Short course in Molecular Biology

2018-2019

Course Program

Genetic information and informational molecules

➢General introduction and historical hints. The chemical structure of DNA and RNA. Three dimensional structure of DNA. Physico-chemical properties of DNA.

DNA, RNA and gene structure

➢Definition of gene coding and regulatory regions. From genes to proteins; messenger RNA, transfer RNA and ribosomal RNA.

Genome organization and evolution

➢DNA content and number of genes. Mutations, DNA rearrangement and genome evolution. The organelle genomes. Interrupted genes; introns. cDNA. Gene families and duplication. DNA repeats.

Transposable elements

➢Transposition mechanisms and control. Retroviruses and retrotransposones. Transposons.

Chromatin and chromosomes

➢Nucleosomes, histones and their modifications. Higher organization levels of chromatin. Heterochromatin and euchromatin. Eukaryotic chromosomes, telomeres and centromeres.

DNA replication

➢DNA polymerases. Proofreading activity of DNA polymerases. Replication mechanism in bacteria and eukaryotic cells.

Introns and RNA splicing

➢Features of spliceosomal introns. Spliceosome and splicing mechanism. Alternative splicing and trans-splicing. Other kinds of introns: group I and group II introns and tRNA introns. The intron movement. RNA editing. Ribozymes and riboswitch.

DNA mutation and repair

➢Spontaneous mutations and mutations caused by physical and chemical mutagens. Pre- and post-replicative repair systems. Recombination in the immunity system cells. Approaches to homologous recombination.

Regulation of gene expression

➢Bacterial promoters. The operon. Activators, repressors and coactivators. Signal transductions and two component regulation systems. Eukaryotic promoters. Activators, repressors and coactivators. Gene expression and chromatin modifications. Epigenetic mechanisms.

RNAs and transcription

➢Different types of RNA: synthesis and maturation. Bacterial RNA polymerase. Sigma factors. Eukaryotic RNA polymerases. Eukaryotic mRNAs: capping, polyadenylation, cytoplasmic localization. The transcription process in bacteria and in eukaryotic cells.

Translation

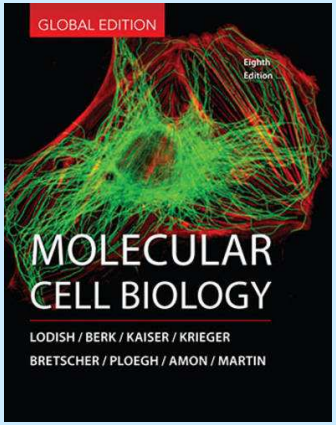
➢Ribosomes. tRNA structure and function. Aminoacyl-tRNA synthesis. Initiation in bacteria and eukaryotic cells. Polypeptide chain synthesis and translation end. Regulation of translation.

Protein localization

6 Credits of Theory→ 48 Hours

Exam Methods

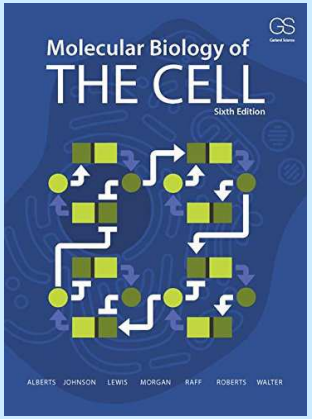
Oral examination



Lodish, Berk, Kaiser, Krieger, Bretscher, Ploegh, Amon and Martin

Molecular Cell Biology, 8e

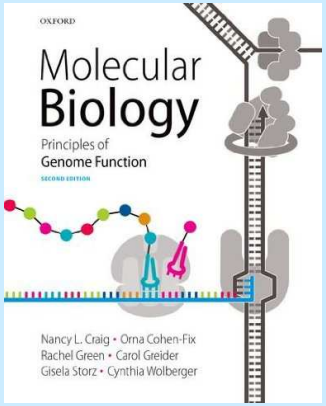
Freeman (€ 88)



Bruce Alberts, et al.

Molecular Biology of the Cell, 6e

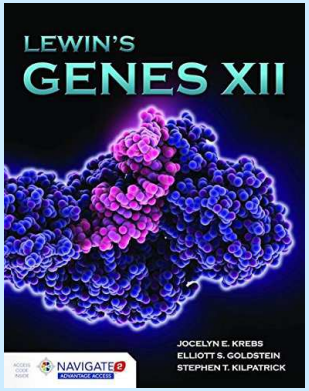
Garland Science (€ 76)



Craig, et al.

Molecular Biology: Principles of Genome Function, 2e

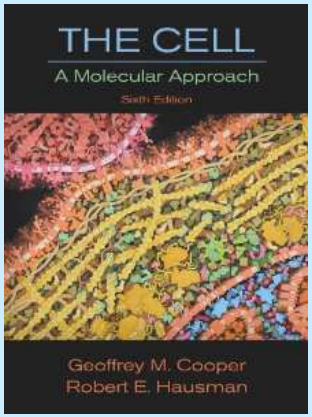
Oxford University Press (€ 62)



Krebs, Goldstein, Kilpatrick

LEWIN'S GENES XII

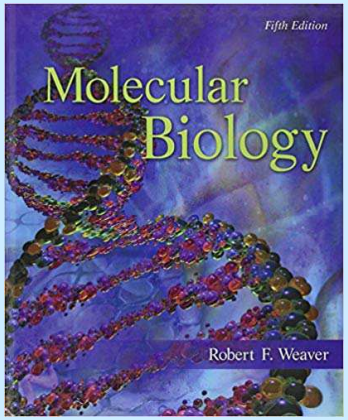
Jones & Bartlett Publ. (€ 82)



Cooper, Hausman

The Cell: A Molecular Approach, 6e

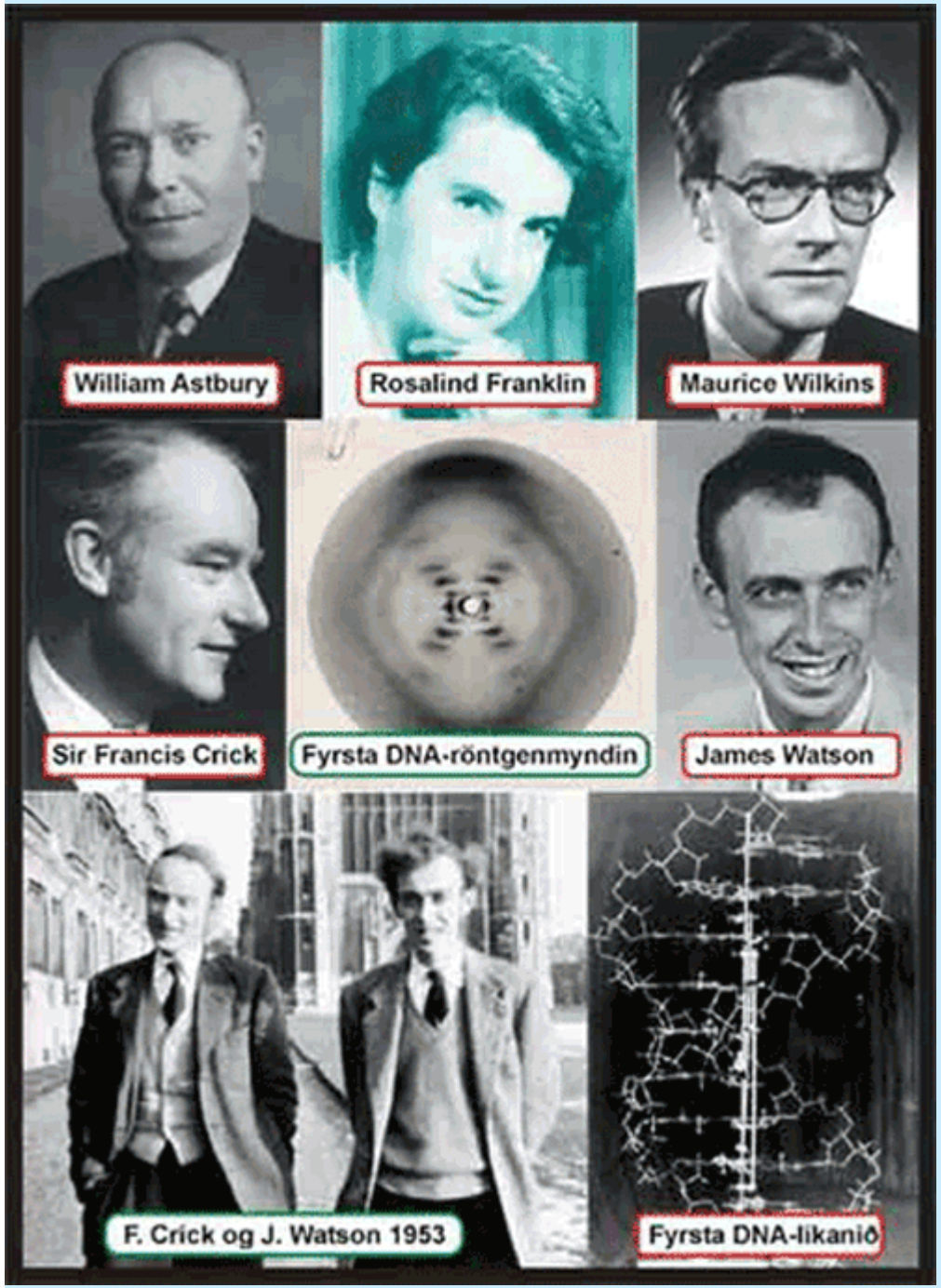
Sinauer Associates, Inc. (€ 65)



Weaver

Molecular Biology, 5e

McGraw-Hill Education (€ 65)



Writing in *Nature*, William Astbury described molecular biology as:
"... not so much a technique as an approach, an approach from the viewpoint of the so-called basic sciences with the leading idea of searching below the large-scale manifestations of classical biology for the corresponding molecular plan. It is concerned particularly with the forms of biological molecules and ..... is predominantly three-dimensional and structural - which does not mean, however, that it is merely a refinement of morphology - it must at the same time inquire into genesis and function."

