Class 12

Genetic Code

Marshall Nirenberg & Heinrich Matthaei - 1961

1

**Main characters involved in the history
(approx. in order of appearance)**

	Nome	Born-Deceas.	Age in 1961	NPW/ area
1	Marshall Nirenberg	1927-2010	34	1968-Med.
2	J. Heinrich Matthaei	1929-	32	
3	Francis Harry Compton Crick	1916-2004	45	1962-Med.
4	James Dewey Watson	1928-	33	1962-Med.
5	George Gamow	1904-1968	57	
6	Har Gobind Khorana	1922-2011	39	1968-Med.
7	Otto Loewi	1873-1961	88	1936-Med.
8	Marianne Grunberg-Manago	1921-2013	40	
9	Christian de Duve	1917-2013	54	1974-Med.
10	Otto Warburg	1883-1970	78	1931-Med.
11	Philip Siekevitz	1918-2009	43	
12	Paul Zamecnik	1912-2009	49	
13	Mahlon Hoagland	1921-2009	40	

2

BACKGROUND
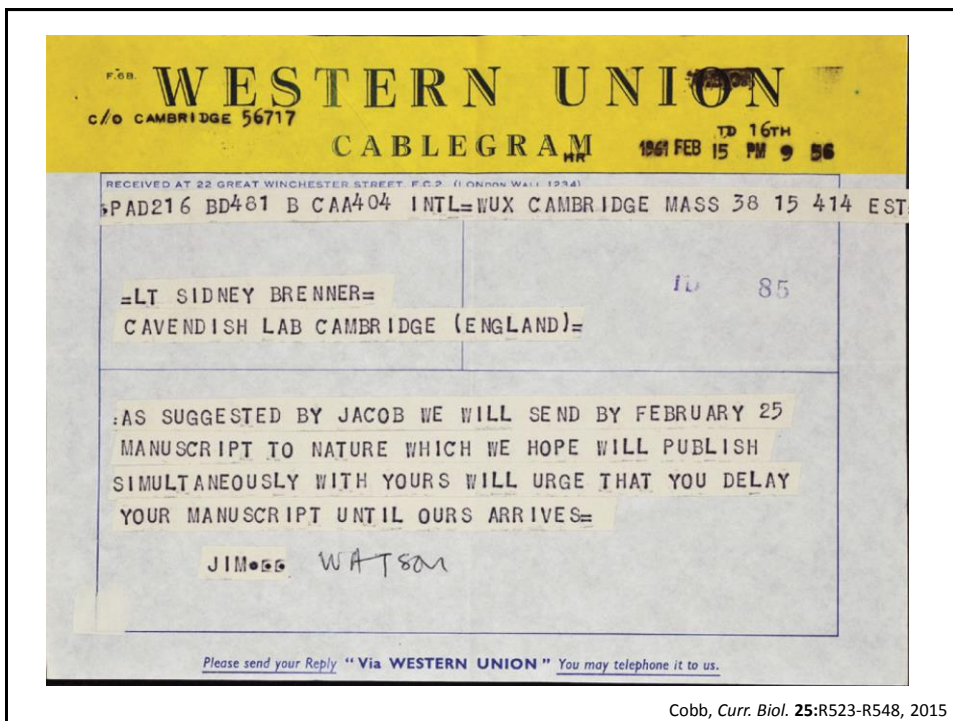
First Ideas about the code

3

In the comparative isolation of Cambridge I must confess that there are times when I have no stomach for decoding.

Francis Crick *early 1955*

Sydney Brenner coined the term "codon" — the trinucleotide unit that specifies one amino acid.
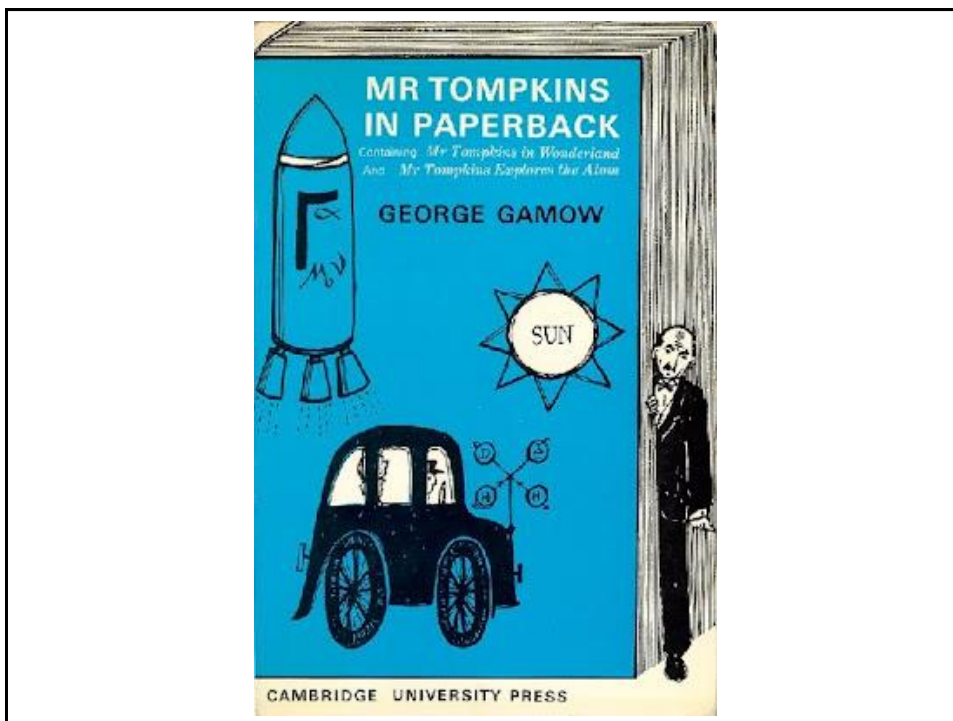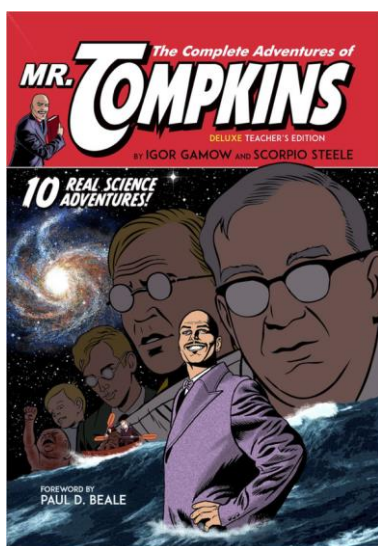
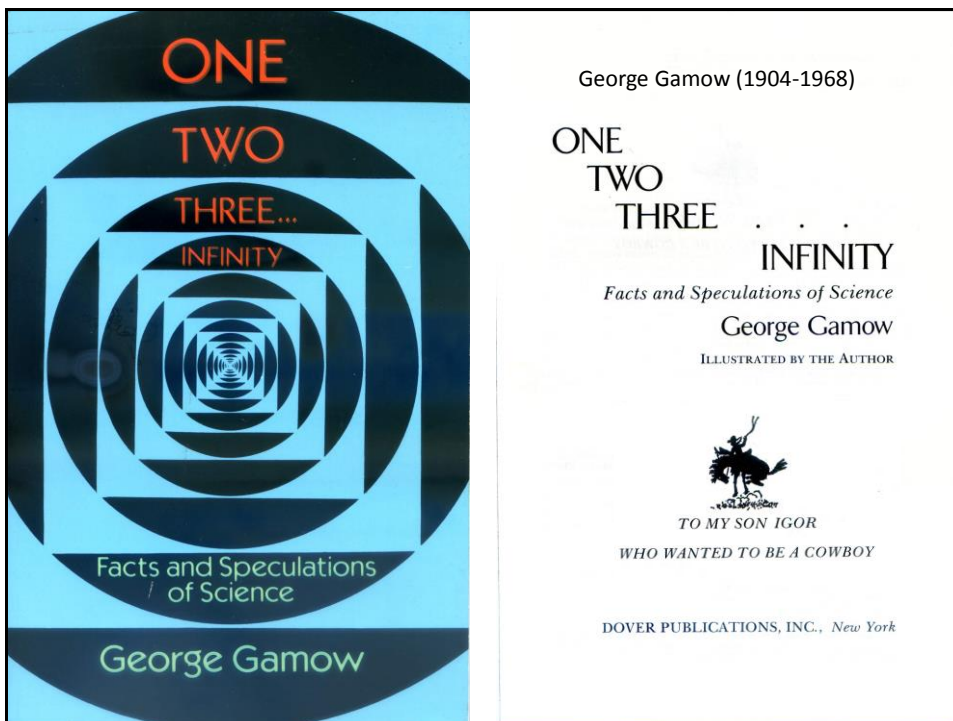4

Cobb, *Curr. Biol.* **25:**R523-R548, 2015

5

Georgiy Antonovich Gamov *aka* George Gamow (1904-1968)
Russian-American physicist: developed Big Bang theory

