Wykład 2

MOLECULAR GENETICS INTRODUCTION

Literature

• **Genetyka Molekularna,** pod red. P. Węglańskiego, Wydawnictwo Naukowe PWN 2000, 2006

• **Genomy,** TA. Brown, Wydawnictwo Naukowe PWN 2001

• **Podstawy Biologii Komórki,** B. Alberts i in., Wydawnictwo Naukowe PWN 1999, 2005

• **Genes,** B. Lewin, Oxford University Press, 1997, 2000

Anatomia człowieka Adam Bochenek Warszawa 2007

Basic concepts of biology

Cell

The basic form of the organization of living matter. It is the main structural and functional element of plants and animals, it can also be an independent organism (eg bacteria, protozoa). Its existence was discovered in the 17th century by the English physicist and biologist Robert Hooke, watching the cork fragments

The beginning

- 1590 discovery of the first optical microscope - microclimate
- 1665 Robert Hooke announces the discovery of the Micrographia cell
- 1676 Antonie van Leeuwenhoek builds an optical microscope for cell research
- 1831 Discovery of the cell nucleus by Robert Brown
- 1838 Formulation of cell theory of the structure of organisms by Matthias Jacob Schleiden
- 1839 Teodor Schwann states that animals and plants are made of cells
- 1861 Max Schultze gives modern cell theory as a nugget of live protoplasmatic mass containing cellular shudder
- 1972 J.F. Kerr, A. H. Wylie and A. R. Currie - the concept of apoptosis

Wstęp do genetyki molekularnej

Genetics - branch of biology dedicated to gene research

- molecular
- cytogenetics
- population

Issues of molecular genetics

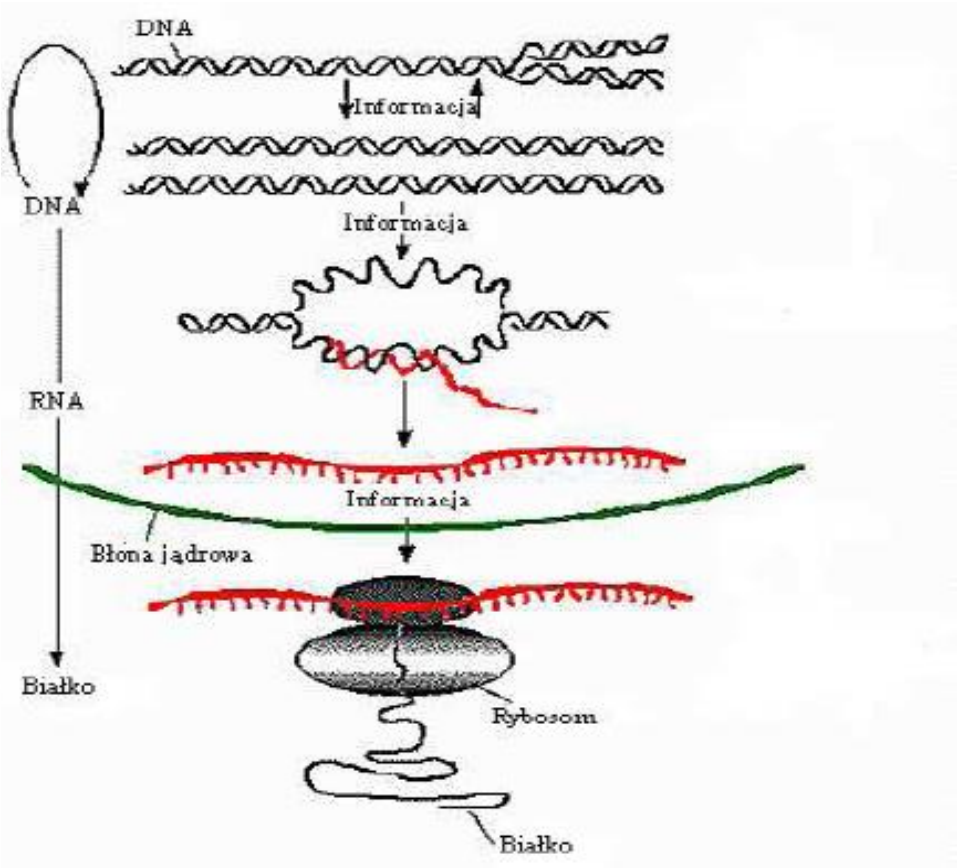
- Nucleic acids: DNA and RNA as genetic material
- Organization of genomes
- DNA metabolism: replication, repair, recombination
- Genetic variation
- Transcription and translation
- Regulation of gene expression
- The participation of genes in the functioning and differentiation of cells and in the development of organisms
- Recombinant DNA technology - practical use of molecular genetics

