA: Competitively binds to ribosome

TABLE 1. CHARACTERISTICS OF AA-sRNA BINDING TO RIBOSOMES

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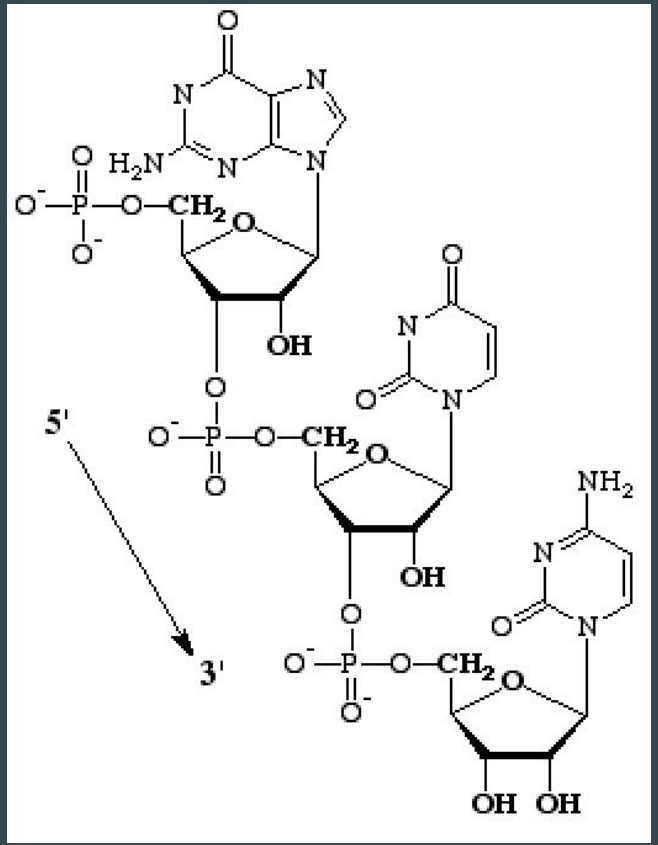
Formation of codon-ribosome-AA-sRNA complexes

Oligionucleotides synthesized using two methods:

i. Polynucleotide phosphorylase (PNPase)
   - UpU + pUp = UpUpU + Pi

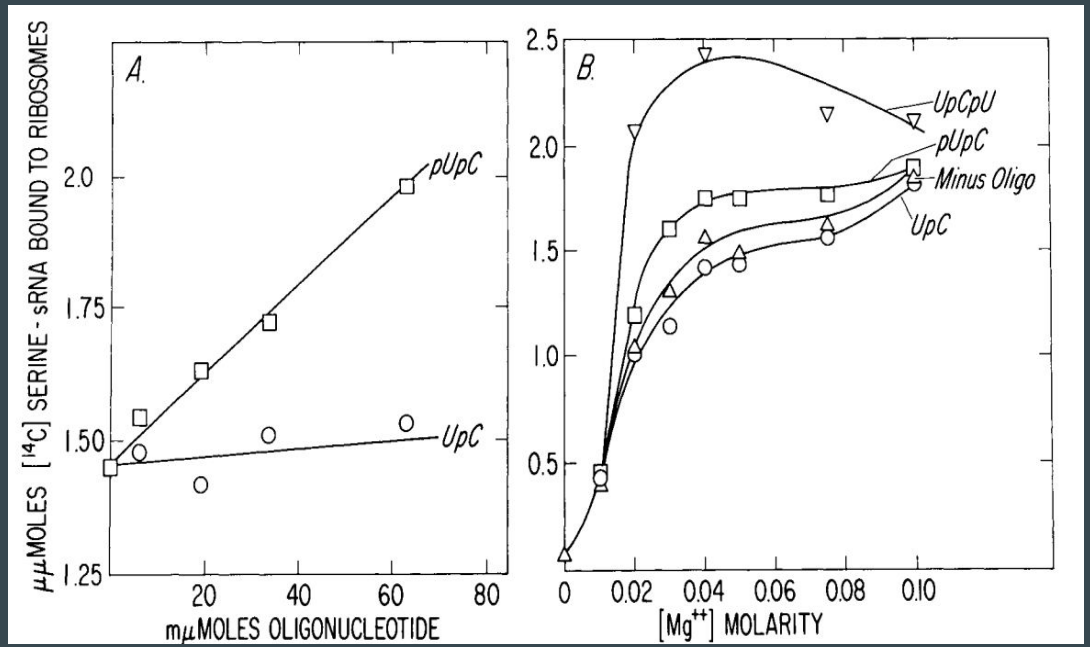
ii. Pancreatic RNase catalysis
   - uridine- or cytidine-2',3' cyclic phosphate



Template Activity of Oligonucleotides with Terminal and Internal Substitutions

Trinucleotides stimulate binding of respective sRNA to a much greater degree than corresponding dinucleotides

- ↪ Demonstrates **triplet code,** 3 sequential bases

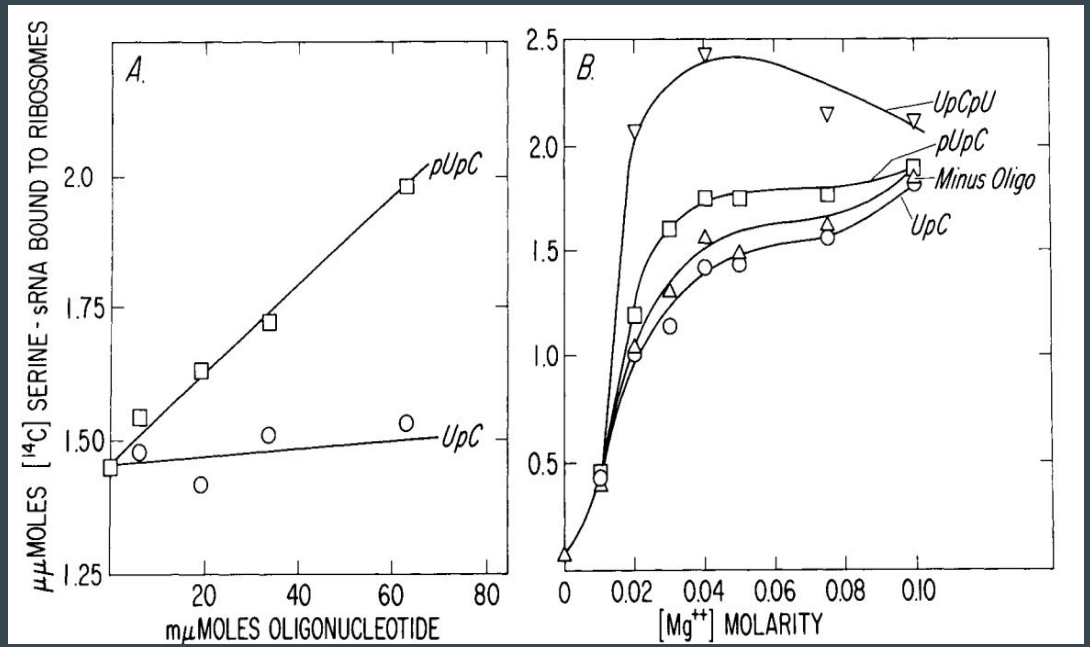


Template Activity of Oligonucleotides with Terminal and Internal Substitutions

Triplets with 5’ terminal phosphate have greater activity than those with 3’ terminal phosphates

Hexa-A nucleotides **more active** than penta-A

- ↪ Two Lys-sRNA bind to hexa-A, only one to penta-A
- ↪ Multiples of 3

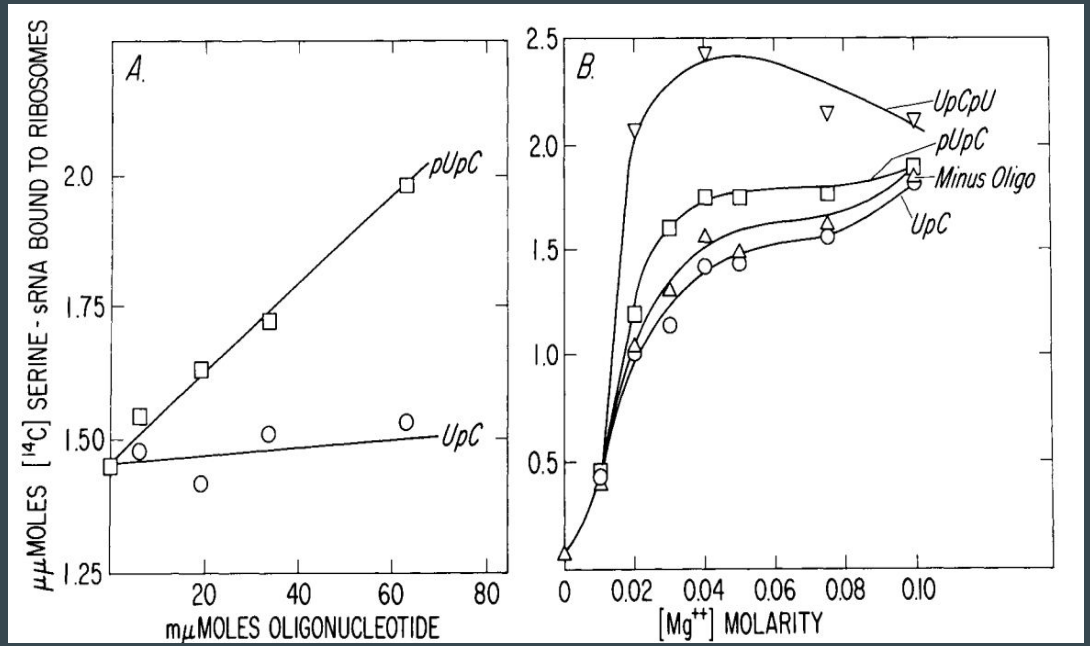


Template Activity of Oligonucleotides with Terminal and Internal Substitutions

Doublet with a 5’ phosphate pUpC templates for Ser-sRNA but **not** Leu-sRNA or Ile-sRNA

