



Universidade Federal de Pelotas
CDTec - Graduação em Biotecnologia
Disciplina de Biologia Molecular



A Estrutura dos Ácidos Nucléicos

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Ácidos Nucleicos

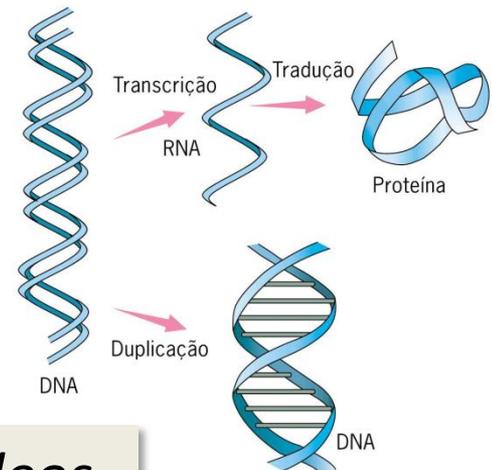
- São macromoléculas **presentes em todos os organismos vivos**;
- Através deles as células recebem informação sobre qual proteína sintetizar – **CÓDIGO GENÉTICO**:

exata sequência de aminoácidos → estrutura → função proteica

- Informação localizada nos **cromossomos** das células;

– Tipos:

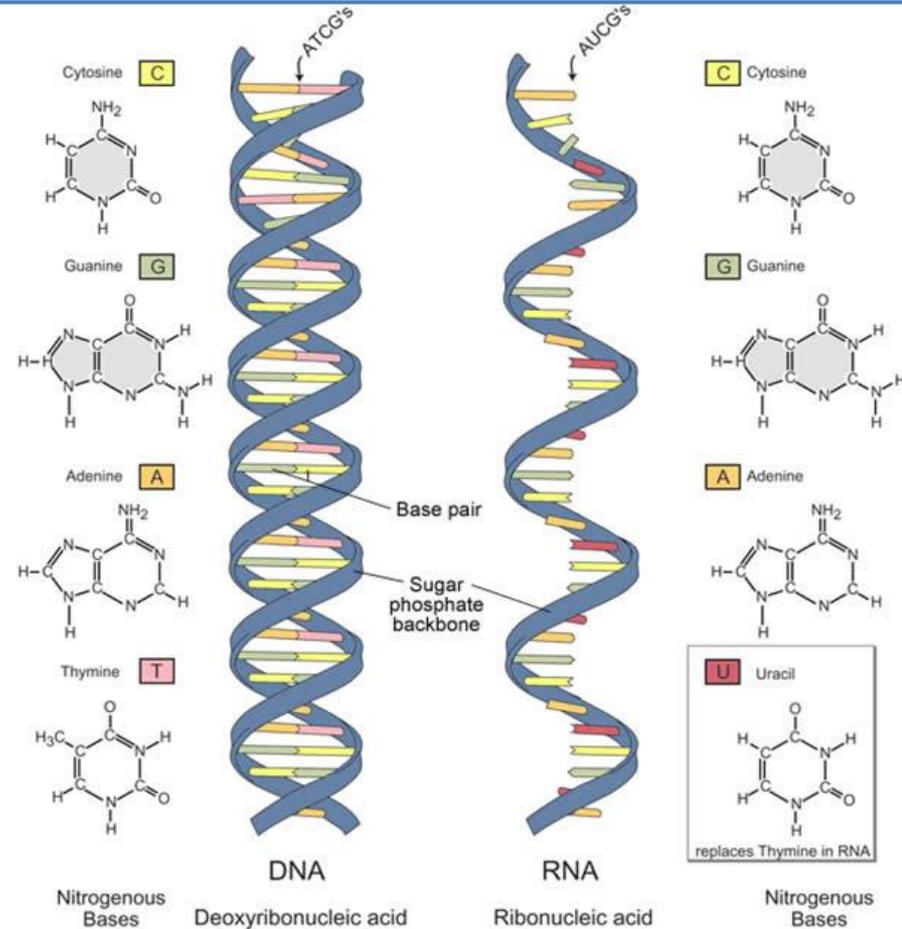
- **Ácido Desoxirribonucleico (DNA)**
- **Ácido Ribonucleico (RNA)**



Ambos são polímeros lineares de nucleotídeos (4 diferentes) unidos por ligações fosfodiéster

Estrutura dos Ácidos Nucléicos

- DNA – Ácido Desoxirribonucléico
- RNA – Ácido Ribonucléico
 - mRNA – RNA mensageiro
 - tRNA – RNA transportador
 - rRNA – RNA ribossômico
 - ✓ hnRNA - RNA nuclear heterogêneo
 - ✓ snRNA - *small nuclear RNA*



❖ Polímeros lineares compostos por 4 diferentes tipos de nucleotídeos.

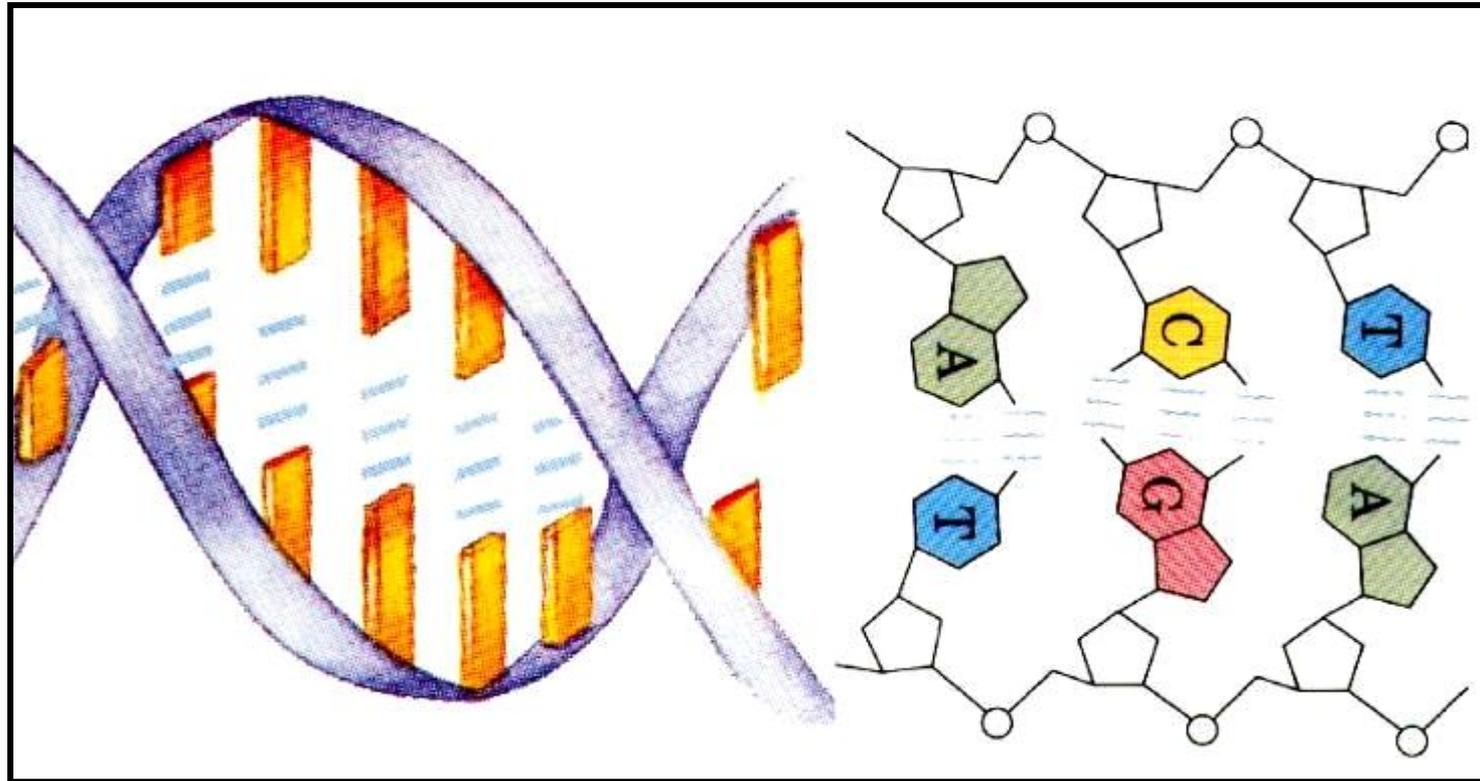
História

Em 1928, **Frederick Griffith** realizou experimentos que forneceram evidências que a informação genética está contida em uma molécula específica

- 1869 - Friedrich Miescher
 - Substância ácida no núcleo = ácido nucléico
- 1944 - Avery, MacLeod e McCarty
 - Sugeriram a função do DNA
- 1949 – Chargaff
 - composição das bases do DNA (A=T, C=G; relação AT/CG variável)
- 1952 – Hershey e Chase
 - Comprovou a função do DNA
- 1953 - Watson e Crick
 - Propuseram a estrutura de hélice dupla do DNA – baseado em dados de difração de raios X

