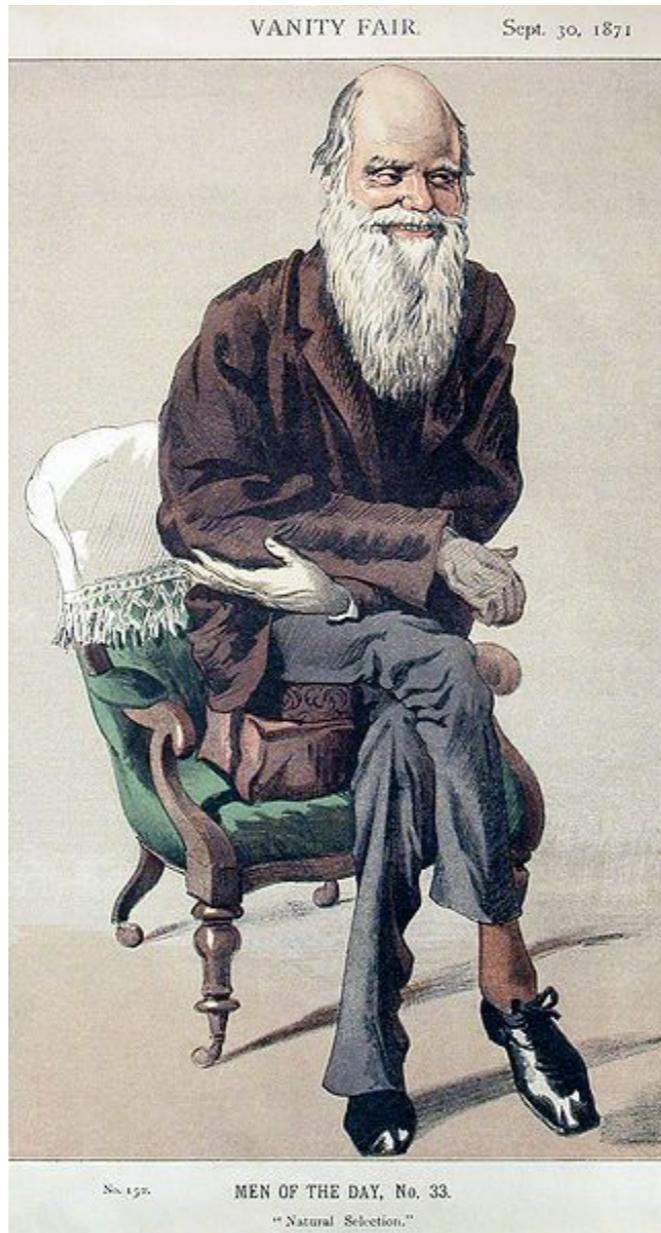


Lecture 1

History: Genetics and Genomics

Charles Darwin

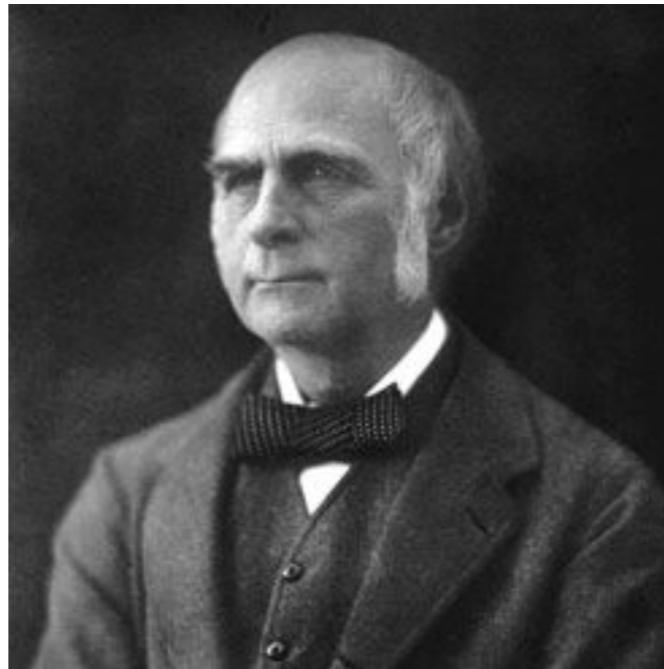
The fabric of biology: 1809 - 1882



Provisional theory of pangenesis. All sperm, egg, buds consisted of multitude of gemmules given off by “*each separate atom of the organism*”.

Francis Galton

Experimental validation: 1822 - 1911



Blood transfusion in rabbits produces no evidence to support provisional theory of pangenesis.

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tions, calls into her councils her men of Science, and becomes a Science-aided State.

EDITOR

PANGENESIS

IN a paper, read March 30, 1871, before the Royal Society, and just published in the Proceedings, Mr. Galton gives the results of his interesting experiments on the inter-transfusion of the blood of distinct varieties of rabbits. These experiments were undertaken to test whether there was any truth in my provisional hypothesis of Pangenesis. Mr. Galton, in recapitulating "the cardinal points," says that the gemmules are supposed "to swarm in the blood." He enlarges on this head, and remarks, "Under Mr. Darwin's theory, the gemmules in each individual must, therefore, be looked upon as entozoa of his blood," &c. Now, in the chapter on Pangenesis in my "Variation of Animals and Plants under Domestication," I have not said one word about the blood, or about any fluid proper to any circulating system. It

Gregor Johann Mendel

Quantifiable heredity: 1822 - 1884



- › Born in Austro-Hungarian Empire (1822)
- › Admitted at the Augustinian monastery in Brno (1843)
- › Studies in Vienna with Doppler, Ettinghausen, Redtenbacher, Fenzl, and Unger (1851 - 1853)
- › Teaches at the Monastery (1853 - 1868)
- › Grows 28,000 plants and analyzed seven pairs of traits

TABLE 1 A Summary of Mendel's Results

Trait studied	Number of plants		Ratio of dominants/recessives
	Dominants	Recessives	
Length of stem: tall/short	787	277	2.84:1
Position of flower: axial/terminal	651	207	3.14:1
Shape of pod: inflated/constricted	882	299	2.95:1
Color of pod: green/yellow	428	152	2.82:1
Shape of seed: round/wrinkled	5474	1850	2.96:1
Color of cotyledons: yellow/green	6022	2001	3.01:1
Color of seed-coat: gray/white	705	224	/ 3.01:1

Results are tabulated from Mendel's results for the F₂ generation in his seven major series of experiments. For each trait studied, the characteristic listed first is the dominant one.



1 HEREDITARY TRAITS ARE GOVERNED BY GENES WHICH RETAIN THEIR IDENTITY IN HYBRIDS. GENES ARE NEVER BLENDED TOGETHER.



ONE FORM ("ALLEL") OF A GENE MAY BE DOMINANT OVER ANOTHER. BUT RECESSIVE GENES WILL POP UP LATER!!



3. EACH ADULT ORGANISM HAS TWO COPIES OF EACH GENE - ONE FROM EACH PARENT. WHEN POLLEN OR SPERM AND EGGS ARE PRODUCED, THEY EACH GET ONE COPY.



DIFFERENT ALLELES ARE SORTED OUT TO SPERM AND EGG RANDOMLY AND INDEPENDENTLY. ALL COMBINATIONS OF ALLELES ARE EQUALLY LIKELY:

AABBCCDDEEFFGGHH
Aa BBCCDDEEFFGGHH
aA BBCCDDEEFFGGHH
aa BBCCDDEEFFGGHH
AA bBCCDDEEFFGGHH
AA BbCCDDEEFFGGHH
Aa BbCCDDEEFFGGHH
aA BbCCDDEEFFGGHH

ETC!

→ WE'LL SEE SHORTLY THAT NOT ALL THESE POINTS ARE EXACTLY CORRECT... DOMINANCE IS SOMETIMES ONLY PARTIAL... THERE ARE ORGANISMS WITH ONLY A SINGLE SET OF GENES... AND SOME WITH FOUR SETS... AND DEVIATIONS FROM INDEPENDENT ASSORTMENT TURN OUT TO BE VERY IMPORTANT...

TABLE 1. F₂ RESULTS, PEA CROSSES

Source	Yellow	Green	Total	Dev. from 3 in 4	Prob. Error	Dev. ÷ P.E.
Mendel, 1866	6,022	2,001	8,023	+ .0024	± .0130	.18
Correns, 1900	1,394	453	1,847	+ .0189	± .0272	.70
Tschermak, 1900	3,580	1,190	4,770	+ .0021	± .0169	.12
Hurst, 1904	1,310	445	1,775	- .0142	± .0279	.51
Bateson, 1905	11,902	3,903	15,806	+ .0123	± .0093	1.32
Lock, 1905	1,438	514	1,952	- .0533	± .0264	2.04
Darbshire, 1909	109,060	36,186	145,246	+ .0035	± .0030	1.16
Winge, 1924	19,195	6,553	25,748	- .0180	± .0125	1.44
Total	153,902	51,245	205,147	+ .0008	± .0038	.21

SOURCE: Johannsen, 1926.

deVreis/Correns/Tschemak



1848-193
5



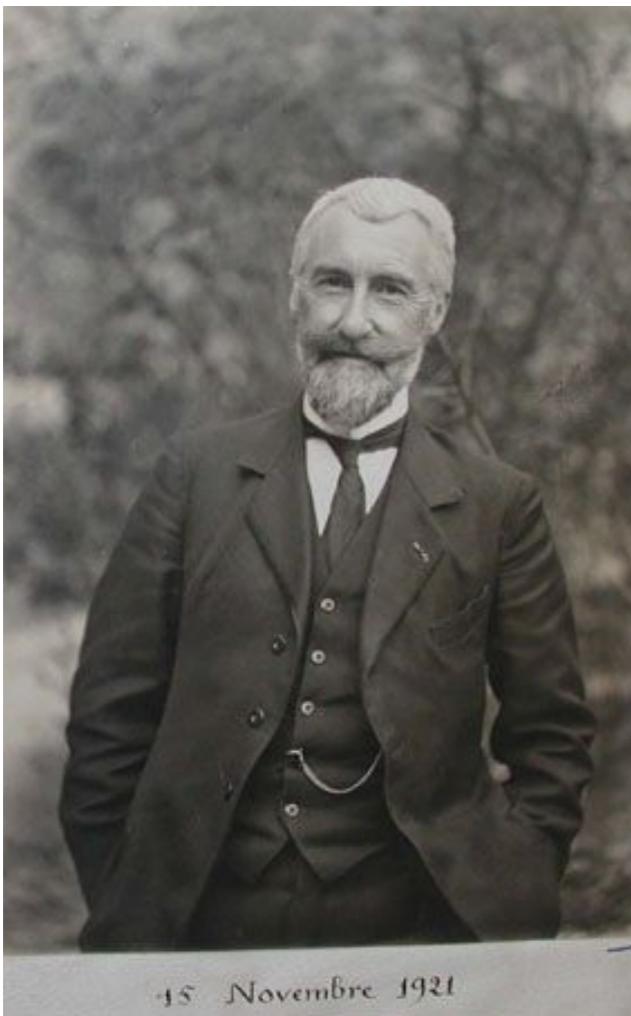
1864-193
5



1871-196
2

The Rediscovery

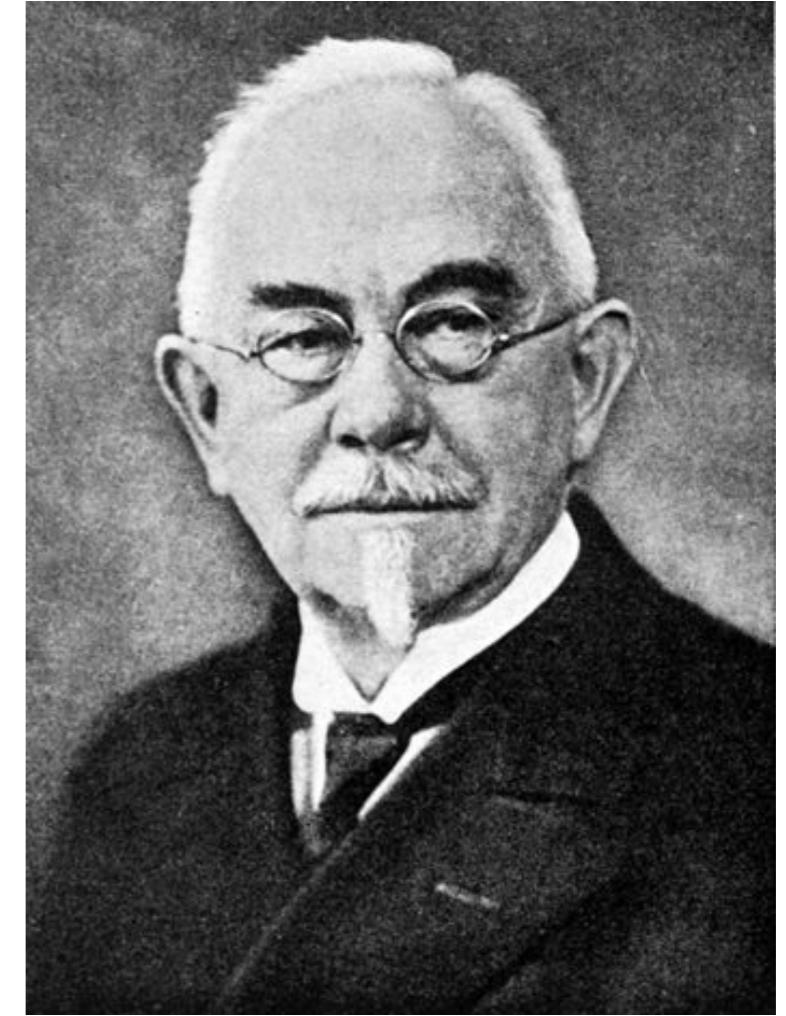
Cuénot/Bateson/Johannsen



1866 -
1951



1861 -
1926



1857 -
1927

Confirmation in animals. **Genetics**, zygote, homozygote, heterozygote, **gene**, genotype, phenotype

State of chromosomal affairs in early 1900s

- Species have a constant number of chromosomes
- Equal numbers come from egg and sperm
- Reduction is accomplished by divisions
- Chromosomes are bearers of hereditary material

The Fly Room

- T. H. Morgan
- Frans-Alfons Janssen
- A. H. Sturtevant
- C. B. Bridges
- H. J. Muller

Morgan

Usefulness of *Drosophila* for study of gene mut. & their inheritance, when intensity studied

orangy
sex-linked genes - with Xchrom.

interchange of the genes
explanation by Janssen theory of diascinotype 1911

degree of linkage dependence distance in chrom. (theory)

Leopold
other group of linked genes which = other chrom.

no crossing over in ♂ 1912

Abnormal abdomen - ovar. influence

various mut. for 1 char. & their interaction (e.g. eye color)

lethal factors in X

postbrevire mutation of w - against presenechabre

Morgan + Bridges - with 1920
Morgan + Bridges - with 1920
a week zone
date on gynandrom.

