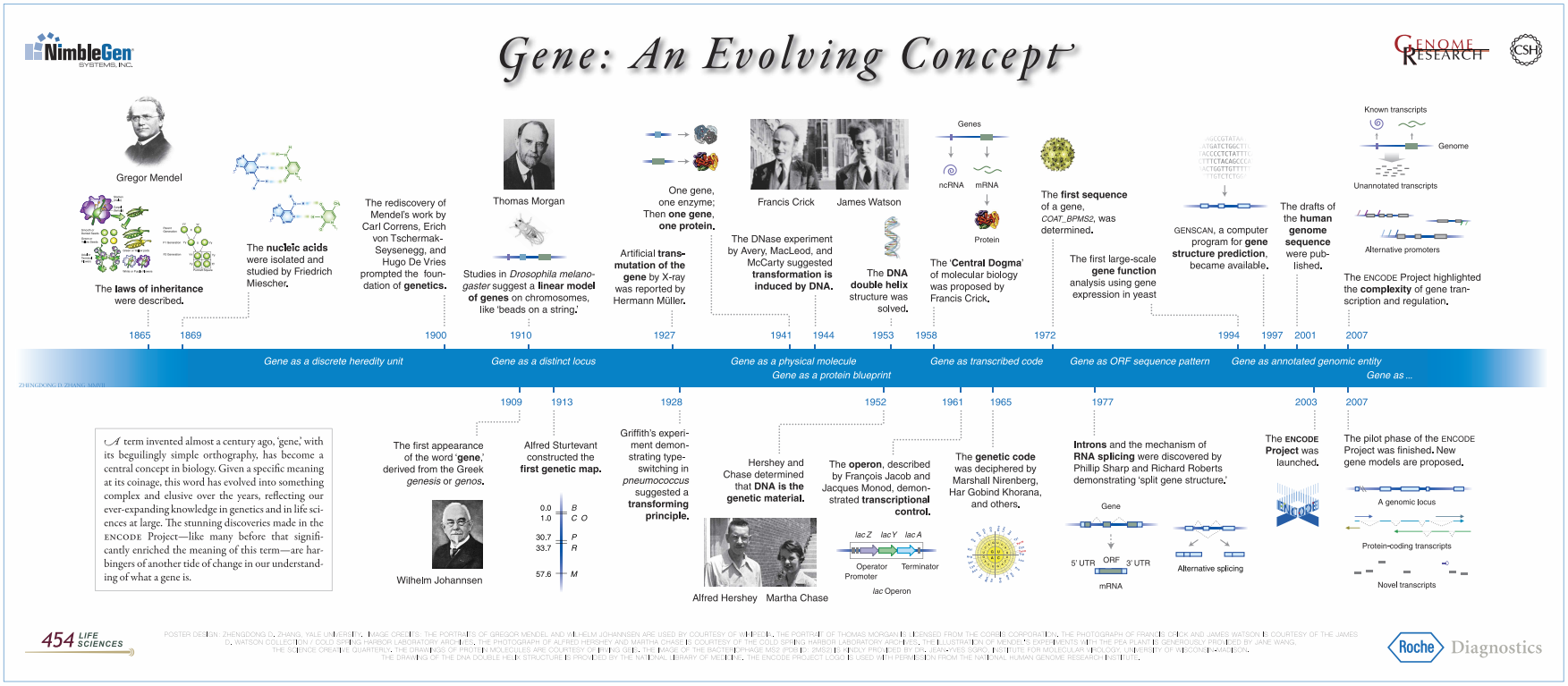
Lecture 3:

The nature of alleles

Course 410

Molecular Evolution

Chronological Review of concepts and finding in genetics and molecular biology



Nuclein: the chemical of the nucleus

Fredrick Miescher bloody bandages (1869)

XLV.

Ueber die chemische Zusammensetzung der Eiterzellen [1]).

Von Dr. **F. Miescher** aus Basel.

Die Chemie des Eiters ist bis vor Kurzem fast nur von den Gesichtspunkten aus studiert worden, die für die Untersuchung von pathologischen Transsudaten massgebend waren. In neuerer Zeit hat man sich mit der Erforschung der Eigenschaften des Protoplasma auch an die Eiterzellen gewandt. Insbesondre musste sich aber seit den bekannten Untersuchungen über die Herkunft der Eiterzellen der Gedanke aufdrängen, dass hier das nächstliegende Material sei zum Studium dieser Zellenspezies, die als constante Grösse nunmehr an so vielen Orten wird eingeführt werden müssen; ein Material, nicht tadelfrei, mit Vorsicht zu verwerthen, aber das einzige leicht zu beschaffende und desshalb zum vorläufigen Ausgangspunkt geeignet.

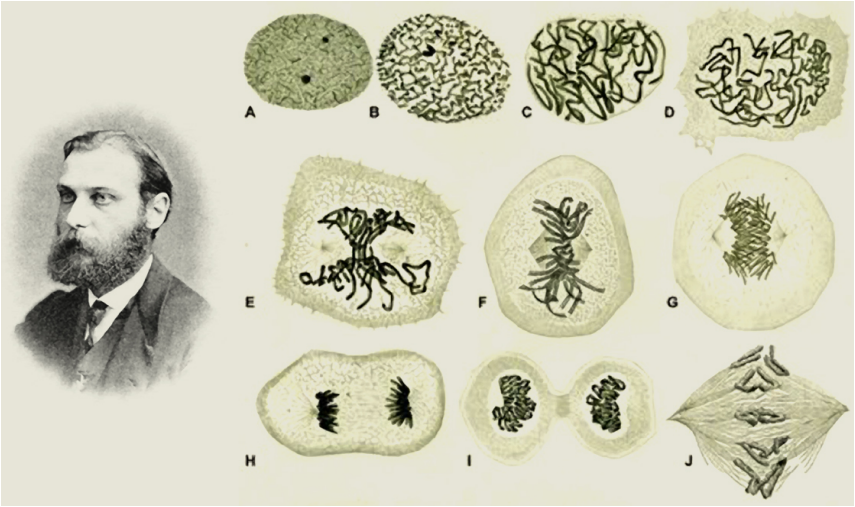
In diesem Sinne habe ich versucht, über die eigentlich gewebsbildenden Stoffe in den Eiterzellen zu einiger Orientierung zu gelangen. Die ganze Reihe der Extractivstoffe, in sofern sie ihrer Menge und Beschaffenheit nach nicht als wesentliche Gewebsbildner zu betrachten sind, habe ich bei Seite gelassen. Das Material zur Untersuchung wurde mir durch dankenswerthe Vermittlung der Herren Assistenzärzte Dr. Bever und Dr. Koch aus der Tübinger chirurgischen Klinik geliefert. Die Verbände, weitaus überwiegend von Operationswunden herrührend, wurden gesammelt, täglich auf das Laboratorium

1) Die Untersuchungen, welche Hr. Miescher in dieser Abhandlung schildert, sind im Tübinger Schlosslaboratorium von Herbst 1868 bis Herbst 1869 ausgeführt und mir kurz darauf zur Veröffentlichung in diesem Hefte übergeben, dessen Erscheinen durch mehre unvorhergesehene Umstände sehr verzögert ist.

Hoppe-Seyler.

Miescher, Friedrich (1871) "On the chemical composition of pus cells", Medicinisch-chemische Untersuchungen, 4 : 441–460.