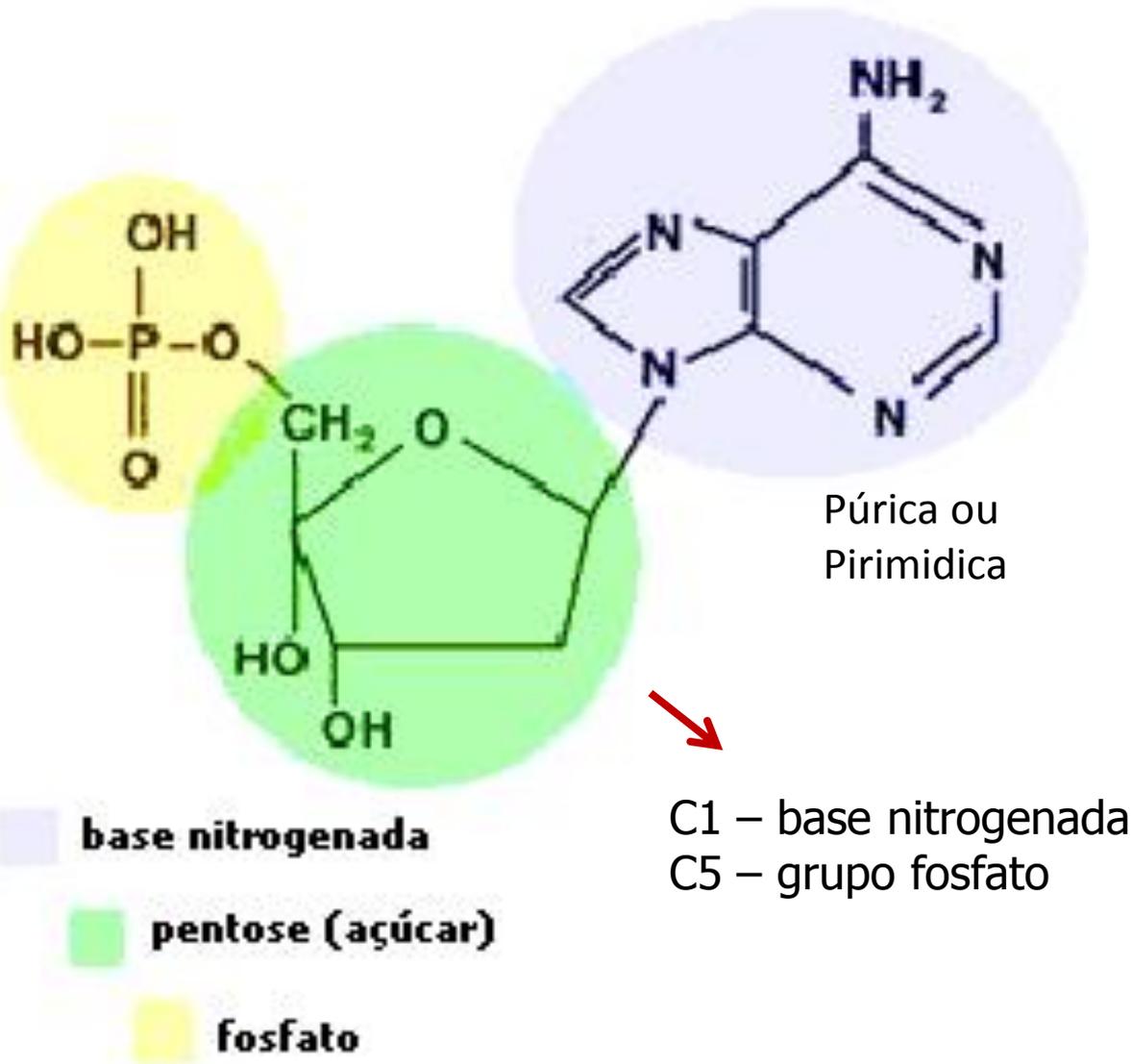


A Estrutura dos Ácidos Nucléicos

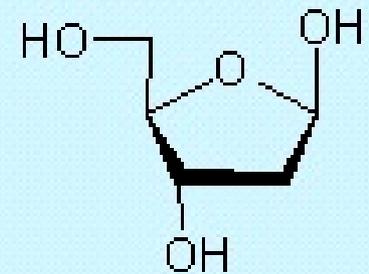


Polímeros lineares de monômeros de nucleotídeos

A Estrutura dos Nucleotídeos

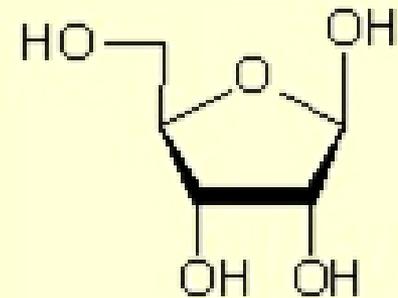


PENTOSE:



Desoxiribose

DNA



Ribose

RNA

≠ Hidroxila no C2

A Estrutura dos Nucleotídeos

O nucleotídeo é composto por:
açúcar
grupo fosfato
base nitrogenada

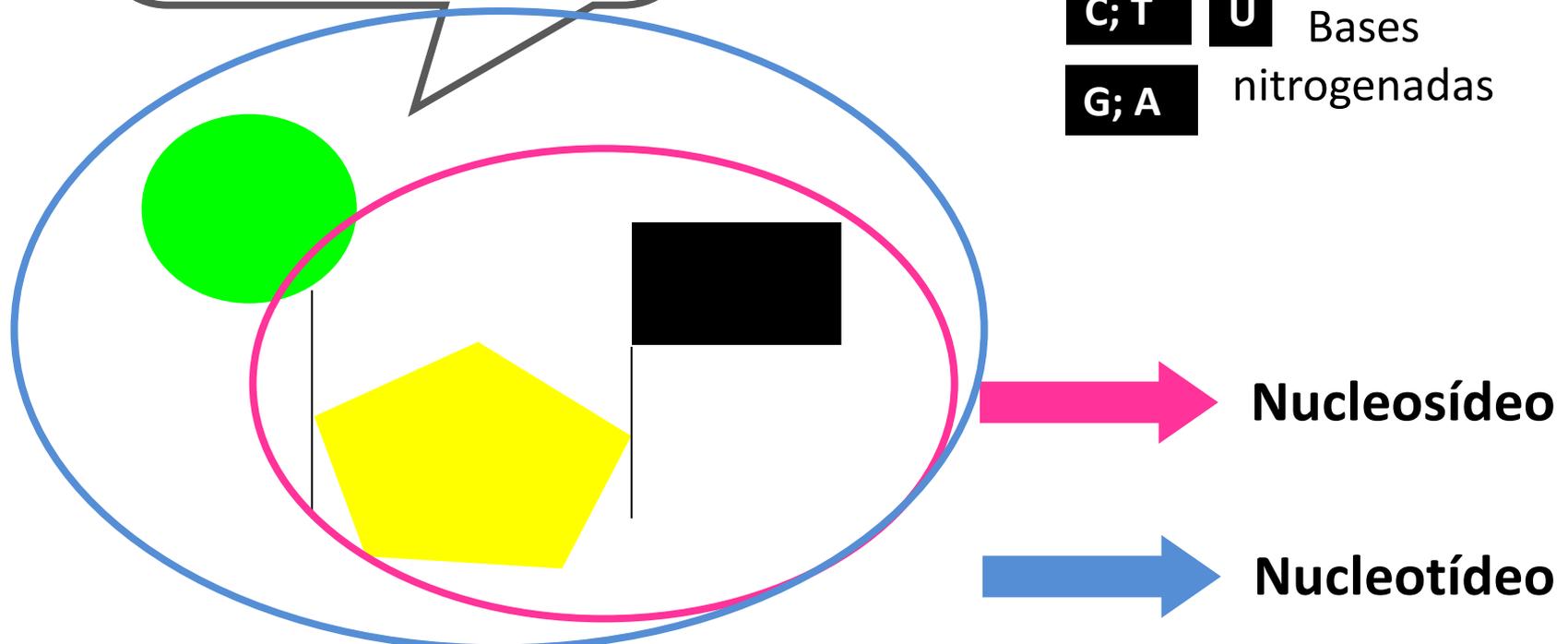
Legenda

 Grupo fosfato

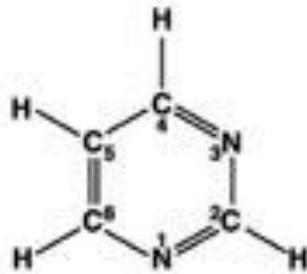
 Açúcar

 C; T  U Bases nitrogenadas

 G; A



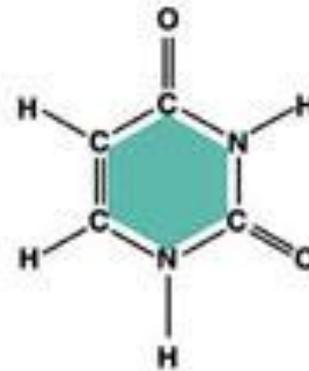
BASES NITROGENADAS



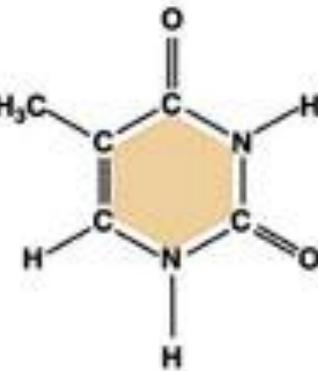
Pyrimidine ring



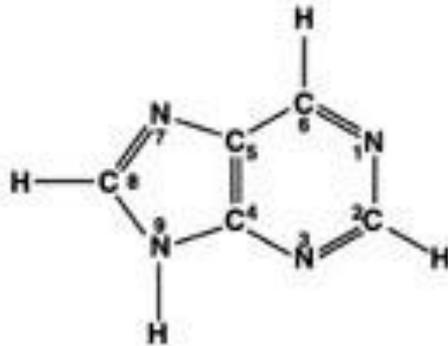
Cytosine



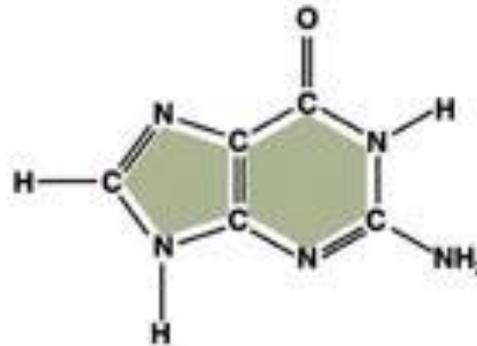
Uracil



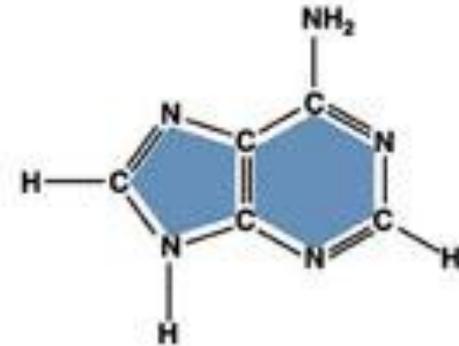
Thymine



Purine ring



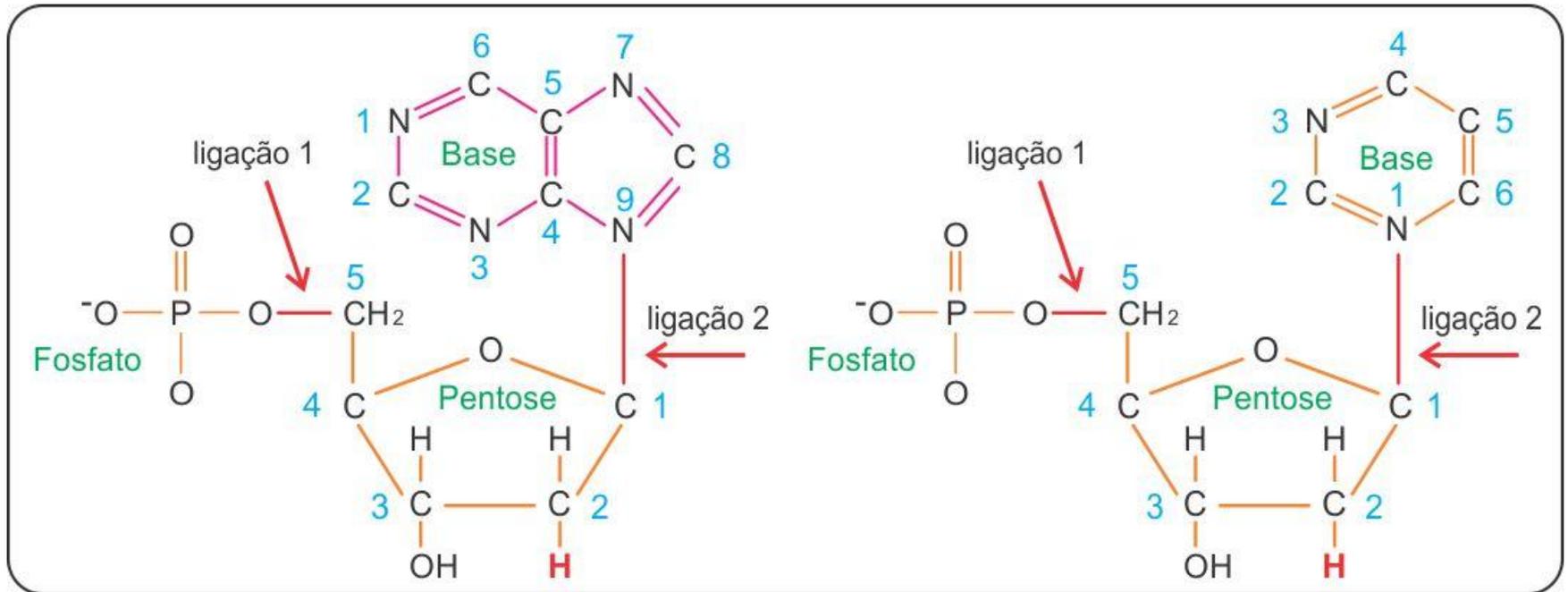
Guanine



Adenine

Base nitrogenada: composto cíclico contendo nitrogênio (grupo funcional
Purinas – possuem anel duplo / Pirimidinas – possuem anel simples
No DNA emparelham-se com as respectivas **bases complementares** (\neq tamanho)

