## Molecular Biology II

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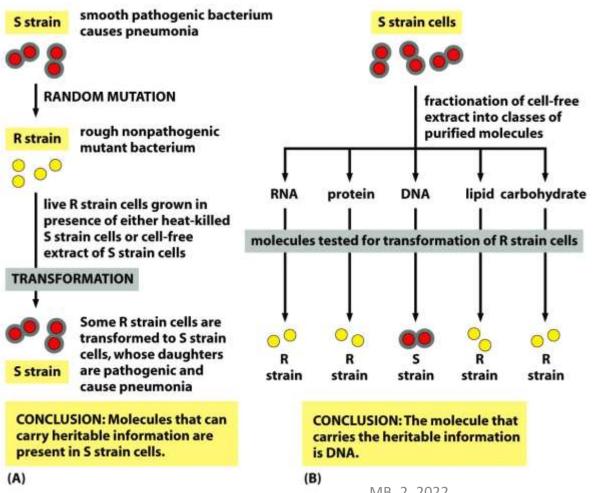
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# The structure and function of biopolymers during the transitions of genetic information

Daniel Renčiuk

#### Genetic information is coded by DNA

The experiment combining two strains of Streptococcus pneumoniae bacteria.



O. Averv C. MacLeod

M.McCarty

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

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

BY OSWALD T. AVERY, M.D., COLIN M. MAGLEOD, M.D., AND MACLYN McCARTY,\* M.D.

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

(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.

#### Nuclein

- Nuclein acidic substance rich in nitrogen and phosphorus
- J. F. Miescher 1864
- Isolated from blood of wounded patients and cleaved by pepsin (proteolytic enzyme)



## Roles of genetic material

**Genotype** – storage of genetic information and its transition to the offspring

**Phenotype** – expression of genetic information to particular properties of an individual

**Evolutionary role** – adaptation of an organism/species to the environment through the changes in genetic information

## Terminology

**Gene** – several "definitions" depending on the point of view:

classic genetics (Mendel) – elementary unit of hereditary genetic information molecular genetics – part of DNA coding for RNA (and as a consequence coding for some property of the individual)

structural genes coding for mRNA/protein (+ regulatory regions) genes coding for functional RNA (miRNA, ...)

strict – structural gene – part of DNA that codes for protein sequence

Allele – particular variant of the respective gene

**Genome** – complete DNA of organism (molecular) x complete genetic information of organism x sum of genes (classic)

**Genotype** – the combination of particular alleles of all genes in individual

**Phenotype** – the sum of actual individual properties (as a result of expression of particular genotype in the respective environment)

**Genophore** – the carrier of genetic information, usually a molecule of DNA (often used for bacteria)

## Information biopolymers

#### Deoxyribonucleic acid (DNA)

- linear heteropolymer composed from 2-deoxyribonucleotides connected by phosphodiester bonds
- usually as a stable and resistant double helix
- serves as a storage of genetic information, as a template for its reproduction (replication) and as a template for the expression of genetic information to the phenotype (transcription)

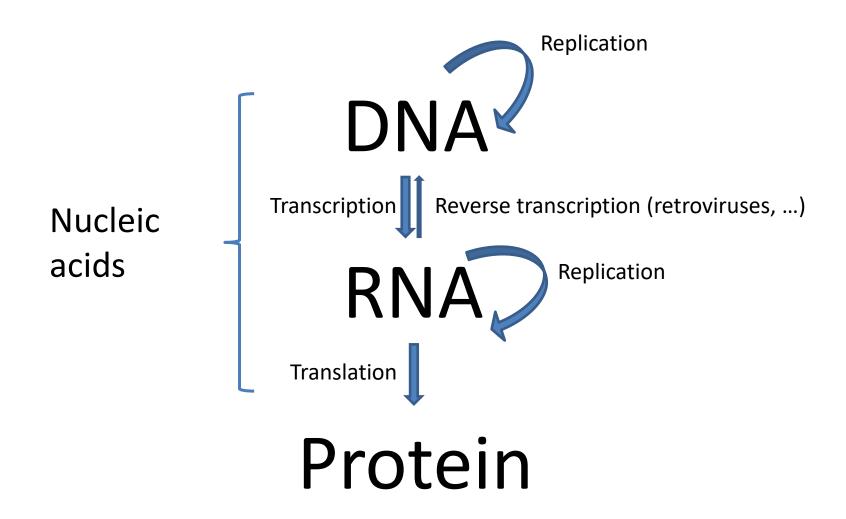
#### Ribonucleic acid (RNA)

- linear heteropolymer composed from ribonucleotides connected by phosphodiester bonds
- usually as a single-stranded structure of variable length, structure and reactivity
- many functions depending on type of RNA

#### **Protein**

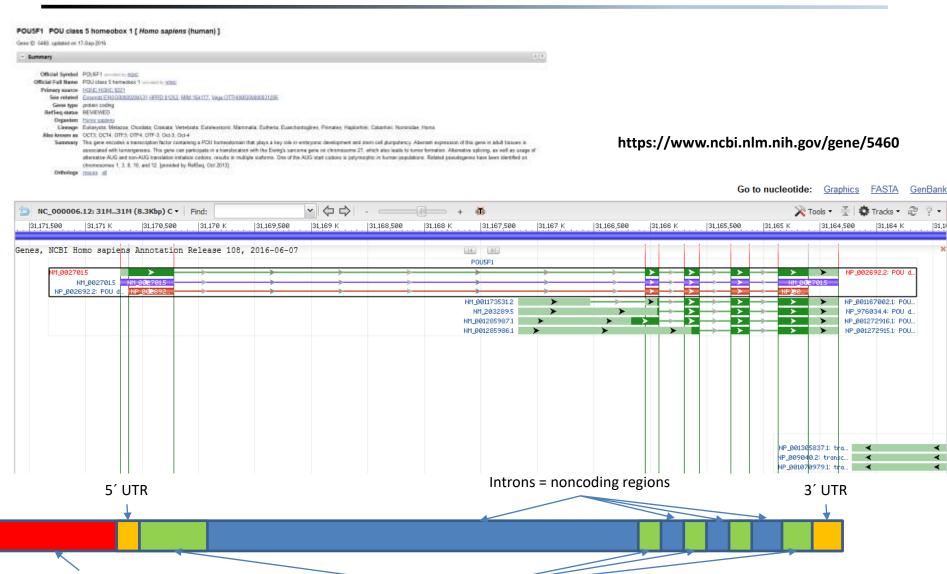
- linear heteropolymer composed from 20 (21) amino acids connected by peptide bonds
- highly variable structures, properties and functions

#### Central dogma of molecular biology



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#### Gene

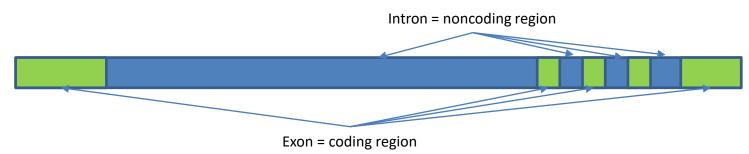


Regulatory regions
Promoter + enhancers

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#### Genes

Most **eukaryotic** genes contain **introns**, that are transcribed into primary RNA transcript and introns are consecutively removed by **splicing** process on **spliceosome** to form final **mRNA**.