- Ser: UCx
- Leu: UCG > UCx
- Ile: AUC

UpCpU **>** pUpC **>>>** UpC

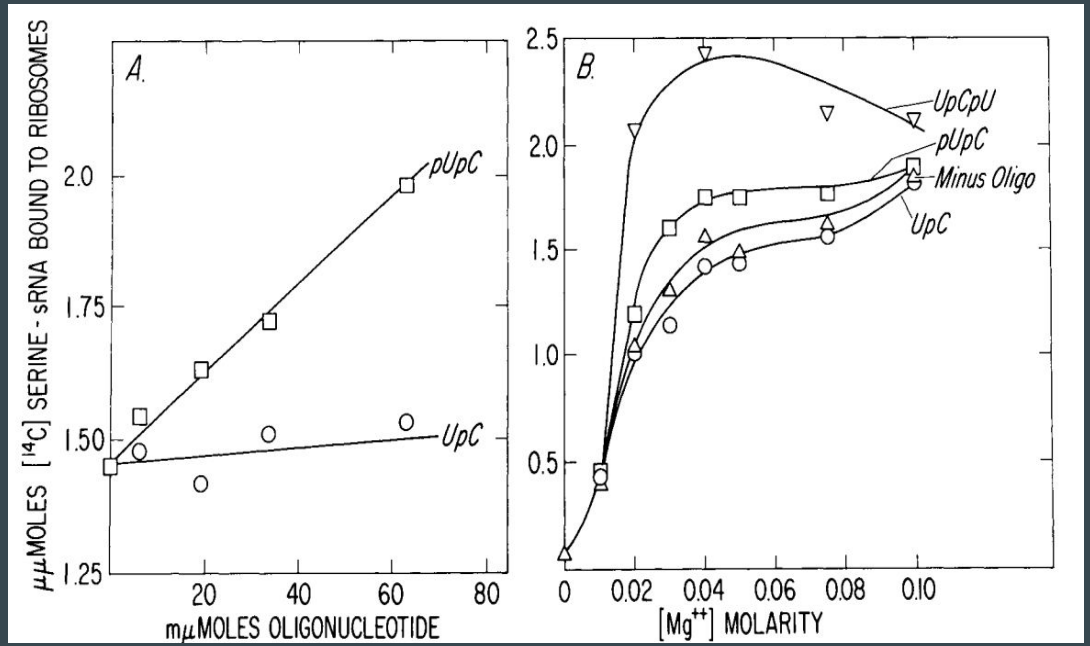


Template Activity of Oligonucleotides with Terminal and Internal Substitutions

A doublet with a 5’ phosphate can serve as a **specific** (though weak) template

Implications:

- Occasional recognition of only **2 of 3** bases during translation
- triplet code made have evolved from a primitive **doublet code**



Template Activity of Oligonucleotides with Terminal and Internal Substitutions

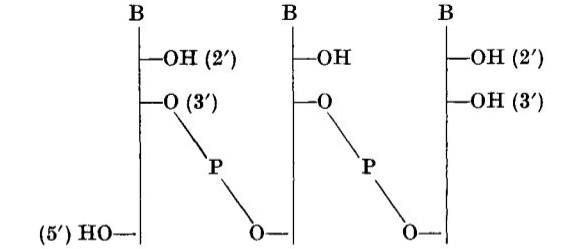
Three classes of codons, differing in structure:

- **5’-terminal**
- **internal**
- **3’-terminal**

The first base of 5’-terminal and last of 3’-terminal may be recognized with **less fidelity**

- ↪ Greater freedom of movement in the absence of a ‘neighbor’
- ↪ Terminal bases may serve as operator regions

TABLE 2. RELATIVE TEMPLATE ACTIVITY OF SUBSTITUTED OLIGONUCLEOTIDES



Oligonucleotide	Relative template activity
p-5′-UpUpU	510
UpUpU	100
CH_3O-pUpUpU	74
UpUpU-3′3-p	48
UpUpUp-OCH_3	18
UpUpU-2′,3′-cyclic p	17
(2′-5′)-UpUpU	0
Oligodeoxy T	0
p-5′-ApApA	181
ApApA	100
ApApA-3′-p	57
ApApA-2′-p	15
(2′-5′)-ApApA	0
Oligodeoxy A	0

Nucleotide Sequences of RNA Codons

Determined by stimulating *E. coli* AA-sRNA binding to *E. coli* ribosomes with trinucleotide templates

Forty-six codon base compositions confirmed using trinucleotide studies

Almost all triplets correspond to amino acids

TABLE 3. NUCLEOTIDE SEQUENCES OF RNA CODONS

1st Base	2nd Base U	C	A	G	3rd Base
U	PHE*	SER*	TYR*	CYS*	U
	PHE*	SER*	TYR*	CYS	C
	leu*?	SER	TERM?	cys?	A
	leu*, f-met	SER*	TERM?	TRP*	G
C	leu*	pro*	HIS*	ARG*	U
	leu*	pro*	HIS*	ARG*	C
	leu	PRO*	GLN*	ARG*	A
	LEU	PRO	gln*	arg	G
A	ILE*	THR*	ASN*	SER	U
	ILE*	THR*	ASN*	SER*	C
	ile*	THR*	LYS*	arg*	A
	MET*, F-MET	THR	lys	arg	G
G	VAL*	ALA*	ASP*	GLY*	U
	VAL	ALA*	ASP*	GLY*	C
	VAL*	ALA*	GLU*	GLY*	A
	VAL	ALA	glu	GLY	G

Nucleotide Sequences of RNA Codons

Alternate bases of degenerate codons usually occupy the third position

Triplet pairs with **3' pyrimidines** (XYU and XYC) usually correspond to the same amino acid

Triplet pairs with **3' purines** (XYA and XYG) often correspond with the same amino acid

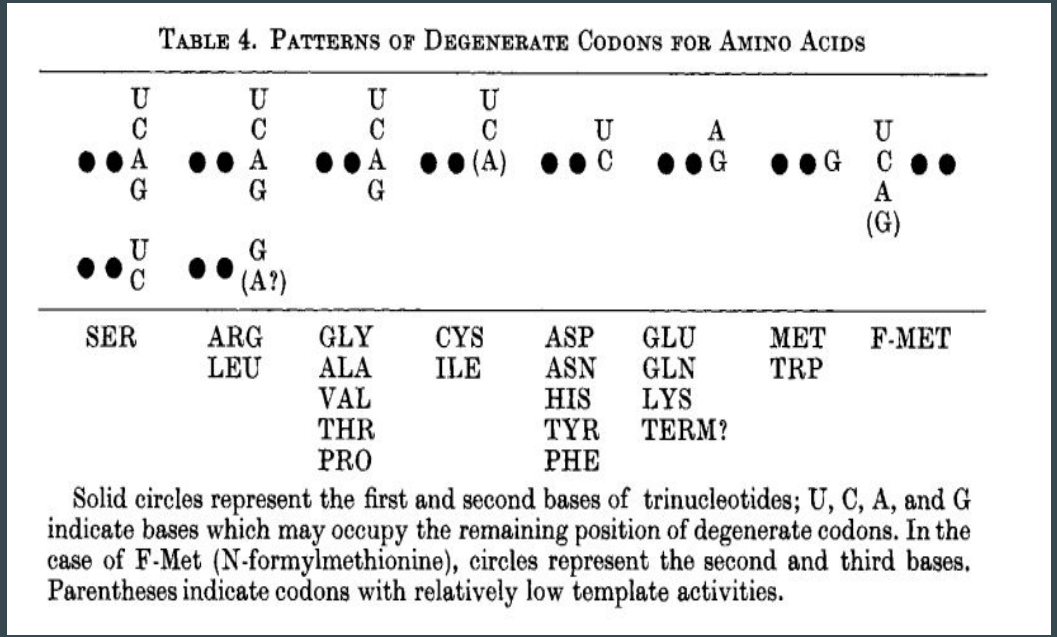


TABLE 4. PATTERNS OF DEGENERATE CODONS FOR AMINO ACIDS

●● U/C/A/G	●● U/C/A/G	●● U/C/A/G	●● U/C/(A)	●● U/C	●● A/G	●● G	U/C/A/(G) ●●
●● U/C	●● G/(A?)						
SER	ARG LEU	GLY ALA VAL THR PRO	CYS ILE	ASP ASN HIS TYR PHE	GLU GLN LYS TERM?	MET TRP	F-MET

Solid circles represent the first and second bases of trinucleotides; U, C, A, and G indicate bases which may occupy the remaining position of degenerate codons. In the case of F-Met (N-formylmethionine), circles represent the second and third bases. Parentheses indicate codons with relatively low template activities.