A Estrutura dos Nucleotídeos



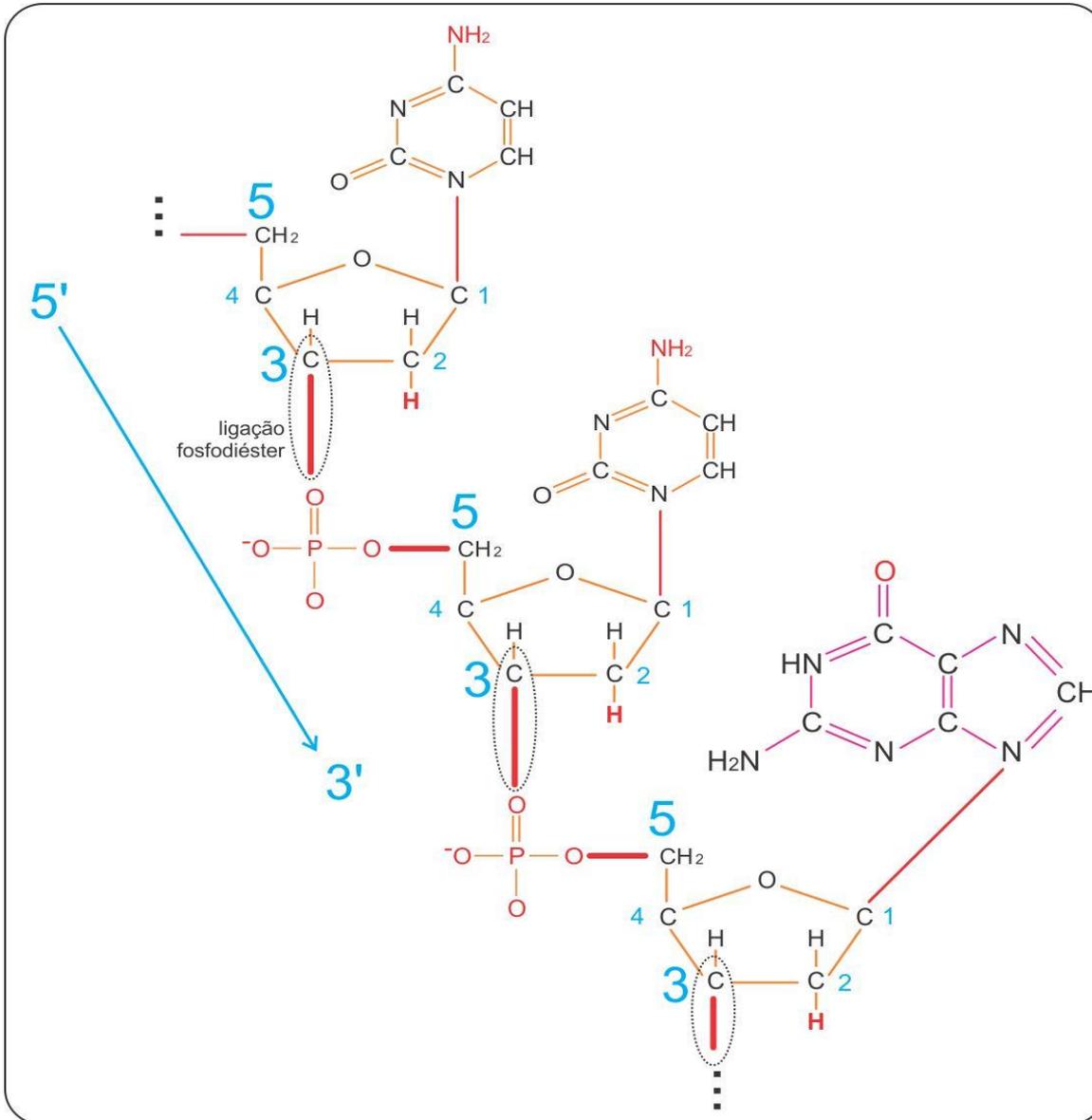
- **Ligação 1 – Ligação Fosfodiéster**

Fosfato + C5 da pentose

- **Ligação 2 – Ligação Glicosídica**

Base Nitrogenada + C1 da pentose

A Estrutura dos Nucleotídeos



1º nucleotídeo: grupo fosfato em C5

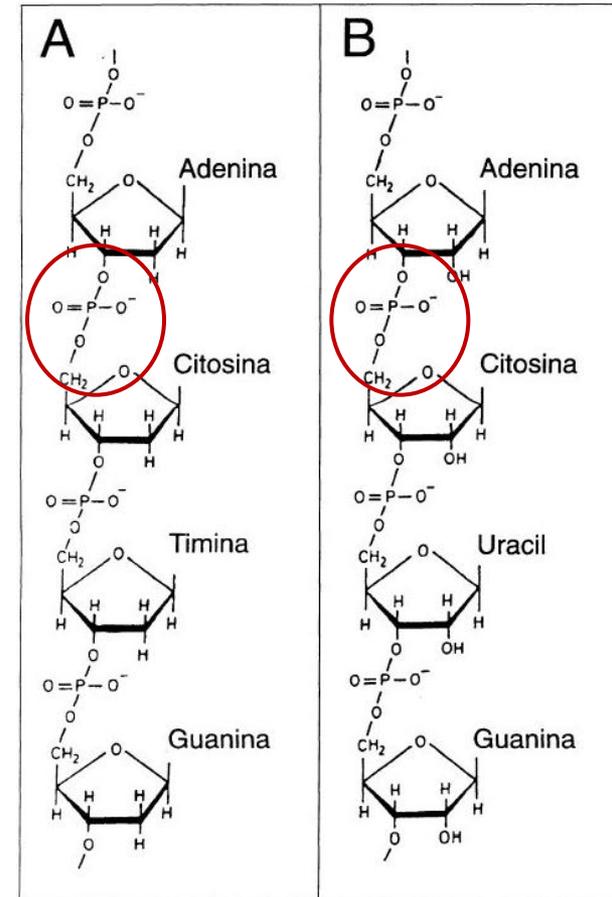
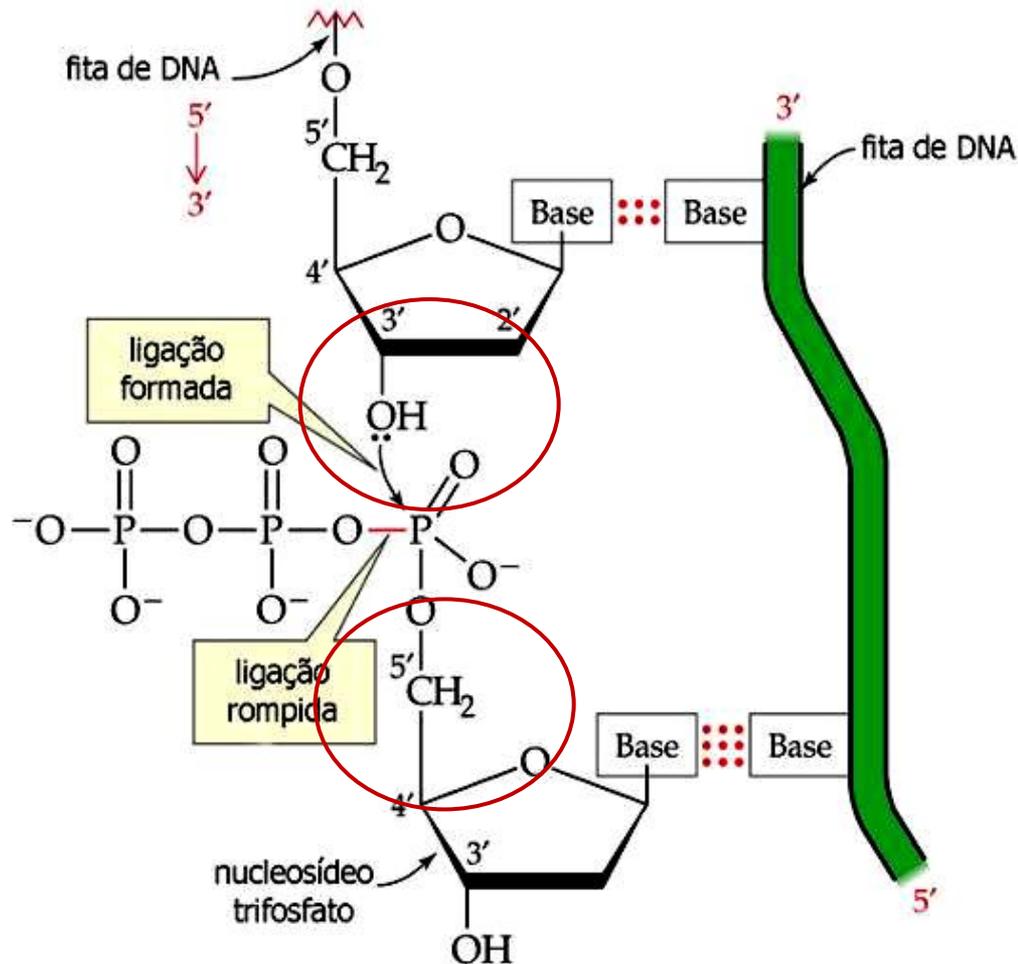
Último nucleotídeo: grupo hidroxílico em C3

Próximo nucleotídeo é adicionado na extremidade 3'-OH

A Estrutura dos Ácidos Nucléicos

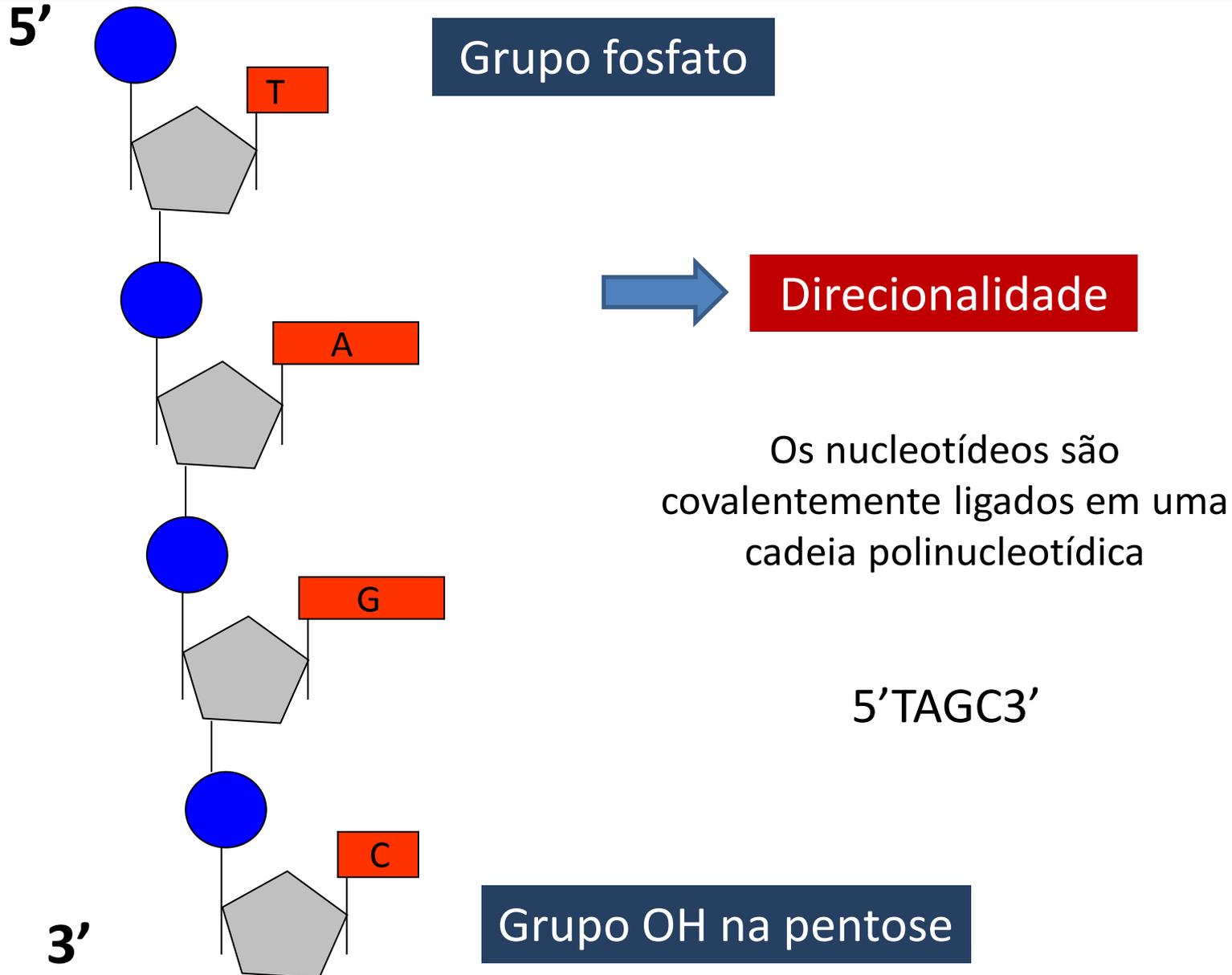
Formação da ligação fosfodiéster

Ligação fosfodiéster



O grupo hidroxila do carbono-3 da pentose do primeiro nucleotídeo se liga ao grupo fosfato ligado a hidroxila do carbono-5 da pentose do segundo nucleotídeo através de uma ligação fosfodiéster.

