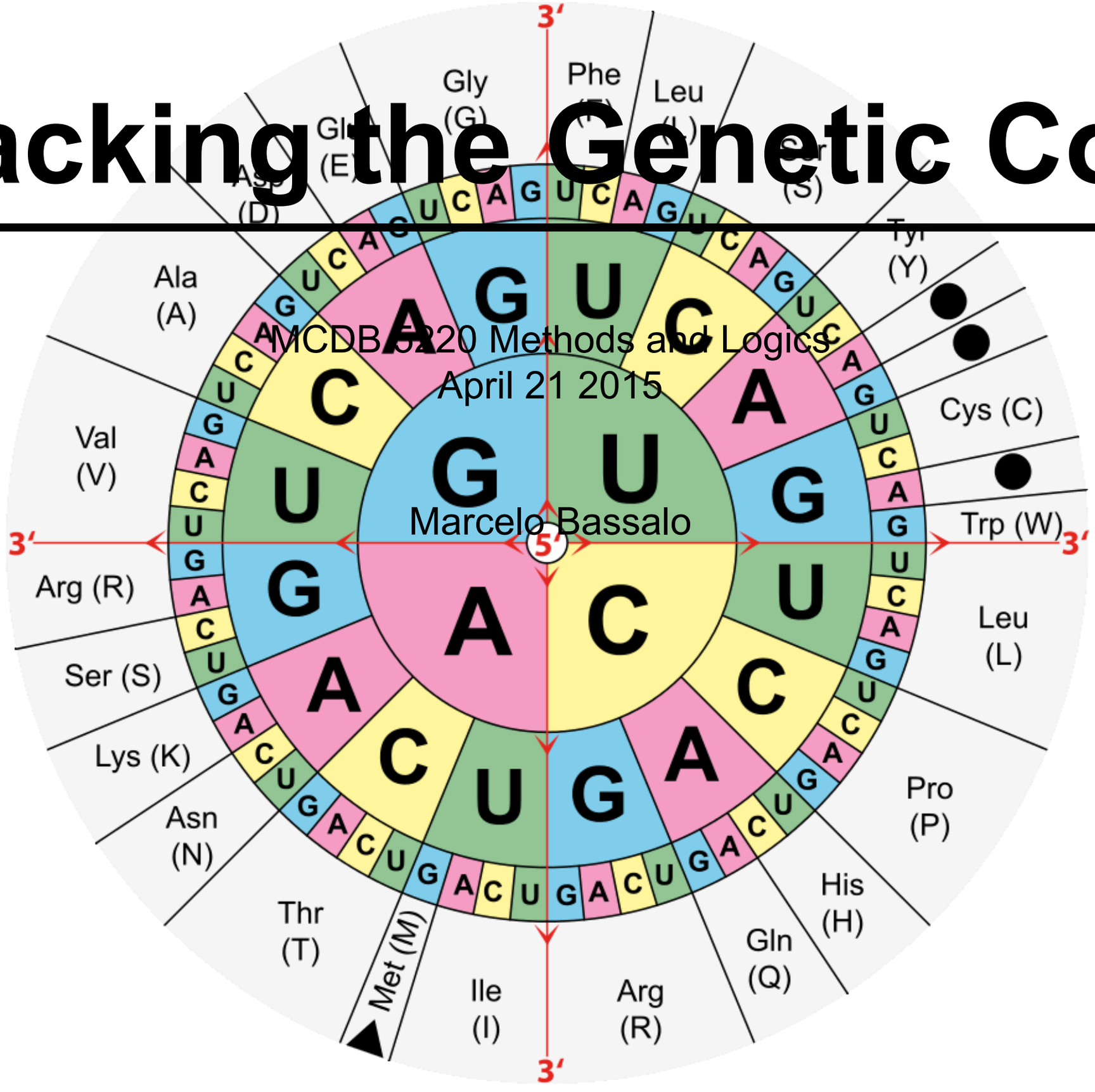
Cracking the Genetic Code



MCDB 5220 Methods and Logics
April 21 2015

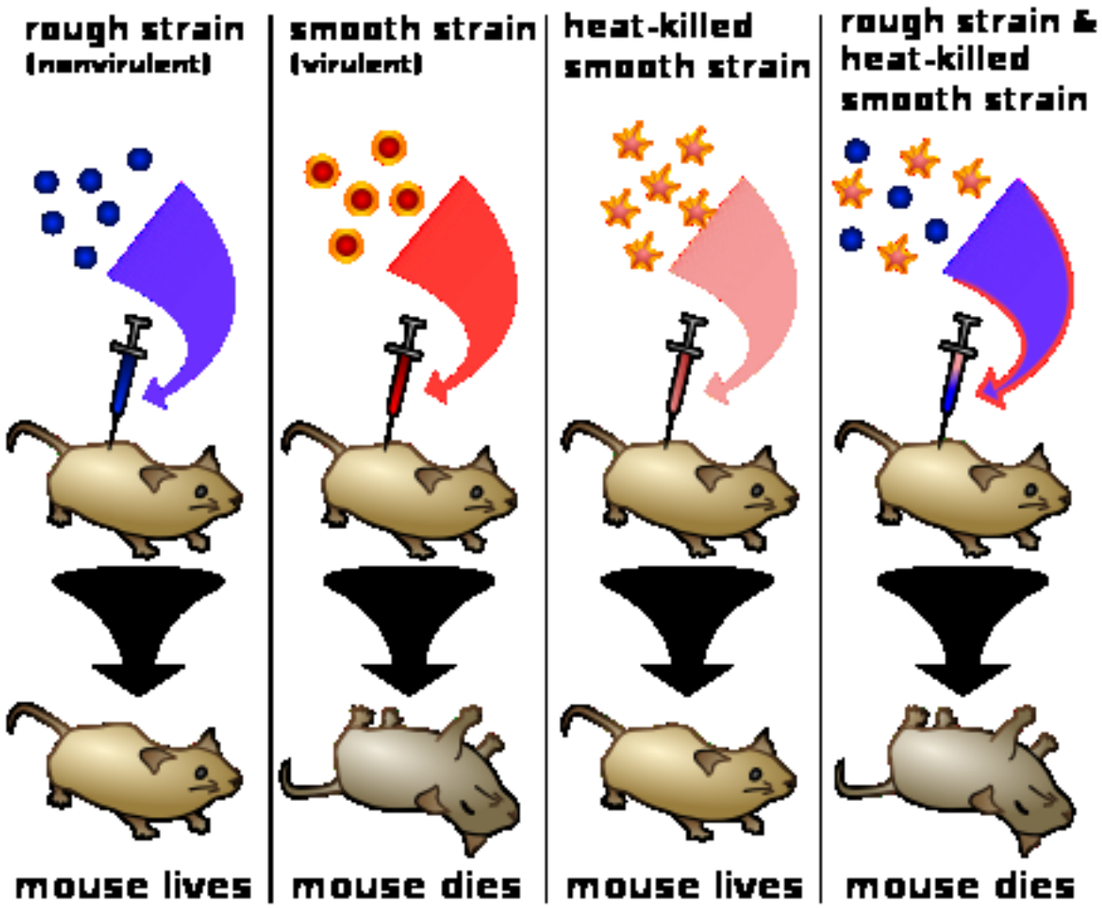
Marcelo Bassalo

The DNA Saga… so far

Important contributions for cracking the genetic code:

- The “transforming principle” (1928)

Frederick Griffith



The DNA Saga… so far

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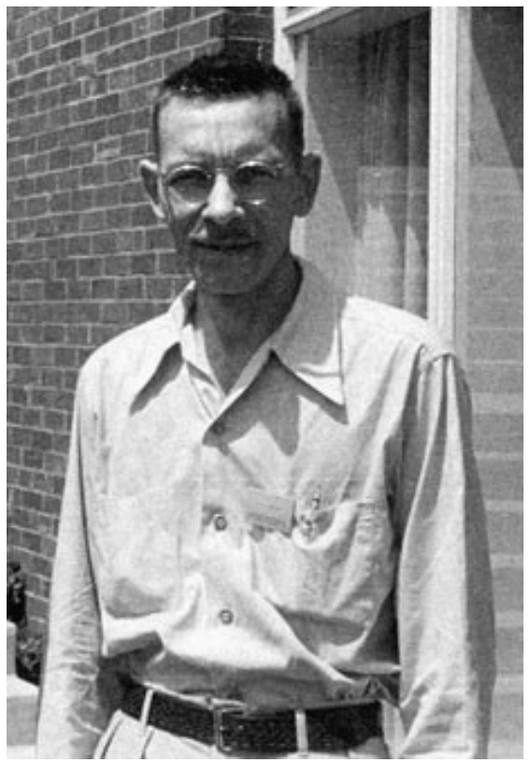
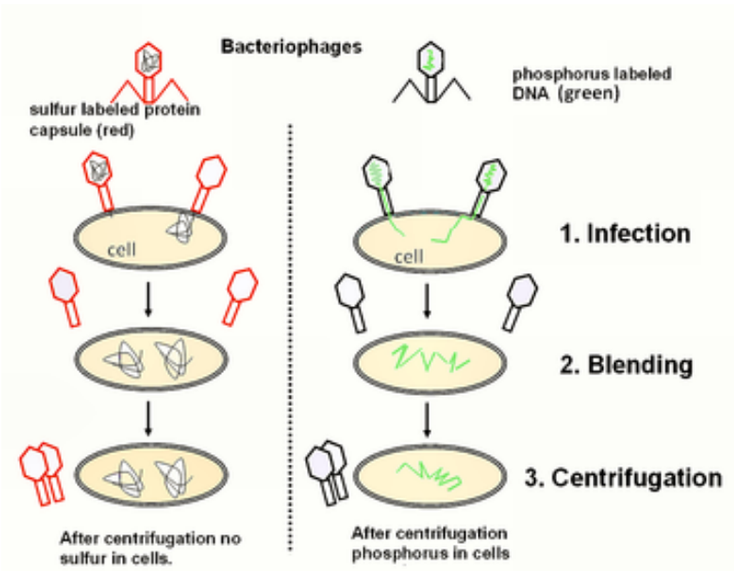
- The nature of the transforming principle: DNA (1944 - 1952)

Oswald Avery



Alfred Hershey

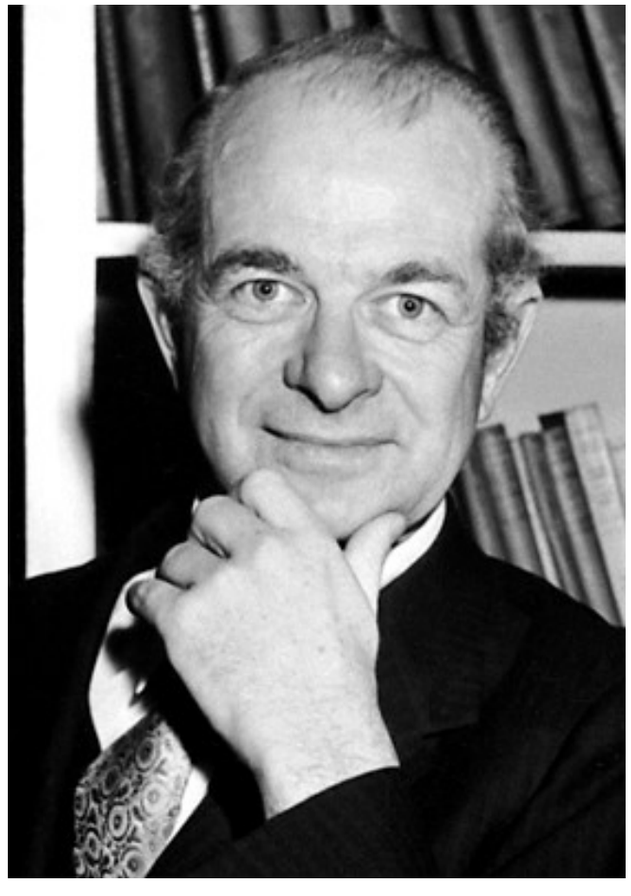
Martha Chase



The DNA Saga… so far

Important contributions for cracking the genetic code:

- The “transforming principle” (1928)
- The nature of the transforming principle: DNA (1944 - 1952)
- **X-ray diffraction and the structure of proteins (1951)**



Linus Carl Pauling

The DNA Saga… so far

Important contributions for cracking the genetic code:

- The “transforming principle” (1928)
- The nature of the transforming principle: DNA (1944 - 1952)
- **X-ray diffraction and the structure of proteins (1951)**
- The structure of DNA (1953)

James Watson and Francis Crick

The DNA Saga… so far

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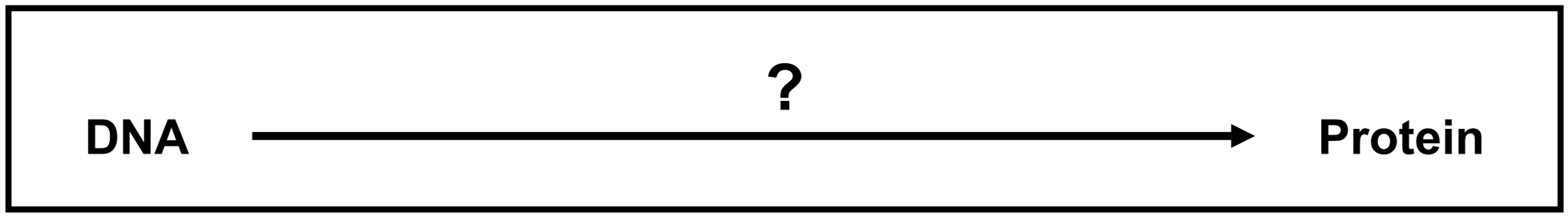
- The “transforming principle” (1928)
- The nature of the transforming principle: DNA (1944 - 1952)
- X-ray diffraction and **the structure of proteins (1951)**
- **The structure of DNA (1953)**

How is DNA (4 nucleotides) the genetic material while proteins (20 amino acids) are the building blocks?

DNA —**?**→ **Protein**

The Coding Craze



What was already known?

- DNA is not the carrier
- DNA resides inside the nucleus
- Protein synthesis occur in the cytoplasm through ribosomes
- Only RNA is associated with ribosomes (no DNA)

- rRNA is not the carrier
- Ribosomal RNA (rRNA) was a homogeneous population

François Jacob

The “messenger RNA” hypothesis

Jacques Monod

The Coding Craze

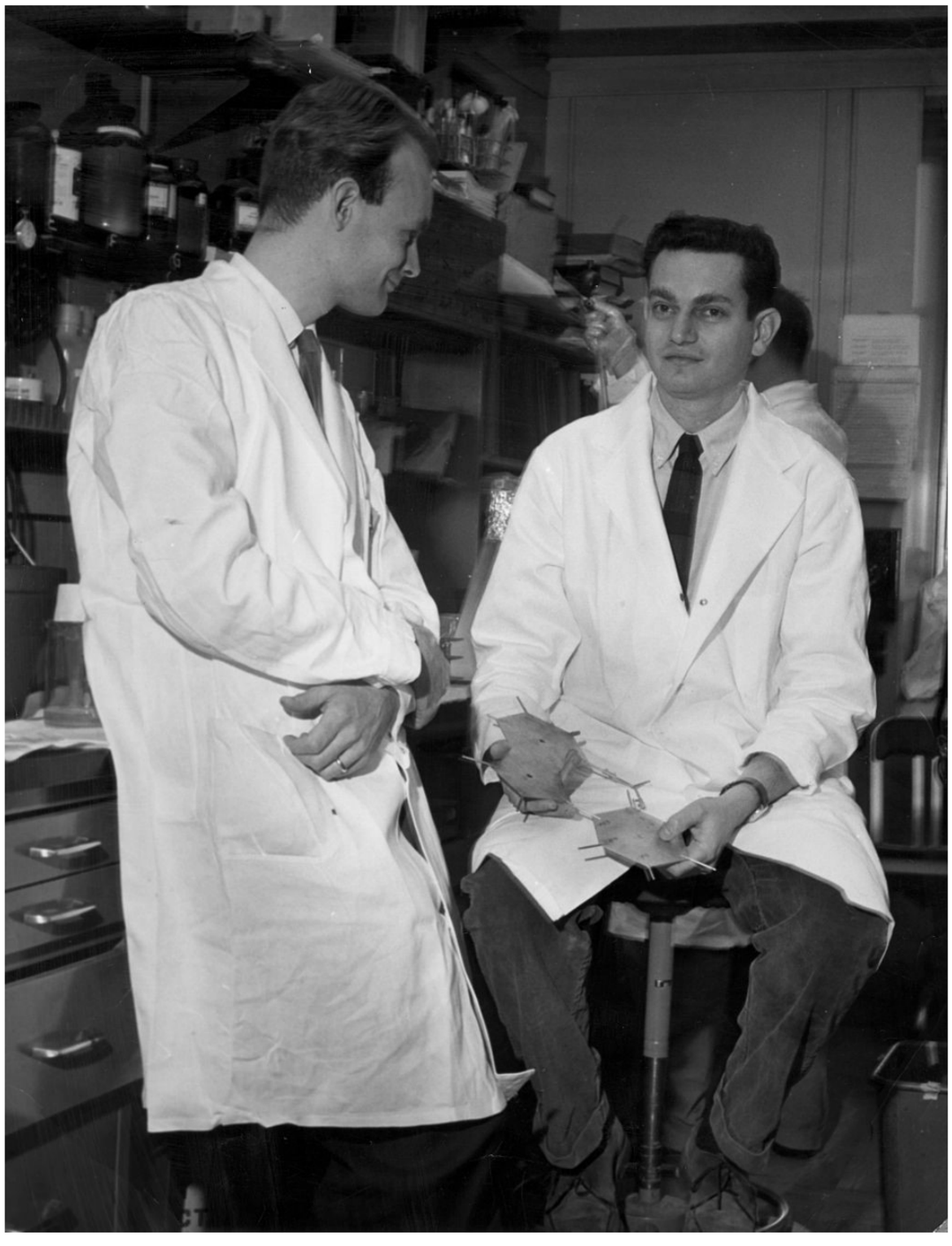
RNA Tie Club

Member	Training	RNA Tie Club Designation
George Gamow	Physicist	ALA
Alexander Rich	Biochemist	ARG
Paul Doty	Physical Chemist	ASP
Robert Ledley	Mathematical Biophysicist	ASN
Martynas Ycas	Biochemist	CYS
Robley Williams	Electron Microscopist	GLU
Alexander Dounce	Biochemist	GLN
Richard Feynman	Theoretical Physicist	GLY
Melvin Calvin	Chemist	HIS
Norman Simons	Biochemist	ISO
Edward Teller	Physicist	LEU
Erwin Chargaff	Biochemist	LYS
Nicholas Metropolis	Physicist, Mathematician	MET
Gunther Stent	Physical Chemist	PHE
James Watson	Biologist	PRO
Harold Gordon	Biologist	SER
Leslie Orgel	Theoretical Chemist	THR
Max Delbrück	Theoretical Physicist	TRY
Francis Crick	Biologist	TYR
Sydney Brenner	Biologist	VAL