Alltogether: Observation of multiple effects of 1 gene, & influence of many genes on same character

attack on previous above expl. of multiple alleles

Mrs. Morgan.

attached X's other exchange deleted X:

X with duplication my chromosome

gynander explanation

Sturtevant

Mapping chromosomes XYY
double crossing over
3rd group of genes (1912, 13)

inherit variation in linkage (1912, 13)

selection through inversions

inversion research (1912, 13)

inegal cr. ov (Bar)

irregularity of similar inversion

mut. on semistar. inversion

systems of Dros.

inter-cell influence in ovariae (Bar, 1912)

supporting T. C. H. Morgan

Lefted Drosophila with translocation, segregation

position effect 1912

suggested explanation of secondary non-disjunction

to Bridges, 1914

specific modifiers of loci, etc.

giant. 1913

non-gran. allel.

(T series, 1912)

cell. mut. (1912)

mut. in tail (1912)

gene mut. (1912, 13)

calc. size & number of seg.

genetic mosaics (1912)

initiated genet. studies (1912)

reciprocal characteristics of mut. (1912)

effect of temperature (1912)

of X-rays (1912)

Bridges

found work out 1911-1913

mutation & action of most of the mutations

accumulated data started by Sturtevant

discover, non-disjunction

and at suggestion of others, did the work myself

disjunction 1913

(proof of chrom.)

linkage variation with age

deficiency (duplication)

translocation

homo - IV 1912

trifoliate - IV

trifoliate - from 1921

Muller's theory of sex-determination

sex-gene balance

trifoliate + homozygous

non-gran. allel.

balanced lethal 1913

explain of Canthar. degen. thru mutation

suggested to Bridges

cofactoristic for deficiency, trifoliate, white, cytol proof of non-disjunction; suggestion of mechanism

conclusion of indeterminacy

linkage var. with X-ray

suggested Ptof's disjunction

environmental

non-gran. allel.

Muller

teaching abilities, work with

most of the mutations

accumulated data started by Sturtevant

discover, non-disjunction

and at suggestion of others, did the work myself

disjunction 1913

(proof of chrom.)

linkage variation with age

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environmental

non-gran. allel.

balanced lethal 1913

specie

attack on previous above expl. of multiple alleles

Mrs. Morgan.

attached X's other exchange deleted X:

X with duplication my chromosome

gynander explanation

for exp. of W. G. Beadle

for exp. of T. H. Morgan

for exp. of G. K. Ciampi

for exp. of H. H. Hopkins

for exp. of R. A. Fisher

for exp. of R. A.

Muller/Bridges/Sturtevant



1890-1967



1889-1936



1891-1970

Elaboration of chromosomal theory | Induced mutations

Mendelism-Morganism

- › Chromosomes contain hereditary information
- › Genes are linearly distributed along chromosome
- › Gene is a unit of information
- › Gene can define characteristics such as eye color, wing shape etc.

But what is the chemical nature of heredity?

- Miescher | nucleic acids
- Kossel | DNA, RNA, histones
- Levene | tetranucleotide theory
- Chargaff | parity rules
- Griffith, Avery, MacLeod, McCarty | nucleic acid is the transforming factor

Friedrich Miescher

1844 - 1895



Analysis of puss cells nuclei yielded *nuclein*, a compound with high phosphorus content that is resistant to pepsin.

Albrecht Kossel

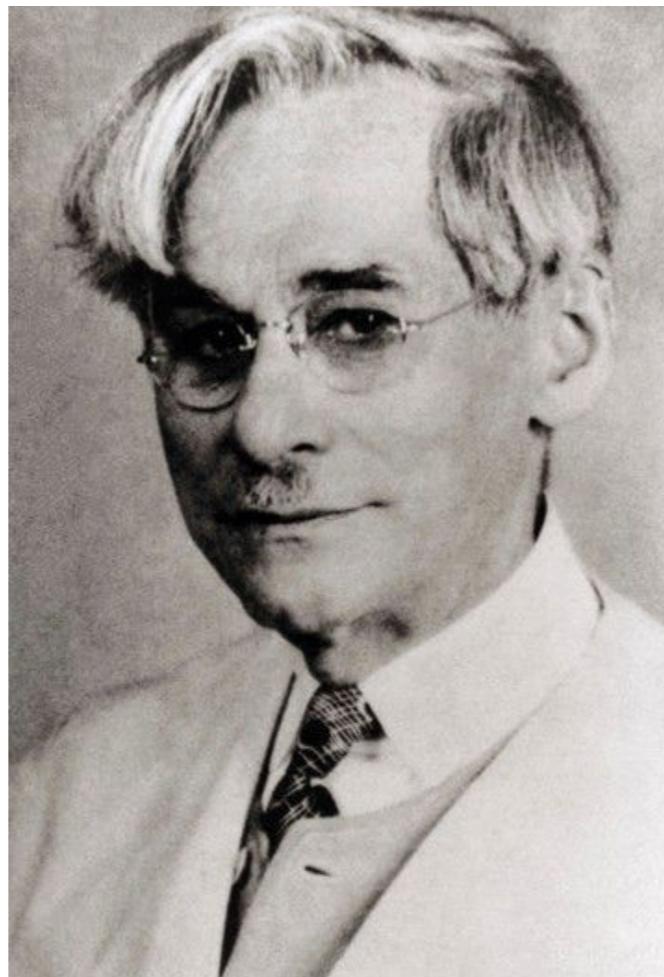
1858 - 1927 (NP 1910)



Five amino-organic compounds adenine, guanine, thymine, cytosine, uracil). Thymus nucleic acid (DNA) and yeast nucleic acid (RNA).

Phoebus Levene

1869 - 1940



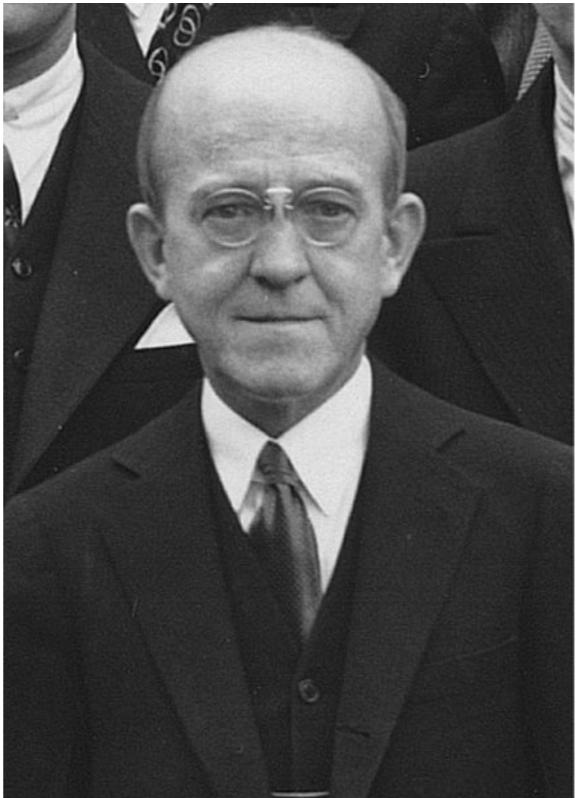
Fundamental work in biochemistry. DNA contains equal amount of all four bases organized as an unchangeable sequence of tetranucleotide units.

1910 – 1930
**Dark age of molecular
biology**

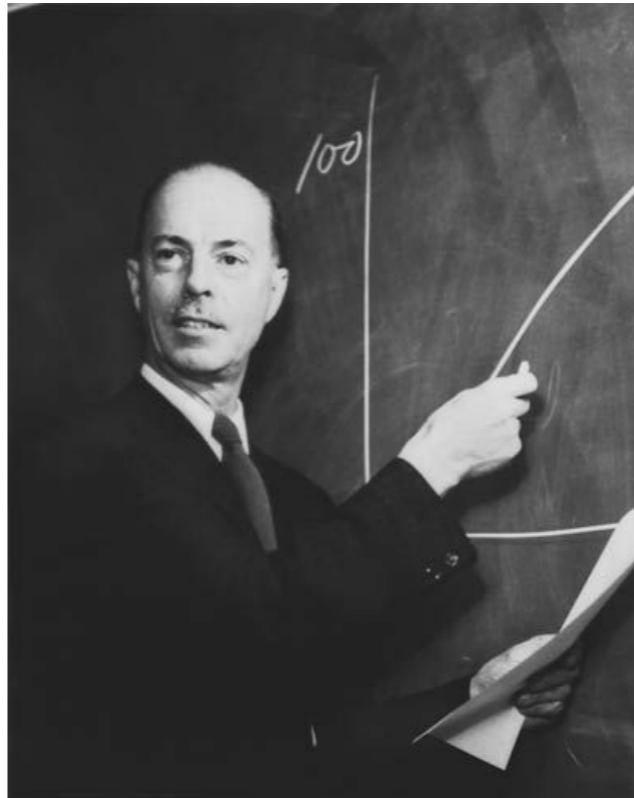
Griffith, Avery, MacLeod, McCarty



1879 - 1941



1877 - 1955



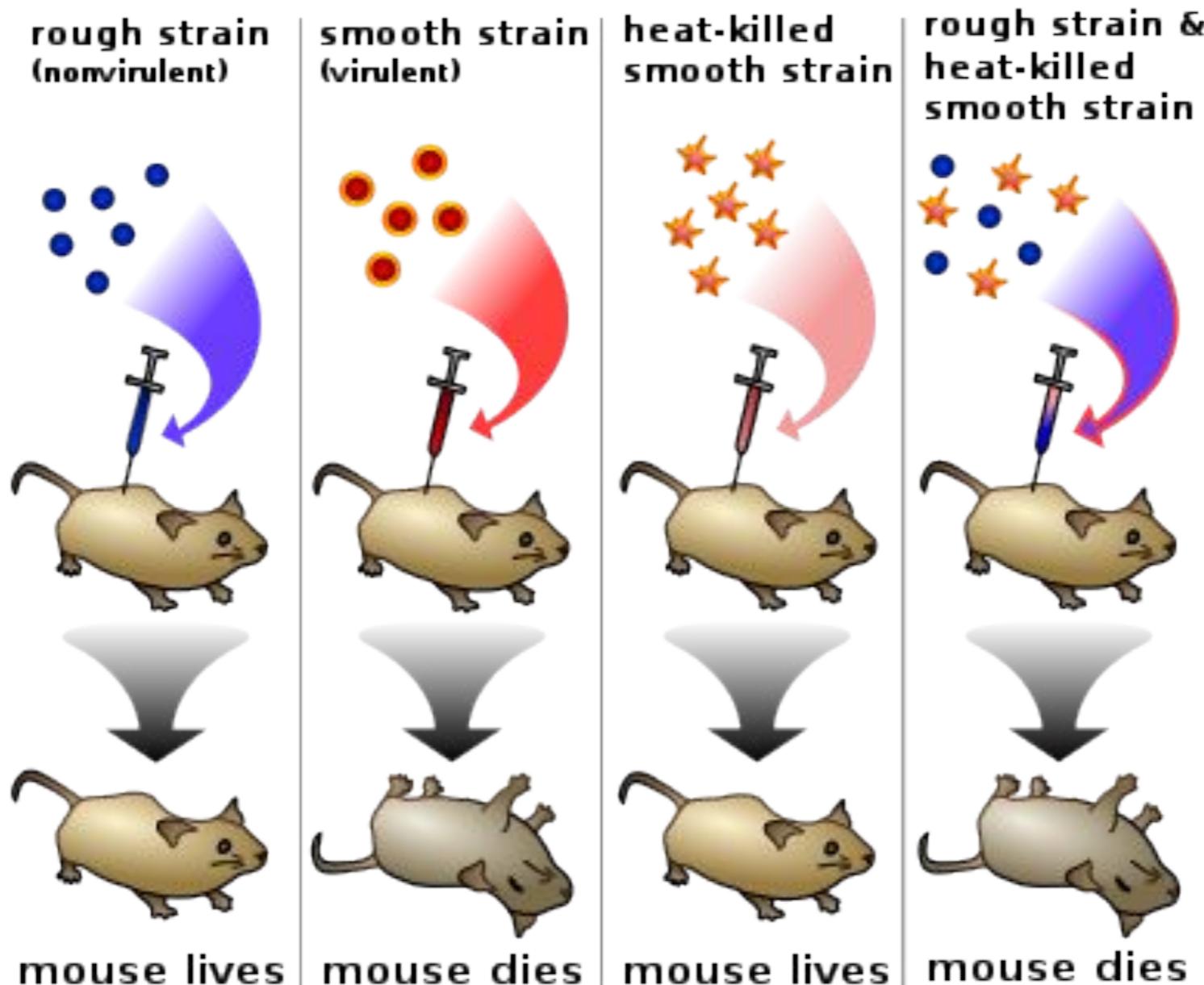
1909 - 1972



1911 - 2005

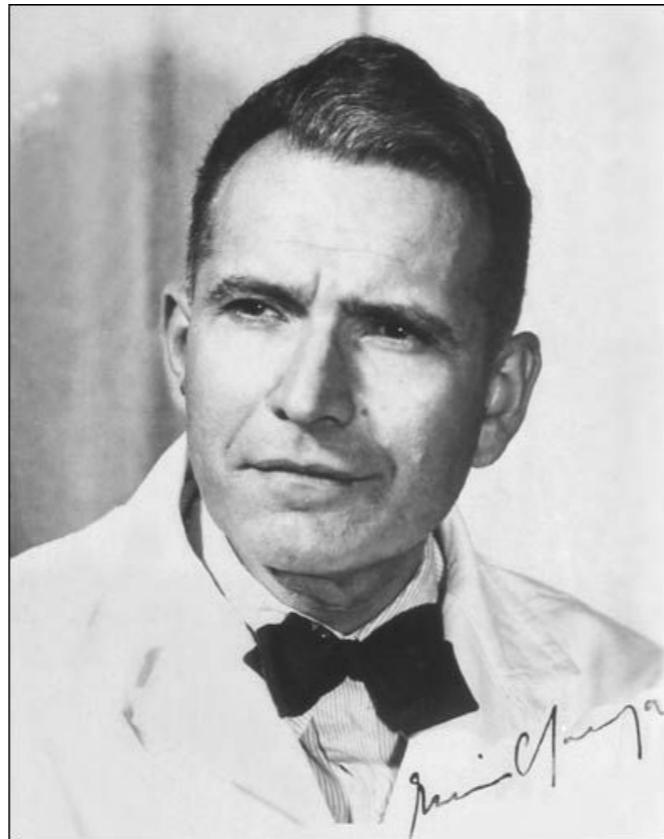
Transforming agent is a highly polymerized form of desoxyribonucleate