Molecular Biology

- **How the genetic material is conserved?**
- **How the information hold in the genetic material is decodified?**

```
  1 gtgcacctga ctcctgagga gaagtctgcc gttactgccc tgtggggcaa ggtgaacgtg
 61 gatgaagttg gtggtgaggc cctgggcagg ctgctggtgg tctacccttg gacccagagg
121 ttctttgagt cctttgggga tctgtccact cctgatgctg ttatgggcaa ccctaaggtg
181 aaggctcatg gcaagaaagt gctcggtgcc tttagtgatg gcctggctca cctggacaac
241 ctcaagggca cctttgccac actgagtgag ctgcactgtg acaagctgca cgtggatcct
301 gagaacttca ggctcctggg caacgtgctg gtctgtgtgc tggcccatca ctttggcaaa
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421 ctggcccaca agtatcac
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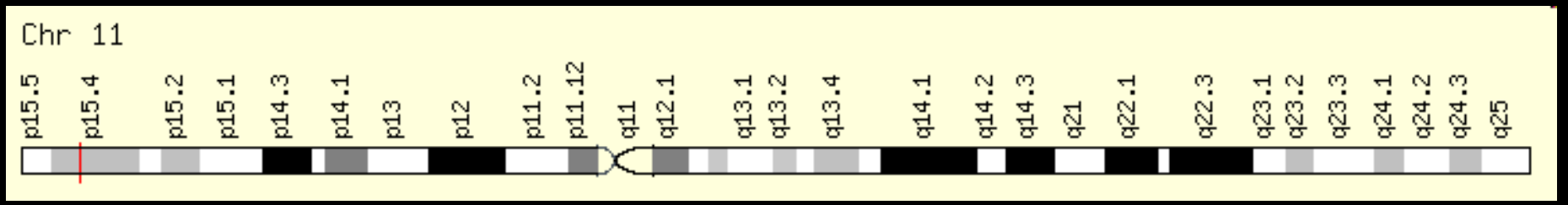
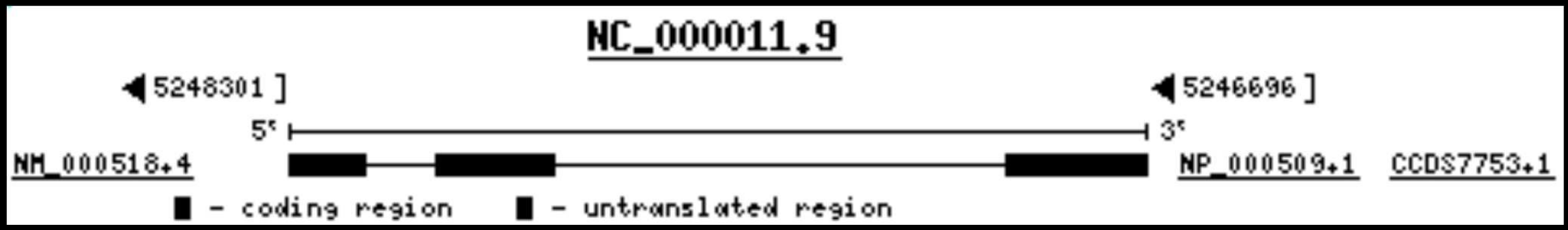




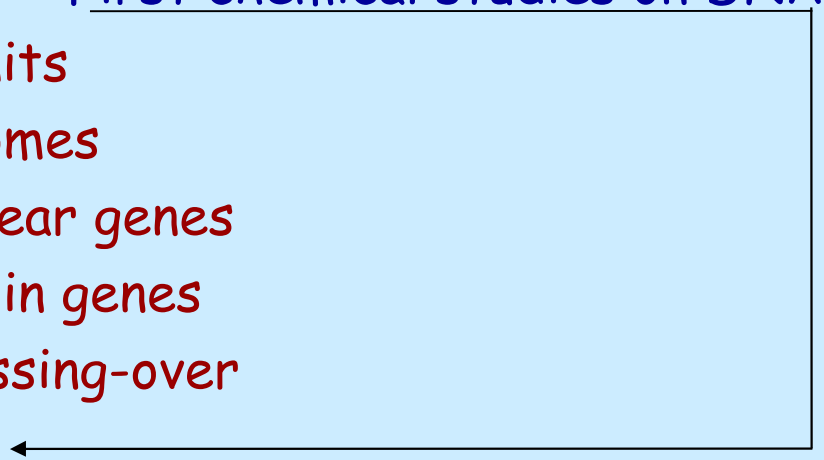
VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFF
ESFGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKG
TFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPV
QAAYQKVVAGVANALAHKYH

Definitions

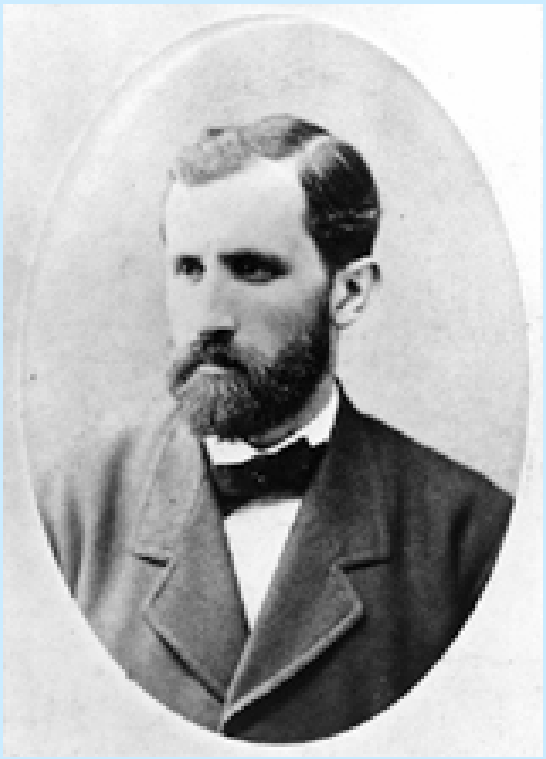
- **Nucleic Acids:** molecules that codify for genetic information. DNA, RNA
- **Gene:** segment of DNA coding for a polypeptide chain.
- **Genome:** whole of DNA sequences of an entire organism.

