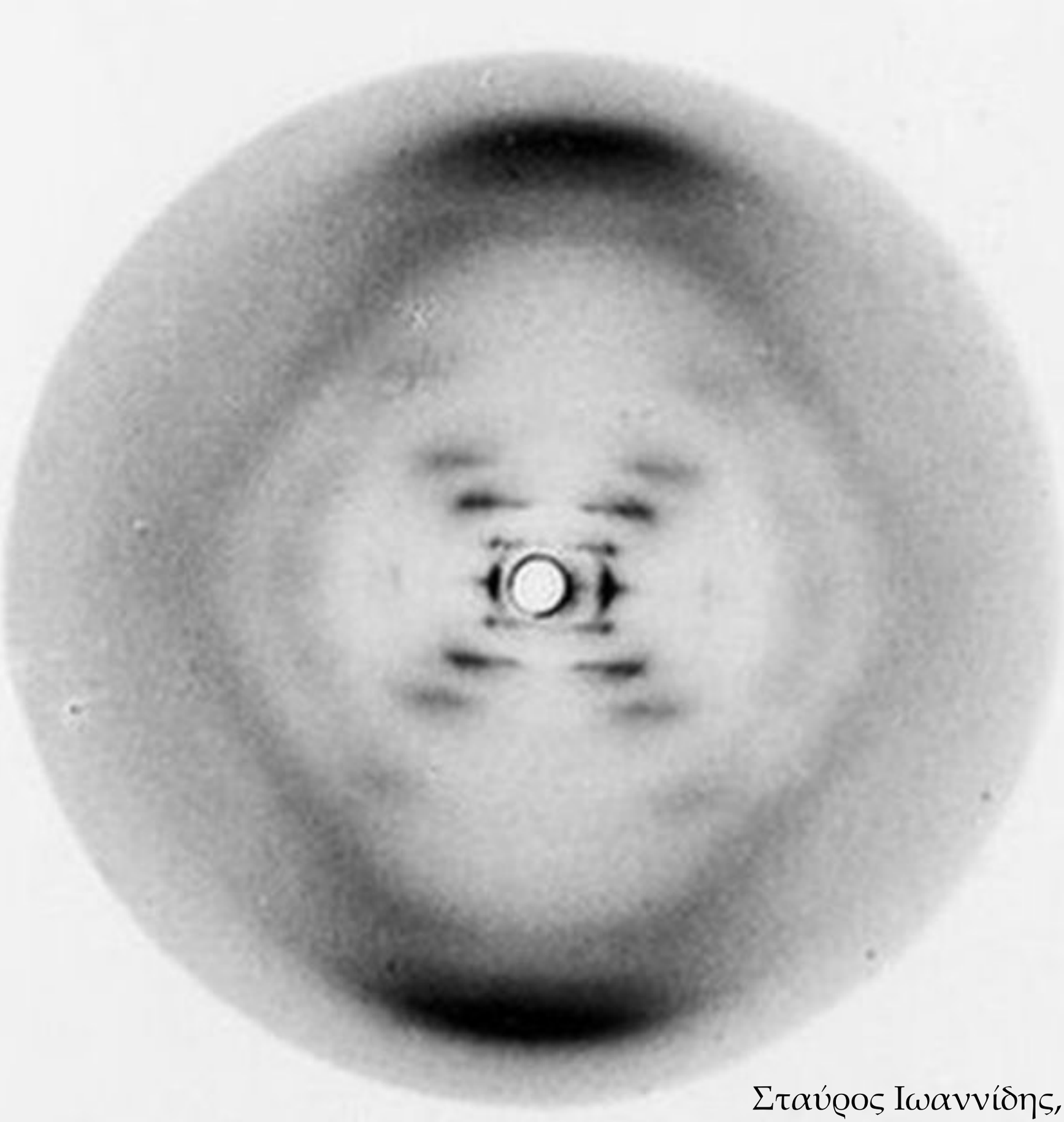
Μοριακή βιολογία:

η ανακάλυψη της δομής του DNA

-

Σύγχρονες εξελίξεις



Σταύρος Ιωαννίδης,
ΙΦΕ/ΕΚΠΑ

Ιστορία της Βιολογίας

Μάθημα 14

Δεν υπάρχει συμφωνία μεταξύ των γενετιστών για το αν τα γονίδια είναι **πραγματικά** ή **υποθετικά**, γιατί στο επίπεδο των πειραμάτων της γενετικής το γονίδιο μπορεί να κατανοηθεί είτε ως μια **υποθετική μονάδα** είτε ως ένα **υλικό σωματίδιο** χωρίς να υπάρχει καμία διαφορά.

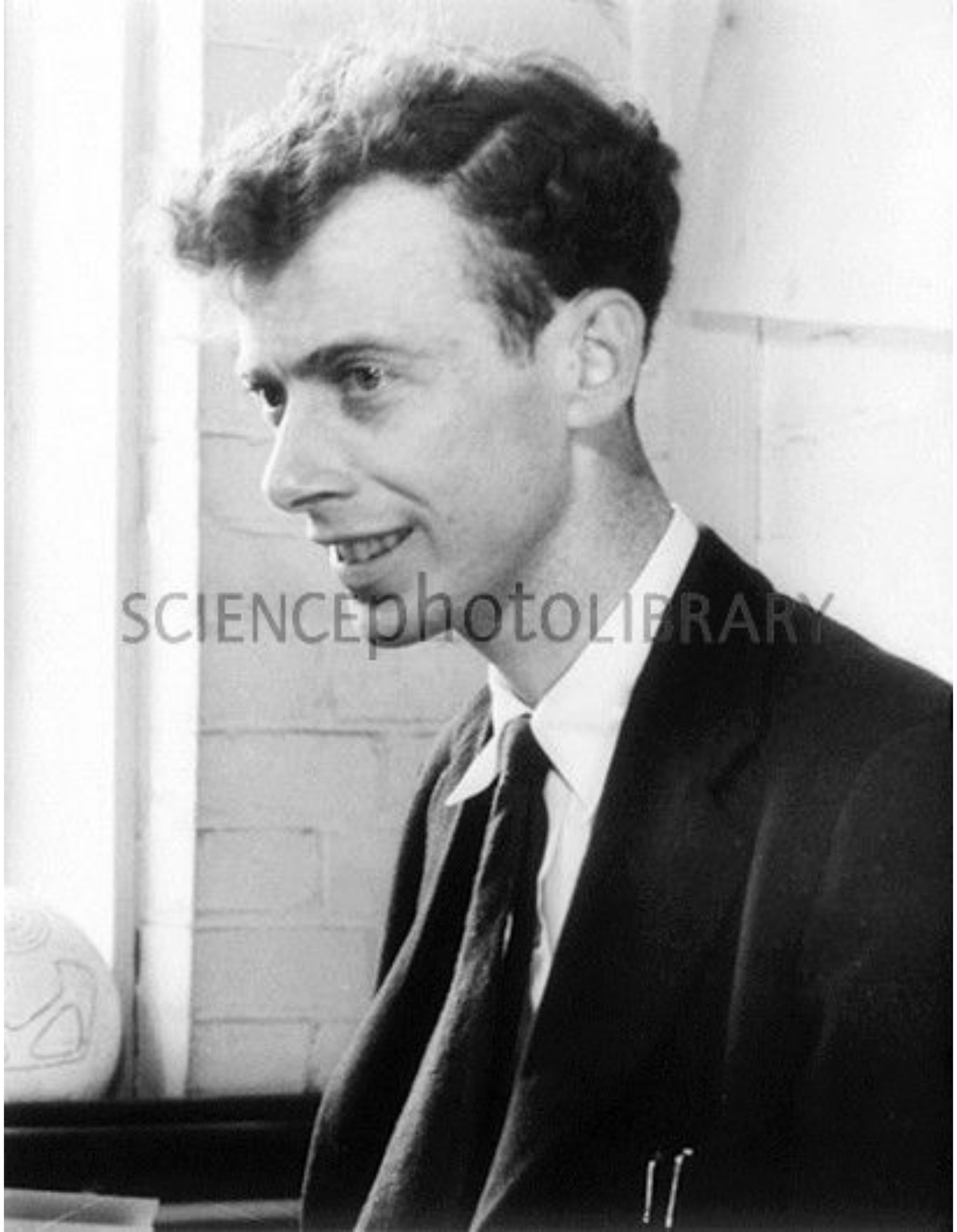
T. H. Morgan (1933)

-γονότυπος - φαινότυπος
-γονίδια πάνω σε χρωμοσώματα
-αλληλόμορφα -> εναλλακτικές μορφές ενός γονιδίου
-genetic locus

Beadle & Tatum (1941) -> ‘one gene-one enzyme hypothesis’

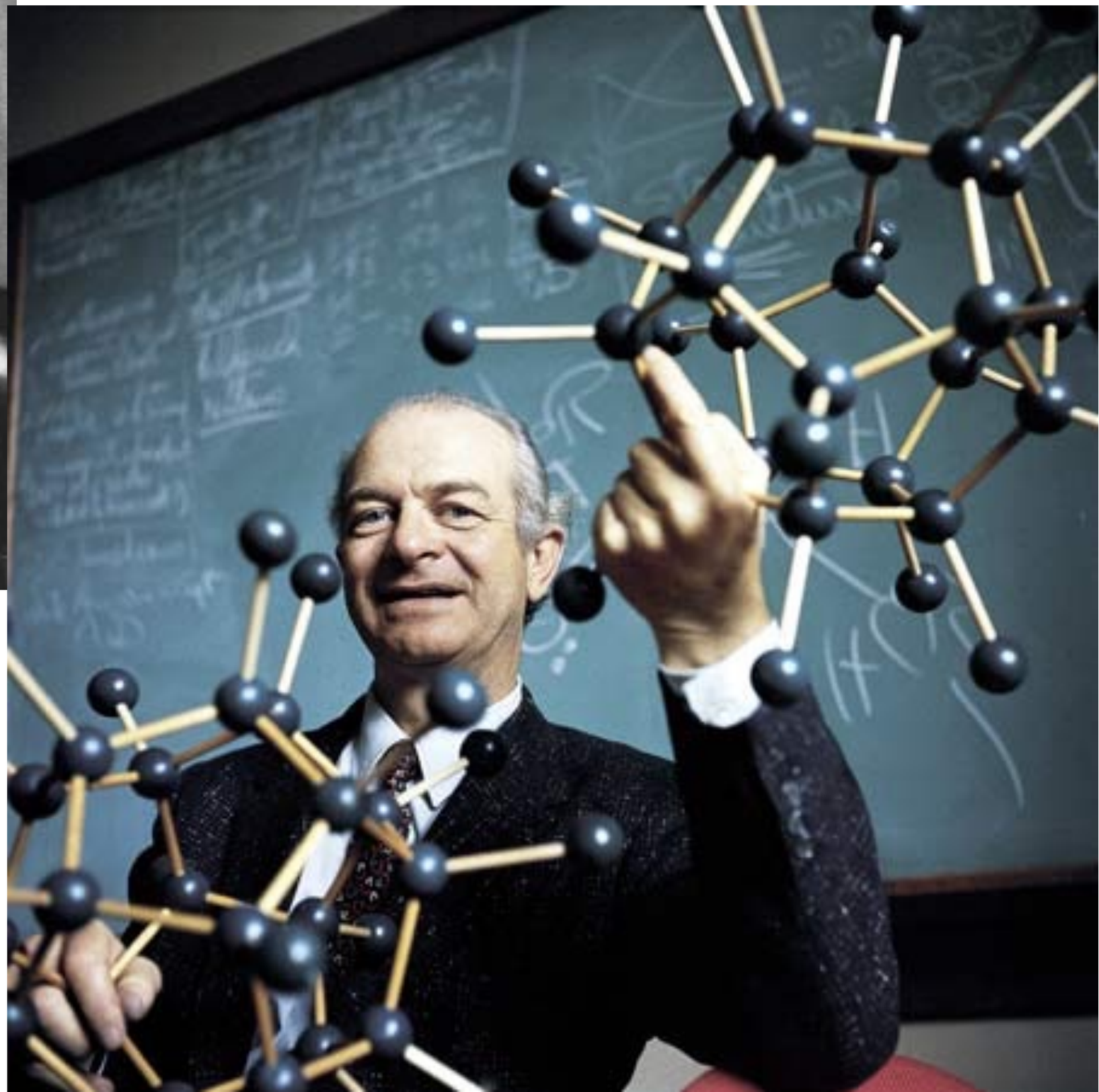
-τα γονίδια περί το **1950**

φυσικά σωματίδια **άγνωστης σύστασης**, διατεταγμένα πάνω στα **χρωμοσώματα**, κληρονομούμενα σύμφωνα με τους **νόμους του Mendel**, και υπεύθυνα για την **κατασκευή ενός ενζύμου**