Griffith, Avery, MacLeod, McCarty



Erwin Chargaff

1905 - 2002



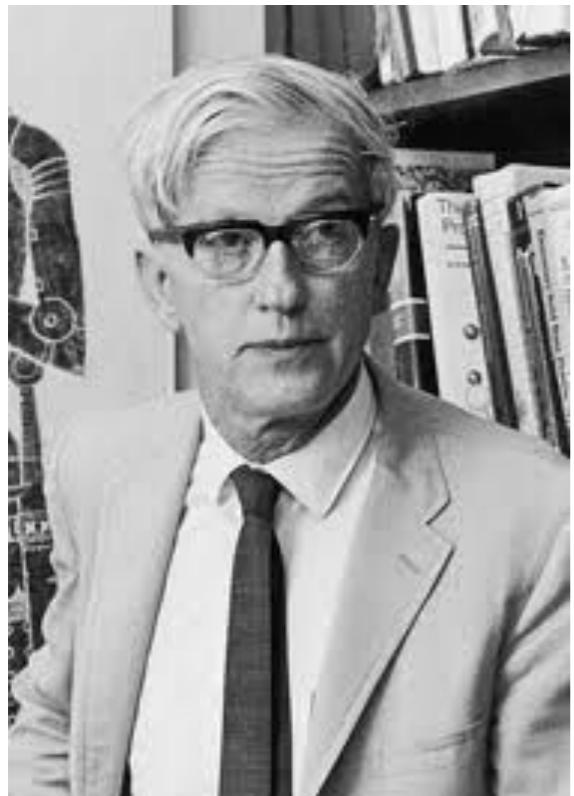
The four DNA bases are NOT in equal amount but follow rules such the ratio of purines to pyrimidines is ~ 1

The Church of Phage

- Delbrück, Luria, Hershey | replication and genetic structure of viruses
- Lwoff | profage and lysogeny
- Lederberg, Hayes | bacterial sex, plasmids
- Hershey, Chase | DNA = heredity
- Watson | ...

Delbrück, Luria, Hershey

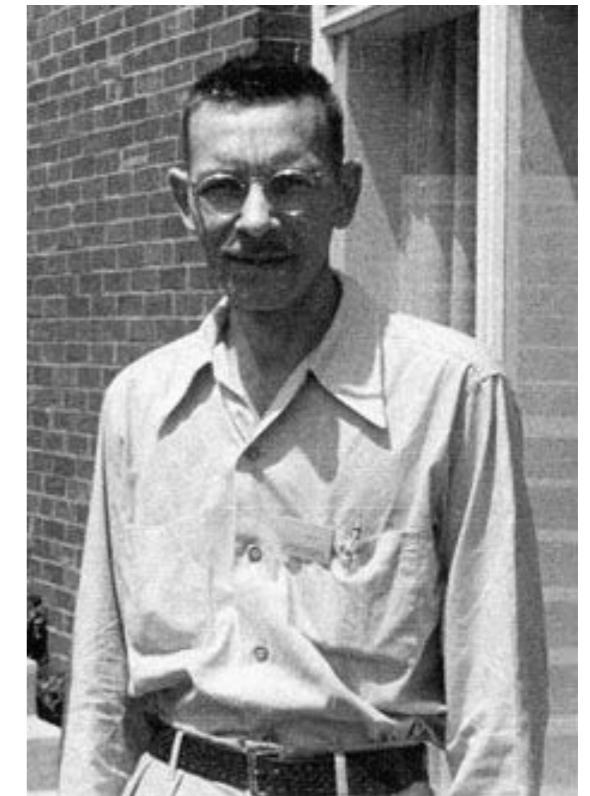
(NP 1969)



1906 -
1981



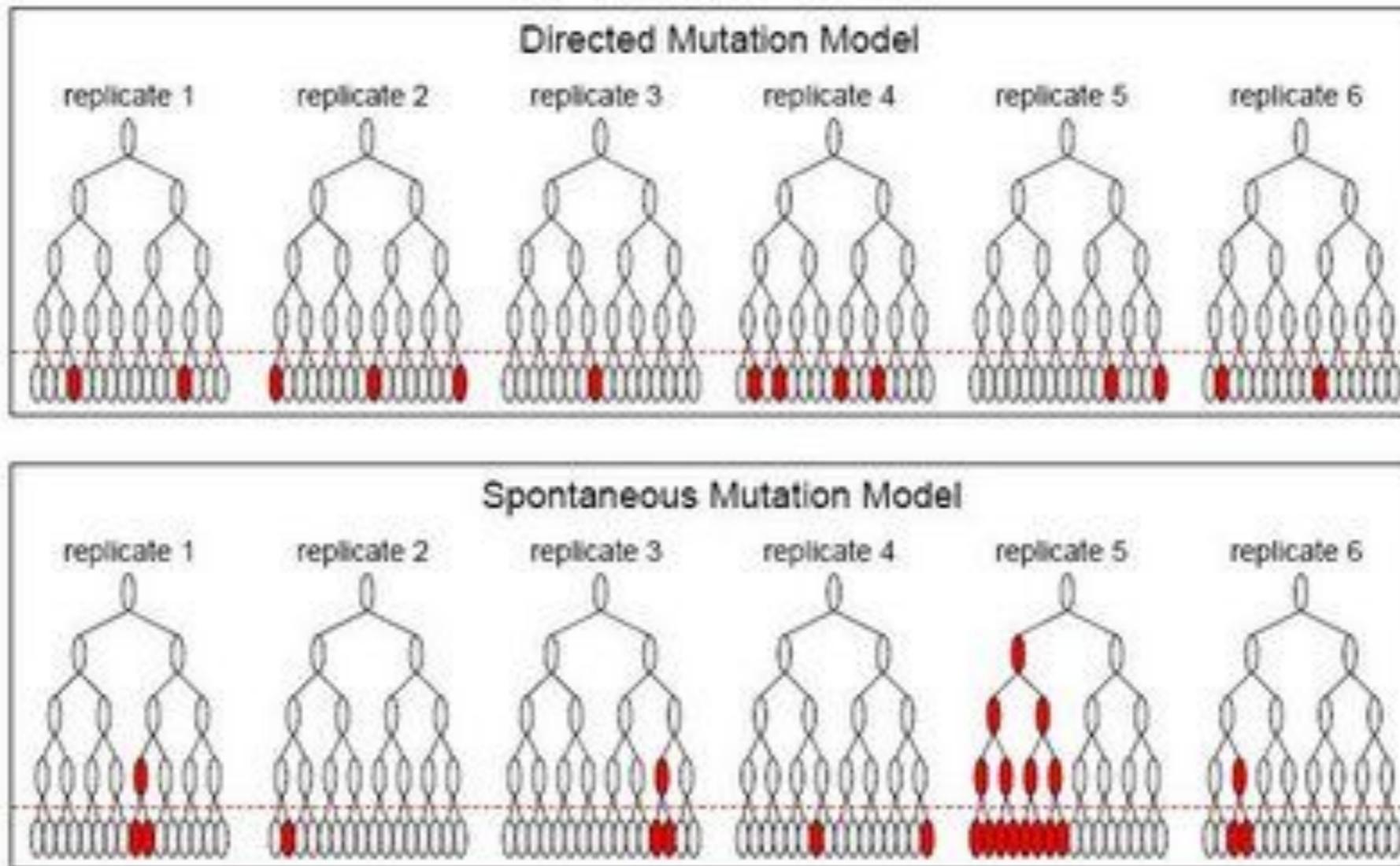
1912 -
1991



1908 -
1997

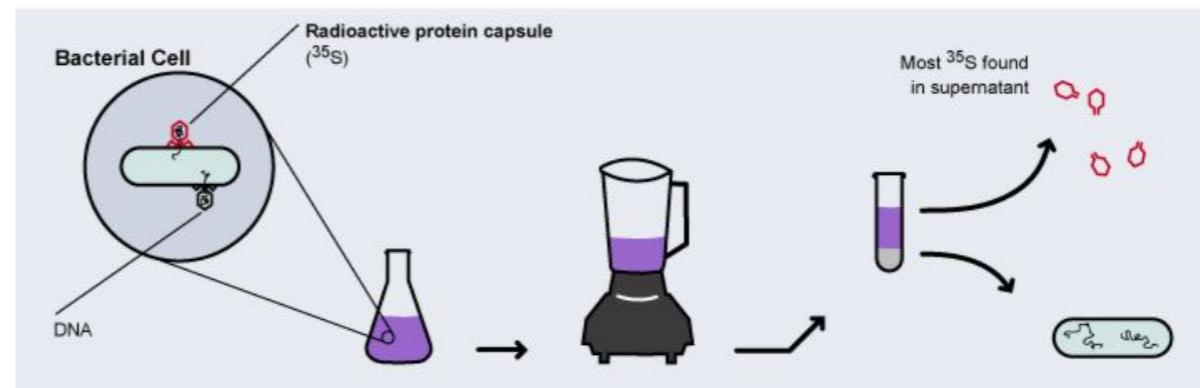
“discoveries concerning the replication mechanism and genetic structure of viruses”.

Delbrück-Luria experiment



Mutations are stochastic

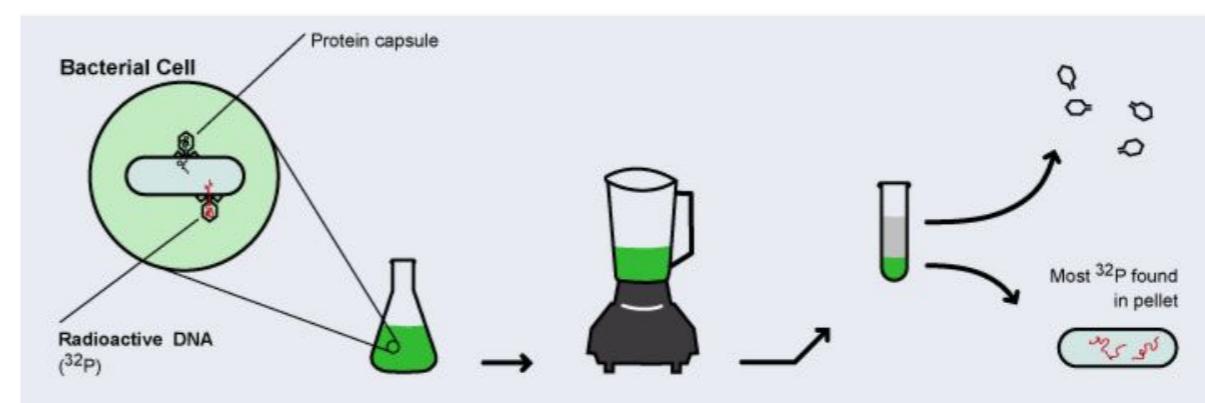
Hershey-Chase experiment



Labeled phages infect bacteria.

Blender separates phages outside the bacteria from the cells and their contents

Cells and Phages are separated by centrifugation.



protective coat that is responsible for the adsorption to bacteria, and functions as an instrument for the injection of the phage DNA into the cell. This protein probably has no function in the growth of intracellular phage. The DNA has some function. Further chemical inferences should not be drawn from the experiments presented.

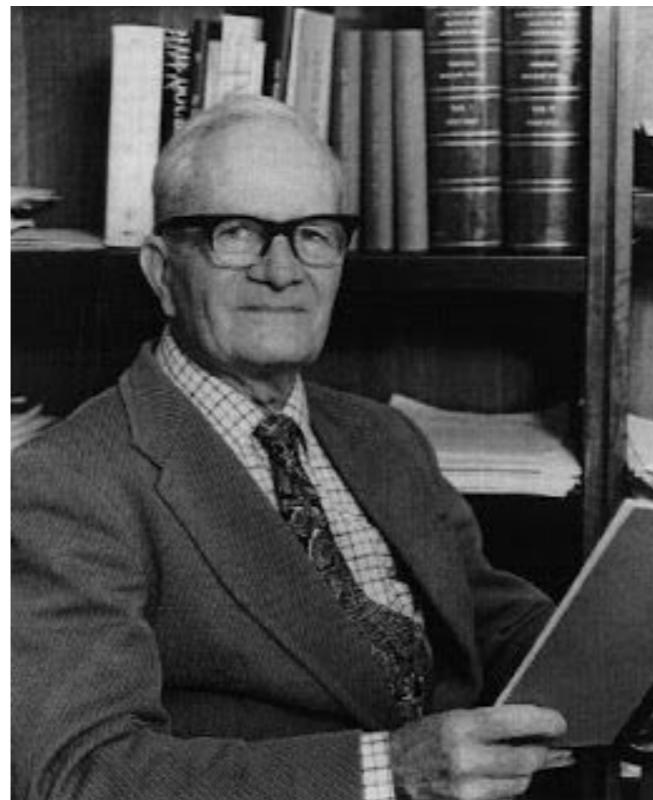
Lederberg Hayes

(NP 1969)



NARA/UPI; NARA/Stanford U.