1865	Genes are particulate factors
1903	Chromosomes are hereditary units
1910	Genes are localized in chromosomes
1913	Chromosomes hold wholes of linear genes
1927	Mutations are physical changes in genes
1931	Recombination is caused by crossing-over
1944	DNA is the genetic material
1945	One gene -> one protein
1953	DNA has a double helix structure
1958	DNA replicates in semiconservative manner
1961	The genetic code is defined by triplets of bases
1977	Eukaryotic genes are interrupted
1977	DNA sequencing
1995	Bacterial genomes sequencig
2001	Human genome sequencig

First chemical studies on DNA



Friedrich Miescher (1844-1895)



The laboratory located in the vaults of an old castle where Miescher isolated nuclein

Johan Friedrich Miescher (13 August 1844, Basel - 26 August 1895, Davos) was a Swiss biologist. He isolated various **phosphate-rich chemicals**, which he called ***nuclein*** (now nucleic acids), from the **nuclei** of **white blood cells** in 1869 at Felix Hoppe-Seyler's laboratory at the University of Tübingen, Germany, paving the way for the identification of DNA as the carrier of inheritance.

Phoebus Levene

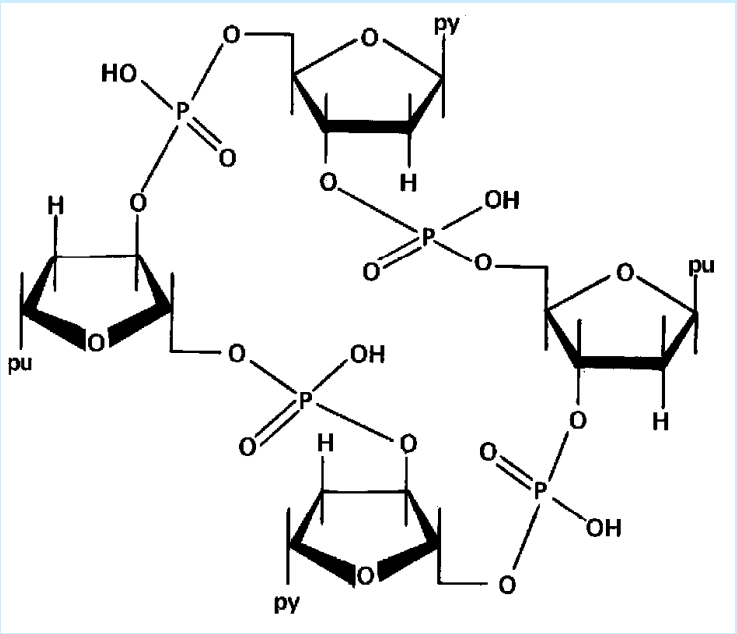
Levene's laboratory with some of his students (1909).

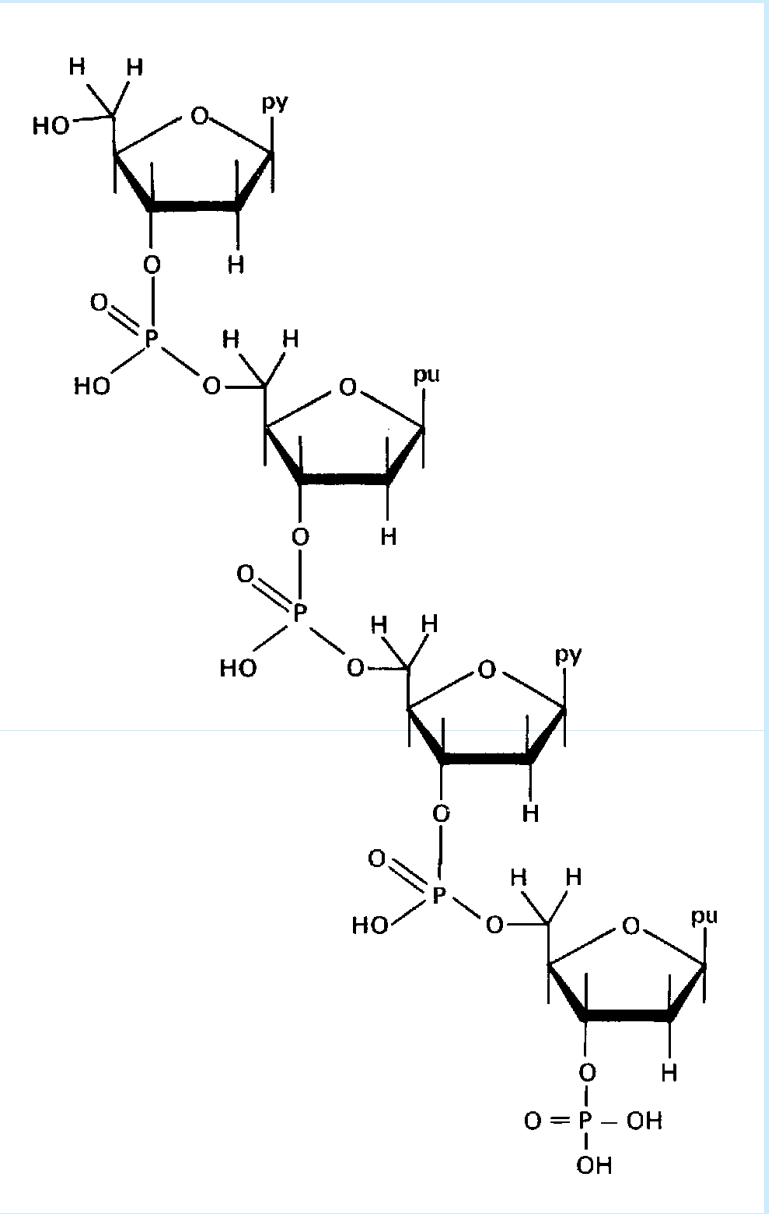
