Topics in Biological Physics

- J. H. Matthaei and M. W. Nirenberg (first paper published at 1961)
  - Decoding the genetic code
  - DNA and RNA influence on protein synthesis

Lecture by : Oren Zarchin, Jan 2007

WEIZMANN INSTITUTE OF SCIENCE

Lets go back in time

- *What did the scientific community know 5 decades ago?*
- *We don't have a magic wand*
- *We do have books and papers*

Introduction 1950-1960

- *DNA (inherited) is shaped by evolution*
- *Structure of DNA*
- *Proteins – Giant molecules constructed from 20 different amino-acids*
- *The code for a protein is carried by a single gene*
- *4 code letters.*
- *How many letters to construct a word?*

1950-1960 – base number

- *George Gamow (1954) – A word in the gene must contain at least 3 letters (4x4=16, 4x4x4=64)*

SINGLET CODE (4 WORDS)

A
G
C
U

DOUBLET CODE (16 WORDS)

AA	AG	AC	AU
GA	GG	GC	GU
CA	CG	CC	CU
UA	UG	UC	UU

TRIPLET CODE (64 WORDS)

AAA	AAG	AAC	AAU
AGA	AGG	AGC	AGU
ACA	ACG	ACC	ACU
AUA	AUG	AUC	AUU
GAA	GAG	GAC	GAU
GGA	GGG	GGC	GGU
GCA	GCG	GCC	GCU
GUA	GUG	GUC	GUU
CAA	CAG	CAC	CAU
CGA	CGG	CGC	CGU
CCA	CCG	CCC	CCU
CUA	CUG	CUC	CUU
UAA	UAG	UAC	UAU
UGA	UGG	UGC	UGU
UCA	UCG	UCC	UCU
UUA	UUG	UUC	UUU

Is the code overlapping?

(e.g. UGUA… results in UGU, GUA, UA_...)

1950-1961

- *DNA **might** not be directly involved in protein synthesis*
- *An Enzyme – RNA polymerase, catalyzes the synthesis of RNA strands from DNA strands*
- *Messenger RNA – carry genetic messages from DNA to ribosomes (Monod & Jacob, 1958)*

1961

- Matthaei & Nirenberg
  - *Cell free protein synthesis*
  - *DNA is not directly involved in protein synthesis*
  - *RNA is directly involved in amino acid incorporation into proteins*
- Crick, Barnett, Brenner and Watts-Tobin

3 letters stand for a word.

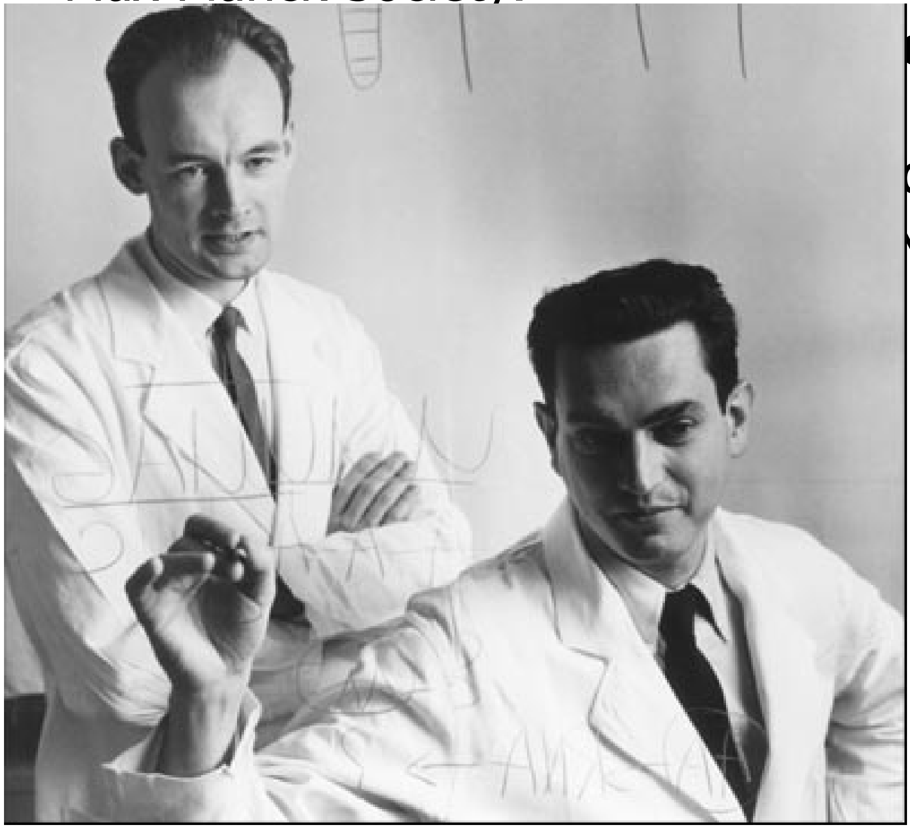
outline

- *Matthaei, Nirenberg and other Nobel prize winners*
- *Cell free protein synthesis*
- *DNA is not directly involved in protein synthesis*
- *RNA is directly involved in amino acid incorporation into proteins*

Matthaei & Nirenberg

Heinrich Matthaei

- A post doc fellow of Nirenberg
- Went to Germany and became a member of the Max Planck Society.



enberg

post doc at NIH, 1957

d on nucleic acids and protein synthesis

Nobel prize (medicine) at 1968

Nirenberg

Nirenberg – *"construct in the test tube a system which uses a nucleic acid as template for the formation of a protein… fed by the scientist with a sentence written in the alphabet of nucleic acids; the machine then translates the sentence into the protein alphabet"*

The Nobel Prize in Physiology or Medicine 1968

"for their interpretation of the genetic code and its function in protein synthesis"

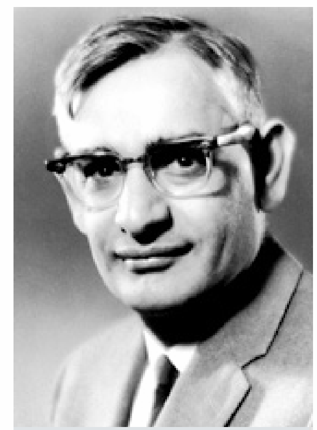
Robert W. Holley

1/3 of the prize

USA

Cornell University
Ithaca, NY, USA

b. 1922
d. 1993



Har Gobind Khorana

1/3 of the prize

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University of Wisconsin
Madison, WI, USA

b. 1922
(in Raipur, India)