Nucleotide Sequences of RNA Codons

Implications:

- Single base replacements may be **silent**
- **Structurally/metabolically related** amino acids have similar codons
  - Asp (GAU and GAC) similar to Glu (GAA GAG)

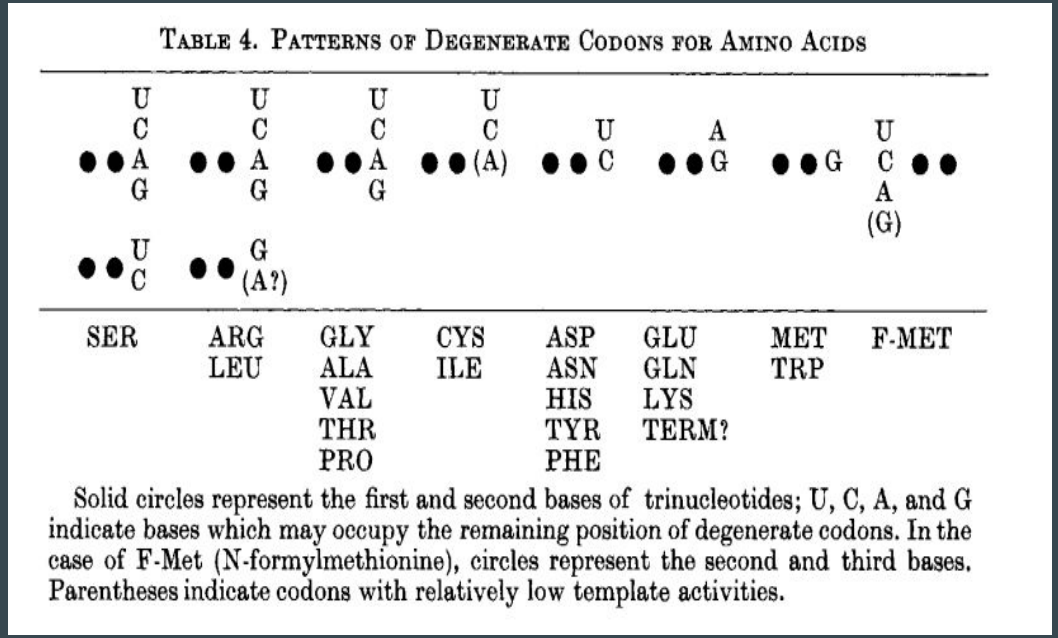


TABLE 4. PATTERNS OF DEGENERATE CODONS FOR AMINO ACIDS

U C A G ● ● U C	U C A G ● ● G (A?)	U C A G ● ● A G	U C ● ● (A)	U C ● ●	A G ● ●	● ● G	U C A (G) ● ●
SER	ARG LEU	GLY ALA VAL THR PRO	CYS ILE	ASP ASN HIS TYR PHE	GLU GLN LYS TERM?	MET TRP	F-MET

Solid circles represent the first and second bases of trinucleotides; U, C, A, and G indicate bases which may occupy the remaining position of degenerate codons. In the case of F-Met (N-formylmethionine), circles represent the second and third bases. Parentheses indicate codons with relatively low template activities.

Nucleotide Sequences of RNA Codons

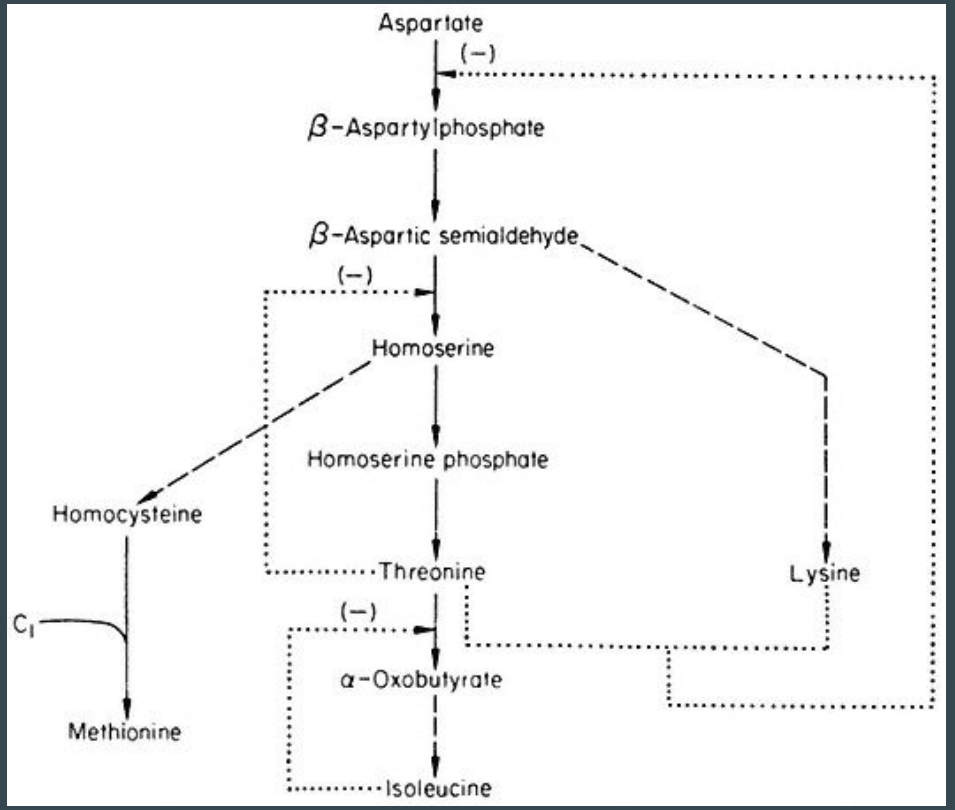
Grouping by **biosynthetic precursor** suggest codon relationships:

Asp: GAU, GAC

- Asn: AAU, AAC
- Lys: AAA, AAG
- Thr: ACU, ACC, ACA, ACG
- Ile: AUU, AUC, AUA
- Met: AUG

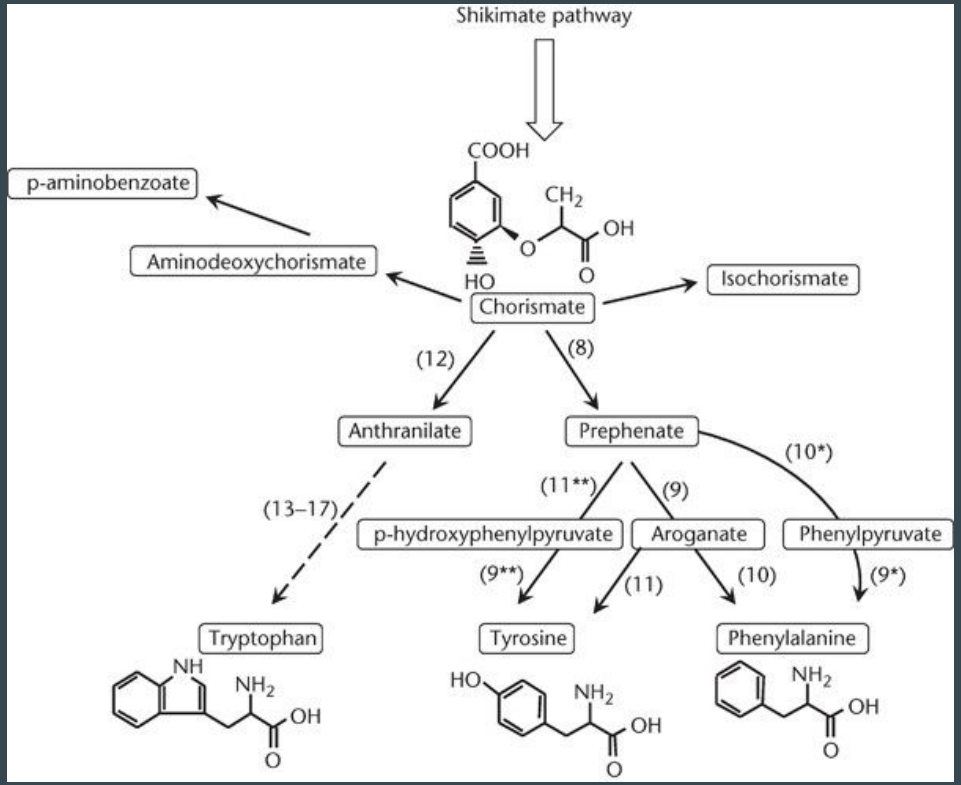
Aromatic amino acids often begin with U

- Phe: UUU, UUC
- Tyr: UAU, UAC
- Trp: UUG



Nucleotide Sequences of RNA Codons

These relationships may be artifacts of evolution or be evidence of **direct** interaction between amino acids and codon bases