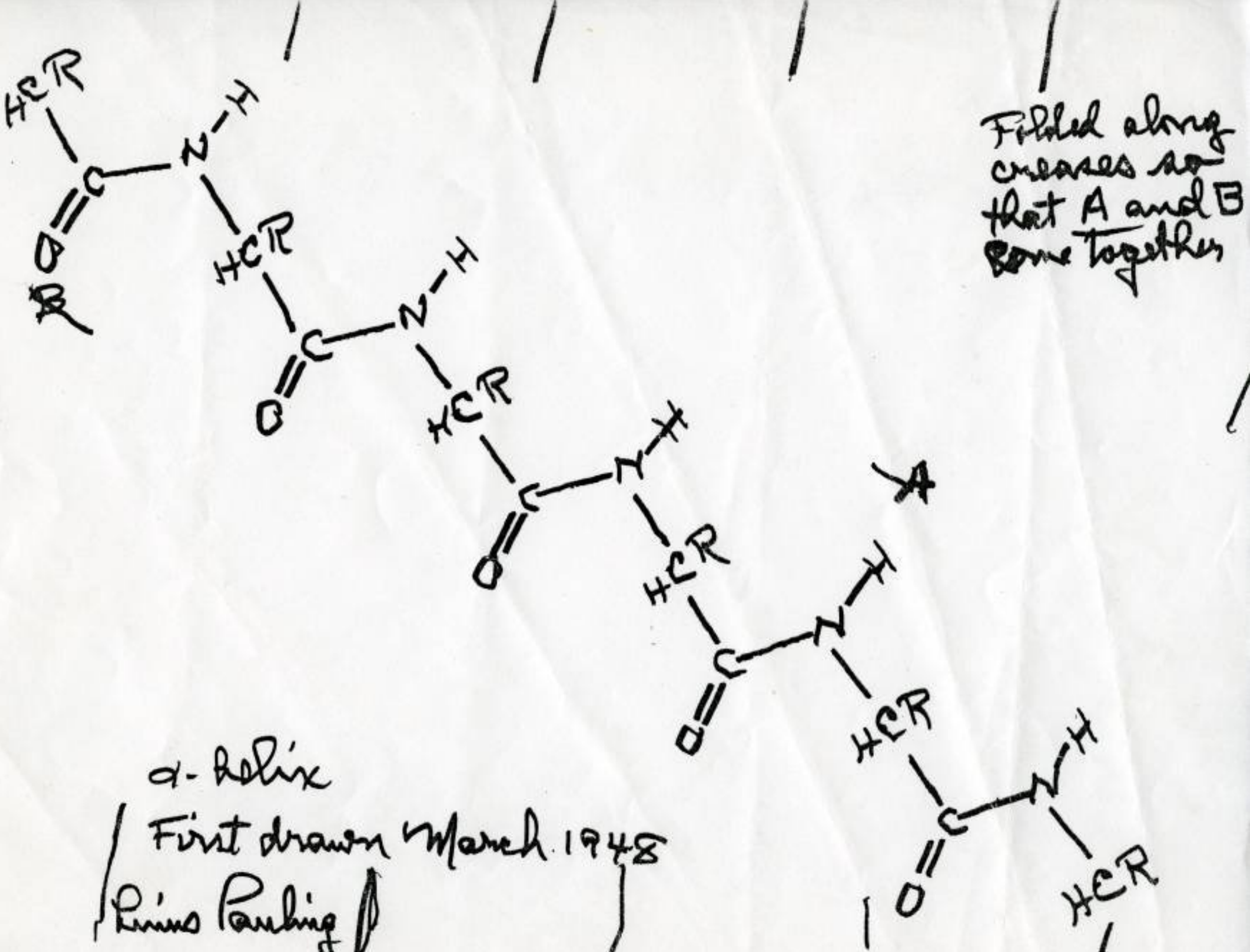


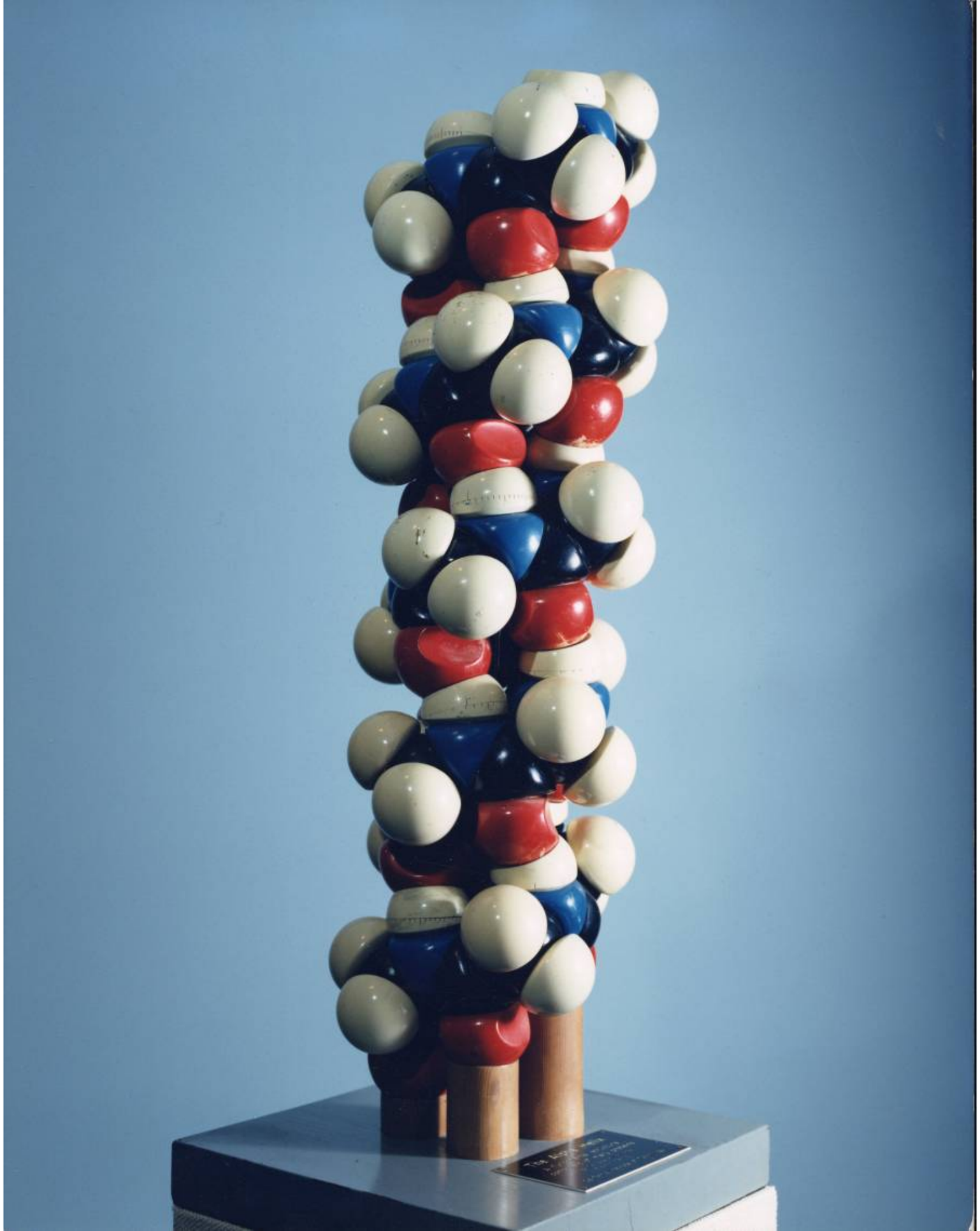

THE EAGLE
CAMBRIDGE

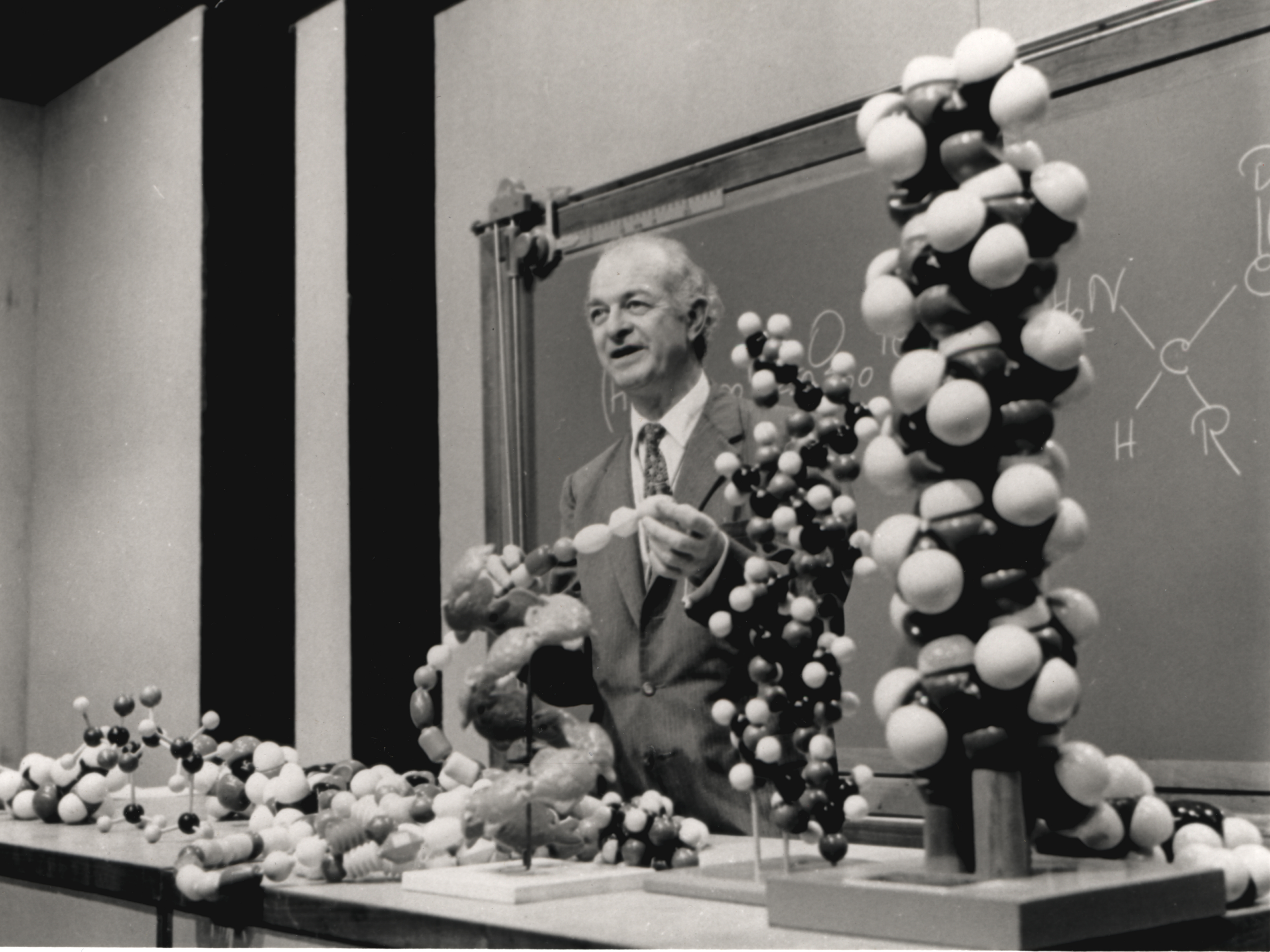

OLIVES



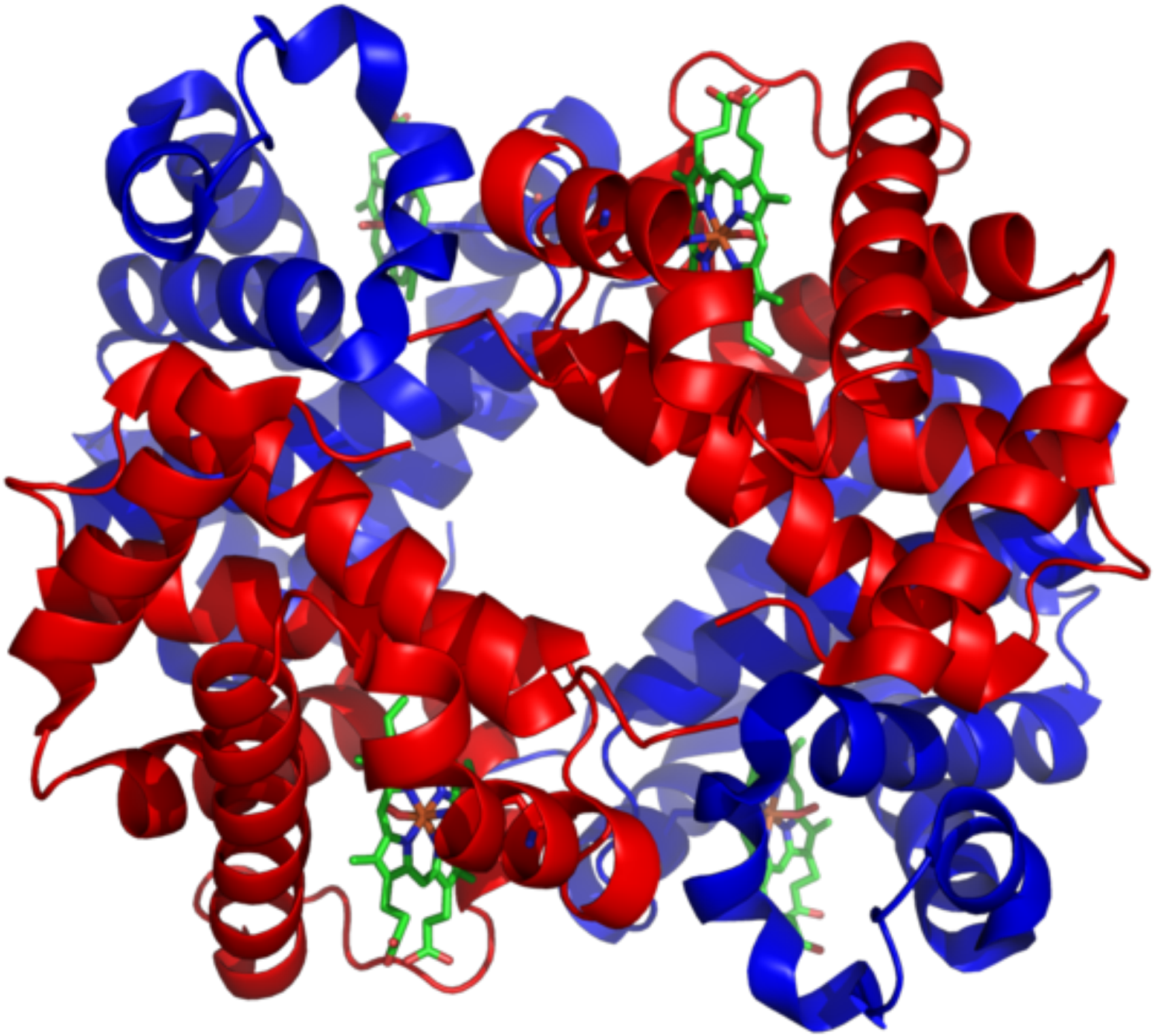
Linus Pauling

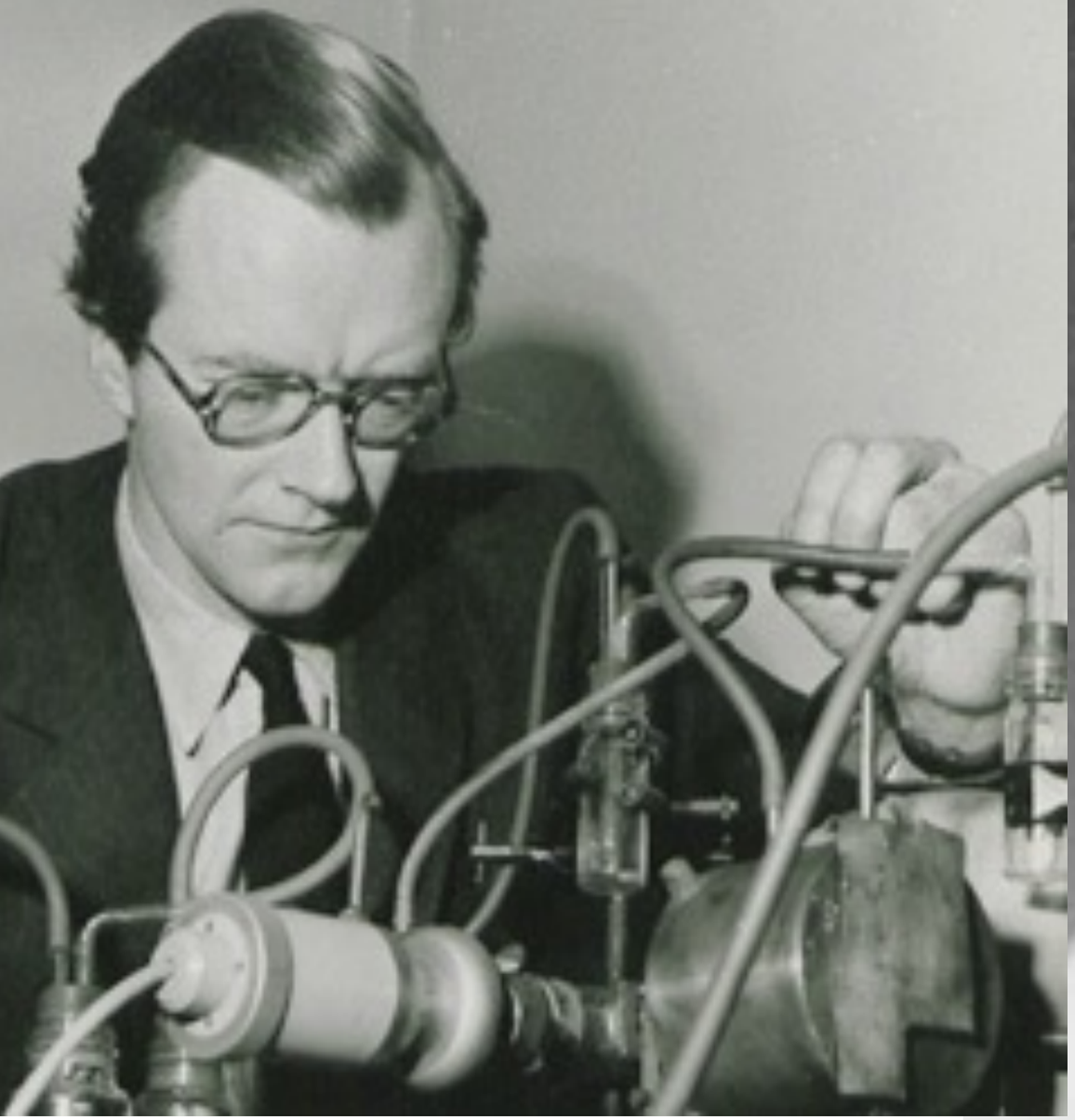

Folded along creases so that A and B come together
A
α-Helix
First drawn March 1948
Linus Pauling



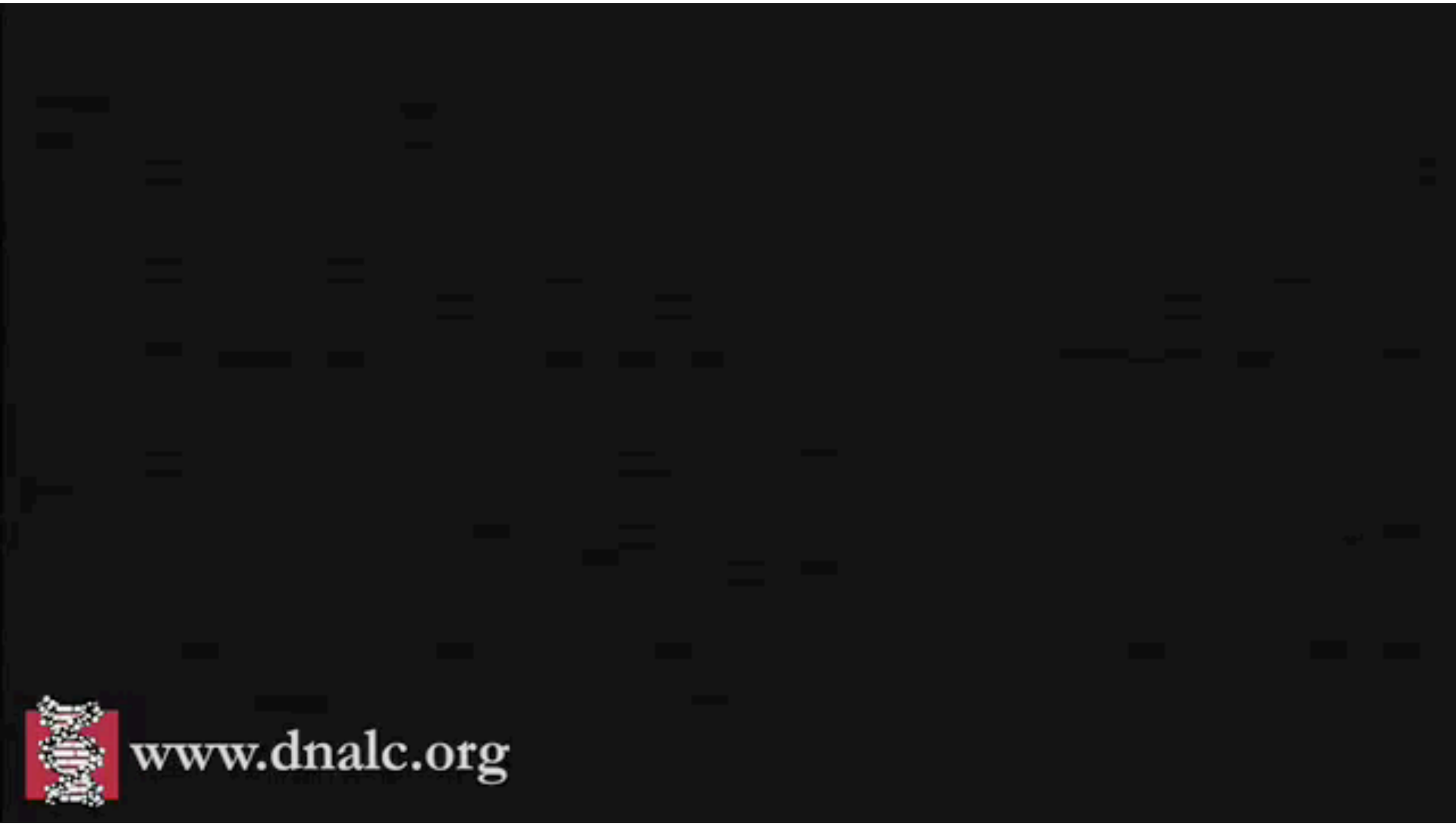


Max Perutz





Πάνω: Maurice Wilkins
Δεξιά: Rosalind Franklin


www.dnalc.org