Table from Wikipedia

The Coding Craze

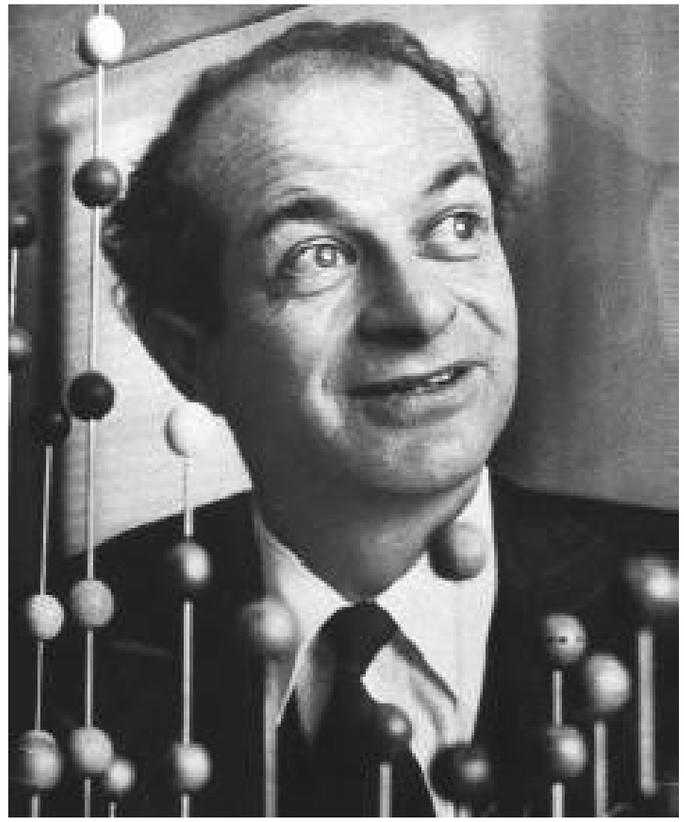
Who won the race



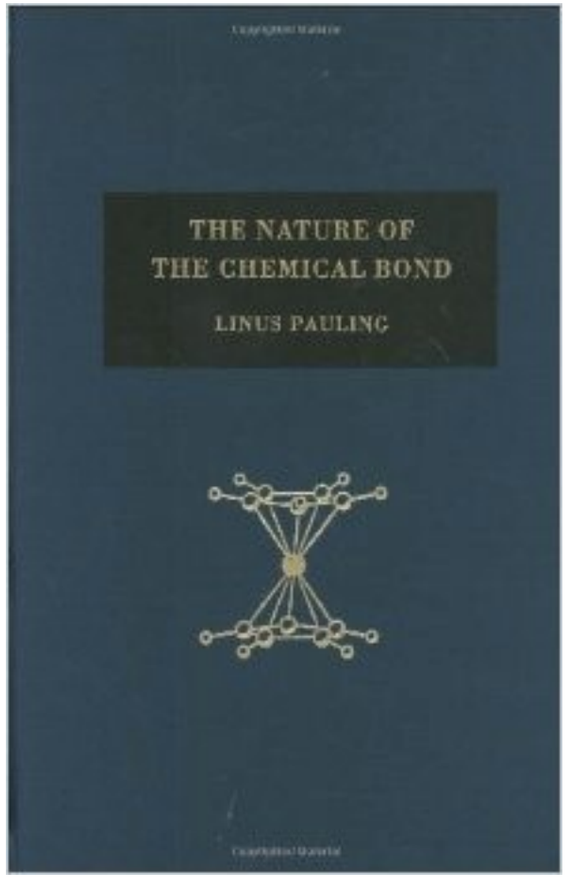
Marshall Nirenberg
J. Heinrich Matthaei

Linus Carl Pauling

- 1901-1994
- Born in Portland, Oregon
- 1922: B.S. Chemical Engineering from Oregon State University
- 1925: PhD Physical Chemistry from Caltech
- Founder of quantum chemistry

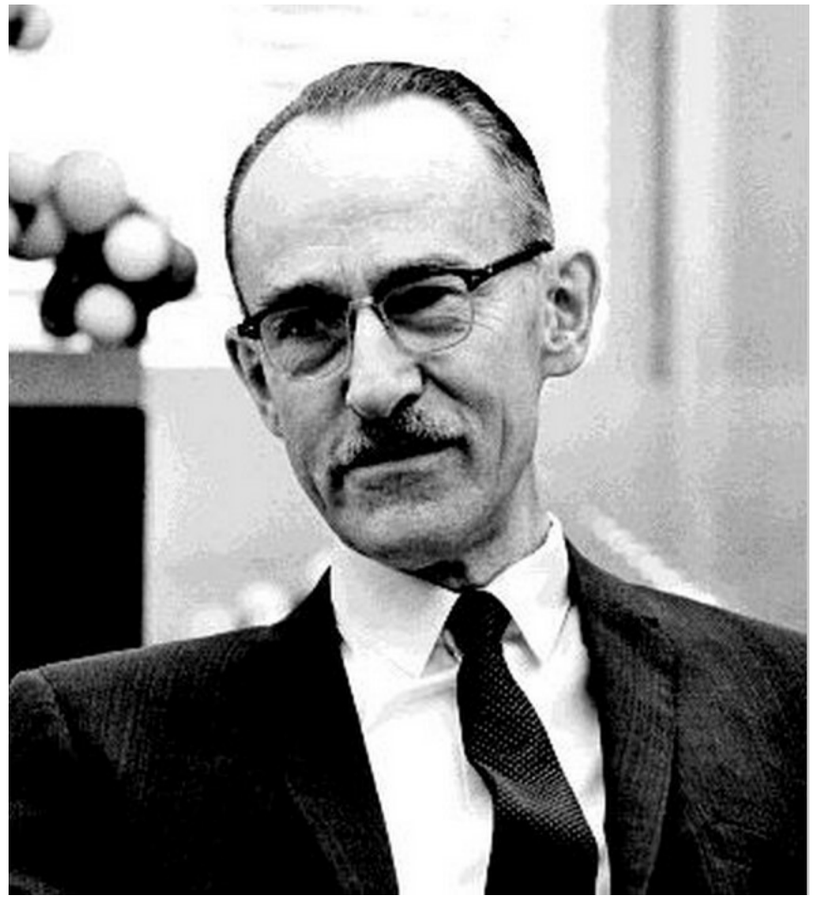


- X-ray diffraction structures
- Nature of the chemical bond — Nobel Prize in Chemistry (1954)
- Peace activist — Nobel Peace Prize (1962)

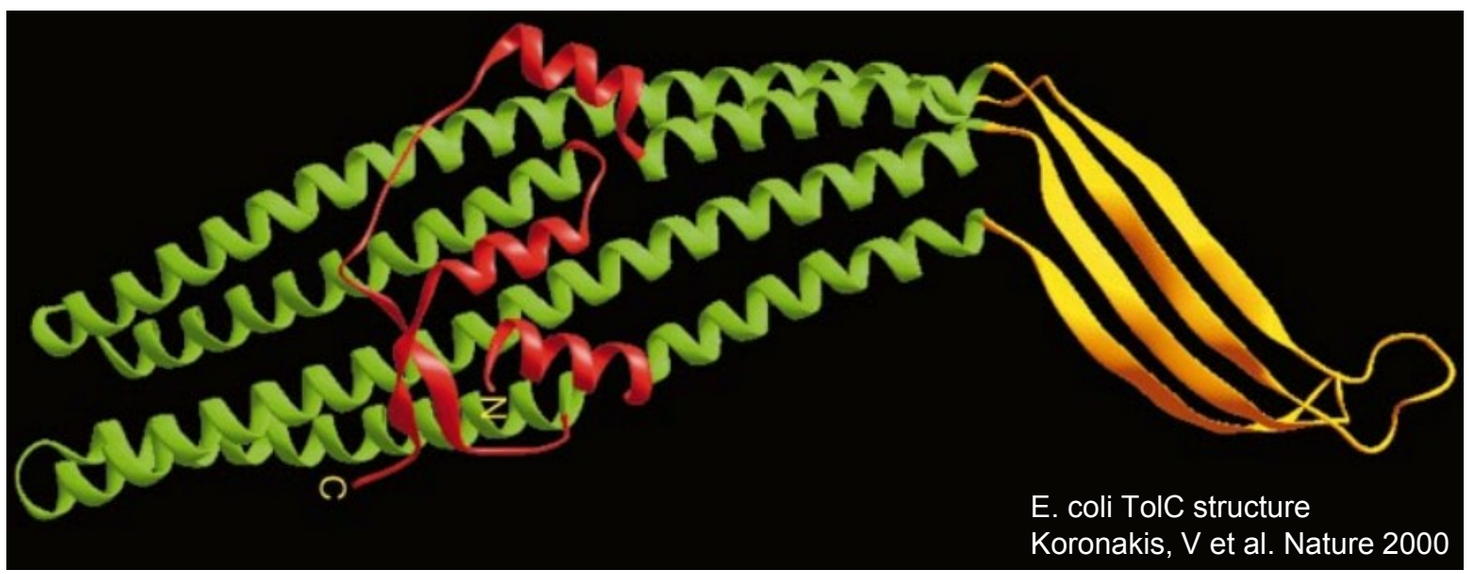


Protein Structure

Hershey Branson



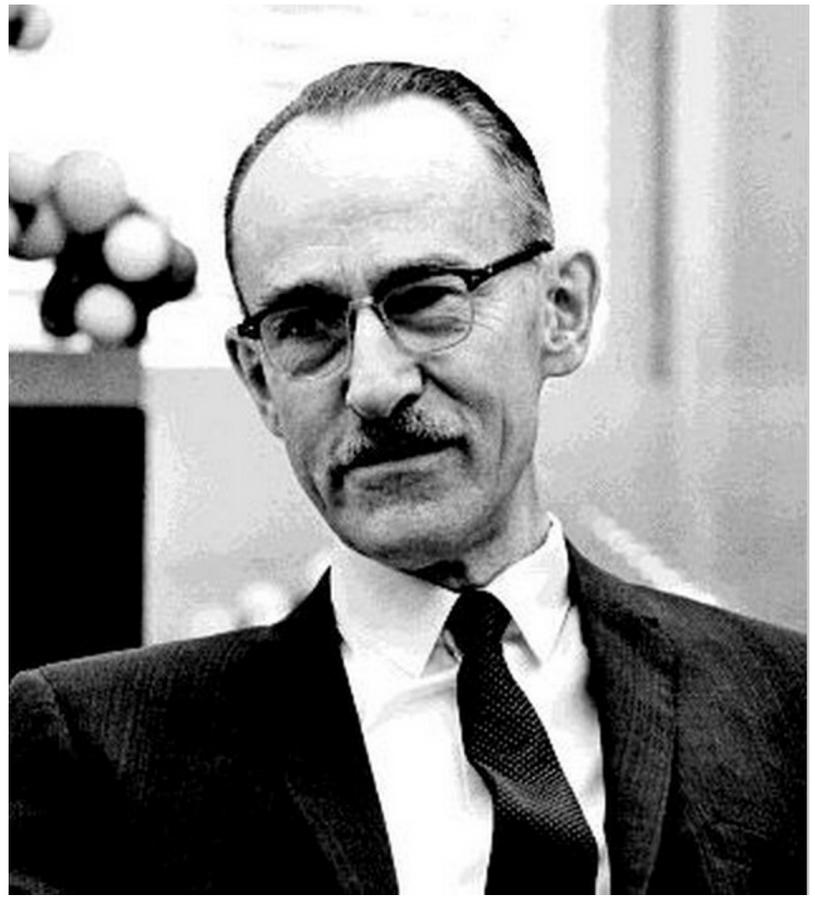
Robert Corey



E. coli TolC structure
Koronakis, V et al. Nature 2000

Protein Structure

Hershey Branson



Robert Corey

PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

May, 1951 Issue

Protein Structure

THE STRUCTURE OF SYNTHETIC POLYPEPTIDES

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology, Pasadena, California

Communicated March 31, 1951

THE PLEATED SHEET, A NEW LAYER CONFIGURATION OF POLYPEPTIDE CHAINS

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology. Pasadena. California

THE STRUCTURE OF FEATHER RACHIS KERATIN

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology, Pasadena, California

Communicated March 31, 1951

Protein Structure

Atomic Coordinates and Structure Factors for Two Helical Configurations of Polypeptide Chains
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 235-240
Full Text (PDF)

The Structure of Synthetic Polypeptides
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 241-250
Full Text (PDF)

The Pleated Sheet, A New Layer Configuration of Polypeptide Chains
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 251-256
Full Text (PDF)

The Structure of Feather Rachis Keratin
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 256-261
Full Text (PDF)

The Structure of Hair, Muscle, and Related Proteins
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 261-271
Full Text (PDF)

The Structure of Fibrous Proteins of the Collagen-Gelatin Group
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 272-281
Full Text (PDF)

The Polypeptide-Chain Configuration in Hbmoglobin and Other Globular Proteins
Linus Pauling and Robert B. Corey
PNAS 1951 37 (5) 282-285
Full Text (PDF)

Protein Structure

THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN

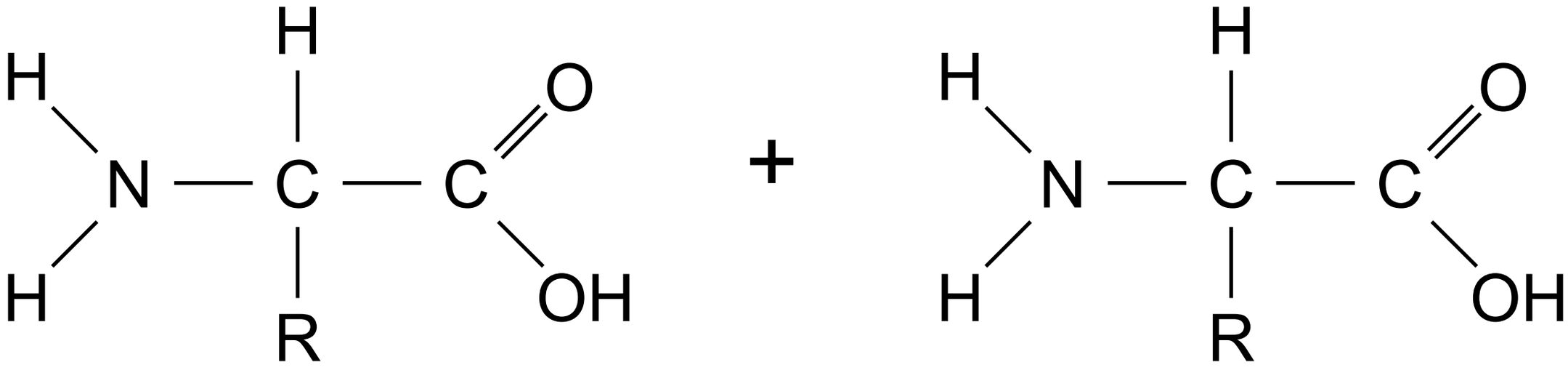
By Linus Pauling, Robert B. Corey, and H. R. Branson*