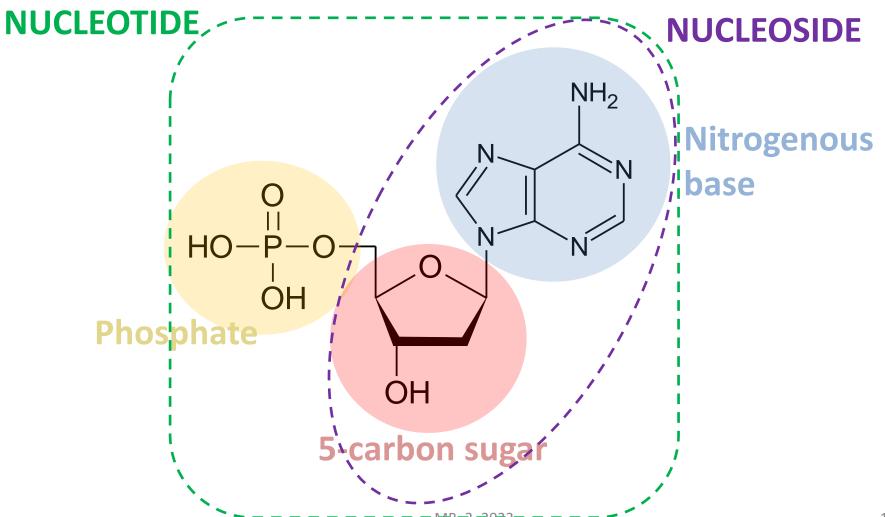


**Prokaryotic** genes do not contain **introns** and they are directly transcribed into mRNA that serves as a template for translation into protein.

Exon = coding region

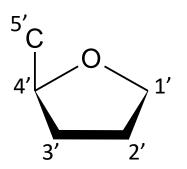
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#### Nucleic acids

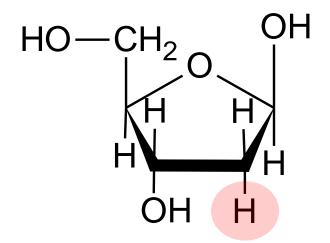


## 5-carbon sugar - pentose

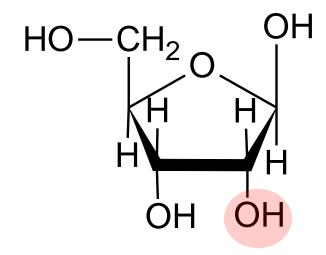
DNA



RNA



β-D-2-deoxyribose

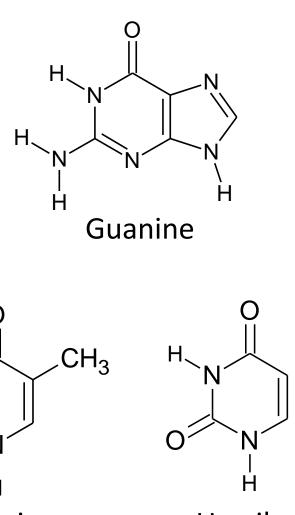


β-D-ribose

12

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#### Base



Pyrimidine

H Cytosine

Thymine MB\_2\_2022

Uracil

#### Nucleic acid nomenclature

	Base	Nucleoside	Nucleotide NTP
G	Guanine	Guanosine	Guanosine triphosphate
Α	Adenine	Adenosine	Adenosine triphosphate
Т	Thymine	Thymidine	Tymidine triphosphate
С	Cytosine	Cytidine	Cytidine triphosphate
U	Uracil	Uridine	Uridine triphosphate

DNA – prefix deoxy-

NMP = monophosphate

NDP = diphosphate

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#### Modified bases

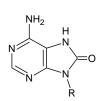
#### tRNA

HN O N R

pseudouracil

dihydrouracil

#### Oxidative damage



H<sub>2</sub>N N R

8-oxo adenine

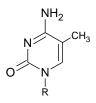
8-oxo guanine

#### Metabolism

# NH NH NH

xanthine

## H NH NH NH



**Epigenetics** 

hypoxanthine i

inosine

MB\_2\_2025-methyl cytosine

5-hydroxymethyl cytosine 15

## Conformation of N-glycosidic bond

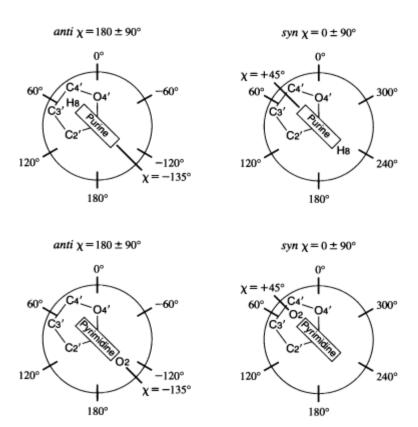
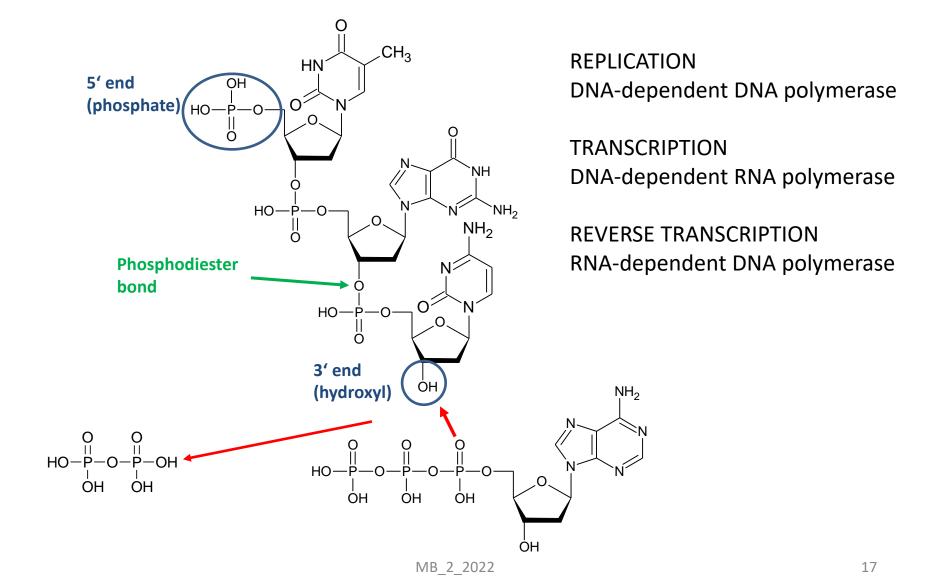


Figure 2-8
The glycosidic torsion angle  $\chi$  is defined by O4'–C1'–N9–C4 for purines and O4'–C1'–N1–C2 for pyrimidines. When  $\chi=0^\circ$  the O4'–C1' is eclipsed by the N9–C4 bond in purines and by the N1–C2 bond in pyrimidines. The syn conformations correspond to  $0^\circ \pm 90^\circ$ ; anti conformations correspond to  $180^\circ \pm 90^\circ$ . In nucleotides steric hindrance limits the conformations actually found to a much narrower range of angles that depend on sugar pucker and base. The syn conformations are usually found with  $\chi=45^\circ \pm 45^\circ$ , anti-

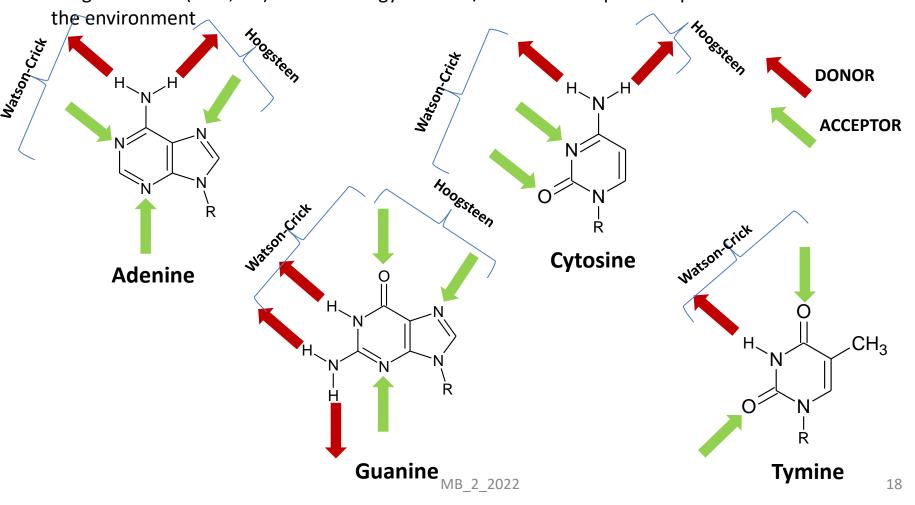
MB conformations are usually found with  $\chi = 45^{\circ} \pm 45^{\circ}$ ; anti-conformations are usually found with  $\chi = -135^{\circ} \pm 45^{\circ}$ .