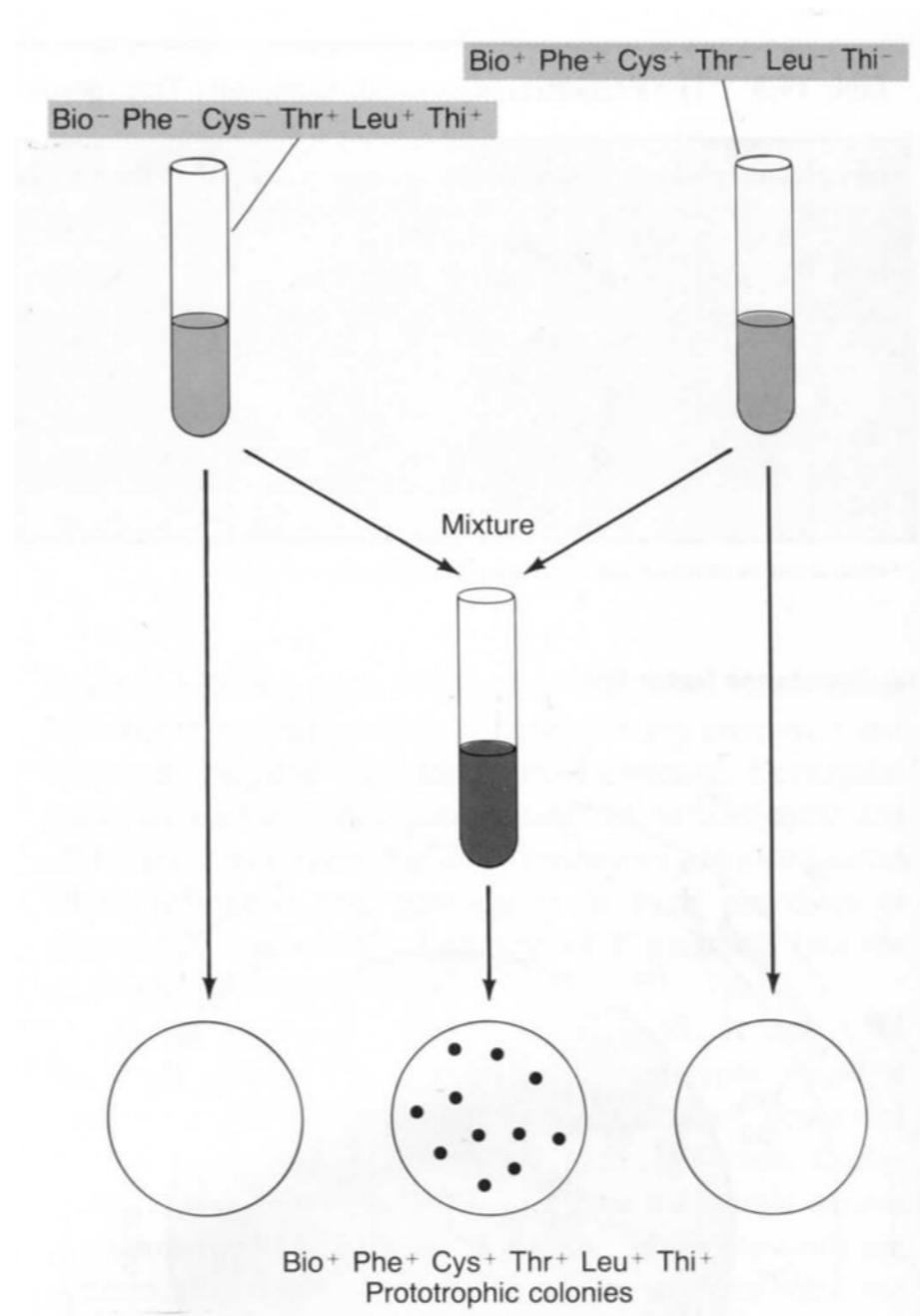
1925 - 2008



1913 - 1994

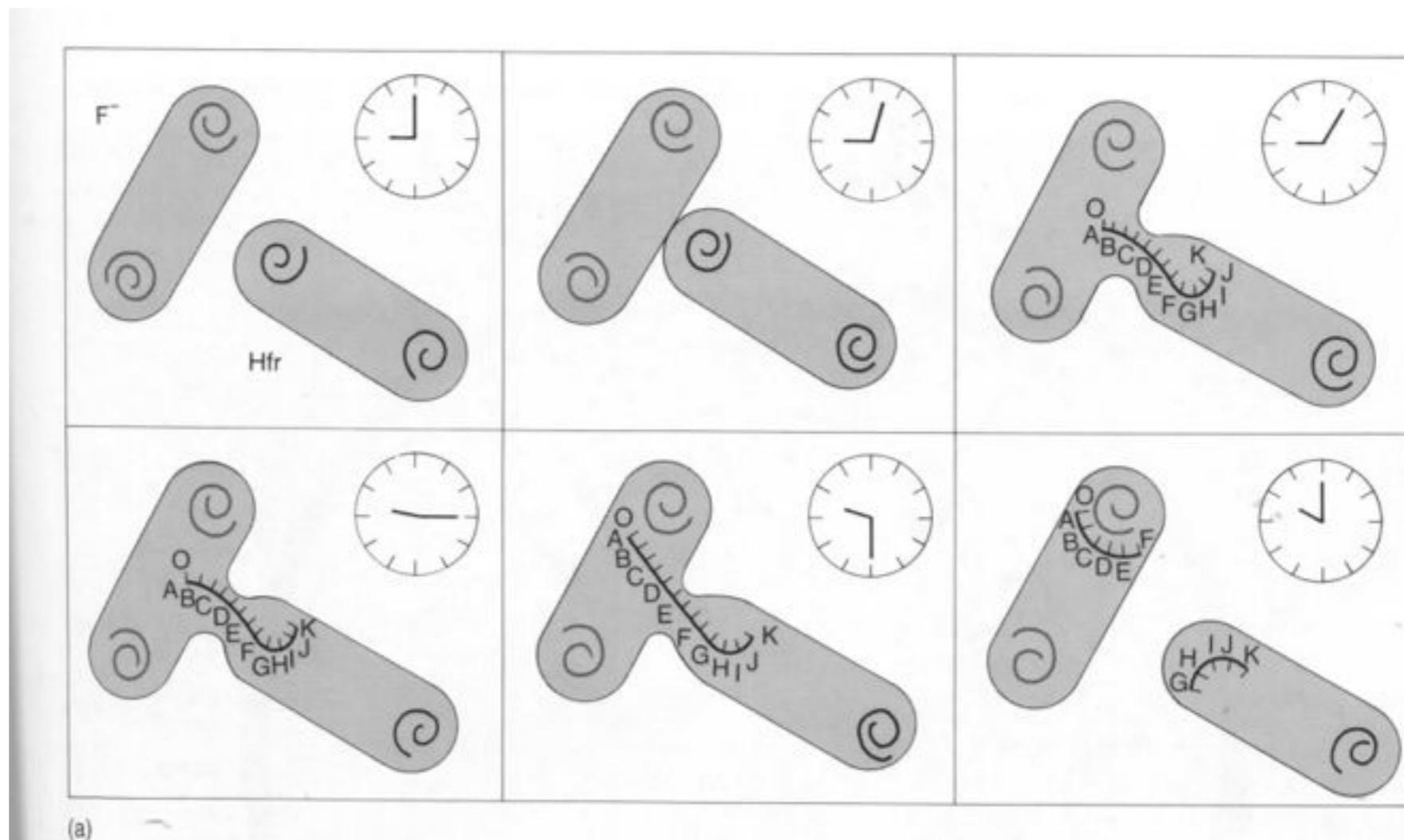
Bacterial mating is unidirectional and is controlled by extrachromosomal factor

Lederberg-Tatum Experiment



genetic recombination in *E. coli*

Blender again...



Elie Wollman (1917-2008)

DNA

- Crick, Franklin, Watson, Wilkins | the structure of DNA
- Meselson, Stahl | semiconservative replication
- Ochoa, Kornberg | enzymatic synthesis of nucleic acids
- Holley, Nirenberg, Khorana | the code

Crick, Franklin, Watson, Wilkins

(NP 1962)



1916 - 2004



1920 - 1958



b. 1928



1916 - 2004

DNA is an antiparallel double helix

Watson & Crick

No. 4356 April 25, 1953

NATURE

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equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

¹ Young, F. B., Gerard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

² Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Suppl.* **5**, 285 (1949).

³ Von Arx, W. S., Woods Hole Papers in Phys. Oceanogr. Meteor., **11** (3) (1950).

⁴ Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

Wilkins, Stokes, Wilson

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King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge.

April 2.

¹ Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Natl. Acad. Sci.*, **39**, 81 (1953).
² Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
³ Chargaff, E., for references see Zamenhof, S., Drawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
⁴ Wyckoff, G. R., *Gen. Physiol.*, **36**, 201 (1922).
⁵ Astbury, J. T., *Symp. Soc. Exp. Biol.*, **1**, Nucleic Acid, **66** (Camb. Univ. Press, 1947).
⁶ Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline^{1,2}, semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner

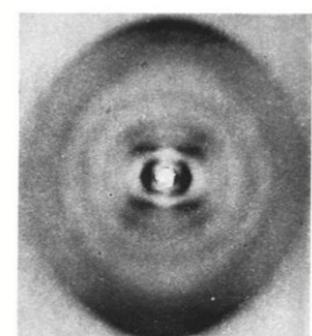


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical.

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_n) on the n th layer line. The helical configuration produces side-bands on this fundamental frequency, the effect³ being to reproduce the intensity distribution about the origin around the new origin, on the n th layer line, corresponding to *C* in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding

Bessel functions gives reinforcement for the inner-

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We wish to thank Prof. J. T. Randall for encouragement; Profs. E. Chargaff, R. Signer, J. A. V. Butler and Drs. J. D. Watson, J. D. Smith, L. Hamilton, J. C. White and G. R. Wyatt for supplying material without which this work would have been impossible; also Drs. J. D. Watson and Mr. F. H. C. Crick for stimulation, and our colleagues R. E. Franklin, R. G. Gosling, G. L. Brown and W. E. Seeds for discussion. One of us (H. R. W.) wishes to acknowledge the award of a University of Wales Fellowship.

M. H. F. WILKINS

Medical Research Council Biophysics Research Unit,

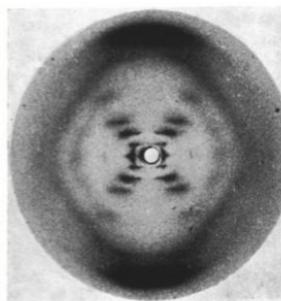
A. R. STOKES

H. R. WILSON

Wheatstone Physics Laboratory,
King's College, London.

April 2.

¹ Astbury, J. T., *Symp. Soc. Exp. Biol.*, **1**, Nucleic Acid (Cambridge Univ. Press, 1947).
² Riley, D. P., and Oster, G., *Biochim. et Biophys. Acta*, **7**, 526 (1951).
³ Wilkins, M. H. F., Gosling, R. G., and Seeds, W. E., *Nature*, **187**, 759 (1960).
⁴ Astbury, J. T., and Bell, F. O., *Cold Spring Harb. Symp. Quant. Biol.*, **6**, 109 (1938).
⁵ Cochran, W., Crick, F. H. C., and Vand, V., *Acta Cryst.*, **5**, 581 (1952).
⁶ Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).



Sodium deoxypentose nucleate from calf thymus. Structure *B*

molecules, each unit being shielded by a sheath of water. Each unit is then free to take up its least-energy configuration independently of its neighbours and, in view of the nature of the long-chain molecules involved, it is highly likely that the general form will be helical. If we adopt the hypothesis of a helical structure, it is immediately possible, from the X-ray diagram of structure *B*, to make certain deductions as to the nature and dimensions of the helix.

The innermost maxima on the first, second, third and fifth layer lines lie approximately on straight lines radiating from the origin. For a smooth single-strand helix the structure factor on the n th layer line is given by:

$$F_n = J_n(2\pi rR) \exp i n(\psi + \frac{1}{2}\pi),$$

where $J_n(u)$ is the n th-order Bessel function of u , r is the radius of the helix, and R and ψ are the radial and azimuthal co-ordinates in reciprocal space²; this expression leads to an approximately linear array of intensity maxima of the type observed, corresponding to the first maxima in the functions J_1 , J_2 , J_3 , etc.

If, instead of a smooth helix, we consider a series of residues equally spaced along the helix, the transform in the general case treated by Crick, Cochran and Vand is more complicated. But if there is a whole number, m , of residues per turn, the form of the transform is as for a smooth helix with the addition, only, of the same pattern repeated with its origin at heights mc^* , $2mc^*$, etc. (c is the fibre-axis period).

In the present case the fibre-axis period is 34 Å. and the very strong reflexion at 3·4 Å. lies on the tenth layer line. Moreover, lines of maxima radiating from the 3·4 Å. reflexion as from the origin are visible on the fifth and lower layer lines, having a J_5 maximum coincident with that of the origin series on the fifth layer line. (The strong outer streaks which apparently radiate from the 3·4 Å. maximum are not, however, so easily explained.) This suggests strongly that there are exactly 10 residues per turn of the helix. If this is so, then from a measurement of R_5 the position of the first maximum on the n th layer line ($n \leq 5$), the radius of the helix, can be obtained. In the present instance, measurements of R_1 , R_2 , R_3 and R_4 all lead to values of r of about 10 Å.

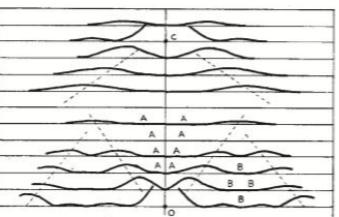
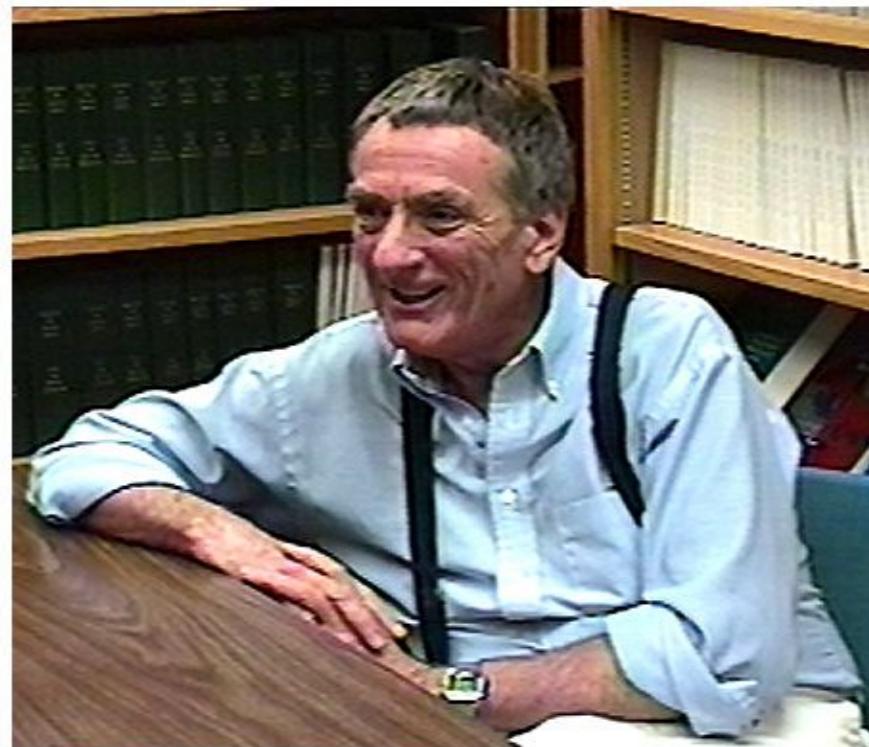


Fig. 2. Diffraction pattern of system of helices corresponding to structure *B*. The squares of Bessel functions of order n on the equator and the first, second, third and fifth layer lines for half of the nucleotide mass at 20 Å. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About C on the tenth layer line the maxima are plotted for an outer diameter of 12 Å.