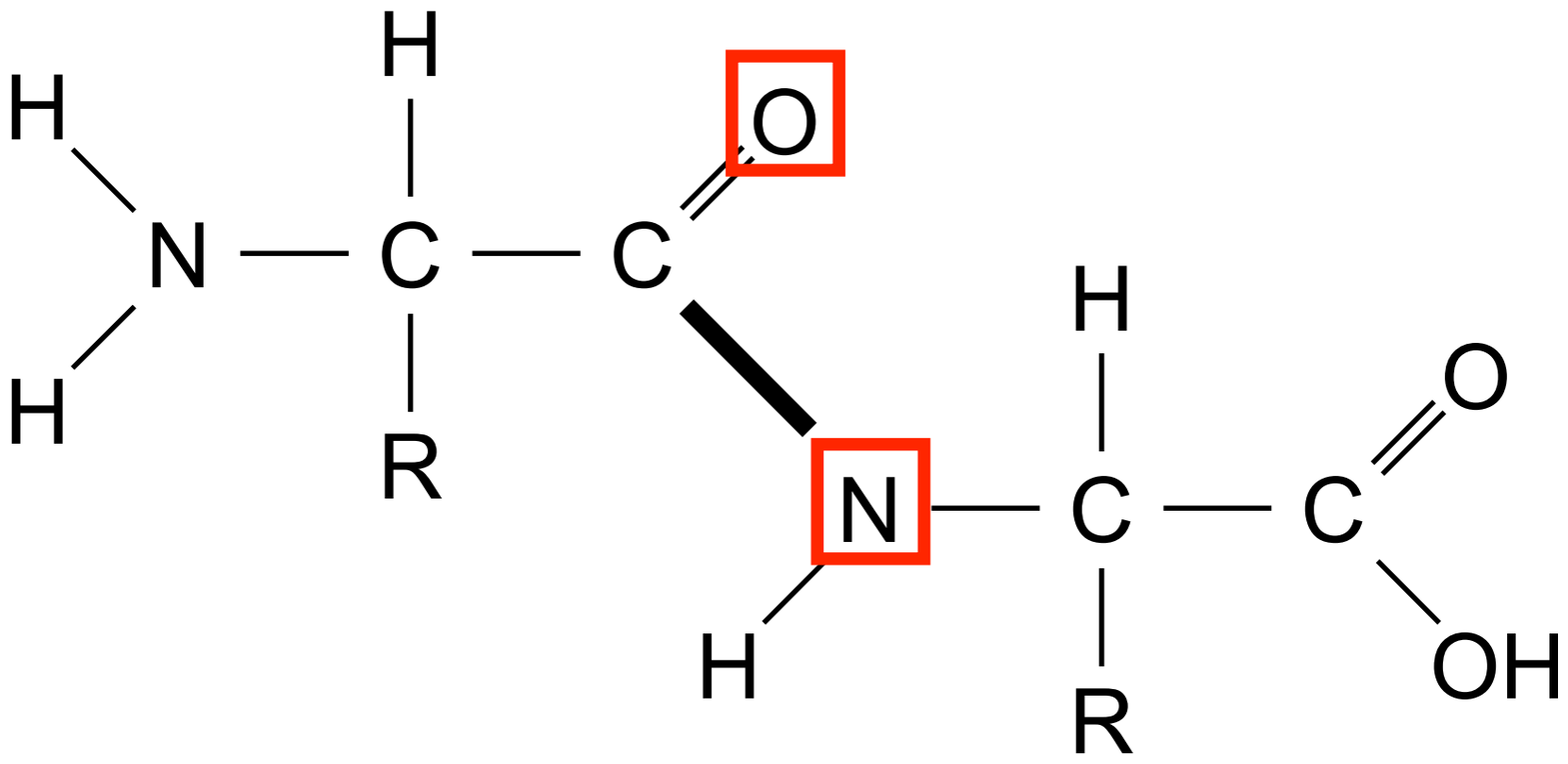
Gates and Crellin Laboratories of Chemistry,
California Institute of Technology, Pasadena, California†

Communicated February 28, 1951

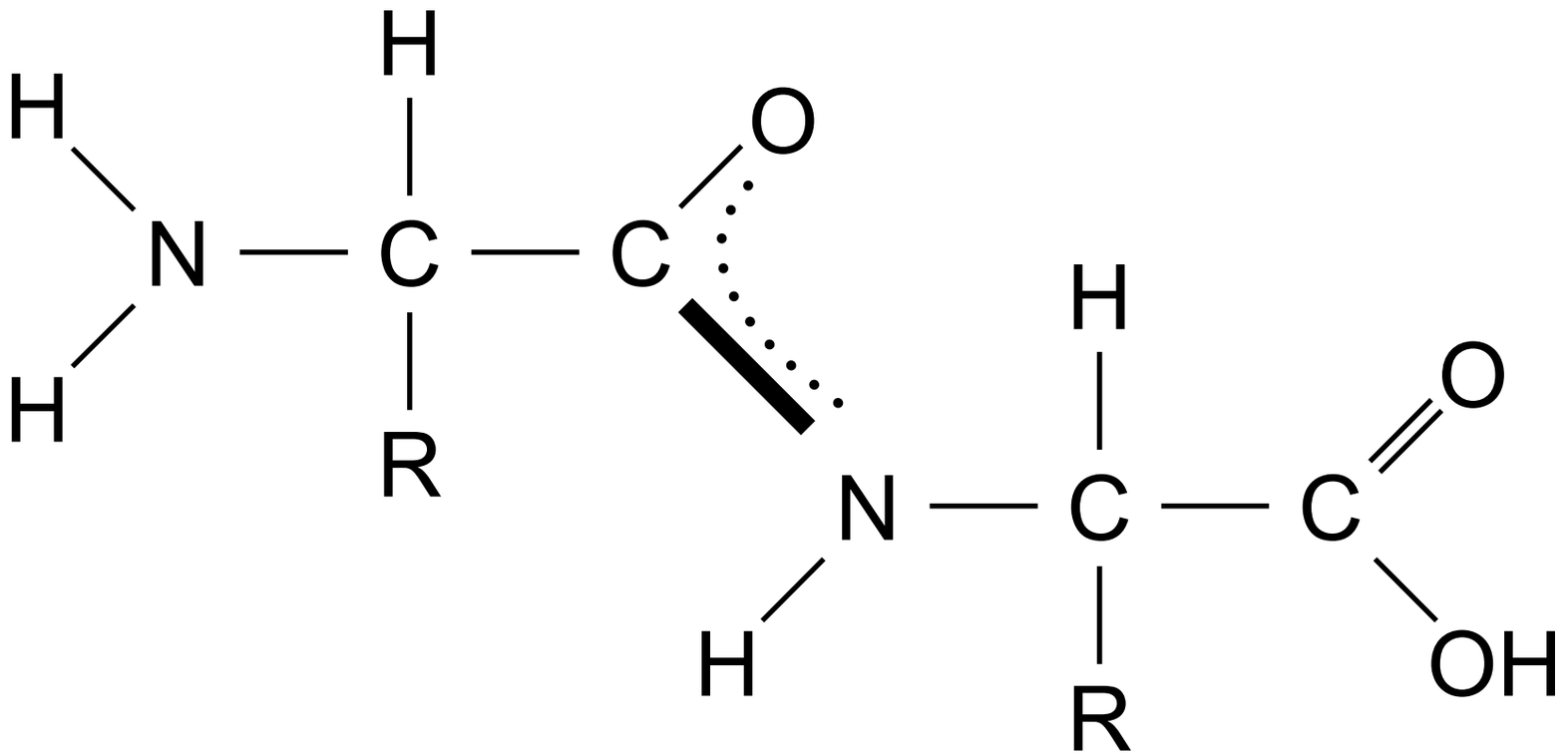
- Focused on single chain rather then whole protein structures
- X-ray pictures from different kinds of protein crystals to look for common features

Protein Structure

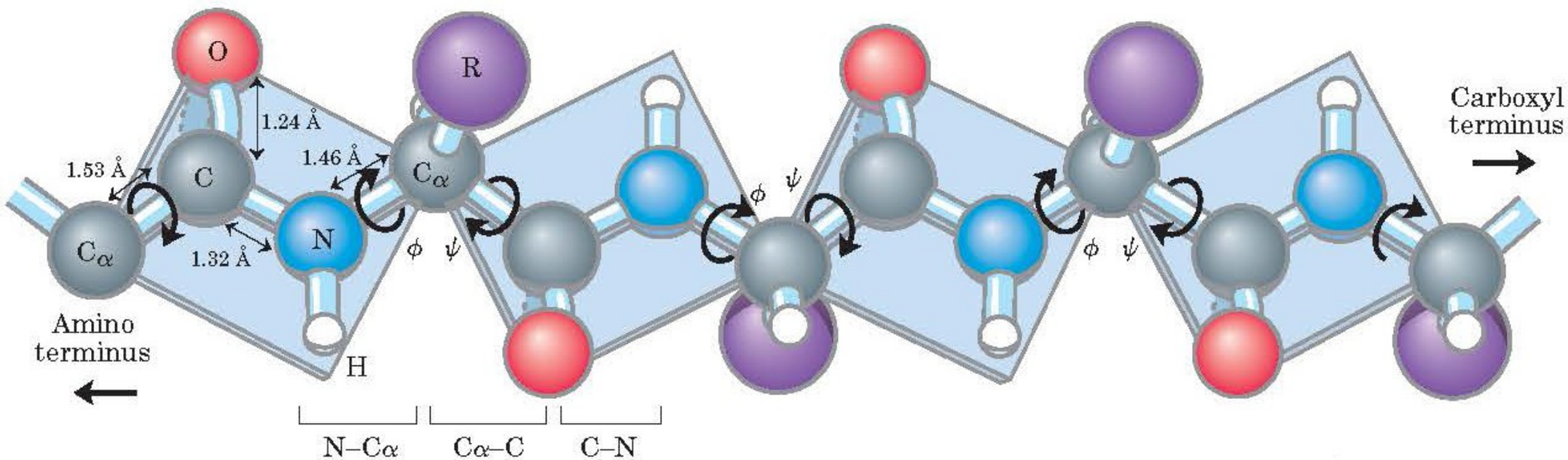




Protein Structure



Electron resonance makes the peptide bond configuration planar



(b)

Protein Structure

- Calculated interatomic distances and bond angles
- Nitrogen atom must form hydrogen bond with oxygen of another residue

- Maximum distance should be 2.72 Å
- Vector angle from NH to Oxygen < 30˚

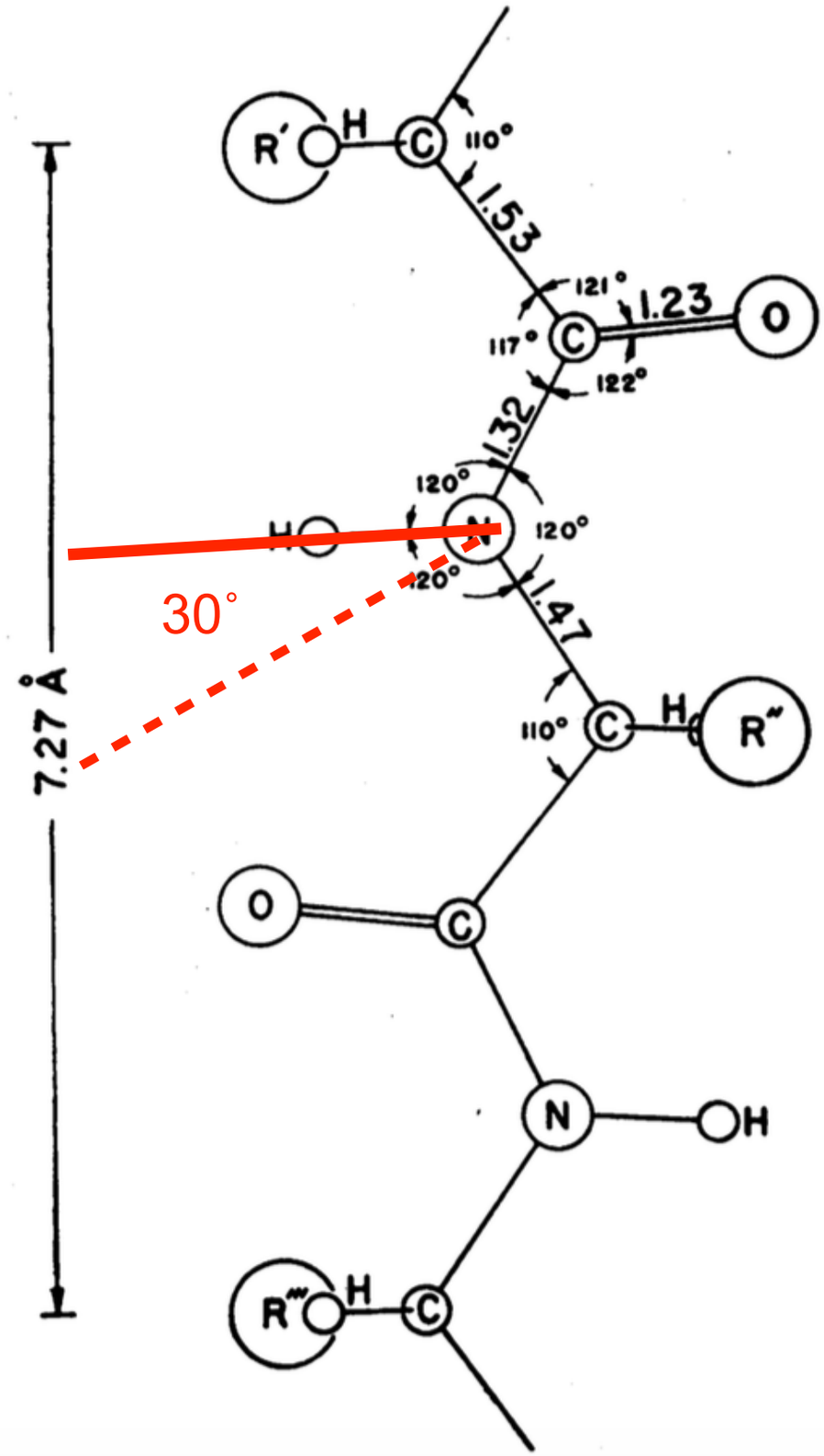
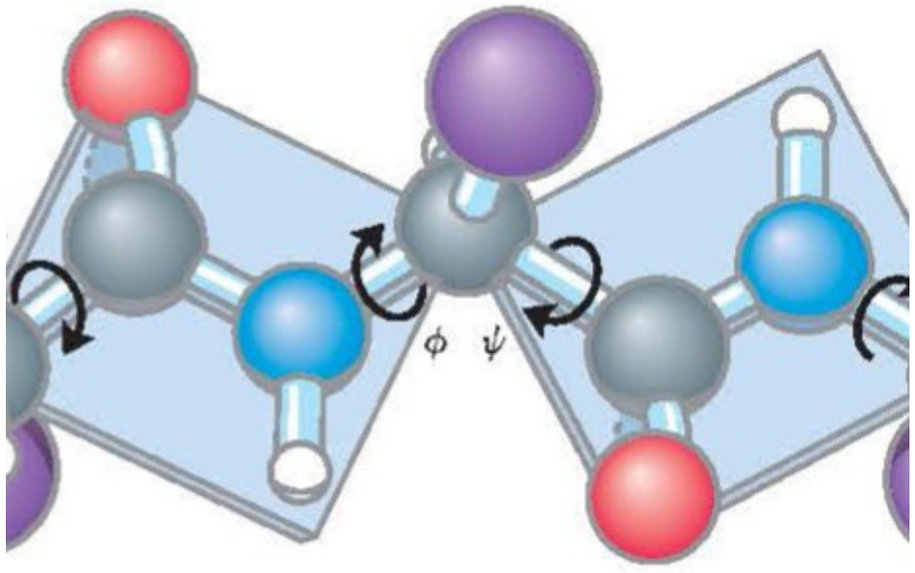


FIGURE 1

Protein Structure

- Residue that will form hydrogen bond with a determined NH depends on the **rotational angle**



- Respecting bond angles and interatomic distances:

- Rotational angle = 97.2˚ **(3.7-residue structure)**
- Rotational angle = 70.1˚C **(5.1-residue structure)**