Patterns of Synonym Codons Recognized by Purified sRNA Fractions

Degenerate codons for the same amino acid may be recognized by specific sRNAs (referred to as **sRNA fractions**)

Fractions were purified using **column chromatography** and **countercurrent distribution**

Patterns of Synonym Codons Recognized by Purified sRNA Fractions

Discernable patterns of recognition in third position synonym codons:

- C = U
- A = G
- G
- U =C = A
- A = G = (U)

Table 5. Codon Patterns Recognized by Purified sRNA Fractions

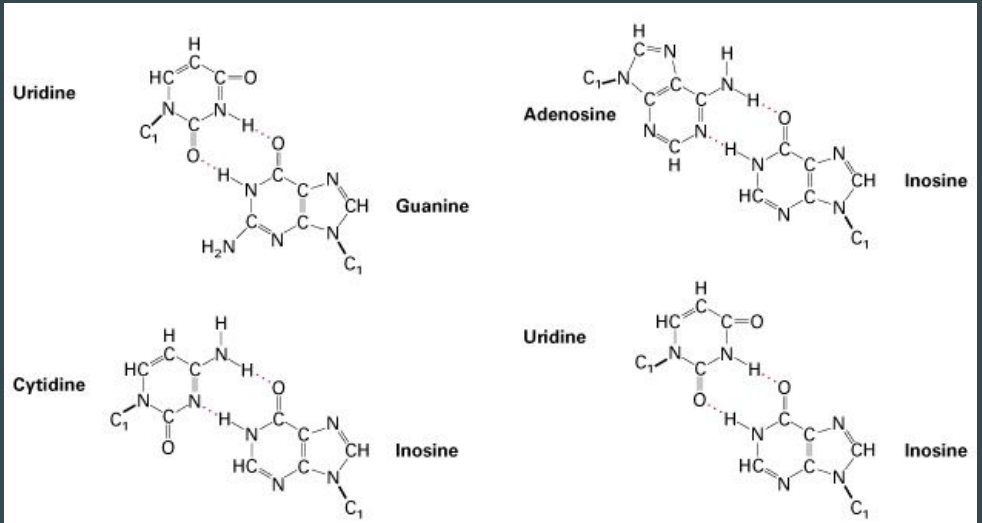
Alternate acceptable bases in 3rd or 1st positions of triplet											
	C U		A G		G		U C A		A G (U)	Possibly only 2 bases recognized	
$TYR_{1,2}$	UA C U	LYS	AA A G	LEU_2	CUG	ALA^{yeast}	U GCC A	ALA_1	A GCG (U)	LEU_3	CU (U) (C)
VAL_3	GU C U			LEU_5	UUG	$SER^{yeast}_{2,3}$	U UCC A	$VAL_{1,2}$	A GUG (U)	$LEU_{4a,b}$	UU (U) (C)
				MET_2	AUG	$F\text{-}MET_1$	U C UG A			LEU_1	(U)UG
						TRP_2	U CGG (A)				

Mechanism of Codon Recognition

Crick (1966) suggests certain anticodon bases form alternate hydrogen bonds with corresponding mRNA bases

↪ “Wobble mechanism”

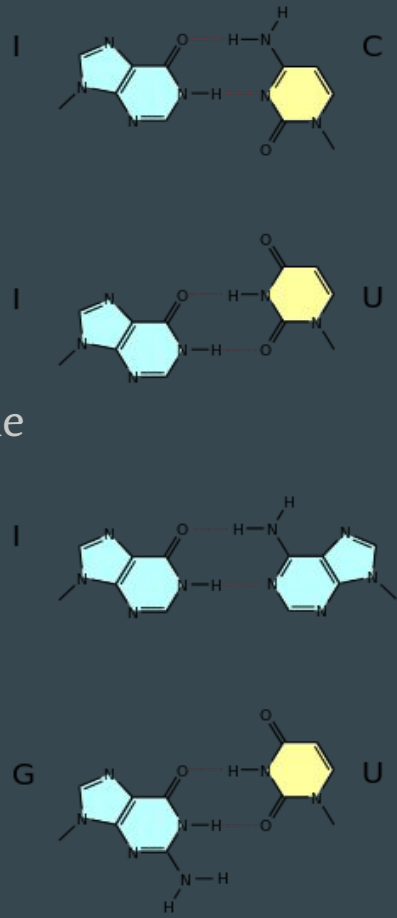
How can this be observed?



tRNA 5' anticodon base	mRNA 3' codon base
A	**U**
C	**G**
G	**C** or *U*
U	**A** or *G*
I	*A* or *C* or *U*

Crick's Wobble Hypothesis

- Pairings in between two nucleotides that do not follow Watson-Crick base pair rules
- Guanine-Uracil, Hypoxanthine-Uracil, Hypoxanthine-Adenine and Hypoxanthine-Cytoseine



Mechanism of Codon Recognition

- Purified yeast (Fig. 2) and unfractionated *E. coli* (Fig. 3) C^{14}-Ala-sRNA response to synonym Ala-codons as a function of [Mg++]
- Different codons may elicit divergent responses

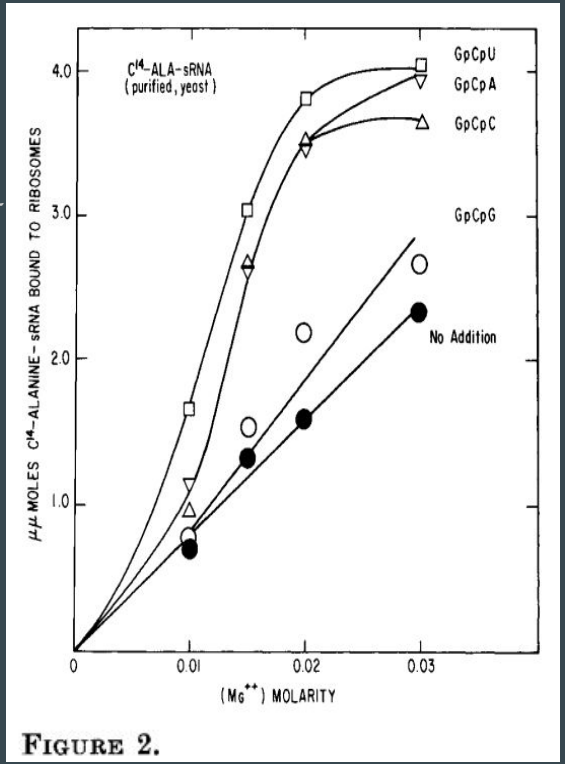


FIGURE 2.

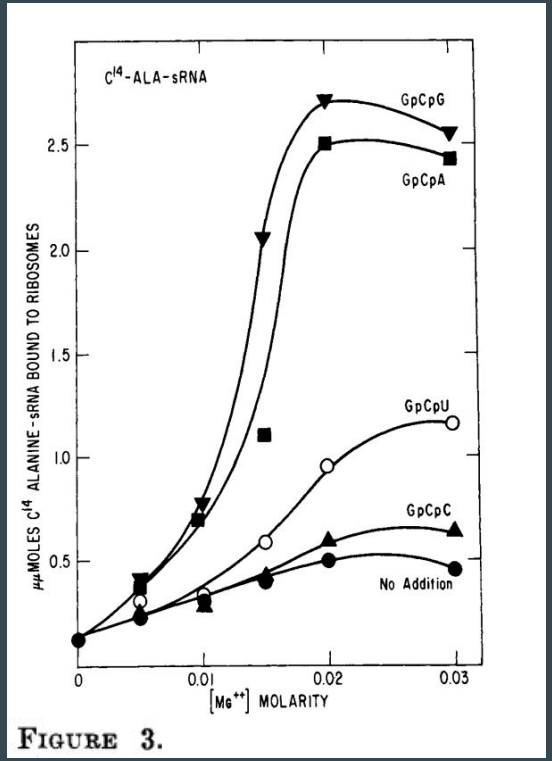


FIGURE 3.