Chromatin

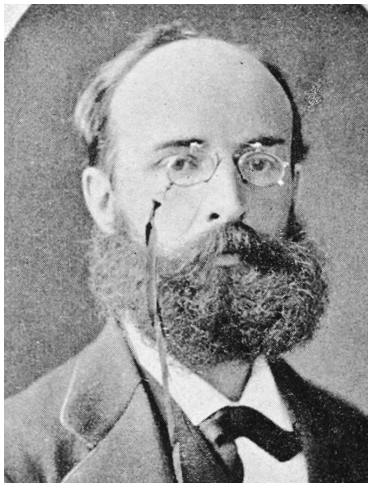
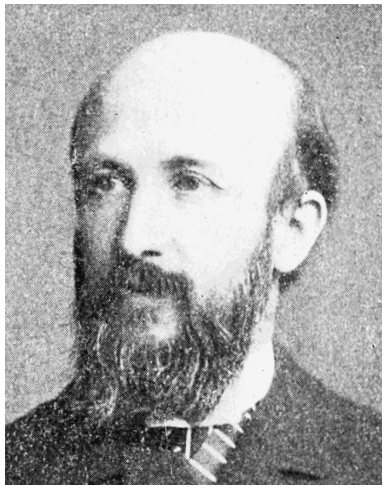
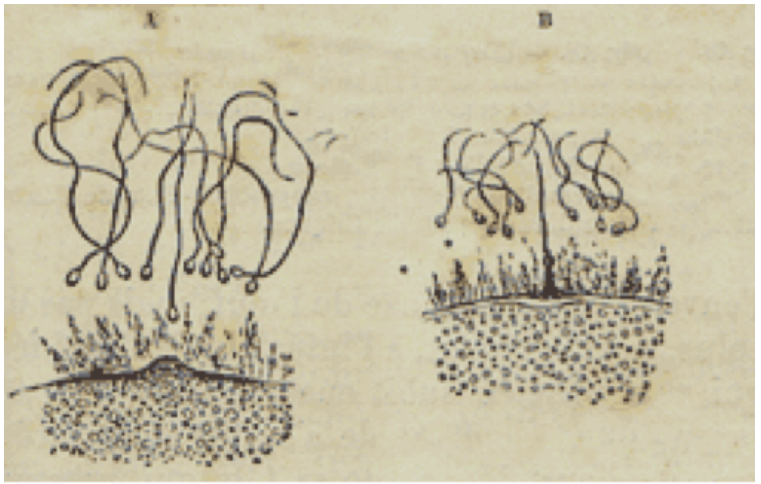


Walther Flemming band structure in dividing cells (1879)

- Used cells of salamanders and staining techniques to study cell division (he called it mitosis).
- The intensely stained parts of the nucleus he called **chromatin** (chroma is Greek for color).

Chromosomes

Hermann Fol and Oscar Hertwig
(1870-1880)

- Observed fertilization and fusion of the eggs and sperms nuclei.
- Chromatin is called **chromosomes**.

Factors on chromosomes

Thomas Morgan

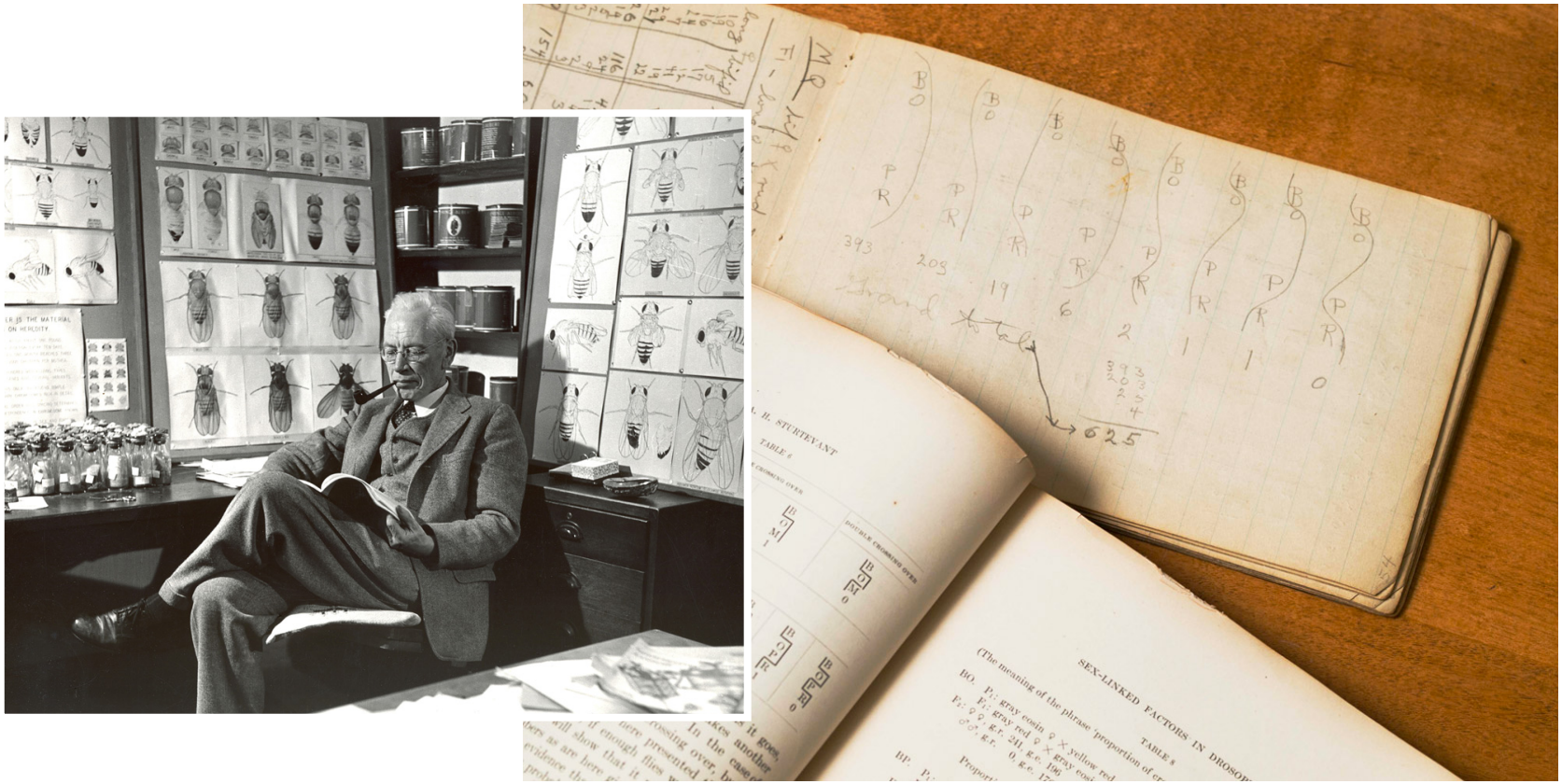
Studies in *Drosophila melanogaster* suggest a **linear model of genes** on chromosomes, like 'beads on a string.'

1910

Morgan and the fly room found evidence to associate factors (genes) to specific chromosome.

How?