#### Formation of sugar-phosphate backbone

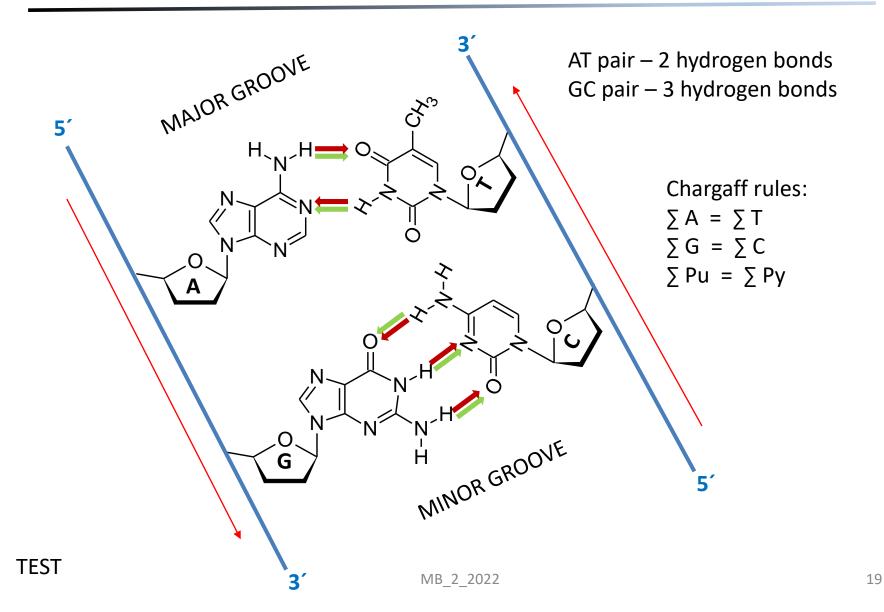


## Base reactivity – hydrogen bonds

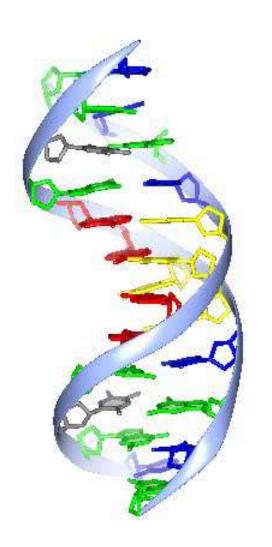
**Hydrogen bond** – weak electrostatic interaction of two polar groups – one covalently binds hydrogen (DONOR – usually -N-H a -O-H); the second (ACCEPTOR) is usually N or O Length:  $\sim 2.8 \text{ A} (2 - 3.4 \text{ A})$  Energy: < 1 kcal/mol both depend on particular atoms and on

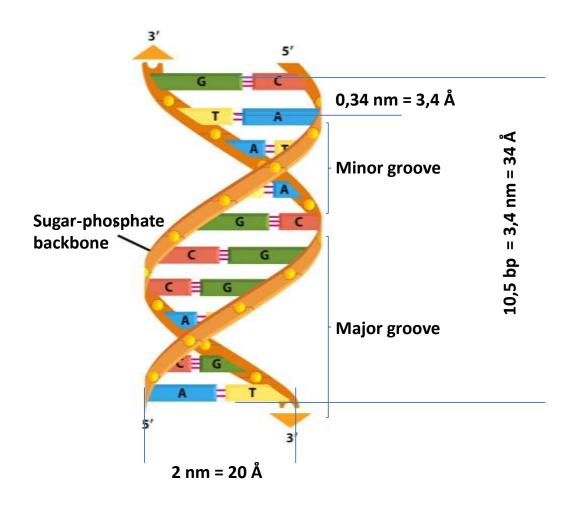


## Watson-Crick base pairing



#### DNA double helix





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#### DNA double helix

- two molecules (strands) of DNA
- the helix is right-handed
- the strands are **antiparallel** their 5´-3´ direction is opposite in the context of double helix direction
- similar content of purines and pyrimidines; content of A = T, G = C
   (Chargaff rules)
- result the strands are complementary i.e. according to the Watson-Crick base pairing rules we can predict the sequence of one strand according to the sequence of the other
- on average the double helix contains **10,5 base pairs** per turn of the helix, which is about **3,4 nm** in length

#### DNA structure –Watson and Crick model



F. Crick

J.D. Watson



M. Wilkins



R. Franklin



E. Chargaff

m =100 April 25, 1953

NATURE

apartment, and to Dr. G. E. B. Denous and the is a residue on each chain every 2-4 A, in the z-direcsoptain and officers of H.R.S. Discovery II for their part in making the observations,

Dots, F. S., timed, H., and Jones, W., Phil. Moc., 45, 144 Chapter Storms, M. S., Nov., Not. Roy. Ame. Ass., Souplan. Supp.,

\* Now Sep. W. S., Woods Stoke Papers in Phys. Strengs, Rosser, 42 PERSONAL S. W., LAWIS Man, Agreen, Print, (Maddeline), \$1511 (1960).

#### MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

Will wish to suggest a structure for the subof decayabase nurses said (D.N.A.). This ateuttime has soved foutures which nee of comulerable

hobigical interest. A attracture for michig acid has strondy been A attacher for invited tend has strongly been proposed by Pauling and Coney. They kindly made that macroscopi available to us in advance of publication. Their model courses of three interewassi chains, with the phosphates near the filter axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two masons: (I) We believe that the material which gives the X escribingment is the suit, not the fero seal. Without the acidio hydrogen utoms it is not clear what forces would hold the structure together, especially as the negatively observed phrephates near the axis will repai cosh other. (2) Some of the van der Weals distance oppose to be too small,

Another three chain structure has also been suggoded by Fower (or the green). In his model the phospharps are on the outside and the bases on the pairts, linked tegration by hydrogen breaks. This structure as described is purber ell-defined, and for this reason we shall not comment.