Mechanism of Codon Recognition

At **limiting** concentrations of C^{14}-Ala sRNA

Yeast:

- GCU - **59%**; GCC - **45%**; GCA - **45%**; GCG - **3%**

E. coli:

- GCU - **18%**; GCC - **2%**; GCA - **38%**; GCG - **64%**

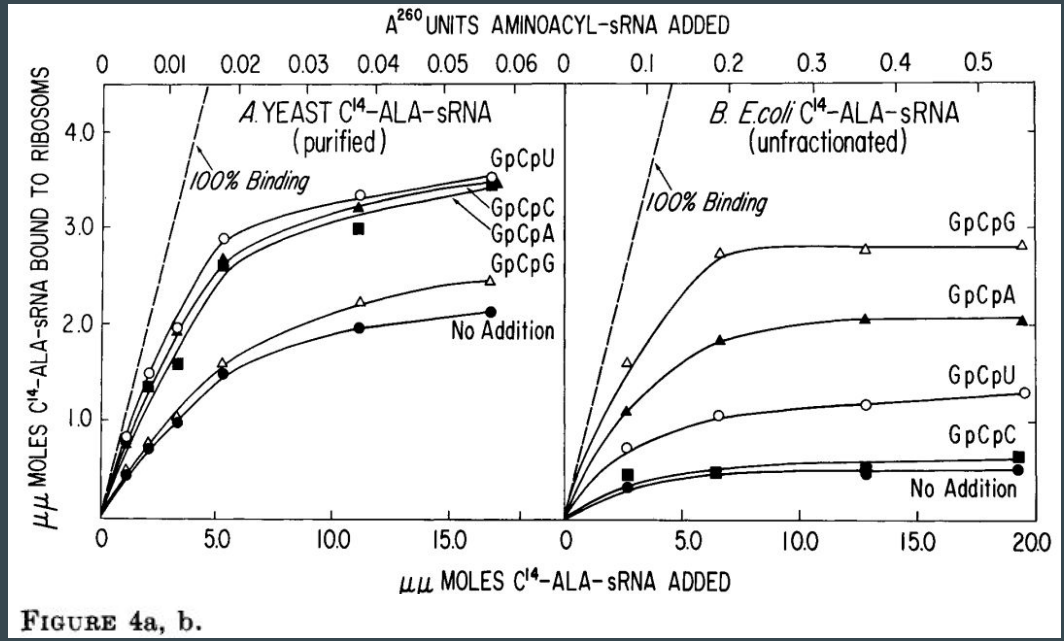


FIGURE 4a, b.

Mechanism of Codon Recognition

The purity of the yeast Ala-sRNA used in these experiments was > 95%

This implies that **one specific molecule** of Ala-sRNA recognizes at least 3 synonym codons

Additionally, there are disparate responses to synonym codons between yeast (Eukaryota) and *E. coli* (Bacteria)

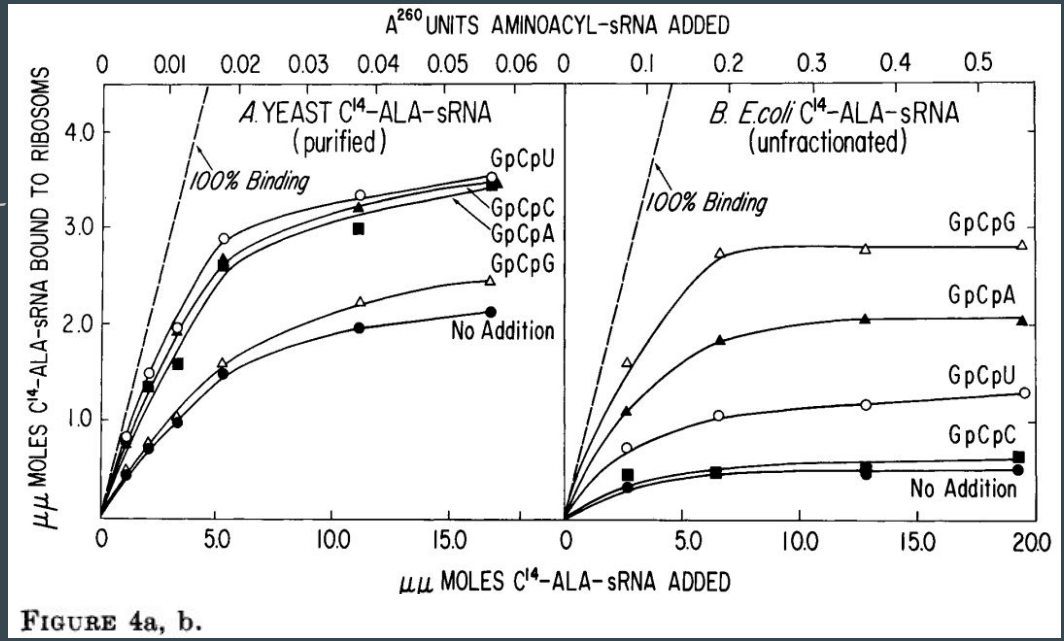


FIGURE 4a, b.

Mechanism of Codon Recognition

To further derive information about the structure of Ala-sRNA and the mechanism of codon recognition, we may relate it to its conjugate mRNA

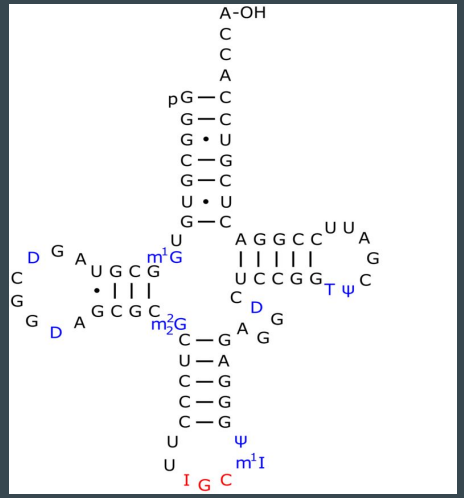
Possible anticodon sequences:

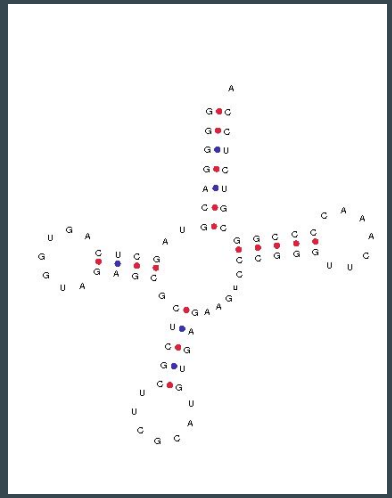
-**IGC** MeI-

or

DiHU-**CGG**-DiHU

* I = hypoxanthine/inosine; DiHU = dihydrouracil





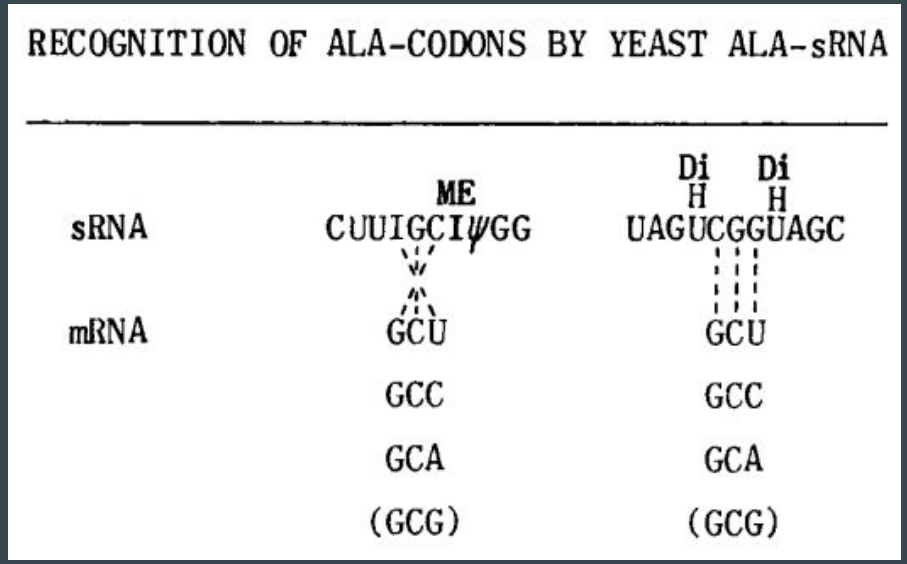
Mechanism of Codon Recognition

If CGG is the anticodon we will observe:

- **parallel** hydrogen bonding with GCU, GCC, and GCA

If IGC is the anticodon we will observe:

- **antiparallel** hydrogen bonds between GC in the anticodon and GC in the first and second position anticodons
- alternate pairing of I in the anticodon with U, C, and A (but not G) in the third position of the Ala-codon



Mechanism of Codon Recognition

Evidence is consistent with an IGC Ala-anticodon

Patterns of codon recognition support wobble hypothesis

Suggest only 2 of 3 bases may be recognized

TABLE 6. ALTERNATE BASE PAIRING

sRNA Anticodon	mRNA Codon
U	A G
C	G
A	U
G	C U
I	U C A
rT	A G
ψ	A G (U)
DiHU	No base pairing

Universality

TABLE 7. NUCLEOTIDE SEQUENCES OF RNA CODONS RECOGNIZED BY AA-sRNA FROM BACTERIA AND AMPHIBIAN AND MAMMALIAN LIVER

	U	C	A	G	
U	PHE	SER	TYR	cys	U
	PHE	SER	TYR	cys	C
	leu?	SER	TERM?	cys	A
	leu, F-MET	SER	TERM?	trp	G
C	leu	PRO	HIS	ARG	U
	leu	PRO	HIS	ARG	C
	leu	PRO	gln	ARG	A
	leu	PRO	gln	ARG	G
A	ILE	THR	asn	SER	U
	ILE	THR	asn	SER	C
	ILE	THR	LYS	ARG†	A
	MET, F-MET?	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	gly	A
	VAL	ALA	GLU	gly	G