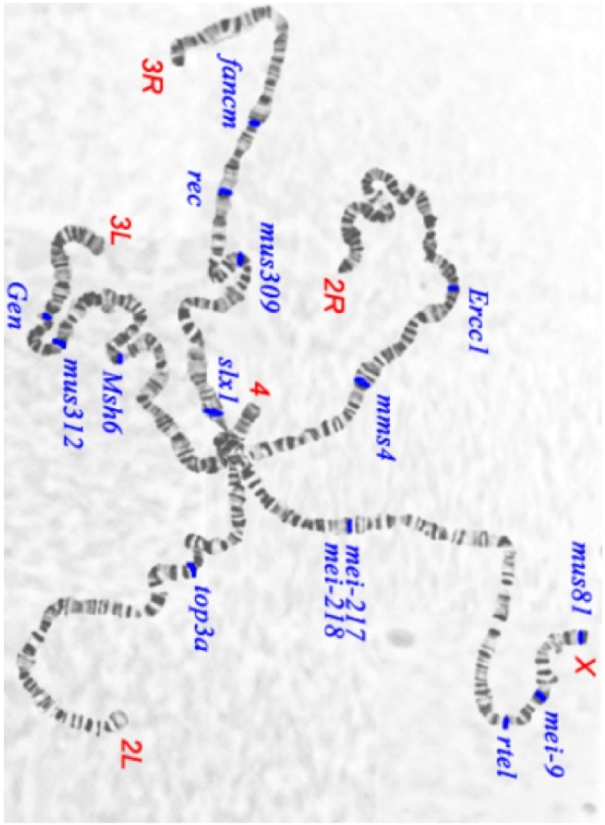
First genetic map



First genetic map constructed by Alfred Sturtevant (1913) - Morgan's student

Fly Room - Significance

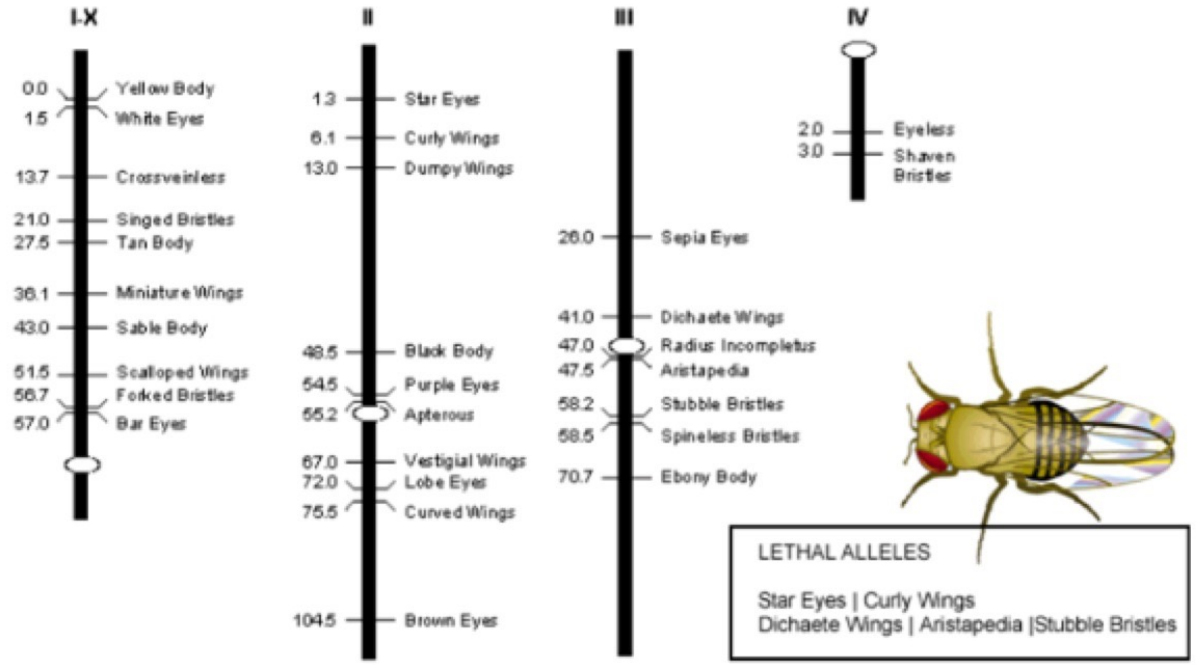
Genes on chromosomes



years, the fly boys began to sketch out a new [illegible] the model that made Morgan's team so historically important. It said that all traits were controlled by genes, and that these genes resided on chromosomes in fixed spots, strung along like pearls on a necklace. Because creatures inherit one copy of each chro-

Fly Room - Significance

Each chromosome - many genes

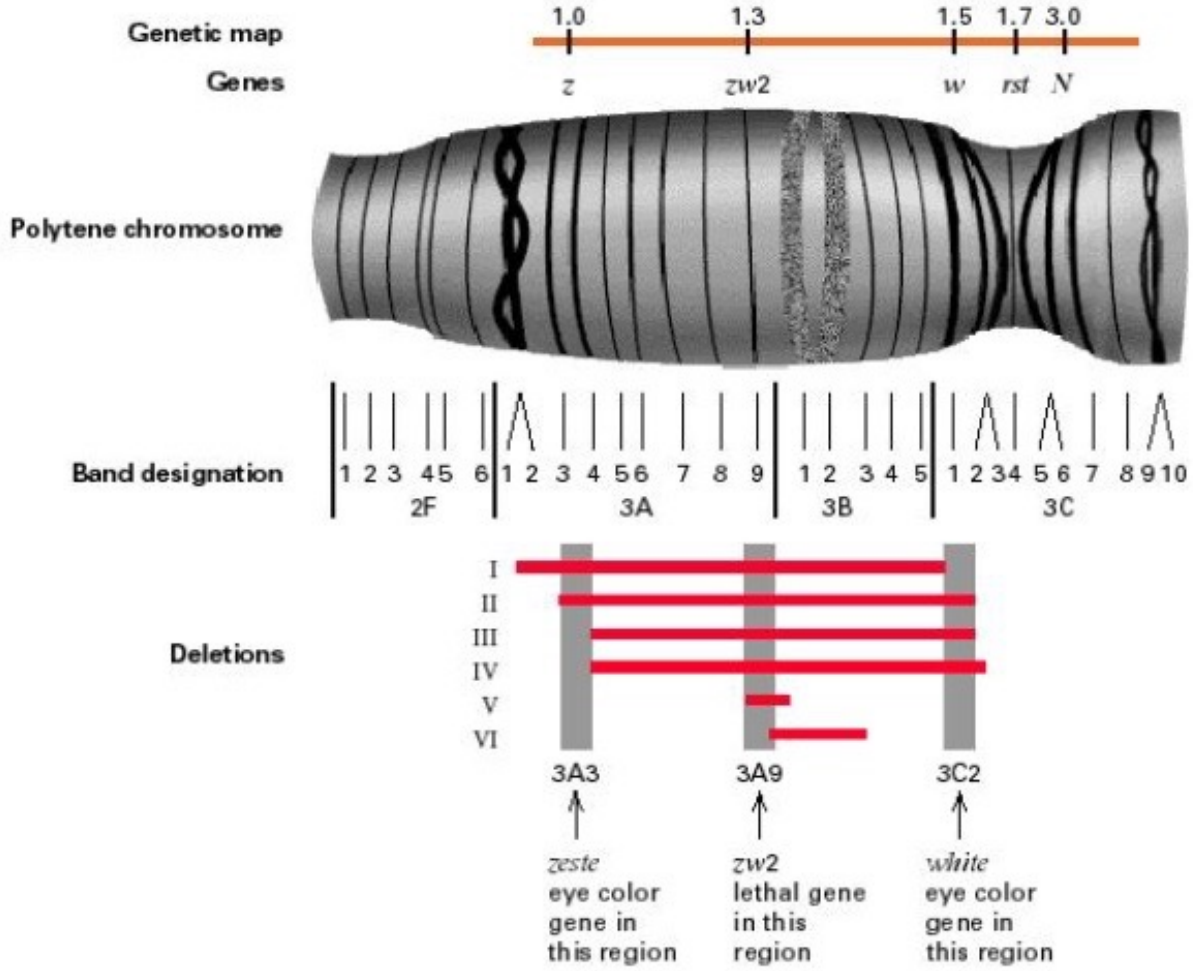


and protect everyone in genetics.