We wish to put forward a radically different structure for the sale of designificar matrix acut. This structure has two belieur cloning each coded round the same axis (see diagram). We have made the award chemical assumptions, amonely, that each closin consists of phosphate dioster groups poining 5-2-dooryethefurances residues with 3',5' limitages. The two chains that nut their based are related by a ifyed perpendicular to the time axis. Both chains follow righthanded believe, but owing to the dyad the sequences of the mone in the two chains run in opposite directions. Each chain benefy escention Furhe haves are on the inside of the helix and the phosphates on the putside. The surdigaration of the sugar and the atoms near it is close to Forley's 'standard configuration', the ought being roughly perpendi-

tion. We have assumed an angle of 50° between adjacent residues in the same chara, 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 sais is 10 A. As the pho-

the outside, estime have The structure is no opis rather high. At for report the bases to til

become more emport. The moved feature or in which the two sha purine and pyrimatine b are perpendicular to th tugether in pairs, a sing chair, so that the two 3-po-ordinates. Our of the other a pyrinodo hydrogen bonds are m I to pyrimidme posipaymentine position &

If it is succeed the etrictum in the mon chian in, with the he figurations) it is foun-(parine) with thymine

Franklin's X-ray photograph shows DNA's 'B'-form (1952)

In other words, if an a a pair, on either chain, then on these assumptions the other member must be thymins; similarly for guanino and optioniss. The sequence of house on a single chain does not uppear to be rescricted in any way. However, if only sportly paint of bosos can be formed, it follows that if the sequence of bosos on one chain is given, then the sequence on the other chain is automatically determined

It has been found experimentally'd that the ratio of the amounts of adenine to thymins, and the ratio of guarante to cytosine, are obways very close to unity for discayribose muchos and,

It is perhably impossible to build this atputtore with a ribous sugar in place of the decayyibous, as the extra expensations would make too close a vander Weals contact.

The proviously published X-ray data<sup>4,4</sup> on decay ribuse markets acid are mentileless for a rigorous tool of our structure. So for as we can tell, it is roughly compatible with the experimental data, but it must be represented as improved until it has been therited against more crack results. Some of these are given in the following communications. We were not neverof the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and succeschemical arguments.

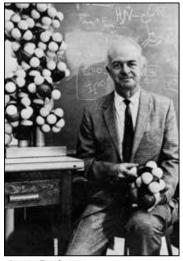
It has not samped our notice that the specific pairing we have postulated immediately suggests a passible copying mechanism for the genetic material

Full donals of the structure, unlading the conditions assumed in building it, together with a set of en-ordinator for the atoms, will be published

We are much indibted to Dr. Jerry Donohus for sensiant advers and criticism, especially on interatomic distances. We have slie been atimplated 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 go-workers as

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## DNA structure – Pauling model



Linus Pauling

A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

BY LINUS PAULING AND ROBERT B. COREY

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,\* CALIFORNIA INSTITUTE OF TECHNOLOGY

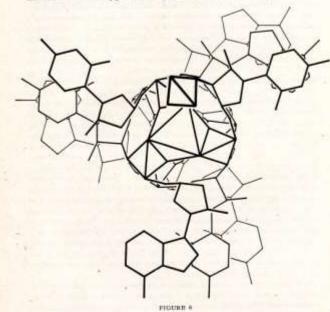
Communicated December 31, 1952

The nucleic acids, as constituents of living organisms, are comparable in importance to the proteins. There is evidence that they are involved in the processes of cell division and growth, that they participate in the transmission of hereditary characters, and that they are important constituents of viruses. An understanding of the molecular structure of the nucleic acids should be of value in the effort to understand the fundamental phenomena of life.

CHEMISTRY: PAULING AND COREY

Proc. N. A. S.

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-



Plan of the nucleic 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  $S_dO_b$ , 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

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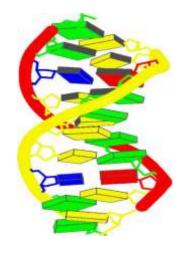
#### Various types of double helix

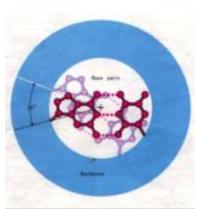
#### **B-DNA**

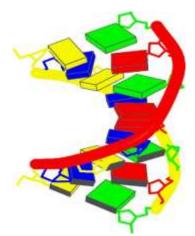
• DNA in water/salt soulutions

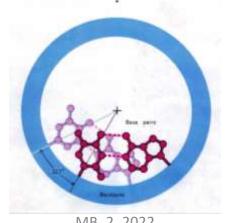
#### **A-DNA**

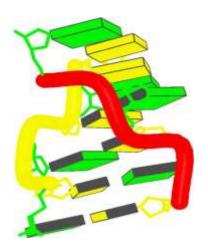
- DNA in crowding solutions CpG sequences in crowding conditions
- RNA







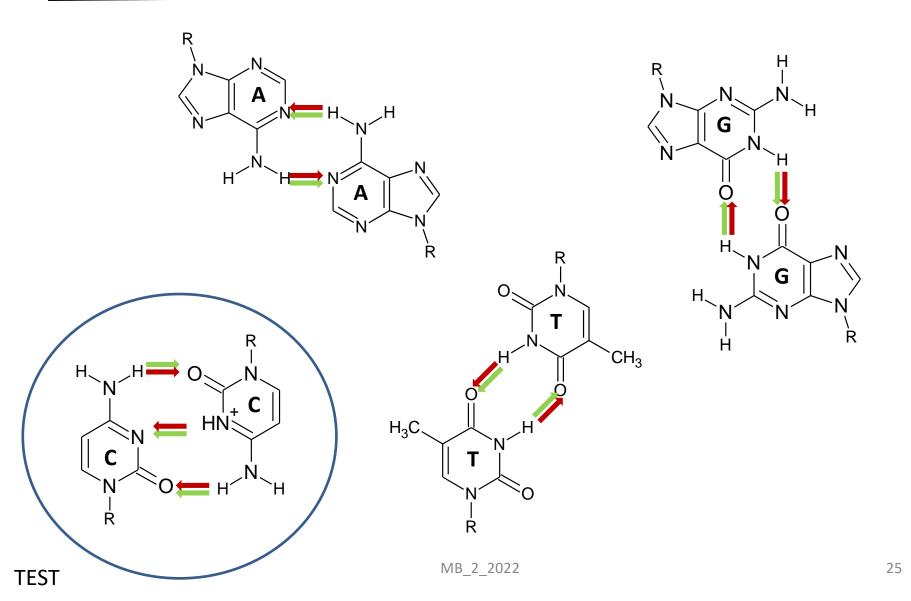




**Z-DNA** 

Left handed Zig-zag step

## Reversed Watson-Crick pairing



#### Base protonation

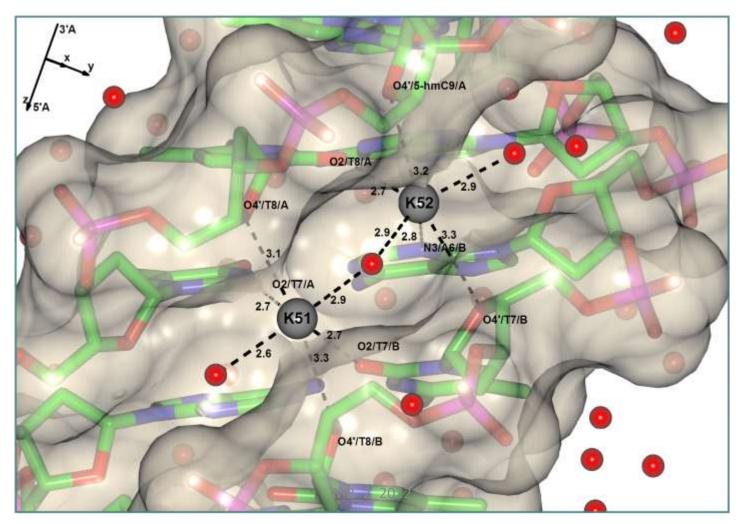
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Cytidine