Polaridade das fitas de DNA



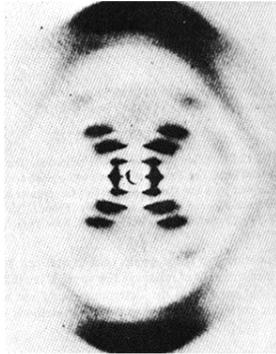
**ttgccgtcccaagcaaatggatgatttgatgctgtccccggacgatattgaacaatggttactgaaga
cccaggtccagatgaagctcccagaatgccagaggctgctcccc[g/c]cgtggccactgcaccagca
gctcctacaccggcggcccctgcaccagccccctcctggccccttgtcattctgtccctccaga**

Table 1. Primers used in qRT-PCR reaction for equine oocytes.

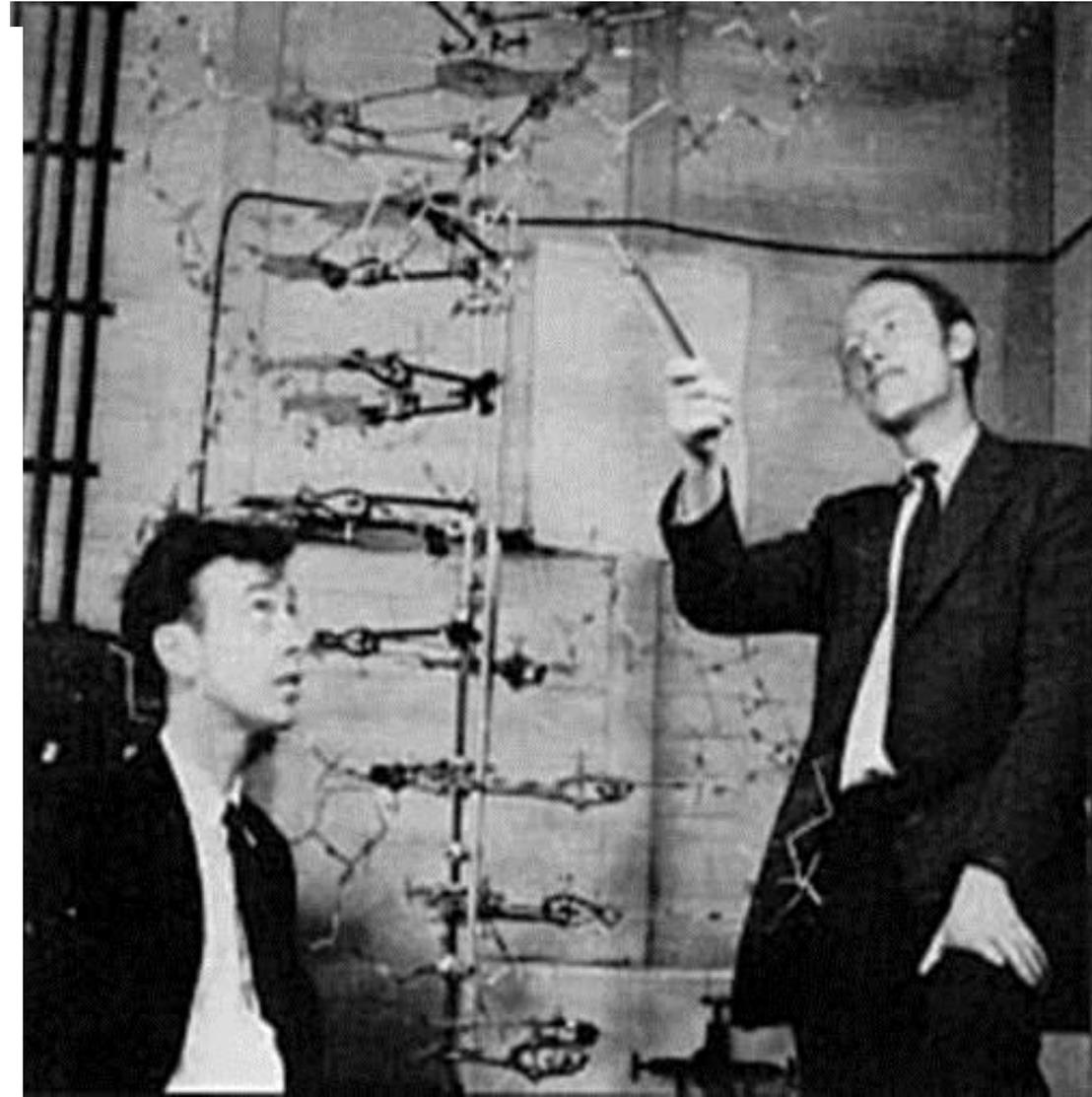
Gene	Acession Number	Sequence	TM	Fragment size	Efficiency (%)	Coorelation (R ²)
Bcl-2 Eq	XM_001499714.1	F 5' GAGACCCCCAGTGCCATCAA 3' R 5' GGGATGTCAGGTCGCTGAAT 3'	57°C	146 bp	99.9%	0.97
BaxEq	XM_001489207.1	F 5' TTTGCTTCAGGGTTTCATCC 3' R 5' ATCCTCTGCAGCTCCATGTT 3'	60°C	162 bp	100%	0.94
p53 Eq	XM_001918153.1	F 5' AAAGGATGCCCATGCTACAGAGGA 3' R 5' AGTAGACTGGCCCTTCTTGGTCTT 3'	63°C	82 bp	90.5%	0.98
SurvivinEq	XM_001915400.1	F 5' TTCATCCACTGTCCCCTACTGA 3' R 5' GTTCCTCTATGGGGTTCGTC 3'	57°C	98 bp	70.5%	0.98
GAPDH	NM_001163856.1	F 5' GCC GTA ACT TCT GTG CTG TG 3' R 5' AAT GAA GGG GTC ATT GAT GG 3'	61°C	150 bp	84.0%	0.98

1953 - Watson e Crick

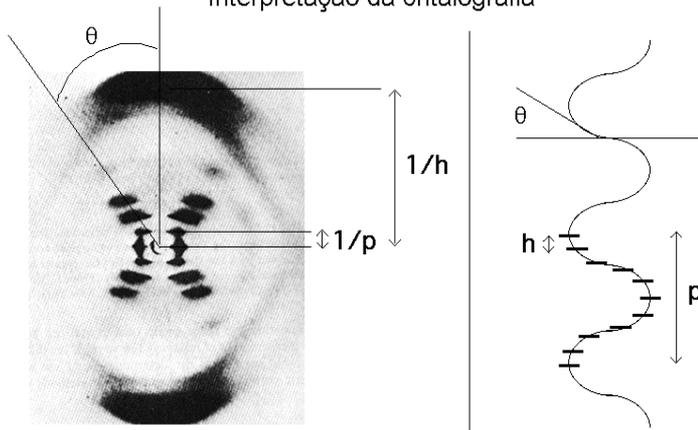
Elucidaram a estrutura do DNA



Padrão de difração de raios-x da forma B do DNA



Interpretação da cristalografia



θ - inclinação da hélice: ângulo perpendicular ao longo do eixo

$h = 3.4 \text{ \AA}$ (distância entre as bases)

$p = 34 \text{ \AA}$ (distância entre uma volta completa da hélice, unidades repetidas da hélice)

equipment, and to Dr. G. E. R. Denson and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

¹ Young, F. B., Gerard, H., and Jensen, W., *Phil. Mag.*, **46**, 149 (1926).

² Langer-Biggar, M. S., *Mon. Not. Roy. Astr. Soc., Geophys. Suppl.*, **4**, 285 (1949).

³ Van Arx, W. S., *World Wide Papers in Fibre Chemistry, Mexico*, **11** (1) (1951).

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 Corey¹. 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 Furbert's² 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 Furbert's 'standard configuration', the sugar being roughly perpendicular to the attached base. There



This figure is partly diagrammatic. The two helices symbolize the two phosphate-sugar chains, and the horizontal rungs the pairs of bases holding the chains together. The vertical line marks the fibre axis.

is a residue on each chain every 3.4 Å. 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 Å. The distance of a phosphate atom from the fibre axis is 10 Å. 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