Dogma of molecular biology

Replication

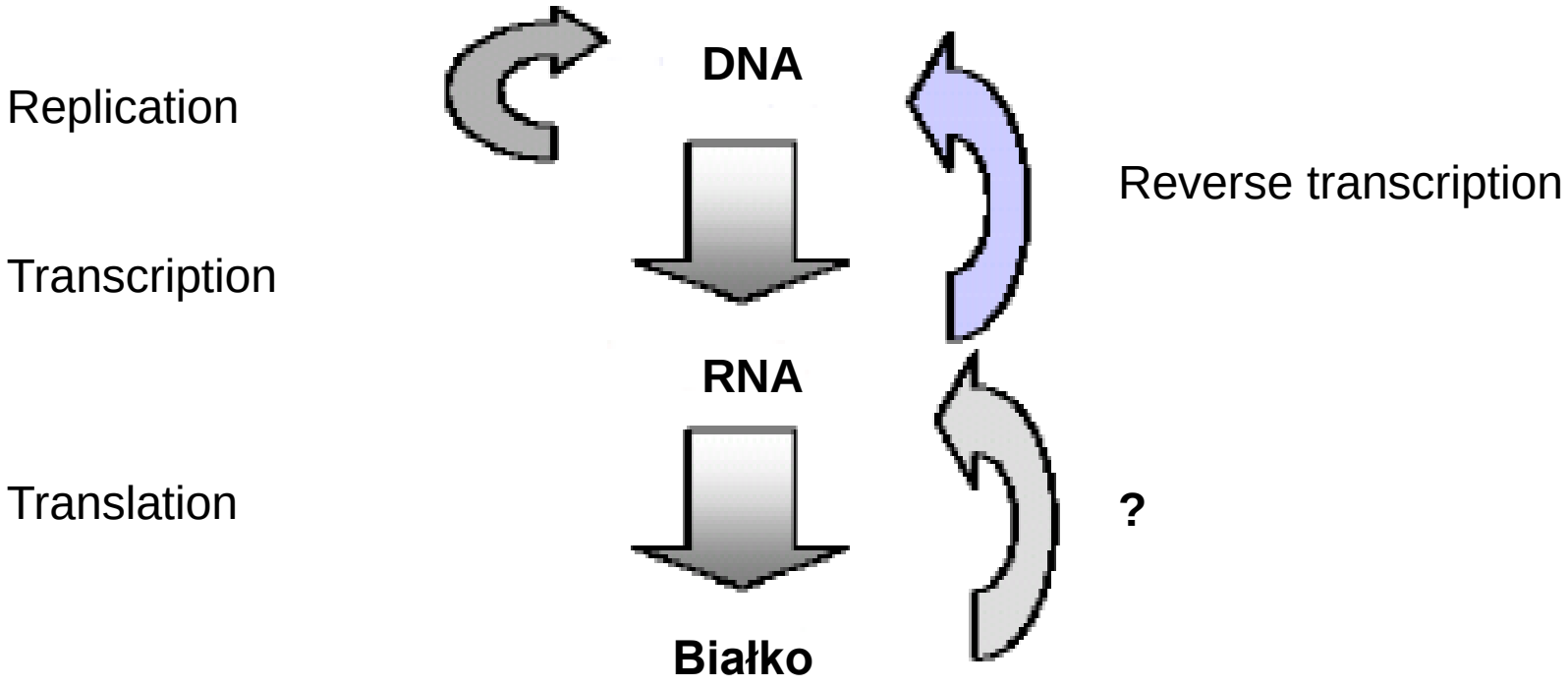
Transcription

Translation



The flow of genetic information takes place in the direction of DNA -> RNA-> protein

Upgrade of the molecular biology dogma



The flow of genetic information takes place in the direction of nucleic acid -> protein

Experimental evidence that DNA is a genetic material

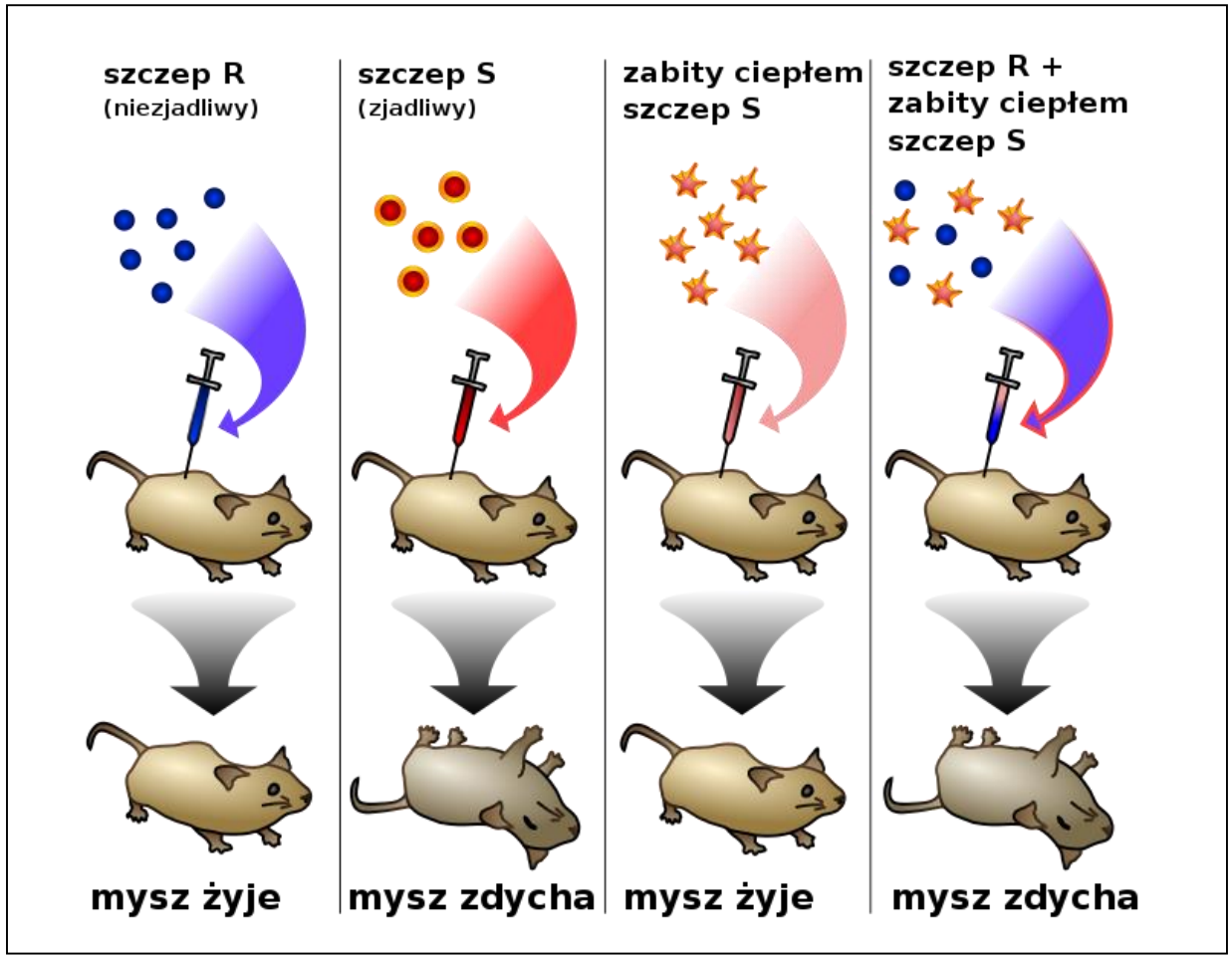
Experimental evidence

Frederick Griffith 1928

An experiment suggesting the possibility
transfer of genetic information
between bacteria