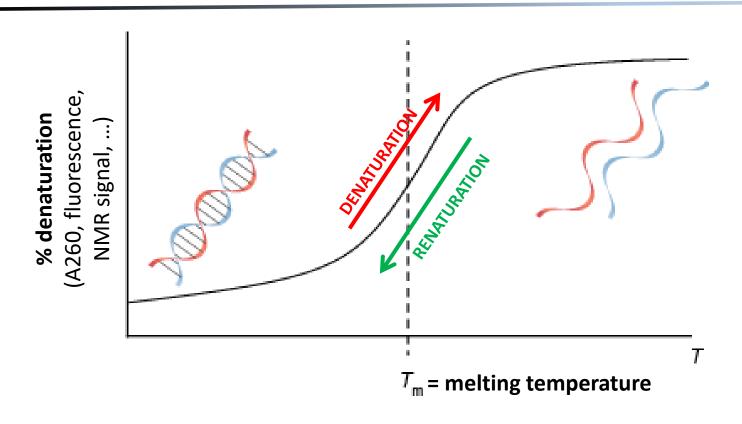
- •base protonation might alter the base reactivity
- free bases have pK far from physiological
- •pK of bases in DNA might be closer to pH 7.4
- cytosine in C<sub>n</sub> sequences
   has pK~7 cytosine i-motif

#### DNA double helix x ions / water

- phosphates in DNA backbone are negatively charged repulsion
- this is compensated by interaction with ions (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, ...) or water (H-H bonds)



## Stability of DNA double helix



hydrogen bonds

$$AT = 2 \times GC = 3$$

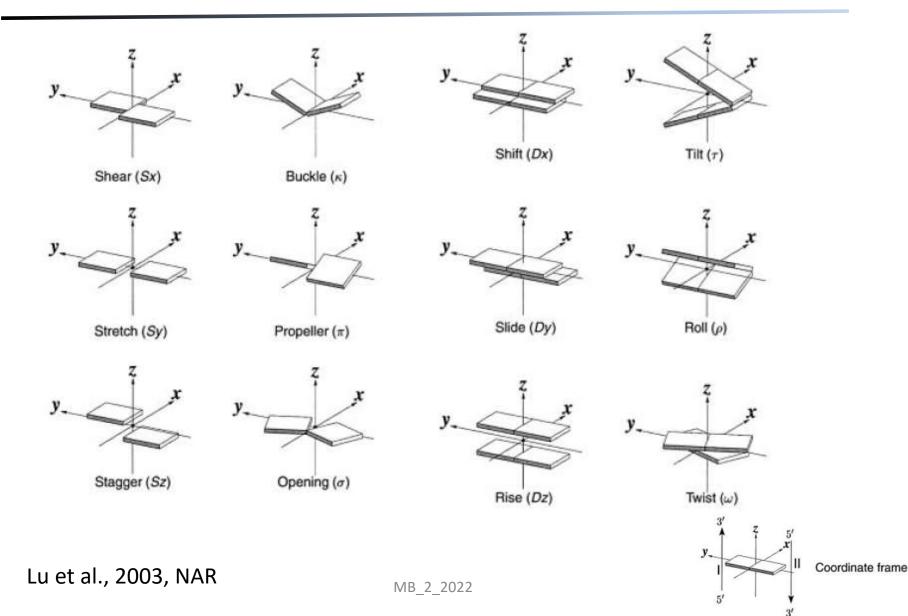
base stacking

various

• repulsion of backbone phosphates Mg<sup>2+</sup>>Na<sup>+</sup>

Tm increases with GC and length Tm increases with length and ions Tm increases with ions

#### Base-pair parameters in double helix

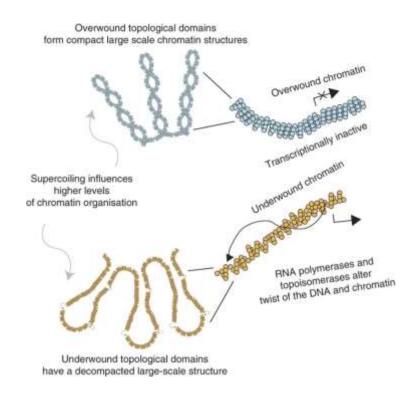


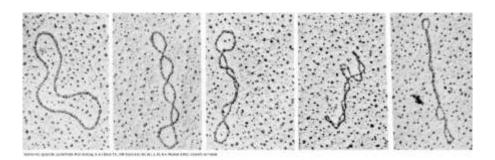
## Types of nucleic acids

- linear (human chromosome) x circular (bacterial genome)
- single-stranded (most RNAs) x double-stranded (human DNA)

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## Superhelicity





Superhelicity happen mostly as a result of transition of polymerase complex and unwinding of DNA (helicase, ...) during replication and transcription.

#### **Topoisomerases**

- Enzymes that relax the superhelicity
- Topo I works on 1 DNA strand
- Topo II works on 2-strand DNA

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#### Reactivity of bases with amino acids

Double-stranded NA: Interaction of Hoogsteen side with amino acid in major groove.

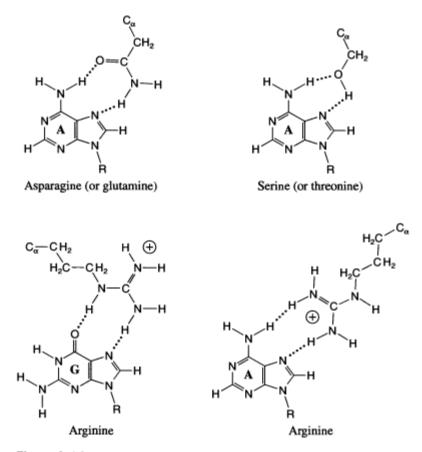


Figure 2-16
Interactions involving two hydrogen bonds between amino acids and bases that can occur through the major groove of a double helix.

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#### Reactivity of bases with amino acids

Single-stranded NA: Interaction of Watson-Crick side with amino acid.

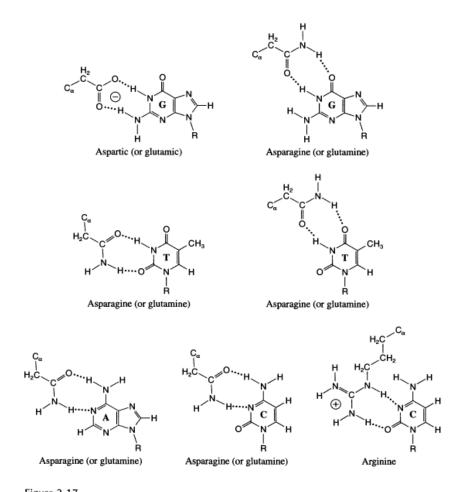
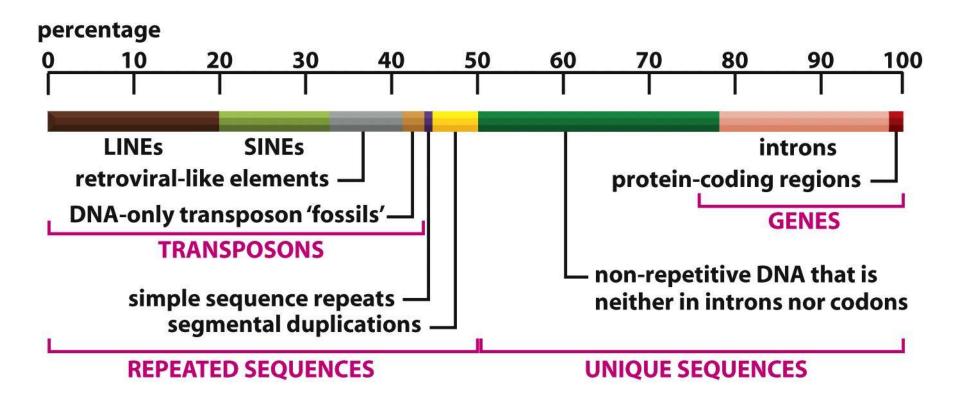


Figure 2-17 Interactions involving two hydrogen bonds between amino acids and bases that take the place of Watson-Crick base pairing.