In 1905, **Levene** was appointed as head of the biochemical laboratory at the Rockefeller Institute of Medical Research. He spent the rest of his career at this institute, and it was there that he identified the components of DNA. (He had discovered **ribose** in 1909 and **deoxyribose** in 1929.) Not only did Levene identify the components of DNA, he also showed that the **components were linked together** in the order **phosphate-sugar-base** to form **units**. He called each of these units a **nucleotide**, and stated that the DNA molecule consisted of a **string of nucleotide units linked together** through the **phosphate groups**, which are the 'backbone' of the molecule. However, his ideas about the structure of DNA were wrong…….. he thought there were only four nucleotides per molecule……..

Tetranucleotide structure proposed in 1935

by Levene e Tips

by Takahashi





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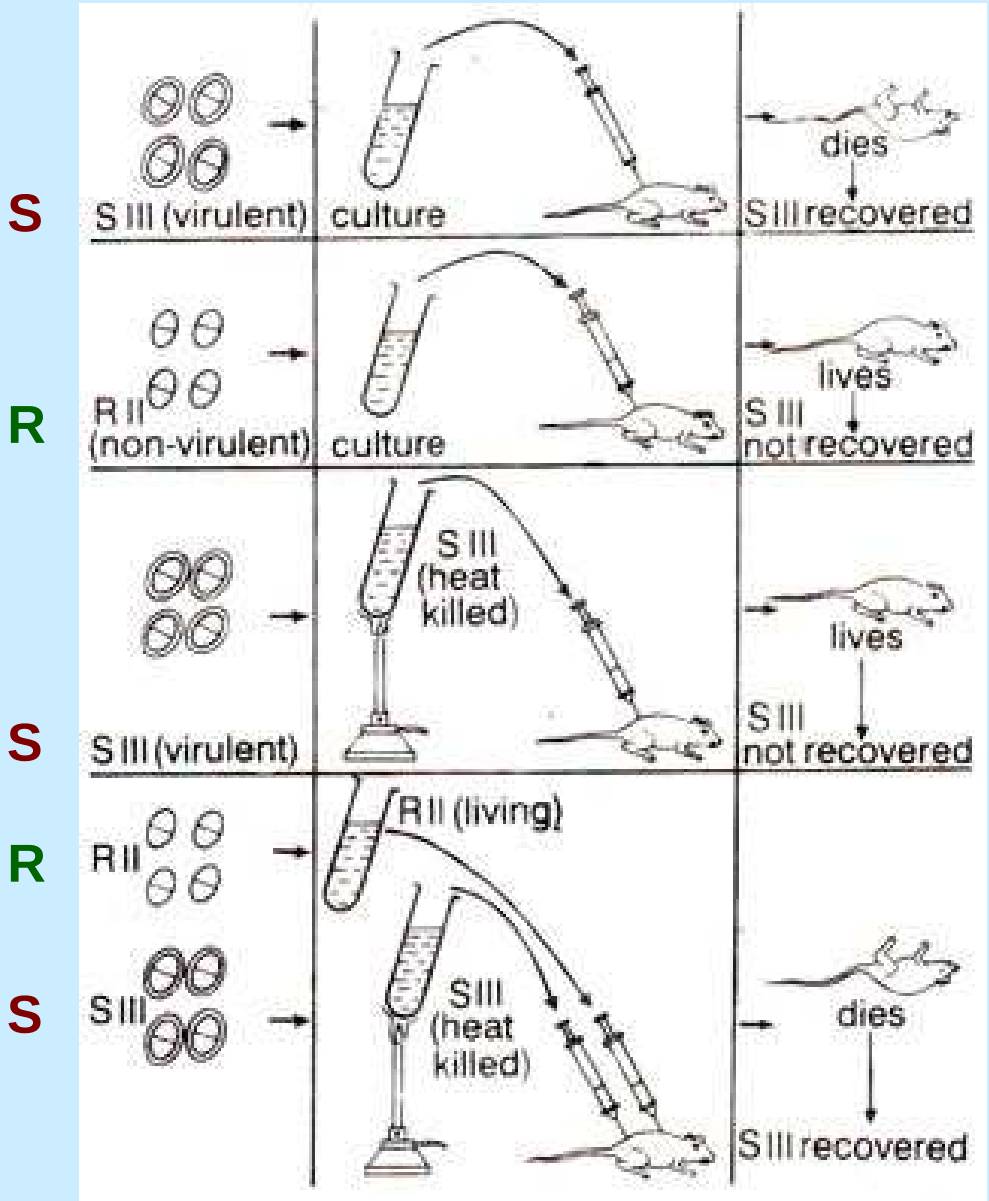
1995	Bacterial genomes sequencig
2001	Human genome sequencig

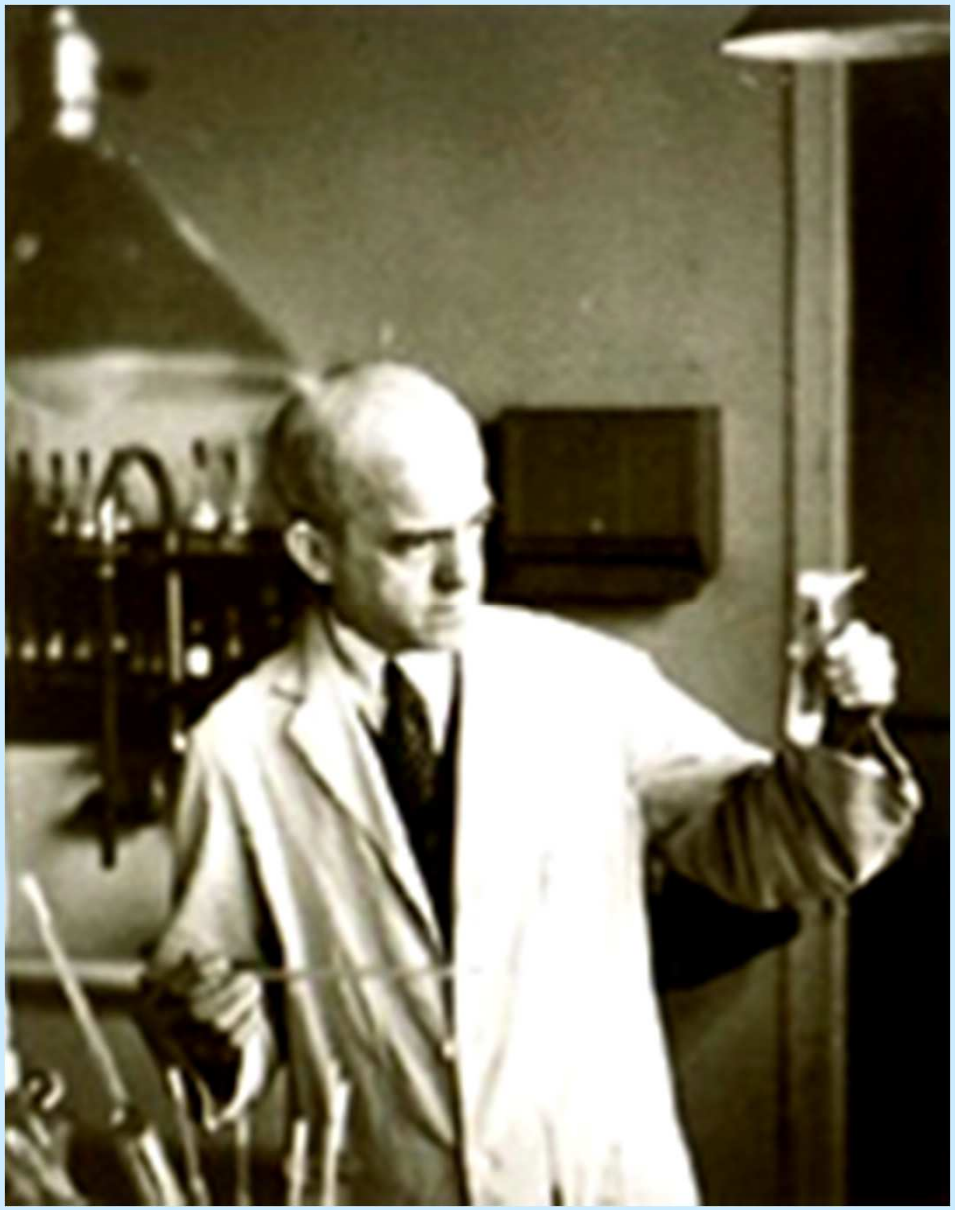
Bacterial “transformation” experiment: Griffith, 1928

Pneumococcus *(Streptococcus pneumoniae)*



- **A “transforming” factor changes the R into the S strain**