6

7

https://bit.ly/3UBtlmN

8
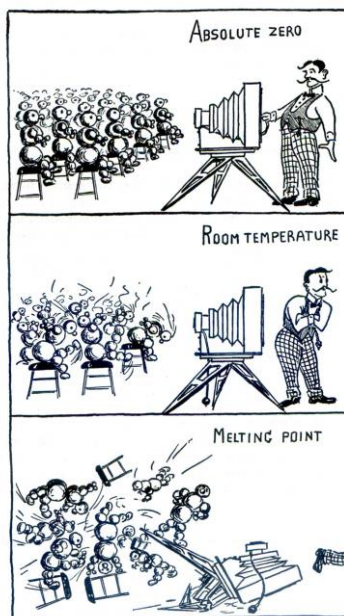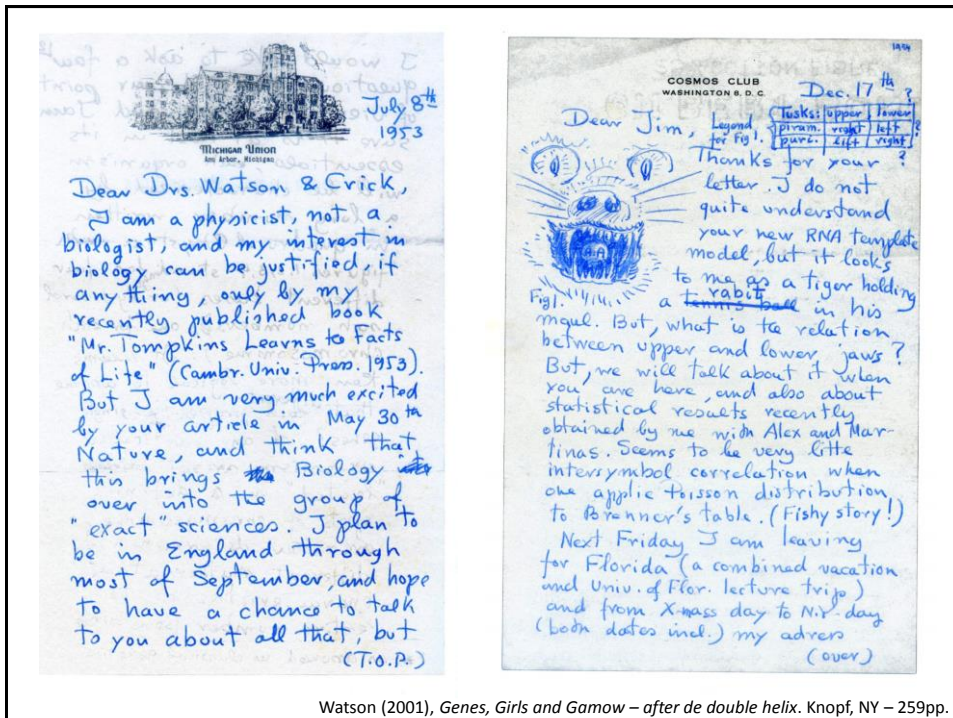
George Gamow (1904-1968)

9

One of the 128 drawings that illustrate the book "1, 2, 3… infinity", by G. Gamow

10

Watson (2001), *Genes, Girls and Gamow – after de double helix*. Knopf, NY – 259pp.
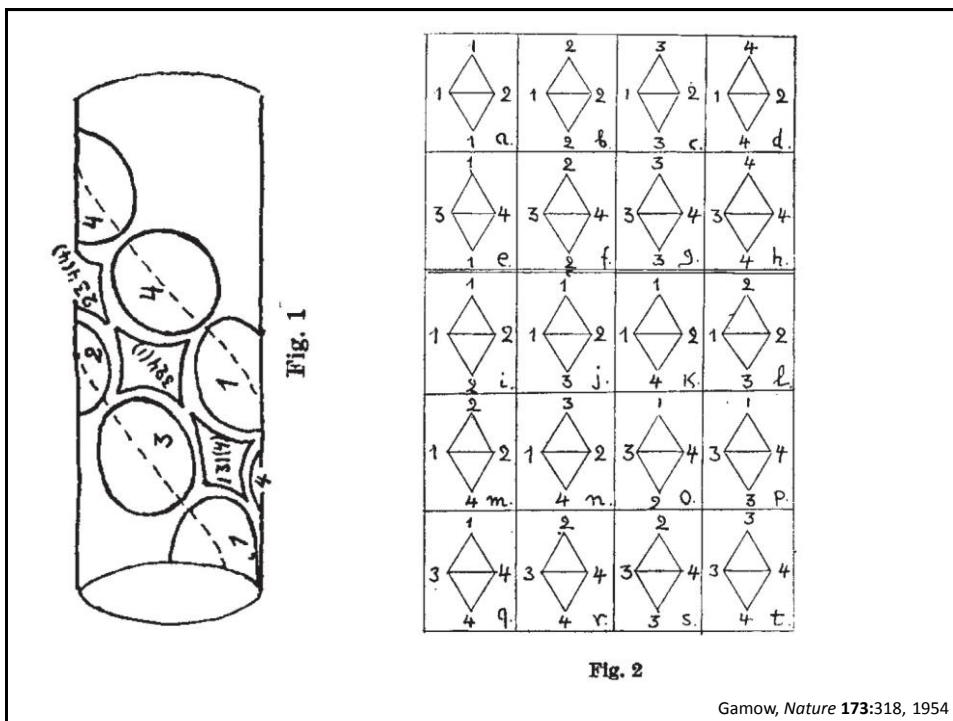
11

Hypothesis of George Gamow for the coding of proteins in DNA

Possible Relation between Deoxyribonucleic Acid and Protein Structures

In a communication in *Nature* of May 30, p. 964, J. D. Watson and F. H. C. Crick showed that the molecule of deoxyribonucleic acid, which can be considered as a chromosome fibre, consists of two parallel chains formed by only four different kinds of nucleotides. These are either (1) adenine, or (2) thymine, or (3) guanine, or (4) cytosine with sugar and phosphate molecules attached to them. Thus the hereditary properties of any given organism could be characterized by a long number written in a four-digital system. On the other hand, the enzymes (proteins), the composition of which must be completely determined by the deoxyribonucleic acid

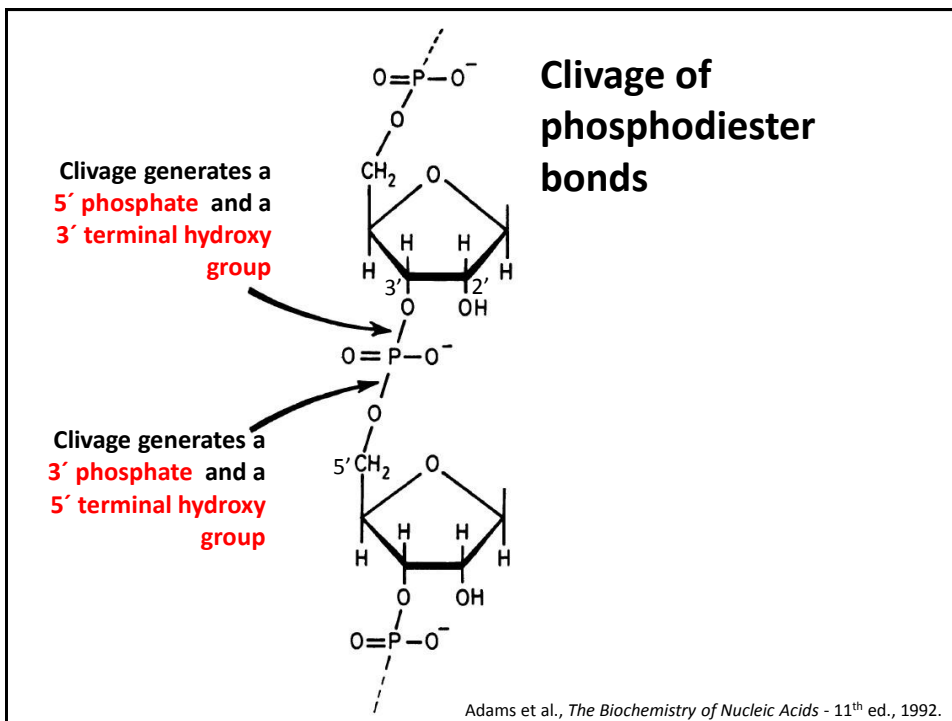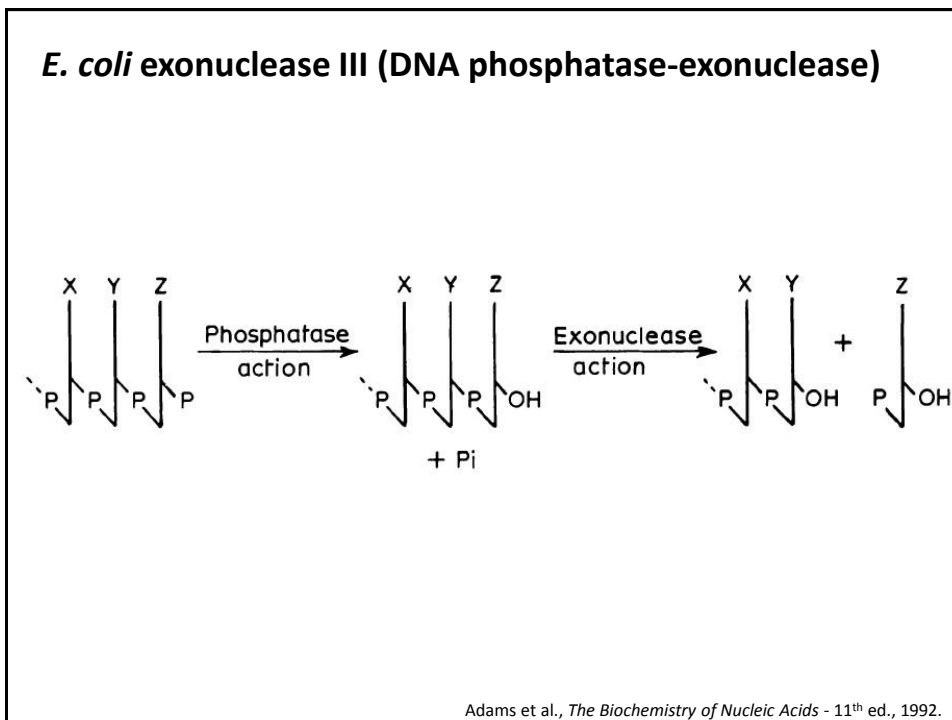Gamow, *Nature* **173:**318, 1954

12

Fig. 1

Fig. 2

Gamow, *Nature* **173:**318, 1954

13

BACKGROUND

A pinch of chemistry

14

Clivage of phosphodiester bonds

Clivage generates a **5´ phosphate** and a **3´ terminal hydroxy group**

Clivage generates a **3´ phosphate** and a **5´ terminal hydroxy group**

Adams et al., *The Biochemistry of Nucleic Acids* - 11th ed., 1992.