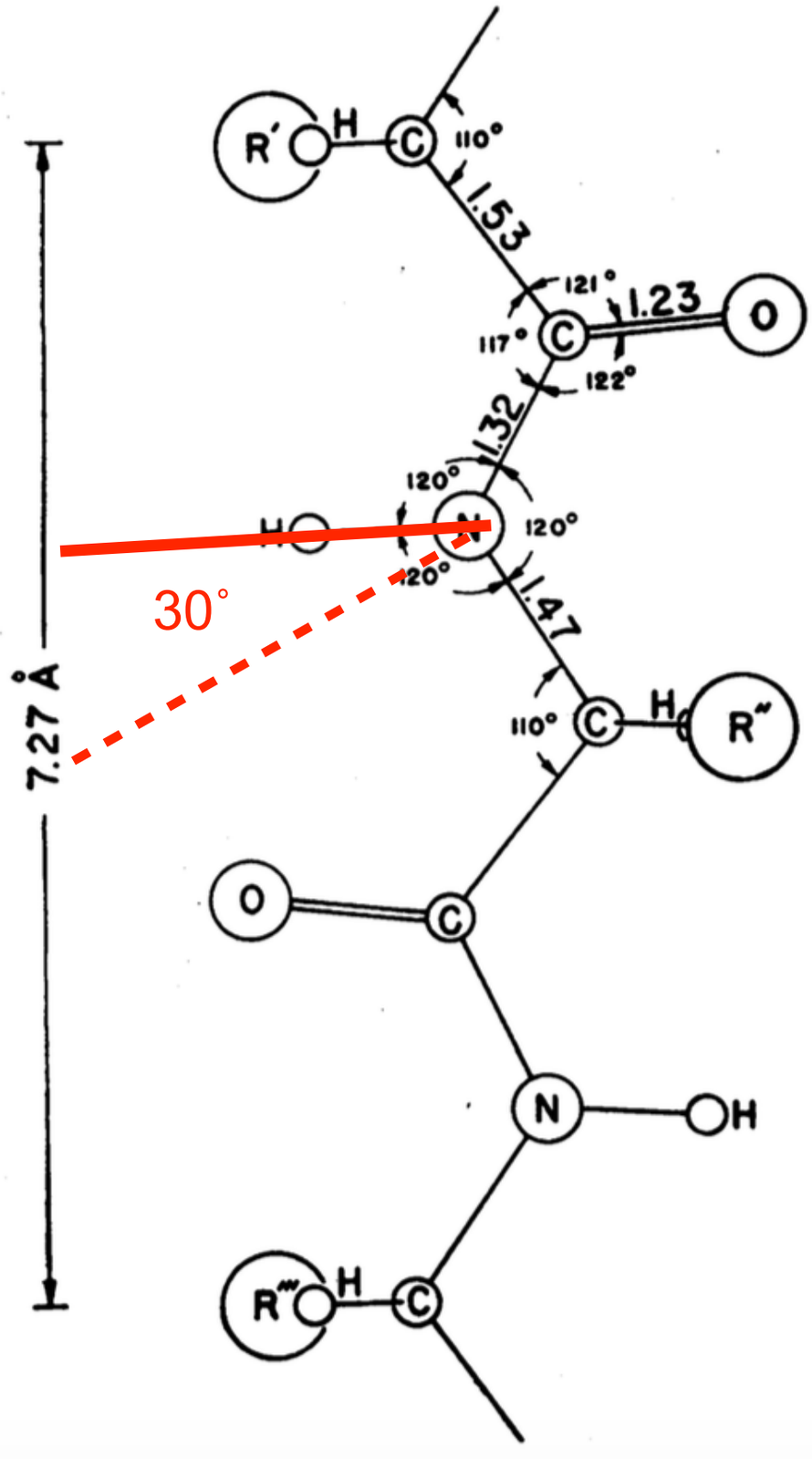


FIGURE 1

Protein Structure

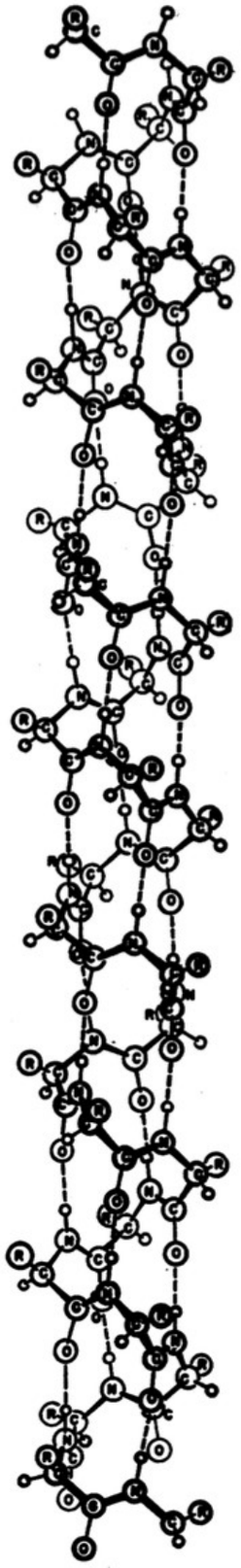


FIGURE 2

The helix with 3.7 residues per turn.

FIGURE 3

The helix with 5.1 residues per turn.

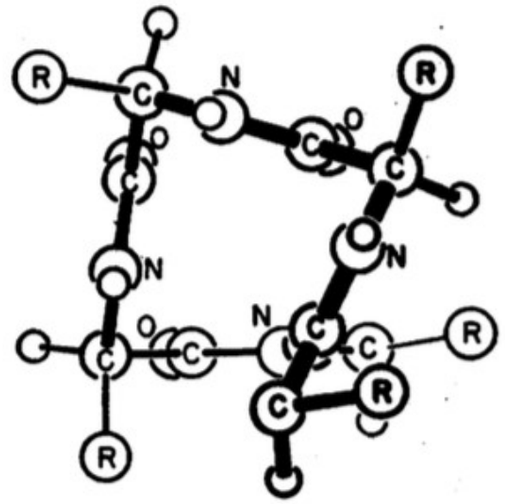


FIGURE 4

Plan of the 3.7-residue helix.

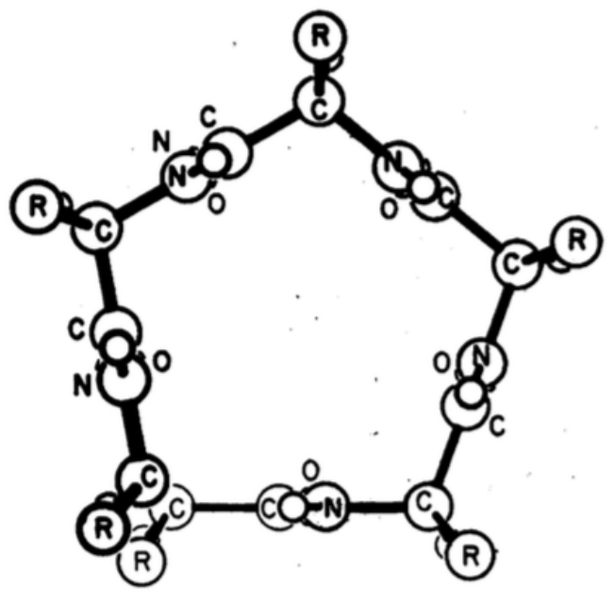


FIGURE 5

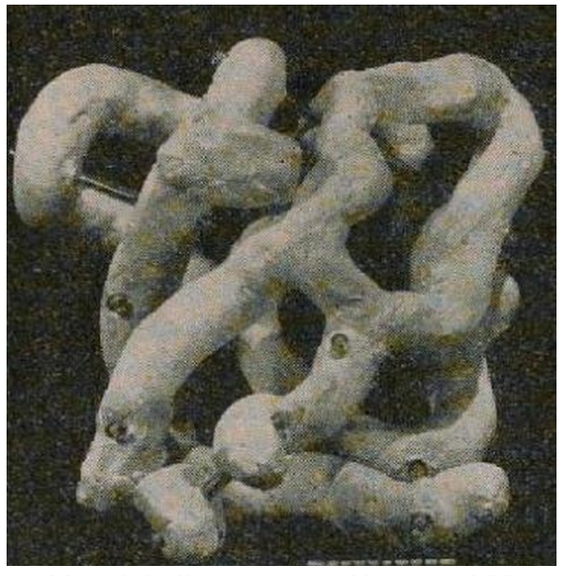
Plan of the 5.1-residue helix.

Protein Structure

Summary:

- Planar structure of peptide bond
- All residues involved in hydrogen bonds
- Helical structure of proteins
- Non-integer number of residues per helix turn was key to elucidate the structure
- Two proposed helical structures:

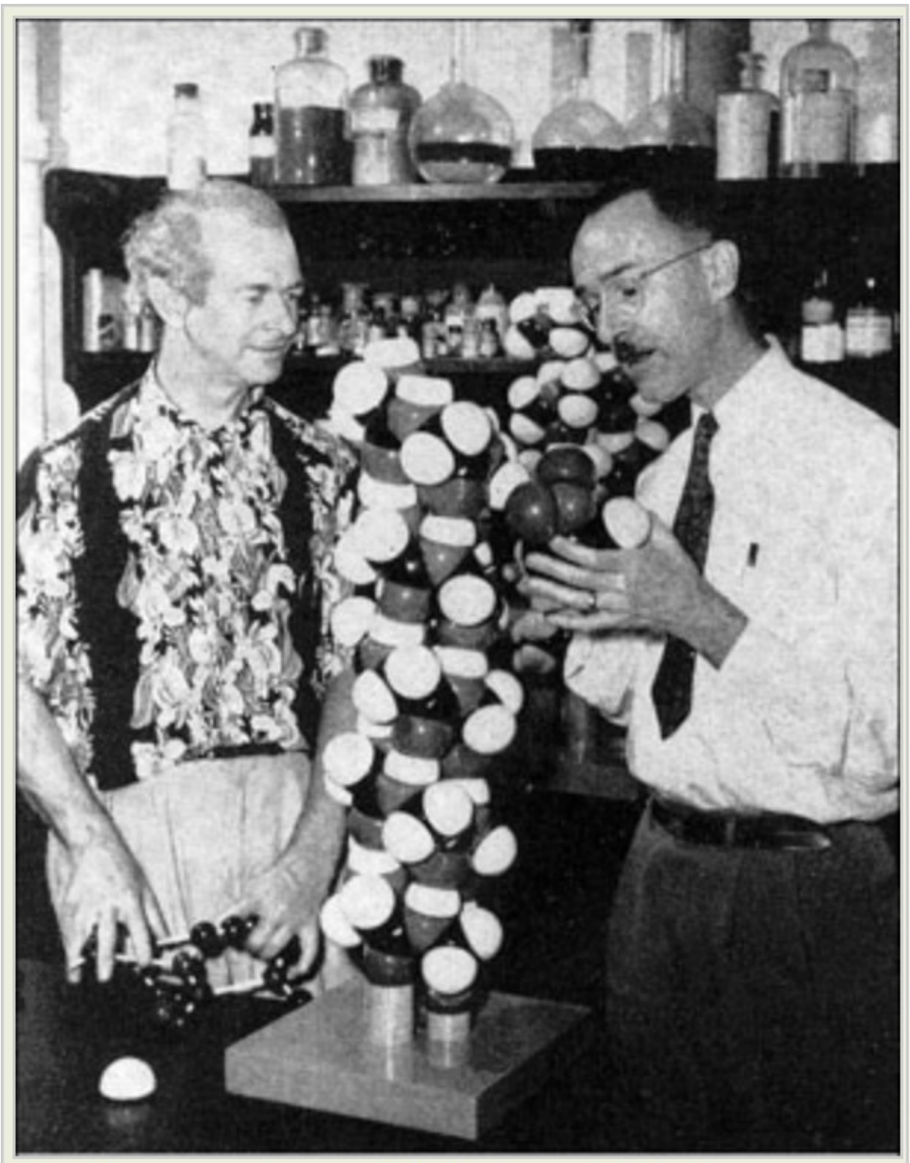
3.7-residues/turn



Myoglobulin structure. Nature 1958

5.1-residues/turn

Never found



Pauling and Corey with their model of the alpha helix, 1951.

Cracking the Genetic Code

Important contributions for cracking the genetic code:

- The “transforming principle” (1928)
- The nature of the transforming principle: DNA (1944 - 1952)
- X-ray diffraction and **the structure of proteins (1951)**
- **The structure of DNA (1953)**

How is DNA (4 nucleotides) the genetic material while proteins (20 amino acids) are the building blocks?

DNA —**?**→ **Protein**

Marshall Warren Nirenberg

- 1927-2010
- Born in New York City
- 1948: B.S. Zoology and Chemistry from University of Florida
- 1952: M.S. Zoology from University of Florida
- 1957: PhD Biochemistry from University of Michigan
- Post-doc / Researcher at NIH
- Wanted to investigate whether DNA or RNA is the template for protein synthesis

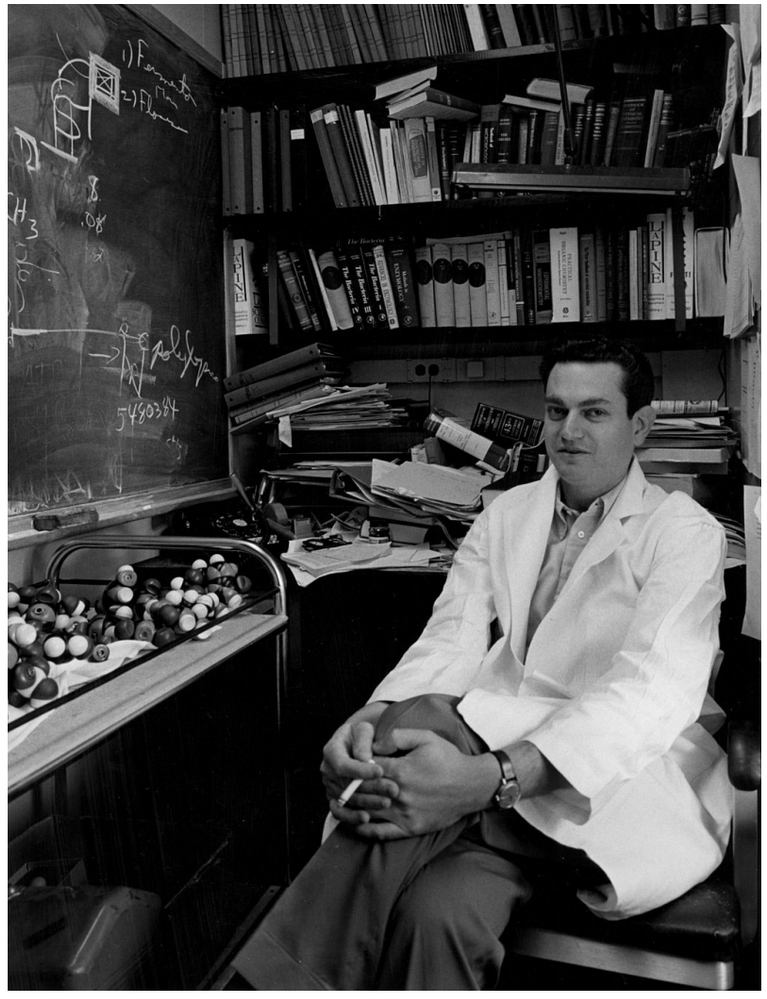


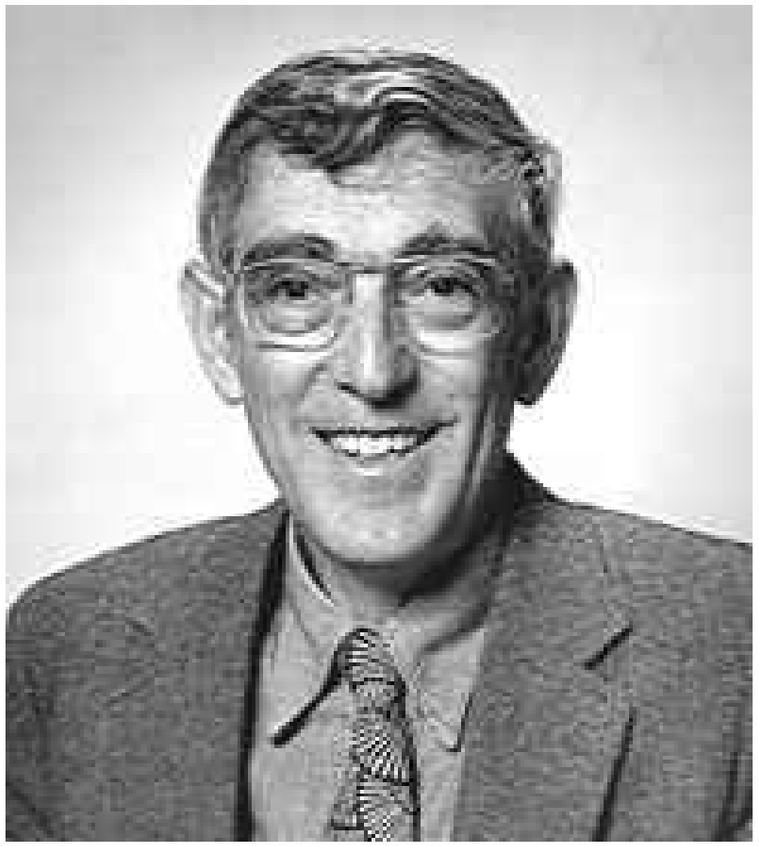
Image from Wikipedia

- Nobel Prize in Physiology or Medicine in 1968 for cracking the genetic code

k that I did only at the NIH. I would never have been awarded a grant to do the work because I ha