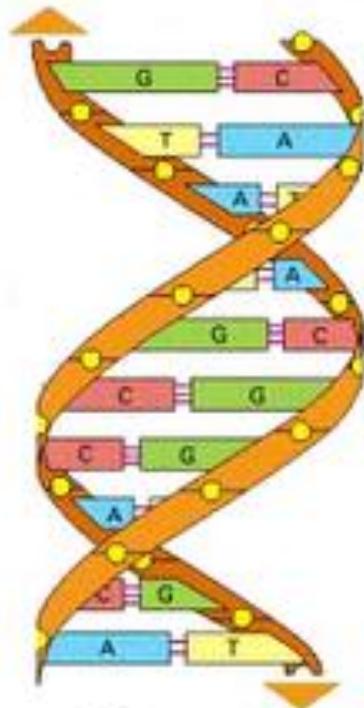


Figura I



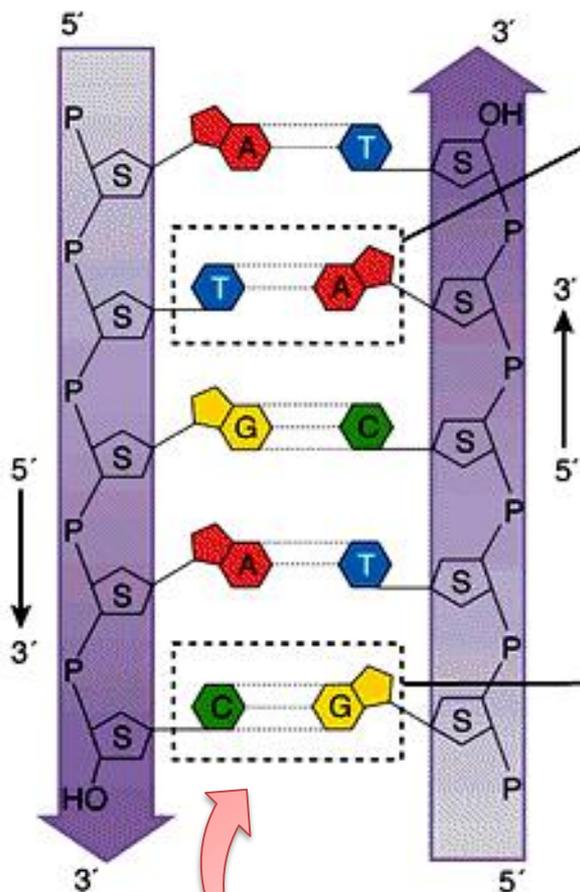
Figura II

**Desenho do trabalho de Watson e Crick
representando a estrutura do DNA**

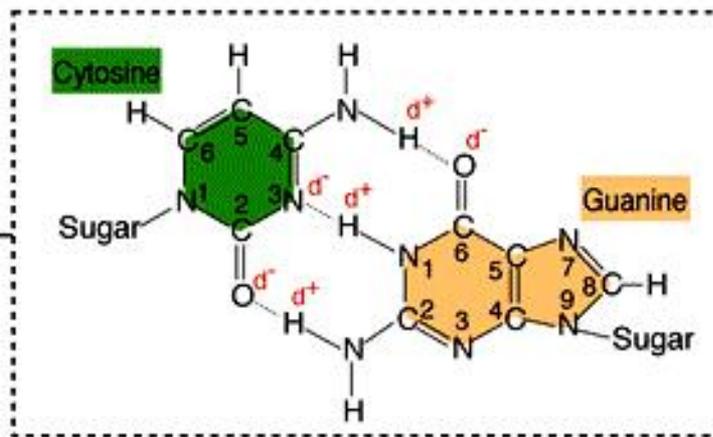
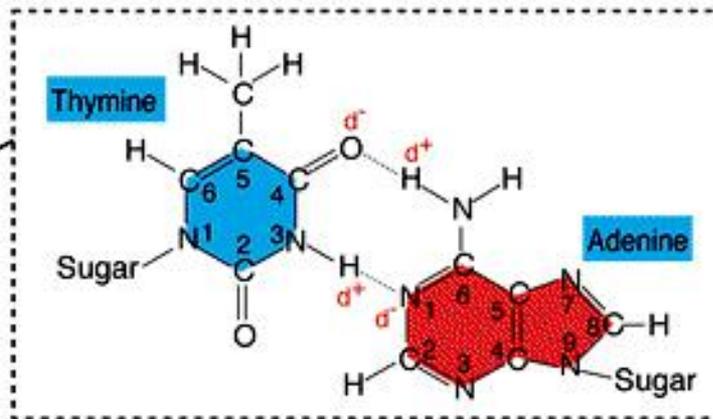
Ácidos nucléicos

Fitas antiparalelas

Opposite polarity of the two strands



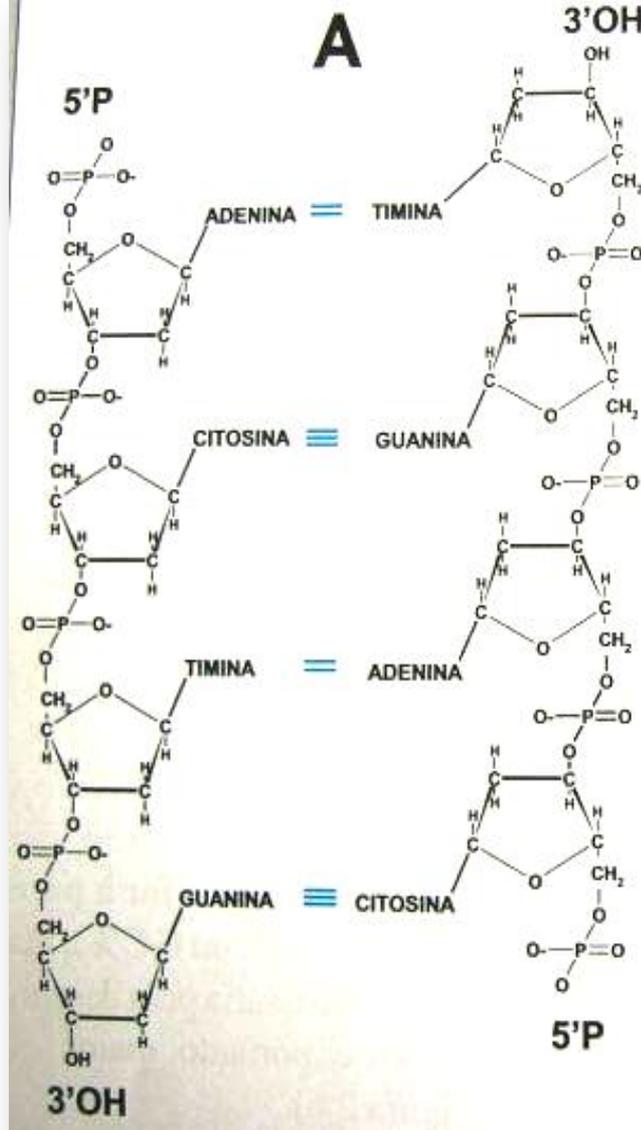
Hydrogen bonding in A-T and G-C base pairs



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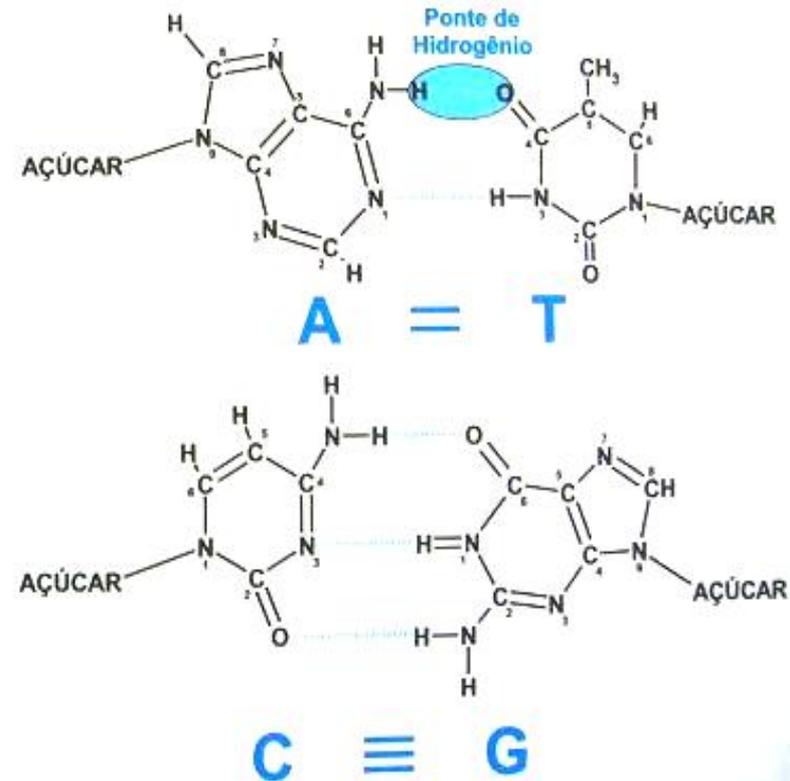
Anéis aromáticos- hidrofóbicos

Ligação
fosfodiéster



GRUPO CETO C=O
GRUPO AMINO C-NH₂

Ponte de Hidrogênio adicional
Nitrogênios dos anéis aromáticos



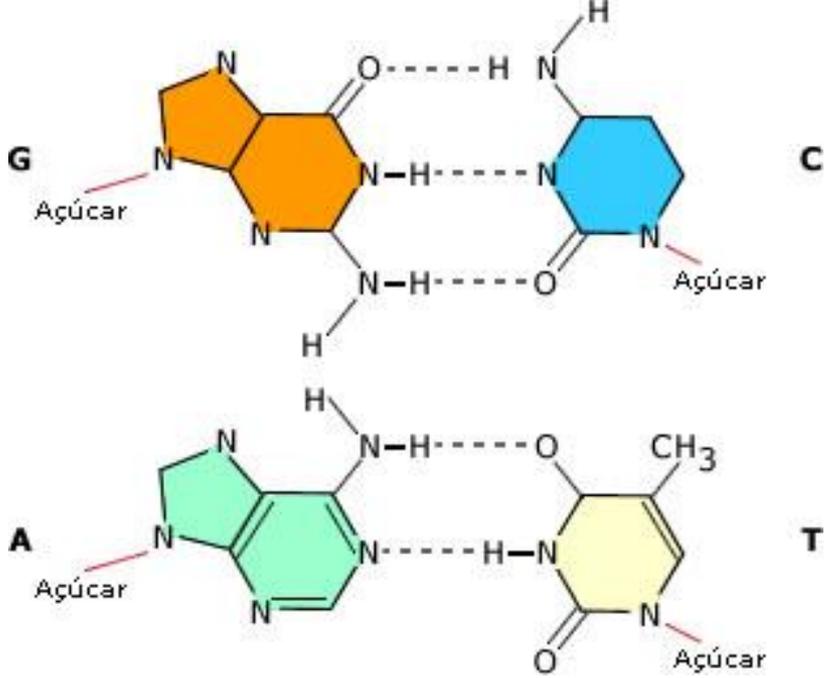
Representação (5' → 3')