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#### Genome composition



#### Repetitive sequences - repeats

Some sequences in genome are **unique**, usually the genomic sequences (both coding and non-coding). In contrast, other sequences exist in many copies – **repetitive sequences** (**repeats**). The length of repeat (microsatelites 2-6 bp x LINE 6-7000 bp), as well as the number of copies (several – 1.5M SINE in human) is highly variable.

#### Structure:

• direct repeats

• inverted repeats + palindromes

#### Position:

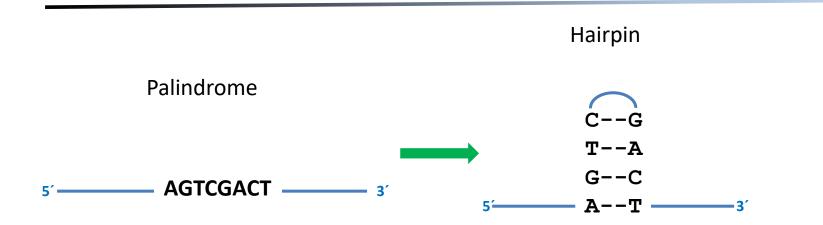
Tandem repeats



• **Interspersed** repeats



#### Inverted repeats

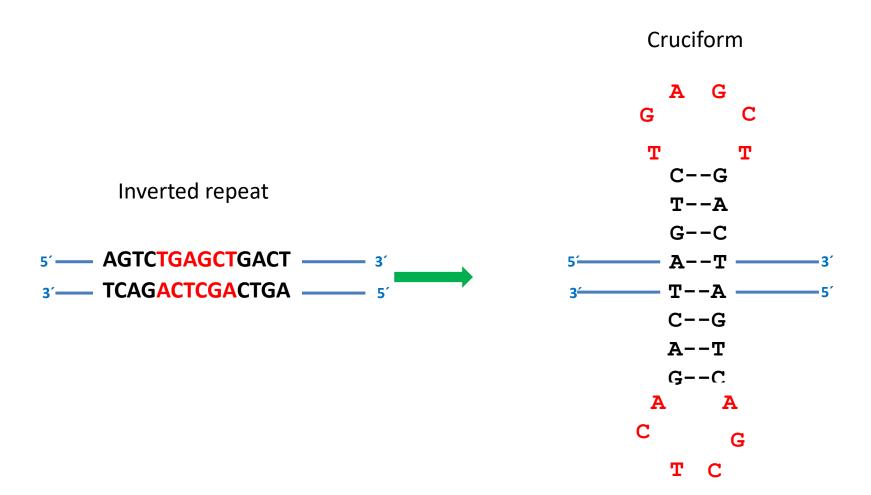


#### Hairpin with loop



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#### Inverted repeats



### Special types of repetitions - transposons

Interspersed repetitions with various lengths and number of copies.

```
LINE – long interspersed nuclear elements – up to 6 kbp – human> 500k copies
```

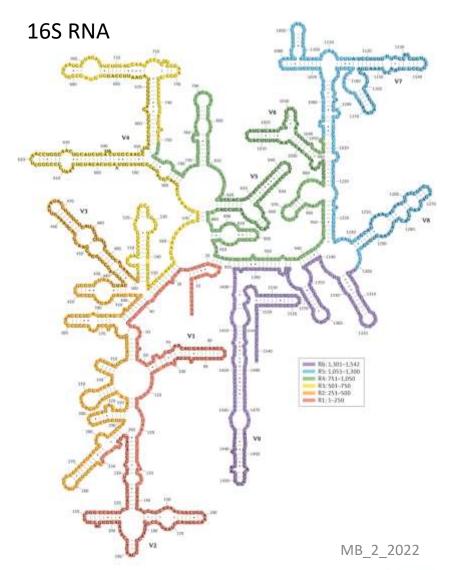
- 3 types (L1, L2, L3) – only some L1 are able to transpose

LTR – long terminal repeat - 100 bp – 5 kbp – variant of retrotransposons

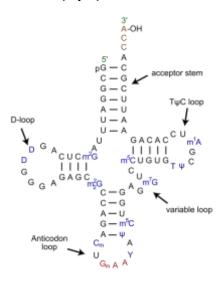
SINE – (Alu, ...) short interspersed nuclear elements – up to 500 bp – human ~ 1,5M copies

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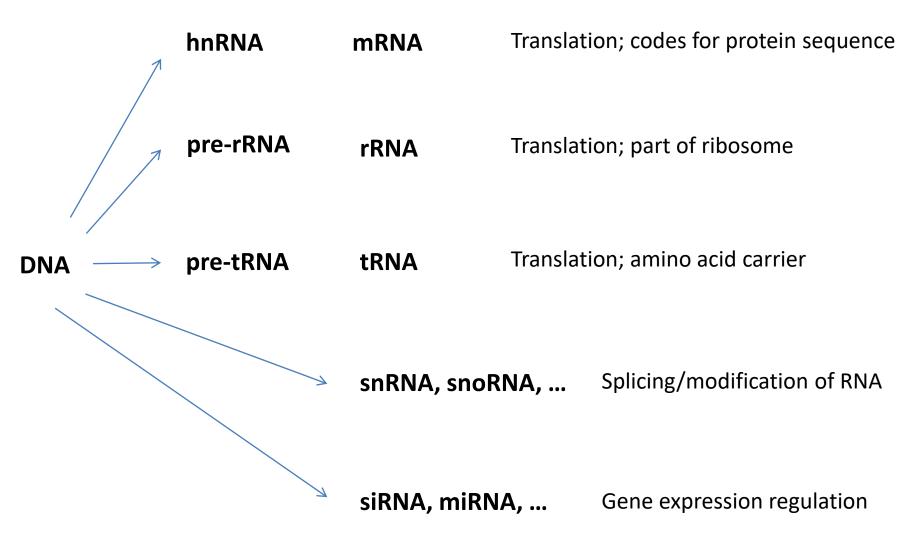
### Loops and hairpins in RNA



#### tRNA (Lys)



# Functional types of RNA



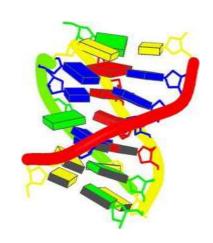
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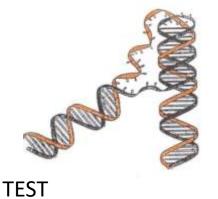
# Hoogsteen pairing - triplexes

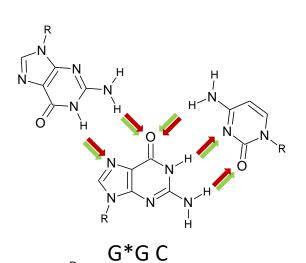
• gene expression regulation

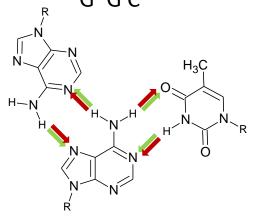
Pu\*Pu Py

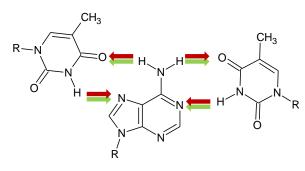
Py\*Pu Py



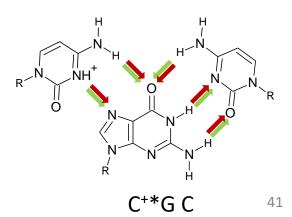








T\*AT



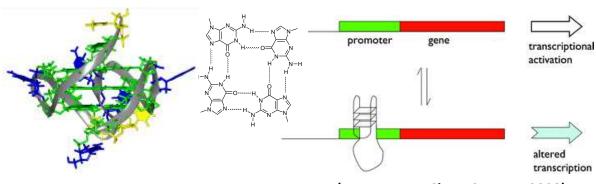
# Hoogsteen pairing – G-quadruplexes

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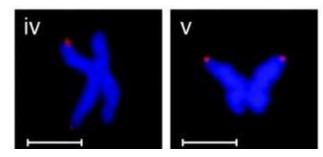
# Guanine quadruplexes