- Marshall Nirenberg

Marshall Warren Nirenberg



Bruce Ames

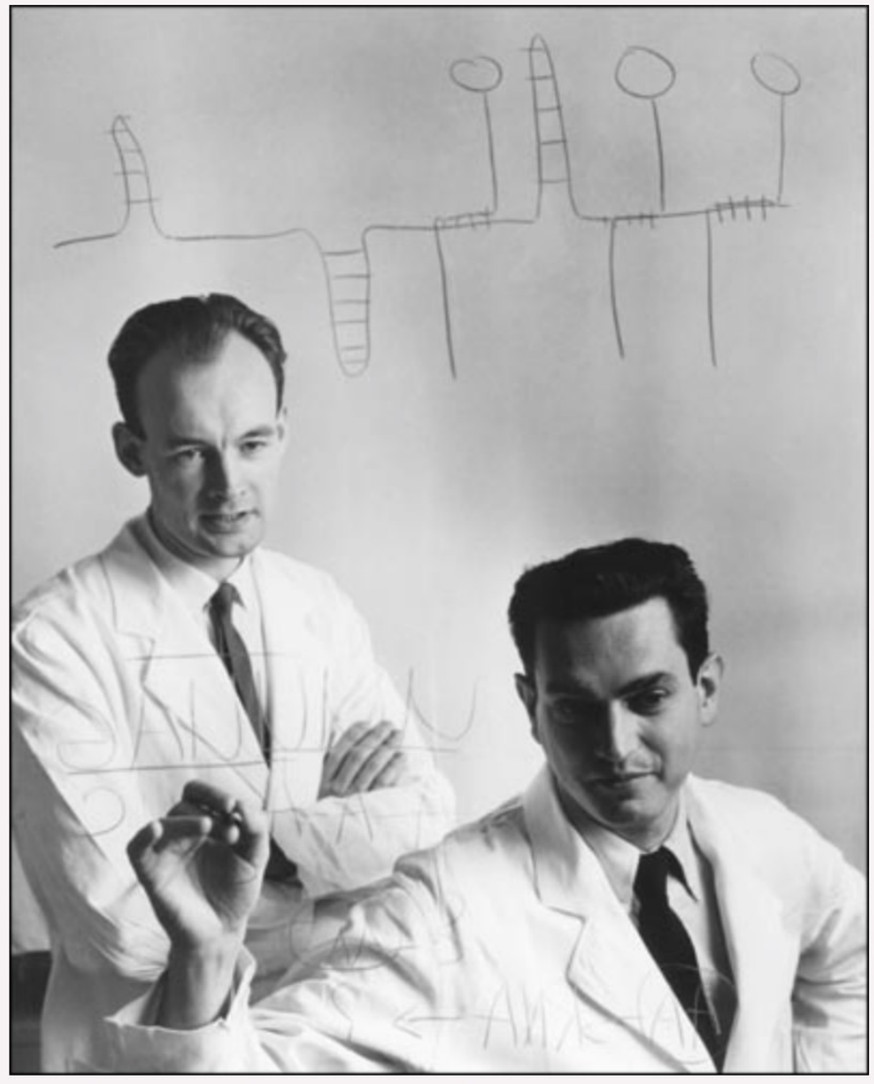
“It’s suicidal to do this”

“Nirenberg is committing professional suicide”

- NIH colleagues

J. Heinrich Matthaei

- 1929-today
- Born in Germany
- 1956: PhD in Biochemistry in Germany
- 1060: Post-doc at NIH
- Experience in generating radioactive amino acids
- No Nobel Prize



Heinrich Matthaei and Marshall Nirenberg (right)

Source: NIH

Cracking the Genetic Code

1) Needed a cell-free system to test different templates

CHARACTERISTICS AND STABILIZATION OF DNAASE-SENSITIVE PROTEIN SYNTHESIS IN E. COLI EXTRACTS

By J. Heinrich Matthaei* and Marshall W. Nirenberg

National Institutes of Health, Bethesda, Maryland

2) Needed to prove that RNA is the template for protein synthesis — **the poly-U experiment**

THE DEPENDENCE OF CELL- FREE PROTEIN SYNTHESIS IN E. COLI UPON NATURALLY OCCURRING OR SYNTHETIC POLYRIBONUCLEOTIDES

By Marshall W. Nirenberg and J. Heinrich Matthaei*

National Institutes of Health, Bethesda, Maryland

3) Identify the amino acid encoded by each codon

*CHARACTERISTICS AND COMPOSITION OF RNA CODING UNITS**

By J. Heinrich Matthaei,† Oliver W. Jones, Robert G. Martin, and Marshall W. Nirenberg

National Institute of Arthritis and Metabolic Diseases, Bethesda

Communicated by Richard Roberts, February 27, 1962

Cracking the Genetic Code

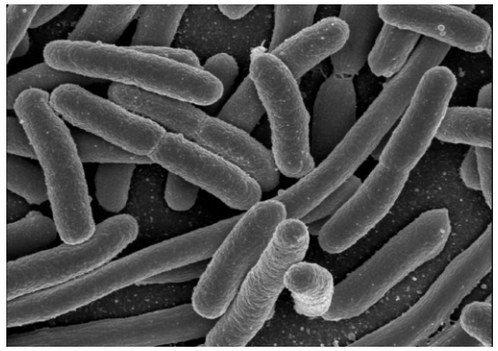
CHARACTERISTICS AND STABILIZATION OF DNAASE-SENSITIVE PROTEIN SYNTHESIS IN E. COLI EXTRACTS

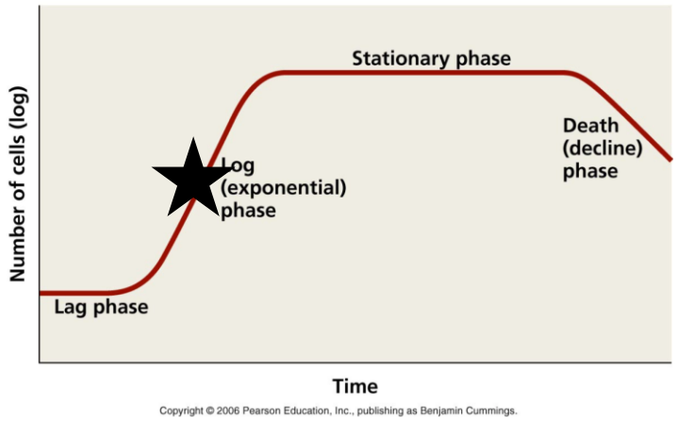
By J. Heinrich Matthaei* and Marshall W. Nirenberg

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by Joseph E. Smadel, August 3, 1961

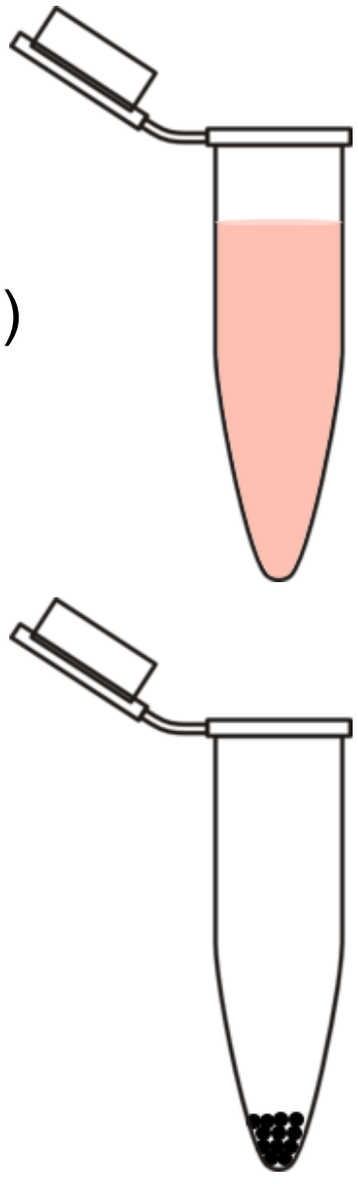
Cracking the Genetic Code



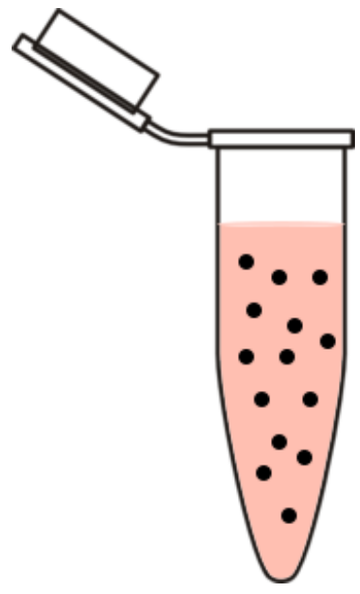


Multiple extraction and centrifugation steps

S-100
(DNA+RNA+Proteins)
(no Ribosomes)



W-Rib
(Ribosomes)

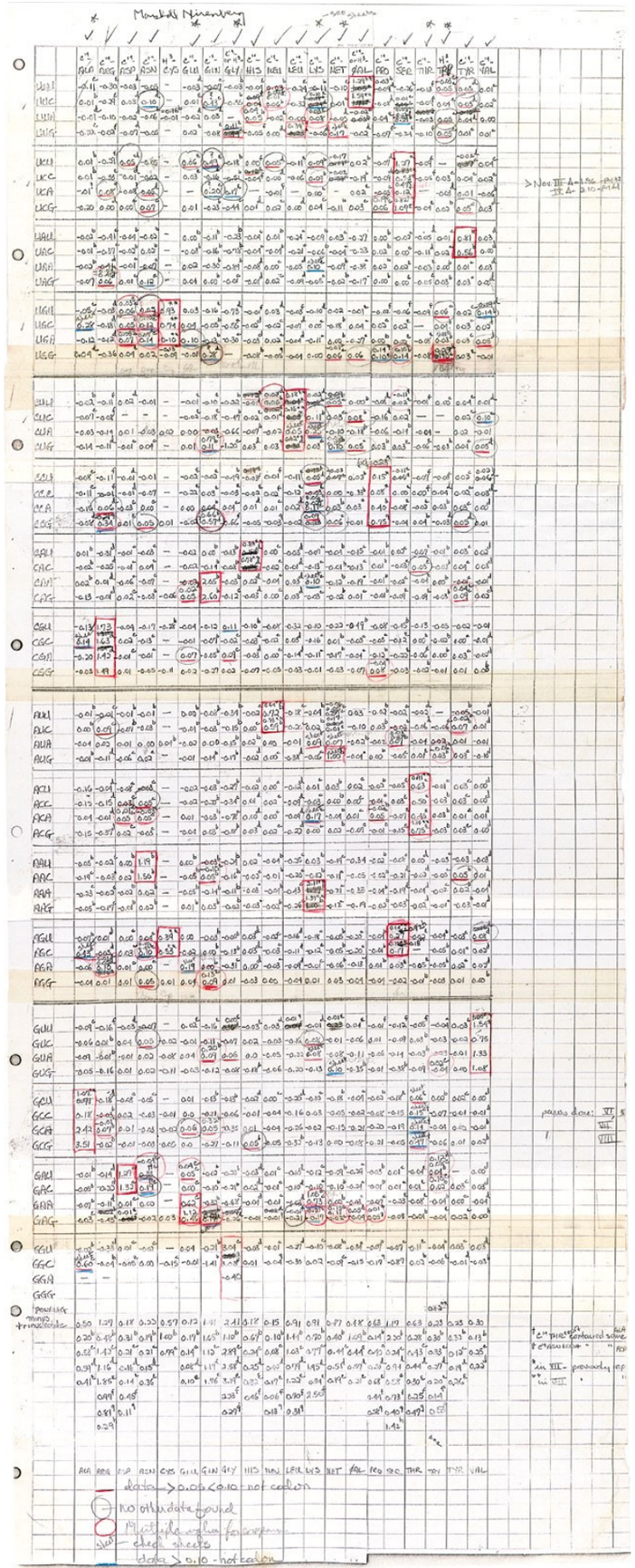


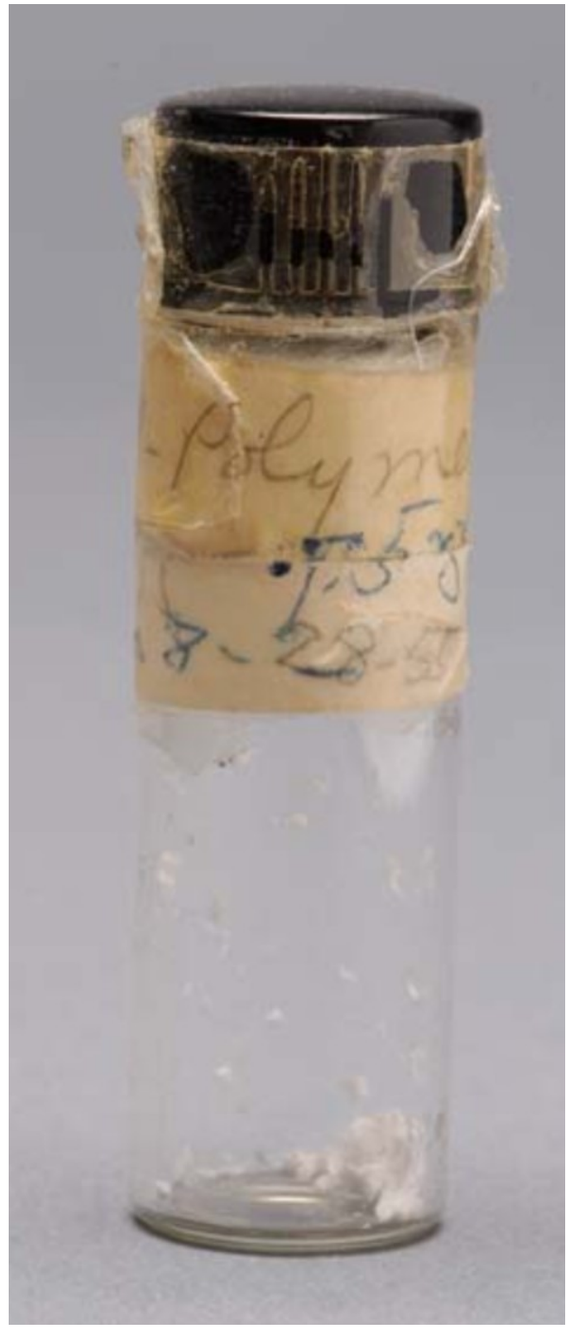
S-30
(DNA+RNA+Proteins+Ribosomes)
(no intact cells)

Cracking the Genetic Code

In vitro translation:

- S-30 / S-100 + W-Rib
- Tris
- Magnesium acetate
- KCl
- Mercaptoethanol
- ATP
- Phosphoenolpyruvate
- PEP kinase
- 19 L-amino acids (except valine)
- GTP/CTP/UTP
- C^{14}-Valine