15

E. coli exonuclease III (DNA phosphatase-exonuclease)

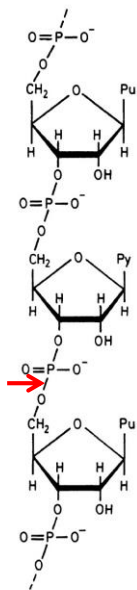Adams et al., *The Biochemistry of Nucleic Acids* - 11th ed., 1992.

16

Action of pancreatic RNase on RNA

Adams et al., *The Biochemistry of Nucleic Acids* - 11th ed., 1992.
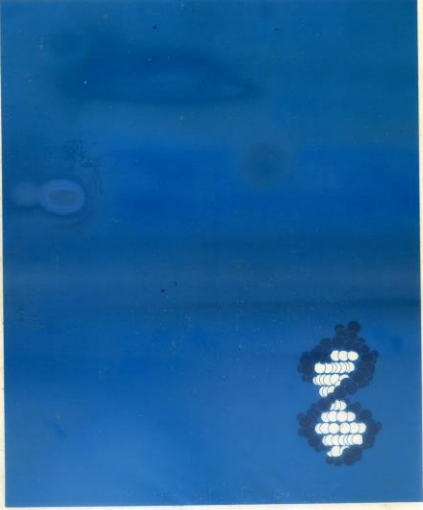
17

BACKGROUND

The code in 1965

18

19

20

TABLE 13–1 *The 64 possible three-letter codons*

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

Watson (1965) *Molecular Biology of the Gene* – 1a. Edição - Benjamin

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POLY U CODES FOR POLYPHENYLALANINE

Poly U was the first synthetic polyribonucleotide discovered to have mRNA activity. None of its bases are normally hydrogen bonded in solution, and it binds well to free ribosomes. It selects phenylalanine sRNA molecules exclusively, thereby forming a polypeptide chain containing only phenylalanine (polyphenylalanine). Thus we know that a codon for phenylalanine is composed of a group of three uridylic acid residues (UUU) (the group number 3 comes from the genetic experiments described in Chapter 9). Similarly, we are able tentatively to assign (CCC) as a proline codon and (AAA) as a lysine codon on the basis of analogous experiments with poly C and poly A. Unfortunately, the guanine residues in poly G firmly hydrogen bond to each other and form multistranded triple helices that do not bond to ribosomes. Thus this type of experiment cannot tell us whether (GGG) is a functional codon.

Watson (1965) *Molecular Biology of the Gene* – 1a. Edição - Benjamin

22

374   MOLECULAR BIOLOGY OF THE GENE

TABLE 13–5   *The genetic code as of May, 1965*[a]

First position (5′ end)	Second position				Third position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Nonsense		A
	Leu	Ser	Nonsense	Try	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
		Pro	GluN	Arg	A
		Pro	GluN	Arg	G
A	Ileu	Thr	AspN	Ser	U
	Ileu	Thr	AspN	Ser	C
	Ileu	Thr	Lys	Arg	A
	Meth	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

[a] The nucleotide order within nonunderlined codons is firmly established. Codons underlined are given on a tentative basis.
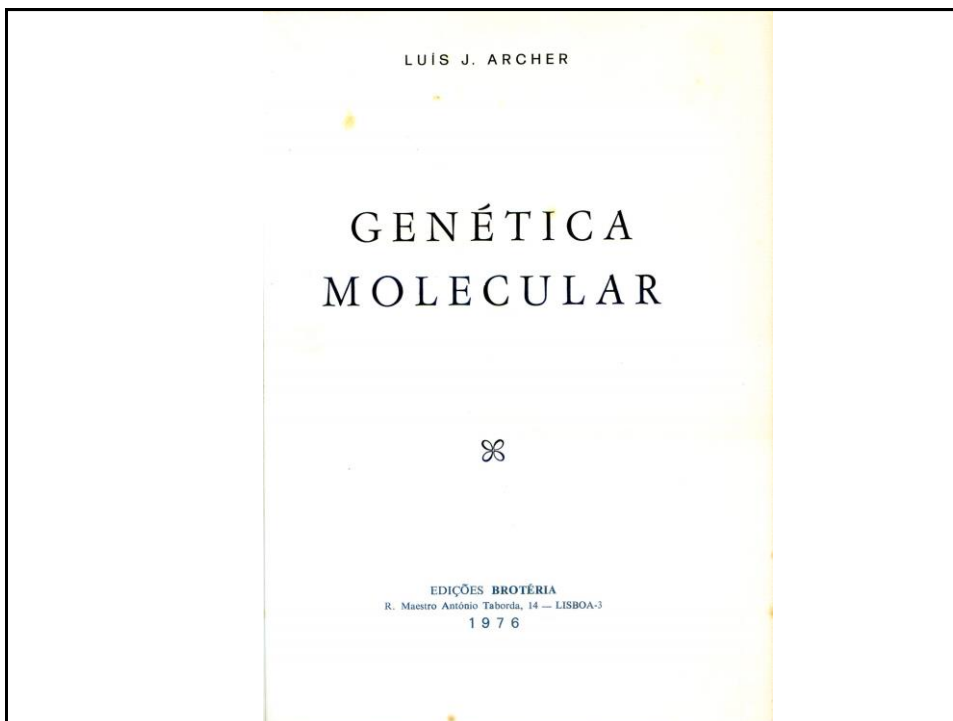
Watson (1965) *Molecular Biology of the Gene* – 1a. Edição - Benjamin

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BACKGROUND

The Code in 1976

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LUÍS J. ARCHER

GENÉTICA MOLECULAR

✿

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CAPÍTULO VI

O CÓDIGO INFORMATIVO

Mesmo depois de se conhecer, nas suas linhas gerais, o mecanismo pelo qual o DNA se transcreve e traduz, ainda era pouco mais que um sonho a pretensão de decifrar em termos químicos o código exacto segundo o qual cada aminoácido é arquivado no DNA.

Entre os anos 60 e 70 esse sonho tornou-se realidade, e vamos percorrer sumariamente o fascinante processo científico que conduziu à revelação do mais íntimo segredo do DNA.

1. O codão é um tripleto não-sobreposto e não-virgulado

a) São 20 os aminoácidos que, em combinações e proporções diferentes, entram na constituição das proteínas dos seres vivos. Por outro lado, são 4 as bases do DNA e do RNA. O número de arranjos completos das 4 bases tomadas duas a duas seria apenas 16 (insuficiente, portanto, para a codificação específica dos aminoácidos). O número de arranjos completos das 4 bases, tomadas 3 a 3, é de 64 (mais que suficiente para os aminoácidos existentes). Estas considerações foram as primeiras a sugerir que a unidade de codificação, ou **codão**, seja formada por 3 nucleotídeos («tripleto»).

b) A hipótese do código **sobreposto** («overlapping code») admitia que na série de bases os tripletos poderiam ser, tal como vai indicado,

A B C D E F

ABC, BCD, CDE, etc.

Se assim fosse,

— deveria verificar-se uma frequente associação dos aminoácidos que se encontram lado a lado nas proteínas, e isso não se verifica.

— a alteração de uma base deveria alterar mais do que um aminoácido. Isto também não se verifica. Conhecem-se, há muito, vários

QUADRO VIII

O CÓDIGO GENÉTICO

SEGUNDA BASE

		U	C	A	G		
P R I M E I R A B A S E	U	UUU UUC } fen UUA UUG } leu	UCU UCC UCA UCG } ser	UAU UAC } tir UAA — UAG —	UGU UGC } cis UGA — UGG : trp	U C A G	**T E R C E I R A B A S E**
	C	CUU CUC CUA CUG } leu	CCU CCC CCA CCG } pro	CAU CAC } his CAA CAG } gln	CGU CGC CGA CGG } arg	U C A G	
	A	AUU AUC } ile AUA AUG : met	ACU ACC ACA ACG } tre	AAU AAC } asn AAA AAG } lis	AGU AGC } ser AGA AGG } arg	U C A G	
	G	GUU GUC GUA GUG } val	GCU GCC GCA GCG } ala	GAU GAC } asp GAA GAG } glu	GGU GGC GGA GGG } gli	U C A G	

7

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BACKGROUND

Har Gobind Khorana

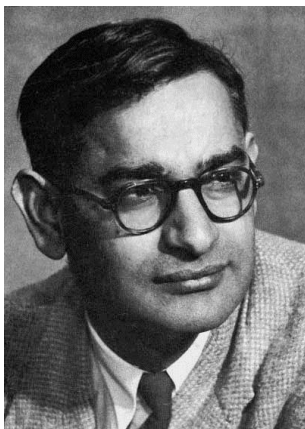**"We must be modest except in our aims"**
Otto Loewi cited by Khorana

27

Har Gobind Khorana (1922-2011)
هار گوبند خورانا

NPW 1968

Master in Chemistry at the University of Punjab in Lahore

Doctorate fellowship (PhD) in organic chemistry at the University of Liverpool, England.