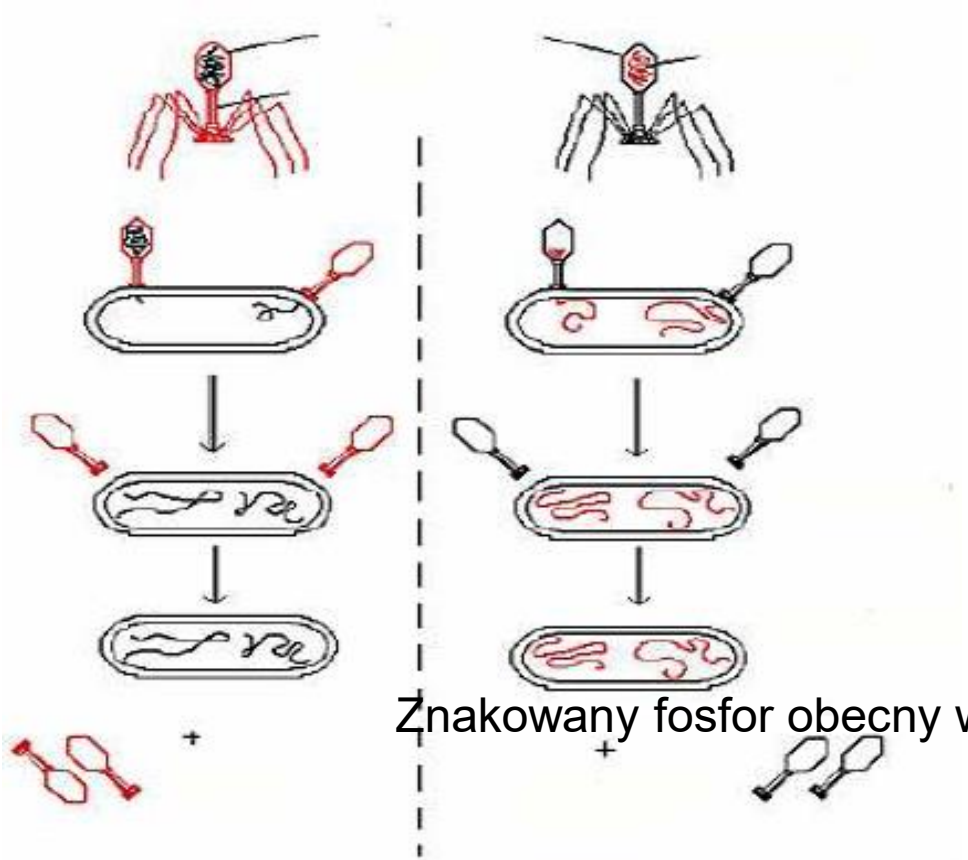
Experimental evidence

Patogeniczność Streptococcus pneumoniae



Experimental evidence

Experiment Hershey & Chasey'a



Brak znakowanej siarki w komórce

Znakowany fosfor obecny w komórce

1. Infekcja
2. Odłączenie otoczki
3. Wirowanie

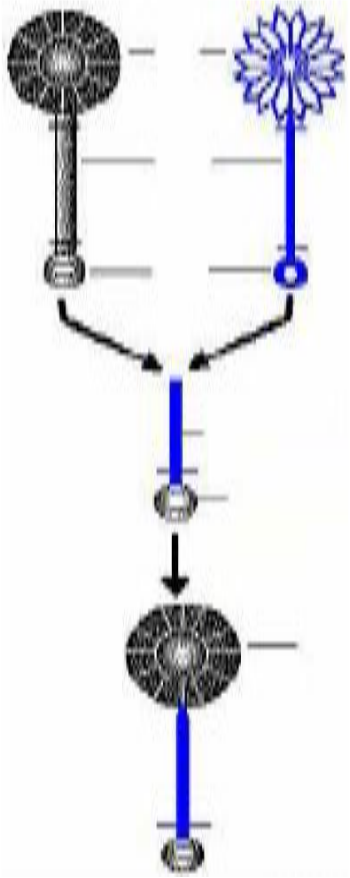
Znakowana siarka obecna w nadsączu

Brak znakowanego fosforu w nadsączu

Experimental evidence

Hammerling's experiment

A. mediterranea A. crenulata



1 – czapeczka
2 – łodyga
3 – podstawa (jądro)

A. crenulata

A. mediterranea

Wzrost

DNA

The basic concept of biology

DNA

A substance that carries genetic information.

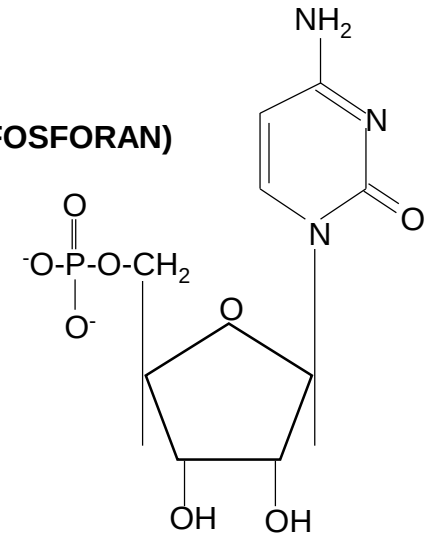
Beginning

- 1869 discovery DNA- J. F. Mischera
- 1944 Avery- DNA is a substance that carries genetic information
- 1953 J.D. Watsona i F. H. C. Cricka DNA double helix model
- 1959 Ochoa & Kronberg explain the mechanism of biological DNA and RNA synthesis
- 1967 Kornberg i Goulian- they synthesize virus DNA
- 1983 PCR- efficient duplication technique DNA

Nucleic Acids

Nucleotide of nucleic acids

All nucleotides have a similar structure, consist of:



Base (ZASADA)

Phosphate (FOSFORAN)

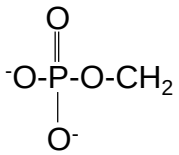
Sugar (CUKIER)

Nucleotides are monomeric units of nucleic acids

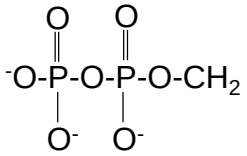
Nucleic Acids

Nucleotide phosphates (Fosforany nukleotydów)

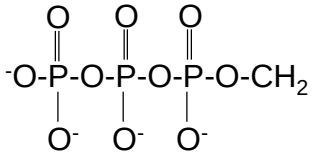
Phosphates are normally linked to the 5 'hydroxyl of ribose or deoxyribose residues. Mono-, di- and triphosphates are common