Meselson, Stahl



b. 1930



b. 1929

DNA replication is semiconservative

Meselson-Stahl experiment

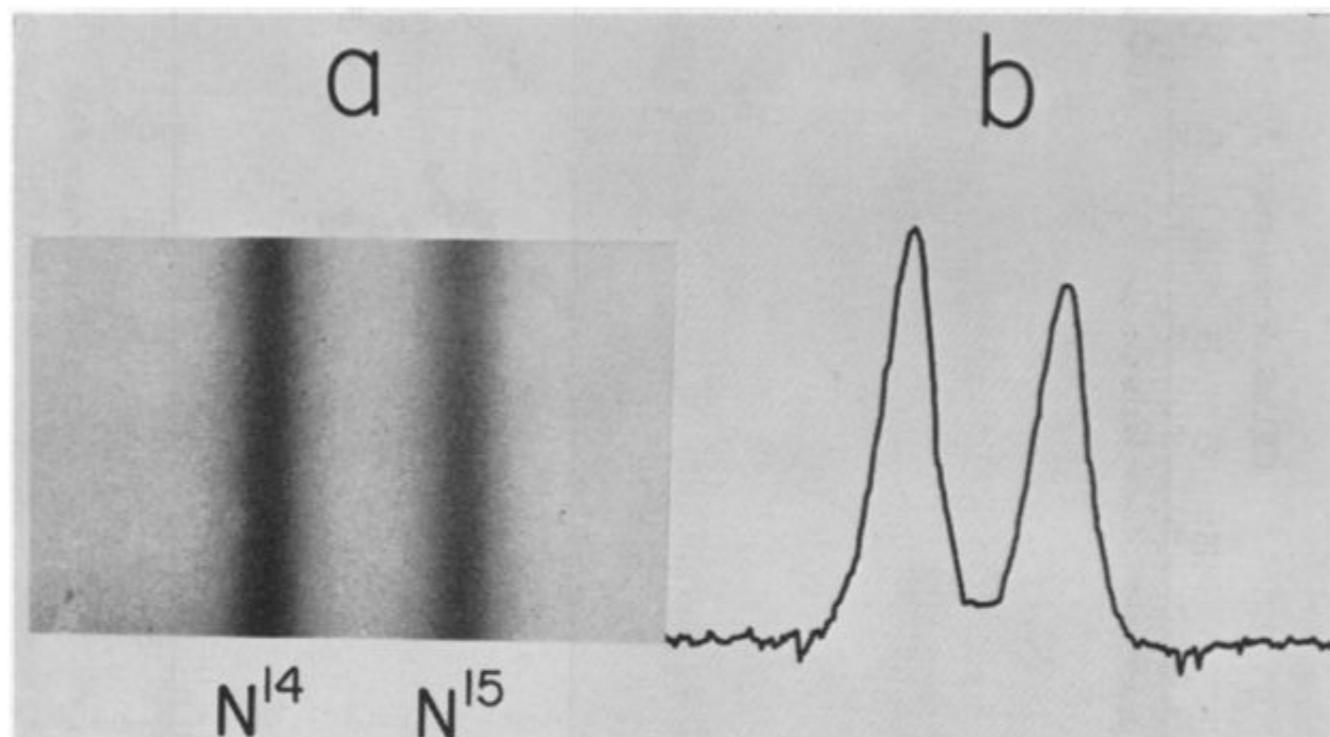
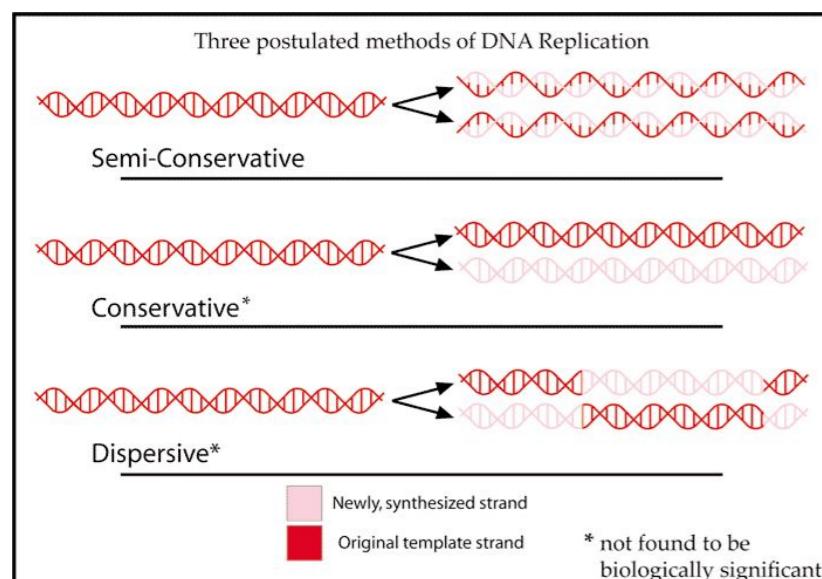


FIG. 2—*a*: The resolution of N^{14} DNA from N^{15} DNA by density-gradient centrifugation. A mixture of N^{14} and N^{15} bacterial lysates, each containing about 10^8 lysed cells, was centrifuged in CsCl solution as described in the text. The photograph was taken after 24 hours of centrifugation at 44,770 rpm. *b*: A microdensitometer tracing showing the DNA distribution in the region of the two bands of Fig. 2*a*. The separation between the peaks corresponds to a difference in buoyant density of 0.014 gm. cm.⁻³

CsCl gradient centrifugation

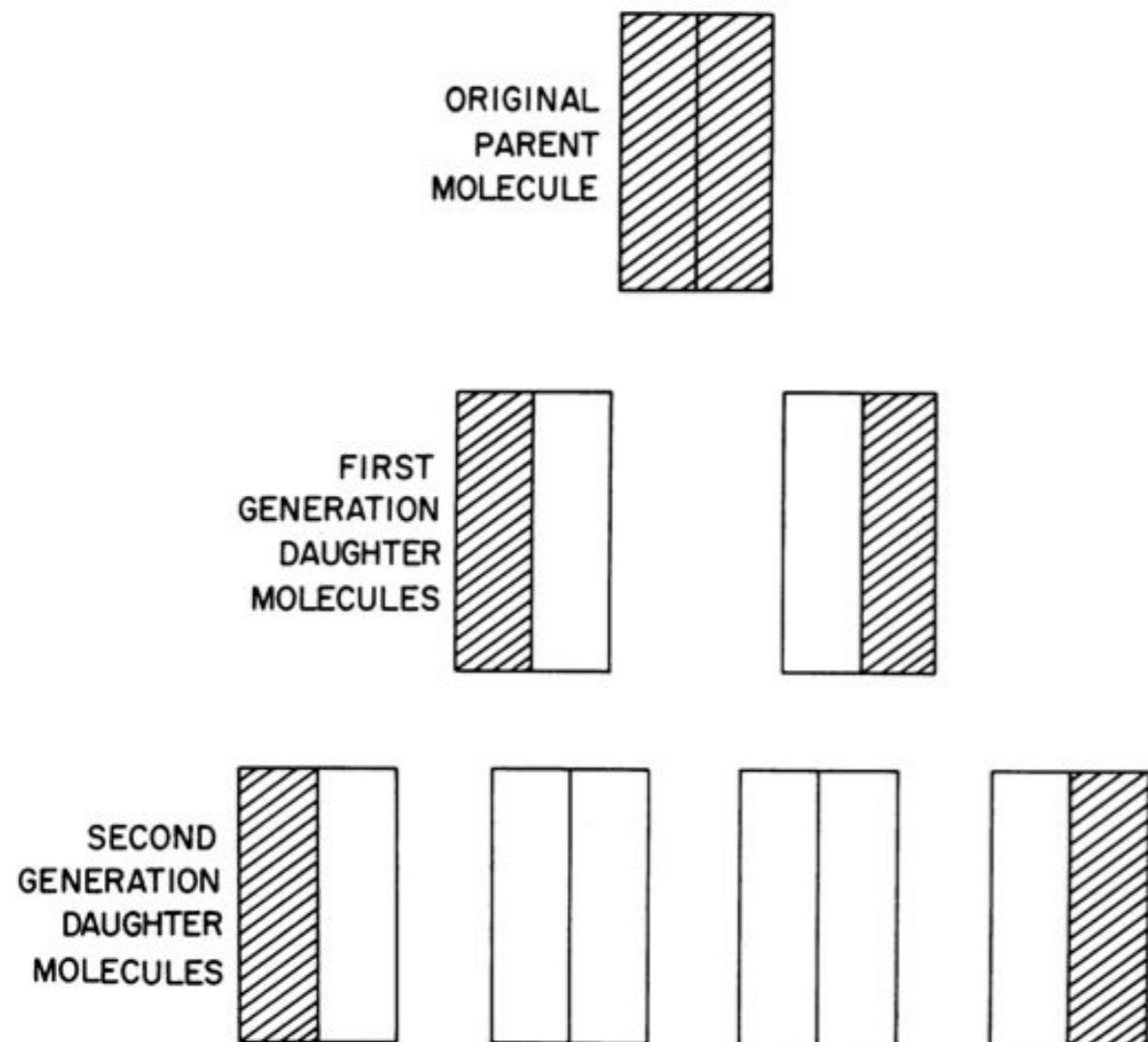
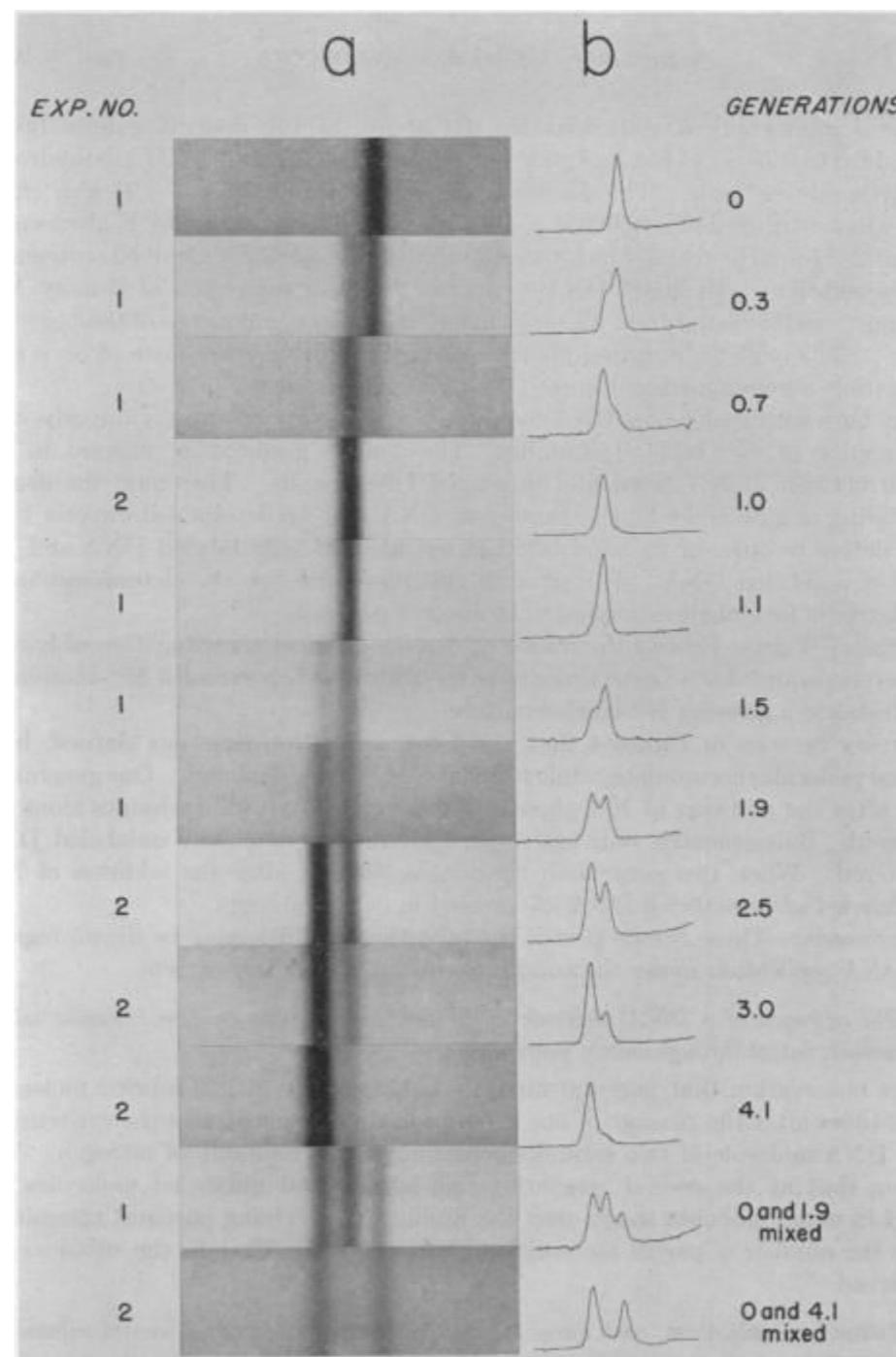


FIG. 5.—Schematic representation of the conclusions drawn in the text from the data presented in Fig. 4. The nitrogen of each DNA molecule is divided equally between two subunits. Following duplication, each daughter molecule receives one of these. The subunits are conserved through successive duplications.