Cracking the Genetic Code

Cell-free translation system was stable

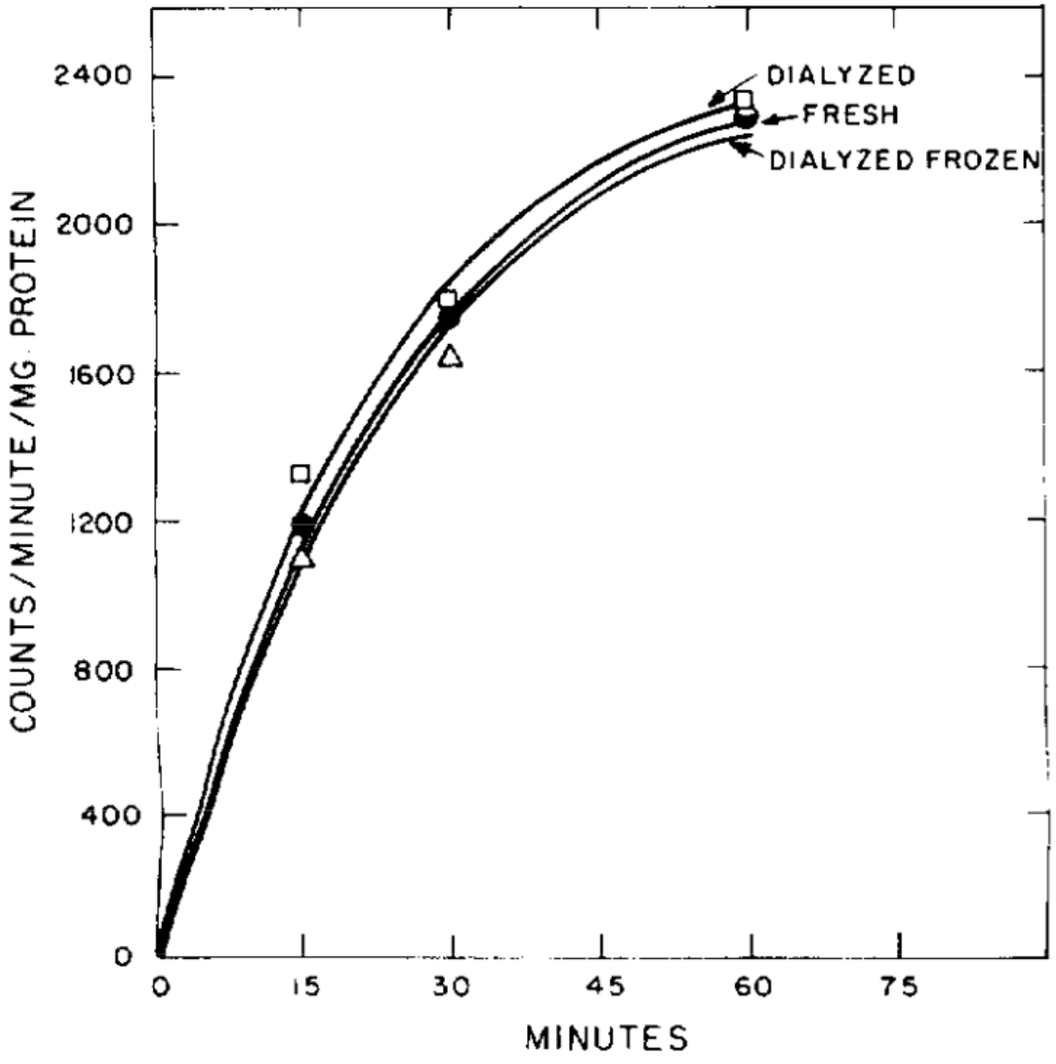


Fig 1: S-30

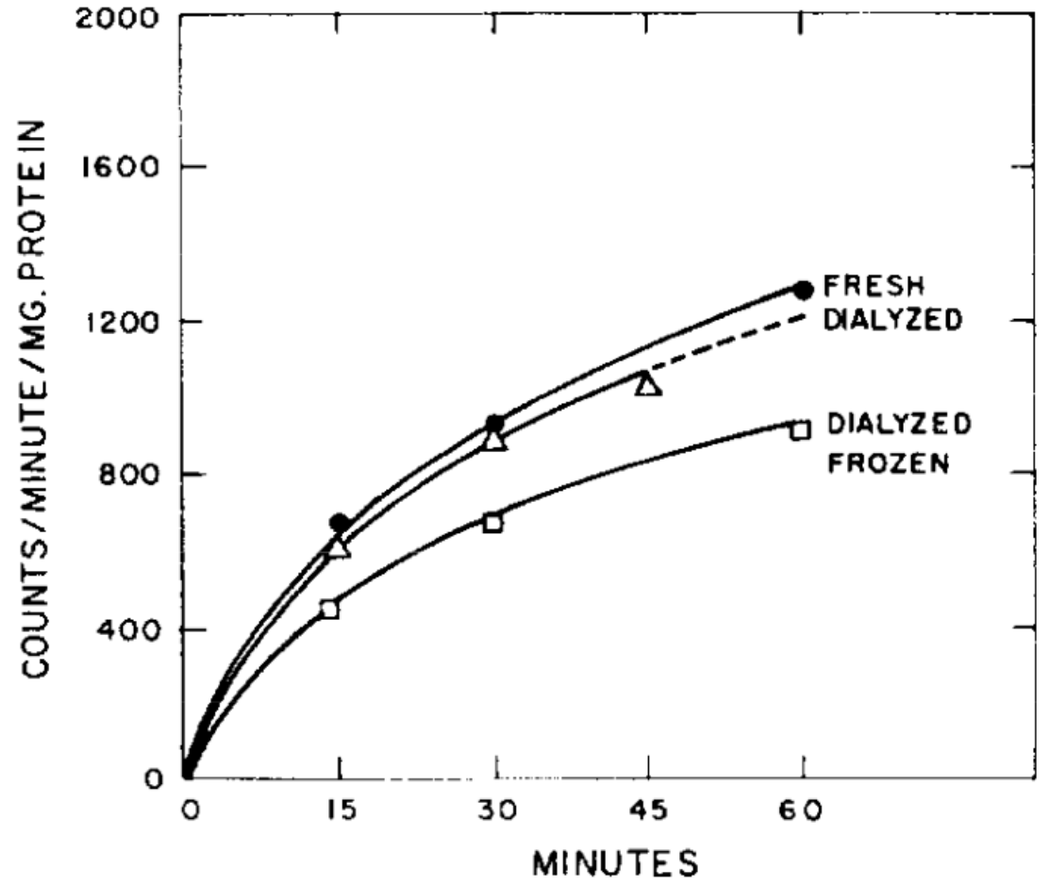


Fig 2: S100 + W-Rib

S-30: DNA, RNA, Proteins, Ribosomes
S-100: DNA, RNA, Proteins (no Ribosomes)
W-Rib: Ribosomes

Cracking the Genetic Code

Both S-100 and W-Rib are required for *in vitro* protein synthesis

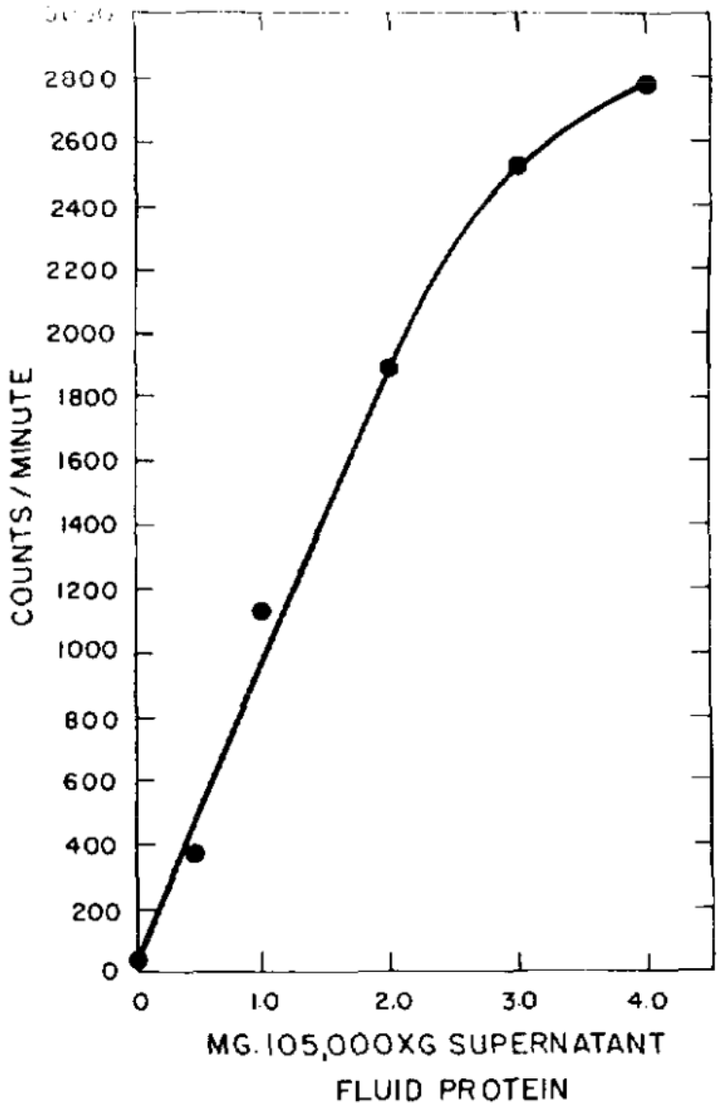


Fig 1: W-Rib + titration of S-100

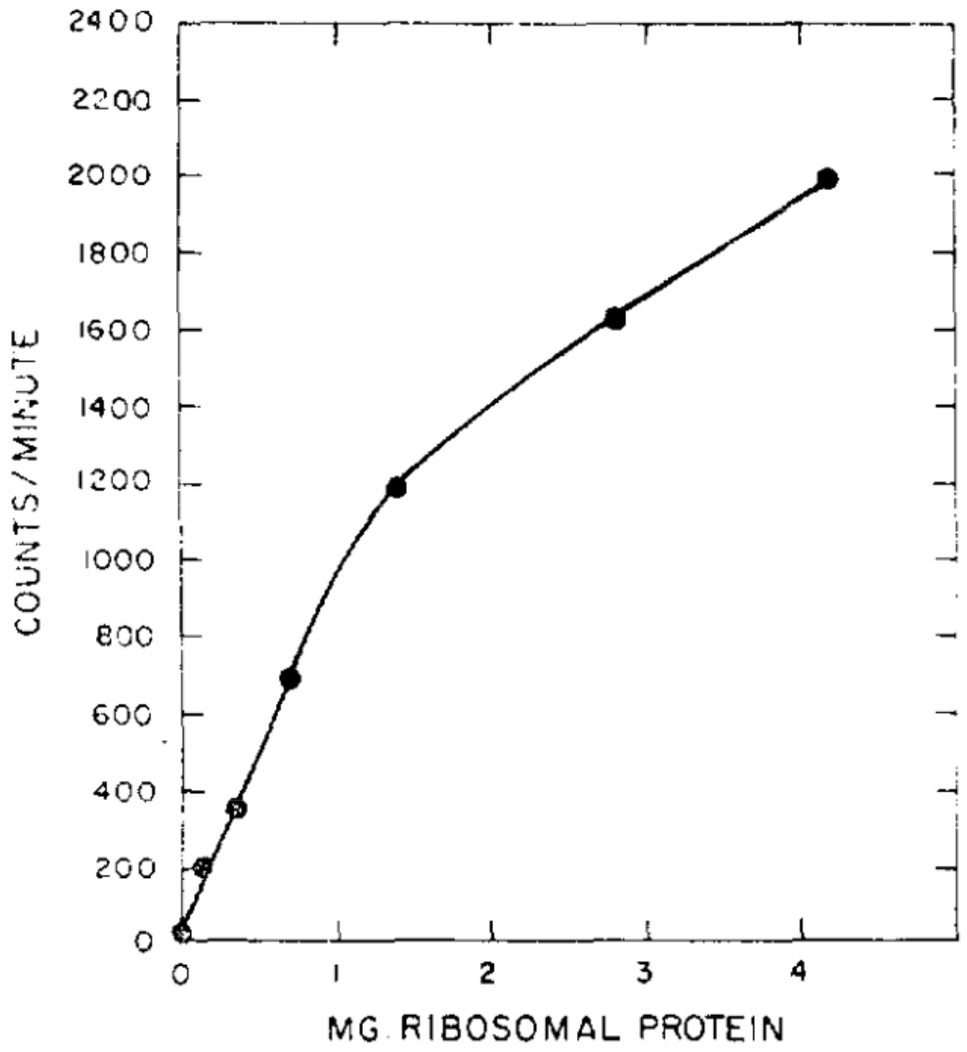


Fig 1: S-100 + titration of W-Rib

S-30: DNA, RNA, Proteins, Ribosomes
S-100: DNA, RNA, Proteins (no Ribosomes)
W-Rib: Ribosomes

Cracking the Genetic Code

TABLE 1

CHARACTERISTICS OF C^{14}-L-VALINE INCORPORATION INTO PROTEIN BY CELL-FREE, *E. coli* ENZYME PREPARATIONS STORED FOR SEVERAL WEEKS AT −15°

Experiment no.		Additions	Counts/min/mg protein
1	Complete		679
S-100 + W-Rib	"	− 105,000 × *g* supernatant solution	74
	"	− Ribosomes	15
	"	− ATP, PEP, PEP kinase	38
	"	+ 10 μg RNAase	9
	"	− Amino acid mixture	365
	"	+ 0.02 μmole Puromycin	38
	"	+ 0.30 μmole Chloramphenicol	82
	"	− 0.03 μmole GTP, CTP, UTP	583
	"	− 0.03 μmole CTP, UTP	652
	"	Deproteinized at zero time	4
2	Complete		2078
	"	+ 10 μg DNAase	1223
S-30	"	Deproteinized at zero time	5

S-30: DNA, RNA, Proteins, Ribosomes
S-100: DNA, RNA, Proteins (no Ribosomes)
W-Rib: Ribosomes

Cracking the Genetic Code

TABLE 3

THE EFFECT OF POLYANIONS UPON THE DNAASE-INHIBITED INCORPORATION OF C^{14}-L-VALINE INTO PROTEIN AND THE EFFECT OF A DNAASE DIGEST OF DNA UPON INCORPORATION

Experiment no		Additions	Counts/min/mg protein
1	Complete		2,040
	"	+ 10 μg DNAase	825
	"	+ " " + 100 μg polyadenylic acid	685
	"	+ " " + 100 μg polyglucose carboxyl derivative	810
	"	+ 100 μg polyadenylic acid	2,430
	"	+ 100 μg polyglucose carboxyl derivative	2,150
	"	Deproteinized at zero time	7
2	Complete		1,842
	"	+ 100 μg DNAase digest of salmon sperm DNA	1,825
3	Complete	+ 10 μg DNAase	604
	"	+ 1.0 ml reaction mixture incubated with 10 μg DNAase for 60 minutes	661

S-100 + W-Rib

S-30

S-30: DNA, RNA, Proteins, Ribosomes
S-100: DNA, RNA, Proteins (no Ribosomes)
W-Rib: Ribosomes

Cracking the Genetic Code

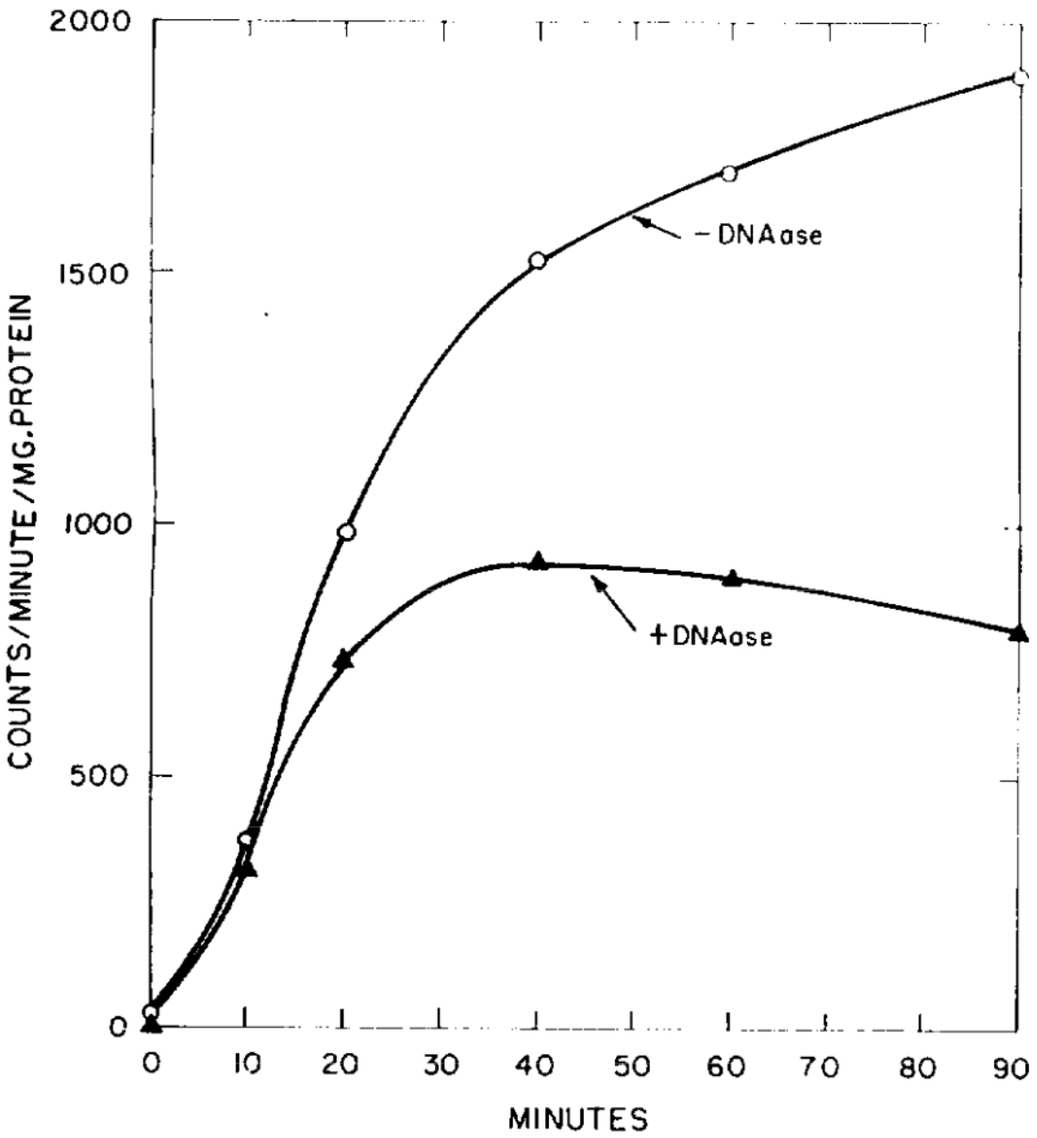


Fig 6: S-30 +/- DNAse

"Inhibition by DNAse observed in this cell-free system may be due to the destruction of DNA and its resultant inability to serve as templates for the synthesis of template RNA"