which are involved in ester linkages. This distortion of the phosphate group from the regular tetrahedral configuration is not supported by direct experimental evidence; unfortunately no precise structure determinations have been made of any phosphate di-esters. The distortion, which corresponds to a larger amount of double bond character for the inner oxygen atoms than for the oxygen atoms involved in the ester linkages, is a reason-

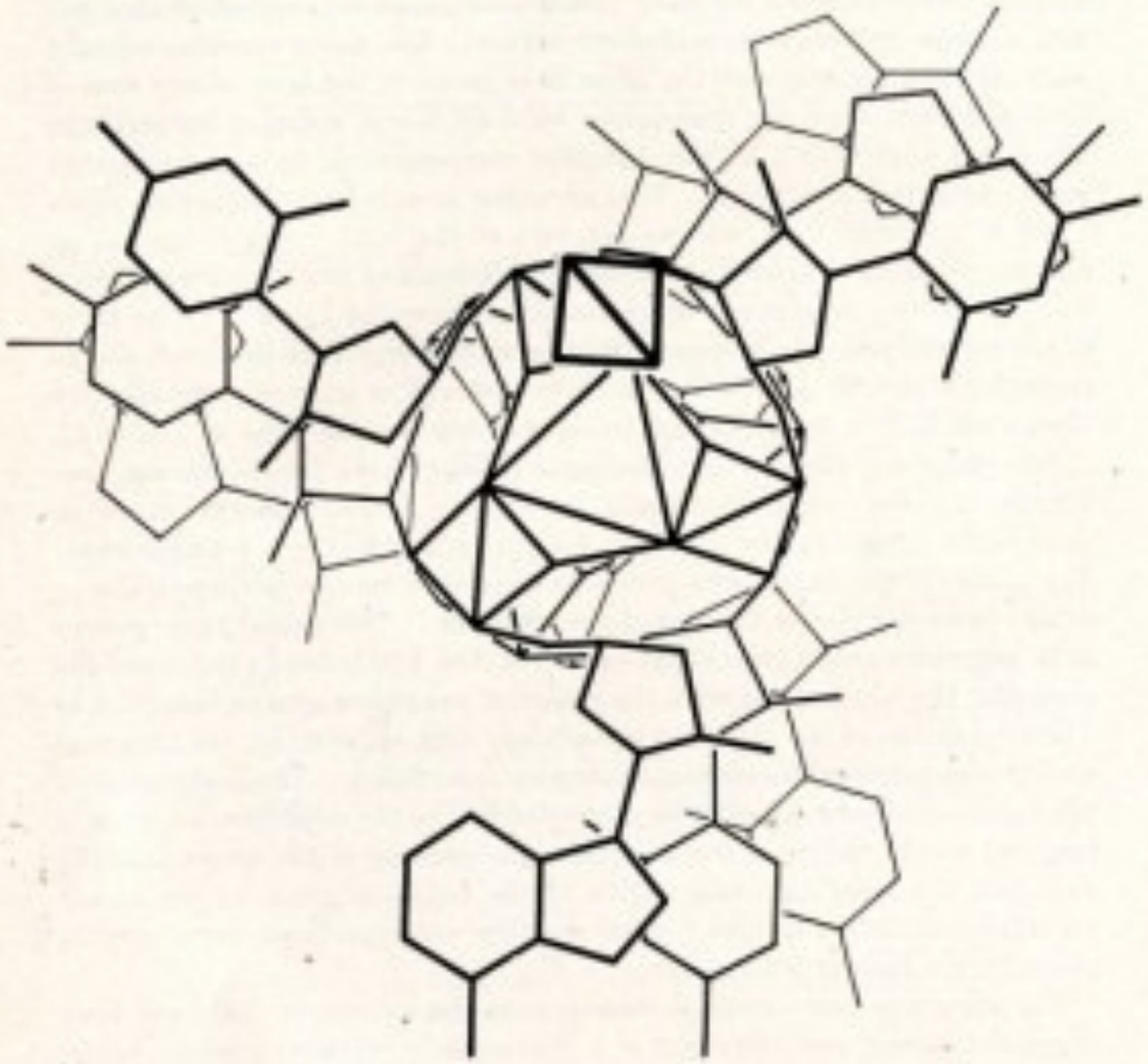
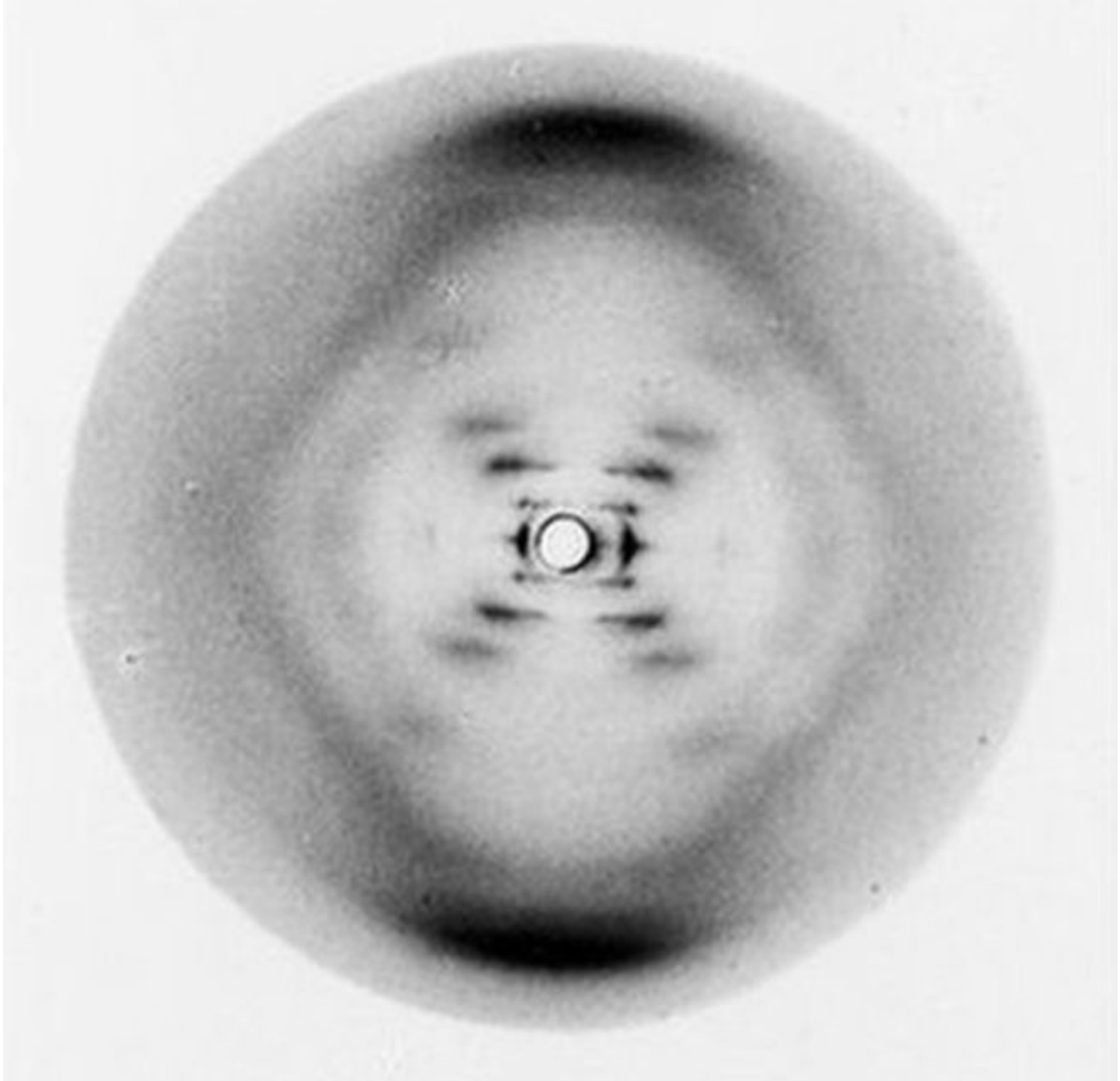