Doctor degree obtained in 1948

Works at the ETH (Eidgenössische Technische Hoshschule) in Zurich - group of Vladimir Prelog (NPW 1975)

Finds the little known paper published by Fritz Zetzsche about the carbodiimides -> will latter use the method for the synthesis of nucleotide cofactors and ATP.

28

A year later runs out of money Zurich.

Fails at concourse in India and obtains a three year fellowship with Alexander Todd, University of Cambridge.

Is introduced to the results obtained by Sanger, Perutz, Kendrew and falls in love with Molecular Biology.

1952 - begins an independent career as non-academic researcher at the "British Columbia Research Council" in Vancouver, Canada. Synthesizes ATP and nucleotide cofactors

1960-1970. Moves to the Institute for Enzyme Research at the University of Wisconsin in Madison.

Shows that CUCUCU codes for a polypeptide with Leucine (CUC) and Serine (UCU).

Synthesizes the gene for tRNA$_{Ala}$ with the regulatory regions and shows that it functions inside a bacterial cell.

Leaves Madison and goes to the MIT where he works with bacteriorhodopsin.

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H. Gobind Khorana   F. Crick   M. Grunberg-Manago

Cold Spring Harbor Symposium on the Genetic Code - 1966

30

Marshall Nirenberg   **H. Gobind Khorana**

2009

31

Chemical synthesis of dinucleotides - Khorana

J. Am. Chem. Soc. **80:**6212–6222, 1958

[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Studies on Polynucleotides. I. A New and General Method for the Chemical Synthesis of the $C_5'-C_3'$ Internucleotidic Linkage. Syntheses of Deoxyribo-dinucleotides[1]

BY P. T. GILHAM AND H. G. KHORANA

RECEIVED APRIL 14, 1958

A new method has been developed for the specific synthesis of the naturally-occurring $(C_5'-C_3')$ internucleotidic linkage; it involves reaction of a suitably protected deoxynucleotide with a second protected deoxy-nucleoside or -nucleotide in the presence of dicyclohexylcarbodiimide or *p*-toluenesulfonyl chloride. By this approach the three dinucleoside phosphates VIIa, VIIb and VIIc have been prepared in good yield. Procedures are described for the synthesis of deoxyribo-dinucleotides bearing 5'- or 3'-phosphoryl end-groups; these are illustrated by the synthesis of the two isomeric dithymidine dinucleotides (XII and XIV), and a mixed dinucleotide (XVI) containing the nucleosides, deoxyadenosine and thymidine. The results of enzymic and acidic degradative experiments are recorded and these provide additional characterization of the synthetic compounds. Some general observations on the scope and mechanism of this method of "phosphodiester" synthesis also are included.
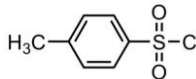
32

Synthesis of polynucleotides by the method developed by Khorana
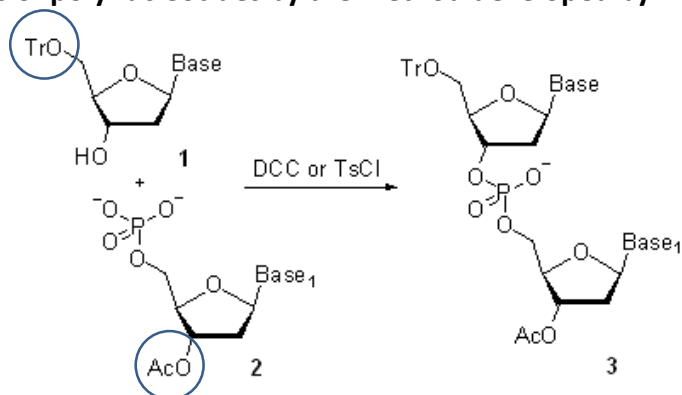
DCC = N,N'-dicyclohexylcarbodiimide
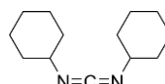
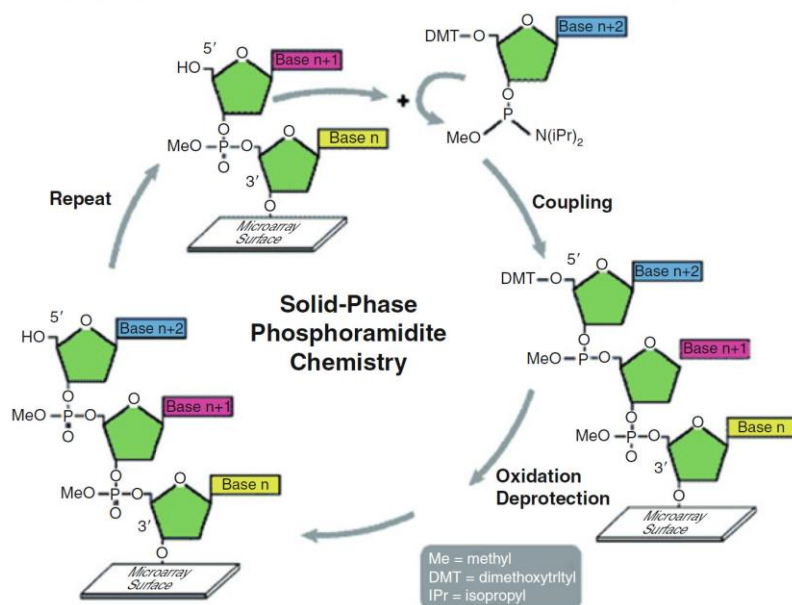TsCl = 4-toluenesulfonyl chloride

Same strategy that was already used for the synthesis of peptides in 1958

Gilham & Khorana, *J. Amer. Chem. Soc.* **80:**6212-6222, 1958.
https://en.wikipedia.org/wiki/Oligonucleotide_synthesis

33

Synthesis of oligonucleotides today

Solid-Phase Phosphoramidite Chemistry

Me = methyl
DMT = dimethoxytrityl
IPr = isopropyl

34

Studies on Polynucleotides

XCVI.† Repair Replication of Short Synthetic DNA's as catalyzed by DNA Polymerases

K. KLEPPE,‡ E. OHTSUKA,§ R. KLEPPE,‡ I. MOLINEUX ∥
AND H. G. KHORANA ∥

Institute for Enzyme Research of the University of Wisconsin
Madison, Wisc. 53706, U.S.A.

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The principles for extensive synthesis of the duplexed tRNA genes which emerge from the present work are the following. The DNA duplex would be denatured to form single strands. This denaturation step would be carried out in the presence of a sufficiently large excess of the two appropriate primers. Upon cooling, one would hope to obtain two structures, each containing the full length of the template strand appropriately complexed with the primer. DNA polymerase will be added to complete the process of repair replication. Two molecules of the original duplex should result. The whole cycle could be repeated, there being added every time a fresh dose of the enzyme. It is however, possible that upon cooling after denaturation of the DNA duplex, renaturation to form the original duplex would predominate over the template-primer complex formation. If this tendency could not be circumvented by adjusting the concentrations of the primers, clearly one would have to resort to the separation of the strands and then carry out repair replication. After every cycle of repair replication, the process of strand separation would have to be repeated. Experiments based on these lines of thought are in progress.

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Issue nr 2 of vol. 72 (1972) of JMB had 13 papers by Khorana´s group on the synthesis of tRNA$_{Ala}$

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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 240, No. 5, May 1965
Printed in U.S.A.

1965

Nucleotide Sequences in the Yeast Alanine Transfer Ribonucleic Acid*

ROBERT W. HOLLEY, GEORGE A. EVERETT, JAMES T. MADISON, AND ADA ZAMIR

From the United States Plant, Soil, and Nutrition Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, United States Department of Agriculture, and the Department of Biochemistry, Cornell University, Ithaca, New York

(Received for publication, November 27, 1964)

The purification of the yeast alanine-, tyrosine-, and valine-transfer ribonucleic acids by countercurrent distribution has been described (1), and preliminary data have been reported on the oligonucleotide compositions of these RNAs (2, 3). The present paper summarizes results of attempts to account quantitatively for all of the fragments obtained by digestion of the alanine-RNA with pancreatic RNase and with Taka-Diastase RNase T1. Results of the analyses of these two digests are consistent, one with the other, and indicate that the alanine-RNA is composed of 77 nucleotides, including nine unusual nucleotides.

water. The column was then eluted with a continuous gradient produced from 240 ml of 0.04 M, 238 ml of 0.20 M, and 228 ml of 1.0 M ammonium carbonate in three chambers of a Varigrad (4). Fractions of approximately 3.4 ml were collected. A flow rate of approximately 10 ml per hour was obtained by placing the Varigrad 20 feet (6 meters) above the top of the column.