Oswald Avery at work in the laboratory, around 1930



Pneumococcus mutants transformation from R in to S strain using a cellular extract

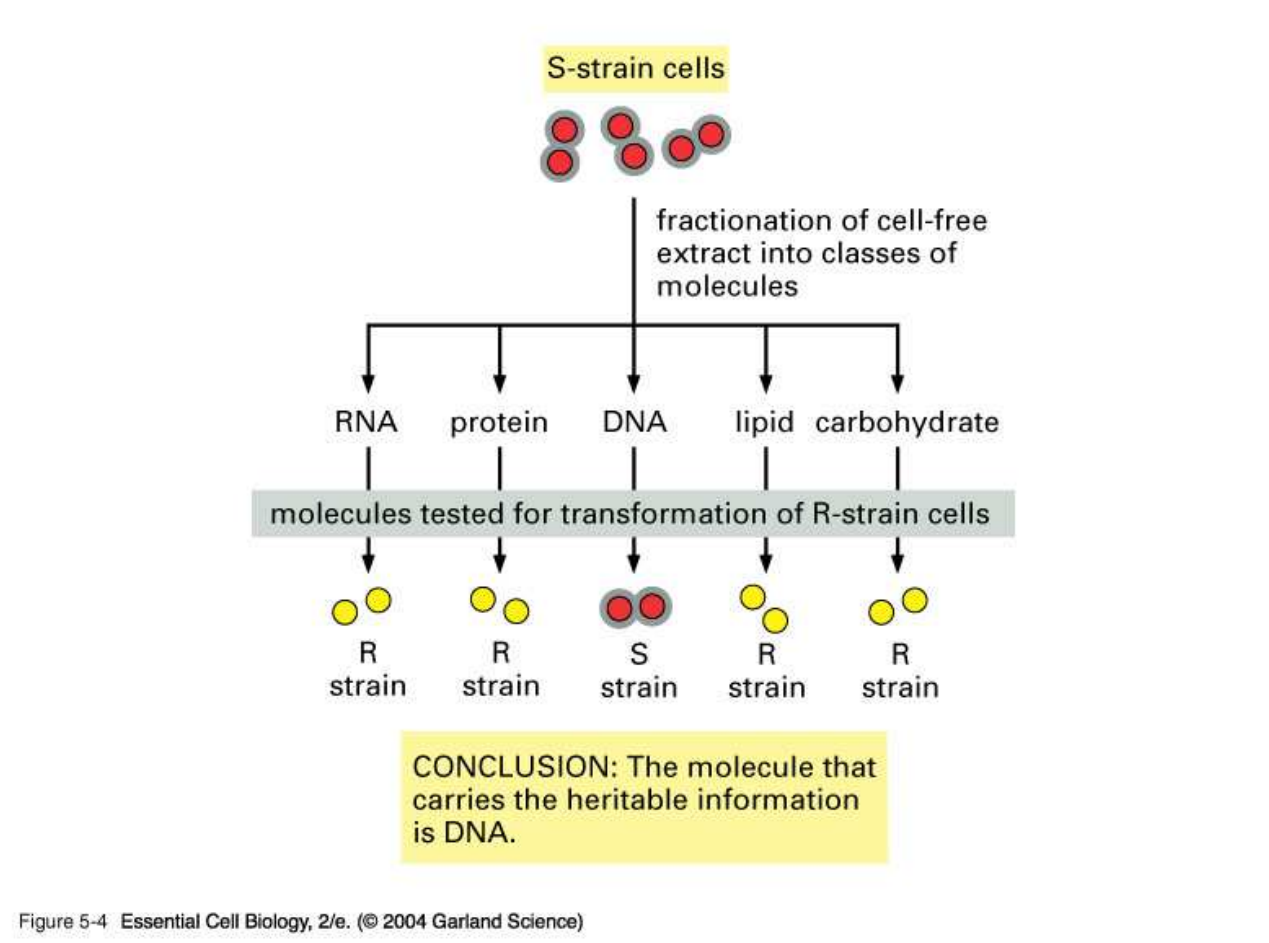
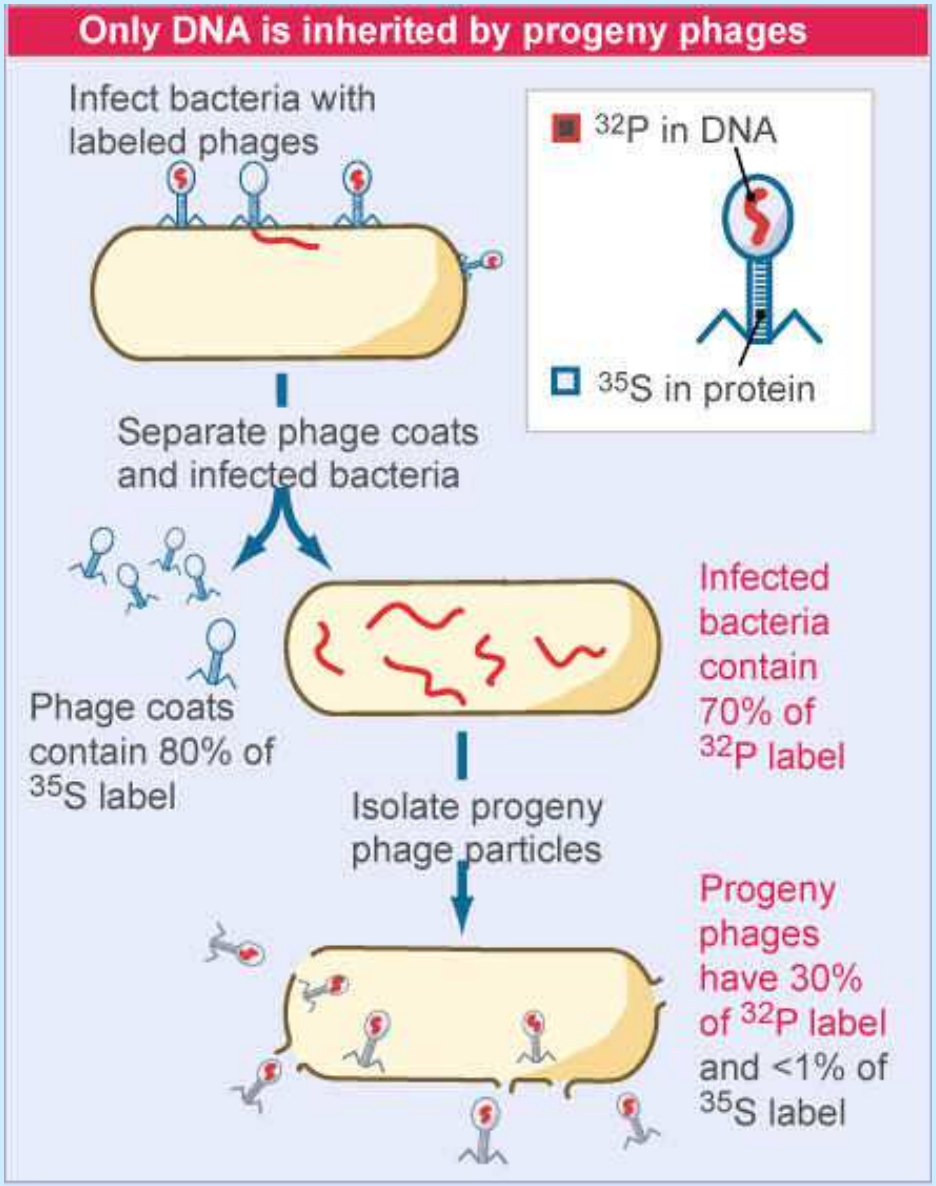


Figure 5-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

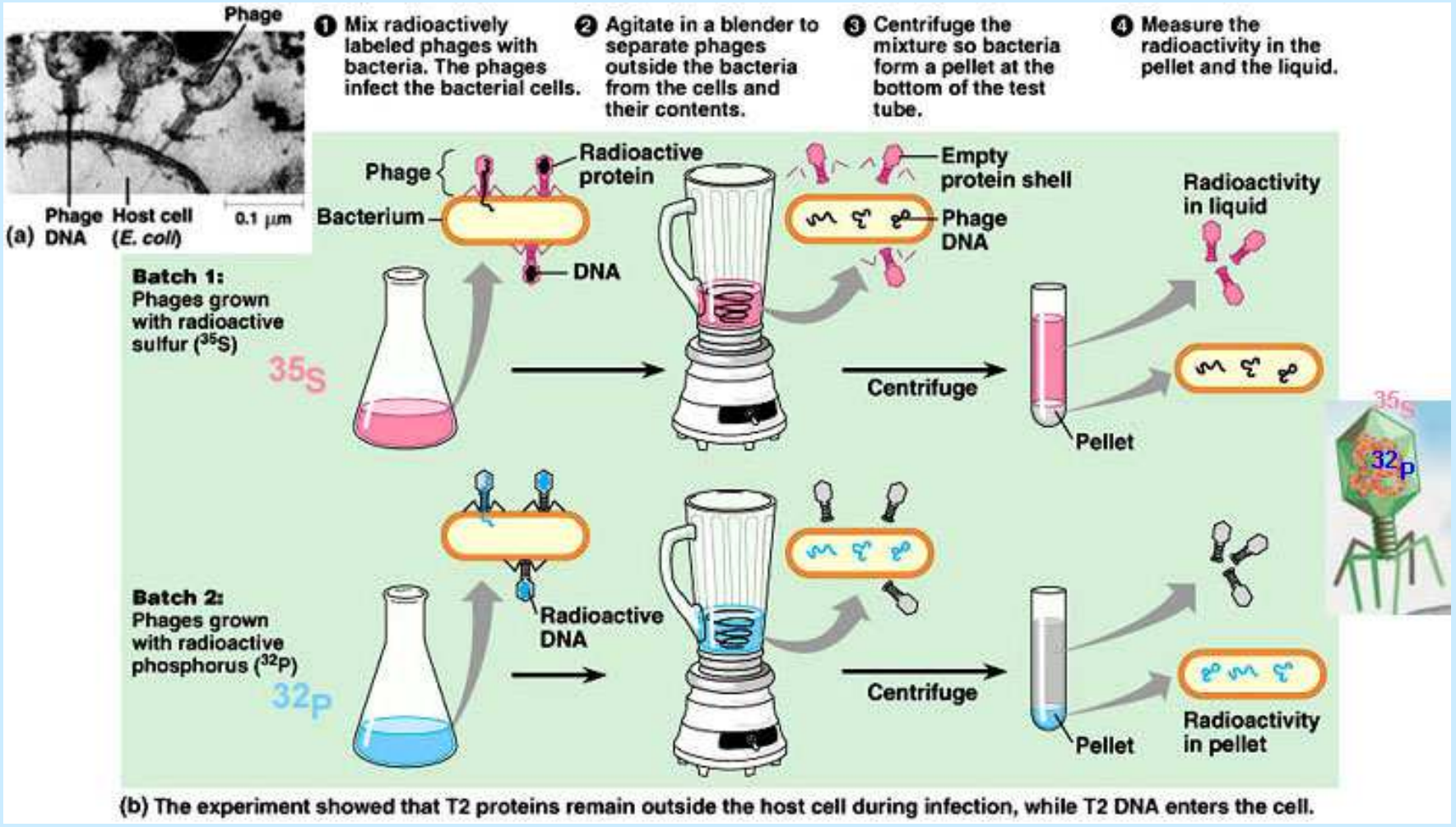
Avery, MacLeod, and McCarty demonstrate that '*DNA is responsible for the transforming activity*' and published that analysis in 1944, just 3 years after Griffith had died in a London air-raid.

Alfred Hershey and Martha Chase (1953)

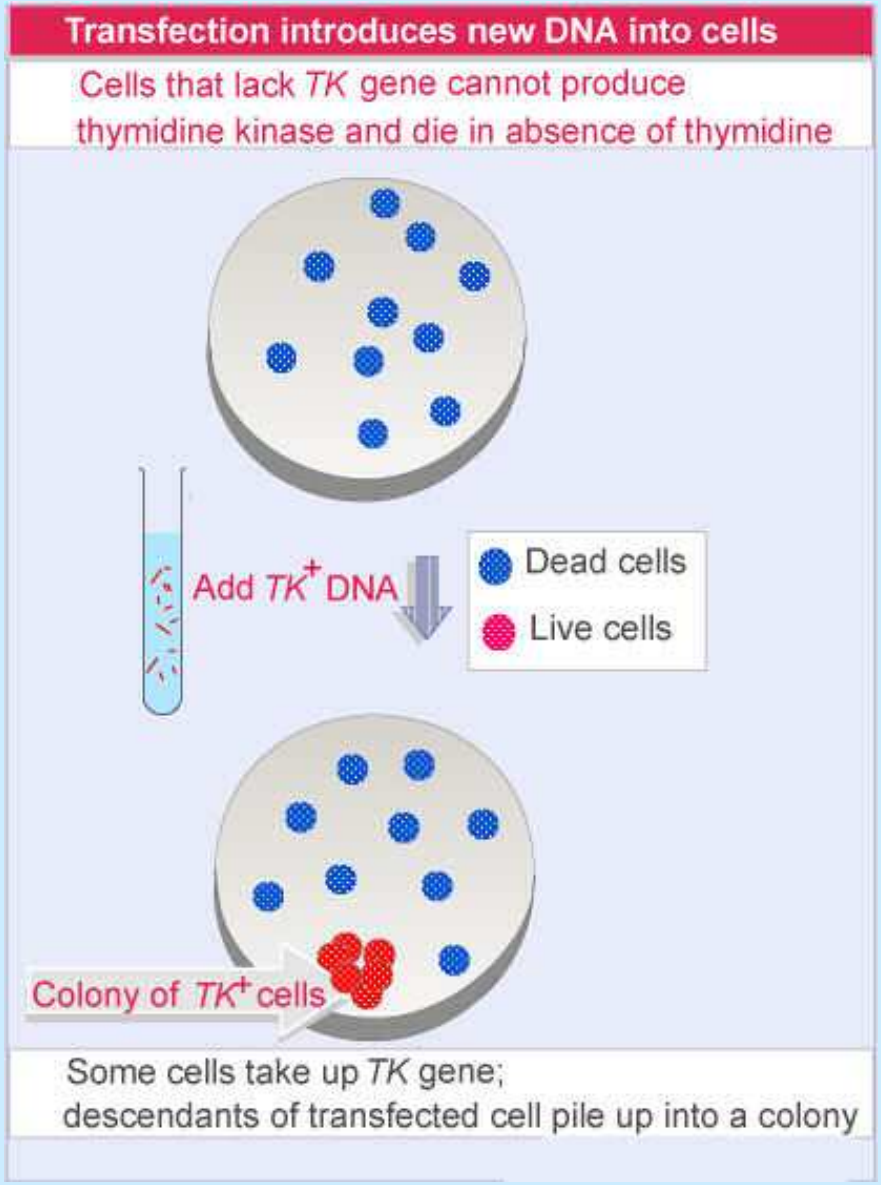
Blender Experiment by Hershey and Chase, 1952



Hershey-Chase experiment furthered work of Avery (1952)

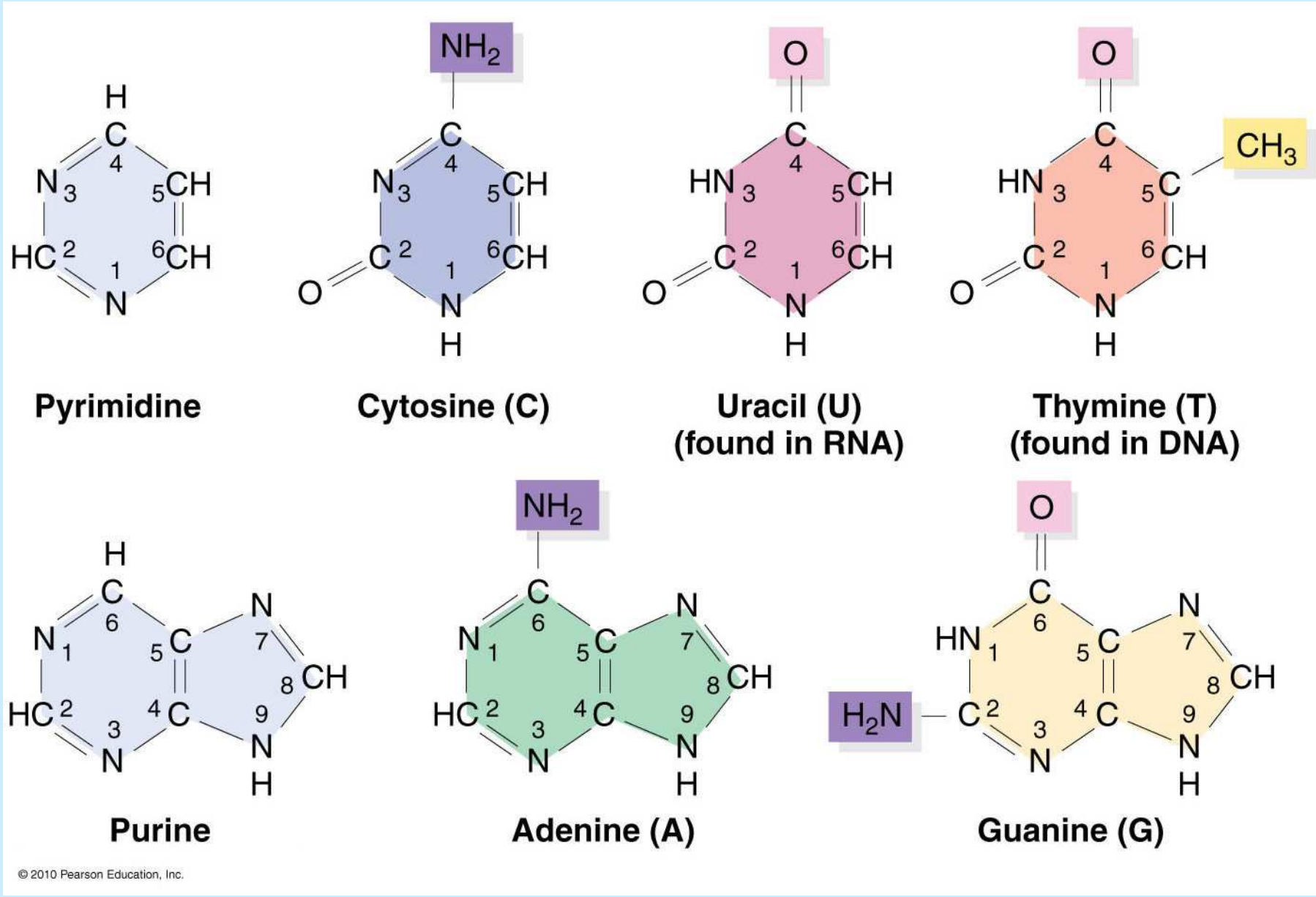


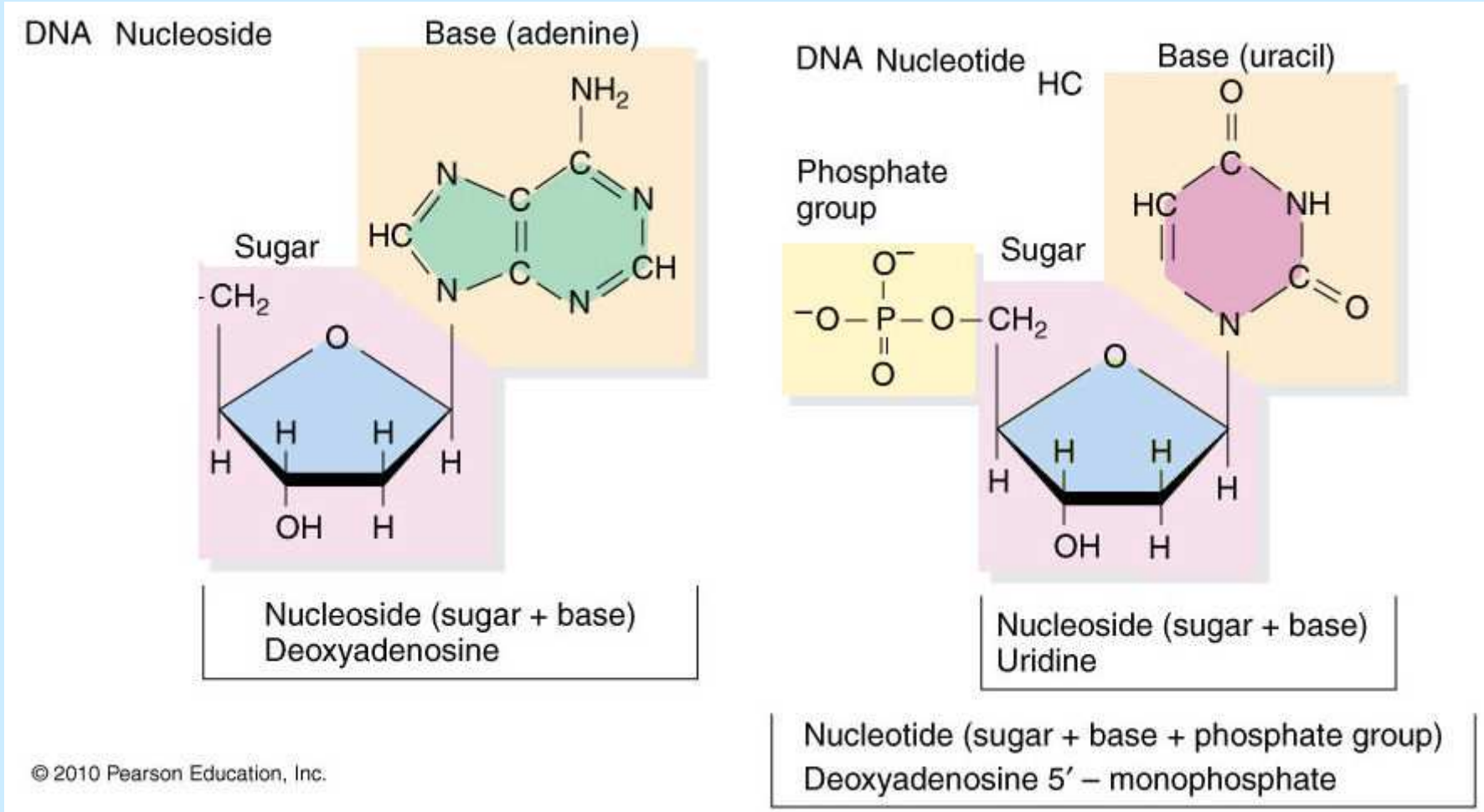
Transfection is the introduction of DNA into a recipient eukaryote cell and its subsequent integration into the recipient cells chromosomal DNA. Transfection is analogous to bacteria transformation but in eukaryotes transformation is used to describe the changes in cultured cells caused by tumor viruses.

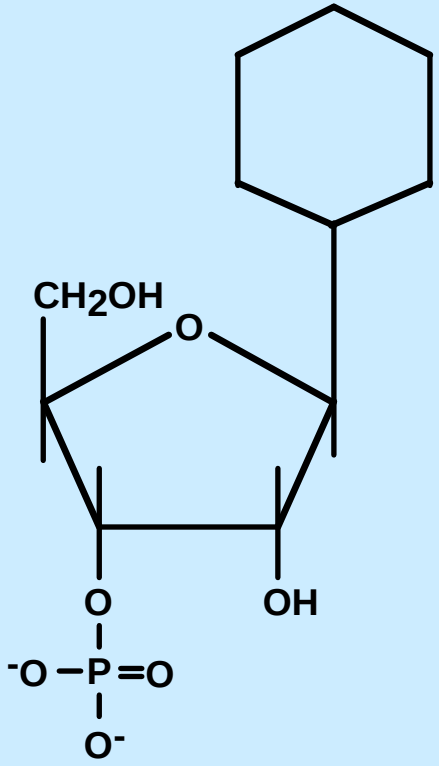


DNA chemical structure

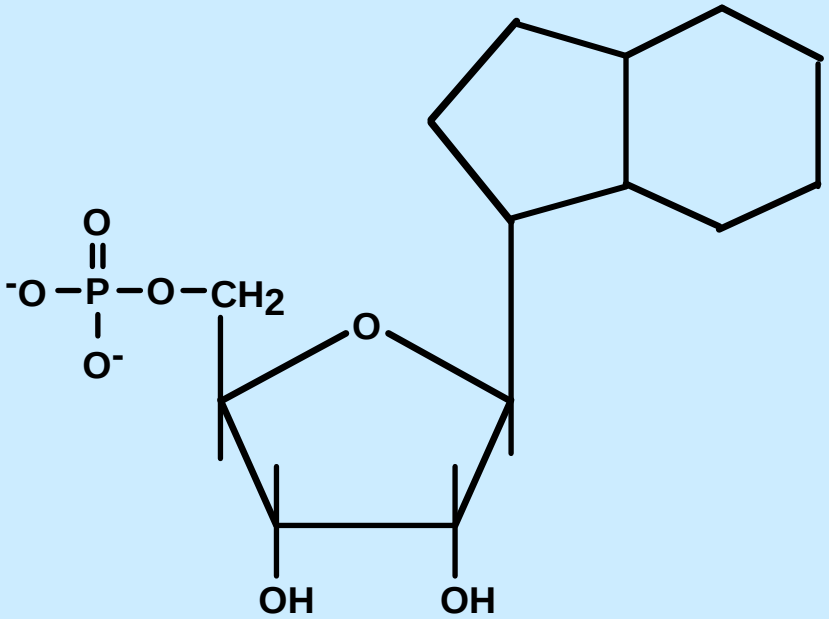
Chemistry of nucleic acids





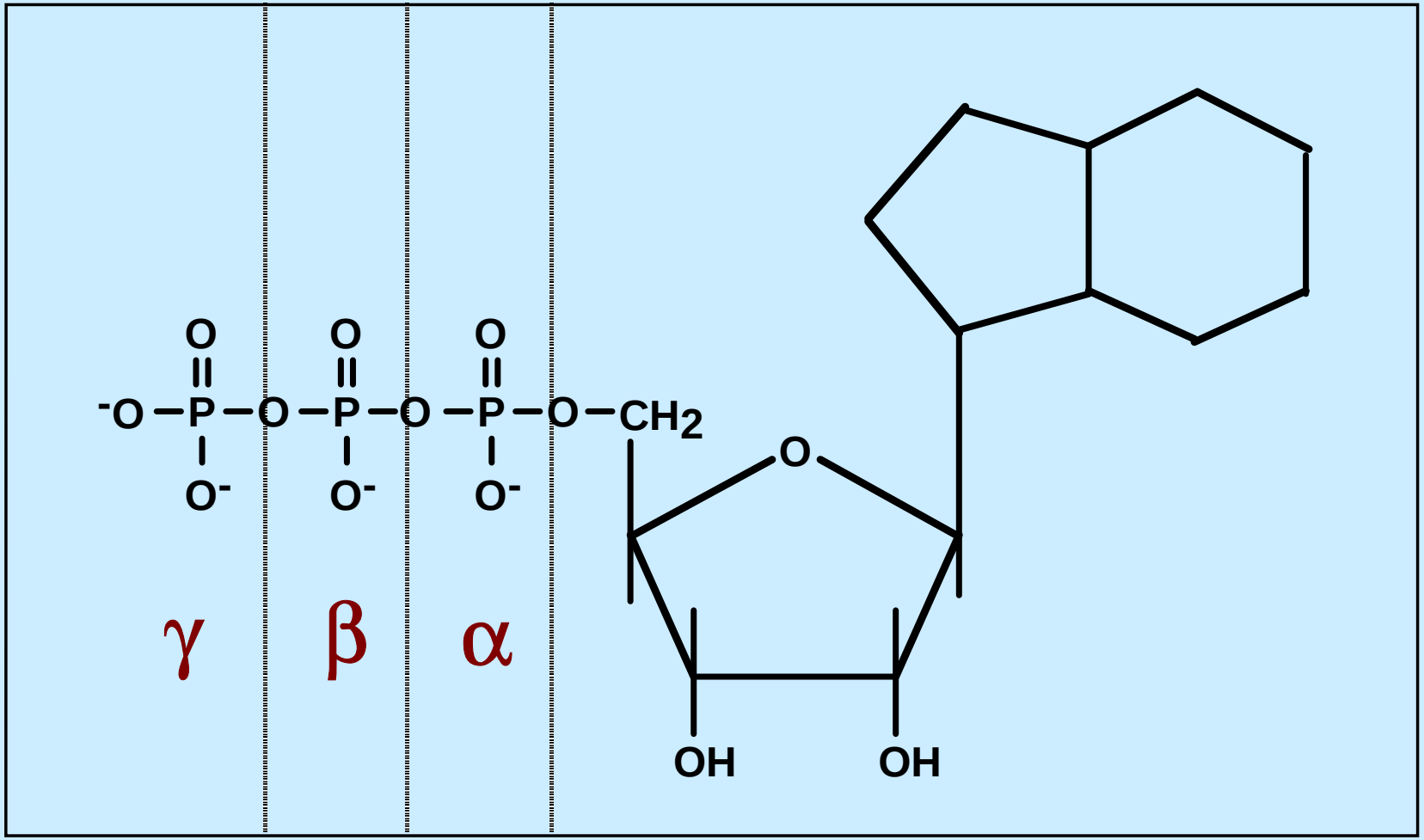