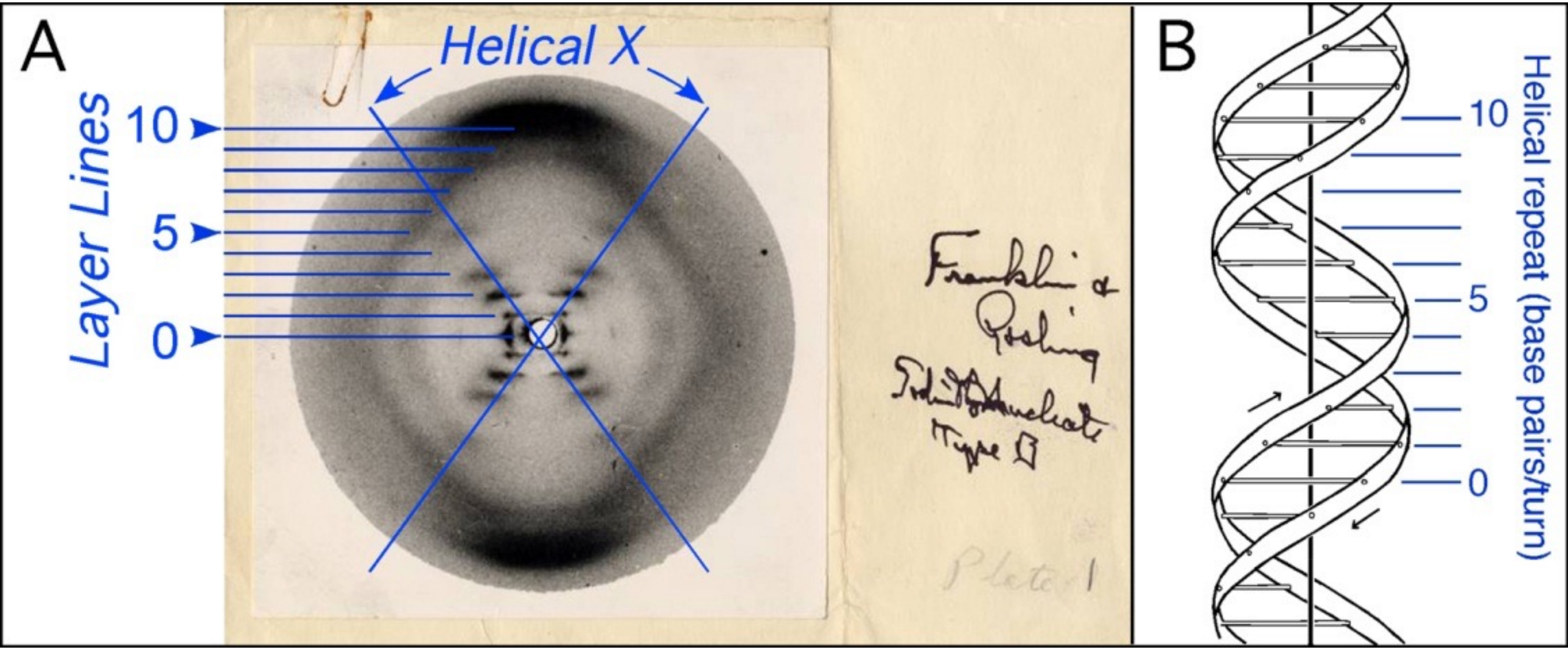
FIGURE 6

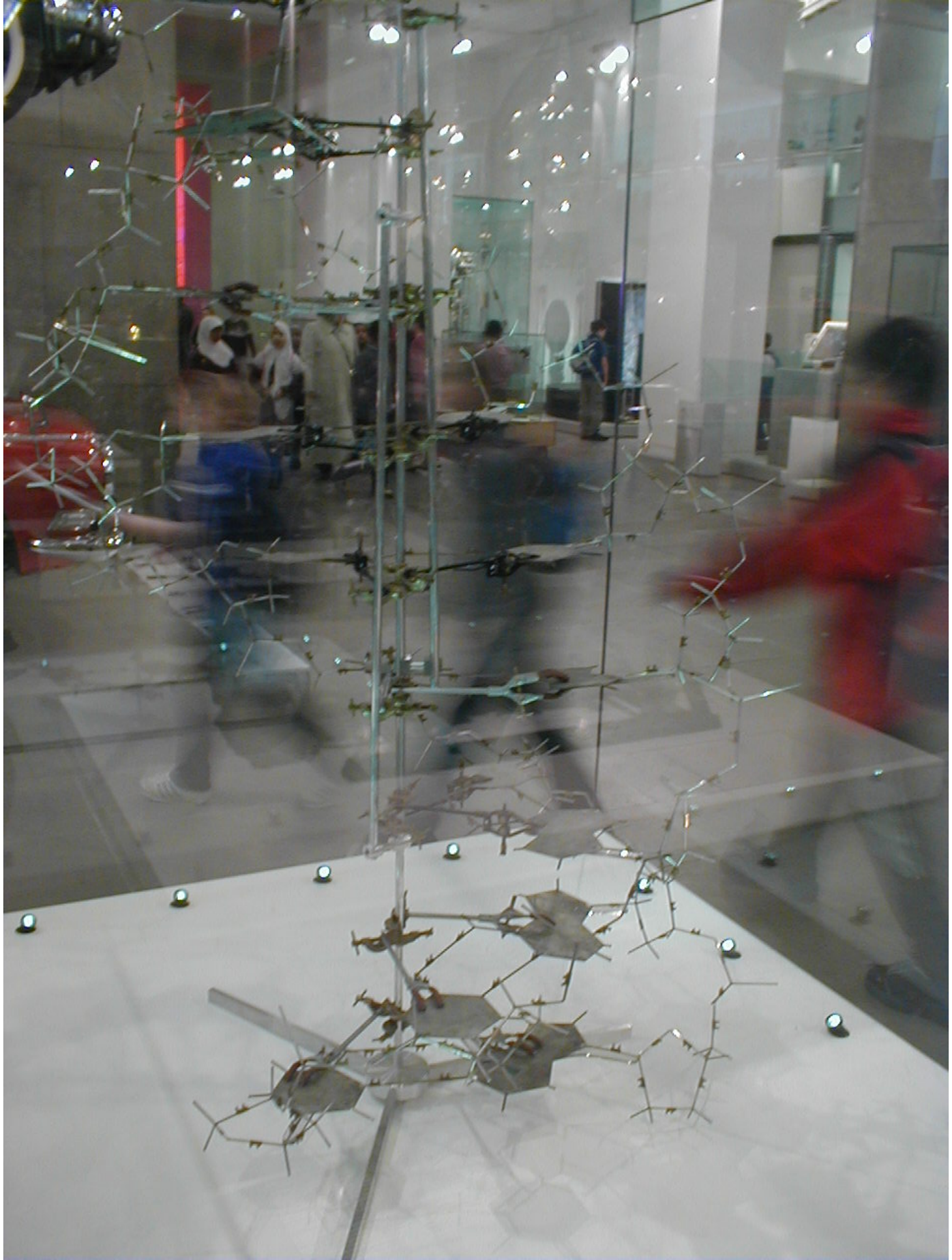
Plan of the nuclein acid structure, showing several nucleotide residues.

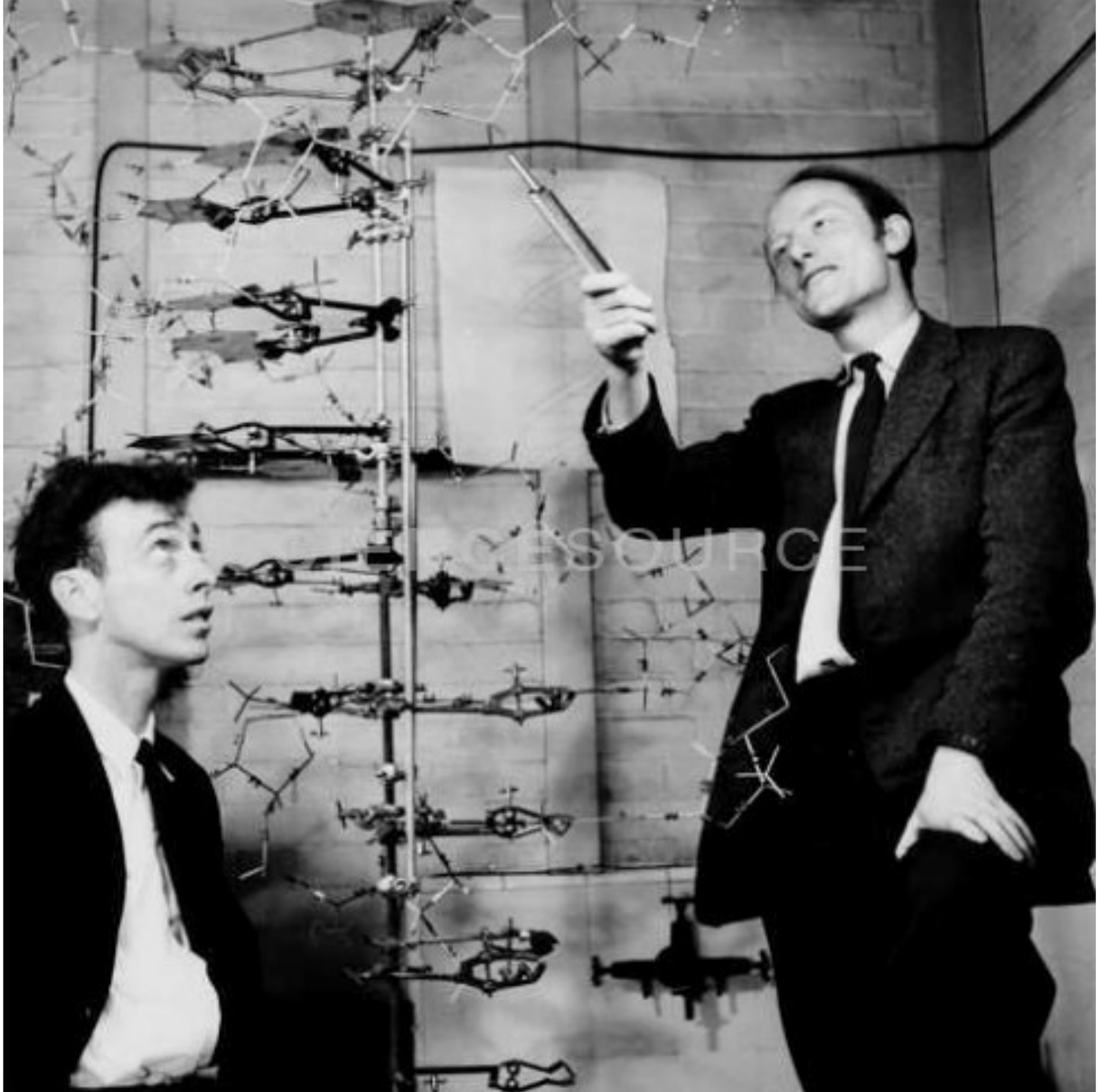
able one, and the assumed distances are those indicated by the observed values for somewhat similar substances, especially the ring compound S_3O_9, in which each sulfur atom is surrounded by a tetrahedron of four oxygen atoms, two of which are shared with adjacent tetrahedra, and two unshared. The O—O distances within the phosphate tetrahedron are 2.32 Å (between the two inner oxygen atoms), 2.46 Å, 2.55 Å, and 2.60 Å. The

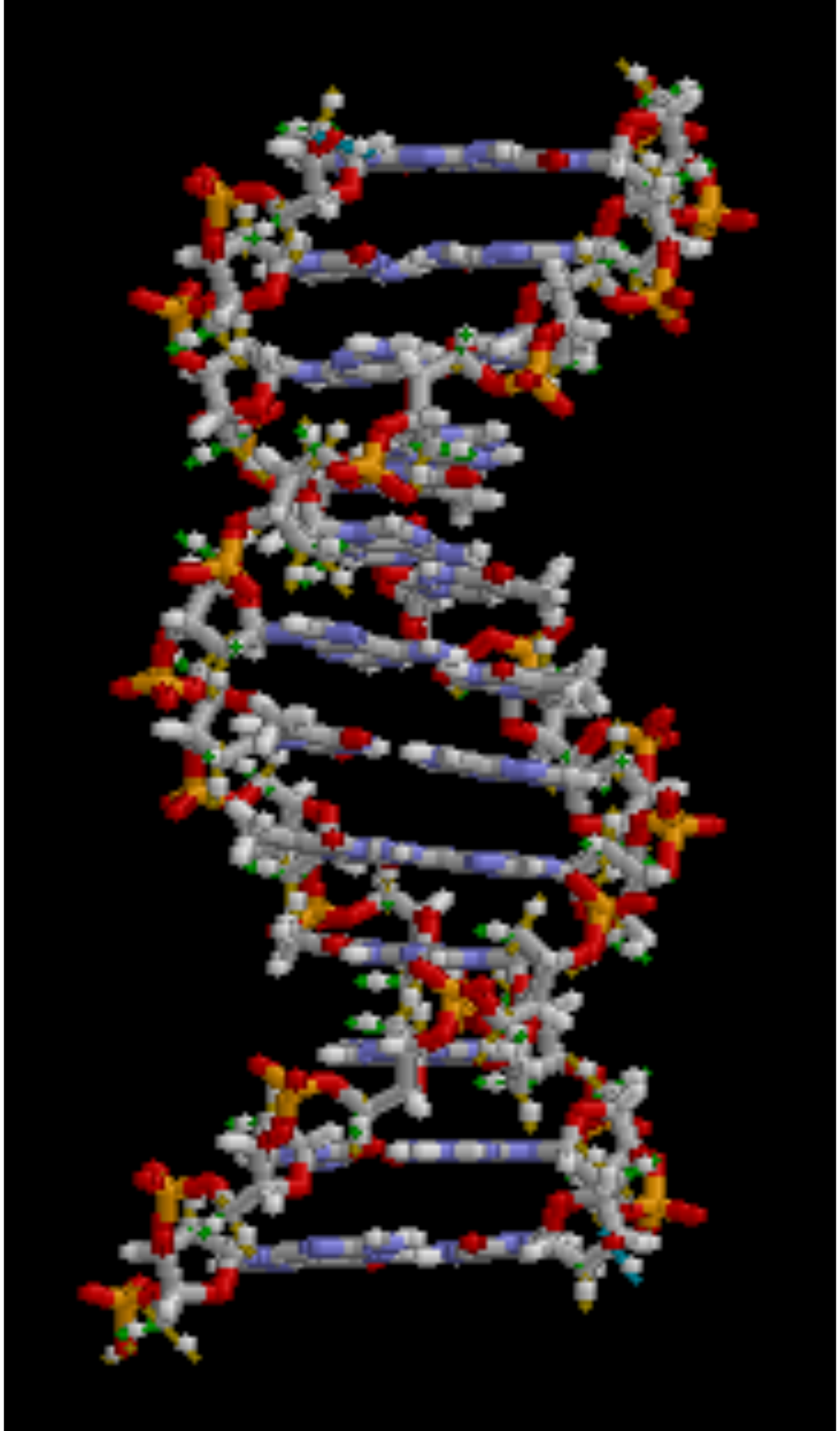
Το μοντέλο του Pauling για το DNA (τριπλή έλικα)




A
Helical X
Layer Lines
10
5
0
Franklin & Gosling
Type B
Plate 1
B
10
5
0
Helical repeat (base pairs/turn)







When we saw the answer we had to pinch ourselves. **Could it really be this pretty?** When we went to lunch [at the Eagle] we realised **it probably was true because it was so pretty**.