Marshall W. Nirenberg

1/3 of the prize

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National Institutes of Health
Bethesda, MD, USA

b. 1927

Nirenberg

Khorana – "*Much of the final work was done by Khorana… he had systematically devised methods which led to the synthesis of well defined nucleic acids*"

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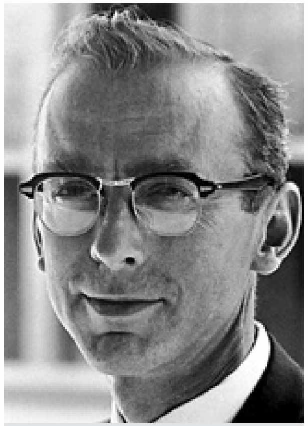

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Nirenberg

Holley – "*succeeded in preparing a transfer-RNA in pure form… established its exact chemical structure*"

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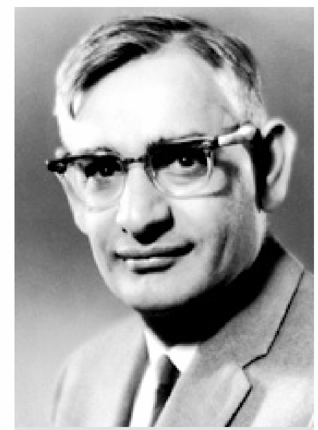

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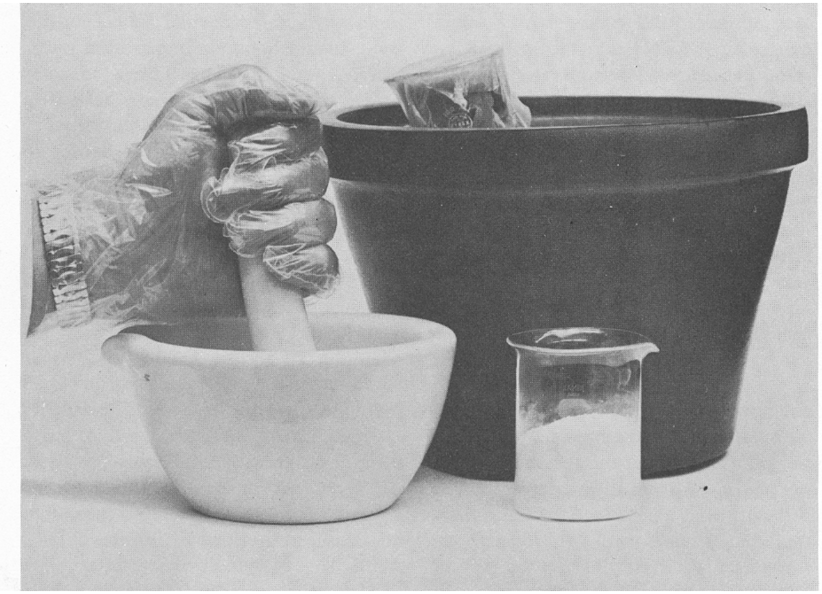
Matthaei & Nirenberg - 1961

Cell free system (1955)

- A mixture of cytoplasmic and nuclear components from cells.
- Used for in vitro protein synthesis, transcription, DNA replication.

Cell free system - mortar

- Homogenization - breaking apart cells releasing organelles and cytoplasm
  (a) A blender breaks the tissue.
  (b) The organelles are then generally separated using differential centrifugation.



EXPERIMENT BEGINS when cells of the colon bacillus are ground in a mortar with finely divided aluminum oxide. "Sap" released from ruptured cells still synthesizes protein.

Sap - sediment

Sap main components - DNA, mRNA, Ribosomes and Enzymes.

Armored chamber *Sedimenting material*

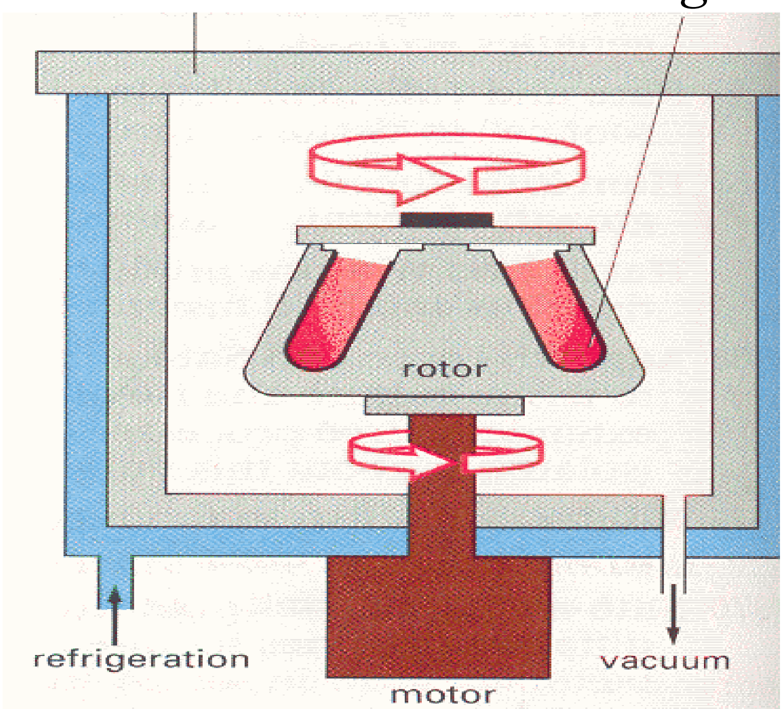
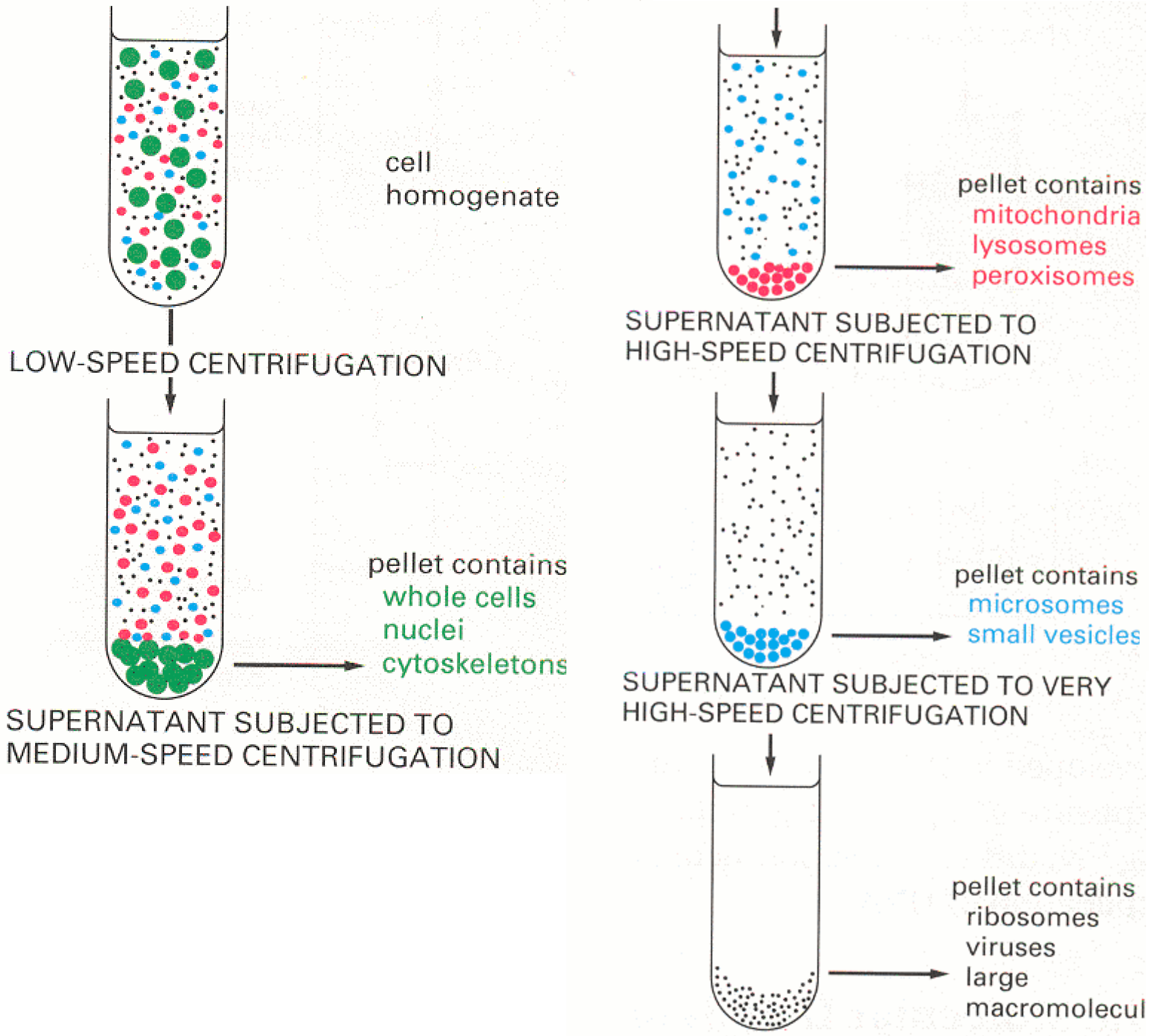