Pareamento

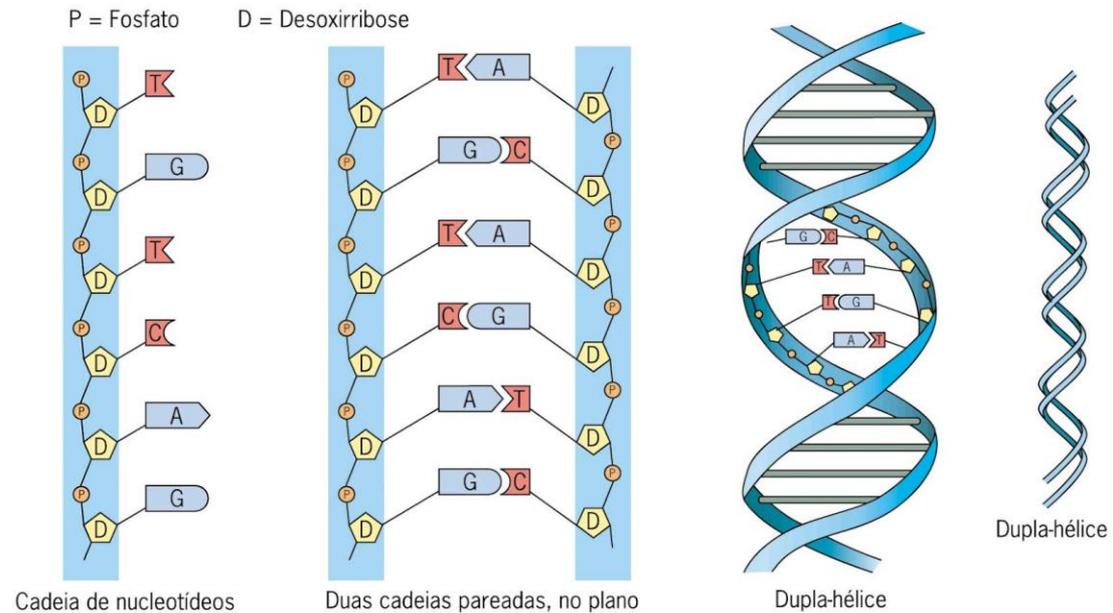
Uniformidade de tamanho

Purinas = Pirimidinas
A+G = C+T

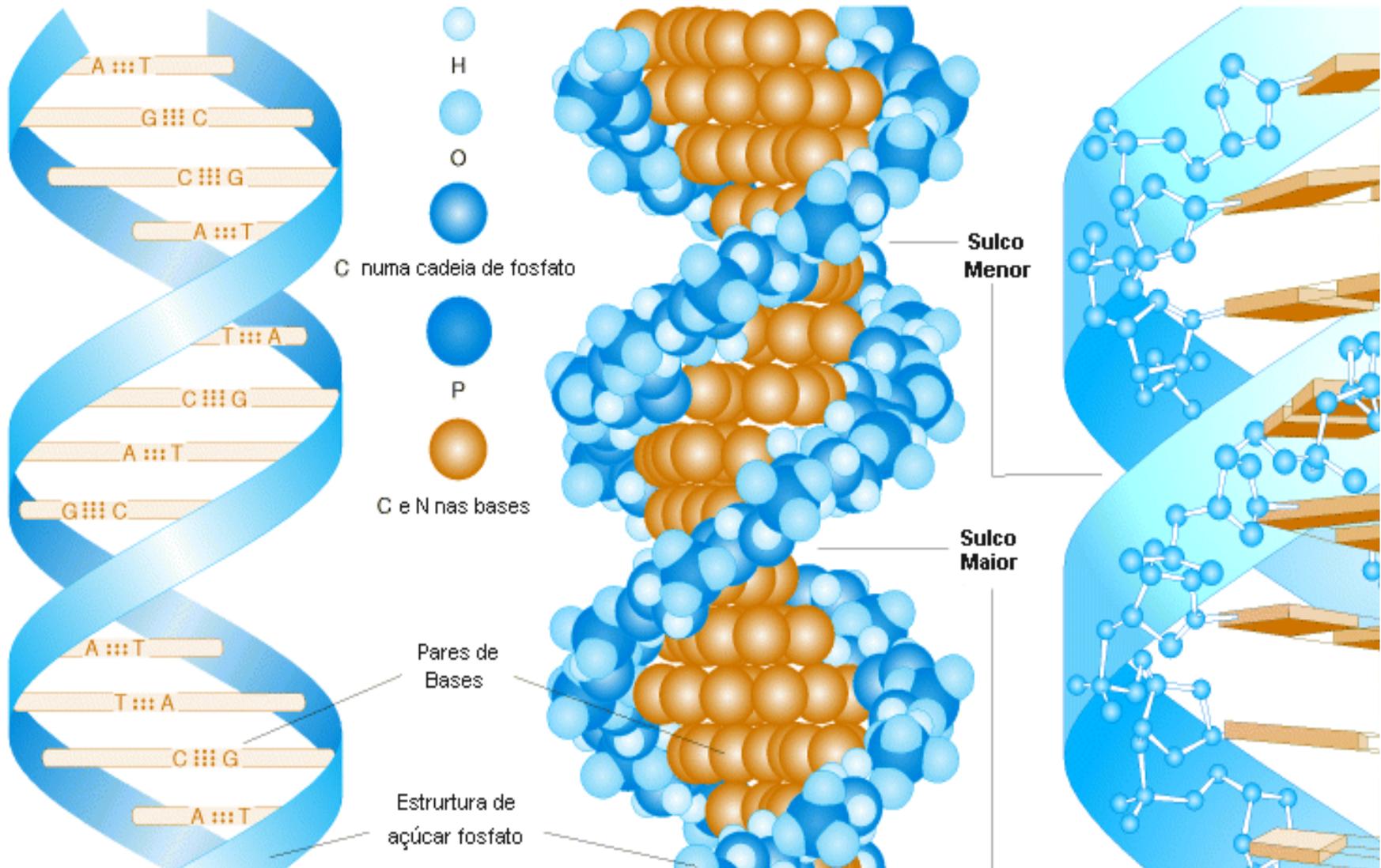
Complementaridade



Esquemas de molécula de DNA, no plano e retorcida



Estrutura do DNA

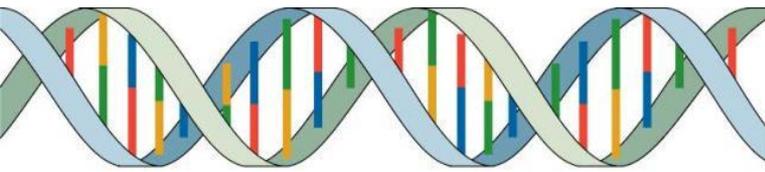


Propriedades do DNA

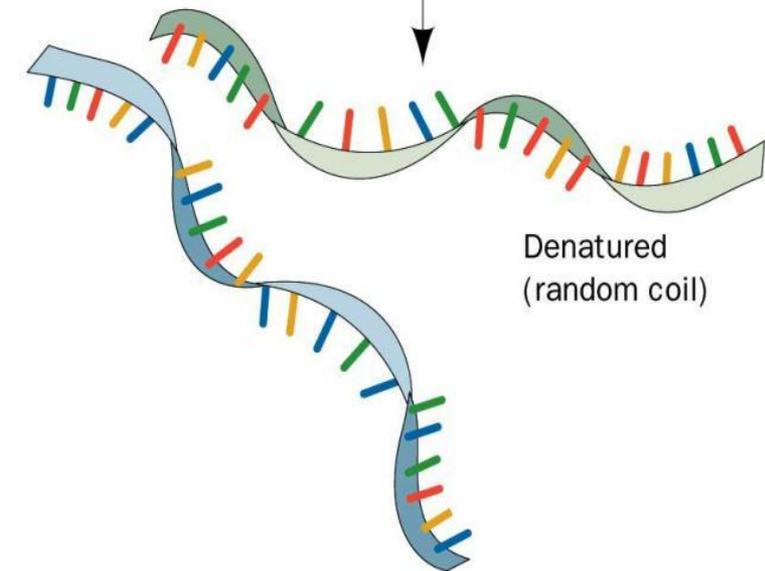
- **Complementariedade** – entre bases nitrogenadas
- **Antiparalelismo** – direcionalidade a cadeias
- **Desnaturação** - pontes de hidrogênio rompem
- **Renaturação** (ou hibridização ou anelamento) – reestabelecimento das pontes de hidrogênio entre as cadeias



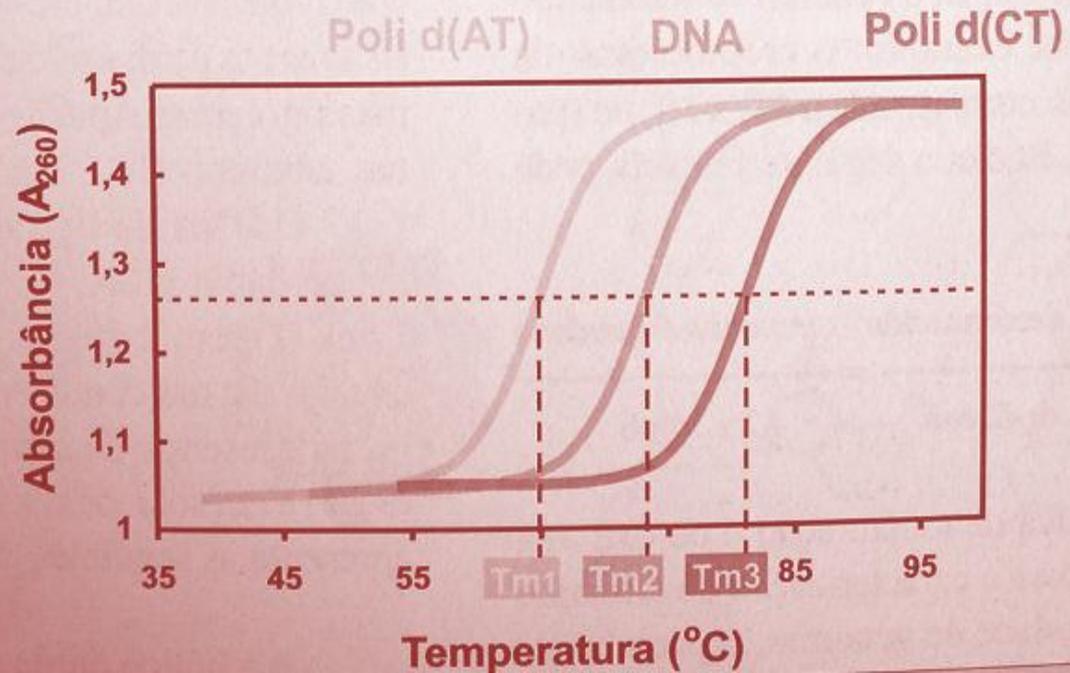
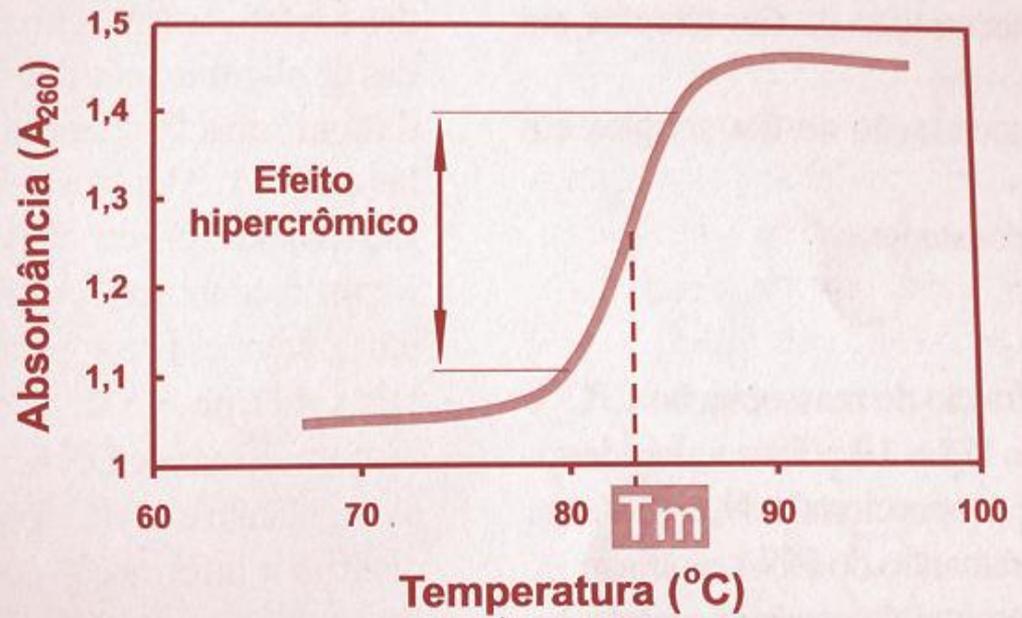
Desnaturação X Renaturação



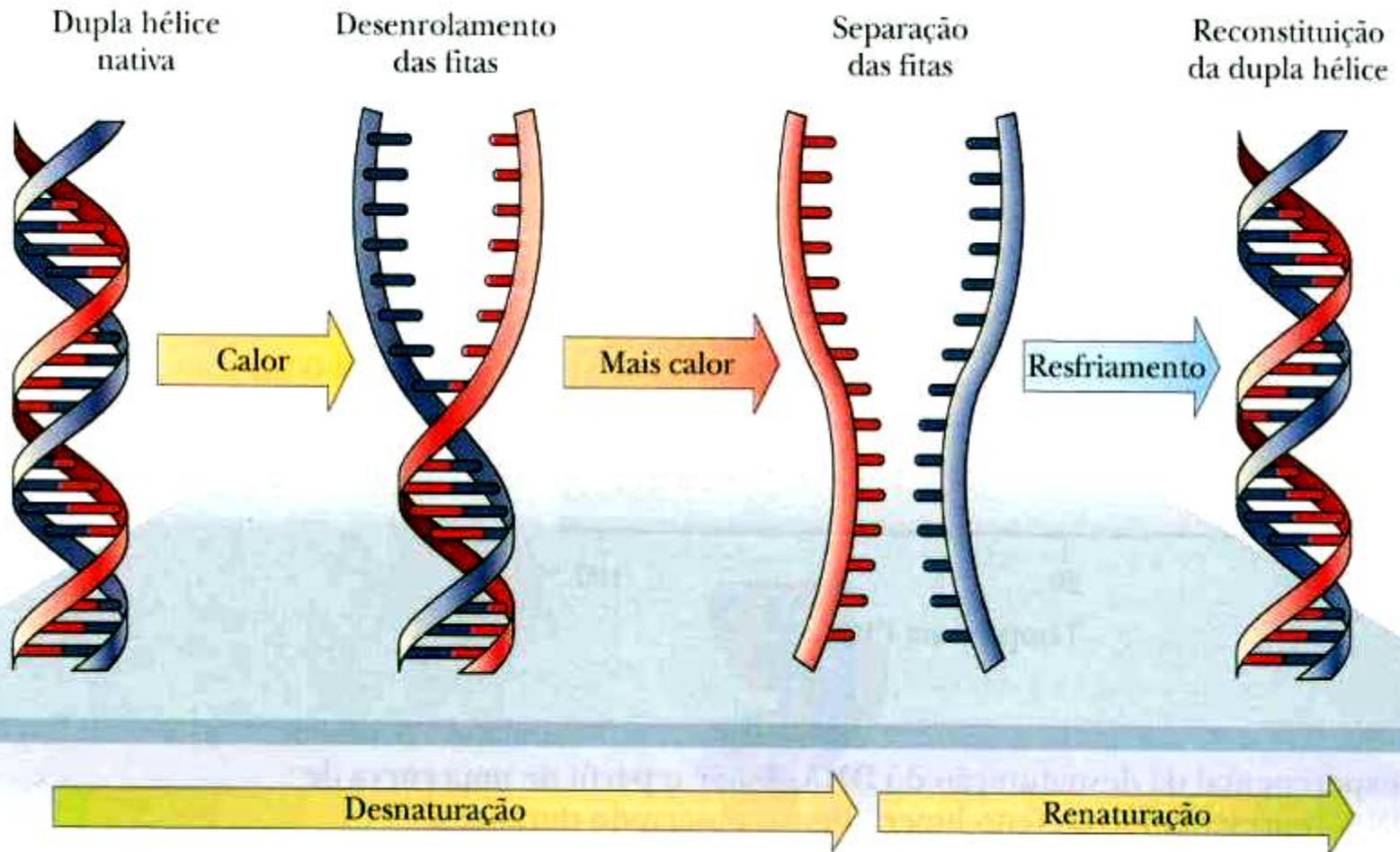
Native (double helix)