The **discovery** was made **on that day**, **not slowly over the course of the week**. It was simple; **instantly** you could explain this idea to anyone - you didn't have to be a high powered scientist to see how the genetic material was copied.

(Watson 2003 BBC interview)

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).

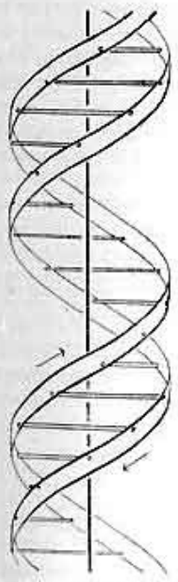
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

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[5,6] 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.

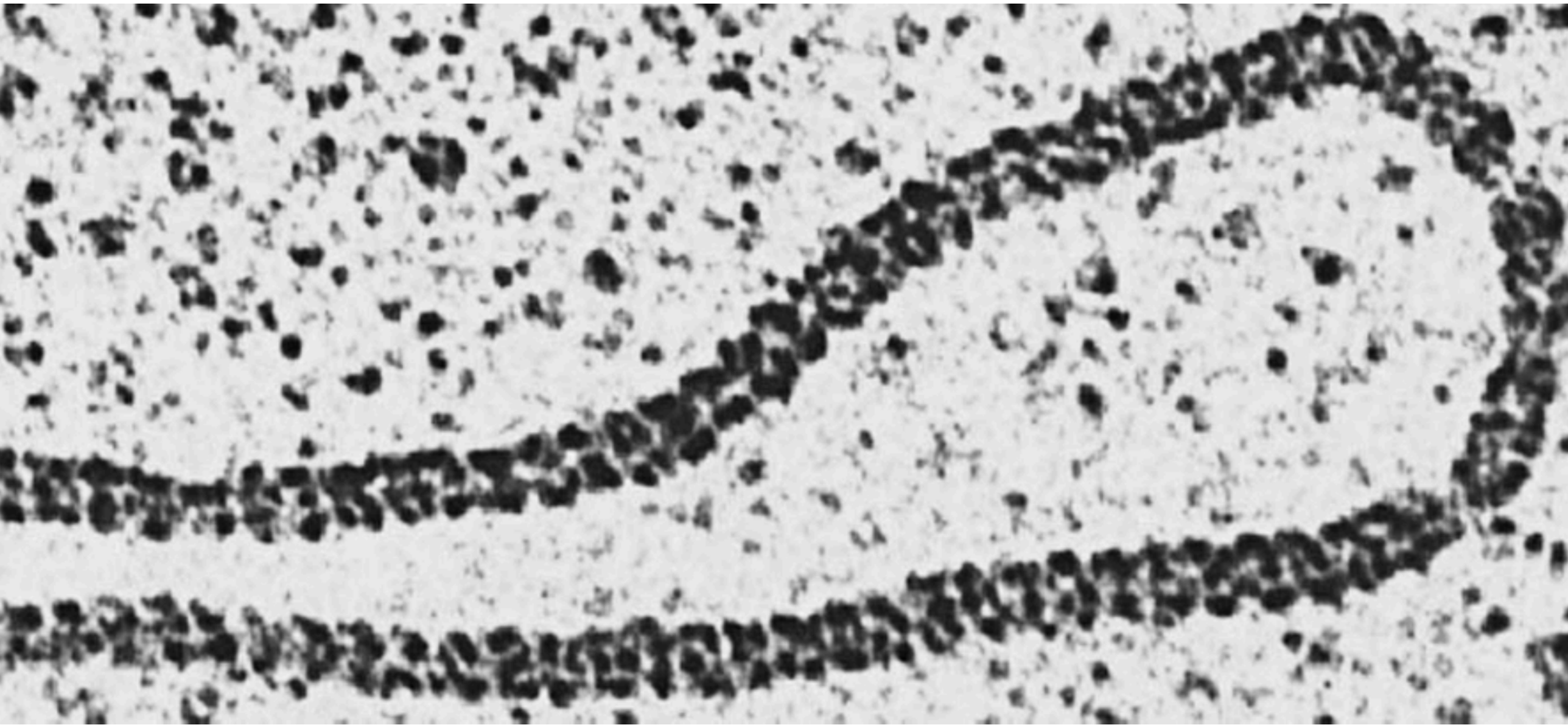
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

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

Nobel Prize in Physiology or Medicine (1962) -Crick, Watson & Wilkins

"for their discoveries concerning the **molecular structure of nucleic acids** and its significance for **information transfer in living material**"

DNA Double Helix 1953
"The secret of life"
For decades the Eagle was the local pub for scientists from the nearby Cavendish Laboratory.
It was here on February 28th 1953 that Francis Crick and James Watson first announced their discovery of how DNA carries genetic information.
Unveiled by James Watson
25th April 2003



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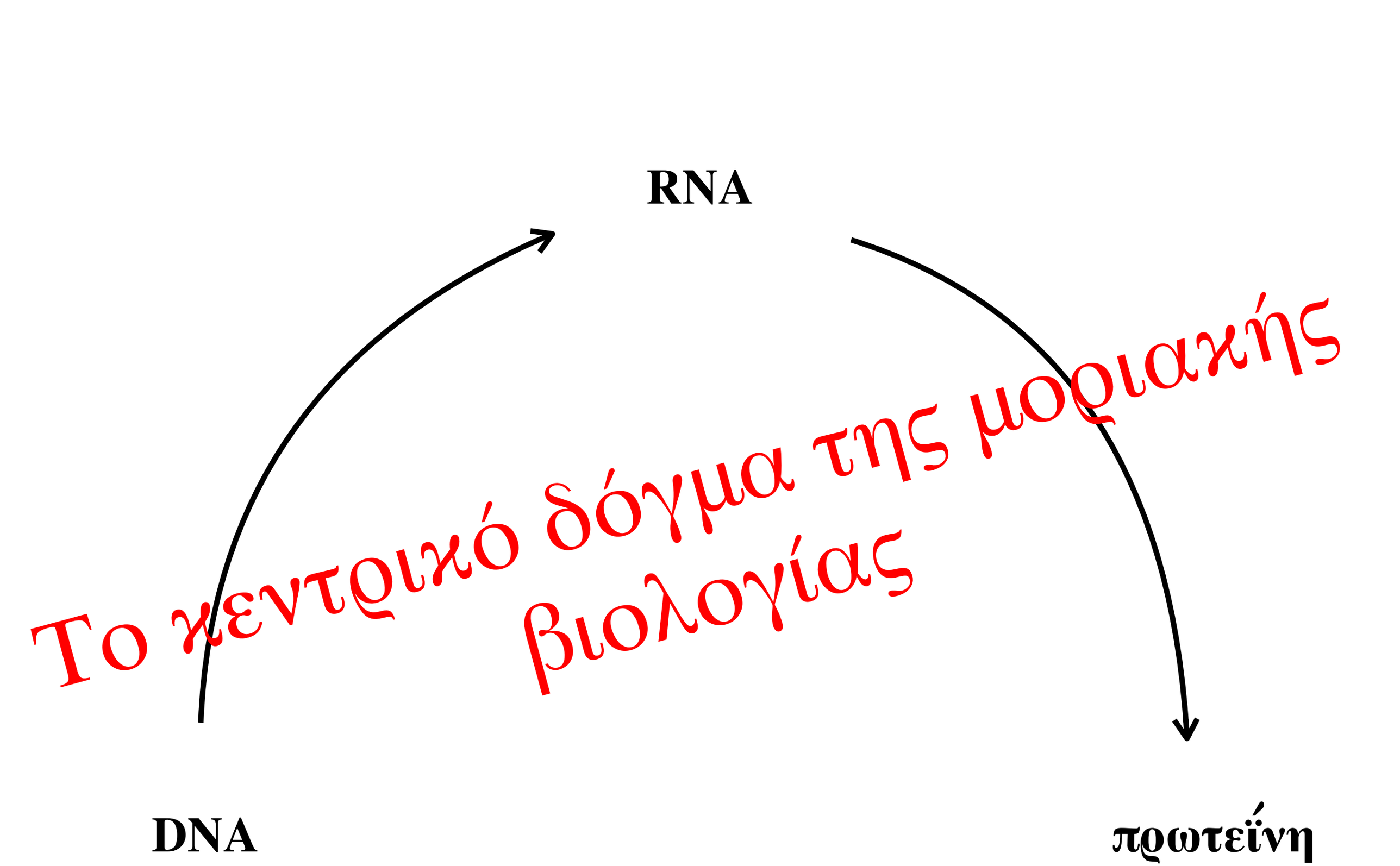

RNA
Το κεντρικό δόγμα της μοριακής βιολογίας
DNA
πρωτεΐνη

δομή
(πρωτεΐνη)

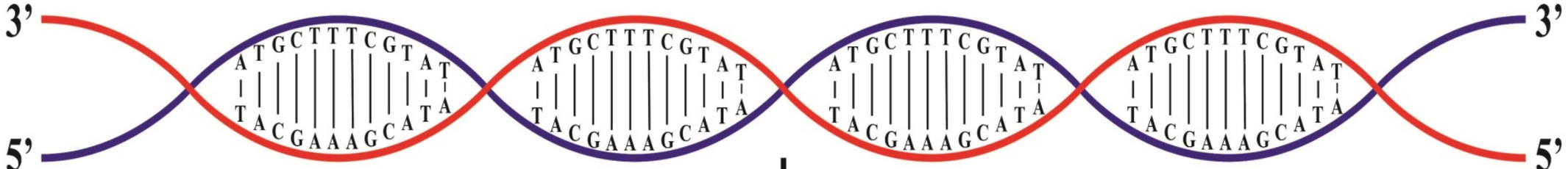
κωδικοποιεί

παρέχει

πληροφορία
(γονίδιο)