Chromatography of Pancreatic RNase Digest on DEAE-cellulose in 7 M Urea (5)—DEAE-cellulose (Carl Schleicher and Schuell, No. 70, standard) was washed thoroughly with 7 M urea and with sodium acetate in 7 M urea. A column (0.35 × 30 cm) was packed by gravity from a suspension of the DEAE-cellulose

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Studies on Polynucleotides†

CIII.‡ Total Synthesis of the Structural Gene for an Alanine Transfer Ribonucleic Acid from Yeast

H. G. Khorana[a], K. L. Agarwal[a], H. Büchi[b], M. H. Caruthers[a],
N. K. Gupta[c], K. Kleppe[d], A. Kumar[e], E. Ohtsuka[f],
U. L. RajBhandary[a], J. H. van de Sande[a], V. Sgaramella[g],
T. Terao[h], H. Weber[i] and T. Yamada[j]

*Institute for Enzyme Research of the University of Wisconsin and the
Departments of Biology and Chemistry, Massachusetts Institute of
Technology, Cambridge, Mass. 02139, U.S.A.*

(*Received 9 December 1971*)

A plan for the total synthesis of the DNA duplex, 77 nucleotide units long, corresponding in sequence to the major yeast alanine transfer RNA, is formulated. The plan involves: (a) the chemical synthesis of 15 polydeoxynucleotide segments ranging in length from five to 20 nucleotide units and (b) ligase-catalyzed covalent joining of several segments to form three parts of the duplex, followed by joining of the three parts to construct the entire duplex. Twelve accompanying papers describe the experimental realization of this objective.

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FRACIONAMENTO CELULAR
(MONTANDO O CENÁRIO)

41

42

Subcellular fractionation through Differential Centrifugation
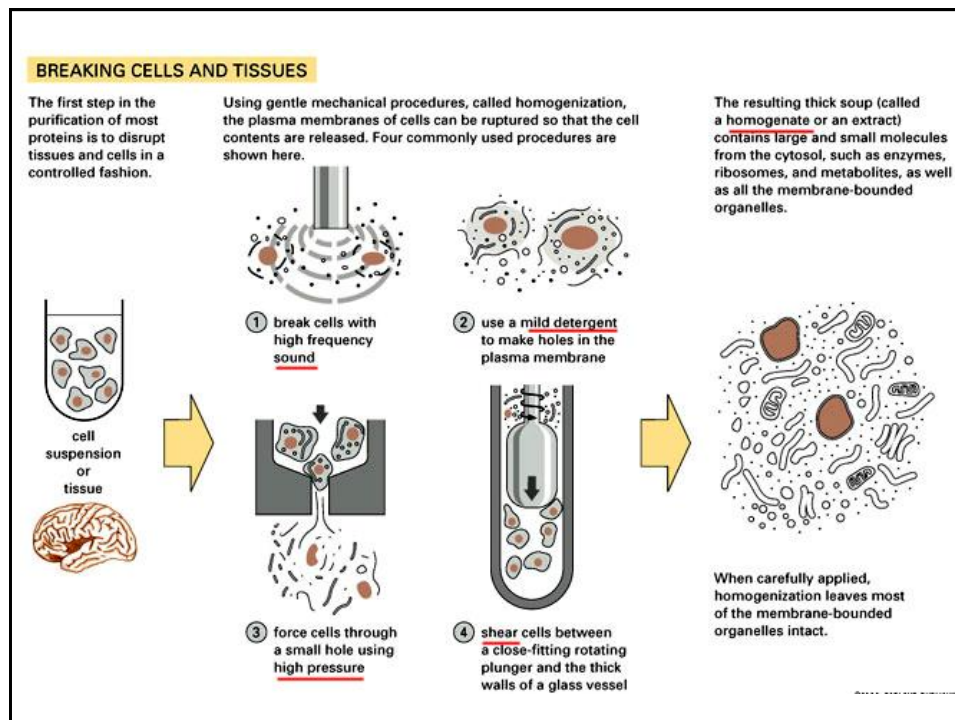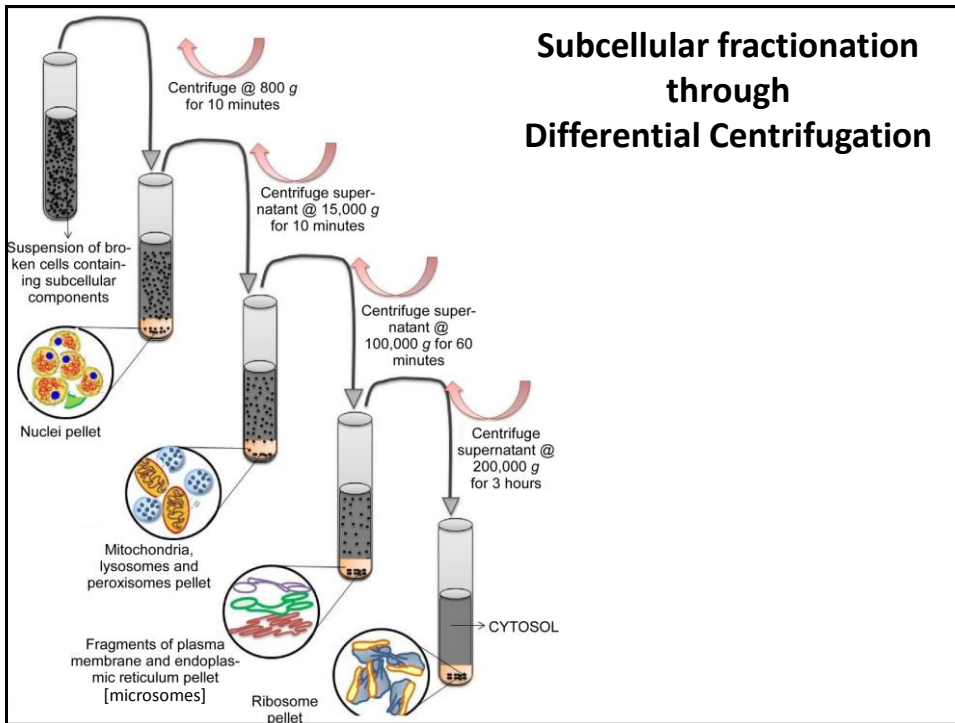
43

Otto Warburg manometric apparatus for metabolic analysis

Otto Warburg

Different manometric arrangements

1931

1970

Sample Flask

44

Warburg flask
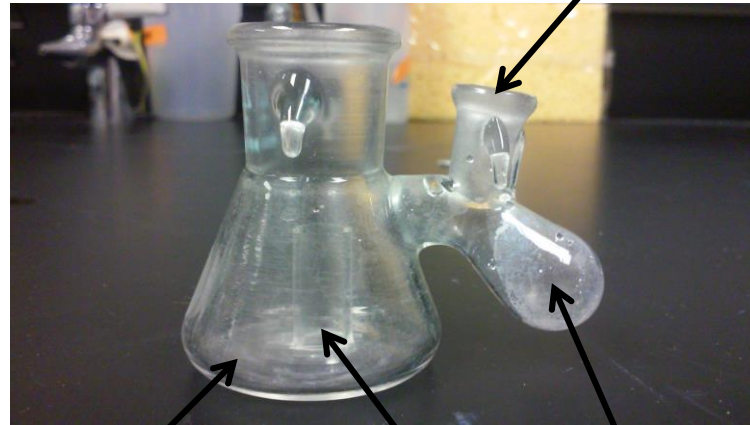
Substrate(s), etc. added here to star reaction

Tissue sample or subcellular fraction to be tested

CO_2 trap

Additional trap

45

Stand for mounting the manometers

46

Original Warburg "machine"