#### GGGN<sub>n</sub>GGGN<sub>n</sub>GGG

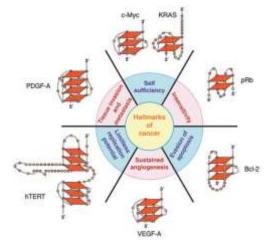
- gene expression regulation
- telomere structure



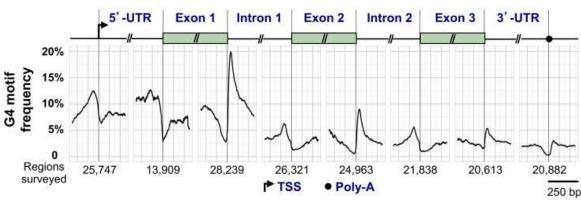
(Huppert J.L., Chem Soc Rev, 2008)



(Biffi G., et al., Nat Chem, 2013)



(Brooks T. A., et al., FEBS J, 2010)

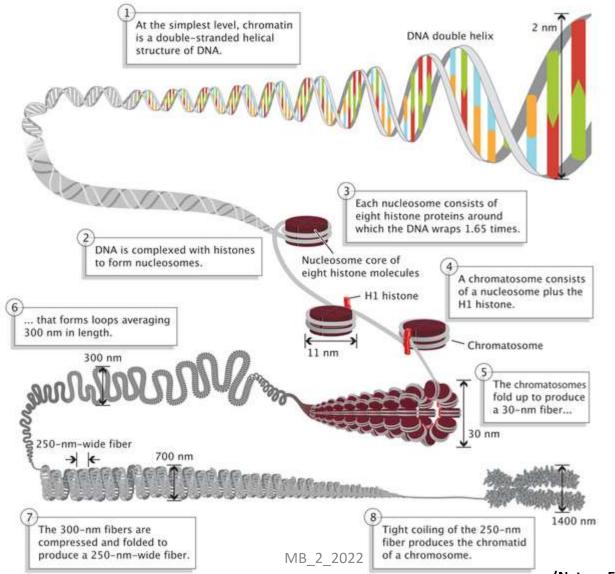


MB\_2\_2022 (Maizels N. and Gray L.T., PloS Genet., 2013)

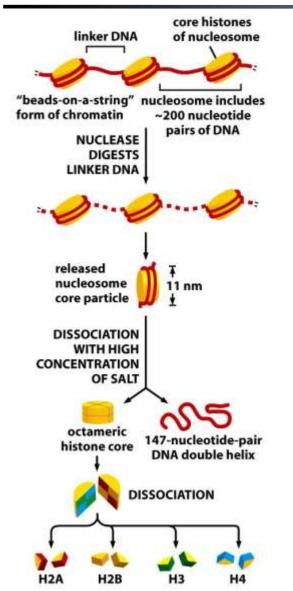
# Base reactivity

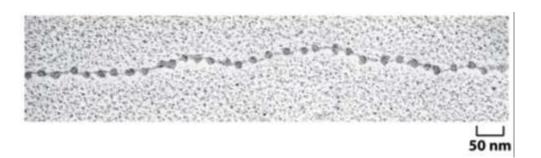
Hydrofobic bases with high ability to form hydrogen bonds are reluctant to be freely expressed into water environment around – if there is any chance to avoid this and lower the base exposition to the environment by any type of base pairing or base stacking, the bases tend to form a structure. Even the "single-stranded" RNA or DNA forms, in fact, compact structure with number of base pairs.

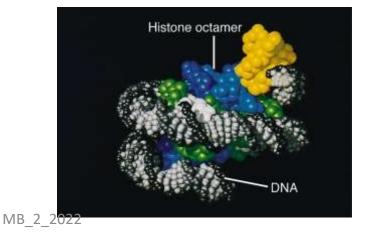
# Packing of DNA into chromosome



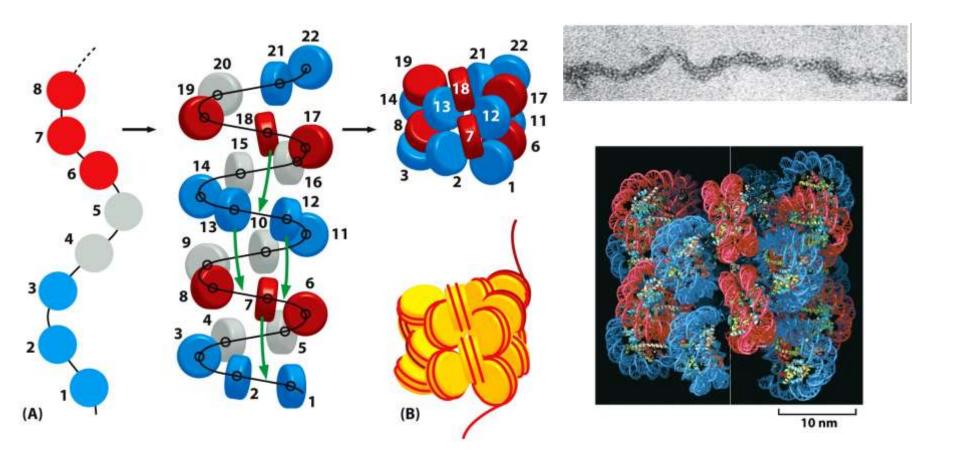
### Binding of DNA to a histone octamer



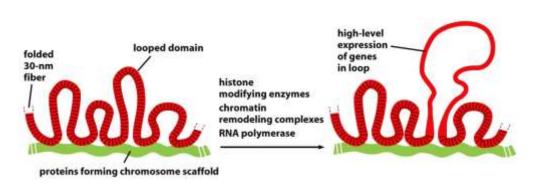


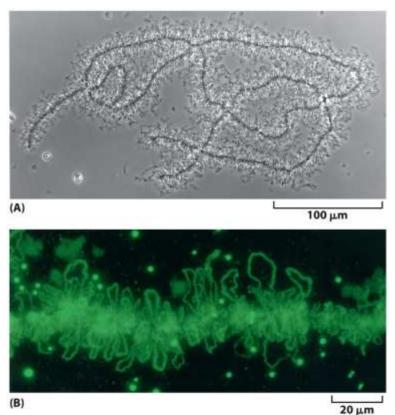


### Folding of nucleosomes into 30 nm fiber

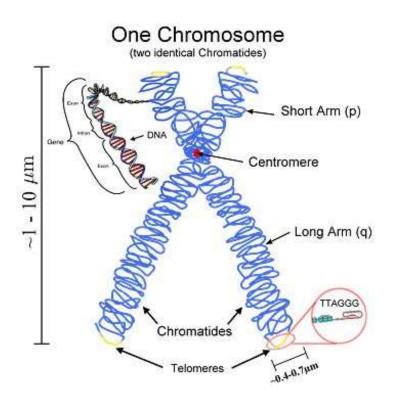


### 30 nm fiber binds to protein scaffold



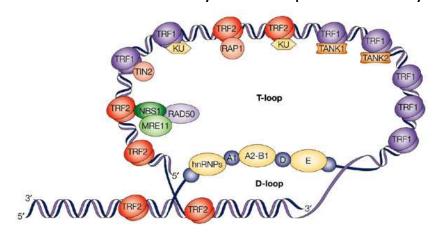


#### Chromosome



Centromere – here are the chromosomes connected to the system of cellular microtubules – important for chromosome segregation during cell division

**Telomere** – terminal part of chromatides that protect the end from being recognised as a double-strand break by a DNA repair machinery



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#### Chromosome

Fully condensed chromosomes are present only during the cell division, otherwise they are more or less decondensed to a lower levels of structure, especially in transcriptionally active sites (**euchromatin**). Transcriptionally inactive parts of DNA, as well as repetitive regions or telomeres are much more condensed (**heterochromatin**). Various types of chromatin differ in **epigenetic** markers of both DNA (5-methyl cytosine) and histones (methylation a acetylation).