λειτουργία

DNA



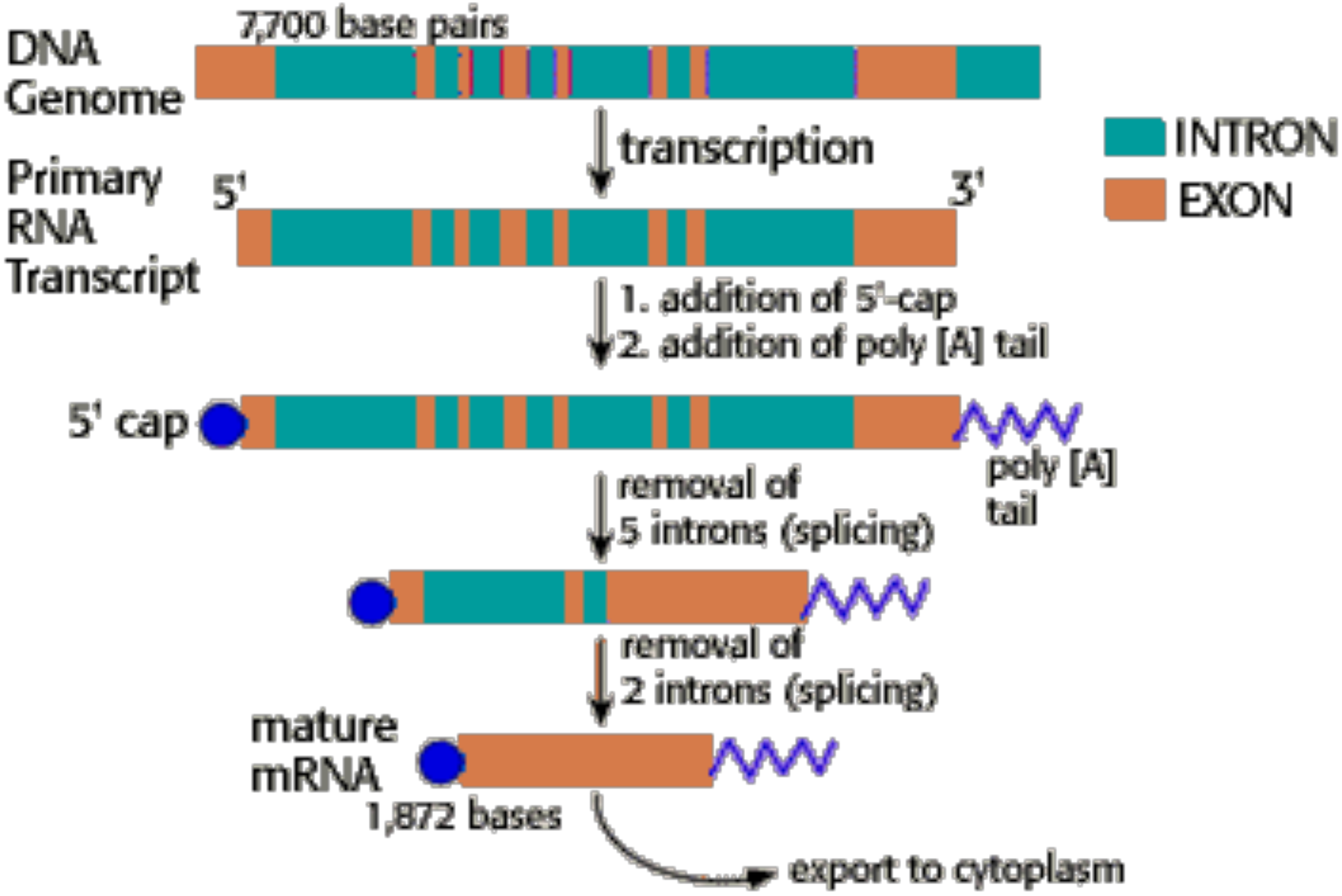
Transcription

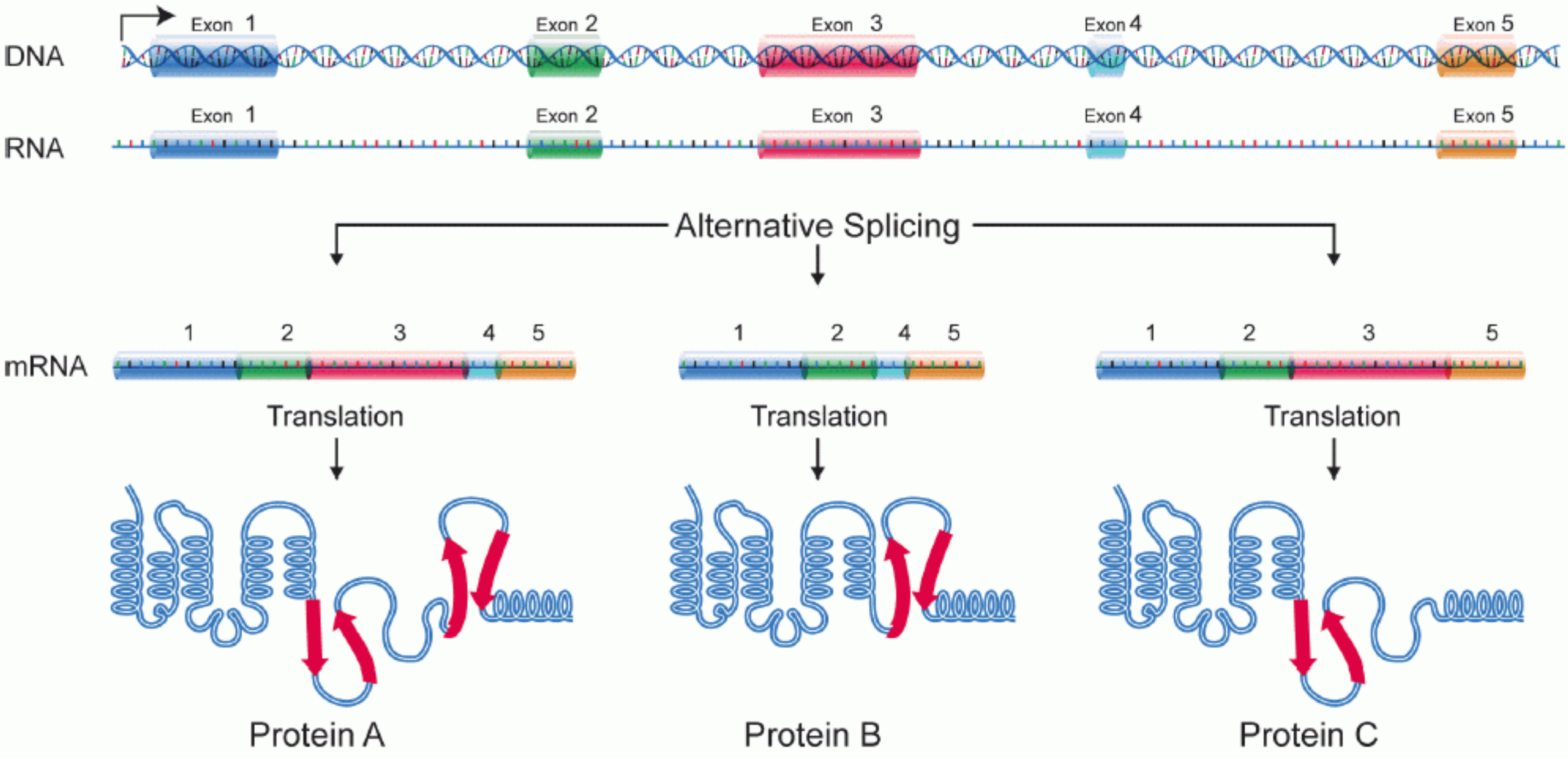
RNA

Translation

Protein

Ovalbumin processing




DNA
RNA
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Alternative Splicing
mRNA
1 2 3 4 5
1 2 4 5
1 2 3 5
Translation
Translation
Translation
Protein A
Protein B
Protein C

-η μοριακή βιολογία έδειξε ότι οι κλασικοί γενετιστές **είχαν εν μέρει δίκιο**:

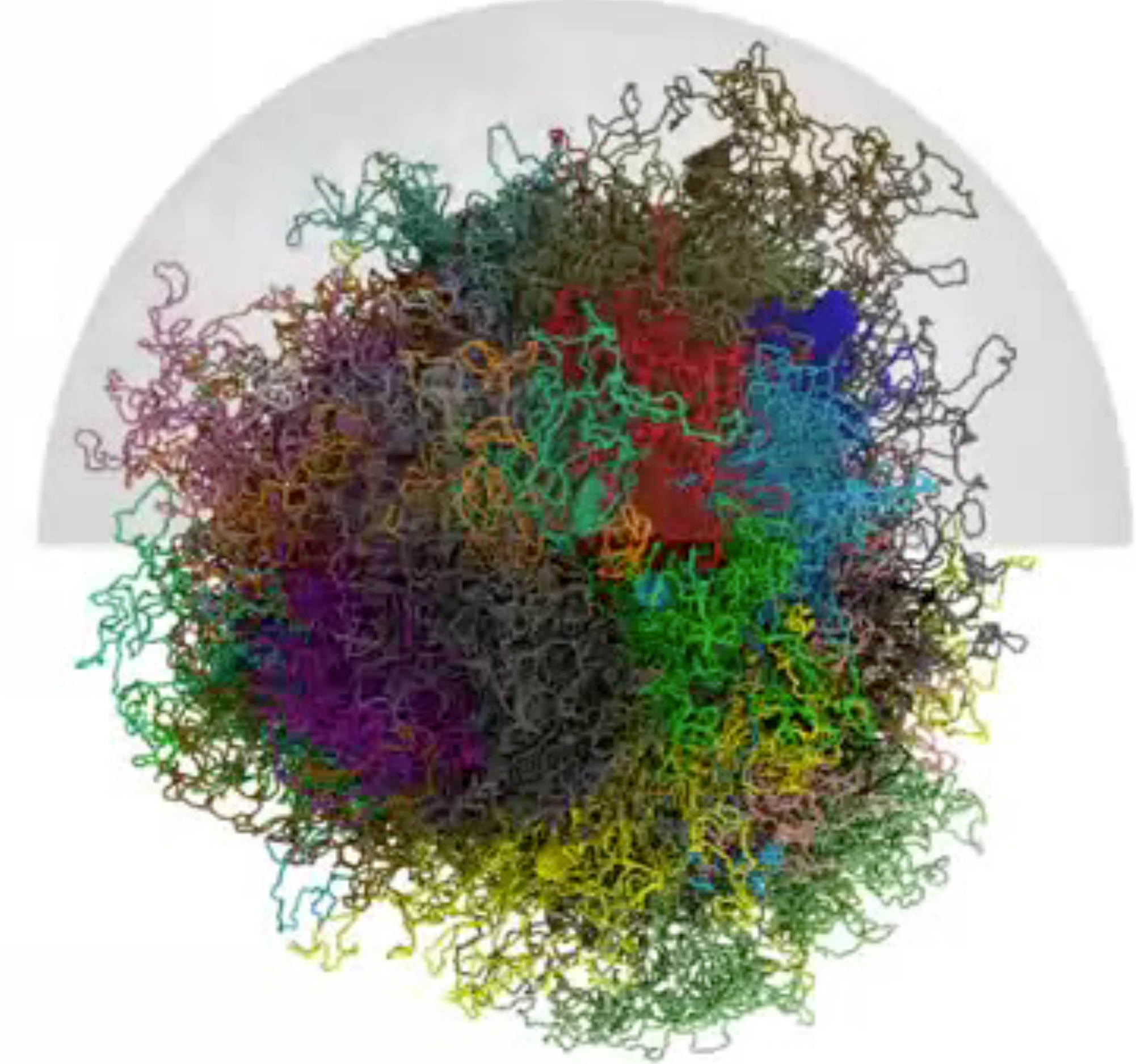
-> τα **γονίδια** είχαν εισαχθεί ως αντικείμενα με **συγκεκριμένο αιτιακό ρόλο**, και οι **μοριακοί βιολόγοι** ανακάλυψαν ότι αποτελούνταν από κομμάτια **DNA** που κωδικοποιούν **πρωτεΐνες**

-> **λειτουργική** μονάδα ≠ **κληρονομική** μονάδα

Lenny Moss: gene-P vs gene-D

-γενική τάση: **διάφορες έννοιες γονιδίου**
-μεγάλη αλλαγή σε έννοια **μεντελιανού κλασικού γονιδίου**

-> γενικά: γενετικά συστήματα **πολύ πιο περίπλοκα** από γονίδια διατεταγμένα σαν '**χάντρες σε κορδόνι**'



ένα γονίδιο

που ρυθμίζει

κωδικοποιεί

μια πρωτεΐνη

ένα μήνυμα

για να
κατασκευαστεί

16 February 2001

Science

Vol. 291 No. 5507
Pages 1145–1434 $9

THE HUMAN GENOME

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

GATCCCTGCC TGGGCTTTGC CTCTGCAGCC CCCCGCCCCA CAGGTTCACA CCTCGGGTCT
TCTCCACCGC TGCCACACGC CAGAGCCTGT AGCGGGGCCT CAGAGTCTGG GAGGTGGGAC
TCCTGCACCT CAGCCATCAT CAGACCCATG GGGCCACCCA GGGAACCTTG GCAGGGACCA
TTACCAGTGA CCTGCCGAGG CCCCGGACTC TGCCAGCCAG CTGTGCCGGC CACCCTGCCC
CGGACAGTGC CGGTTCATGT GGGAACTAGG GGACGATGTG GTTCTTCGCA TCTGATGATG
AAGGCCCTGG GCCACTTGGC ACGGGCGGGC GCTCCCGAGA TGGATATGAG GAGCCCCCTC
TGCCCAACTC CCAGAAAGGC CGAGGCTCTG CAGCGGGAGG AAGTCCTGCG ATGTCCTGGG
GGGCAGCAGC GCAGGGCACA GGGACAGCCC CCCTCCACAG CTCTTCCTGG CCAGCCCTCC
CCACTATCTG CCAGGAGGTT GCTTCTTCCA GGAGGCTTTT CCCGACCAGC CCAGGGGTCC
AGGGTCTGGG GCTCCCAGCT GCTGTGAGTG CTGCACATTC TCTTGAGGAC AGCCCCCTCC
CTCCCCCACC CACTTCTGGT GCCCACTGTG GCCACAGCAA GCACTGGGGC CTGCACTCAG
GGACCTCGGG GCCTCCTGGG GAGCTGCTGA CCCTAGGCAG AGAGATTGCA CATCCCTAAG
AGTCTACAGA CACCCCAGTG TTTGCCAGTG TTTGCCCGTG TTCACCAGTG TTTGCCAGTG
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GTTTGGGCGG GAAGTTGCAG GTCCCTCCAG GACAGTTGGC CGATGACGTG GAGACAGACC
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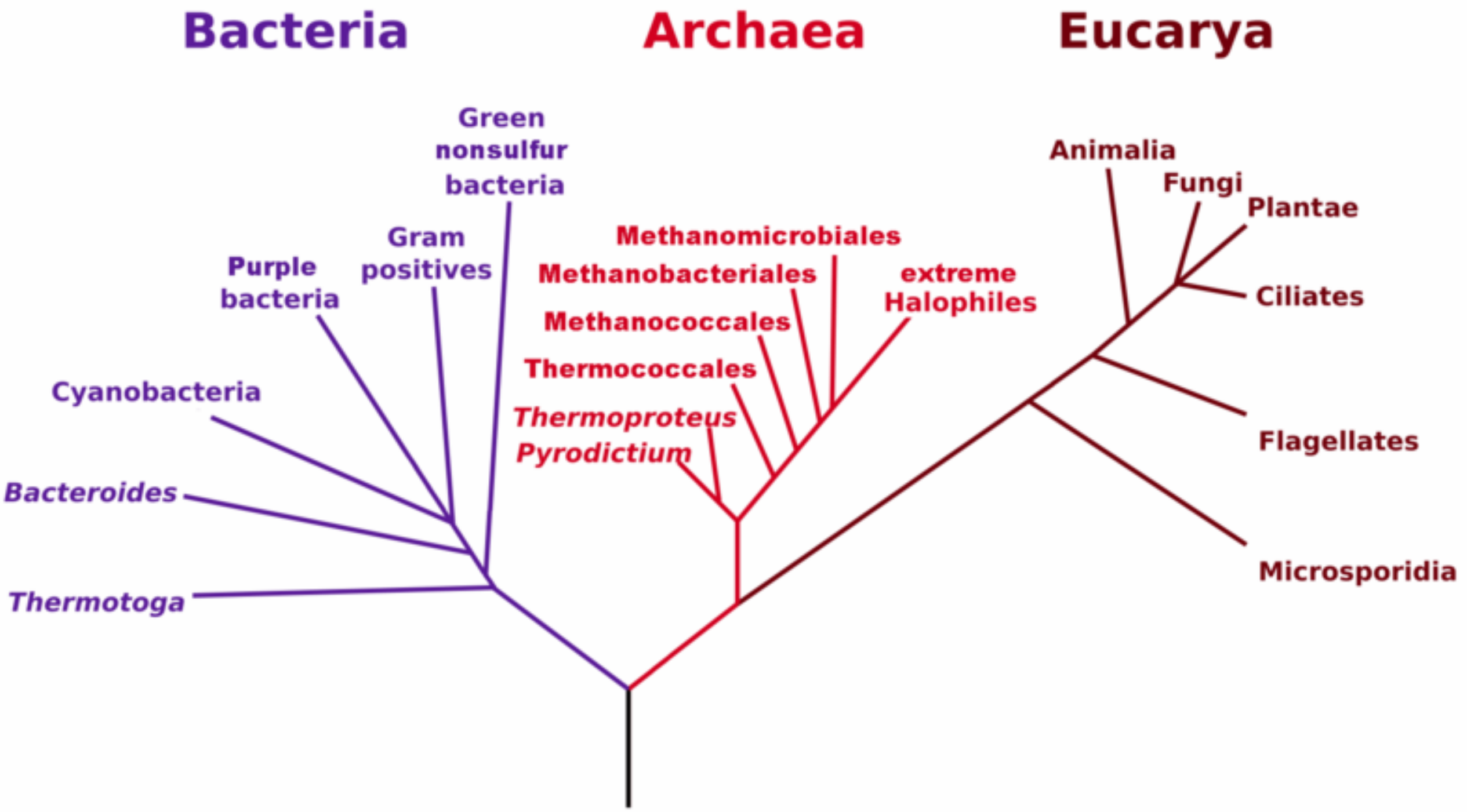
GCACGACGGG CACCTCCGTG TTCACCAGTC CAATGGGCAC GGAGCGTGGC TTTATTTGCA
TGTCTGGATT CCTAACGACT TCAGCCTCTG CACCTCCTGG GTTTTCCCTG CTGCAAATTG
CCATTTGGCG TCGTCCCCAA TTTCCGGCCA AGGCCGCGTC GTCGTGCTGC TGTGTAATTT
GATGTGTGGA GTTCTAGATA CCAAGTGTCT GTCGGTTTTA GACATCGCAA ACGTCCTTCC
CAGTGTGGCC CGTCCATTCG CTTCTGTGCA GCAAAATCTT TAATTATTTG ATGGCATCAA
AATGTGTGTC CAGTTTTACC TTCTAGTTTA TACTTTCGAA CATTTGTTTG AGAAATCTTT
CTCCCACCTG TGGCTGATAG TGACGTCTTC TAACTTCCCA TTTACTATGT TACATTCAGA
CCCATCATCT TCAGGAAGAC GCTTGTGTGC GAGACGGGTA TGAGGCCCCC ACACCCCGCC