Different samples

Water bath for temperature control

MOVEMENT

Electric motor for sample shaking in the water bath

47

"Modern" Warburg manometers and water bath

48

Setting up a "modern" Warburg apparatus with several samples

49

Sir Hans Krebs (NPW 1953)(1900-1981) and his students

50
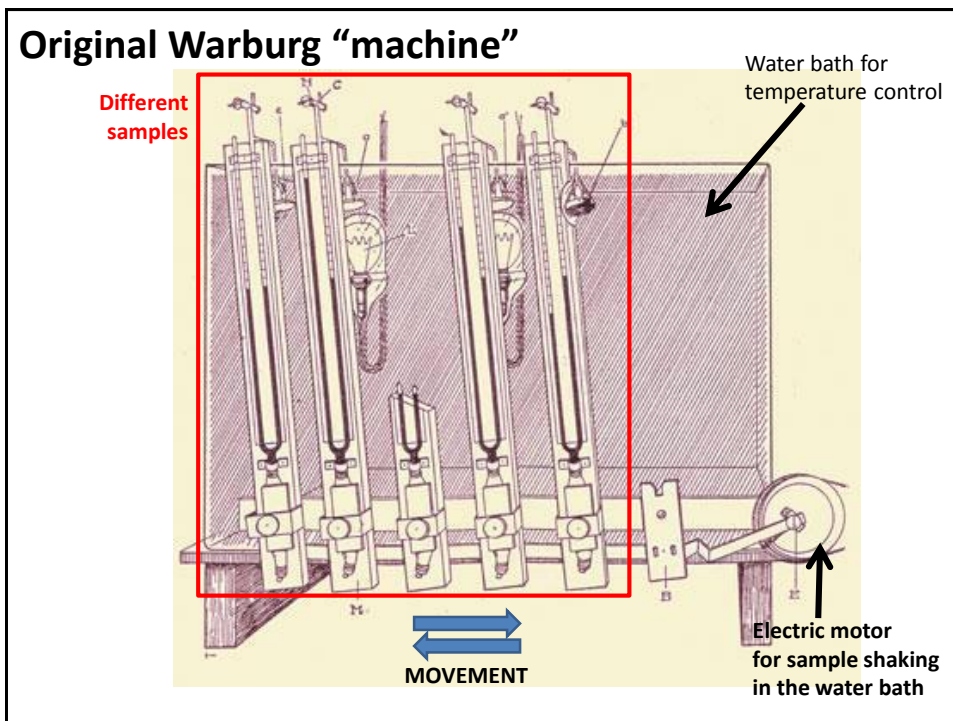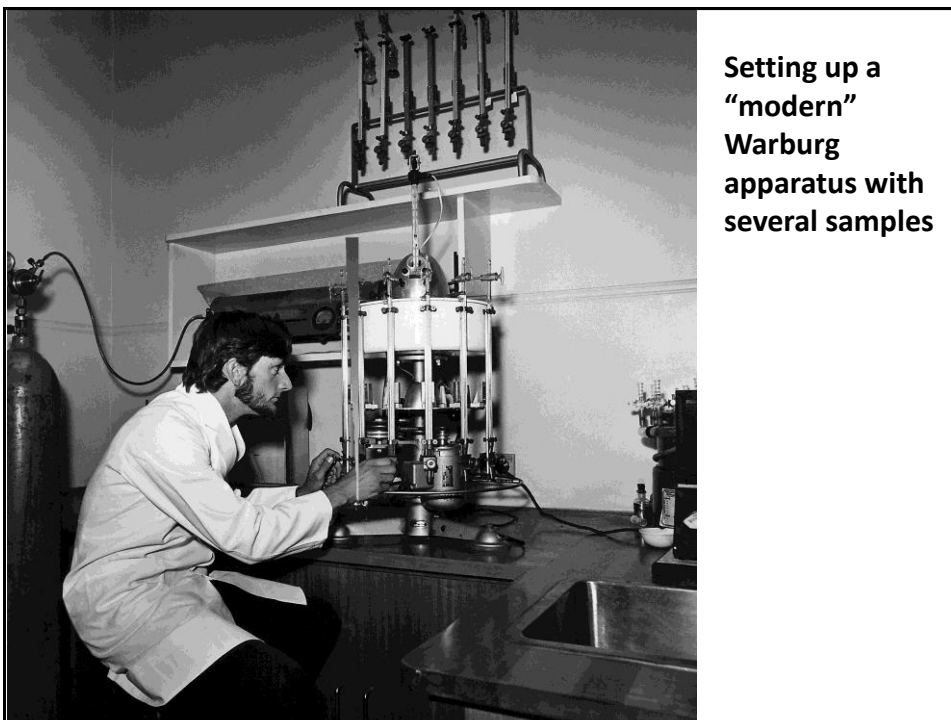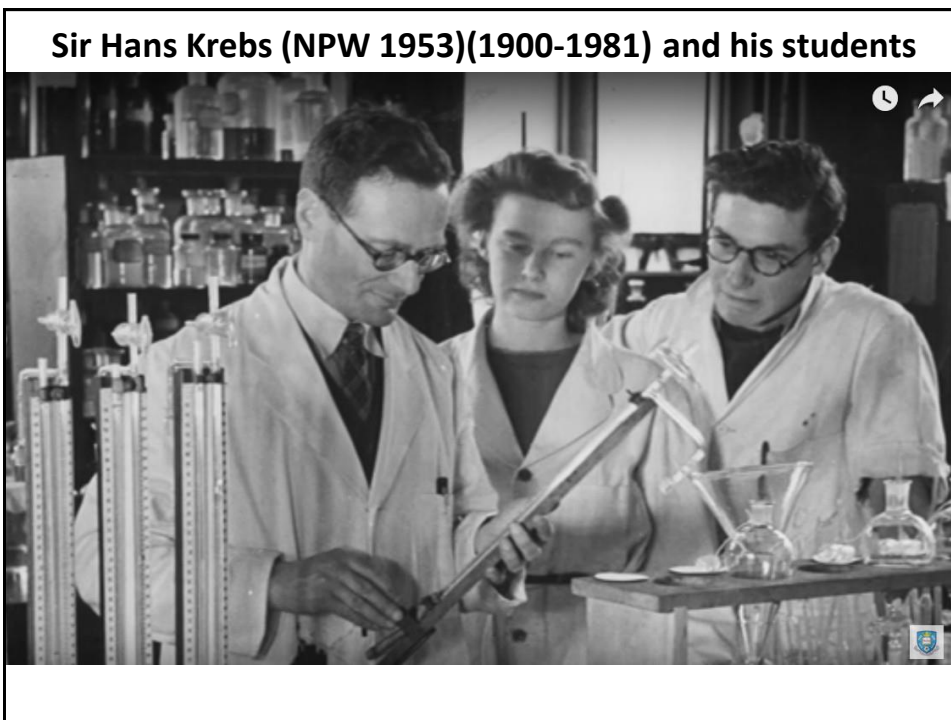
Some movies in YouTube about the Warburg apparatus setup and use

Warburg Manometer setup
https://www.youtube.com/watch?v=M-HYbZwN43o
https://www.youtube.com/watch?v=TI41djHlh_o

51

Incorporation of radioactive alanine into the proteins of the various fractions of a rat liver homogenate (1952)
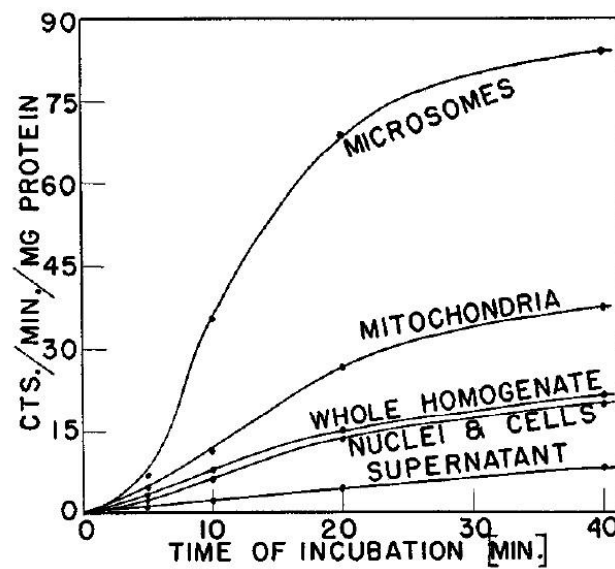
Siekevitz J. Biol. Chem. **195:**549-565, 1952

52

Relation between metabolism of phosphorilated compounds (ATP-like) and protein synthesis

RAT LIVER

Fraction	Protein per 100 mg. wet weight tissue	Per cent weight	O₂ per hr.	Q_{O_2}	PO₄ esterified per 5 min.	α-Keto-glutarate disappearing per 60 min.	C.p.m. per mg. protein	
							Minus α-keto-glutarate	Plus α-keto-glutarate
	mg.		micro-atoms		μM	μM		
Homogenate.....................	17.6	100	37.3	24	3.6	19.1	1.4	10.8*
Nuclei + cells....................	3.3	18	1.6	5	0.0		1.2	2.9
Mitochondria....................	2.5	15	7.3	36	3.4	4.0	0.9	1.3
Mixed fraction..................	2.2	14	1.1	6	0.0		1.7	1.1
Microsomes.....................	1.5	12	0.1	1	0.0	0.0	1.6	1.0
Supernatant....................	7.8	40	1.2	1	0.0	0.0	0.1	0.4
Mitochondria + microsomes......	4.0	24	13.8	39	3.0	6.6	1.1	4.2
" + supernatant.....	10.3	55	17.1	19	4.6	7.9	1.0	6.6
" + microsomes + supernatant	11.8	64	20.8	20	3.5	10.7	0.8	9.8
All fractions	17.3	98	38.9	25	3.4	18.8	0.8	10.5

* 0.012 μM of L-alanine per gm. of protein per 30 minutes.

Siekevitz *J. Biol. Chem.* **195:**549-565, 1952

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Biochemical and molecular biological sketches for protein synthesis
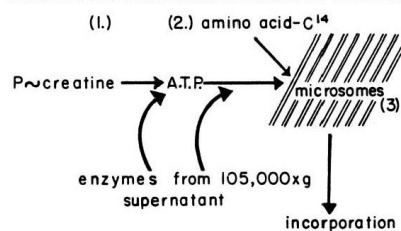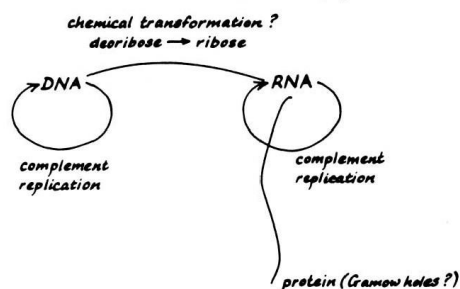
Darden & Craver, *Stud. Hist. Phil. & Biomed. Sci.* **33:**1-28, 2002

54

Mahlon Hoagland & Paul Zamecnik, ca. 1984

55

Fracionamento e recombinação do extrato de fígado de rato
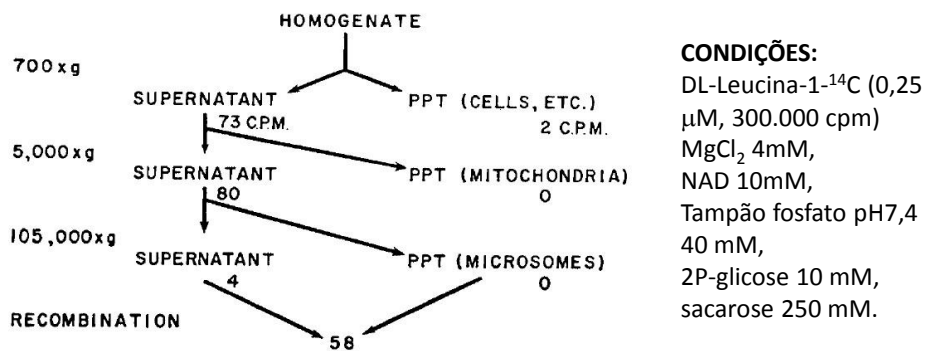
CONDIÇÕES:
DL-Leucina-1-^{14}C (0,25 µM, 300.000 cpm)
$MgCl_2$ 4mM,
NAD 10mM,
Tampão fosfato pH7,4 40 mM,
2P-glicose 10 mM,
sacarose 250 mM.