Universality

RNA code is largely universal

Cell may may differ in specificity of codon translation

Near identical translations in bacteria, mammalia and amphibia

↪ Similarity suggests functional genetic code may be > 3 billion years old

TABLE 8. SPECIES DEPENDENT DIFFERENCES IN RESPONSE OF AA-sRNA TO TRINUCLEOTIDE CODONS

		sRNA		
Codon		Bacterial (*E. coli*)	Amphibian (*Xenopus laevis*)	Mammalian (Guinea pig liver)
ARG	AGG	±	++++	+++
	CGG	±	++++	++++
MET	UUG	++	±	±
ALA	GCG	++++	±	++
ILE	AUA	±	++	++
LYS	AAG	±	++++	++++
SER	UCG	++++	±	++
	AGU	±	+++	+++
	AGC	±	+++	+++
CYS	UGA	±		+++

Possible differences: ACG, THR; AUC, ILE; CAC, HIS; GUC, VAL; and GCC, ALA

No differences found: ASP, GLY, GLU, PHE, PRO, and TYR.

Unusual Aspects of Codon Recognition as potential indicators of special codon functions

- Introduction
- Codon Frequency and Distribution
- Codon Position
- Template Activity
- Codon Specificity
- Conclusion

Introduction

- Codons can serve multiple functions other than corresponding to amino acids; such as initiation & termination codons or the regulation of protein synthesis.
- Some codons can exhibit special properties related to codon position, template activity/specificity, stability of codon-ribosome-tRNA complexes, etc.
- These topics will be discussed to explain how they are possible indicators of special codon function.

Codon Frequency and Distribution

- Multiple species of tRNA can correspond to the same amino acid, differing only in the 3rd base of the anticodon
- Since a different tRNA is required for each codon it can be concluded that protein synthesis may be regulated by the frequency and distribution of codons (as there's a limited abundance of each tRNA) as well as recognition of degeneracies.

Codon Position

- They discussed how reading of the mRNA is probably initiated at the 5’ terminal end to the 3’ end.
- N-formyl-Met-tRNA may act as an initiator of protein synthesis (done in *E. coli*), binding primarily to AUG.
- In *E. coli* protein synthesis can be initiated by start codons specifying the N-formyl-Met-tRNA or by other means that do not involve the N-formyl-Met-tRNA (may be codons with a high Mg^{++} concentration).
- UAA and UAG trinucleotides seem to function as terminator codons because they do not stimulate binding of the tRNA to the ribosomes.

Codon Position Continued

- Extragenic suppressors can affect the specificity of these terminator codons (UAA and UAG).
- Amber mutation - UAG codon
- Ochre mutation - UAA codon
- The amber suppressor mutates the tRNA to override the stop codon (UAG) and continue reading the strand (ochre suppressors working in much the same way). The amber suppressor has a higher efficiency than the ochre suppressor, therefore ochre mutations (UAA codons) are more frequent in vivo.
- Protein synthesis can be regulated by the position of the codon in respect to the amber suppressors.

Template Activity

- UAA, UAG, & UUA show little template activity for AA-tRNA, while other codons are active templates for tRNA in some organisms but not others.
- Possible explanations for low template activity can be: codon position, abundance of appropriate tRNA, high ratio of deacylated to AA-tRNA, low Mg++ concentrations, special codon function, etc.

Codon Specificity

- Synonym trinucleotides differ in template specificity and can change depending on the concentration of Mg++ present (Shown in Table 9).
- At 0.010-0.015M Mg ++ trinucleotide specificity is high but at 0.03M Mg ++ there's so much Mg++ present that the specificity is reduced and recognition of trinucleotides become ambiguous.
- In some cases one or two codons in a synonym set are active at 0.01 m Mg++ and all degeneracies are active at 0.03 m Mg++. Other times all synonym trinucleotides are active at both concentrations (ex: Valine) or only active at the 0.03 m Mg++ concentration (ex: Tyrosine).
- Codon-ribosome-AA-tRNA complexes (formed with degeneracies) therefore have varying stability.

TABLE 9. TEMPLATE ACTIVITY OF TRINUCLEOTIDES IN 0.01 OR 0.03 M Mg^{++}

	U	C	A	G	
U	PHE	**SER**	TYR	**CYS**	U
	PHE	SER	TYR	**CYS**	C
		(SER)			A
	F-MET	**SER**		(TRP)	G
C		PRO	HIS	**ARG**	U
		PRO	HIS	ARG	C
		(PRO)	GLN	**ARG**	A
	LEU	(PRO)	**GLN**	ARG	G
A	ILE	**THR**	ASN	SER, CYS	U
	ILE	THR	ASN	SER, CYS	C
		THR	**LYS**		A
	MET	**THR**	LYS		G
G	**VAL**	ALA	**ASP**	**GLY**	U
	VAL	ALA	**ASP**	**GLY**	C
	VAL	**ALA**	**GLU**	(GLY)	A
	VAL	**ALA**	GLU	(GLY)	G

Legend:

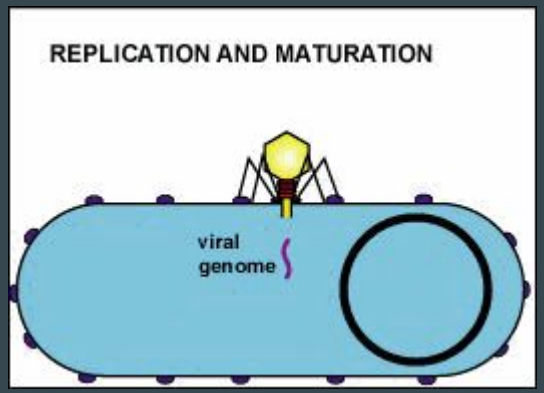
	0.01 M Mg	0.03 M Mg
Box =	+	+
No Box =	−	+
( ) =	not tested	

Relative template activities of trinucleotides in reactions containing 0.01 or 0.03 M Mg^{++}. A plus (+) sign in the legend means that the trinucleotide stimulates AA-sRNA binding to ribosomes at that magnesium concentration; a minus (−) sign means it is relatively inactive as a template. The results refer to AA-sRNA from *E. coli* strains B and/or W3100. The data are from Anderson, Nirenberg, Marshall, and Caskey (1966).

Conclusion

- Codons can have alternate meanings, in that the location of the codon in the strand will affect what amino acid is produced.
- A codon can have multiple functions
- These functions are subject to change
- Degenerate codons usually exhibit differences in their template properties

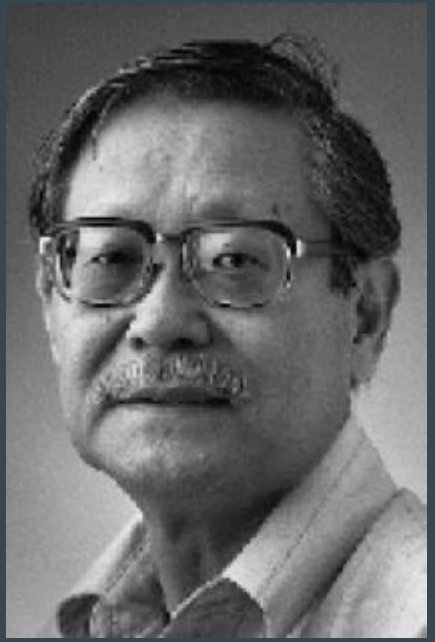
MODIFICATION OF CODON RECOGNITION DUE TO PHAGE INFECTION



Discovering the changes that a bacteriophage can make in host cell's protein synthesis.