Figure 4–34 The preparative ultracentrifuge. The sample is contained in tubes that are inserted into a ring of cylindrical holes in a metal *rotor*. Rapid rotation of the rotor generates enormous centrifuga forces, which cause particles in the sample to sediment. The vacuum reduces friction, preventing heating of the rotor and allowing the refrigeration system to maintain the sample at 4°C.

Sap - separation



Cell fractionation by centrifugation. Repeated centrifugation at progressively higher speeds will fractionate homogenates of cells into their components. In general, the smaller the subcellular component, the greater is the centrifugal force required to sediment it.

Sap does not last long – 1961

A major difficulty in the study of cell-free protein synthesis in *E. coli* systems had been the necessity for preparing fresh enzyme extracts for each experiment. Techniques were not available for stabilization and storage of these enzyme extracts

MARSHALL W. NIRENBERG, J. HEINRICH MATTHAEI,[1]
OLIVER W. JONES, ROBERT G. MARTIN, AND
SAMUEL H. BARONDES
National Heart Institute, National Institutes of Health, Bethesda, Maryland

- *Needed to make fresh extracts for each experiment.*
- *Takes a long time !!*

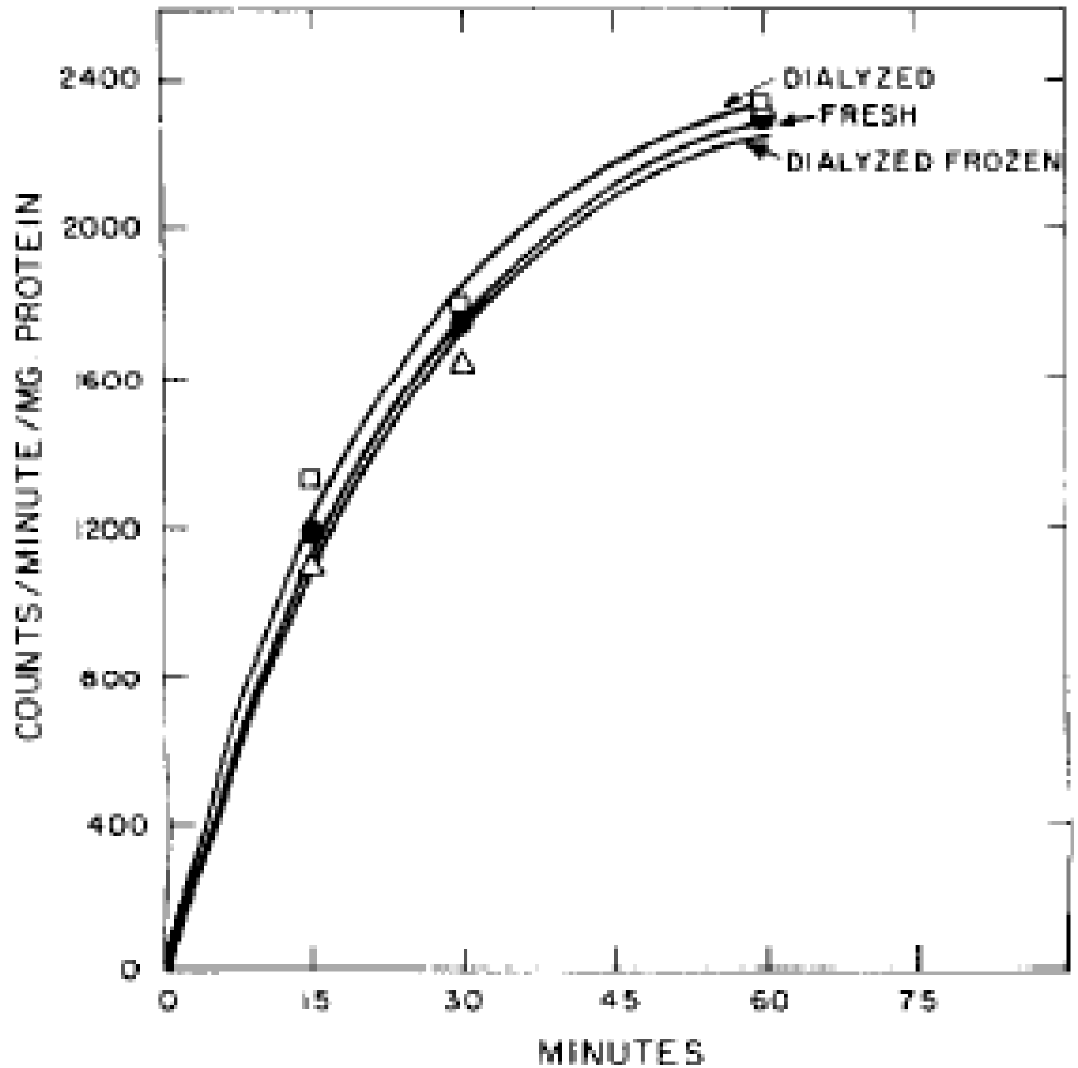
Sap stabilization & storage

Matthaei & Nirenberg - 1961

- *Prepared the sap (mortar)*
- *Separated components (Enzymes and ribosomes)*
- *Dialyzed with mercaptoethanol (antioxidant)*
- *Stored at -15^{0}c*

Sap activity graph

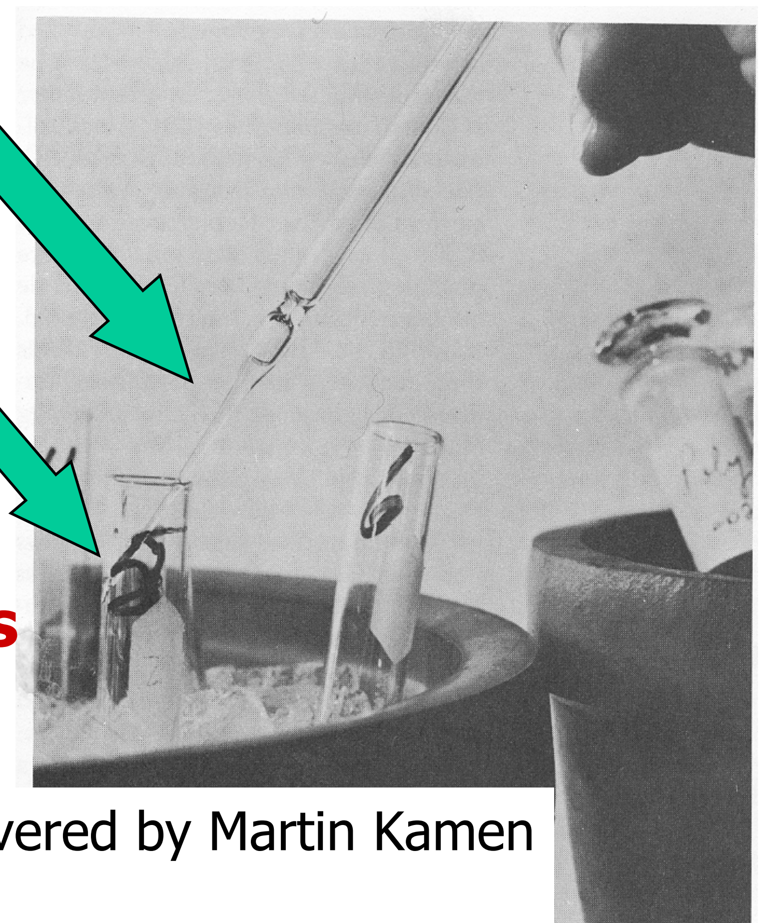
The effects of dialysis and freezing on amino acid incorporation into protein



Methods (stimulating)

add mRNA and amino acids

cell-free bacterial system



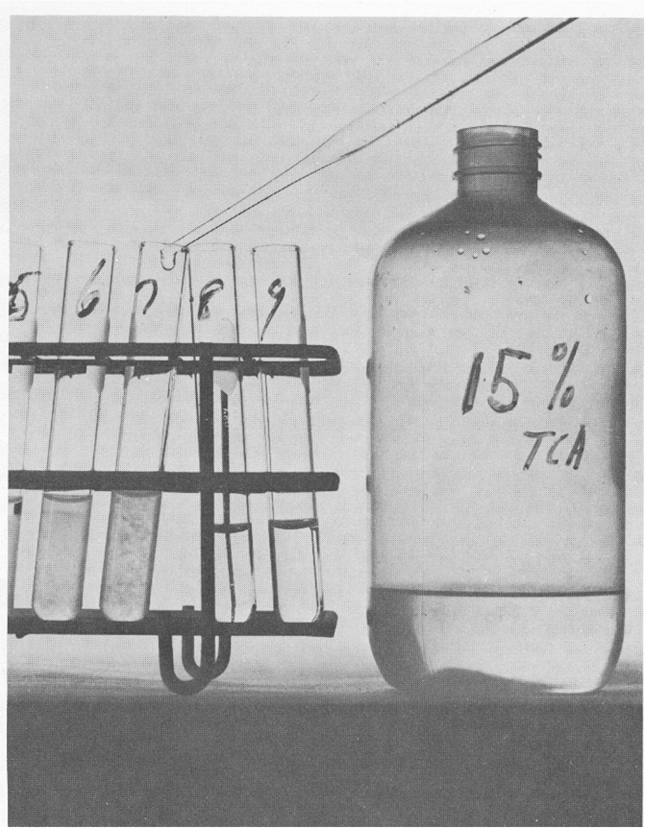
Stimulating protein synthesis

(one amino acid is **radioactive**)

Carbon-14- A radioactive isotope discovered by Martin Kamen and Sam Ruben (1940).