S-30: DNA, RNA, Proteins, Ribosomes
S-100: DNA, RNA, Proteins (no Ribosomes)
W-Rib: Ribosomes

Cracking the Genetic Code

THE DEPENDENCE OF CELL- FREE PROTEIN SYNTHESIS IN E. COLI UPON NATURALLY OCCURRING OR SYNTHETIC POLYRIBONUCLEOTIDES

By Marshall W. Nirenberg and J. Heinrich Matthaei*

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by Joseph E. Smadel, August 3, 1961

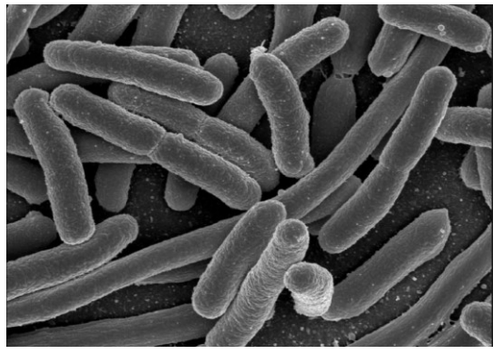
- Two species of RNA were known at the time:

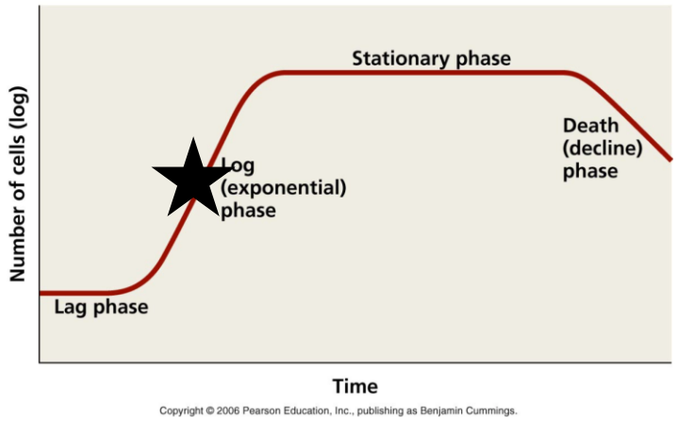
- Soluble RNA (RNA present in extraction after ribosomes were precipitated)
- Ribosomal RNA (RNA associated with ribosomes) —> Higher concentrations!

- Where would messenger RNA be?

- Nirenberg thought that rRNA would could have small amounts of template RNA (higher concentration)

Cracking the Genetic Code

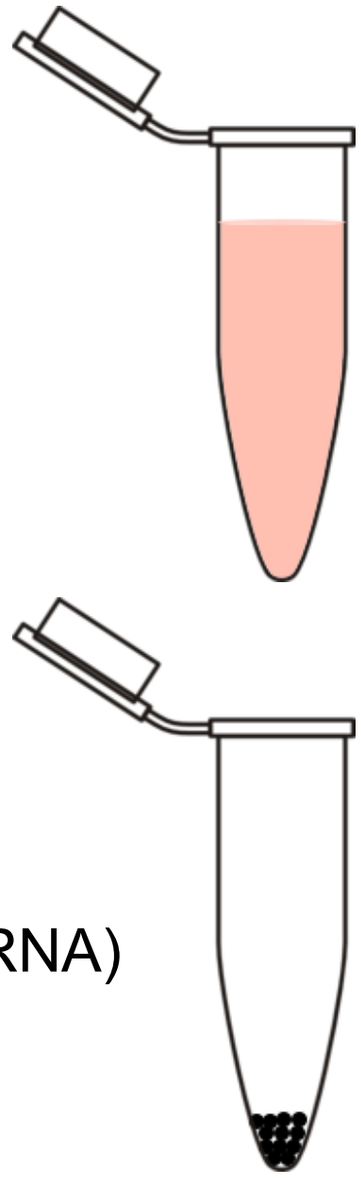




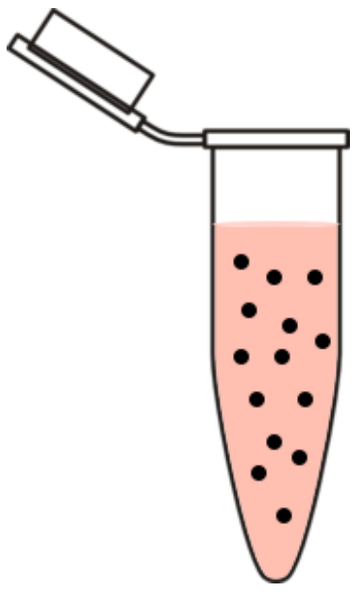
Multiple extraction and centrifugation steps

+DNAse

S-100
(Soluble RNA)
-tRNA-



W-Rib
(Ribosomes, ribosomal RNA)



S-30
(RNA+Proteins+Ribosomes)
(no intact cells, no DNA)

Cracking the Genetic Code

Effect of soluble vs ribosomal RNA in stimulating protein synthesis

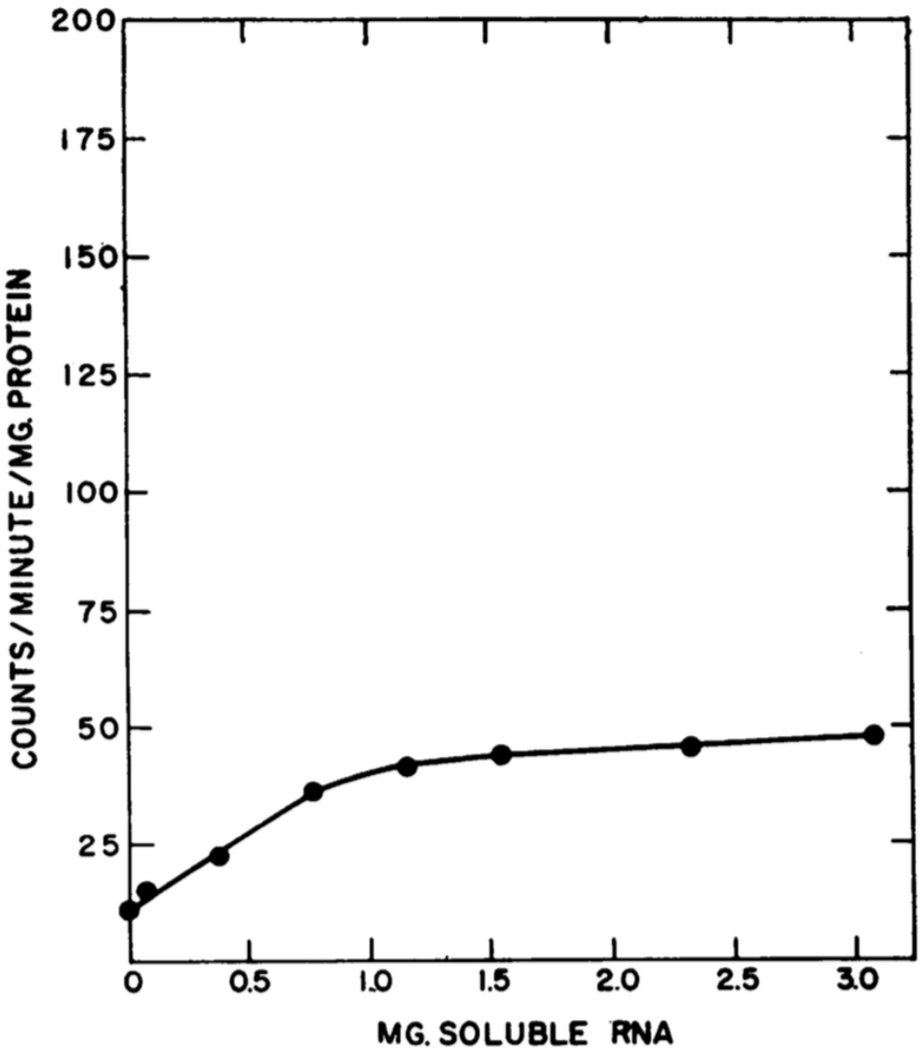


Fig 1: S-30 supplemented with S-100

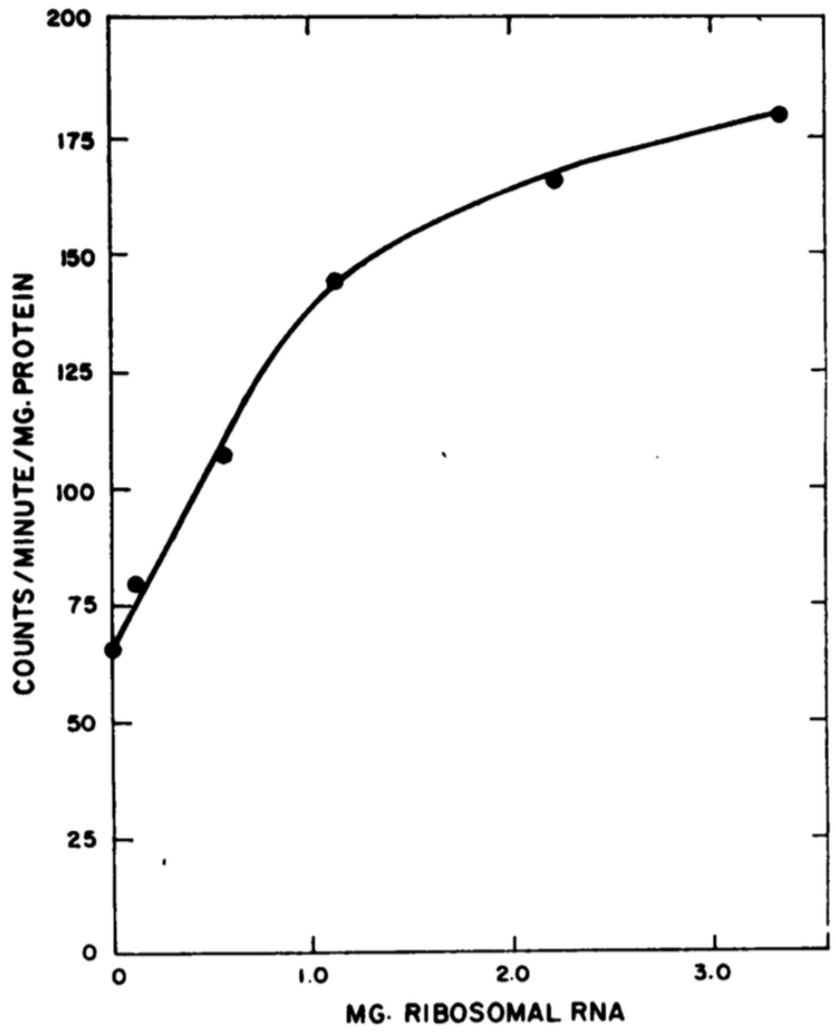


Fig 2: S-30 supplemented with W-Rib

S-30: Soluble + Ribosomal RNA
S-100: Soluble RNA
W-Rib: Ribosomal RNA

Cracking the Genetic Code

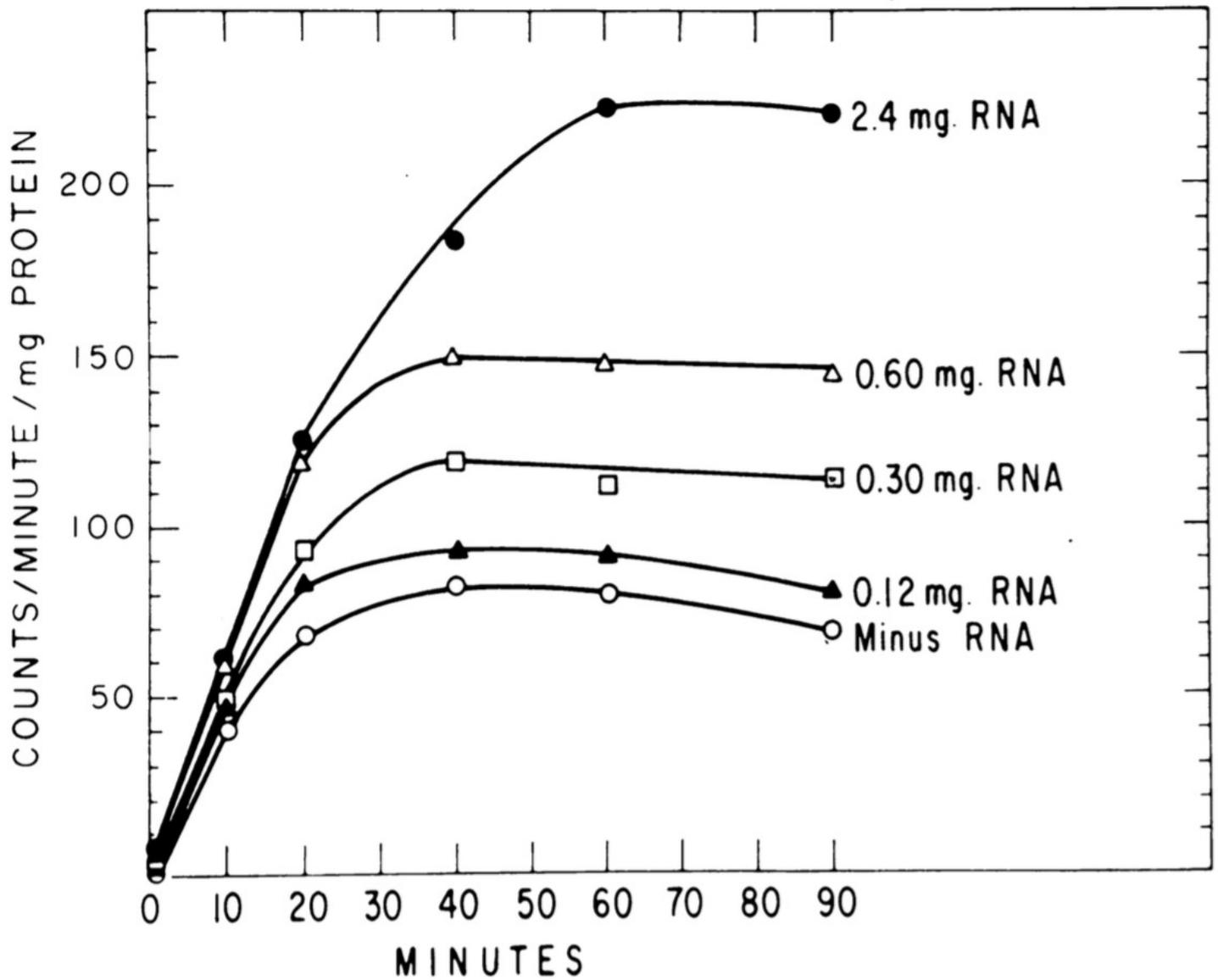


Fig 3: S-30 protein synthesis stimulation by W-Rib

S-30: Soluble + Ribosomal RNA
S-100: Soluble RNA
W-Rib: Ribosomal RNA

Cracking the Genetic Code

- Ribosomal RNA contained RNA species that seemed to work as template for protein synthesis (C^{14}-Valine incorporation)

TABLE 1

CHARACTERISTICS OF C^{14}-L-VALINE INCORPORATION INTO PROTEIN

Experiment no.	Addition			Counts/min/mg protein
1	− Ribosomal RNA			42
	+ " "			204
Sensitive to protein synthesis inhibitors	+ " "		+ 0.15 μmole Chloramphenicol	58
	+ " "		+ 0.20 μmole Puromycin	7
	+ " "		deproteinized at zero time	8
2	− Ribosomal RNA			35
	+ " "			101
Sensitive to ATP levels and RNAse. Not to DNAse.	+ " "		− ATP, PEP, PEP kinase	7
	+ " "		+ 10 μg RNAase	6
	+ " "		+ 10 μg DNAase	110
	+ Boiled Ribosomal RNA			127
	+ Ribosomal RNA, deproteinized at zero time			8
3	− Ribosomal RNA			34
Sensitive to amino acids levels	− " "		− 20 L amino acids	21
	+ " "			99
	+ " "		− 20-L-amino acids	52