Noboru Sueoka - Molecular Biologist

- born April 12 1929 in Kyoto Japan
- Undergraduate (1953) and Masters degrees from Kyoto University, PhD (1955) from California Institute of Technology
- Research fellow at Harvard, Cambridge and Massachusetts
- Professor at The University of Illinois, Princeton and Colorado
- Member of the American Academy of Arts and Science
- Contributor to over 140 articles on genetics and molecular biology
- Daughter and Wife
- Enjoys Fly Fishin and Skiiing in his spare time

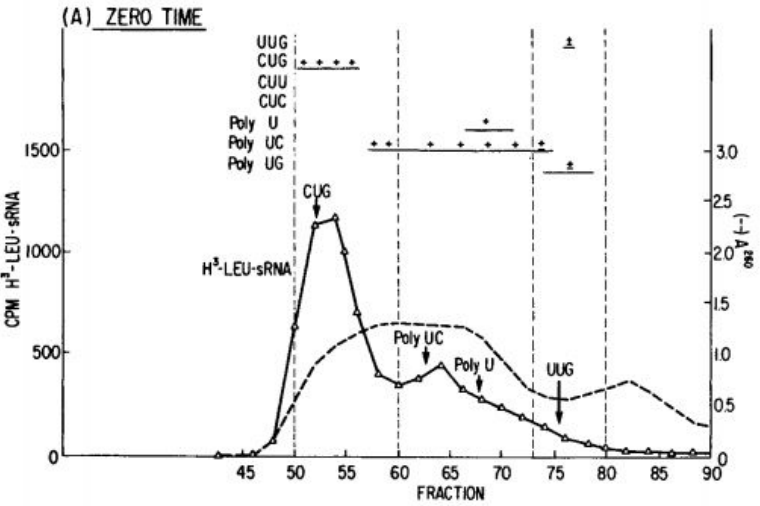


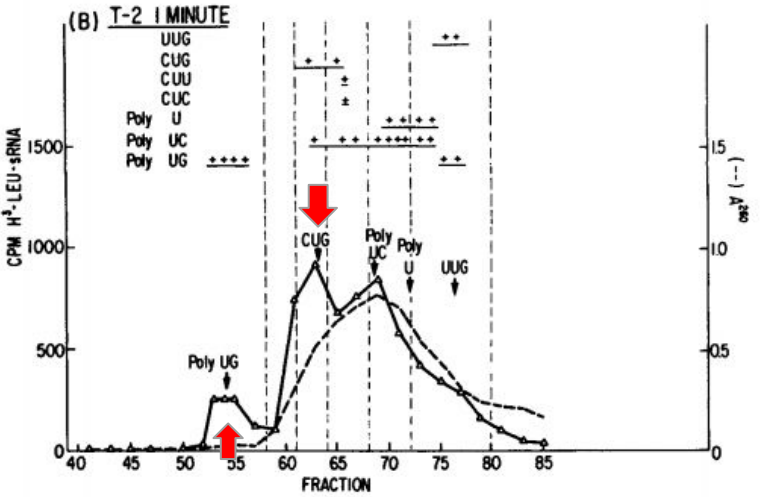
The Original Experiment That led to Helping Nirenberg

- Completed at Princeton University
- Knew that phage infection causes differentiation in gene expression within the host cell, but How?
- Maybe sRNAs are involved!
- Compared aminoacyl-sRNAs for 17 amino acids before and after infection
- Using MAK (methylated albumin-kieselguhr) column fractionation technique
- Only leucyl-sRNA showed a significant change after infection, and with even closer analysis only certain components of the sRNAs were being altered
- With further experimentation, it was also found that the phage DNA must be injected into the host and protein synthesis for the host cell must occur after the infection
- In the end, the host cell's protein synthesis is inhibited and the virus' continues

Sueoka & Nirenberg working together

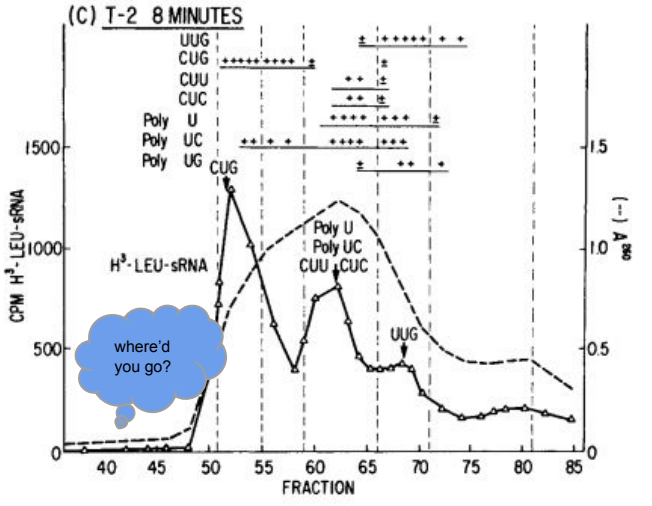
- What does this mean for the modified Leu-sRNAs codon recognition?
- sRNA preparations were isolated before the phage infection and at 1 minute and 8 minutes after the infection
- sRNA was then acylated with H^3 leucine by *E. coli* or Yeast synthetase (yeast allows both anticodon recognition and enzyme recognition sites to be monitored)
- MAK chromatography was then used to purify the Leu-sRNA preparations
- this allowed the observation of the differential binding to ribosome templates between each of the fractions of Leu-sRNA

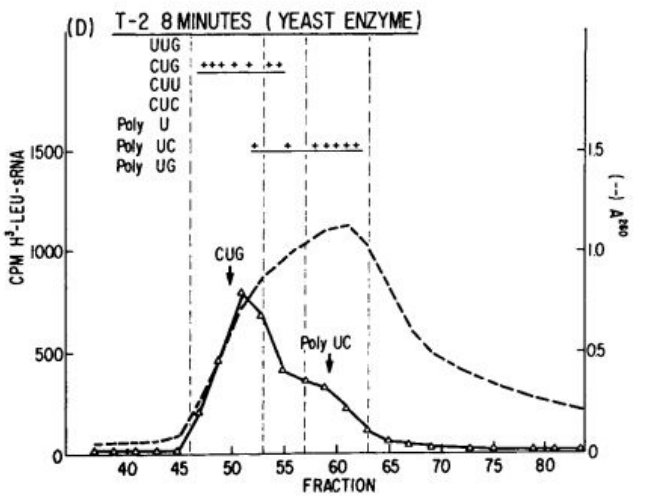


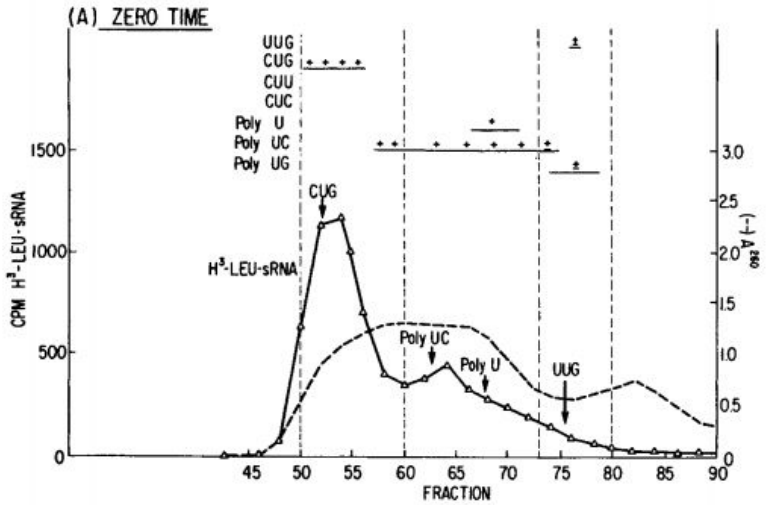


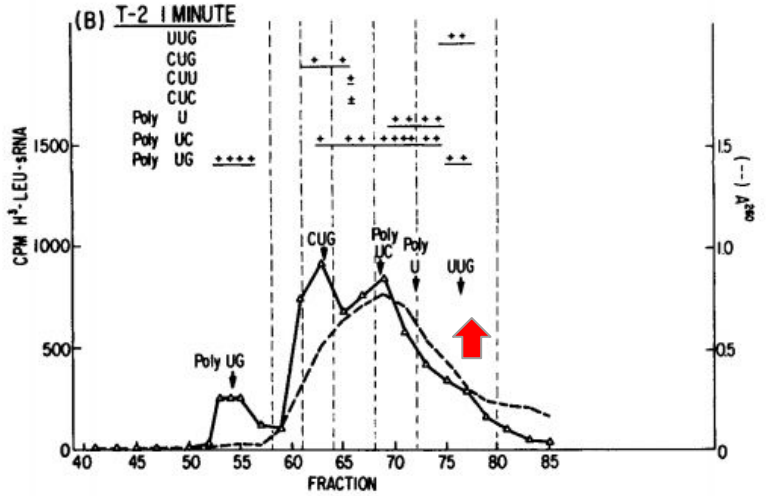
- after 1 minute of infection, Leu-sRNA$_2$ decreased in its response to CUG

- correspondingly, Leu-sRNA$_1$ had an increase in response to poly UG but not to the trinucleotides and was completely undetected after 8 minutes