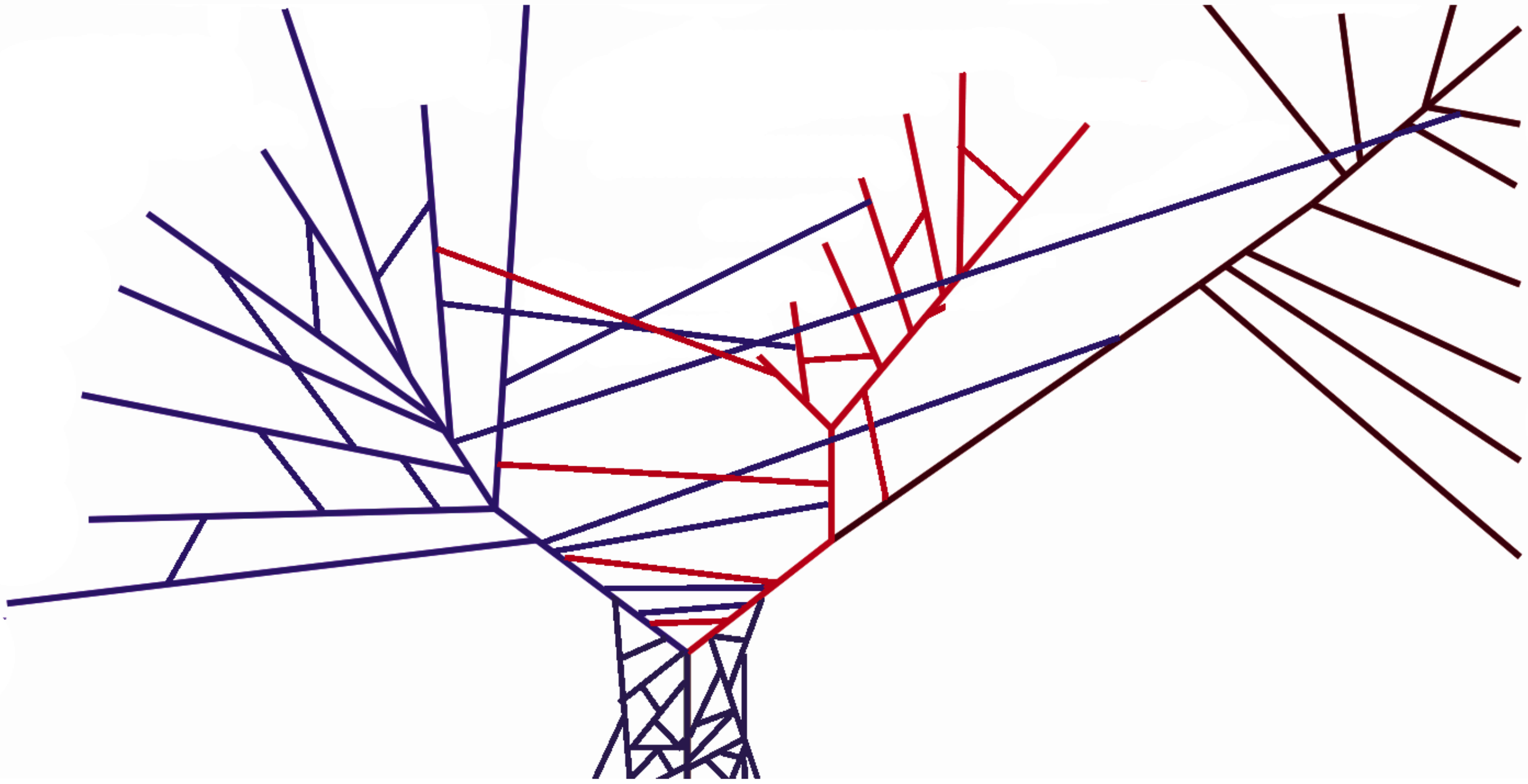
Phylogenetic Tree of Life



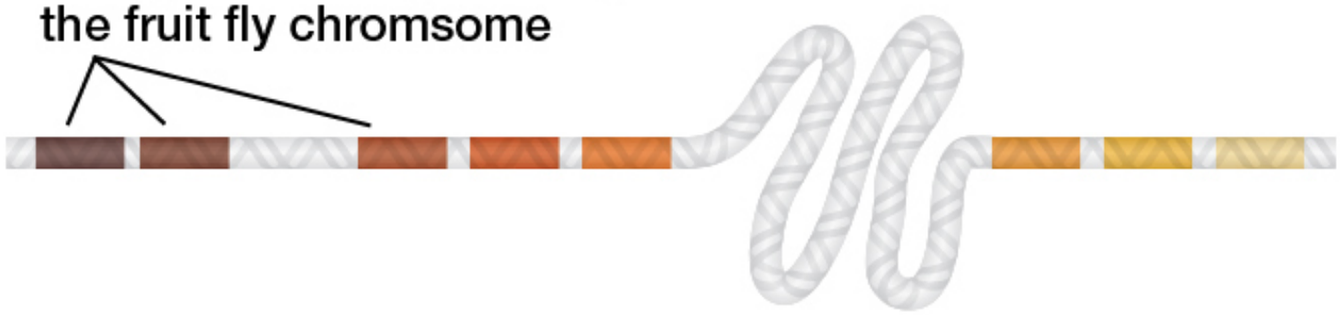
Bacteria

Archaea

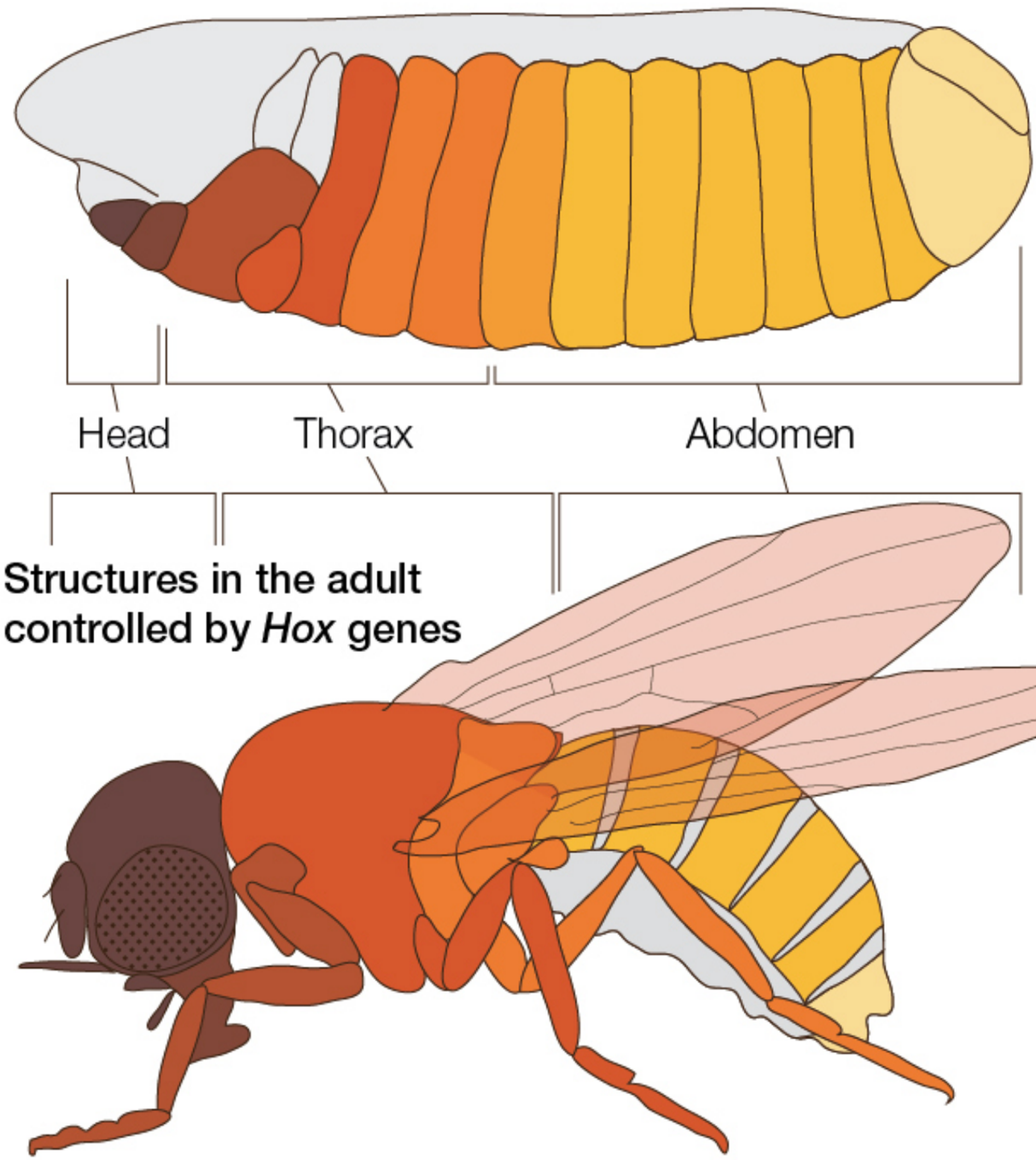
Eucarya

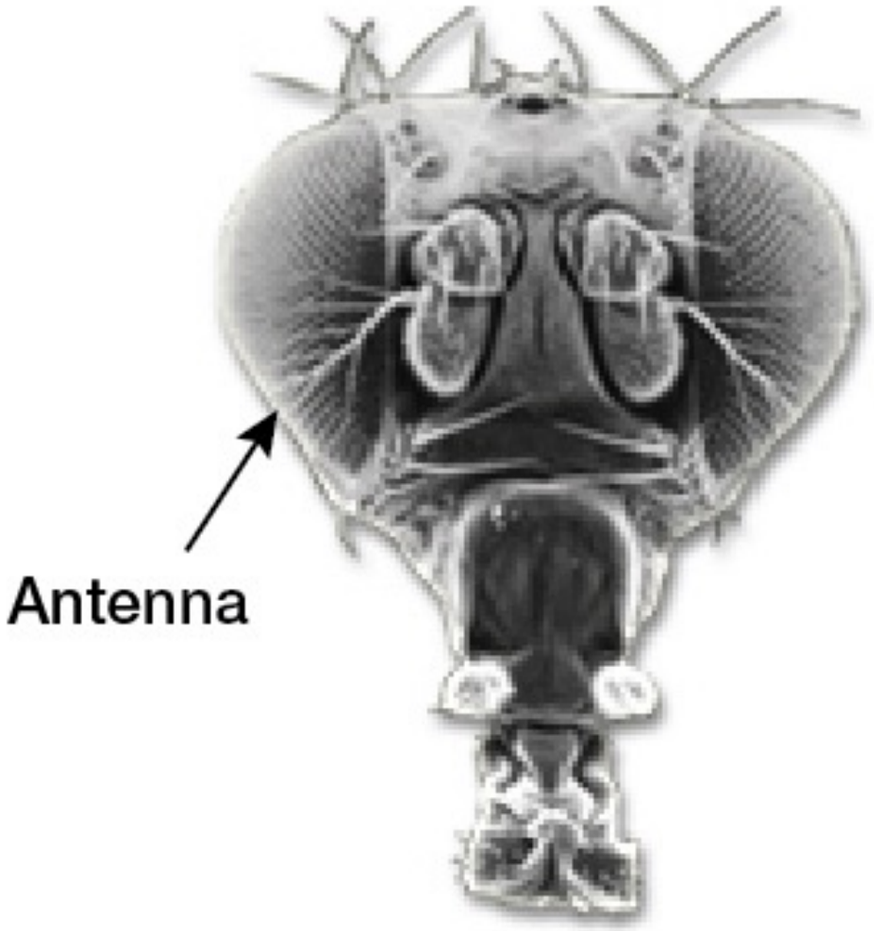


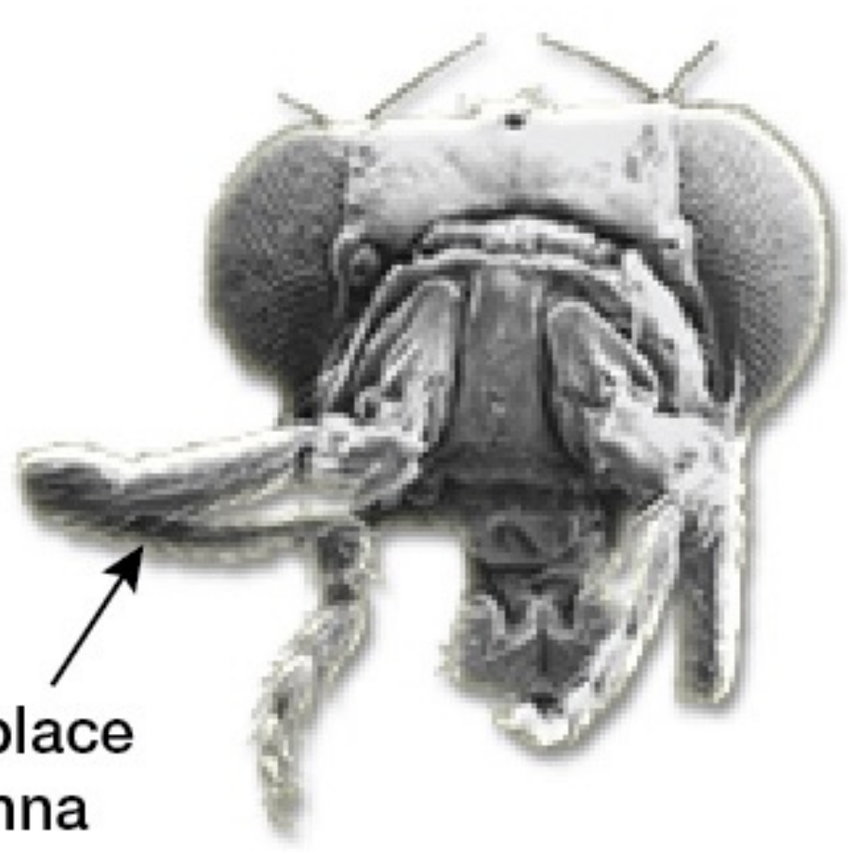
Hox genes arranged along the fruit fly chromsome

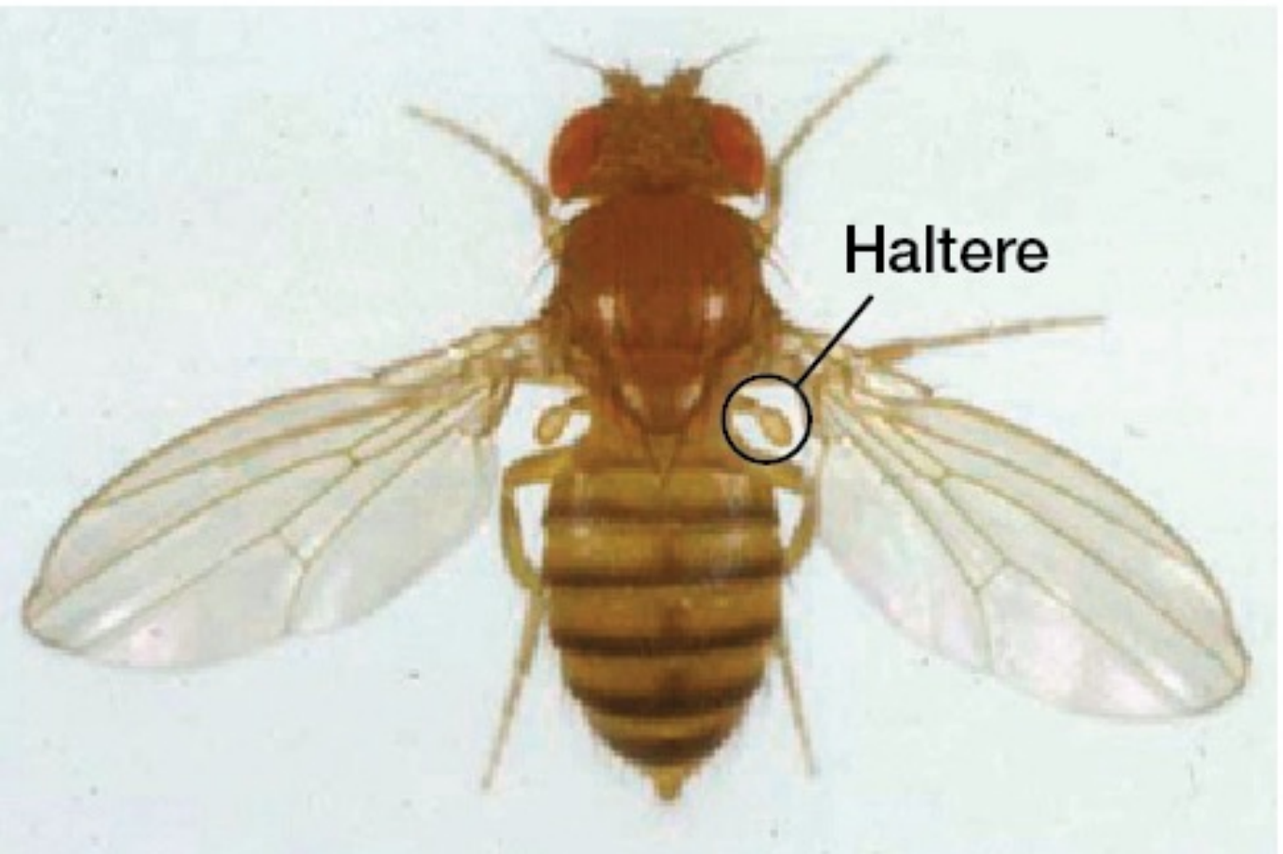


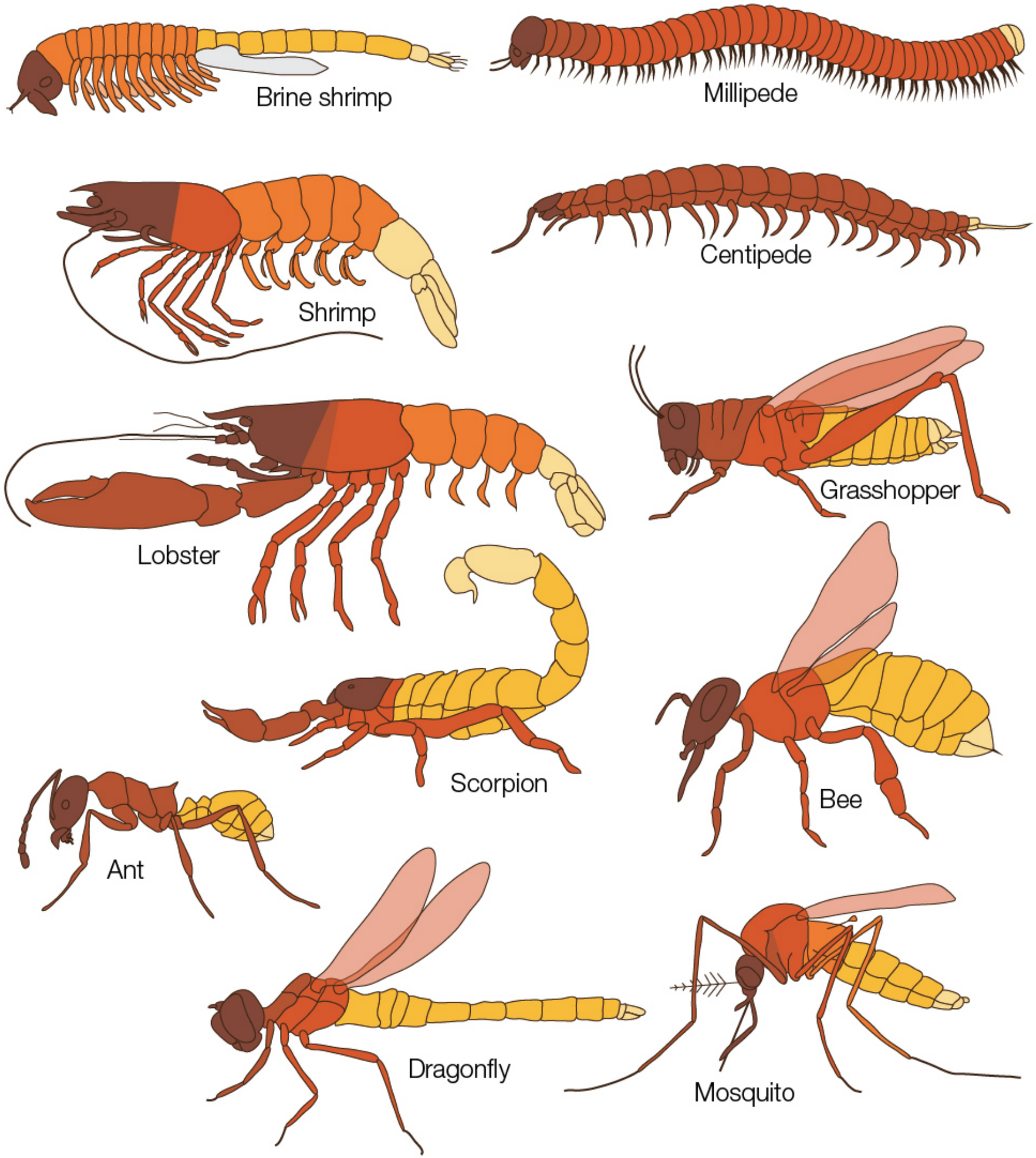