(Pyrimidine)
3'- monofosfate



(Purine)
5'- monofosfate

The phosphate groups form bonds with either the 5' or 3' position (also 2')

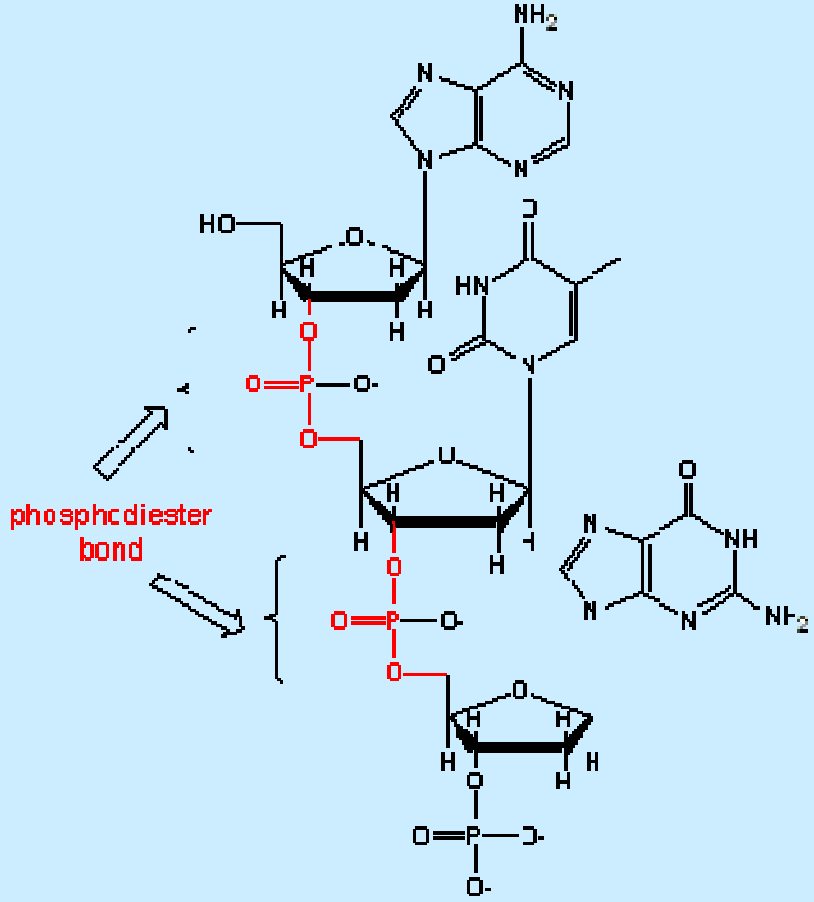


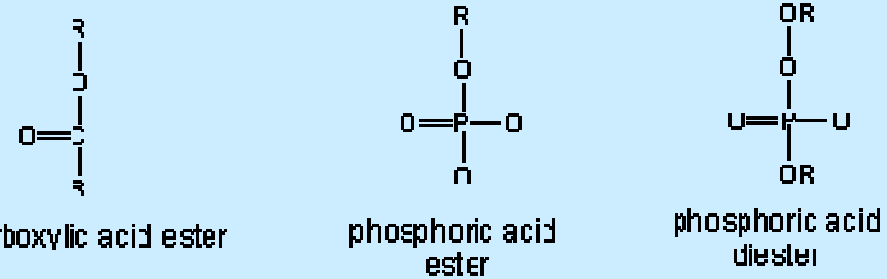
AMP + P → ADP + P → ATP

Bases, nucleosides and nucleotides

Base	Nucleoside	Nucleotide	RNA	DNA
Adenine	Adenosine	Adenosine-5'-phosphate (Adenilic Acid)	AMP	dAMP
Guanine	Guanosine	Guanosin-5'- phosphate (Guanylic Acid)	GMP	dGMP
Cytosine	Cytidine	Citidin-5'- phosphate (Cytidylic Acid)	CMP	dCMP
Thymine	Thymidine	Thymidine-5'- phosphate (Thymidylic Acid)		dTMP
Uracil	Uridine	Uridine-5'- phosphate (Uridylic Acid)	UMP	

Part of a DNA chain





carboxylic acid ester

phosphoric acid ester

phosphoric acid diester

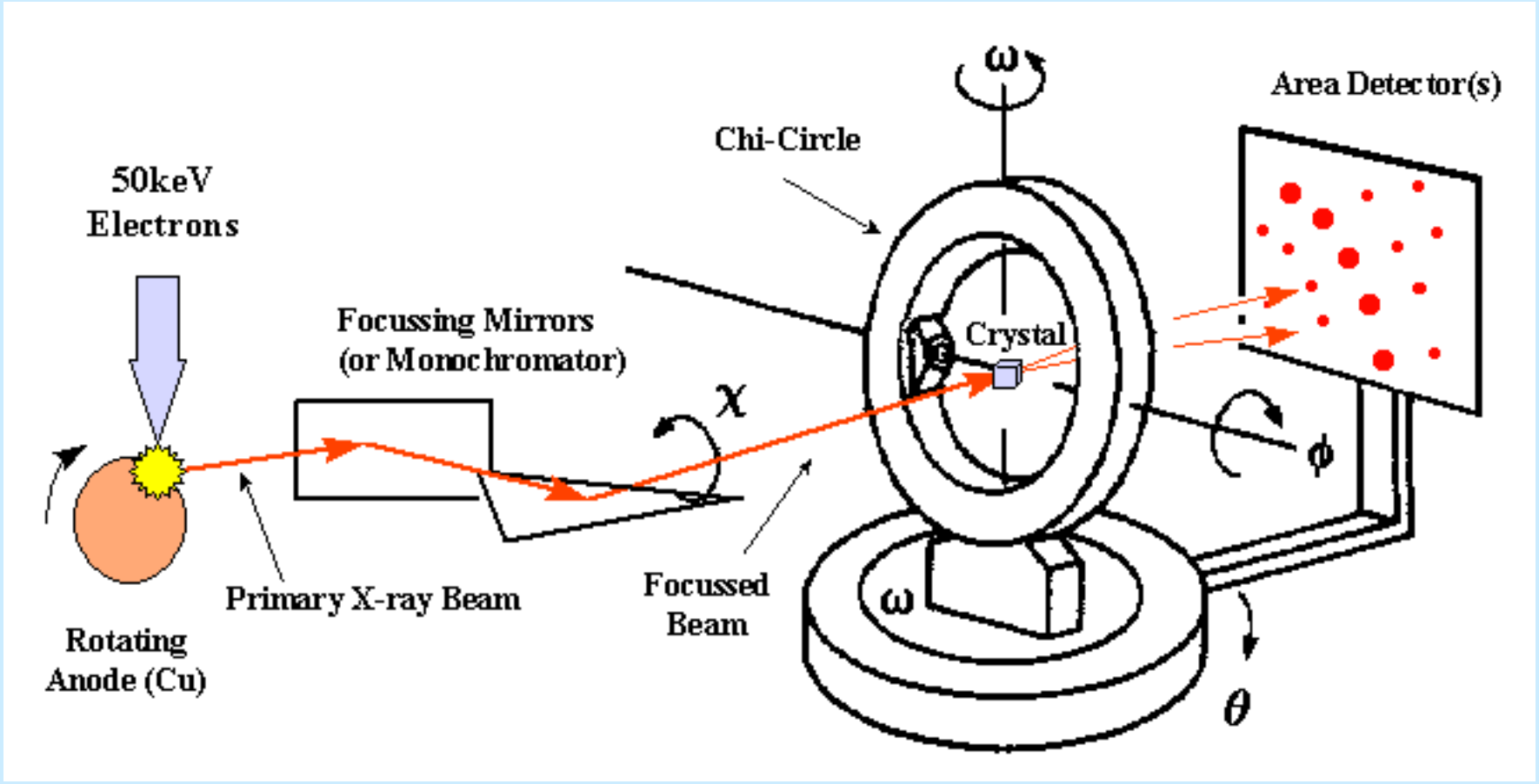
Erwin Chargaff

The first who precisely measured the percentage composition of the four nucleotides in DNA

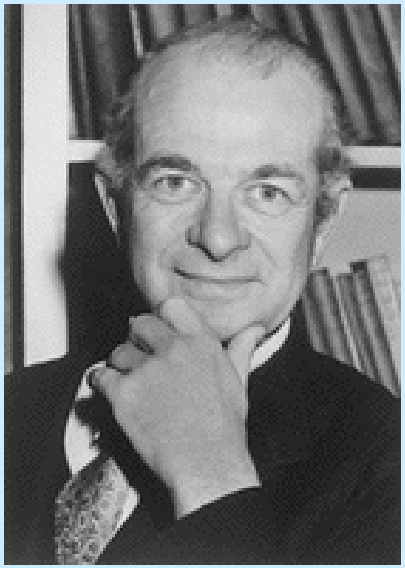
DNA base composition in different spieces

Organism	A	G	C	T	A/T	G/C	G+C	Py/Pu
Escherichia coli	26.0	24.9	25.2	23.9	1.08	0.99	50.1	1.04
Mycobact. tuberc.	15.1	34.9	35.4	14.6	1.03	0.99	70.3	1.00
Yeast	31.7	18.3	17.4	32.6	0.97	1.05	35.7	1.00
Bovine	29.0	21.2	21.2	28.7	1.01	1.00	42.4	1.01
Pig	29.8	20.7	20.7	29.1	1.02	1.00	41.4	1.01