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Dissection of the rat-liver cellfree system for the incorporation of [^{14}C]-amino acids into protein

I.

HOMOGENATE

aa* ⟶ protein*

end of the 1940s

II.

SOLUBLE ENZYMES
aa*

ATP ⟶

MICROSOMES
protein*

ca. 1952

III.

pH 5 ENZYMES
ATP + E + aa* ⇌
E(AMP–aa*) + PP

GTP ⟶

RNP PARTICLE
protein*

ca. 1955

IV.

pH 5 ENZYMES
ATP + E + aa* ⇌
E(AMP–aa*) + PP

⟶ SOLUBLE RNA
RNA–aa*

GTP

RNP PARTICLE
protein*

end of 1950s

Hoagland, *Rec. Trav. Chim.* **77:**623-633, 1958
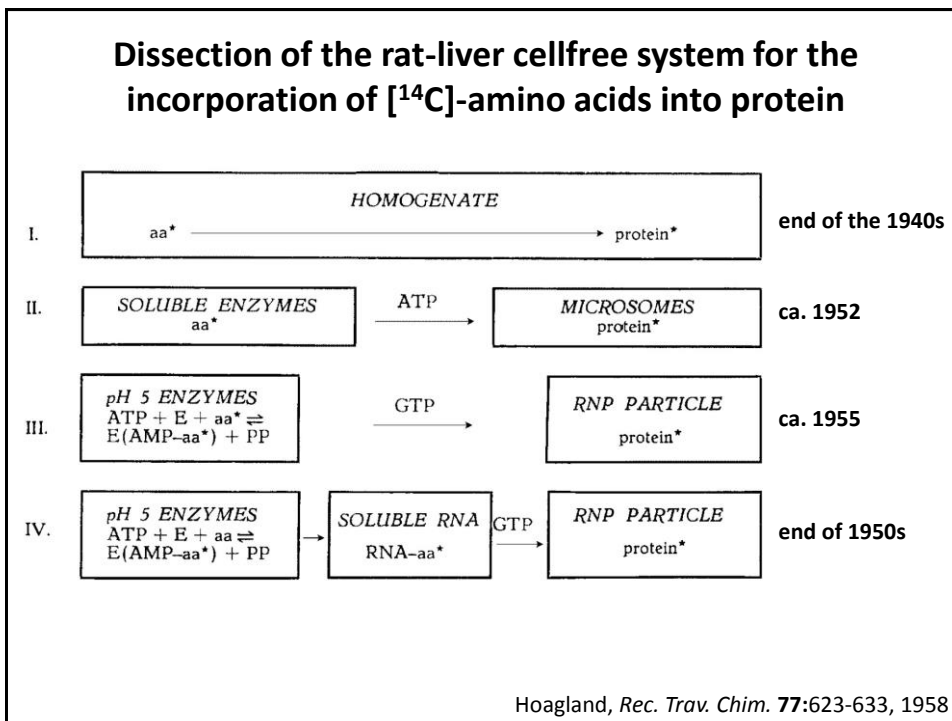
57

NIRENBERG et al.

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Marshall Nirenberg

1927 – is born on April 10th in NYC

**1941 – Rheumatic fever ->
moves to Orlando, FL**

1948 - BS (Zoology and Chemistry)

1952 - MS (Zoology)

1957 - PhD (Biological Chemistry)

1959-60 - Postdoc

2002

**1961 - Describes the experiment with poly U in Moscow at
the International Congress of Biochemistry (August)
(Nirenberg & Matthaei)**

1963-66 - Completes the genetic code
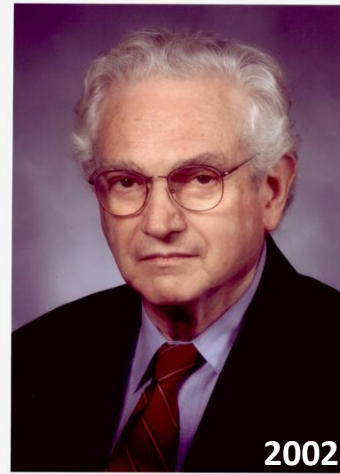
59

1965-69 – Go to study neurobiology

1967 – begins the study of neuroblastoma.

1968 – receives the Nobel Prize with RW Holey e HG Khorana

**1969 - publishes his first paper on neurobiology with Philip
Nelson.**

1973 – Studies the efect of morphine in the Nervous System

**1976 - Begins the work with receptors of nerve cells in retina
of chicks.**

2010 - dies on January 15

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President Arthur Costa E Silva
Palacio Da Alvorada
Brasilia

Dear Mister President:

The recent decision of your government to remove a number of Brazil's leading scientists and scholars from their University positions is a matter of grave concern to us. We feel that to deprive Brazil of the benefit of the intellecutal and scientific leadership provided by such internationally eminent scientists as Profs. Isaias Raw, Alberto Carvalho da Silva and Helio Lourenco deOliveira among others, will cause inestimable damage to the progress of science and education in Brazil. Moreover, the anti-intellectual image of the government, created by this unfortunate action, is certain to have serious repercussions among scientists in the world community, whose sympathy and cooperation is essential to the continued technological development of Brazil.

We appeal to you to look personally into this matter in order that these scholars are returned to their institutions and encouraged to pursue their work in a climate of intellectual freedom.

[signatures]

61

Marshall Nirenberg e Heinrich Matthaei – aprox. 1961

Primeiro pos-doc de Nirenberg com quem publicou o famoso experimento do poli-U

62
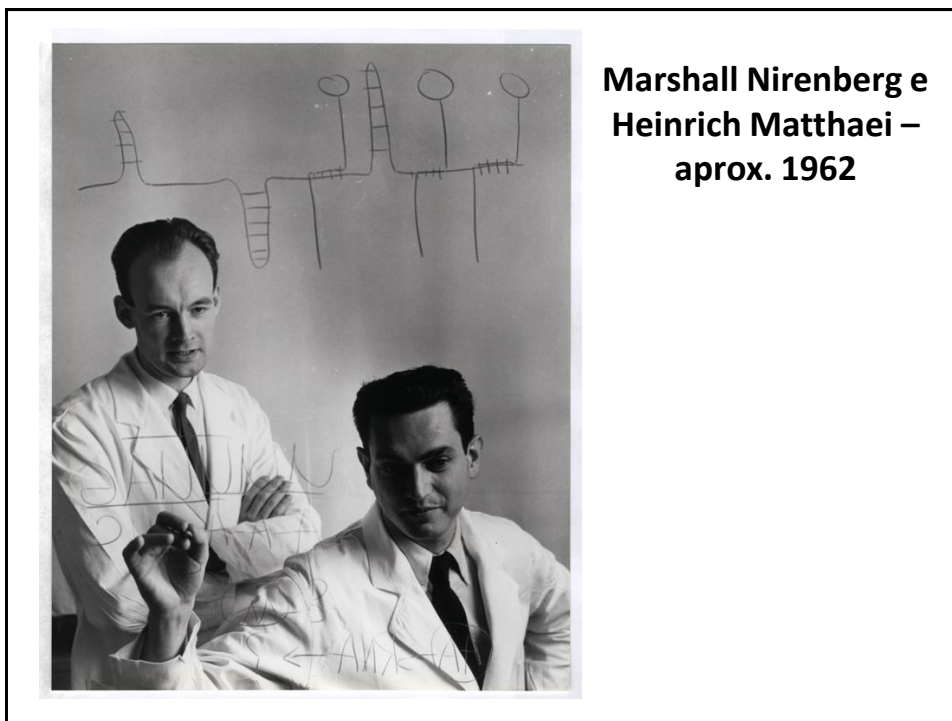
Marshall Nirenberg e Heinrich Matthaei – aprox. 1962

63

We have obtained a stable, cell-free E. coli system which

incorporates C14 into protein at a rapid rate, and which

has many characteristics of protein synthesis. Conditions have

been found which demonstrate a novel characteristic of this system;

that is, a requirement for RNA, needed even in the presence

of excess soluble RNA and ribosomes. Naturally occurring RNA,

a synthetic polynucleotide, were active in this system.

The synthetic polynucleotide appears to contain the code for the

synthesis of a protein containing only one amino acid.

First slide, please. Each reaction mixture contains these

constitutents in micromoles per milliliter. The enzyme extract

consisted of E. coli ribosomes and 100,000 x g supernatant solution.

Complete details are presented in Biochemical and Biophysical Research

Communications 4 (1961).