as in AMP



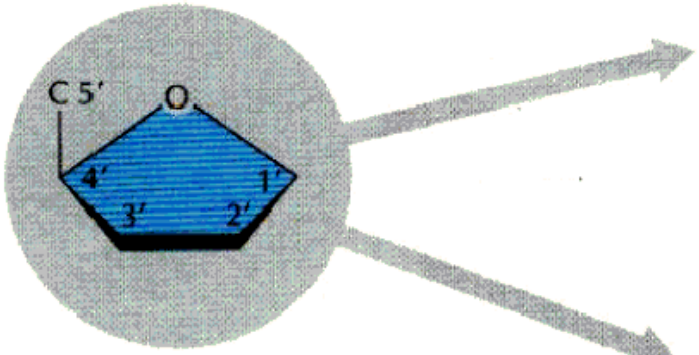
as in ADP



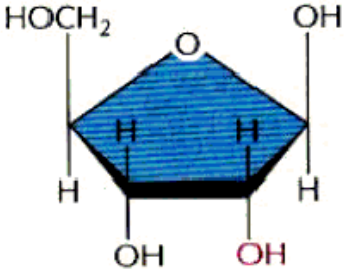
as in ATP

Nucleic Acids

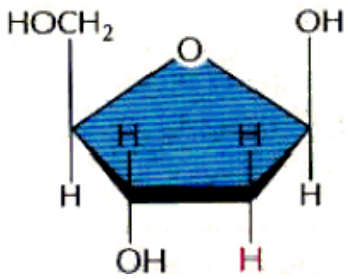
Sugars in nucleotides



PENTOZA
cukier pięciowęglowy



β-D-ryboza
występuje w kwasach
rybonukleinowych



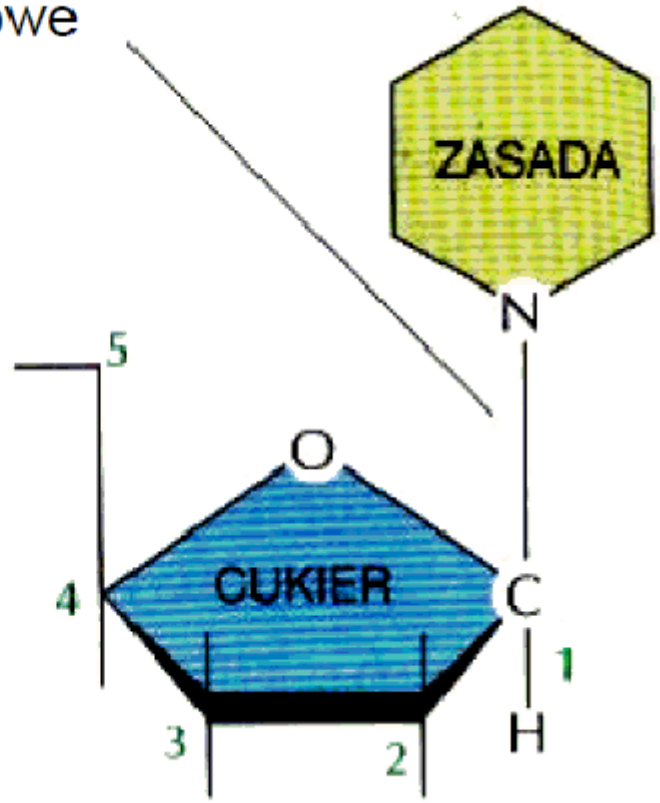
β-D-2-deoksyryboza
występuje w kwasach
deoksyrybonukleinowych

Nucleic Acids

Binding sugar- base

N-glycosidic bond

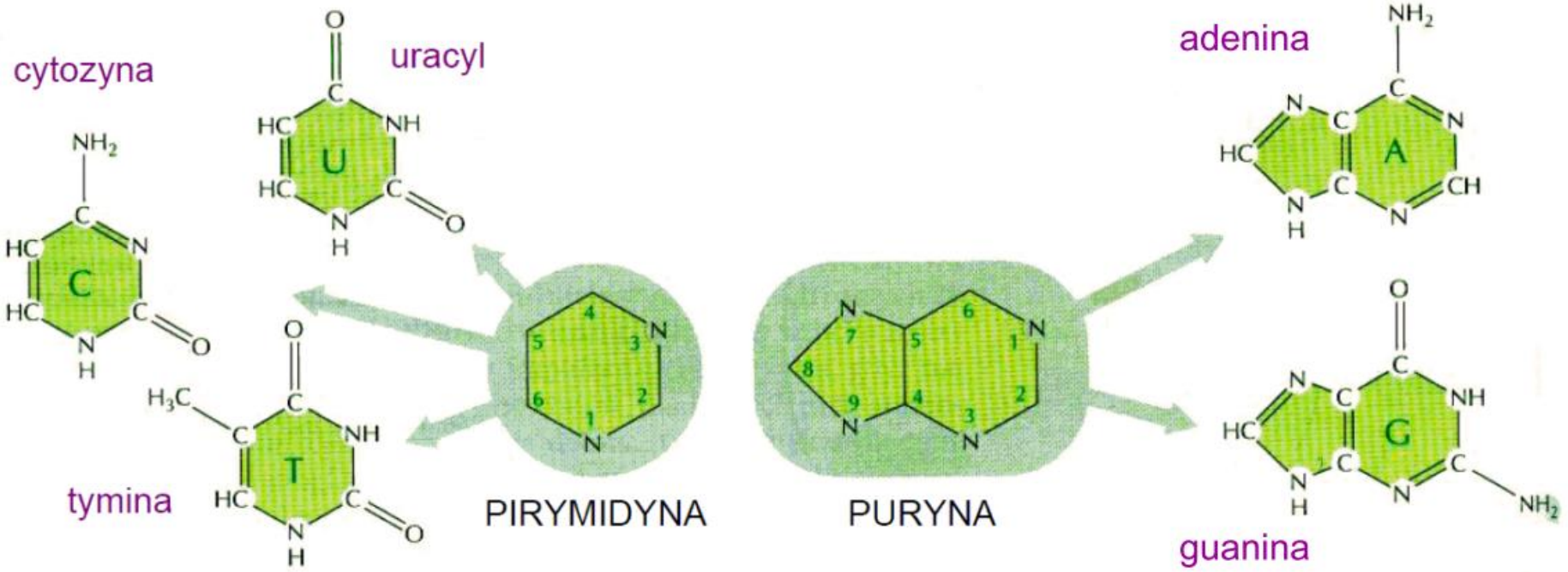
wiązanie *N*-glikozydowe



Nucleic Acids

Nucleobases, also known as *nitrogenous bases* or often simply *bases*

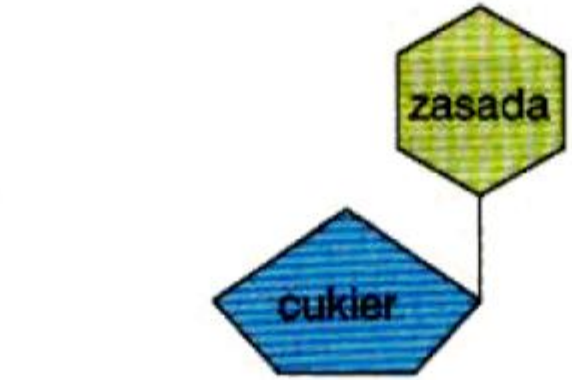
(Zasady azotowe nukleotydów)



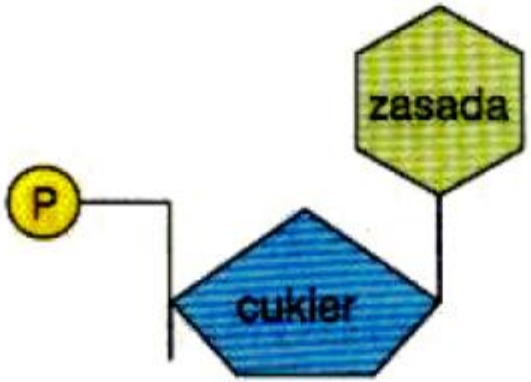
Nucleic Acids

Nomenclature

Base (ZASADA)	nucleoside (NUKLEOZYD)
adenina	adenozyna
guanina	guanozyna
cytozyna	cytydyna
tymina	tymidyna
uracyl	urydyna