Methods (precipitate the protein)

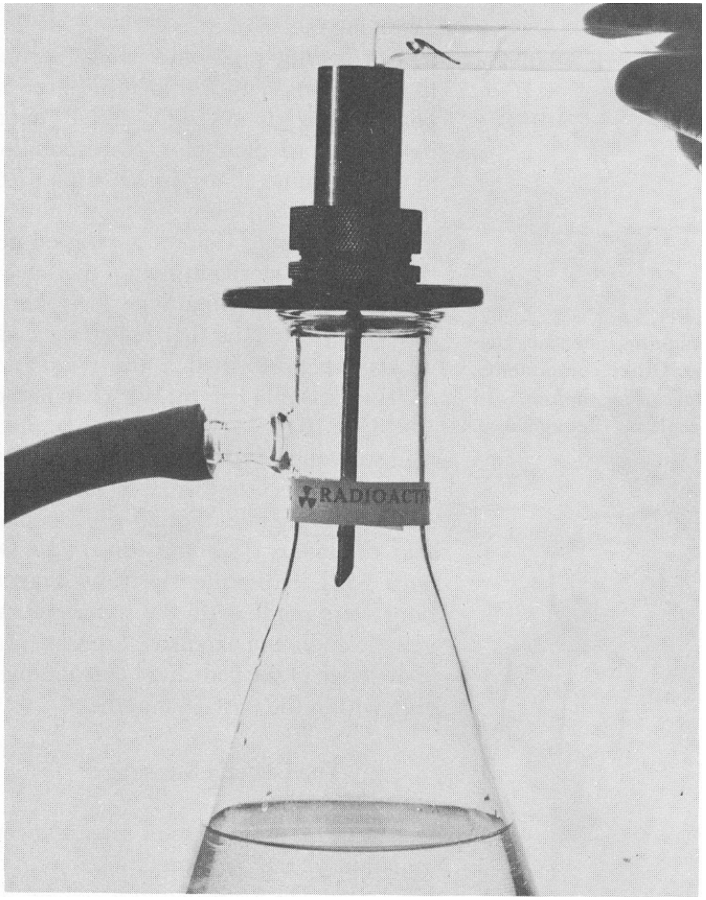
- Add trichloroactic acid (TCA).
  free amino acids cannot be precipitated with TCA while proteins can



Methods (filter)

- Solution is transferred through a filter paper disk, placed inside a carrier.

Proteins are left on the filter paper

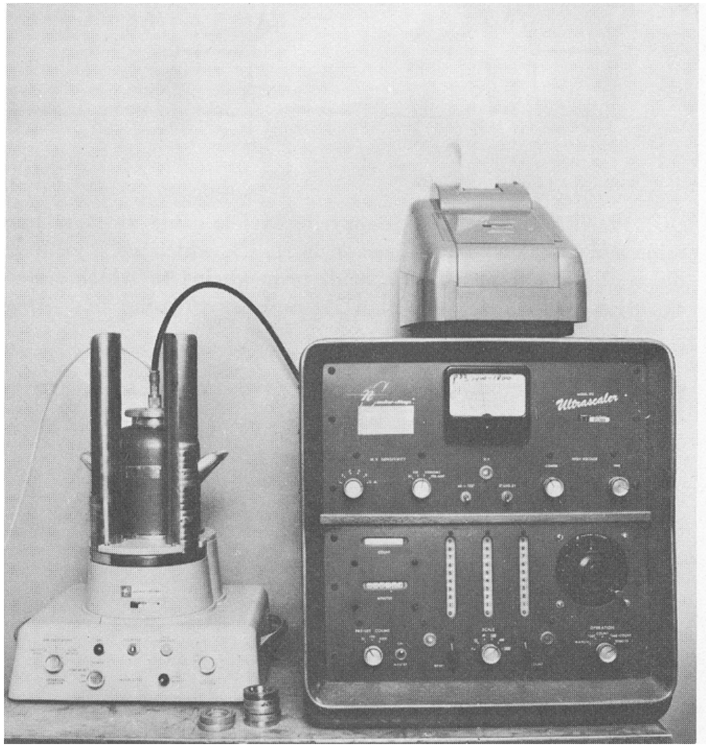


Methods (counting)

- Carrier is stacked in a radiation counting unit,

  indicating how well a given sample of mRNA has directed amino acids into protein.

Counts / minute / mg protein



Understanding graph

m - material for protein incorporation

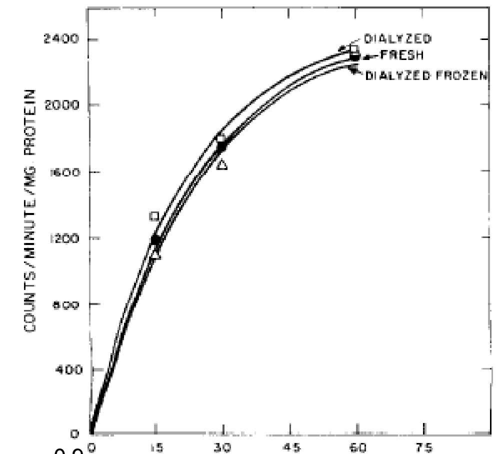
λ - Rate on incorporation

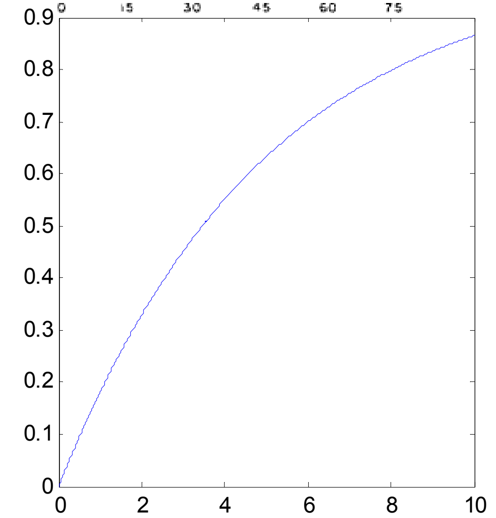
$$\frac{dm}{dt} = -\lambda m \Rightarrow m(t) = m_0 e^{-\lambda t}$$