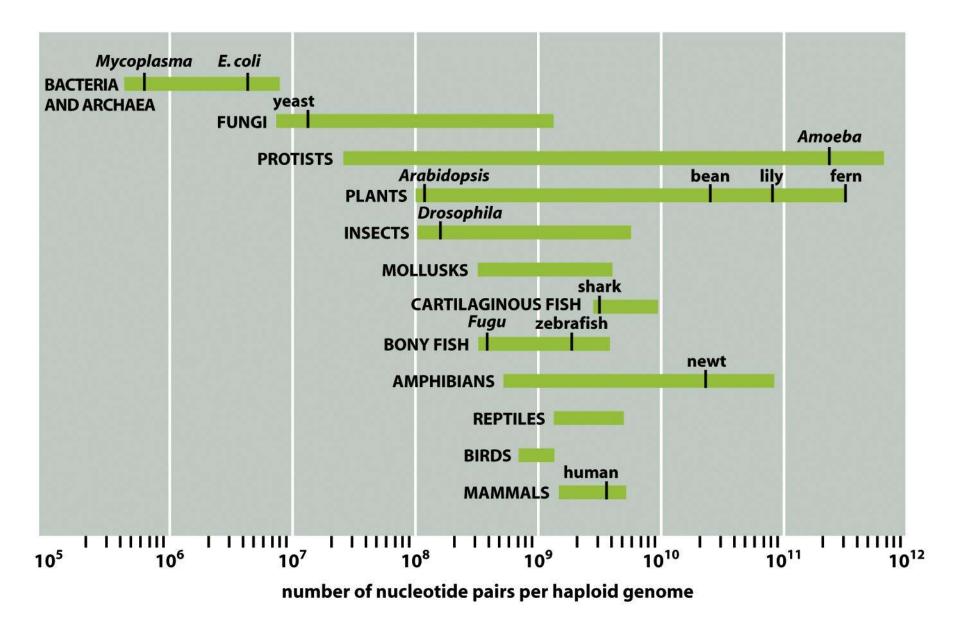


Table 1-1 Some Genomes That Have Been Completely Sequenced

SPECIES	SPECIAL FEATURES	HABITAT	GENOME SIZE (1000s OF NUCLEOTIDE PAIRS PER HAPLOID GENOME)	ESTIMATED NUMBER OF GENES CODING FOR PROTEINS
ARCHAEA				
Methanococcus jannaschii	lithotrophic, anaerobic, methane-producing	hydrothermal vents	1664	1750
Archaeoglobus fulgidus	lithotrophic or organotrophic, anaerobic, sulfate-reducing	hydrothermal vents	2178	2493
Nanoarchaeum equitans	smallest known archaean; anaerobic; parasitic on another, larger archaean	hydrothermal and volcanic hot vents	491	552
EUCARYOTES				
Saccharomyces cerevisiae (budding yeast)	minimal model eucaryote	grape skins, beer	12,069	~6300
Arabidopsis thaliana (Thale cress)	model organism for flowering plants	soil and air	~142,000	~26,000
Caenorhabditis elegans (nematode worm)	simple animal with perfectly predictable development	soil	~97,000	~20,000
Drosophila melanogaster (fruit fly)	key to the genetics of animal development	rotting fruit	~137,000	~14,000
Homo sapiens (human)	most intensively studied mammal	houses	~3,200,000	~24,000

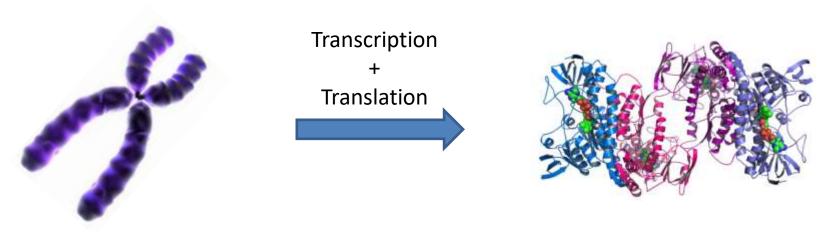
Genome size and gene number vary between strains of a single species, especially for bacteria and archaea. The table shows data for particular strains that have been sequenced. For eucaryotes, many genes can give rise to several alternative variant proteins, so that the total number of proteins specified by the genome is substantially greater than the number of genes.

# Levels of structure of biopolymers

DNA **RNA** Protein Ser-Asp-Val-Gin-Leu UCCGACGUUCAGCUA AGGCTGCAAGTCGAT **Primary** Secondary **Tertiary** 53 MB 2 2022

#### Genetic code

Set of rules that assign a sequence of aminoacids in the protein to the sequence of nucleotides in DNA or RNA.





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MB\_2\_2022

RNA CODEWORDS AND PROTEIN SYNTHESIS, III. ON THE NUCLEOTIDE SEQUENCE OF A CYSTEINE AND A LEUCINE RNA CODEWORD

By Philip Leder and Marshall W. Nihenberg national heast institute, national institutes of health Communicated by Richard B. Roberts, October 1, 1984

Previous studies utilizing randomly ordered synthetic polynucleotides to direct amino acid incorporation into protein in E. coli extracts indicated that RNA codewords corresponding to valine, leucine, and cysteine contain the bases (UUG). <sup>1-4</sup> The activity of chemically defined trinucleotides in stimulating the binding of a specific C<sup>14</sup>-aminoacyl-sRNA to ribosomes, prior to peptide bond formation,<sup>1</sup> provided a means of investigating base sequence of RNA codewords and showed that the sequence of a valine RNA codeword is GpUpU.<sup>5</sup>

# Properties of genetic code

• genetic code is based on **triplets** – one aminoacid in protein is coded by a sequence of three nucleotides in DNA (RNA)

mRNA CGUGGUACGAUUGGAUGU
Protein Arg Gly Thr Ile Gly Cys

Triplet = Codon x anticodon = complementary sequence on particular tRNA that carries the respective aminoacid

• genetic code is **universal** – individual triplets code for the same aminoacid in almost all organisms (x mitochondria)



CGU = Arginine

CGU = Arginine

CGU = Arginine

55

• genetic code is **degenerated** – one aminoacid might be coded by several different triplets (but the opposite is not true)



### Genetic code

First nt	U	С	Α	G	Third nt
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	С
	Leu	Ser	STOP	STOP/Sel	Α
	Leu	Ser	STOP	Trp	G
С	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	С
	Leu	Pro	Gln	Arg	Α
	Leu	Pro	Gln	Arg	G
A	lle	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	С
	lle	Thr	Lys	Arg	Α
	Met/START	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	С
	Val	Ala MR 2	<b>Glu</b> 2022	Gly	Α
	Val	Ala	Glu	Gly	G

#### Reading frames

Genetic code is based on triplets – three possible ways of reading (reading frames), but only one is correct.

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#### Genetic code

Although the genetic code is universal, the usage of particular codons, as well as the amount of particular tRNAs and aminoacyl transferases differ

Modification of synthetic genes for recombinant protein production according to the expression system used (Bacteria, human, ...) might be highly beneficial.