But historians can assign credit for some things. All the fly boys helped determine which clusters of traits got inherited together. More important, they discovered that four distinct clusters existed in flies—exactly the number of chromosome pairs. This was a huge boost for chromosome theory because it showed that every chromosome harbored multiple genes.

Fly Room - Significance

Linkage maps + cytogenetic maps



The chemical identity of the gene

What is the gene made of?

Griffith's transformation experiment

VOLUME XXVII JANUARY, 1928 No. 2

THE SIGNIFICANCE OF PNEUMOCOCCAL TYPES.

BY FRED. GRIFFITH, M.B.
(*A Medical Officer of the Ministry of Health.*)

(*From the Ministry's Pathological Laboratory.*)

CONTENTS.

Griffith, F. (1928). The Significance of Pneumococcal Types. The Journal of Hygiene, 27(2), 113–159.

Avery's transformation experiment

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND MACLYN MCCARTY,* M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research*)

PLATE 1

(Received for publication, November 1, 1943)

Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microörganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

Griffith found that mice injected subcutaneously with a small amount of a living R culture derived from Pneumococcus Type II together with a large inoculum of heat-killed Type III (S) cells frequently succumbed to infection, and that the heart's blood of these animals yielded Type III pneumococci in pure culture. The fact that the R strain was avirulent and incapable by itself of causing fatal bacteremia and the additional fact that the heated suspension of Type III cells contained no viable organisms brought convincing evidence that the R forms growing under these conditions had newly acquired the capsular structure and biological specificity of Type III pneumococci.

The original observations of Griffith were later confirmed by Neufeld and Levinthal (2), and by Baurhenn (3) abroad, and by Dawson (4) in this laboratory. Subsequently Dawson and Sia (5) succeeded in inducing transformation *in vitro*. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system. Later, Alloway (6) was able to cause

* Work done in part as Fellow in the Medical Sciences of the National Research Council.

Avery, O. T., MacLeod, C. M., & McCarty, M. (1944). STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES : INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III. The Journal of Experimental Medicine, 79(2), 137–158.

Hershey and Chase experiment

INDEPENDENT FUNCTIONS OF VIRAL PROTEIN AND NUCLEIC ACID IN GROWTH OF BACTERIOPHAGE*

By A. D. HERSHEY and MARTHA CHASE

(*From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island*)

(Received for publication, April 9, 1952)

The work of Doermann (1948), Doermann and Dissosway (1949), and Anderson and Doermann (1952) has shown that bacteriophages T2, T3, and T4 multiply in the bacterial cell in a non-infective form. The same is true of the phage carried by certain lysogenic bacteria (Lwoff and Gutmann, 1950). Little else is known about the vegetative phase of these viruses. The experiments reported in this paper show that one of the first steps in the growth of T2 is the release from its protein coat of the nucleic acid of the virus particle, after which the bulk of the sulfur-containing protein has no further function.

Materials and Methods.—Phage T2 means in this paper the variety called T2H (Hershey, 1946); T2*h* means one of the host range mutants of T2; UV-phage means phage irradiated with ultraviolet light from a germicidal lamp (General Electric Co.) to a fractional survival of 10^{-5}.

Sensitive bacteria means a strain (H) of *Escherichia coli* sensitive to T2 and its *h* mutant; resistant bacteria B/2 means a strain resistant to T2 but sensitive to its *h* mutant; resistant bacteria B/2*h* means a strain resistant to both. These bacteria do not adsorb the phages to which they are resistant.

"Salt-poor" broth contains per liter 10 gm. bacto-peptone, 1 gm. glucose, and 1 gm. NaCl. "Broth" contains, in addition, 3 gm. bacto-beef extract and 4 gm. NaCl.

Glycerol-lactate medium contains per liter 70 mM sodium lactate, 4 gm. glycerol, 5 gm. NaCl, 2 gm. KCl, 1 gm. NH_4Cl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 gm. gelatin, 10 mg. P (as orthophosphate), and 10 mg. S (as $MgSO_4$), at pH 7.0.

Adsorption medium contains per liter 4 gm. NaCl, 5 gm. K_2SO_4, 1.5 gm. KH_2PO_4, 3.0 gm. Na_2HPO_4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.01 gm. gelatin, at pH 7.0.

Veronal buffer contains per liter 1 gm. sodium diethylbarbiturate, 3 mM $MgSO_4$, and 1 gm. gelatin, at pH 8.0.

The HCN referred to in this paper consists of molar sodium cyanide solution neutralized when needed with phosphoric acid.

* This investigation was supported in part by a research grant from the National Microbiological Institute of the National Institutes of Health, Public Health Service. Radioactive isotopes were supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, United States Atomic Energy Commission.

Hershey A, Chase M (1952). "Independent functions of viral protein and nucleic acid in growth of bacteriophage" . J Gen Physiol 36 (1): 39–56.

What is nucleic acid made of?

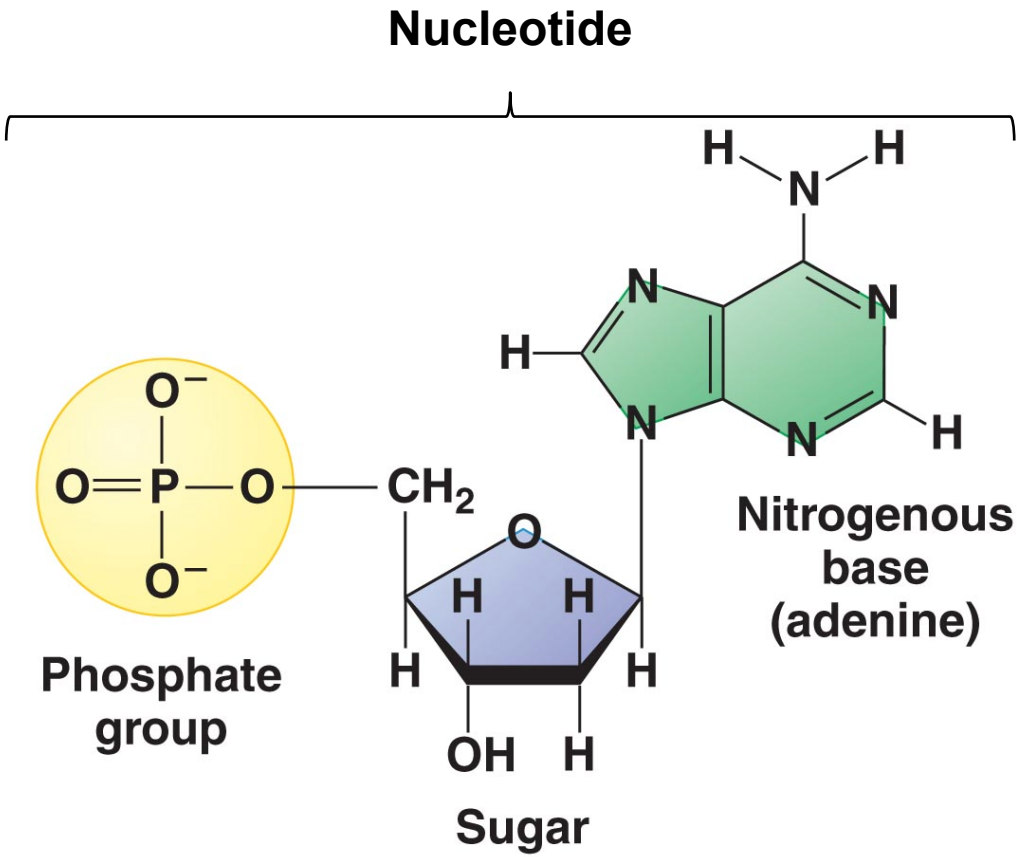
What is the chemical composition of nucleic acids (DNA and RNA)?