In slide No. 2, counts per minute per milligram protein is

plotted against time and minutes. In the absence of added DNAase,

valine was rapidly incorporated into protein. At the end of 90 minutes

Apresentação de Nirenberg em Moscou (Agosto de 1961) sobre a incorporação de ^{14}C-Phe num sistema "cell-free" com poli-U

64

**Apresentação de Nirenberg em
Moscou (Agosto de 1961) sobre a incorporação de ^{14}C-Phe
num sistema "cell-free" com poli-U**

65
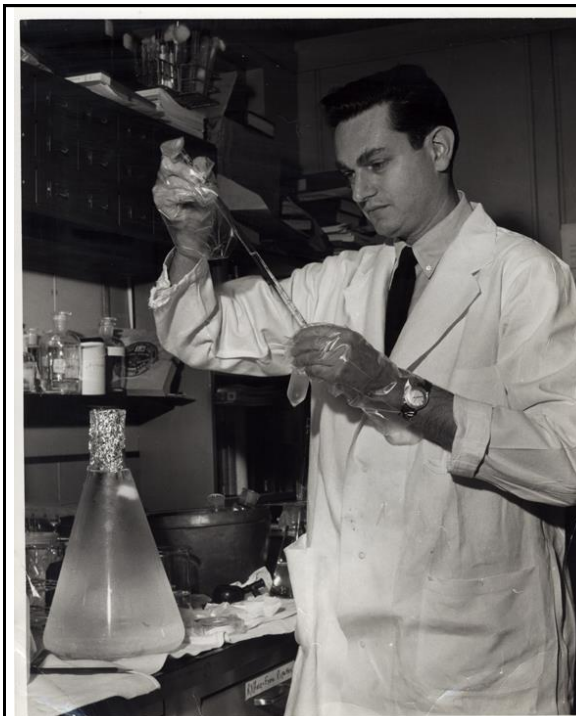
**Marshall Nirenberg
no laboratório –
aprox. 1962**

66

33

CSH Symposium on protein synthesis **1966**

Courtesy of Cold Spring Harbor Laboratory Archives. Noncommercial, educational use only.

Phil Leder discussing with coleagues

http://www.dnaftb.org/images/22/16501_leder2.jpg

67

Declaration of Marshall Nirenberg about the code deciphering

http://www.dnaftb.org/22/av.html

68

Codon usage table of *E. coli* based on all ORFs of the genome

CODON USAGE IN *E. COLI* GENES[1]

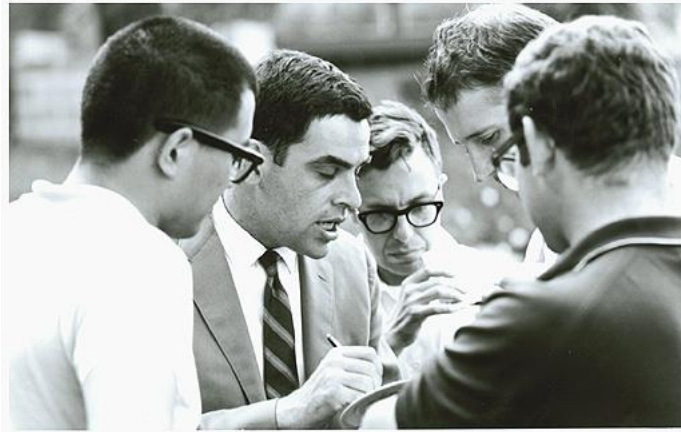|	Codon	Amino acid[2]	%[3]	Ratio[4]	Codon	Amino acid	%	Ratio	Codon	Amino acid	%	Ratio	Codon	Amino acid	%	Ratio	
U	UUU	Phe (F)	1.9	0.51	UCU	Ser (S)	1.1	0.19	UAU	Tyr (Y)	1.6	0.53	UGU	Cys (C)	0.4	0.43	U
	UUC	Phe (F)	1.8	0.49	UCC	Ser (S)	1.0	0.17	UAC	Tyr (Y)	1.4	0.47	UGC	Cys (C)	0.6	0.57	C
	UUA	Leu (L)	1.0	0.11	UCA	Ser (S)	0.7	0.12	UAA	STOP	0.2	0.62	UGA	STOP	0.1	0.30	A
	UUG	Leu (L)	1.1	0.11	UCG	Ser (S)	0.8	0.13	UAG	STOP	0.03	0.09	UGG	Trp (W)	1.4	1.00	G
C	CUU	Leu (L)	1.0	0.10	CCU	Pro (P)	0.7	0.16	CAU	His (H)	1.2	0.52	CGU	Arg (R)	2.4	0.42	U
	CUC	Leu (L)	0.9	0.10	CCC	Pro (P)	0.4	0.10	CAC	His (H)	1.1	0.48	CGC	Arg (R)	2.2	0.37	C
	CUA	Leu (L)	0.3	0.03	CCA	Pro (P)	0.8	0.20	CAA	Gln (Q)	1.3	0.31	CGA	Arg (R)	0.3	0.05	A
	CUG	Leu (L)	5.2	0.55	CCG	Pro (P)	2.4	0.55	CAG	Gln (Q)	2.9	0.69	CGG	Arg (R)	0.5	0.08	G
A	AUU	Ile (I)	2.7	0.47	ACU	Thr (T)	1.2	0.21	AAU	Asn (N)	1.6	0.39	AGU	Ser (S)	0.7	0.13	U
	AUC	Ile (I)	2.7	0.46	ACC	Thr (T)	2.4	0.43	AAC	Asn (N)	2.6	0.61	AGC	Ser (S)	1.5	0.27	C
	AUA	Ile (I)	0.4	0.07	ACA	Thr (T)	0.1	0.30	AAA	Lys (K)	3.8	0.76	AGA	Arg (R)	0.2	0.04	A
	AUG	Met (M)	2.6	1.00	ACG	Thr (T)	1.3	0.23	AAG	Lys (K)	1.2	0.24	AGG	Arg (R)	0.2	0.03	G
G	GUU	Val (V)	2.0	0.29	GCU	Ala (A)	1.8	0.19	GAU	Asp (D)	3.3	0.59	GGU	Gly (G)	2.8	0.38	U
	GUC	Val (V)	1.4	0.20	GCC	Ala (A)	2.3	0.25	GAC	Asp (D)	2.3	0.41	GGC	Gly (G)	3.0	0.40	C
	GUA	Val (V)	1.2	0.17	GCA	Ala (A)	2.1	0.22	GAA	Glu (E)	4.4	0.70	GGA	Gly (G)	0.7	0.09	A
	GUG	Val (V)	2.4	0.34	GCG	Ala (A)	3.2	0.34	GAG	Glu (E)	1.9	0.30	GGG	Gly (G)	0.9	0.13	G
		U				C				A				G			

69

Codon usage table of *E. coli* based on all ORFs of the genome

CODON USAGE IN *E. COLI* GENES[1]

	Codon	Amino acid[2]	%[3]	Ratio[4]	Codon	Amino acid	%	Ratio	Codon	Amino acid	%	Ratio	Codon	Amino acid	%	Ratio	
U	UUU	Phe (F)	1.9	**UUU = 0,51**		(S)	1.1	0.19	UAU	Tyr (Y)	1.6	0.53	UGU	Cys (C)	0.4	0.43	U
	UUC	Phe (F)	1.8	**UUC = 0,49**		(S)	1.0	0.17	UAC	Tyr (Y)	1.4	0.47	UGC	Cys (C)	0.6	0.57	C
	UUA	Leu (L)	1.0			Ser (S)	0.7	0.12	UAA	STOP	0.2	0.62	UGA	STOP	0.1	0.30	A
	UUG	Leu (L)	1.1	0.11	UCG	Ser (S)	0.8			OP	0.03	0.09	UGG	Trp (W)	1.4	1.00	G
C	CUU	Leu (L)	1.0	0.10	CCU	Pro (P)	0.7	**CCU = 0,16**		(H)	1.2	0.52	CGU	Arg (R)	2.4	0.42	U
	CUC	Leu (L)	0.9	0.10	CCC	Pro (P)	0.4	**CCC = 0,10**		(H)	1.1	0.48	CGC	Arg (R)	2.2	0.37	C
	CUA	Leu (L)	0.3	0.03	CCA	Pro (P)	0.8	**CCA = 0,20**		(Q)	1.3	0.31	CGA	Arg (R)	0.3	0.05	A
	CUG	Leu (L)	5.2	0.55	CCG	Pro (P)	2.4	**CCG = 0,55**		(Q)	2.9	0.69	CGG	Arg (R)	0.5	0.08	G
A	AUU	Ile (I)	2.7	0.47	ACU	Thr (T)	1.2			Asn (N)	1.6	0.39	AGU	Ser (S)	0.7	0.13	U
	AUC	Ile (I)	2.7	0.46	ACC	Thr (T)	2.4	0.43	AAC	Asn (N)	2.6	0.61	AGC	Ser (S)	1.5	0.27	C
	AUA	Ile (I)	0.4	0.07	ACA	Thr (T)	0.1	0.30	AAA	Lys (K)	3.8	**AAA = 0,76**		(R)	0.2	0.04	A
	AUG	Met (M)	2.6	1.00	ACG	Thr (T)	1.3	0.23	AAG	Lys (K)	1.2	**AAG = 0,24**		(R)	0.2	0.03	G
G	GUU	Val (V)	2.0	0.29	GCU	Ala (A)	1.8	0.19	GAU	Asp (D)	3.3	0.59	GGU	Gly (G)	2.8	0.38	U
	GUC	Val (V)	1.4	0.20	GCC	Ala (A)	2.3	0.25	GAC	Asp (D)	2.3	0.41	GGC	Gly (G)	3.0	0.40	C
	GUA	Val (V)	1.2	0.17	GCA	Ala (A)	2.1	0.22	GAA	Glu (E)	4.4	0.70	GGA	Gly (G)	0.7	0.09	A
	GUG	Val (V)	2.4	0.34	GCG	Ala (A)	3.2	0.34	GAG	Glu (E)	1.9	0.30	GGG	Gly (G)	0.9	0.13	G
		U				C				A				G			

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Nirenberg Lab – 1968

https://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/JJBCCQ

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