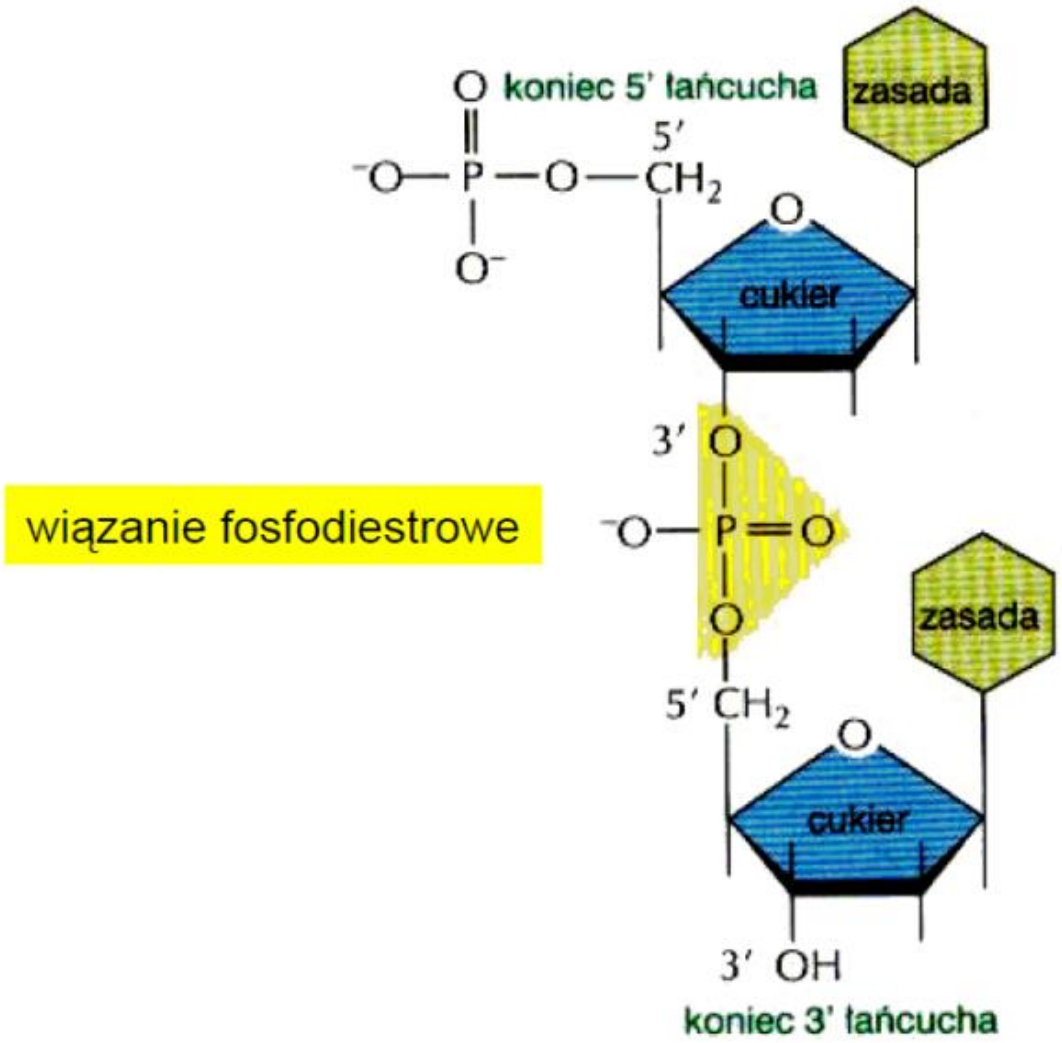
ZASADA + CUKIER = NUKLEOZYD



ZASADA + CUKIER + FOSFORAN = NUKLEOTYD

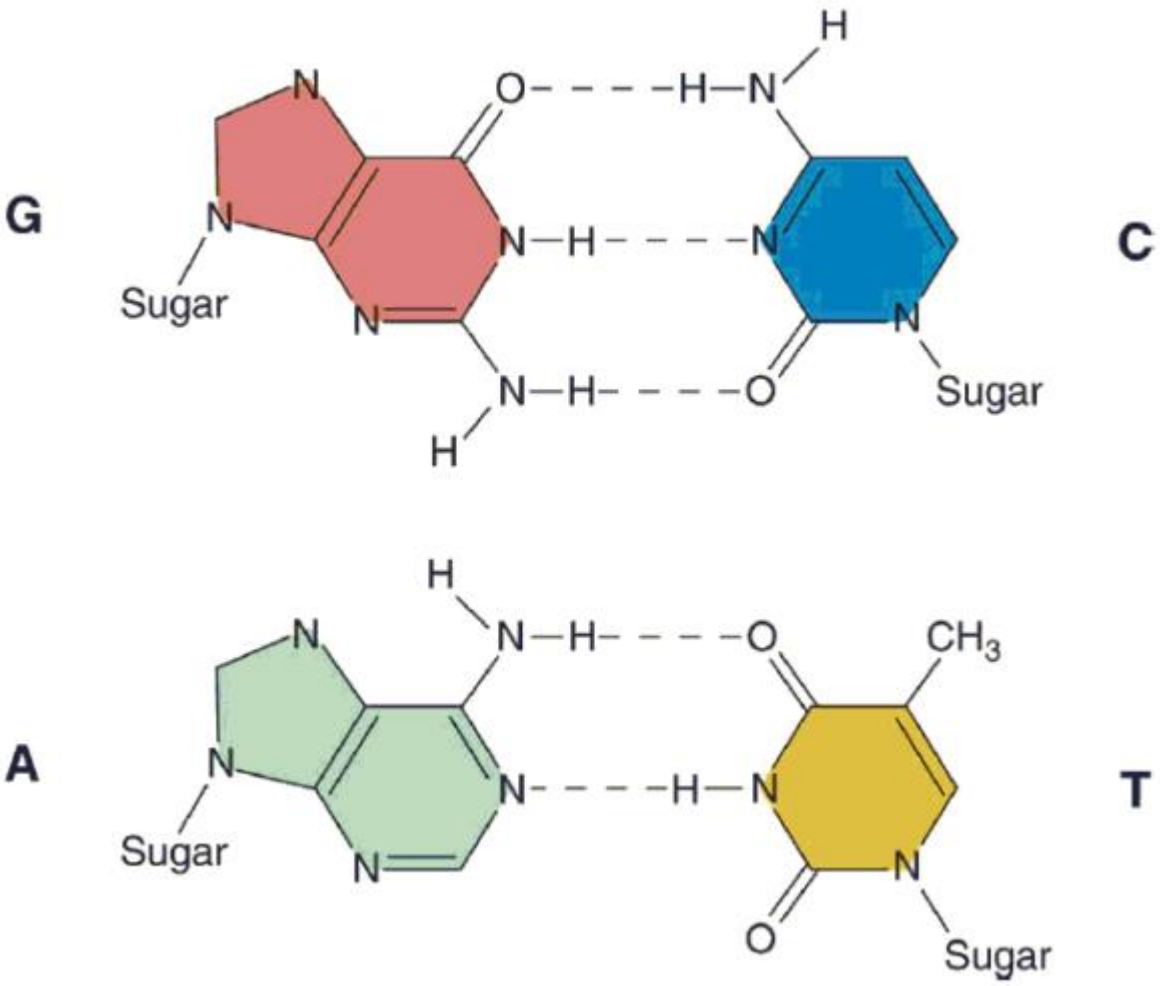
Nucleic Acids

Combination of nucleotides



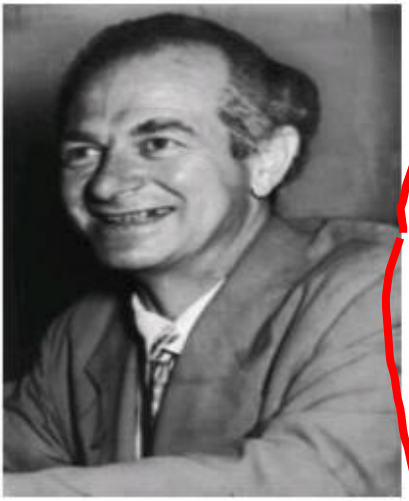
Nucleic Acids

Nitrogenous base pairing (Parowanie zasad)



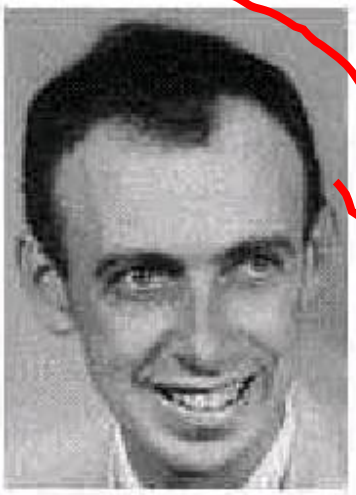
Nucleic Acids

DNA structure



Linus Pauling