DNA and RNA chemical unit

- The chemical unit that makes nucleic acids (DNA and RNA) is called **Nucleotide.**

- A **nucleotide** is composed of:

1. Sugar
2. Phosphate group
3. Nitrogenous base



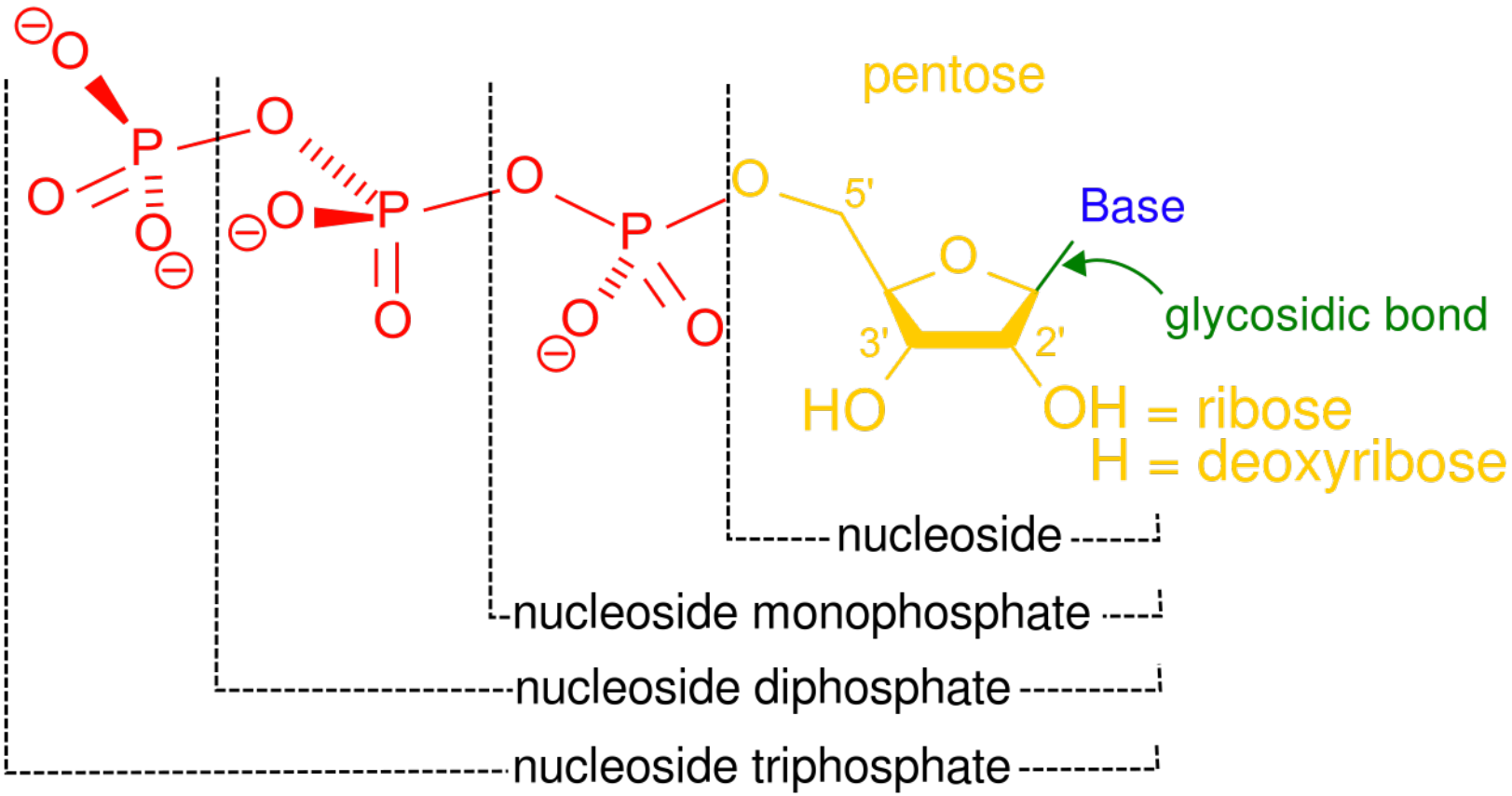
DNA and RNA chemical unit

Do you remember what a nucleoside is?

only for fun :-)

The phosphate group

- A number of phosphate groups can be attached to carbon 5 of the sugar.
- NTPs? dNTPs?



The phosphate group

What is the function os the phosphate groups?

Any relation to replication/sequencing?