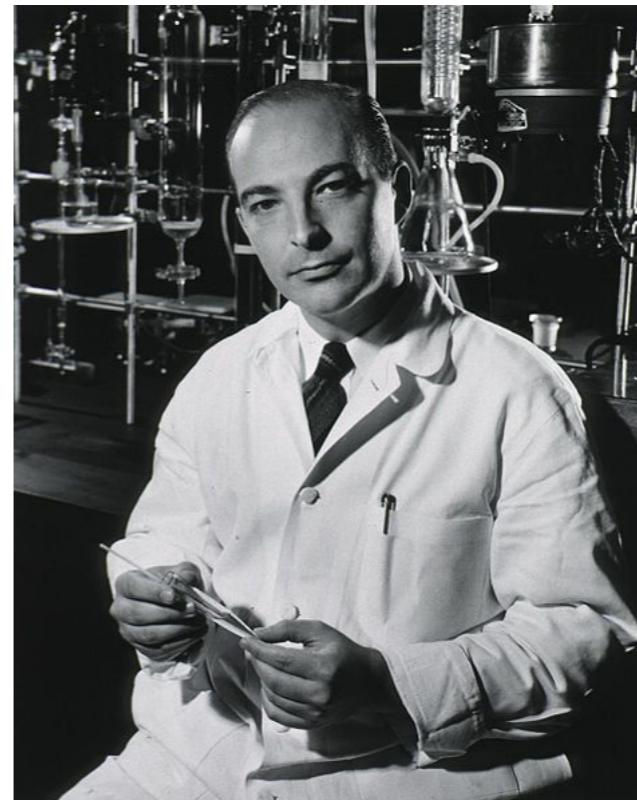


Ochoa, Kornberg

(NP
1959)



1905 -
1993



1918 -
2007

Enzymes for RNA and DNA synthesis

Holley, Khorana, Nirenberg

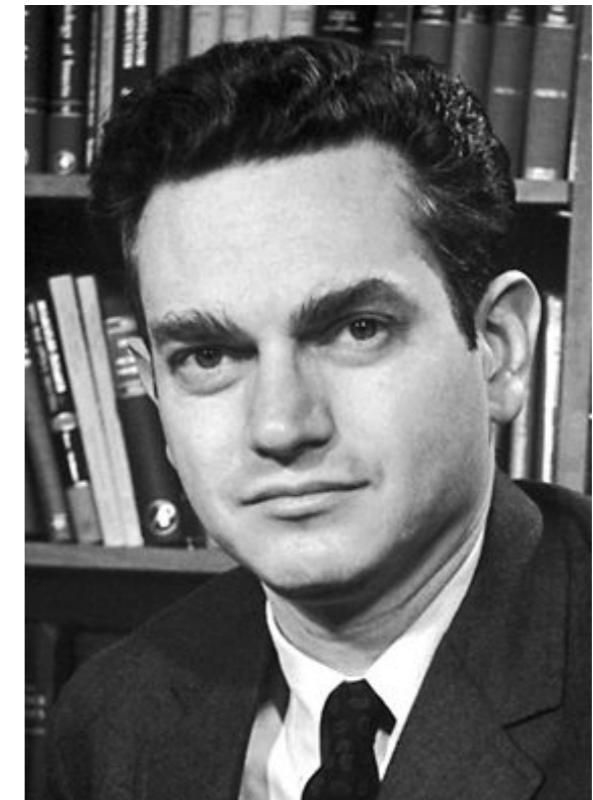
(NP
1959)



1922 -
1993



1922 -
2011



1927 -
2010

tRNA, 64 codons

Gene function

- Lwoff, Jacob, Monod | genetic map and gene regulation
- Beadle, Tatum | one gene = one protein
- Pauling, Ingram | molecular disease

Lwoff, Jacob, Monod

(NP 1965)



1902 - 1994



1920 - 2013



1910 - 1976

Lysogeny, prophage, genetic maps, gene regulation

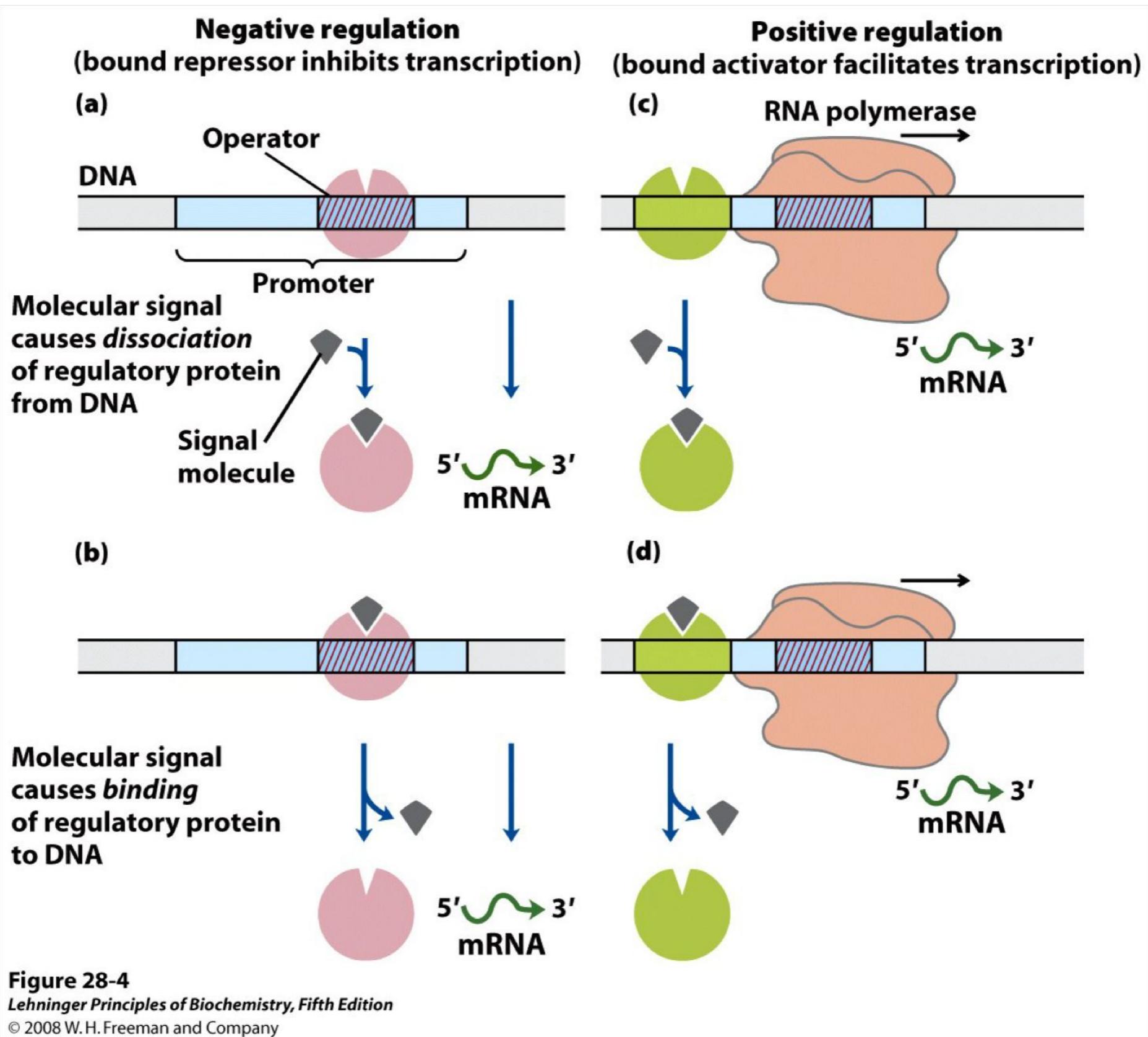


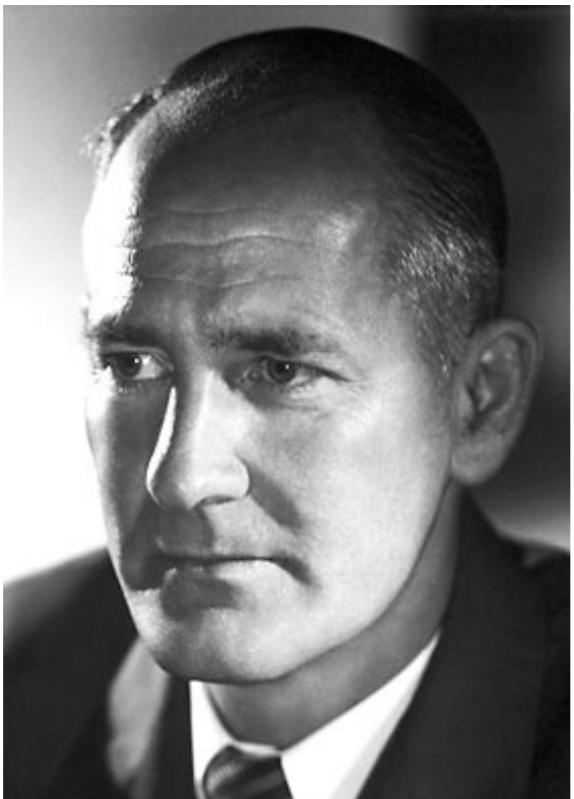
Figure 28-4

Lehninger Principles of Biochemistry, Fifth Edition

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Beadle, Tatum

(NP 1958)