Francis Crick



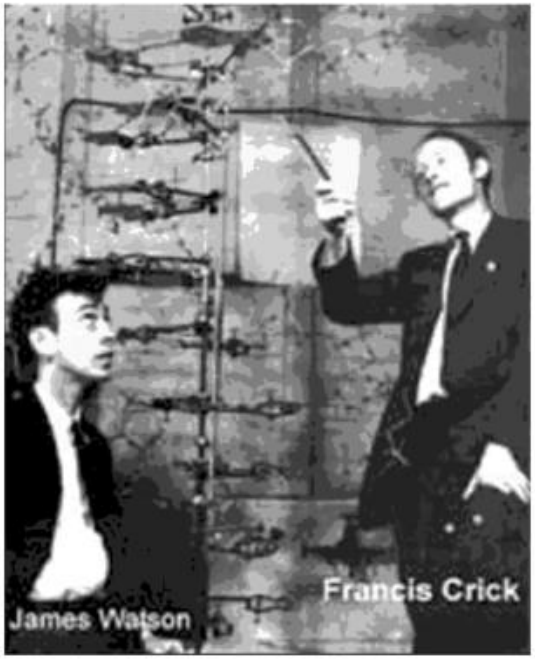
James Watson

Maurice Wilkins

Rosalind Franklin

Nucleic Acids

DNA structure



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**, 169 (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. 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 interwined 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