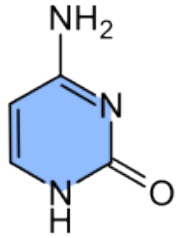
The bases

Nitrogenous bases

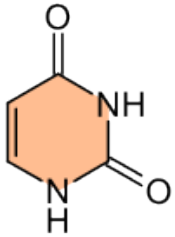
Small bases

Pyrimidines
One ring

Cytosine **C**



Uracil **U**

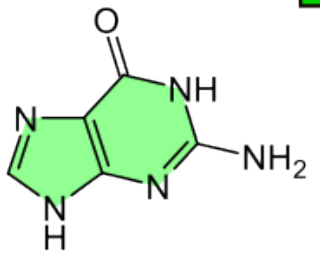


Found in RNA

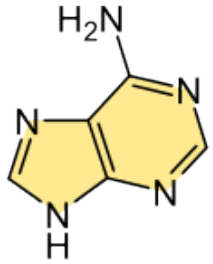
Large bases

Purines
Two rings

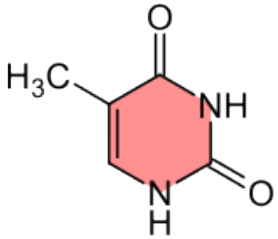
Guanine **G**



Adenine **A**



Thymine **T**

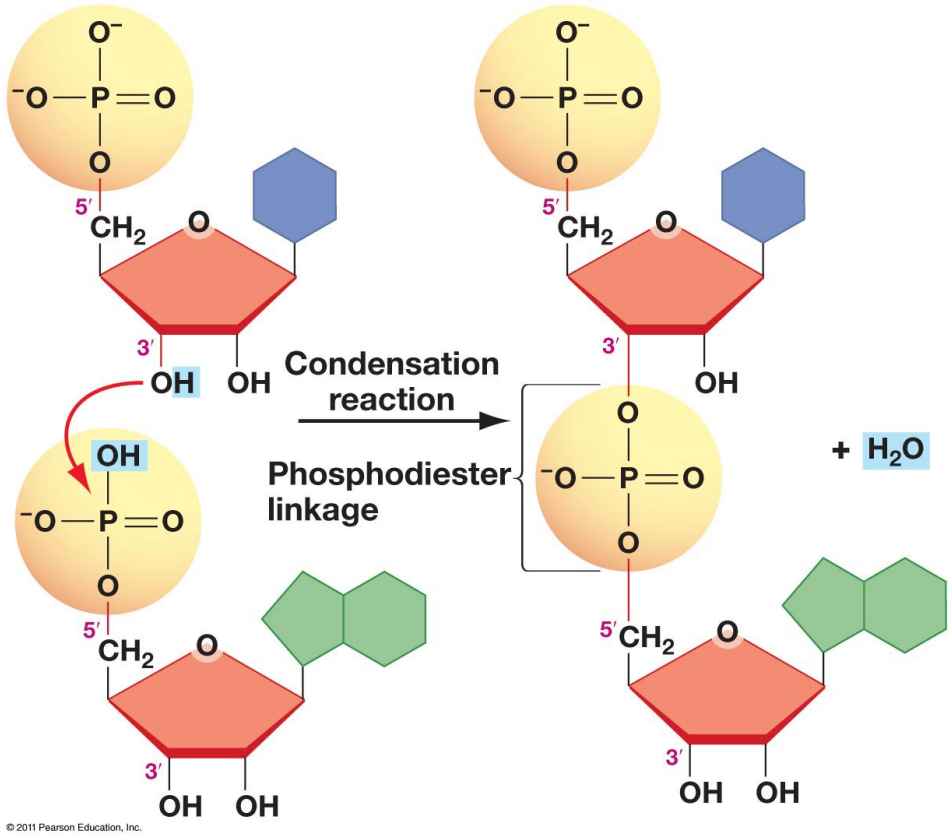


Found in DNA

Found in DNA and RNA

Nucleotide linking

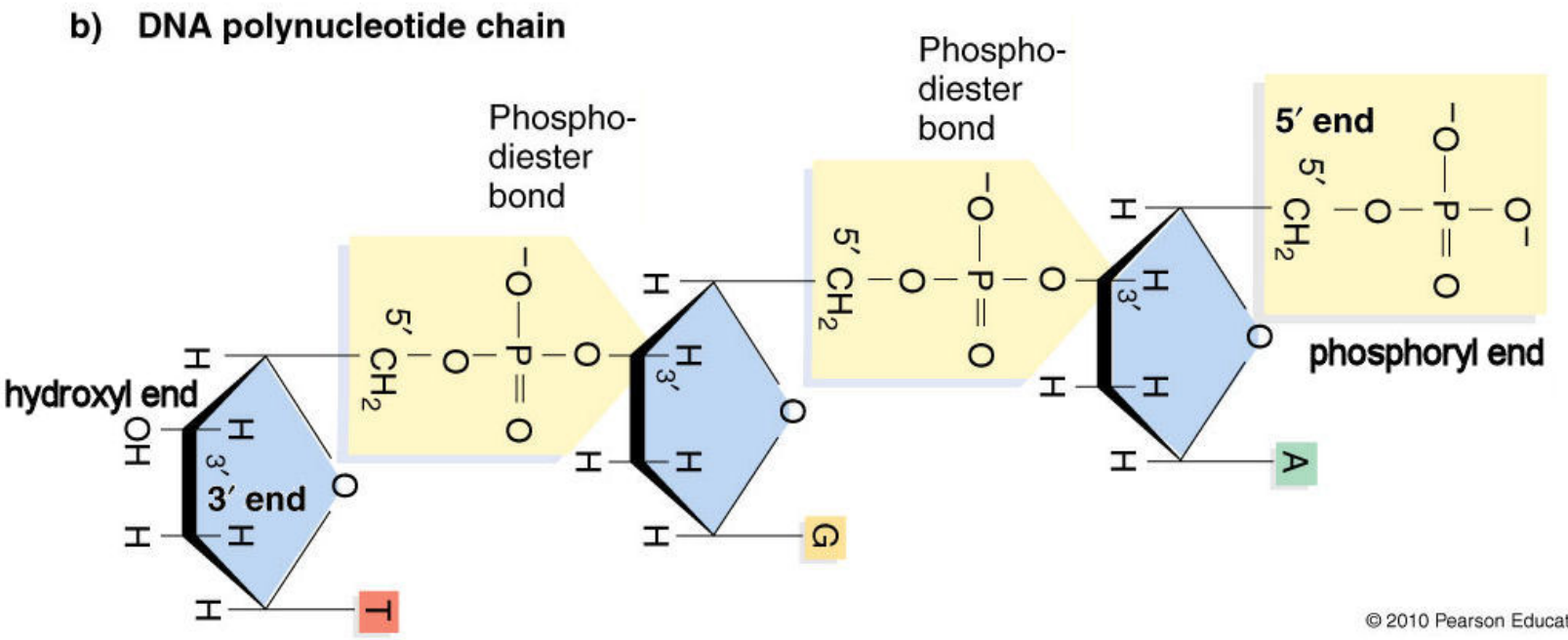
- Nucleotides are linked via **phosphodiester bond.**
- A covalent bond links the phosphate group of one nucleotide to the 3’ carbon of the sugar of another.



Polarity

- **5’ end:** where the 5’ carbon at one end of the molecule has a phosphate group.

- **3’ end:** where the 3’ carbon at the other end of the molecule has a hydroxyl group.



The Race to DNA structure

The story of the discovery of the double helix involves these key actors

Watson and Crick

- They published a 900 words paper and Franklin and Wilkin also published on the same issue of Nature.

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., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

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

³ Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. 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 Corey[1]. 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.

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

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[2] 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 is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[3,5] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

DNA structure

1) DNA is a double helix.

2) Two polynucleotides chains.

3) The two chains wind around right handedly - right handed double helix.

4) The two chains are in an anti-parallel orientation. One strand 5’ – 3’ orientation and the other 3’ – 5’).

5) Sugar-phosphate backbone is located on the outside of the helix.

6) The nitrogenous bases located on the inside of the helix.

DNA structure

7) The bases are stacked flat and perpendicular to the axis of the helix. The bases are on top of each other following the twist of the helix.

8) The bases of the two polynucleotides are bonded together via hydrogen bonds on the inside of the helix.

9) Bases of the two polynucleotide chains are base-pairing in a combination that maintains similar diameter of the double helix.

10) A Pyrimidine always basepair with Purine forming **complementary base pairs.**