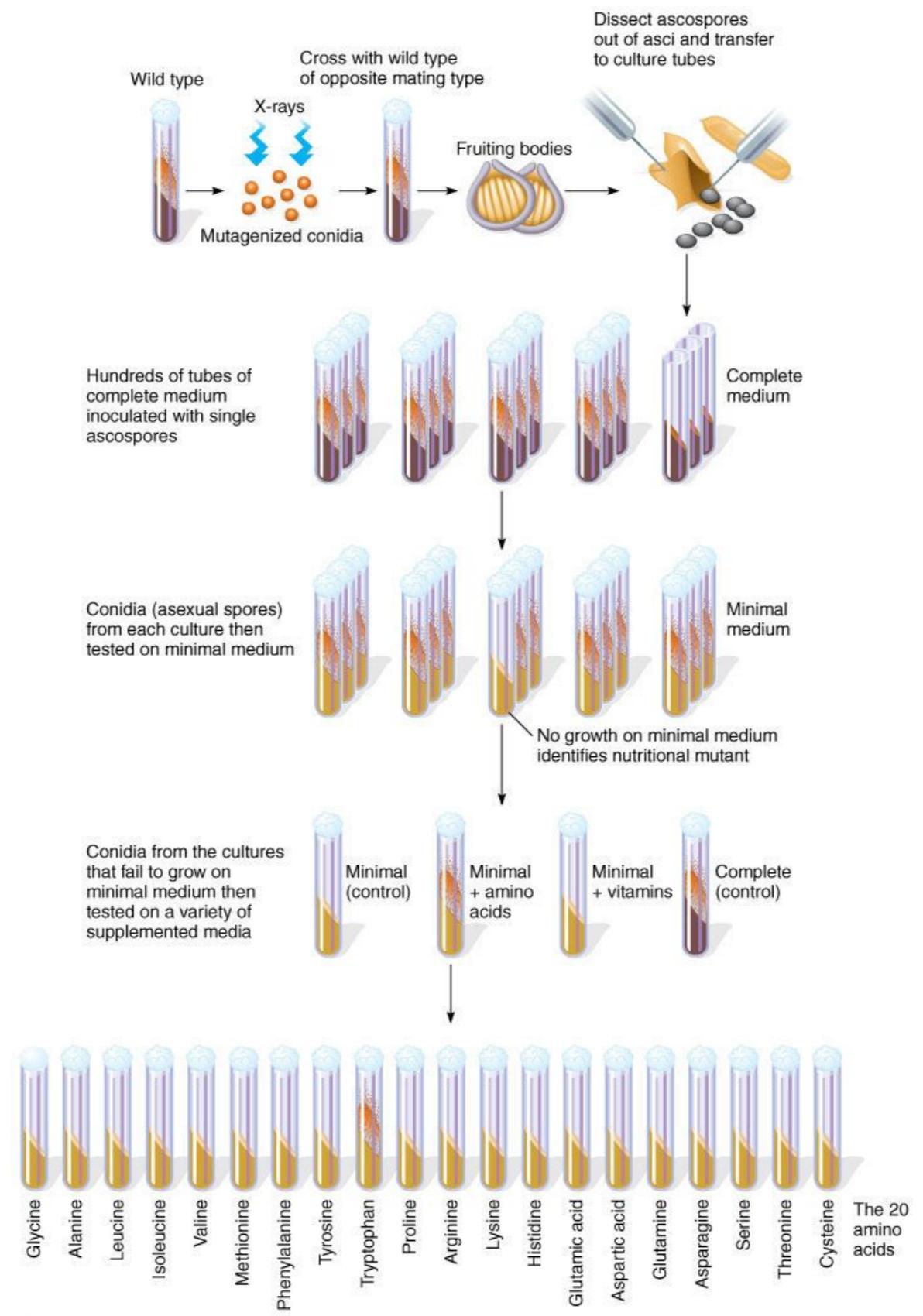


1903 -
1989

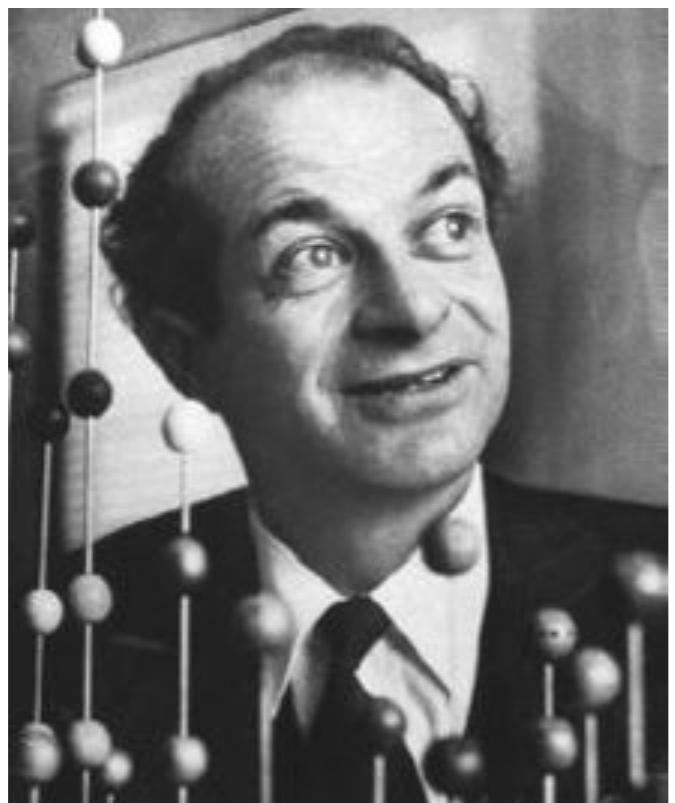


1909 -
1975

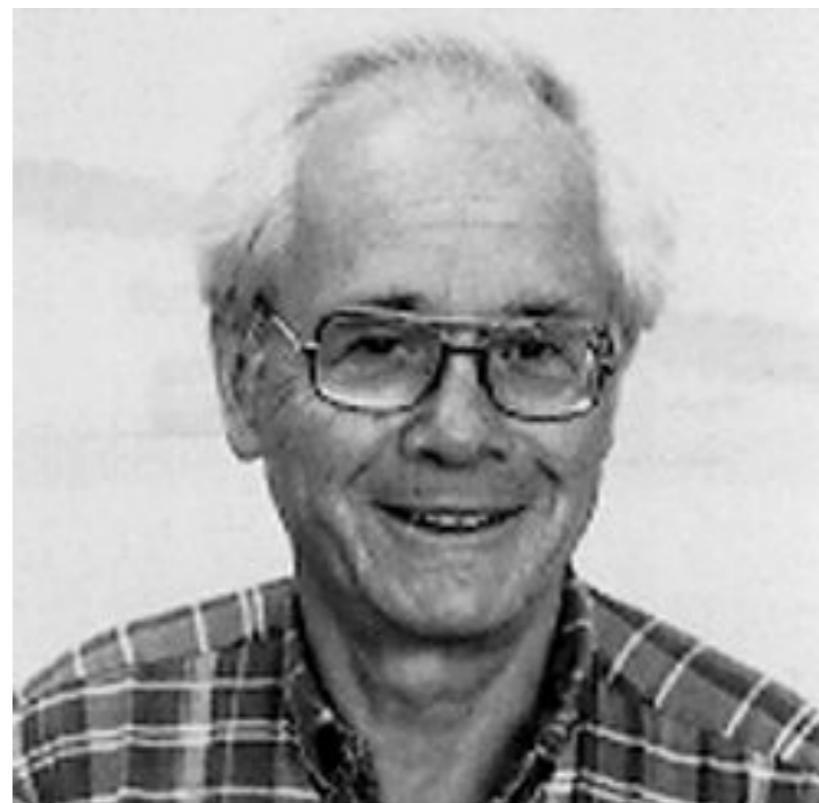
One gene one enzyme



Pauling, Ingram



1901 -
1994



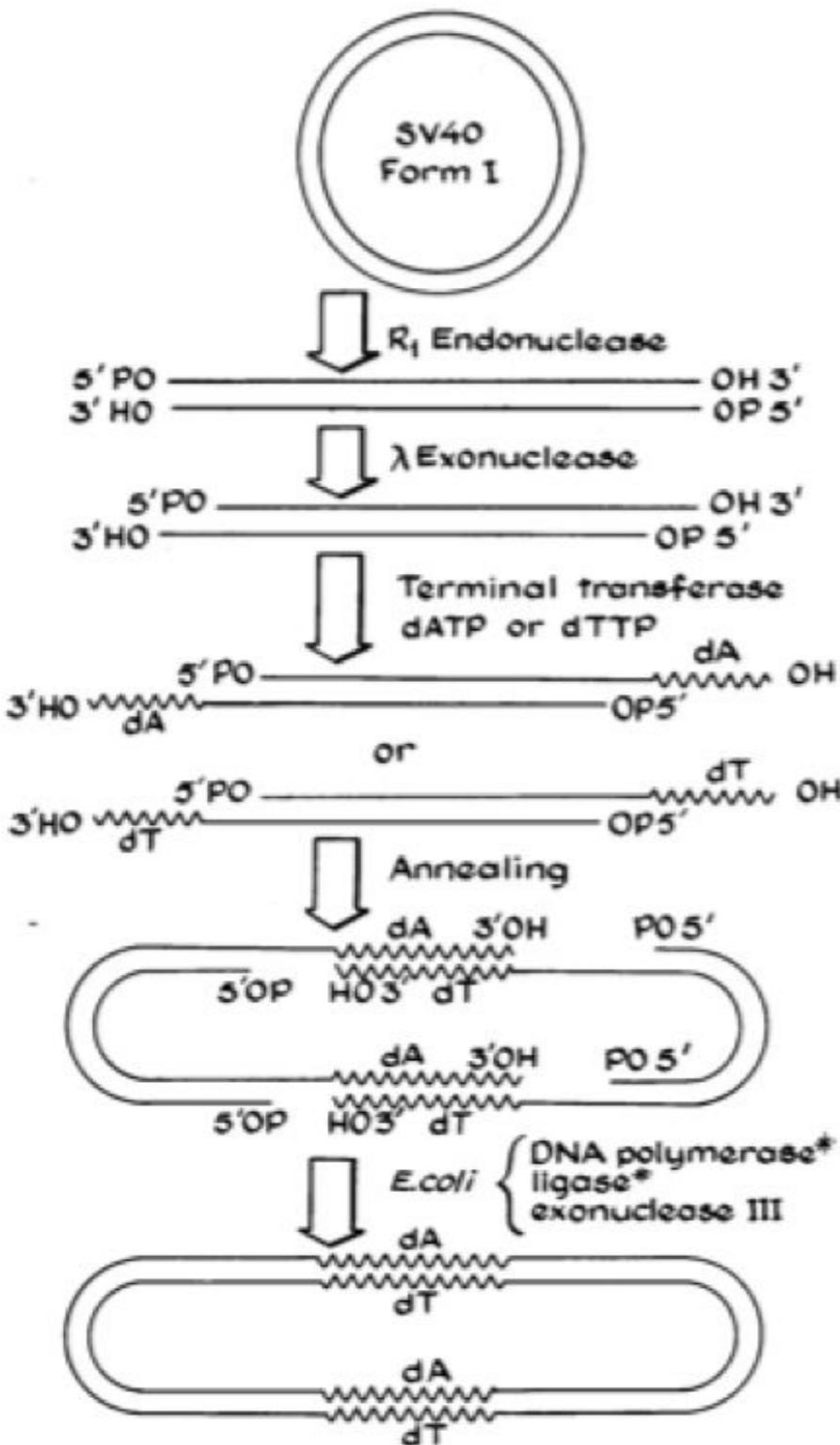
1924 -
2006

Sickle cell anemia is first molecular disease

Reverse Transcription, Recombinant Age

- Temin, Baltimore | reverse transcription
- Cohen, Boyer | recombinant methodologies
- Berg | SV40
- Maxam, Gilbert, Sanger | sequencing is here

1972: Berg experiment



Enzymes. The circular SV40 and λ -*dgal* DNA molecules were cleaved with the bacterial restriction endonuclease RI (Yoshimori and Boyer, unpublished; the enzyme was generously made available to us by these workers). Phage λ -exonuclease (given to us by Peter Lobban) was prepared according to Little *et al.* (5), calf-thymus deoxynucleotidyl terminal transferase (terminal transferase), prepared according to Kato *et al.* (6), was generously sent to us by F. N. Hayes; *E. coli* DNA polymerase I Fraction VII (7) was a gift of Douglas Brutlag; and *E. coli* DNA ligase (8) and exonuclease III (9) were kindly supplied by Paul Modrich.

One important feature of this method, which is different from some other techniques that can be used to join unrelated DNA molecules to one another (16, 19), is that here the joining is directed by the homopolymeric tails on the DNA. In our protocol, molecule A and molecule B can only be joined to each other; all AA and BB intermolecular joinings and all A and B intramolecular joinings (circularizations) are prevented. The yield of the desired product is thus increased, and subsequent purification problems are greatly reduced.

1977: Maxam-Gilbert/Sanger

A Maxam-Gilbert sequencing (Chemical sequencing)

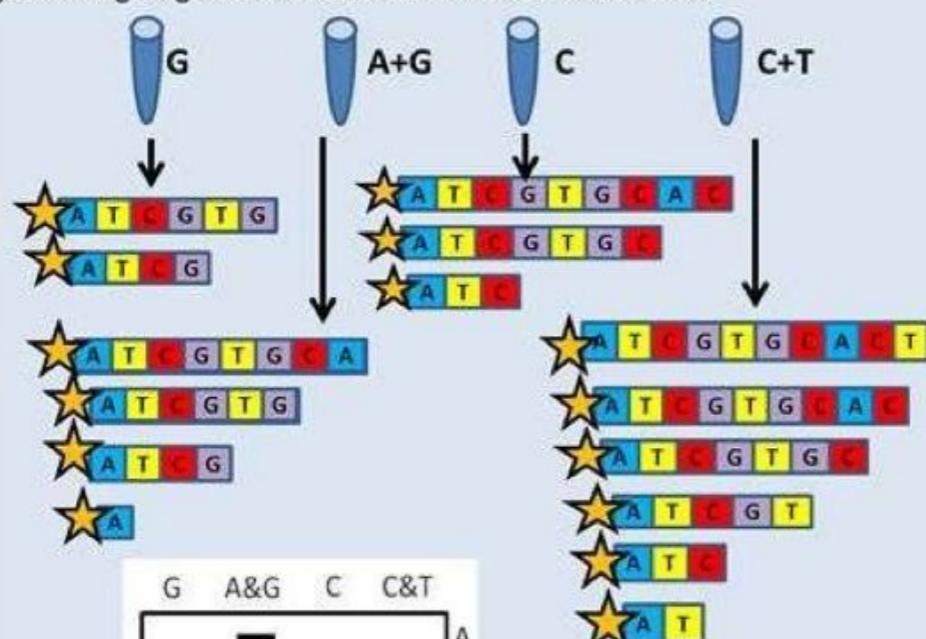
1. Double stranded DNA libraries radioactively labelled



2. 5' End labelled double strands de-natured to form single strands



3. DNA cleaved at specific bases by four base-specific reactions generating fragments ended with each individual base

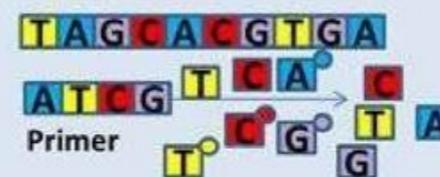


	G	A&G	C	C&T
G	-	-	-	A
A	-	-	T	T
T	-	-	C	C
C	-	-	G	G
G	-	-	T	T
T	-	-	C	C
C	-	-	A	A
A	-	-	C	C
T	-	-	T	T

4. Each reaction separated side by side on a polyacrylamide gel allowing reading of up to 50bp per reaction

B Sanger Dideoxy Sequencing

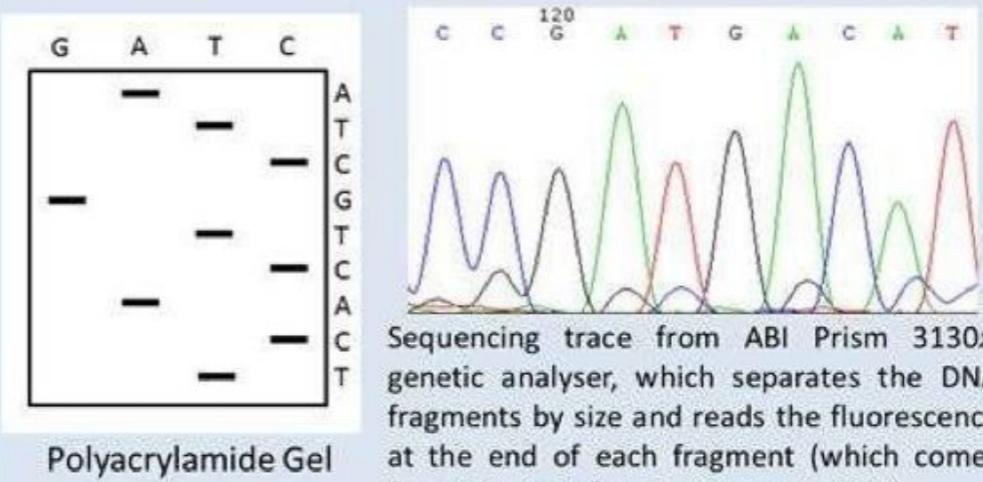
1. Four DNA synthesis reactions incorporating chain-terminating dideoxynucleotides lead to ending of the sequence at each A, T, C or G each labelled with a separate nucleotide.



2. Each reaction thus generates fragments of increasing size, ending at the base specified by the reaction i.e. each A, T, C or G.



3. Fragments resolved on a gel or automated sequencing machine.



Kary Mullis



1944 - 2019

PCR

Enzymatic Amplification of β -Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia

Randall K. Saiki, Stephen Scharf, Fred Falloona, Kary B. Mullis
Glenn T. Horn, Henry A. Erlich, Norman Arnheim

Recent advances in recombinant DNA technology have made possible the molecular analysis and prenatal diagnosis of several human genetic diseases. Fetal DNA obtained by amniocentesis or chorionic villus sampling can be analyzed by restriction enzyme digestion, with subsequent electrophoresis, Southern transfer, and specific hybridization to cloned gene or oligonucleotide probes. With

This disease results from homozygosity of the sickle-cell allele (β^S) at the β -globin gene locus. The S allele differs from the wild-type allele (β^A) by substitution of an A in the wild-type to a T at the second position of the sixth codon of the β chain gene, resulting in the replacement of a glutamic acid by a valine in the expressed protein. For the prenatal diagnosis of sickle cell anemia, DNA ob-

Abstract. Two new methods were used to establish a rapid and highly sensitive prenatal diagnostic test for sickle cell anemia. The first involves the primer-mediated enzymatic amplification of specific β -globin target sequences in genomic DNA, resulting in the exponential increase (220,000 times) of target DNA copies. In the second technique, the presence of the β^A and β^S alleles is determined by restriction endonuclease digestion of an end-labeled oligonucleotide probe hybridized in solution to the amplified β -globin sequences. The β -globin genotype can be determined in less than 1 day on samples containing significantly less than 1 microgram of genomic DNA.

polymorphic DNA markers linked genetically to a specific disease locus, segregation analysis must be carried out with restriction fragment length polymorphisms (RFLP's) found to be informative by examining DNA from family members (1, 2).

Many of the hemoglobinopathies, however, can be detected by more direct methods in which analysis of the fetus alone is sufficient for diagnosis. For example, the diagnosis of hydrops fetalis (homozygous α -thalassemia) can be made by documenting the absence of any α -globin genes by hybridization with an α -globin probe (3–5). Homozygosity for certain β -thalassemia alleles can be determined in Southern transfer experiments by using oligonucleotide probes that form stable duplexes with the normal β -globin gene sequence but form unstable hybrids with specific mutants (6, 7).

Sickle cell anemia can also be diagnosed by direct analysis of fetal DNA.

tained by amniocentesis or chorionic villus sampling can be treated with a restriction endonuclease (for example, Dde I and Mst II) that recognizes a sequence altered by the β^S mutation (8–11). This generates β^A - and β^S -specific restriction fragments that can be resolved by Southern transfer and hybridization with a β -globin probe.