Nucleic Acids

Nobel Prize AD 1962

Francis Crick

James Watson

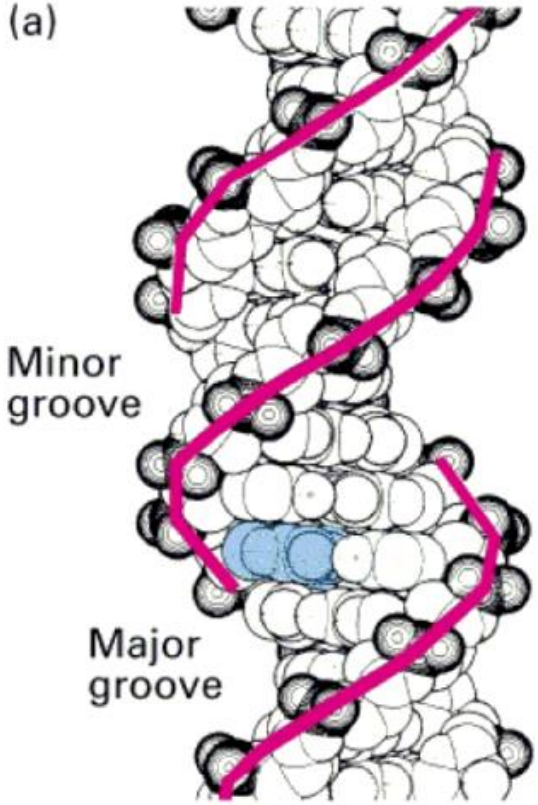
Maurice Wilkins

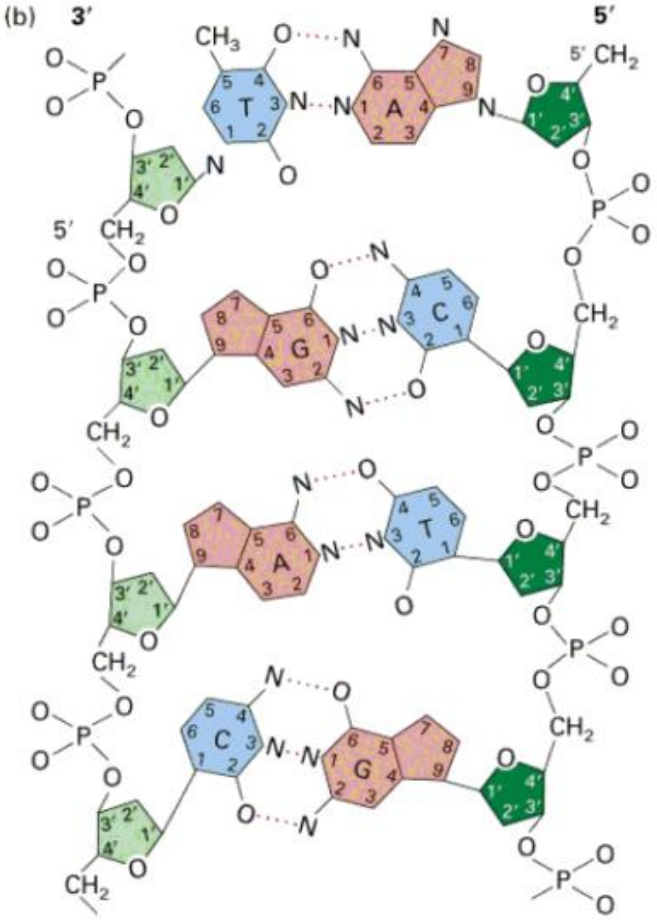
Rosalind Franklin

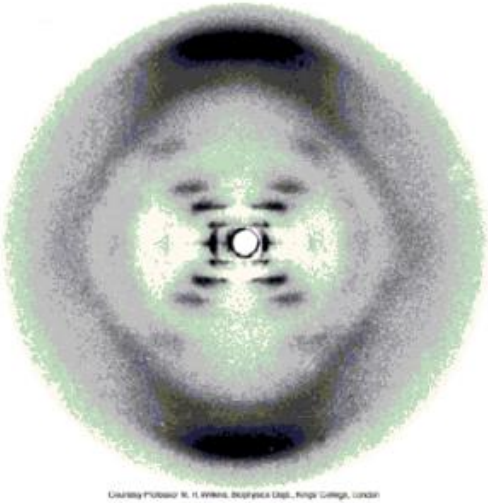
1958

Nucleic Acids

DNA structure



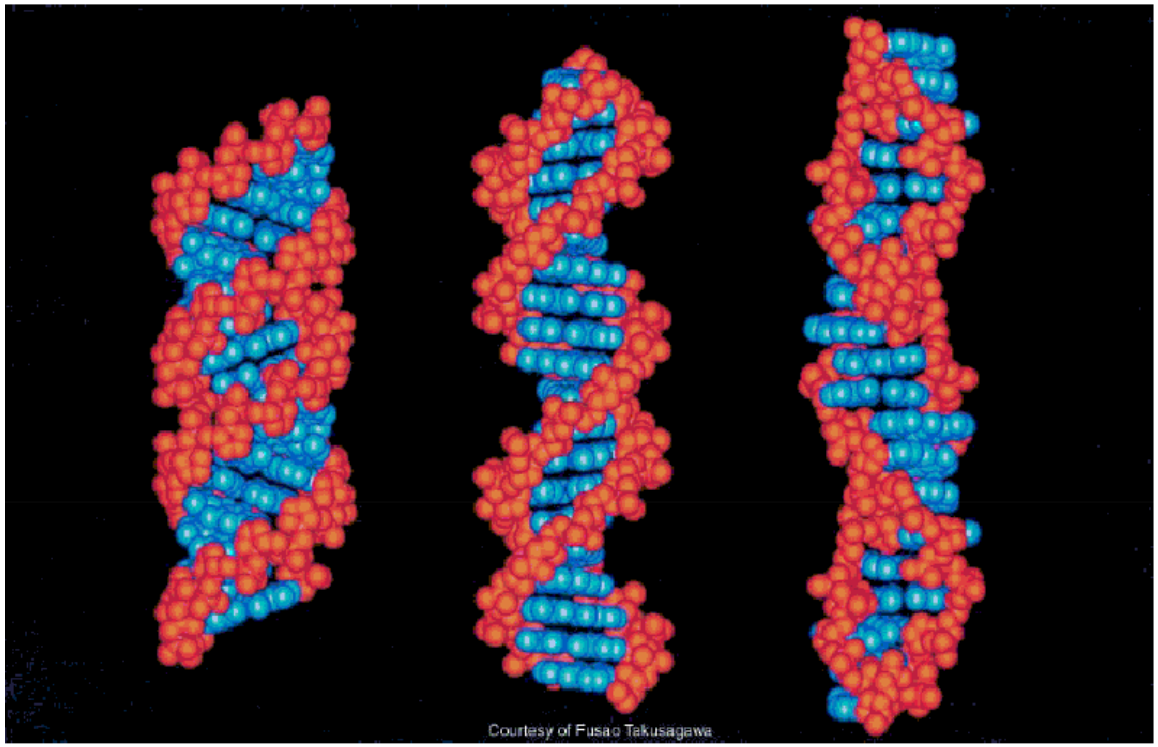




Zdjecie 51, B-DNA, 2 Maj 1952, Franklin, Gosling

Nucleic Acids

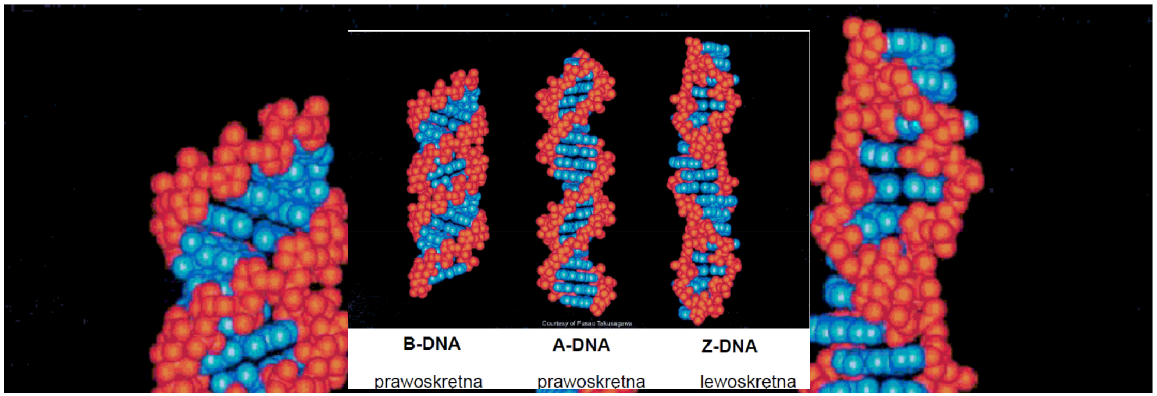
Different forms of DNA helix



B-DNA	A-DNA	Z-DNA
prawoskrętna	prawoskrętna	lewoskrętna

Nucleic Acids

Different forms of DNA helix



Typ helisy	prawoskrętna	prawoskrętna	lewoskrętna
Średnica helisy (nm)	2.37	2.55	1.84
Przyrost na pz (nm)	0.34	0.29	0.37
Liczba zasad na skręt	10	11	12
Topologia większego rowka	szeroki, głęboki	wąski, głęboki	płaski
Topologia mniejszego rowka	wąski, płytki	szeroki, płytki	wąski, głęboki