Human	30.4	19.9	19.9	30.1	1.01	1.00	39.8	1.01

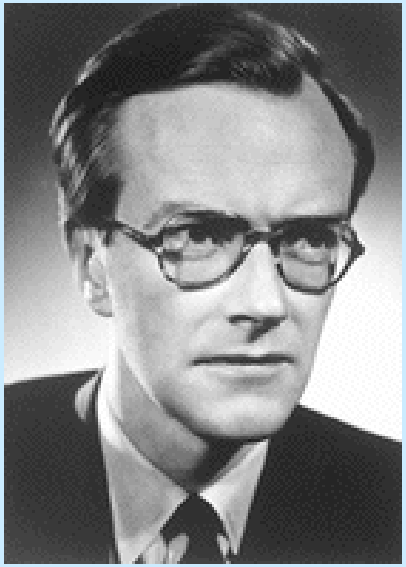
Physical-chemical structure of DNA


ω
Area Detector(s)
Chi-Circle
50keV
Electrons
Focussing Mirrors
(or Monochromator)
Crystal
χ
ϕ
Primary X-ray Beam
Focussed
Beam
ω
Rotating
Anode (Cu)
θ

Linus Pauling
(1901 - 1994)



Maurice Wilkins
(1916 - 2004)



Rosalind Franklin
(1920 - 1958)

Francis Crick
(1916 - 2004)

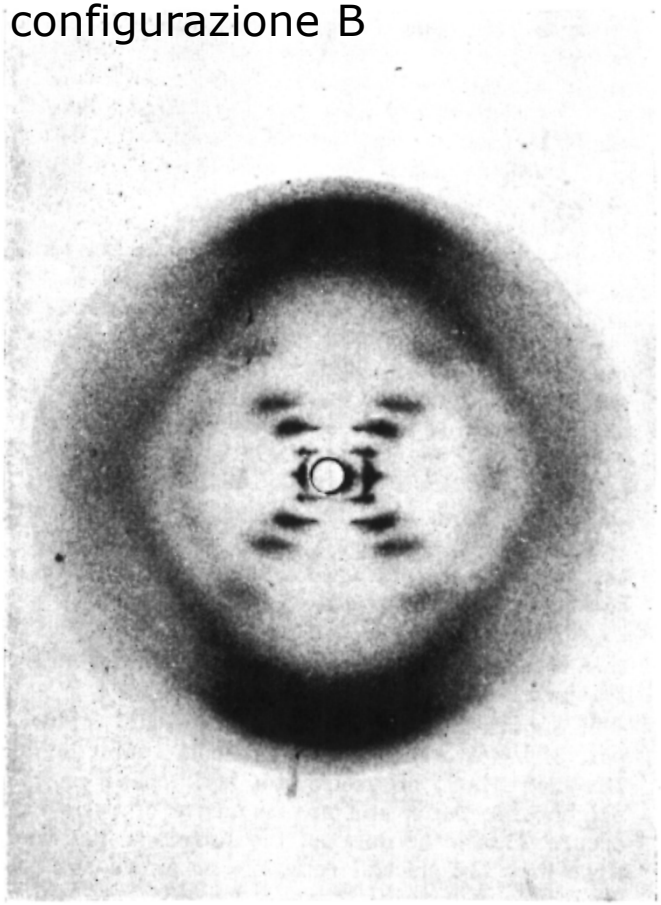
James Watson
(1928 -)

DNA structural data known before Watson & Crick structure

- Is formed by two chains
- Base percentage: G=C e A=T
- Phosphodiester bonds between nucleotides

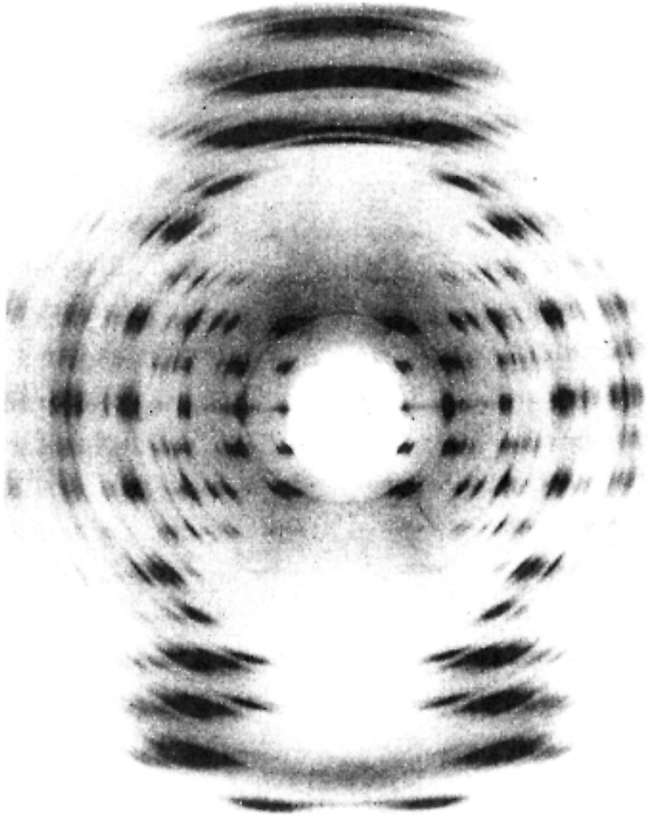
X-ray diffraction patterns from DNA fibres

configurazione B



Franklin R.E. & Gosling R., 1953

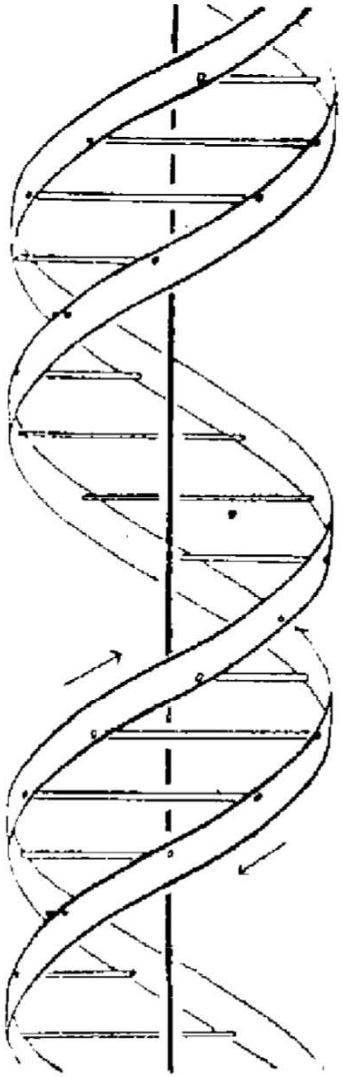
configurazione A



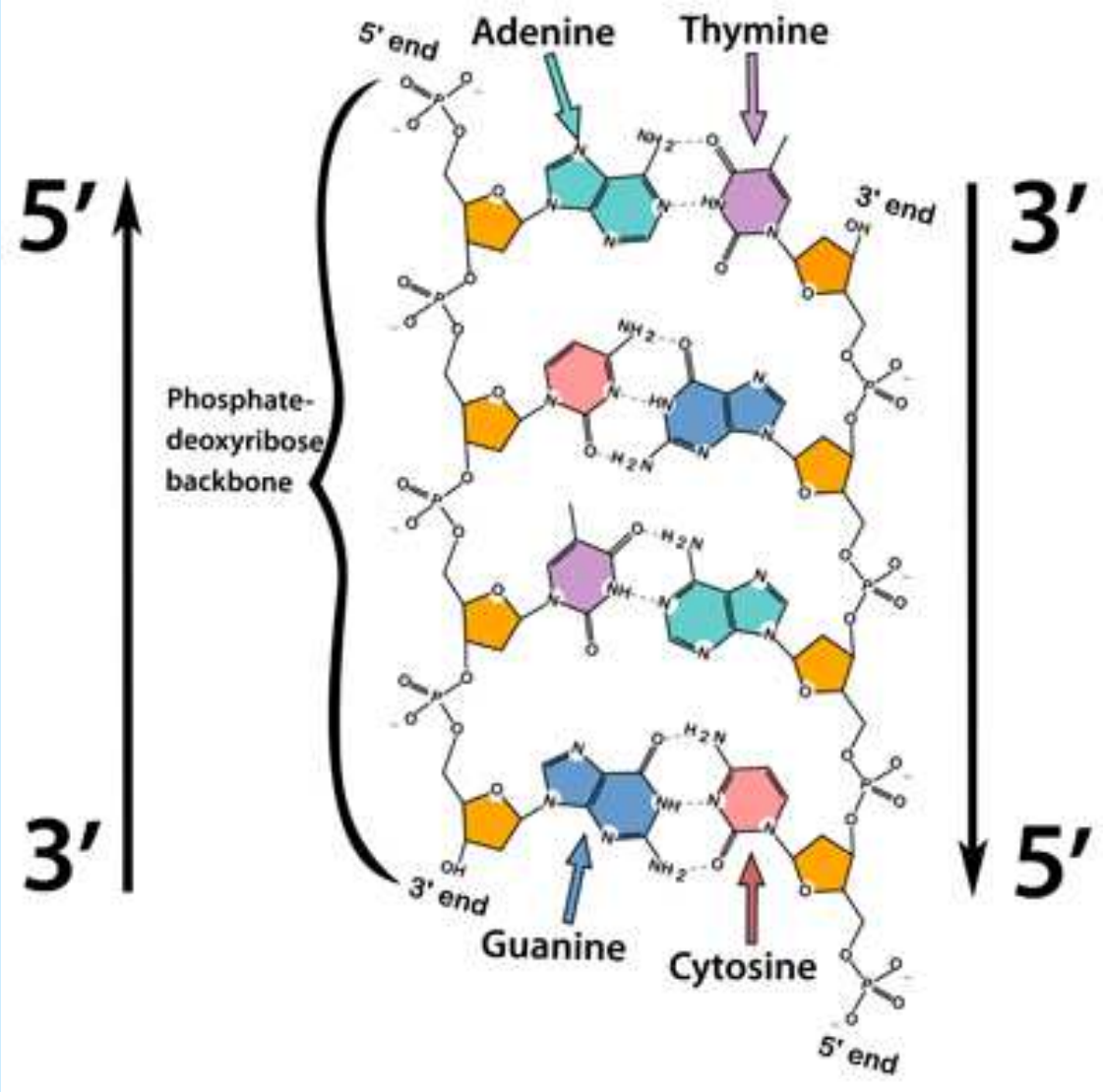
Wilkins M.H.F., 1956

Original figure from Watson e Crick paper

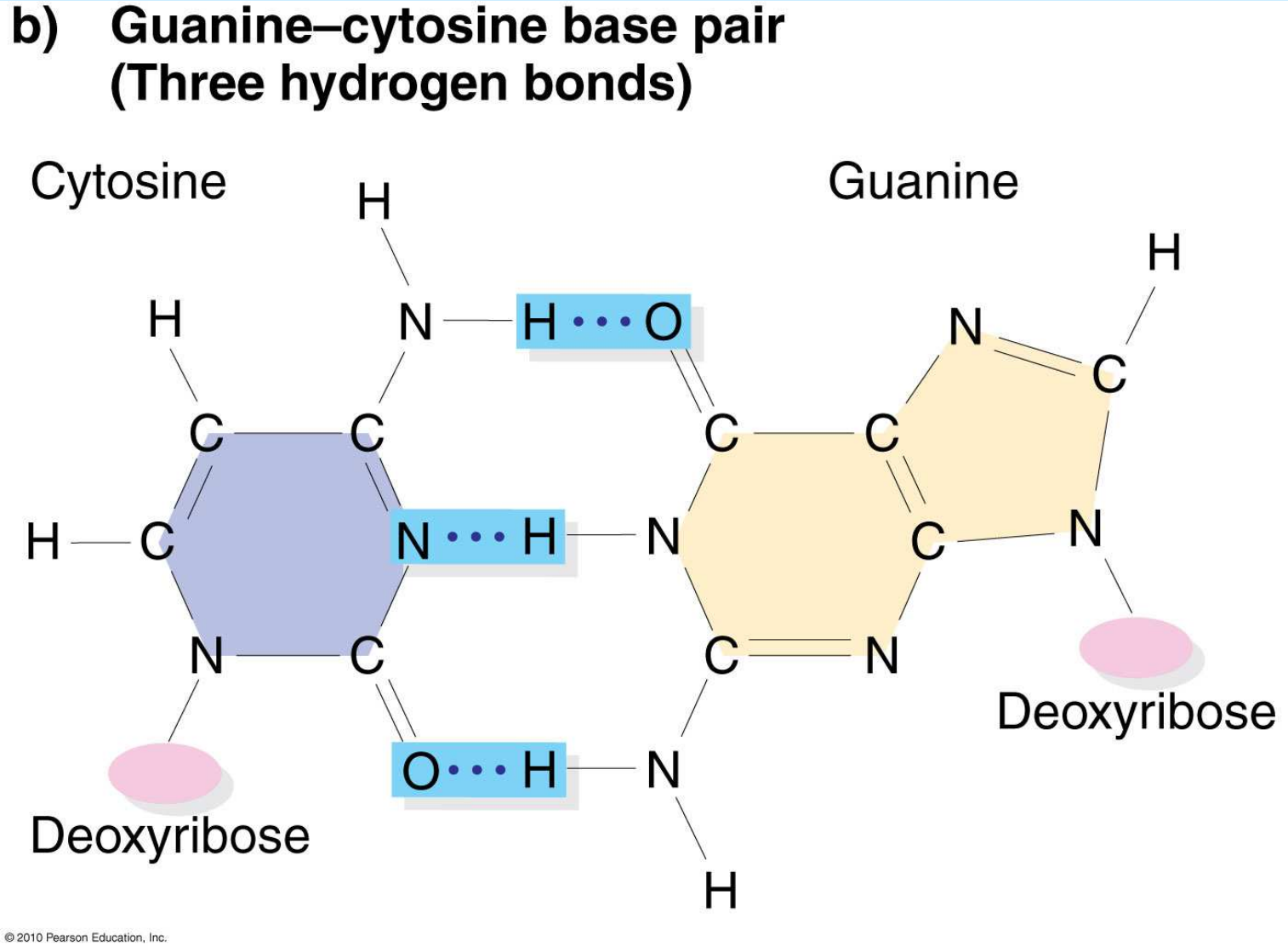
Nature, April 1953

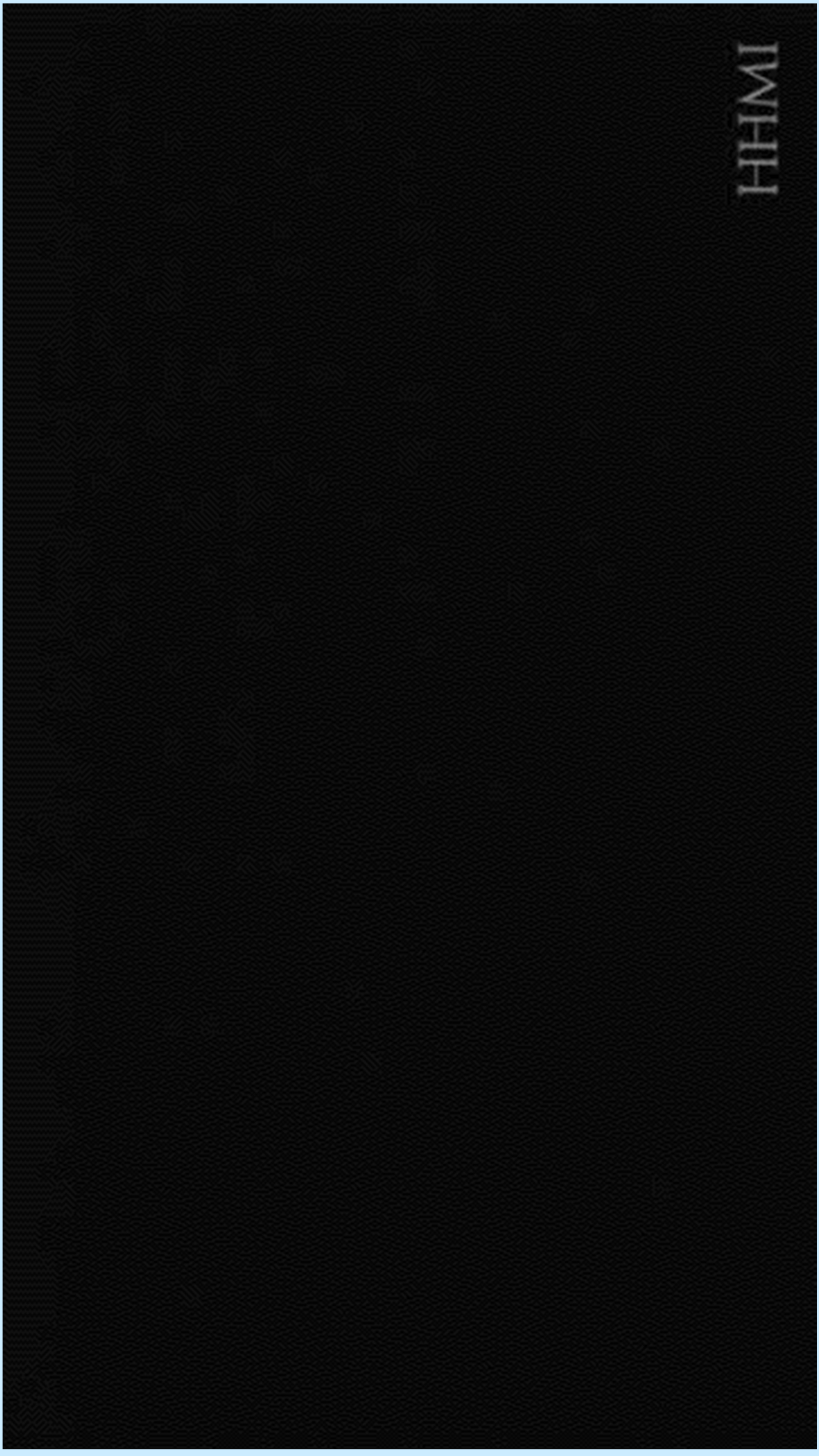


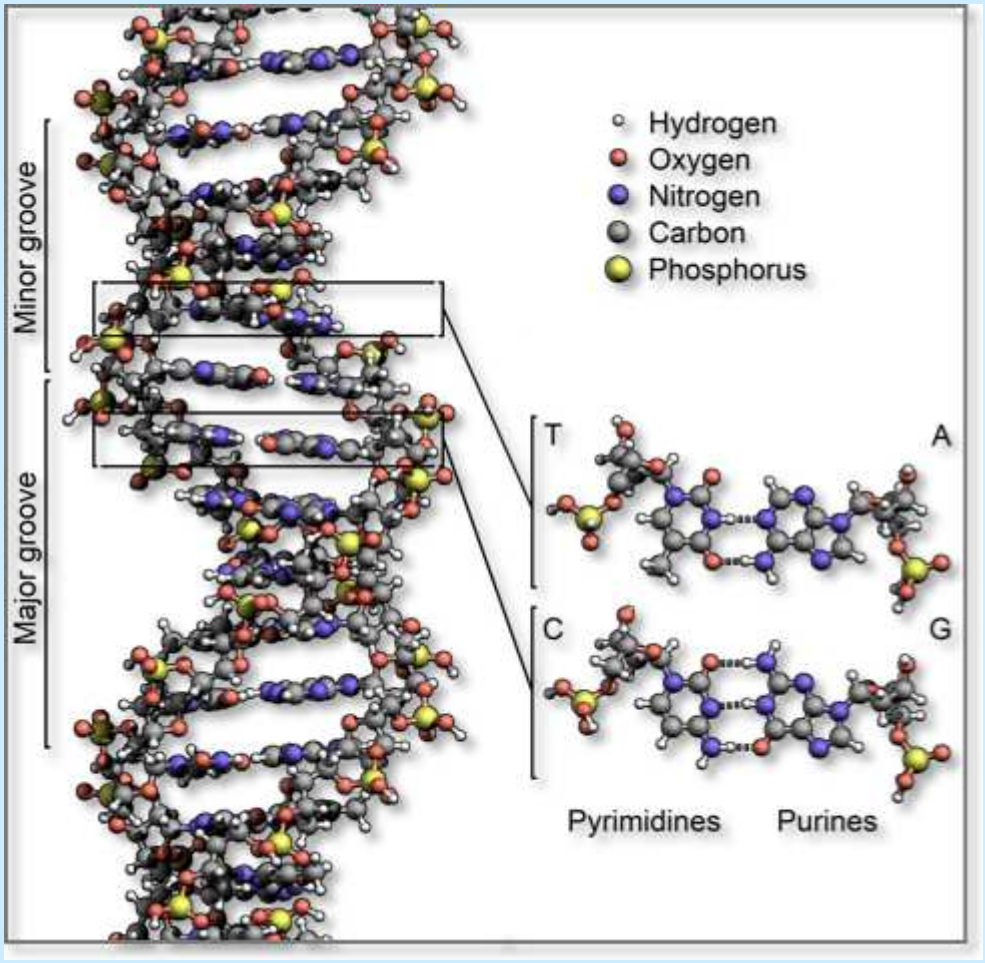
This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

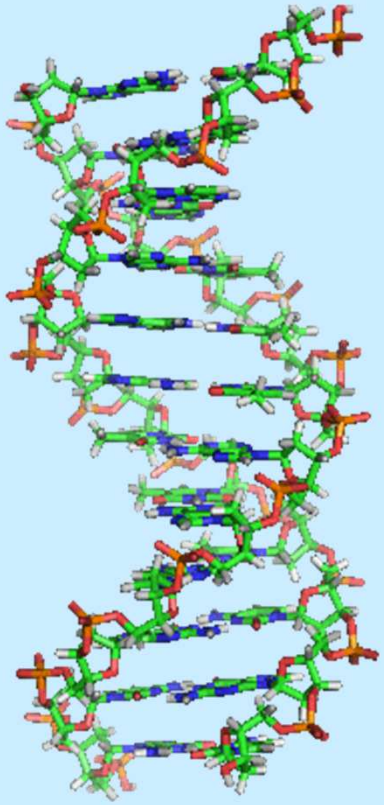

5′ end
Adenine
Thymine
5′
3′ end
3′
Phosphate-
deoxyribose
backbone
3′
3′ end
5′
Guanine
Cytosine