DNA structure

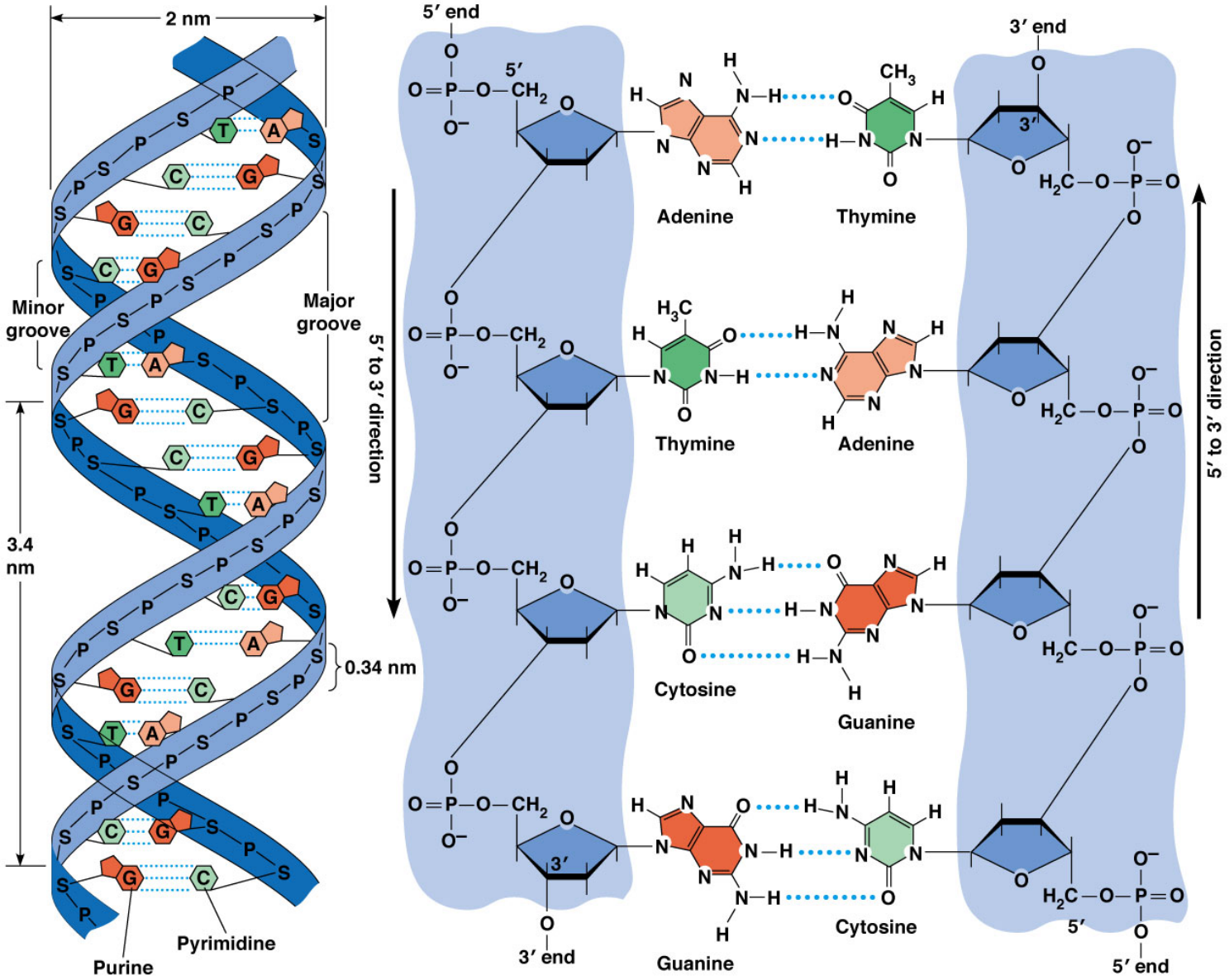
11) Thymine (T) basepair with Adenine (A), and Cytosine basepair with Guanine (G).

12) Two hydrogen bonds involve the base-pairing of (A and T) and three hydrogen bonds between (G and C).

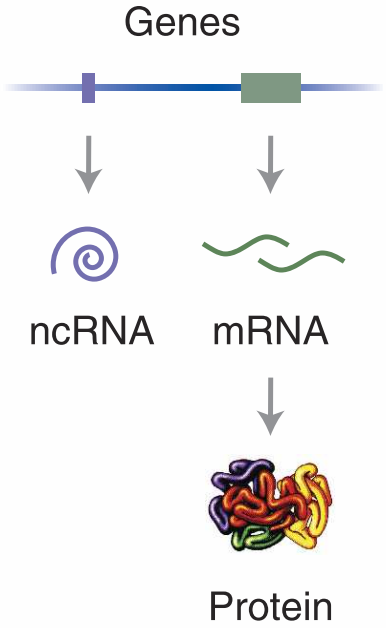
13) The sequence of one chain (strand) is enough to predict the complementary one in the other orientation.

14) A major and minor groove result from the unequal spacing of the phosphate-sugar backbone.

Summary

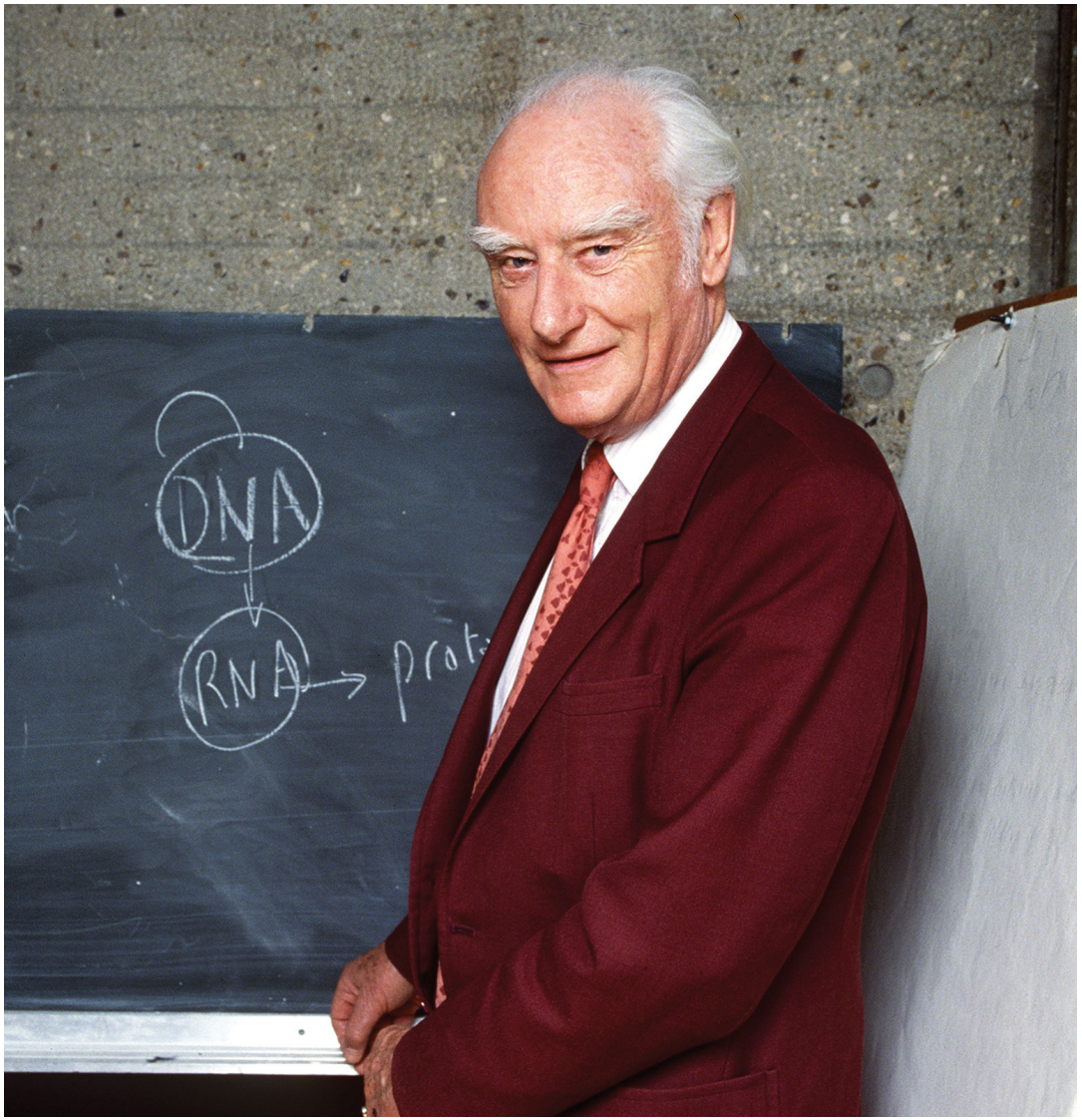


The Central Dogma



The '**Central Dogma**' of molecular biology was proposed by Francis Crick.