S-30: Soluble + Ribosomal RNA

Cracking the Genetic Code

Ribosomal RNA stimulation required both ribosomes (W-Rib) and S-100

TABLE 2

THE INEFFECTIVENESS OF RIBOSOMAL RNA IN STIMULATING C^{14}-L-VALINE INCORPORATION INTO PROTEIN IN THE PRESENCE OF RIBOSOMES OR 105,000 × *g* SUPERNATANT SOLUTIONS ALONE

	Additions	Counts/min
Complete		51
"	+ 2.1 mg Ribosomal RNA	202
"	− Ribosomes	17
"	− Ribosomes + 2.1 mg Ribosomal RNA	20
"	− Supernatant solution	36
"	− Supernatant solution + 2.1 mg Ribosomal RNA	45
"	Deproteinized at zero time	25

S-100 + W-Rib

S-30: Soluble + Ribosomal RNA
S-100: Soluble RNA
W-Rib: Ribosomal RNA

Cracking the Genetic Code

Ribosomal RNA in *E. coli* does not contain a lot of template/messenger RNA

TABLE 5

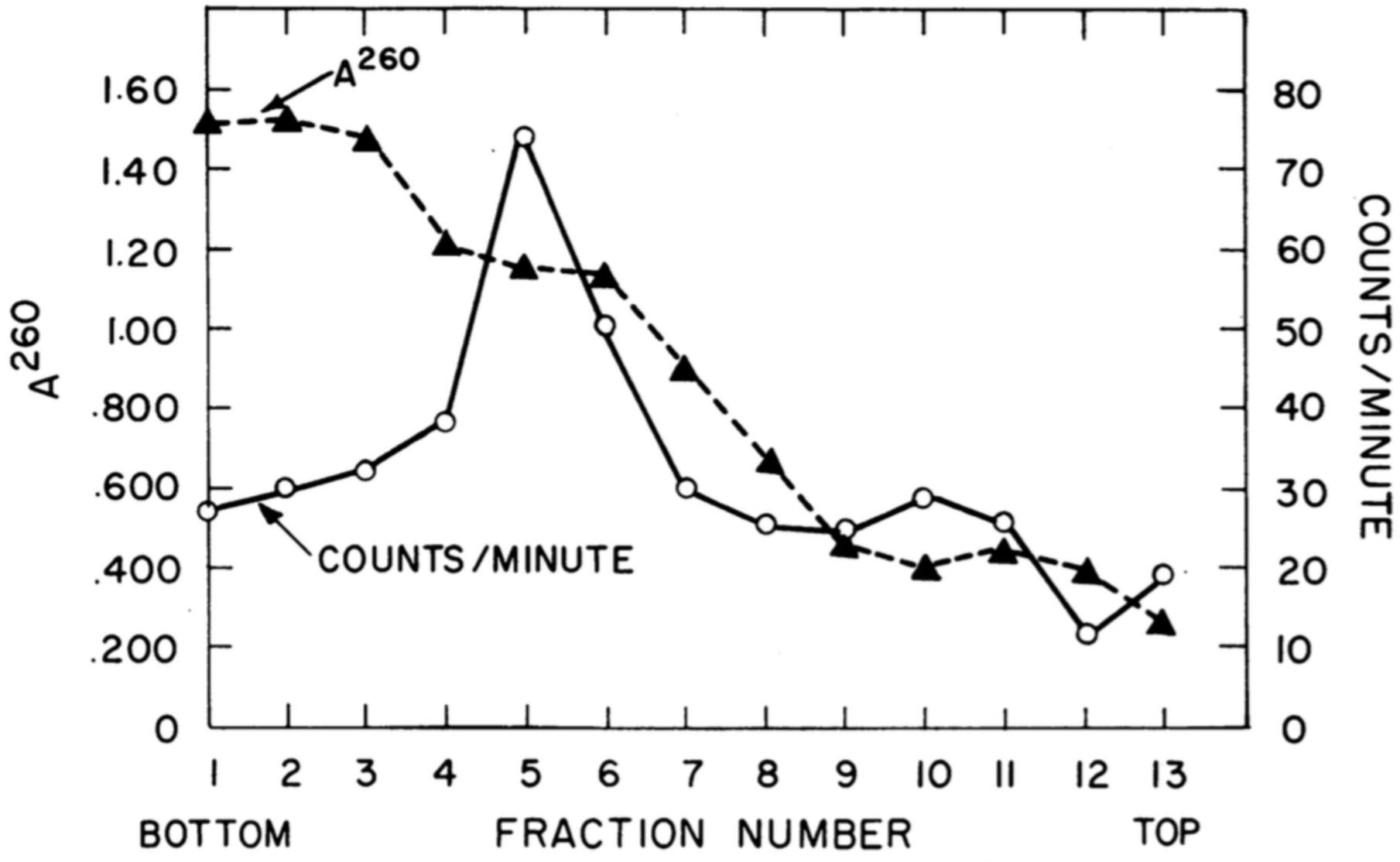
STIMULATION OF AMINO ACID INCORPORATION BY RNA FRACTIONS PREPARED FROM DIFFERENT SPECIES

Additions	Counts/min/mg protein
None	42
+ 0.5 mg *E. coli* ribosomal RNA	75
+ 0.5 mg Yeast ribosomal RNA	430
+ 0.5 mg Tobacco mosaic virus RNA	872
+ 0.5 mg Ehrlich ascites tumor microsomal RNA	65

The components of the reaction mixtures and the incubation conditions are presented in Table 1. Reaction samples contained 1.9 mg Incubated-S-30 protein.

Cracking the Genetic Code

How much of the Ribosomal RNA is actually working as template RNA?



Sucrose-density gradient centrifugation of Ribosomal RNA

Cracking the Genetic Code

So far:

- Ribosomal RNA stimulated C^{14}-Valine incorporation in *vitro*
- Ribosomal RNA seems to contain small amounts of “template/messenger” RNA

How to confirm that RNA is indeed the template?

- Synthetic RNA

Cracking the Genetic Code

Effect of synthetic RNAs in stimulating protein synthesis (Phenylalanine incorporation)

TABLE 6

POLYNUCLEOTIDE SPECIFICITY FOR PHENYLALANINE INCORPORATION

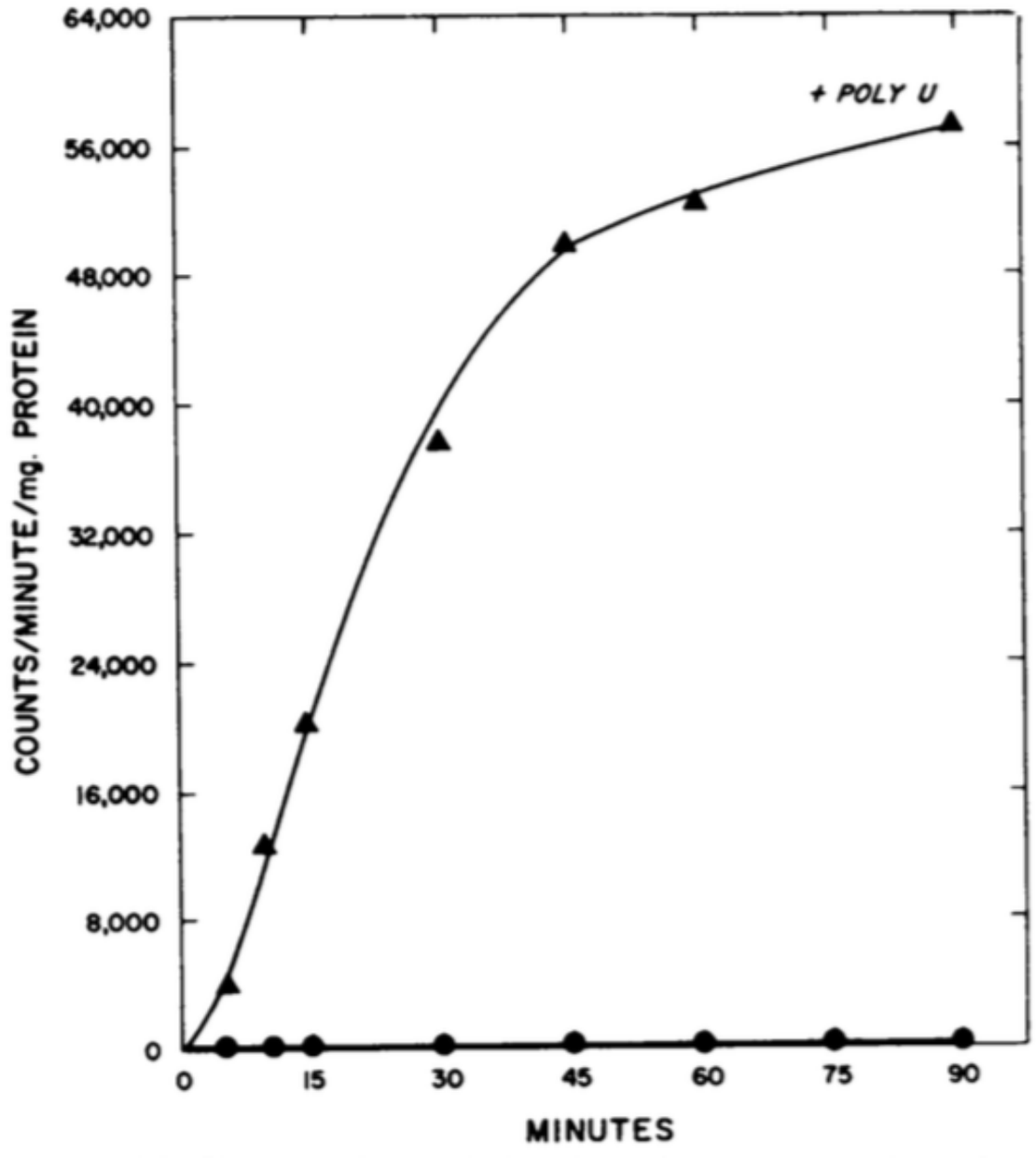
Experiment no.	Additions	Counts/min/mg protein
1	None	44
	+ 10 μg Polyuridylic acid	39,800
	+ 10 μg Polyadenylic acid	50
	+ 10 μg Polycytidylic acid	38
	+ 10 μg Polyinosinic acid	57
	+ 10 μg Polyadenylic-uridylic acid (2/1 ratio)	53
	+ 10 μg Polyuridylic acid + 20 μg polyadenylic acid	60
	Deproteinized at zero time	17
2	None	75
	+ 10 μg UMP	81
	+ 10 μg UDP	77
	+ 10 μg UTP	72
	Deproteinized at zero time	6

S-30

S-30

Cracking the Genetic Code

Poli-U strongly stimulates Phenylalanine incorporation



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Poli-U stimulation is restricted to Phenylalanine

TABLE 8

SPECIFICITY OF AMINO ACID INCORPORATION STIMULATED BY POLYURIDYLIC ACID

Experiment no.	C^{14}-amino acids present	Additions	Counts/min/mg protein
1	Phenylalanine	Deproteinized at zero time	25
		None	68
		+ 10 μg polyuridylic acid	38,300
2	Glycine, alanine, serine, aspartic acid, glutamic acid	Deproteinized at zero time	17
		None	20
		+ 10 μg polyuridylic acid	33
3	Leucine, isoleucine, threonine, methionine, arginine, histidine, lysine, tyrosine, tryptophan, proline, valine	Deproteinized at zero time	73
		None	276
		+ 10 μg polyuridylic acid	899
4	S^{35}-cysteine	Deproteinized at zero time	6
		None	95
		+ 10 μg polyuridylic acid	113

Cracking the Genetic Code

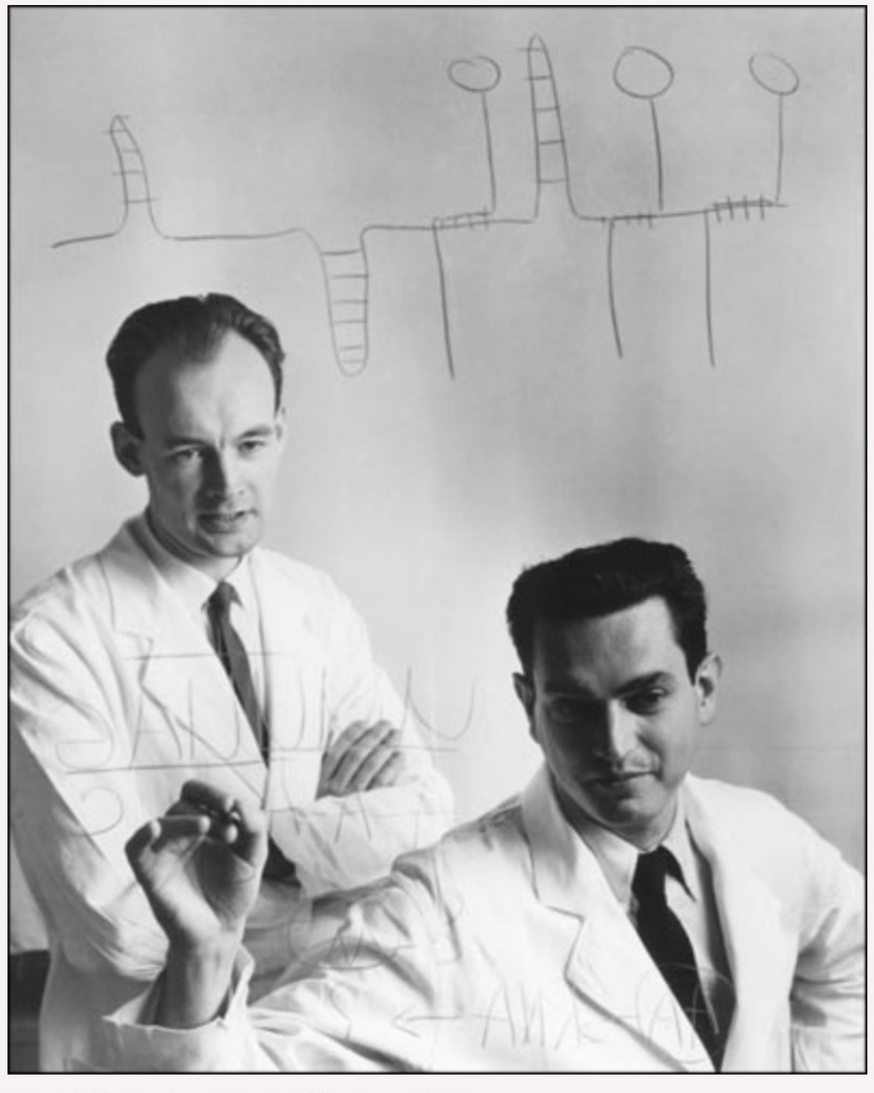
TABLE 7

CHARACTERISTICS OF POLYURIDYLIC ACID–DEPENDENT PHENYLALANINE INCORPORATION

Additions	Counts/min/mg protein
Minus polyuridylic acid	70
None	29,500
Minus 100,000 × *g* supernatant solution	106
Minus ribosomes	52
Minus ATP, PEP, and PEP kinase	83
+ 0.02 μmoles puromycin	7,100
+ 0.31 μmoles chloramphenicol	12,550
+ 6 μg RNAase	120
+ 6 μg DNAase	27,600
Minus amino acid mixture	31,700
Deproteinized at zero time	30

Cracking the Genetic Code

“One or more uridylic acid residues therefore appear to be the code for phenylalanine. Whether the code is of the singlet, triplet, etc., type has not yet been determined. Polyuridylic acid seemingly functions as a synthetic template or messenger RNA”



Heinrich Matthaei and Marshall Nirenberg (right)

Next Steps

Using cell-free system + synthetic RNAs

GENERAL NATURE OF THE GENETIC CODE FOR PROTEINS

By Dr. F. H. C. CRICK, F.R.S., LESLIE BARNETT, Dr. S. BRENNER and Dr. R. J. WATTS-TOBIN

Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, Cambridge

Thursday!

*CHARACTERISTICS AND COMPOSITION OF RNA CODING UNITS**

By J. Heinrich Matthaei,† Oliver W. Jones, Robert G. Martin, and Marshall W. Nirenberg

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, BETHESDA

Communicated by Richard Roberts, February 27, 1962

Thanks!


GOD... THE HUMAN GENOME CODE'S BEEN UNRAVELLED
DAMN HACKERS!!! NOW, I HAVE TO CHANGE THE PASSWORD