5′ end

Base pairing comes with hydrogen bond formation

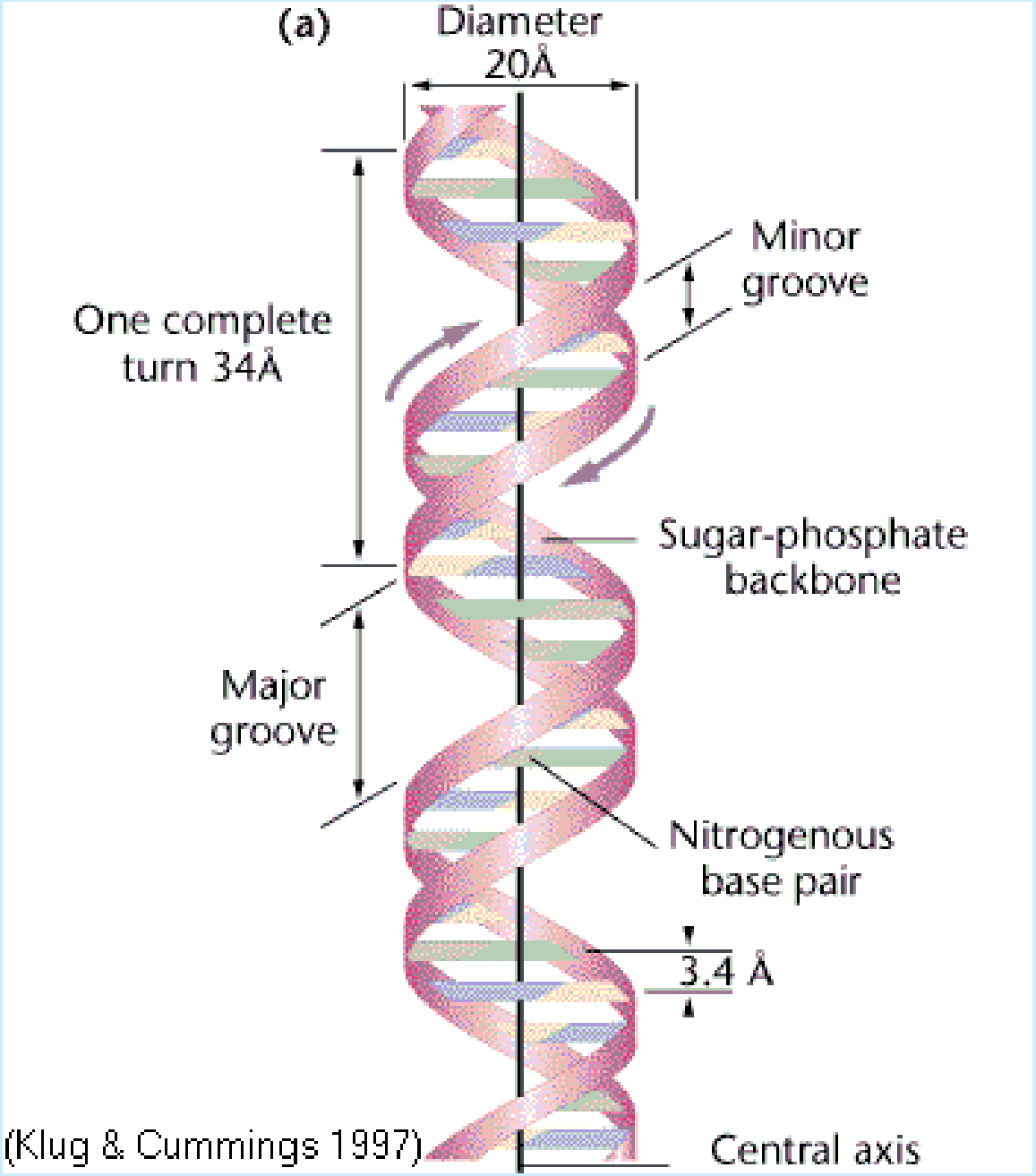



HHMI





Base pairs are flat


(a)
Diameter
20Å
Minor
groove
One complete
turn 34Å
Sugar-phosphate
backbone
Major
groove
Nitrogenous
base pair
3.4 Å
(Klug & Cummings 1997)
Central axis

DNA (B) structure

- DNA is a regular double helix, 20Å diameter, 34Å one turn of helix.
- Hydrogen bonds between bases.
- Base stacking is determined by hydrophobic interactions (pie-pie interactions).
- Sequent base pairs turned by 36°.
- Major groove (22Å) and minor groove (12Å).
- Coiled in a clockwise manner (right handed helix).

Nature, Vol. 171, p.737, April 25, 1953

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.

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 every 3.4 A. in the z-direction. We have assumed an angle of 36° between adjacent

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

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. The are joined together in pairs, 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 ration of guanine to cytosine, are always bery 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 King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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