1958



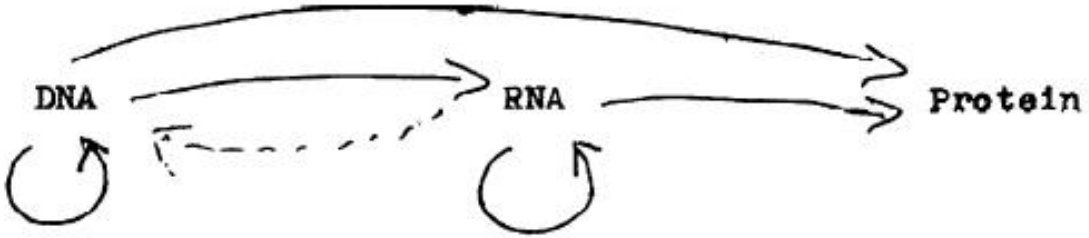
The Central Dogma

Ideas on Protein Synthesis (Oct. 1956)

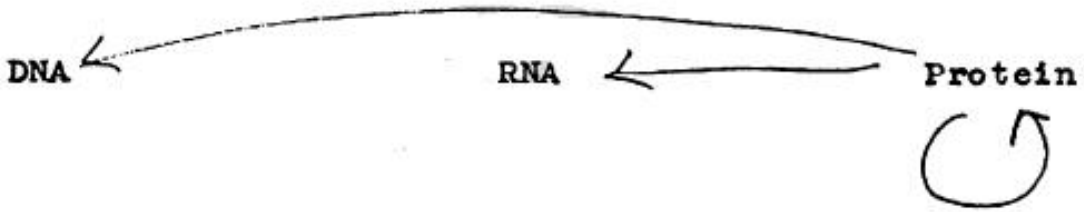
The Doctrine of the Triad.

The Central Dogma: "Once information has got into a protein it can't get out again". Information here means the sequence of the amino acid residues, or other sequences related to it.

That is, we may be able to have



but never



where the arrows show the transfer of information.

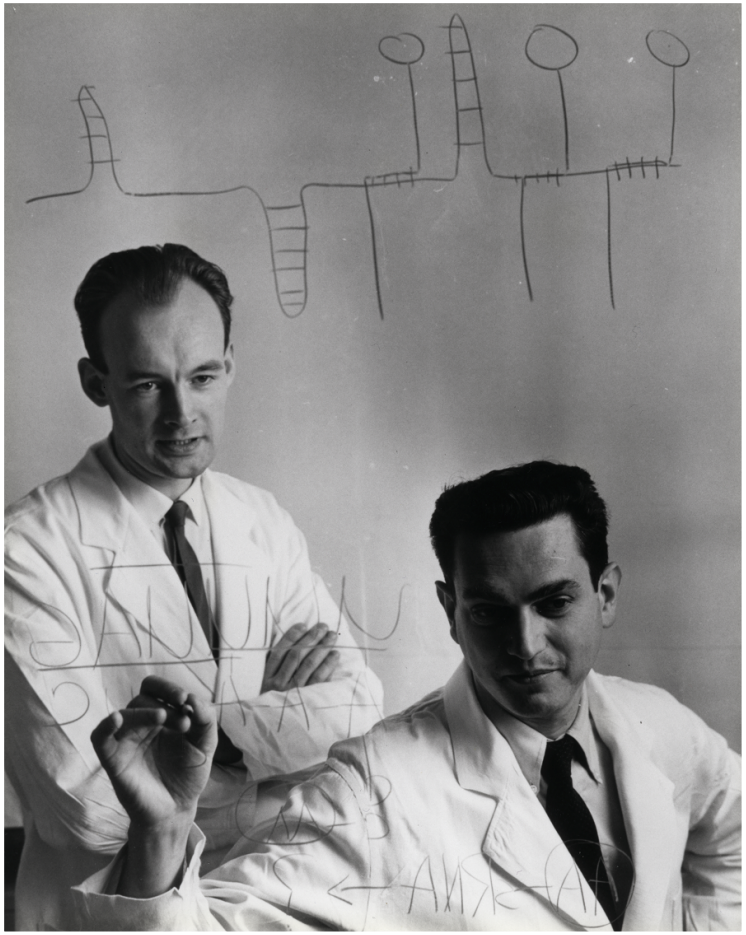
The Central Dogma

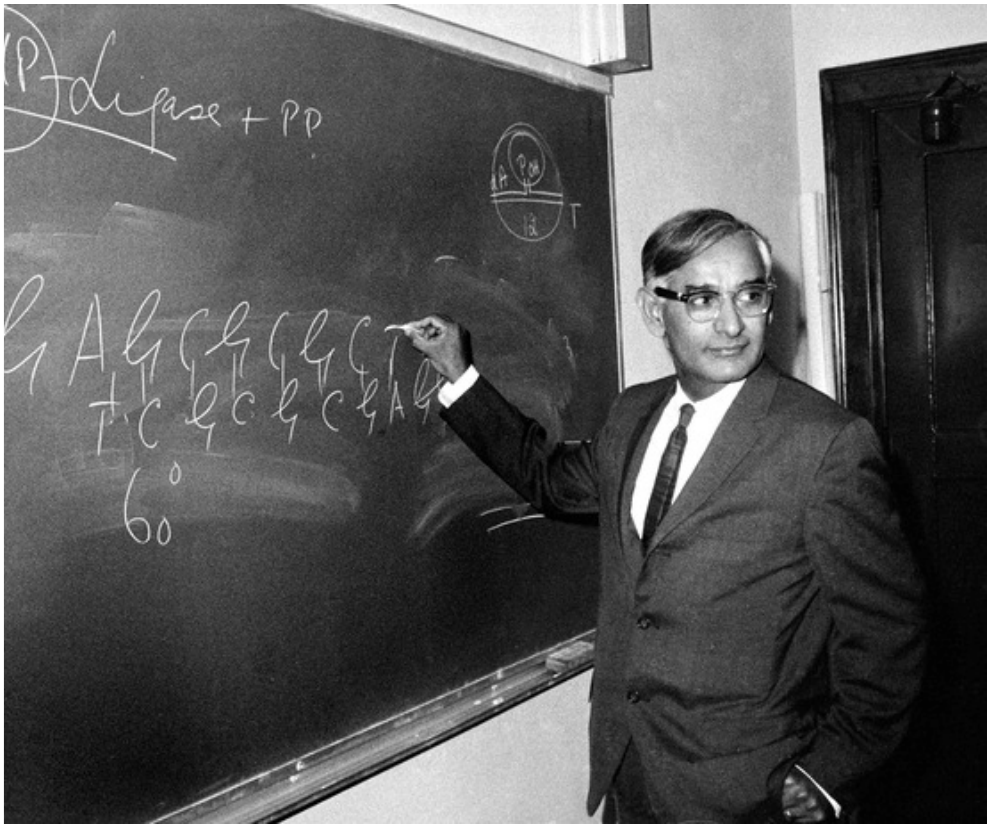
Molecules as information and information flow

a hypothesis with regard to the function of the 'bases' – the four kinds of molecule that are spaced along each strand of the double helix and which bind the two strands together. They wrote: 'it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information.'

This phrase, which was almost certainly the work of Crick, must have seemed both utterly strange and completely familiar to those

The code





1965: Marshall Nirenberg and Har Gobind Khorana (and others)

The genetic code

The genetic code is composed of 64 codons

61 amino acid coding codons

- Start codon (AUG) methionine (Met)
- 60 codons code for 19 other amino acids

Three codons code for the stop of translation

- UAA
- UAG
- UGA

Characteristics of the genetic code

1. The genetic code is made of triplets of nucleotides (3nts) called codons.
2. The genetic code is continuous (no skipping).
3. The code is not overlapping. Every three nucleotides in a sequence code for one codon.
4. The genetic code is universal (almost).
5. The code has specific signals for start of translation and stop of translation.
6. The genetic code is “degenerate”.
7. The Wobble effect of the third base in the codon.

Disclaimer

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