Denatured
(random coil)



Desnaturação e Renaturação



Supertorção

- Enrolamento da hélice dupla sobre si mesmo
- Conformação predominante do DNA na célula
- Fundamental para o empacotamento do DNA nos genomas
- Envolvido na replicação, transcrição e na recombinação
- Característica de quase todos os DNAs circulares e lineares
- Importante para a sua funcionalidade

Cadeia Polinucleotídica – Estrutura Primária

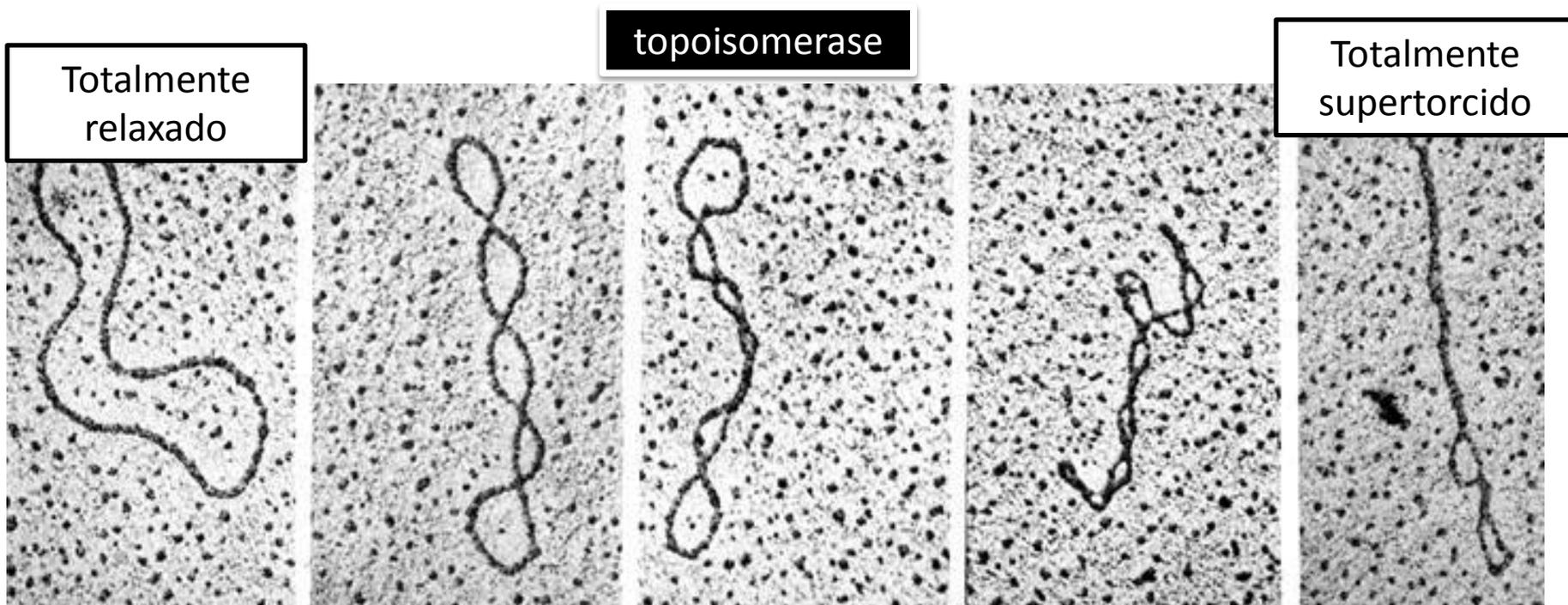
Dupla Hélice – Estrutura Secundária

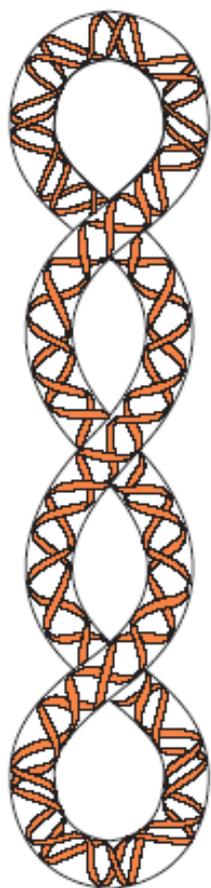
Supertorção – Estrutura Terceária

Supertorcida / Superenrolada / Super Helicoidal

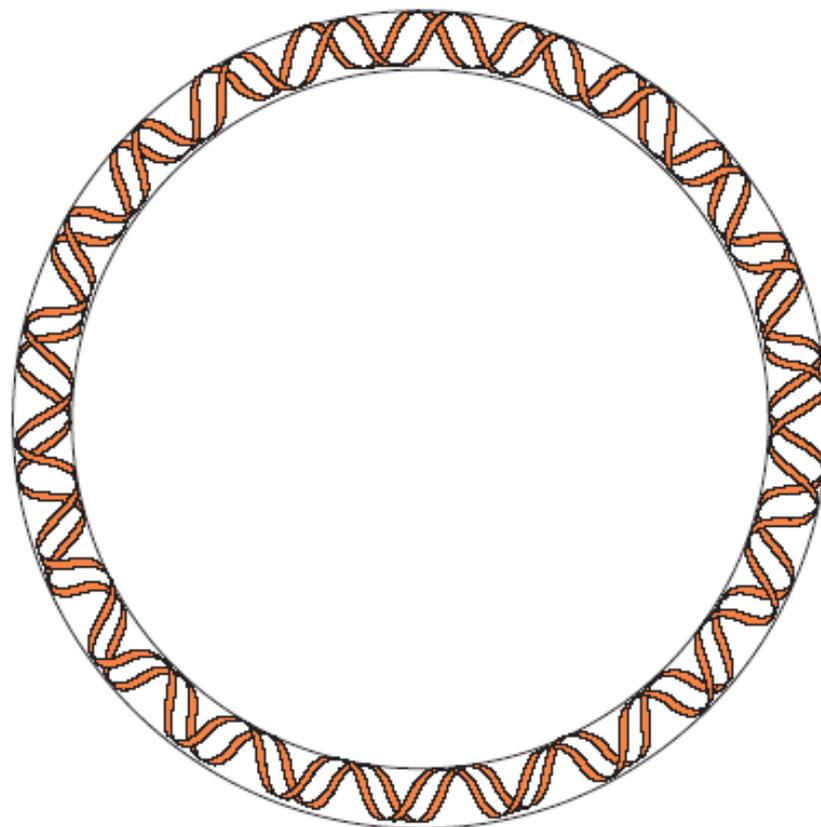
Supertorção

- Extremidades ligadas covalentemente
- Supertorcida, superenrolada, superhelicoidal
 - Superenrolamento negativo
 - Superenrolamento positivo
- Relaxada





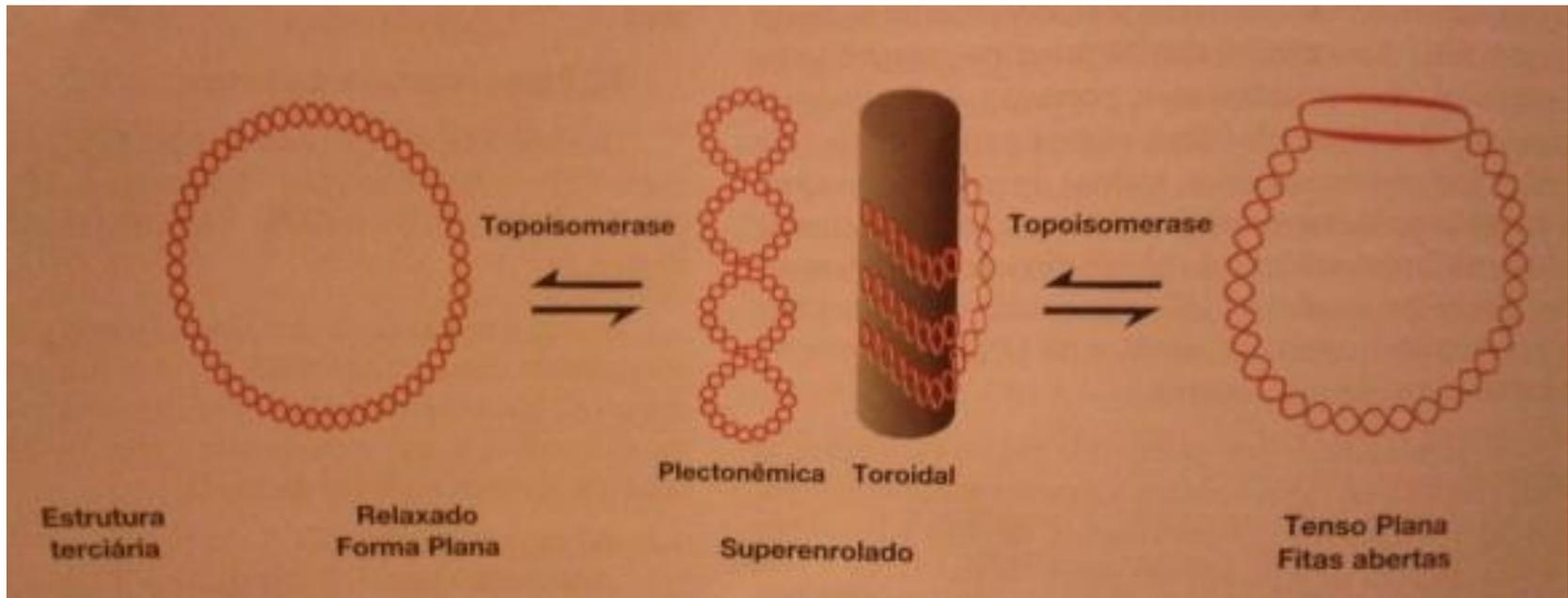
**Super-hélice para
direita (negativa)**