We have developed a procedure for the detection of the sickle cell mutation that is very rapid and is at least two orders of magnitude more sensitive than standard Southern blotting. There are two special features to this protocol. The first is a method for amplifying specific β -globin DNA sequences with the use of oligonucleotide primers and DNA polymerase (12). The second is the analysis of the β -globin genotype by solution hybridization of the amplified DNA with a specific oligonucleotide probe and subsequent digestion with a restriction endonuclease (13). These two techniques increase the speed and sensitivity, and

lessen the complexity of prenatal diagnosis for sickle cell anemia; they may also be generally applicable to the diagnosis of other genetic diseases and in the use of DNA probes for infectious disease diagnosis.

Sequence amplification by polymerase chain reaction. We use a two-step procedure for determining the β -globin genotype of human genomic DNA samples. First, a small portion of the β -globin gene sequence spanning the polymorphic Dde I restriction site diagnostic of the β^A allele is amplified. Next, the presence or absence of the Dde I restriction site in the amplified DNA sample is determined by solution hybridization with an end-labeled complementary oligomer followed by restriction endonuclease digestion, electrophoresis, and autoradiography.

The β -globin gene segment was amplified by the polymerase chain reaction (PCR) procedure of Mullis and Falloona (12) in which we used two 20-base oligonucleotide primers that flank the region to be amplified. One primer, PC04, is complementary to the (+)-strand and the other, PC03, is complementary to the (-)-strand (Fig. 1). The annealing of PC04 to the (+)-strand of denatured genomic DNA followed by extension with the Klenow fragment of *Escherichia coli* DNA polymerase I and deoxynucleotide triphosphates results in the synthesis of a (-)-strand fragment containing the target sequence. At the same time, a similar reaction occurs with PC03, creating a new (+)-strand. Since these newly synthesized DNA strands are themselves template for the PCR primers, repeated cycles of denaturation, primer annealing, and extension result in the exponential accumulation of the 110-base pair region defined by the primers.

An example of the degree of specific gene amplification achieved by the PCR method is shown in Fig. 2A. Samples of DNA (1 μ g) were amplified for 20 cycles and a fraction of each sample, equivalent to 36 ng of the original DNA, was subjected to alkaline gel electrophoresis and transferred to a nylon filter. The filter was then hybridized with a 32 P-labeled 40-base oligonucleotide probe, RS06, which is complementary to the target sequence (Fig. 1A) but not to the PCR primers. The results, after a 2-hour autoradiographic exposure, show that a fragment hybridizing with the RS06 probe

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migrates at the position expected of the amplified target DNA segment (110 bases) (lanes 1 and 2). No hybridization with the RS06 probe could be detected in unamplified DNA (lane 4). When PCR amplification was performed on a DNA sample derived from an individual with hereditary persistence of fetal hemoglobin in which both the δ - and β -globin genes are deleted (14), again no 110-base fragment was detected (lane 3). To estimate the yield and efficiency of 20 cycles of PCR amplification, we prepared a Southern blot that contained 36 ng of an amplified genomic DNA sample and a dilution series consisting of various amounts of cloned β -globin sequence. The efficiency was calculated according to the formula: $(1 + X)^n = Y$, where X is the mean efficiency per cycle, n is the number of PCR cycles, and Y is the extent of amplification (yield) after n cycles (for example, a 200,000-fold increase after 20 cycles). The amounts of cloned β -globin sequences used in this experiment were calculated to represent efficiencies of 70 to 100 percent.

The reconstructions were prepared by digesting the β -globin plasmid, pBR328:: β^A , with the restriction enzymes Hae III and Mae I. Both of these enzymes cleave the β -globin gene within or very near to the 20 base regions that hybridize to the PCR primers, generating a 103-base pair (bp) fragment that is almost identical in size and composition to the 110-bp segment created by PCR amplification. After hybridization with the RS06 probe and autoradiography, the amplified genomic sample was compared with the known standards, and the result indicated an overall efficiency of approximately 85 percent (Fig. 2B), representing an amplification of about 220,000 times (1.85²⁰).

Distinguishing the β^A and β^S alleles by the oligomer restriction method. We have previously described a rapid solution hybridization method that can indicate whether a genomic DNA sample contains a specific restriction enzyme site at, in principle, any chromosomal location (13). This method, called oligomer restriction (OR), involves the stringent hybridization of a 32 P end-labeled oligonucleotide probe to the specific segment of the denatured genomic DNA which spans the target restriction site. The ability of a mismatch within the restriction site to prevent cleavage of the duplex formed between the probe and the target genomic sequence is the basis for detecting allelic variants. The presence of the restriction site in the target DNA is revealed by the appearance of a specific

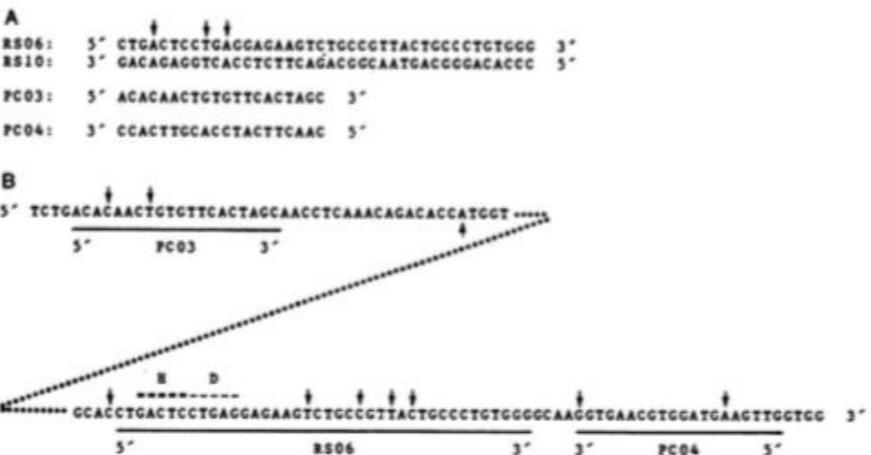


Fig. 1. Sequence of synthetic oligonucleotide primers and probe and their relation to the target β -globin region. (A) The primer PC03 is complementary to the (-)-strand and the primer PC04 is complementary to the (+)-strand of the β -globin gene. The probe RS06 is complementary to the (-)-strand of the wild-type (β^A) sequence of β -globin. RS10 is the "blocking oligomer", an oligomer complementary to the RS06 probe except for three nucleotides, indicated by the downward arrows. It is added before enzyme digestion to the OR reaction to anneal to the excess RS06 oligomer and prevent nonspecific cleavage products due to hybridization of RS06 to nontarget DNA (13). Because of the mismatches within the Dde I and Hinf I restriction sites, the RS06/RS10 duplex is not cleaved by Dde I and Hinf I digestion. (B) The relation between the primers, the probe, and the target β -globin sequence. The upward arrow indicates the β -globin initiation codon. The downward arrows indicate nucleotide differences between β - and δ -globin. The polymorphic Dde I site (CTCAG) is represented by a single horizontal dashed line (D), and the invariant Hinf I (GACTC) site is represented by double horizontal dashed lines (H).

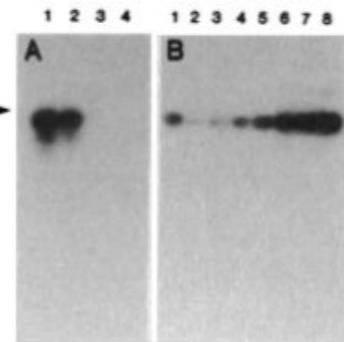


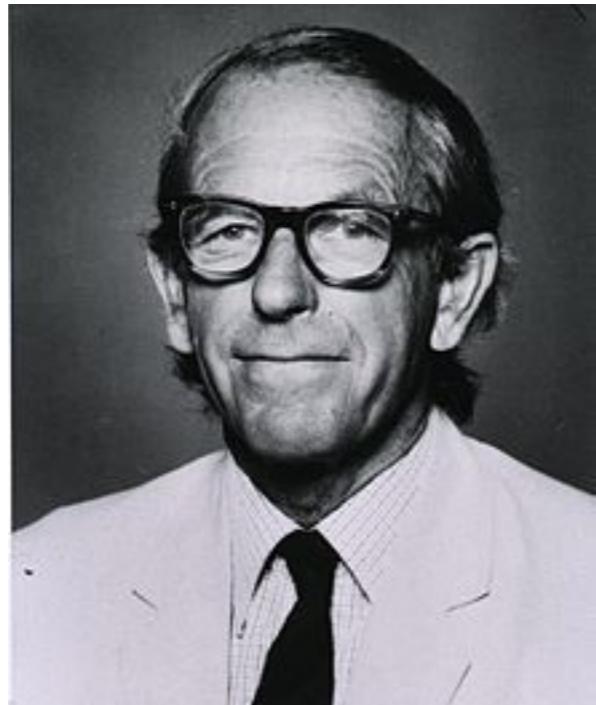
Fig. 2. Southern analysis of PCR amplified genomic DNA with the RS06 probe. (A) Samples (1 μ g) of genomic DNA were dispensed in microcentrifuge tubes and adjusted to 100 μ l in a buffer containing 10 mM tris, pH 7.5, 50 mM NaCl, 10 mM MgCl₂, 1.5 mM deoxynucleotide triphosphate [(dNTP) each of all four was used], 1 μ M PC03, and 1 μ M PC04. After heating for 5 minutes at 95°C (to denature the genomic DNA), the tubes were centrifuged for 10 seconds in a microcentrifuge to remove the condensation. The samples were immediately transferred to a 30°C heat block for 2 minutes to allow the PC03 and PC04 primers to anneal to their target sequences. At the end of this period, 2 μ l of the Klenow fragment of *E. coli* DNA polymerase I (Biolabs, 0.5 unit/ μ l in 10 mM tris, pH 7.5, 50 mM NaCl, 10 mM MgCl₂) was

added, and the incubation was allowed to proceed for an additional 2 minutes at 30°C. This cycle—denaturation, centrifugation, hybridization, and extension—was repeated 19 more times, except that subsequent denaturations were done for 2 instead of 5 minutes. (The final volume after 20 cycles was 140 μ l.) Thirty-six nanograms of the amplified genomic DNA (5 μ l) were applied to a 4 percent Nusieve (FMC) alkaline agarose minigel and subjected to electrophoresis (50 V), for 2 hours until the bromoresol green dye front reached 4 cm. After neutralization and transfer to Genetrans nylon membrane (Plasco), the filter was "prehybridized" in 10 ml 3 \times SSPE (1 \times SSPE is 0.18M NaCl, 10 mM NaH₂PO₄, 1 mM EDTA, pH 7.4), 5 \times DET (1 \times DET is 0.02 percent each polyvinylpyrrolidone, Ficoll, and bovine serum albumin; 0.2 mM tris, 0.2 mM EDTA, pH 8.0), 0.5 percent SDS, and 30 percent formamide for 4 hours at 42°C. Hybridization with 1.0 pmol of phosphorylated (with $[\gamma]^{32}$ P]ATP) RS06 (~5 μ Ci/pmol) in 10 ml of the same buffer was carried out for 18 hours at 42°C. The filter was washed twice in 2 \times SSPE, 0.1 percent sodium dodecyl sulfate (SDS) at room temperature for 30 minutes, and autoradiographed at -70°C for 2 hours with a single intensification screen. (Lanes 1 to 3) DNA isolated from the cell lines Molt4, SC01, and GM2064, respectively. Molt4 is homozygous for the normal, wild-type allele of β -globin ($\beta^A\beta^A$), SC-1 is homozygous for the sickle cell allele ($\beta^S\beta^S$), and GM2064 is a cell line in which the β - and δ -globin genes have been deleted ($\Delta\Delta$) (13). (Lane 4) Contains 36 ng of Molt4 DNA that was not PCR amplified. The horizontal arrow indicates the position of a 114-base marker fragment obtained by digestion of pBR328 with Nar I. (B) Thirty-six nanograms of 20-cycle amplified Molt4 DNA (see above) was loaded onto a Nusieve gel along with measured amounts of Hae III-Mae I digested pBR328:: β^A (13) calculated to represent the molar increase in β -globin target sequences at PCR efficiencies of 70, 75, 80, 85, 90, 95, and 100 percent (lanes 2 to 8, respectively). DNA was transferred to Genetrans and hybridized with the labeled RS06 probe as described above. (Lane 1) Molt4 DNA (36 ng); (lanes 2 to 8) 7.3 \times 10⁻⁴ pmol, 1.3 \times 10⁻³ pmol, 2.3 \times 10⁻³ pmol, 4.0 \times 10⁻³ pmol, 6.8 \times 10⁻³ pmol, 1.1 \times 10⁻² pmol, and 1.9 \times 10⁻² pmol of pBR328:: β^A , respectively (20).

Evolution of DNA sequencing: Wu, Sanger, Gilbert, Mullis



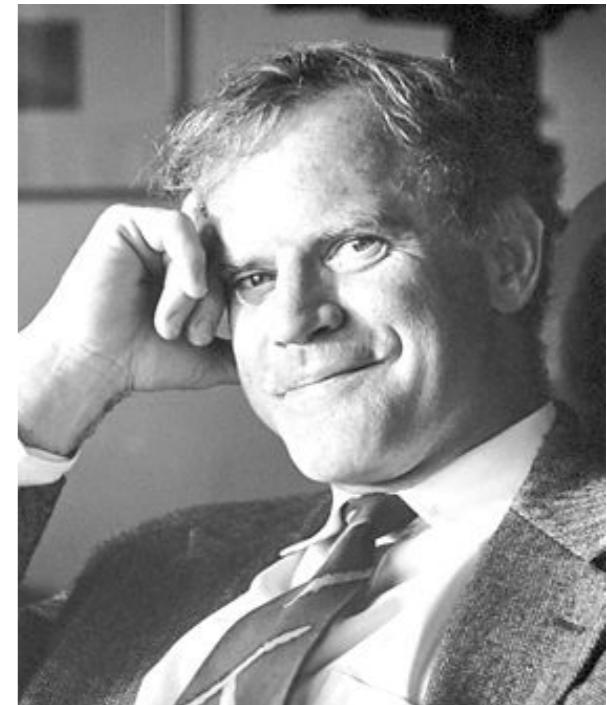
1928 - 2008



1918 - 2013



b. 1932



1944 - 2019

Sequencing will be covered in great detail
in **Lecture 15**