p - Protein concentration

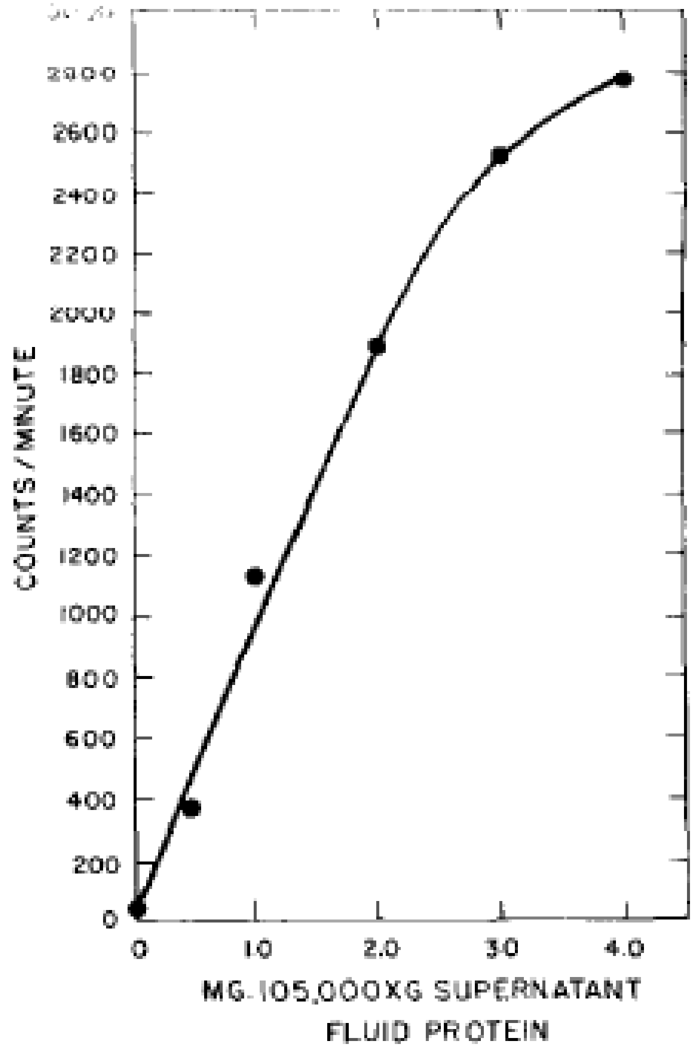
$$p(t) \propto \int_0^t m(t)\,dt \Rightarrow$$

$$p(t) \propto m_0 \left(1 - e^{-\lambda t}\right)$$





Incorporation dependence on sap

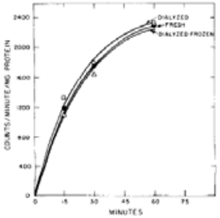


The dependence of amino acid incorporation into protein on the supernatant solution

(after 60 minutes of incubation)

Outline – reaching DNA

– *Matthaei, Nirenberg and other Nobel prize winners*

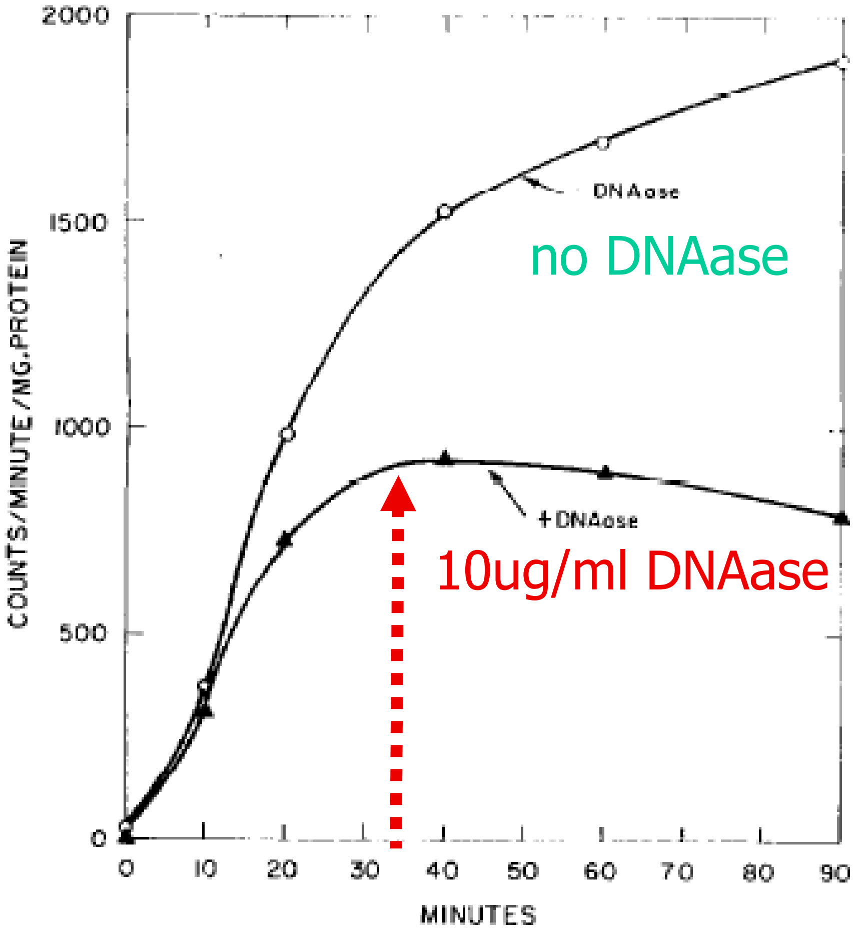


– *Cell free protein synthesis*

– *DNA is not directly involved in protein synthesis*

– *RNA is directly involved in amino acid incorporation into proteins*

DNAase effect



The effect of DNAase upon amino acid incorporation into protein

without DNA and after ~30min incorporation into protein ceases

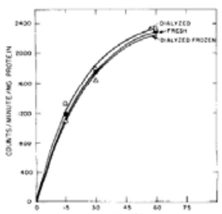
- Contamination by RNAase?
- RNAase effect on incorporation?
- 0.001ug/ml RNAase depresses incorporation !!!
- DNAase had 0.0001ug/ml RNAase (has no effect on incorporation)

Experiment conclusion

- It is not possible to say whether intact, DNA is necessary
- One possibility… the initial rate of amino acid incorporation is primarily due to the completion of partially finished peptides linked to RNA templates.
- Inhibition by DNAase …may be due to the destruction of DNA and its resultant inability to serve as templates for the synthesis of template RNA.
- If template RNA were used only once, amino acid incorporation would cease as soon as the peptide chains were finished.
- Other explanations, however, are fully plausible, and it is not possible at this state to rule out alternative interpretations.

Outline – addressing RNA

– *Matthaei, Nirenberg and other Nobel prize winners*



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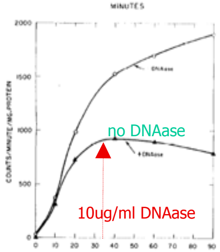