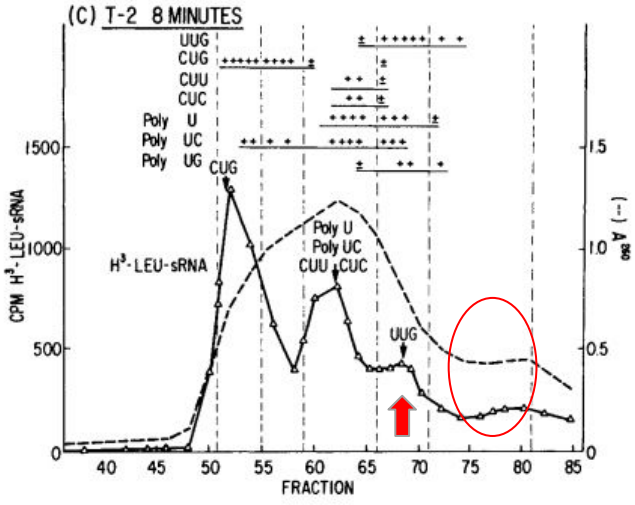


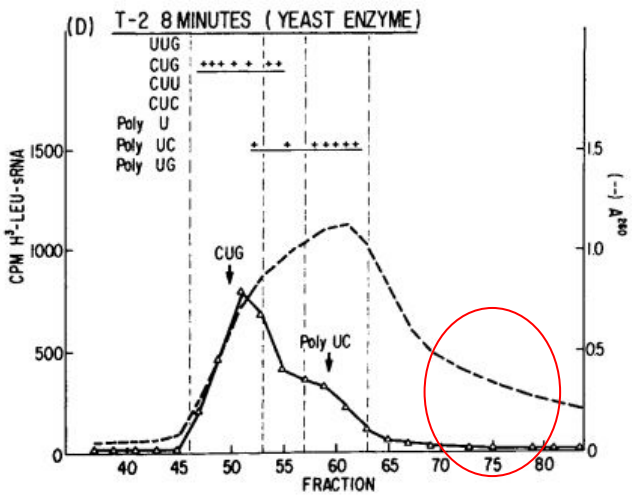




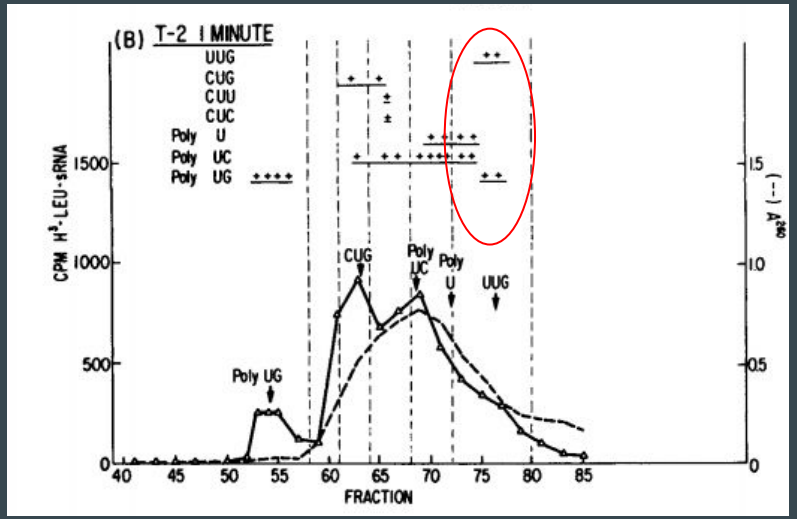
- an increase in Leu-sRNA$_5$ response to UUG was observed at 1 minute after infection and was even greater at 8 minutes

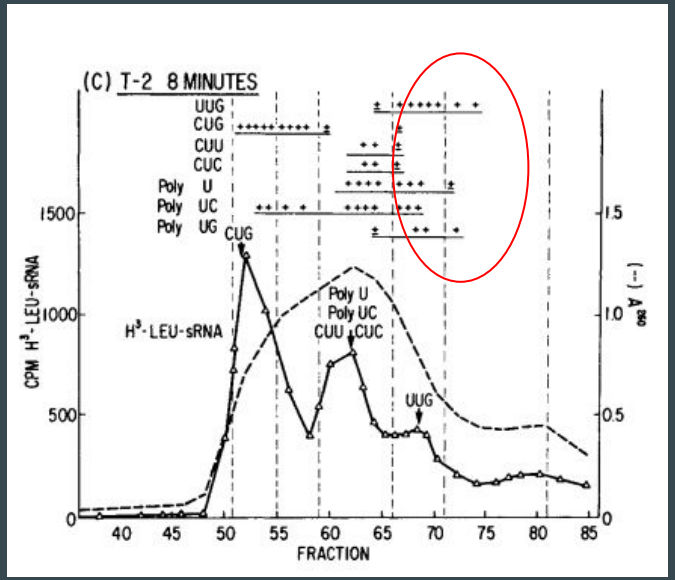
- both Leu-sRNA$_3$ and Leu-sRNA$_{4a,b}$ had greater response to poly UC 8 minutes after infection but they also had varying responses in yeast and *E. coli*





- this suggests that a fraction of Leu-sRNA$_3$ must differ from the Leu-sRNA$_{4a,b}$ even though they both respond to poly UC
- and the multiple responses of Leu-sRNA$_{4a,b}$ to poly U, poly UC and the trinucleotides CUU and CUC suggests that the fractions may be from two different species of Leu-sRNA



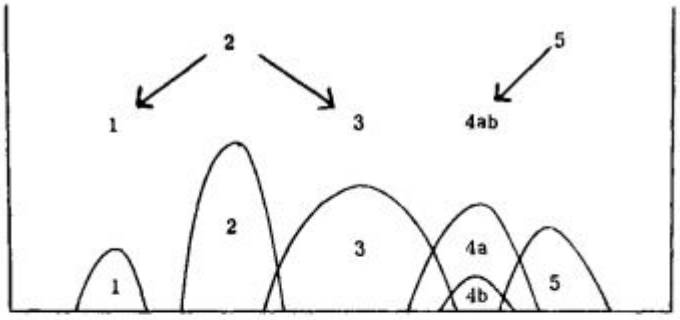


Why are these fractions responding so differently?

- Leu-sRNA fractions 1,2 and 3 respond to both E. coli and Yeast Leu-sRNA synthetase
- Leu-sRNA$_5$ and Leu-sRNA$_{4a,b}$ are only recognized by E. coli synthetase
- This suggests that there are two separate cistrons for Leu-sRNA
- fractions 1, 2 and 3 in one cistron and fractions 4 a, b and 5 in another

- the corresponding decrease in Leu-sRNA$_2$ and increase in Leu-sRNA$_1$ suggests that Leu-sRNA$_2$ is the precursor of Leu-sRNA$_1$ and the data also suggests it is the precursor of Leu-sRNA$_3$

Cistron "A" includes the Leu-sRNA fractions 1, 2 and 3

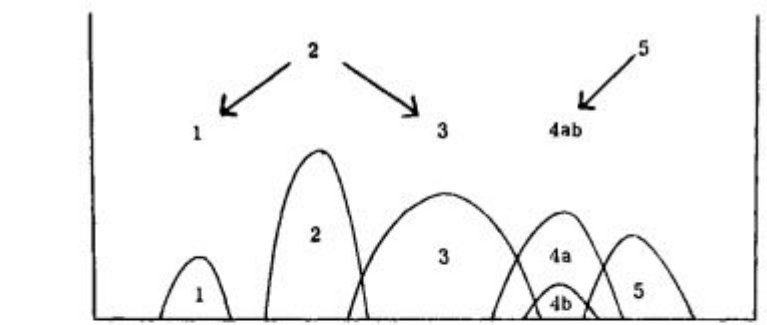


CODON	(-)UG POLY UG	CUG	CU(-) POLY CU	UU(-) CUU CUC POLY U POLY CU	UUG POLY UG
ZERO TIME	±	++++	++	+	+
T-2 1"	++	++	++	+	++
T-2 8"	±	+++	+++	+++	+++
AA-sRNA SYNTHETASE					
YEAST	+	+	+	—	—
E.COLI	+	+	+	+	+

- Leu-sRNA$_2$ shows a relationship with the CUG codon
- Leu-sRNA$_3$ to the CU(-) codons, (can be subsituted with multiple end bases)
- Leu-sRNA$_1$ to the (-)UG codons

Cistron “B” includes the Leu-sRNA fractions 4 a, b and 5

- Leu-sRNA$_5$ differs from Leu-sRNA$_2$ in both anitcodon and synthase recognition sites
- Data suggests that Leu-sRNA$_5$ is the precursor to Leu-sRNA$_{4a, b}$
- Leu-sRNA$_5$ demonstrates a relationship with the codon UUG
- Leu-sRNA$_4$ with the codons UU(-), UC(-), UA(-), CU(-), and AU(-)



CODON	(-)UG POLY UG	CUG	CU(-) POLY CU	UU(-) CUU CUC POLY U POLY CU	UUG POLY UG
ZERO TIME	±	++++	++	+	+
T-2 1"	++	++	++	+	++
T-2 8"	±	+++	+++	+++	+++
AA-sRNA SYNTHETASE					
YEAST	+	+	+	—	—
E.COLI	+	+	+	+	+

So what does this mean?

- we know that modification of Leu-sRNA after infection requires protein synthesis to occur (from Sueoka's prior experiment), which suggests that specific enzymes may be needed to modify the bases in Leu-sRNA fractions
- the inhibition of the E.coli's protein synthesis but not the virus' suggests that the modifications to Leu-sRNA may be to blame
- the initiator of protein synthesis in E. coli responds to the same trinucleotides as the Leu-sRNA fractions (UUG and CUG)
- the modification of Leu-sRNA must result in the prevention of E. coli protein synthesis initiation but must leave the viral protein synthesis unaffected

Further studies were required…

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