Zones of *Hox* gene activity in the embryo

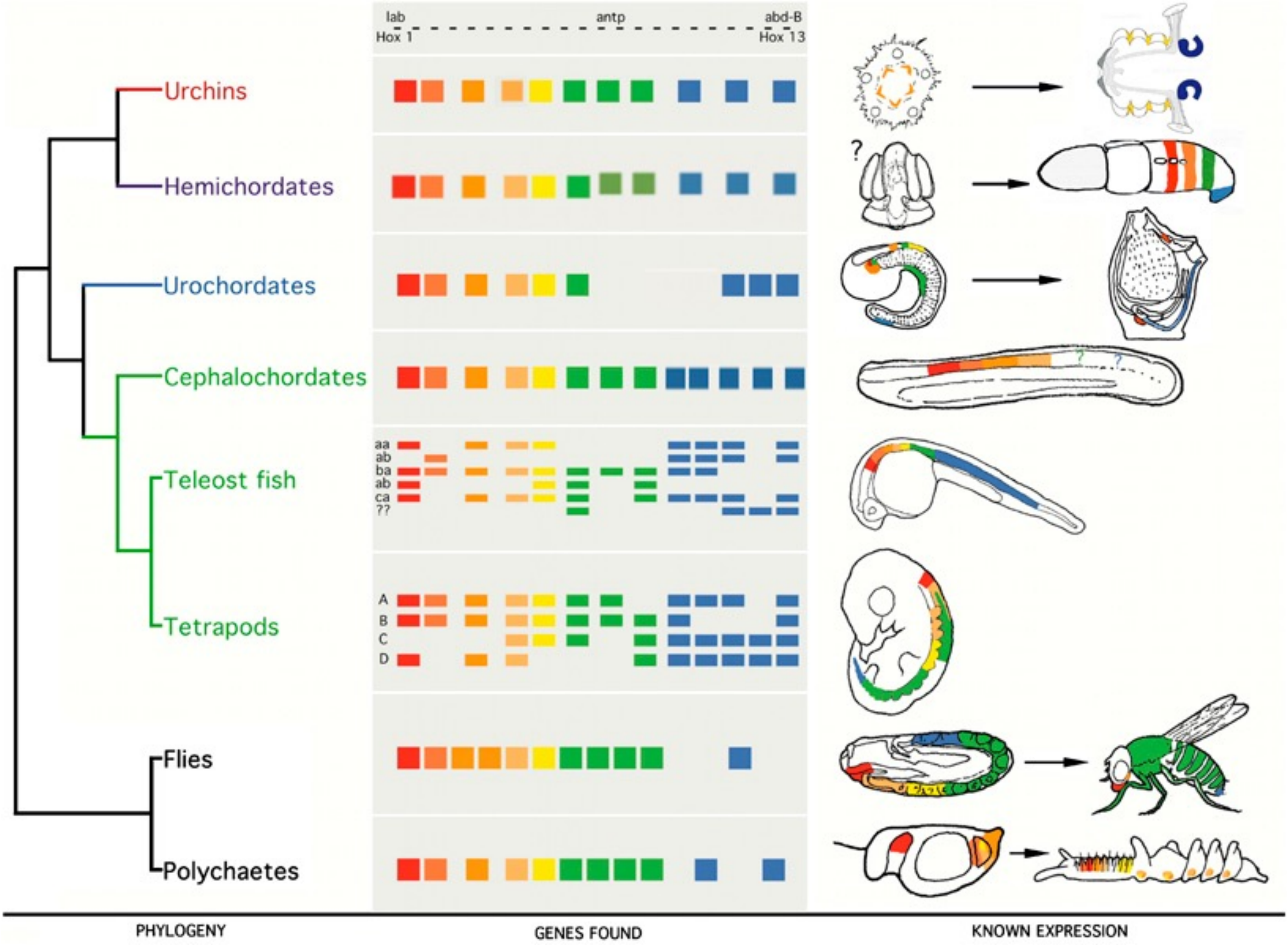



Antenna


Leg in place
of antenna


Haltere


Brine shrimp
Millipede
Shrimp
Centipede
Grasshopper
Lobster
Scorpion
Bee
Ant
Dragonfly
Mosquito


lab
Hox 1
antp
abd-B
Hox 13
Urchins
Hemichordates
Urochordates
Cephalochordates
Teleost fish
Tetrapods
Flies
Polychaetes
aa
ab
ba
ab
ca
??
A
B
C
D
?
PHYLOGENY
GENES FOUND
KNOWN EXPRESSION