– *DNA is not directly involved in protein synthesis*

– *RNA is directly involved in amino acid incorporation into proteins*

DNAase & RNAase

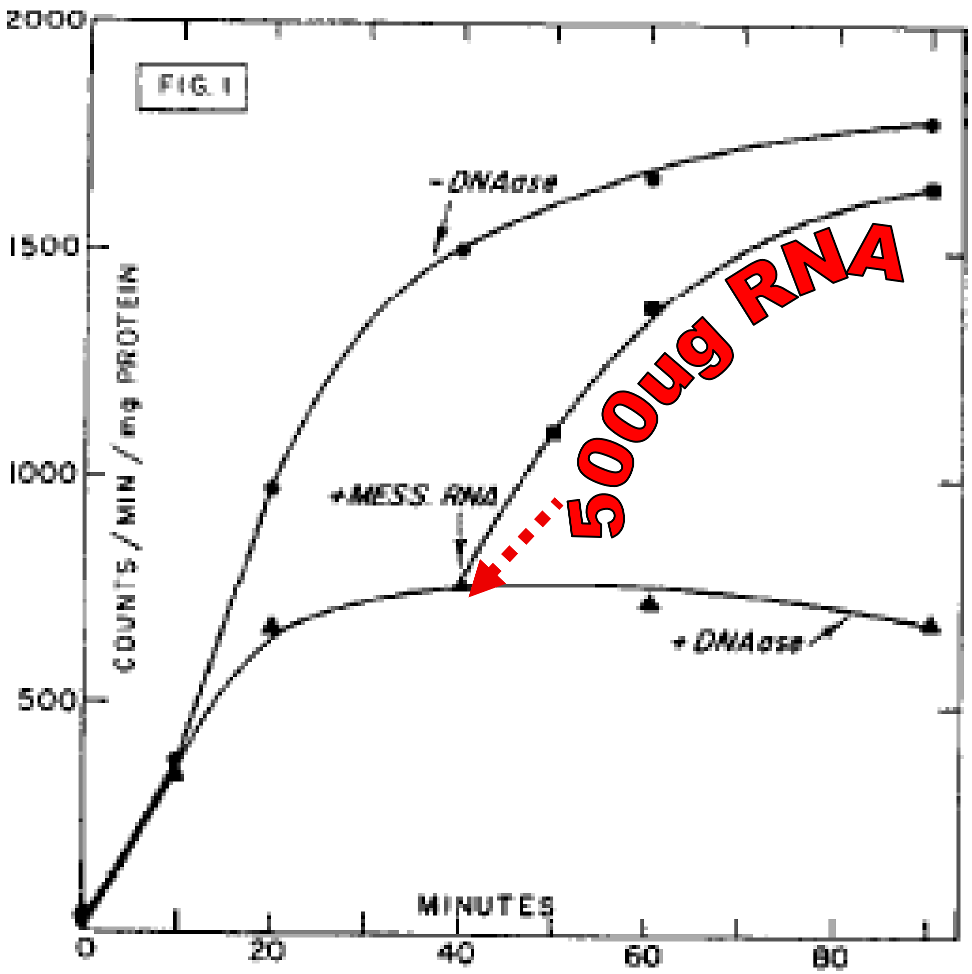


FIG. 1. Cell-free *E. coli* assay for messenger RNA: ● Minus deoxyribonuclease, ▲ 10 μg deoxyribonuclease, ■ 500 μg of crude messenger RNA prepared from yeast ribosomes added at indicated time.

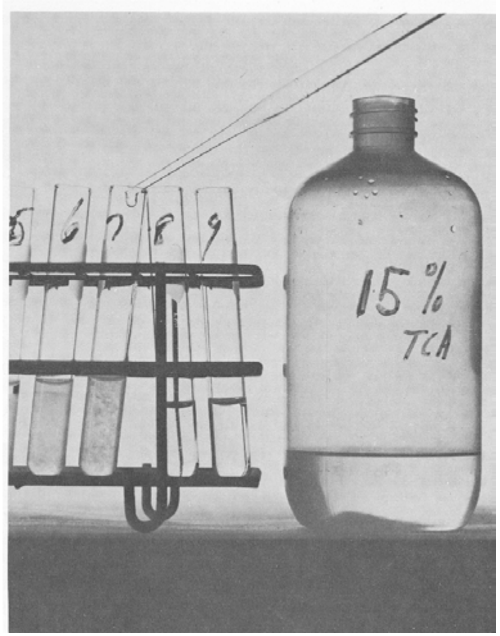
Step by step advancement

- Cell free system (1955)
  -Added mercaptoethanol and cooled to -150c
- DNA is involved but not directly
- **RNA is directly involved**
- Synthetic Polyuridylic acid (polyuncleotide phosphtylase) discovered by Grunberg-Manago and Ochoa (1955).

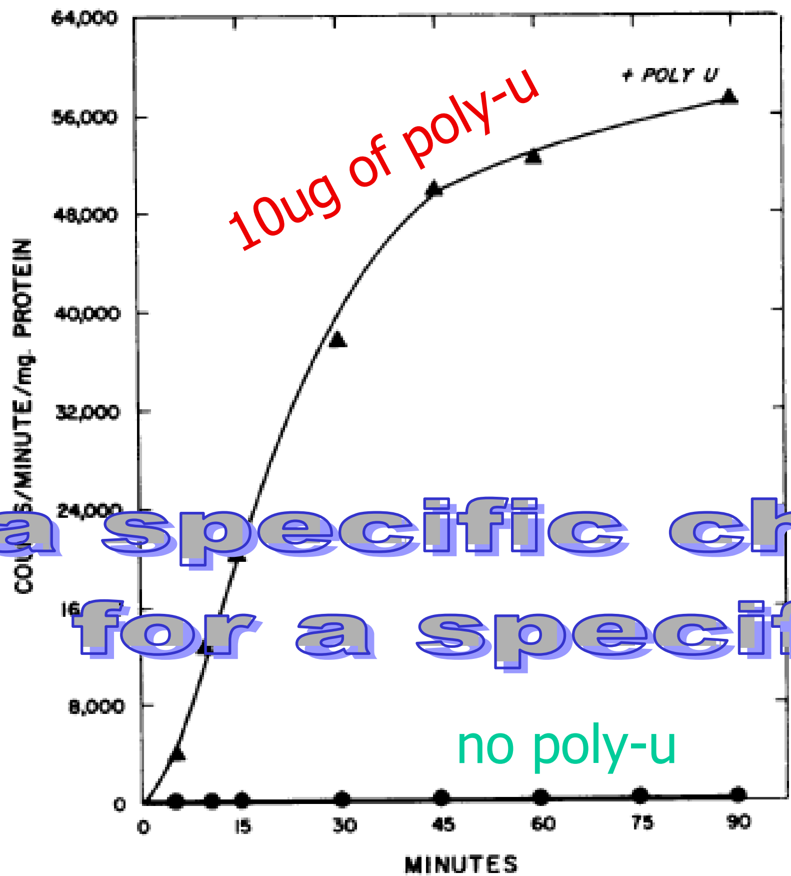
  - Matthaei and Nirrenberg used this chain as mRNA

Poly-U effect - method

- 20 cell free system tubes, filled with 20 amino acids.
- In each tube, one amino acid is radioactive
- Poly-U is added to all the tubes.
- Check in which tube the proteins are radioactive



Polyuridylic acid as mRNA



Polyuridylic acid incorporates ***Phenylalanine*** *into protein*

a specific character codes for a specific amino acid

FIG. 6.—Stimulation of U-C[14]-L-phenylalanine incorporation by polyuridylic acid. ● without polyuridylic acid; ▲ 10 μg polyuridylic acid added.