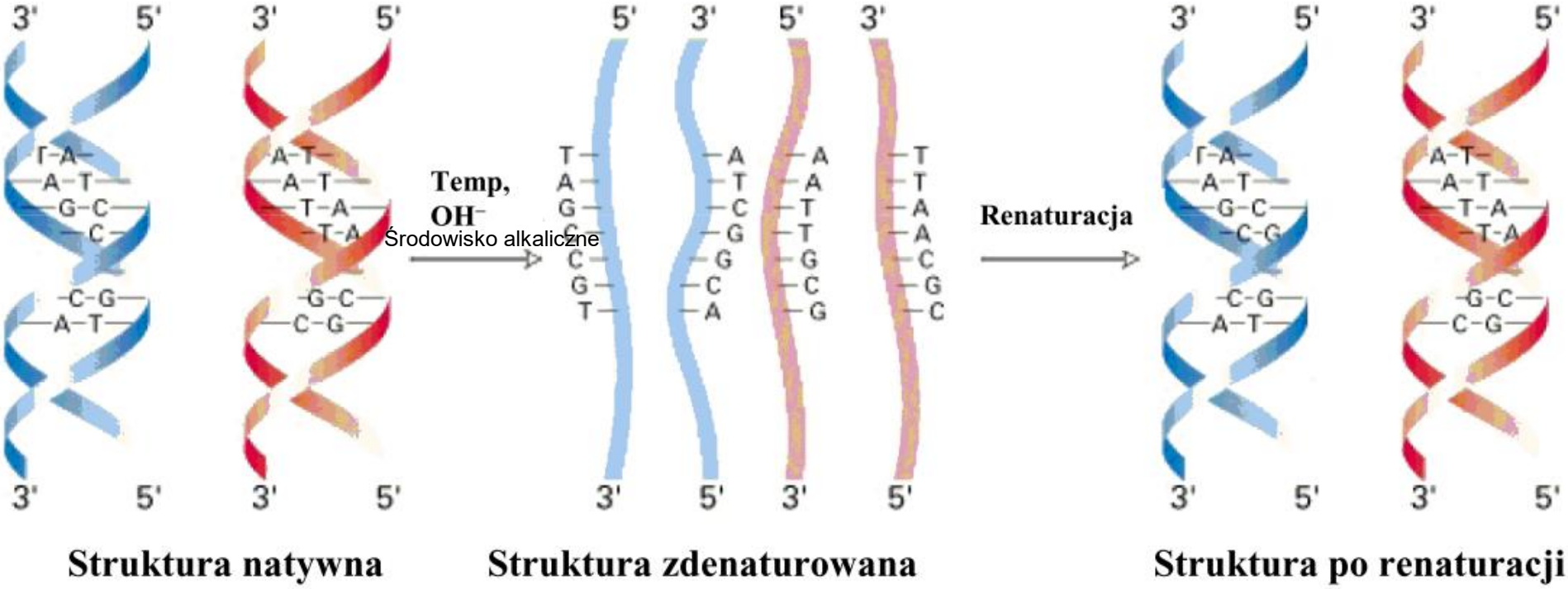
Inne formy: B', C, C', C'', D, E i T

prawoskrętna prawoskrętna lewoskrętna

Nucleic Acids

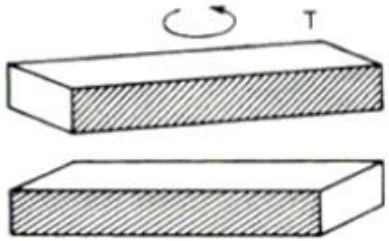
In DNA, reversible thread separation can occur

(W DNA może dojść do odwracalnego rozdzielenia nici)

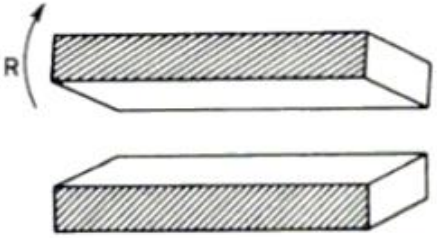


Nucleic Acids

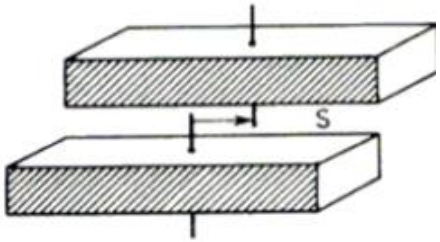
Parameters used to describe the local structure of the double helix



T (ang. Twist)= (skręt): Torsion: Specifies the torsion angle of the planes relative to each other along the longitudinal axis of the helix (*określa kąt skręcenia w stosunku do siebie płaszczyzn wzdłuż podłużnej osi helisy*)



R (ang. Roll)= (obrót): defines the angle of inclination of adjacent planes relative to the longitudinal axis of the helix (*określa kąt rozchylenia sąsiadujących płaszczyzn względem osi podłużnej helisy*)



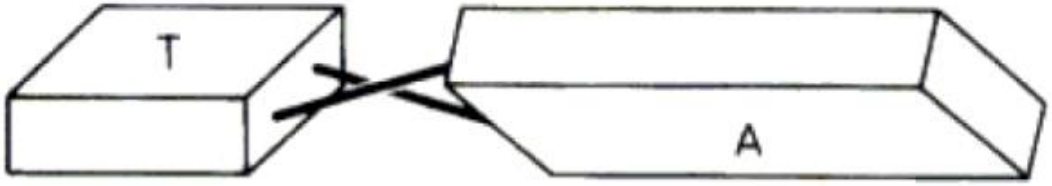
S (ang. Slide)= (przesunięcie): wskazuje wielkość przesunięcia płaszczyzn zasad w warstwach w stosunku do siebie, względem osi podłużnej helisy (*indicates the amount of shift of base planes in layers relative to each other, relative to the longitudinal axis of the helix*)

Nucleic Acids

Propeller twist

Propeller twist (*Skręcenie śmigłowe*) base planes relative to each other caused by the pursuit of maximum strong hydrophobic interactions in adjacent layers

(*Skręcenie płaszczyzn zasad w stosunku do siebie wywołane dążeniem do maksymalnie silnych oddziaływań hydrofobowych w sąsiadujących warstwach*)



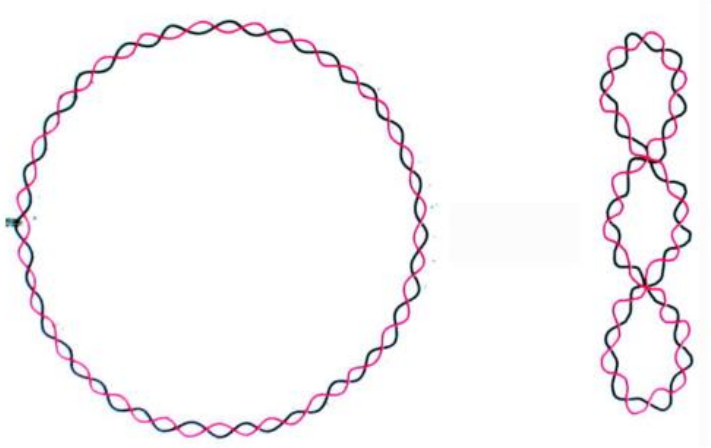
Nucleic Acids

Super helical forms of DNA curled circular spaces of DNA molecules
(Superhelikalne formy DNA zwinięte przestrzenie koliste cząsteczek DNA)

Lk (ang. linking number)- *liczba opleceń*

Lk=Wr+Tw

Wr (ang. writhe)- *liczba zwojów wskazuje ile razy oś podwójnej helisy owija (krzyżuje) się ze sobą*
Tw (ang. twist)- *liczba skrętów- wskazuje ile razy kolisty DNA został skręcony wokół osi helisy*



Nucleic Acids

Liczba zasad przypadająca na skok podwójnej spirali (h)

h= N/Lk-Wr lub h=N/Tw

N- liczba zasad w cząsteczce DNA
Lk- liczba opleceń
Wr- Liczba zwojów