Experiment conclusion

- We have demonstrated that template RNA is a requirement for cell-free amino acid incorporation.

- The function of ribosomal RNA remains an enigma, although at least part of the total RNA is thought to serve as templates for protein synthesis and has been termed **"messenger" RNA**. Alternatively, a part of the RNA may be essential for the synthesis of active ribosomes from smaller ribosomal particles.

- Polyuridylic acid contains the information for the synthesis of a protein having many of the characteristics of poly-L-phenylalanine.

Code map - 1963

AMINO ACID	RNA CODE WORDS			
ALANINE	CCG	UCG■		
ARGININE	CGC	AGA	UCG■	
ASPARAGINE	ACA	AUA		
ASPARTIC ACID	GUA			
CYSTEINE	UUG△			
GLUTAMIC ACID	GAA	AGU■		
GLUTAMINE	ACA	AGA	AGU■	
GLYCINE	UGG	AGG		
HISTIDINE	ACC			
ISOLEUCINE	UAU	UAA		
LEUCINE	UUG	UUC	UUA	UUU□
LYSINE	AAA	AAG●	AAU●	
METHIONINE	UGA■			
PHENYLALANINE	UUU			
PROLINE	CCC	CCU▲	CCA▲	CCG▲
SERINE	UCU	UCC	UCG	
THREONINE	CAC	CAA		
TRYPTOPHAN	GGU			
TYROSINE	AUU			
VALINE	UGU			

△ UNCERTAIN WHETHER CODE IS UUG OR GGU

■ NEED FOR U UNCERTAIN

□ CODES PREFERENTIALLY FOR PHENYLALANINE

● NEED FOR G AND U UNCERTAIN

▲ NEED FOR U A,G UNCERTAIN

Code map

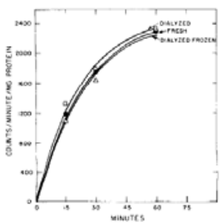
http://www.geneticengineering.org/chemis/Chemis-NucleicAcid/Graphics/Code.gif

The Genetic Code

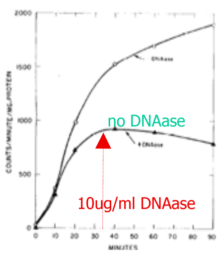
	U	C	A	G	
U	UUU Phenyl alanine	UCU Serine	UAU Tyrosine	UGU Cysteine	U
	UUC	UCC	UAC	UGC	C
	UUG Leucine	UCA	UAA Stop	UGA Stop	A
	UUA	UCG	UAG	UGG Tryptophan	G
C	CUU Leucine	CCU Proline	CAU Histidine	CGU Arginine	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Glutamine	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Isoleucine	ACU Threonine	AAU Asparagine	AGU Serine	U
	AUC	ACC	AAC	AGC	C
	AUA	ACA	AAA Lysine	AGA Arginine	A
	AUG Methionine	ACG	AAG	AGG	G
G	GUU Valine	GCU Alanine	GAU Aspartic acid	GGU Glycine	U
	GUC	GCC	GAC	GGC	C
	GUA	GCA	GAA Glutamic acid	GGA	A
	GUG	GCG	GAG	GGG	G

Summary (a)

– *Matthaei, Nirenberg and other Nobel prize winners*



– *Cell free protein synthesis*



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– *RNA is directly involved in amino acid incorporation into proteins*

Overlapping code?

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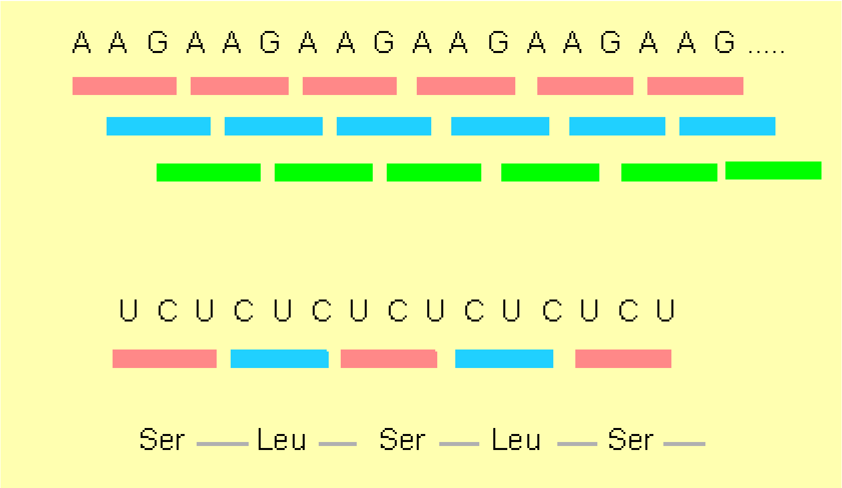
GCA	GCG	GCC	GCU
GUA	GUG	GUC	GUU
CAA	CAG	CAC	CAU
CGA	CGG	CGC	CGU
CCA	CCG	CCC	CCU
CUA	CUG	CUC	CUU
UAA	UAG	UAC	UAU
UGA	UGG	UGC	UGU
UCA	UCG	UCC	UCU
UUA	UUG	UUC	UUU

Is the code overlapping?

(e.g. UGUA… results in UGU, GUA, UA_...)

Overlapping code?



- AAA can be followed by AAX (only 4 options)
- AAA can be followed by AXY (16 options)

S. Brenner (Cambridge, 1957)

Code mutations

- Use code mistakes (mutations) to understand the code.
- Nitrous acid induces C→U or A→G transformation.

- Tobacco Mosaic Virus.
- Has 158 amino acid (Schramn *et al. 1959*)

TMV experiment

- Wittman, Tsugita and Fraenkel-Conrat
  - produced a large number of nitrite-induced mutants.
  - isolated single ones
  - analyzed the amino acid sequences of their proteins.

- Neighboring amino acids did not change !

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Summary

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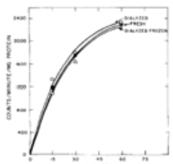

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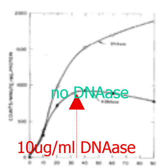

– *DNA is not directly involved in protein synthesis*

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phenilalanine

– *RNA is directly involved in amino acid incorporation into proteins*

TMV

– *No code ovelaping*

Bibliography

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