**Hélice circular
normal**



**Super-hélice para
a esquerda (positiva)**

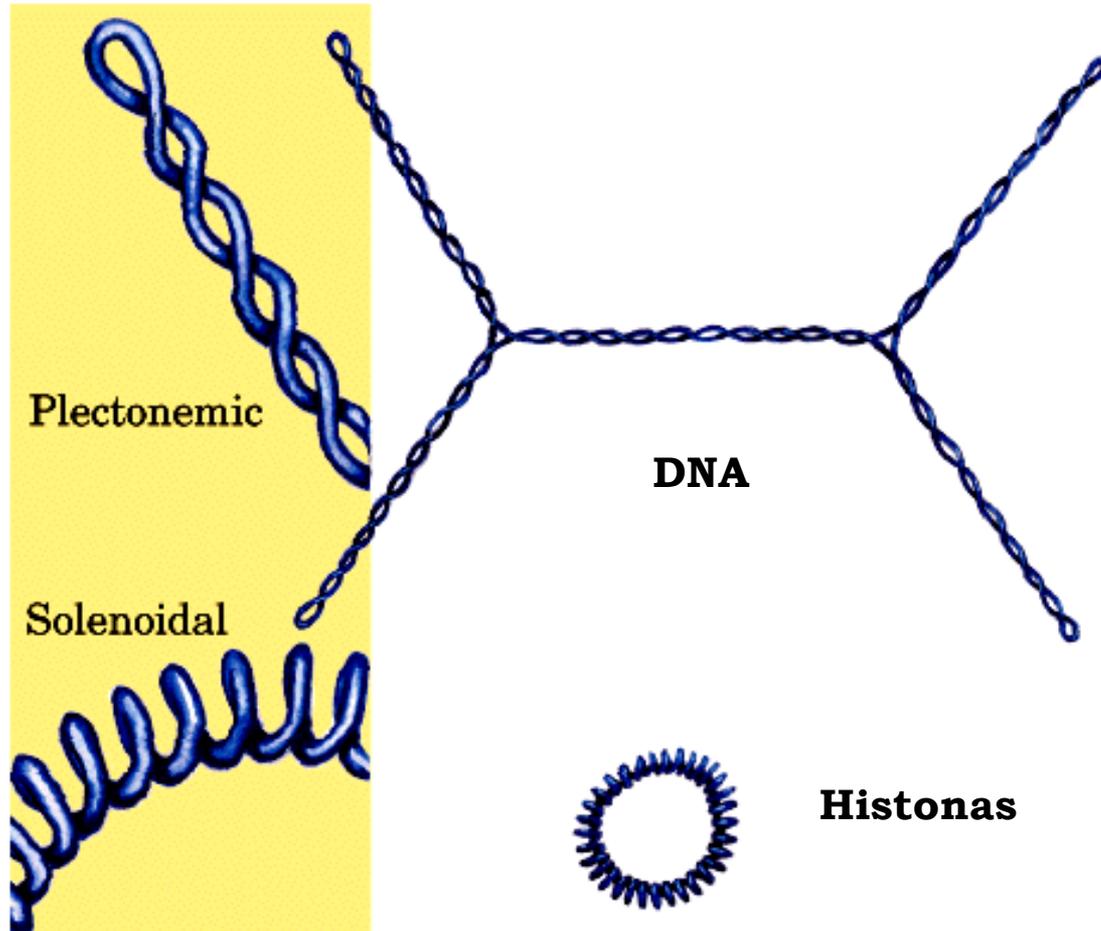


Superenrolamento negativo: gerado pelo desenrolamento da dupla hélice

Plectonêmico: encontrado no DNA em solução

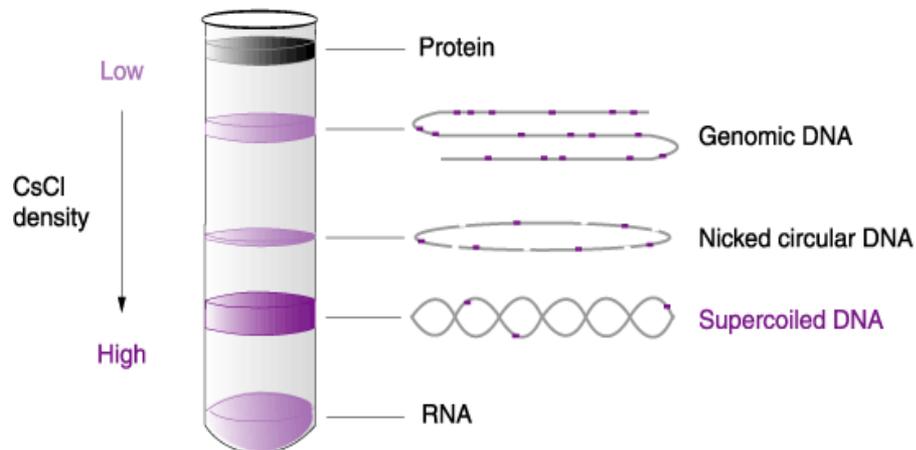
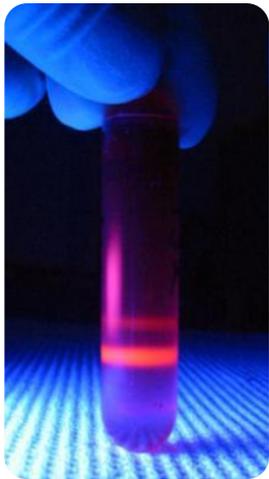
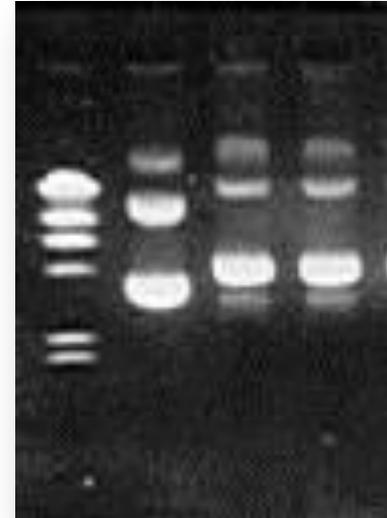
Toroidal: DNA enrolado em proteínas como as histonas, que formam os nucleossomos.

Tipos de superenrolamento



Efeitos do Superenrolamento

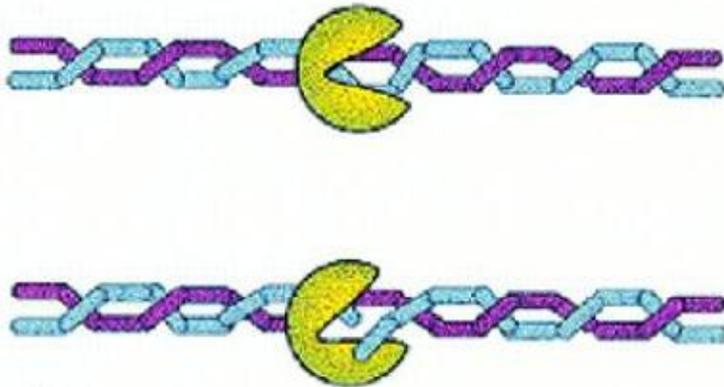
- Eletroforese em gel de agarose
- Microscopia eletrônica
- Velocidade de sedimentação



Topoisomerases

Enzimas que introduzem ou removem superenrolamentos no DNA

TOPOISOMERASE I



Fundamentais para:

- Transcrição
- Replicação
- Recombinação
- Remodelamento da cromatina

Sistema complexo de enzimas – Topoisomerases

Células Eucarióticas e Procarióticas

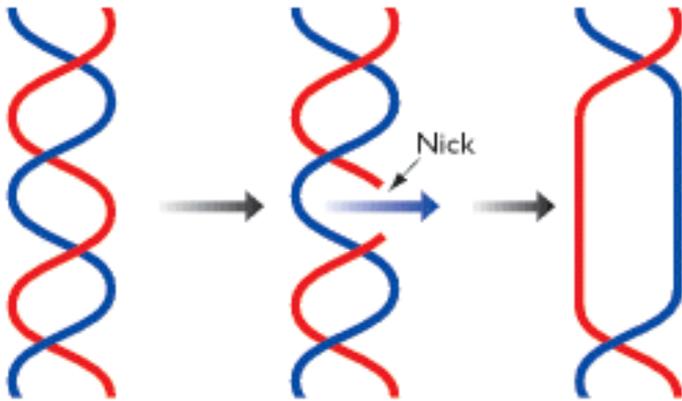
Manutenção da homeostasia do superenrolamento do DNA

Promovem a quebra transitória das Ligações Fosfodiéster na fita de DNA

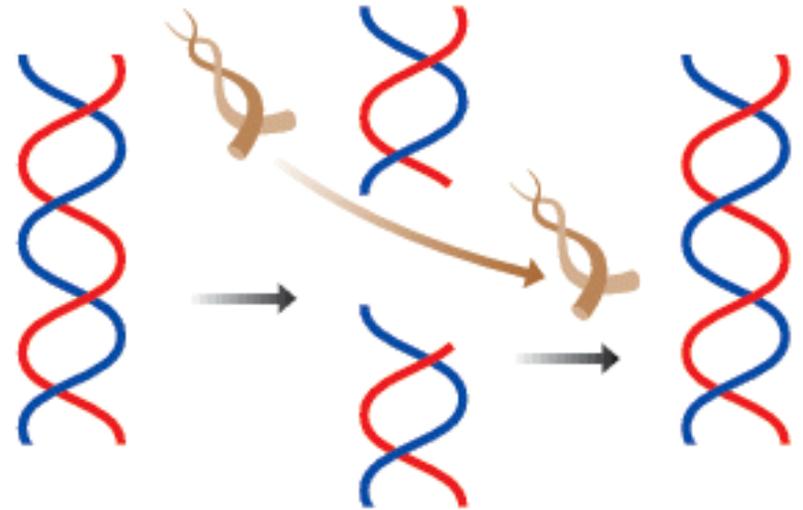
Topoisomerases

Enzimas que introduzem ou removem superenrolamentos no DNA

(A) Type I



(B) Type II



Tipos de DNA

Composição de bases, meio em que se encontram ou sua ligação com proteínas

Se diferenciam pela sua espessura, número de pares de base por volta da hélice e a exposição das bases ao meio externo

- **DNA B**

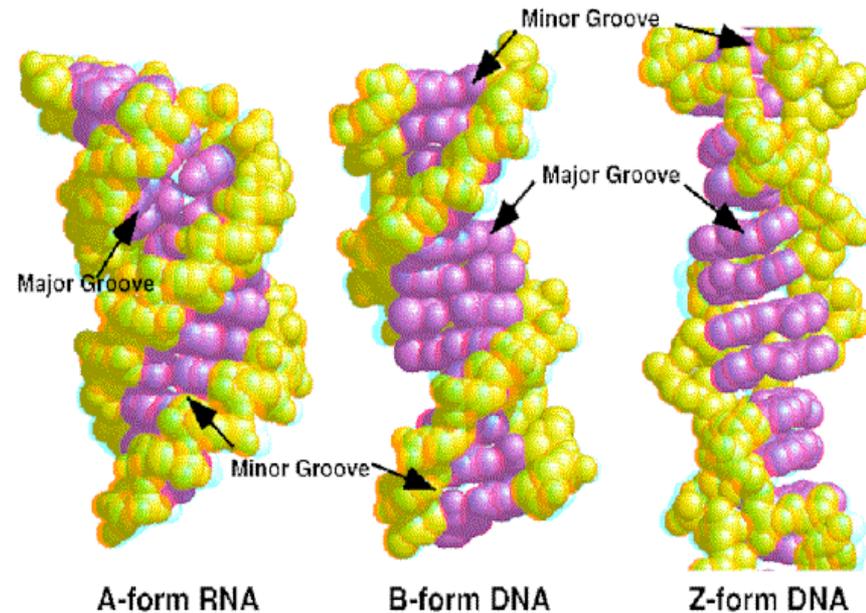
- É a forma mais abundante na célula (fisiológico)
- É a forma clássica do DNA
- A dupla hélice gira para a direita
- Volta completa a cada 10,4 pb

- **DNA A**

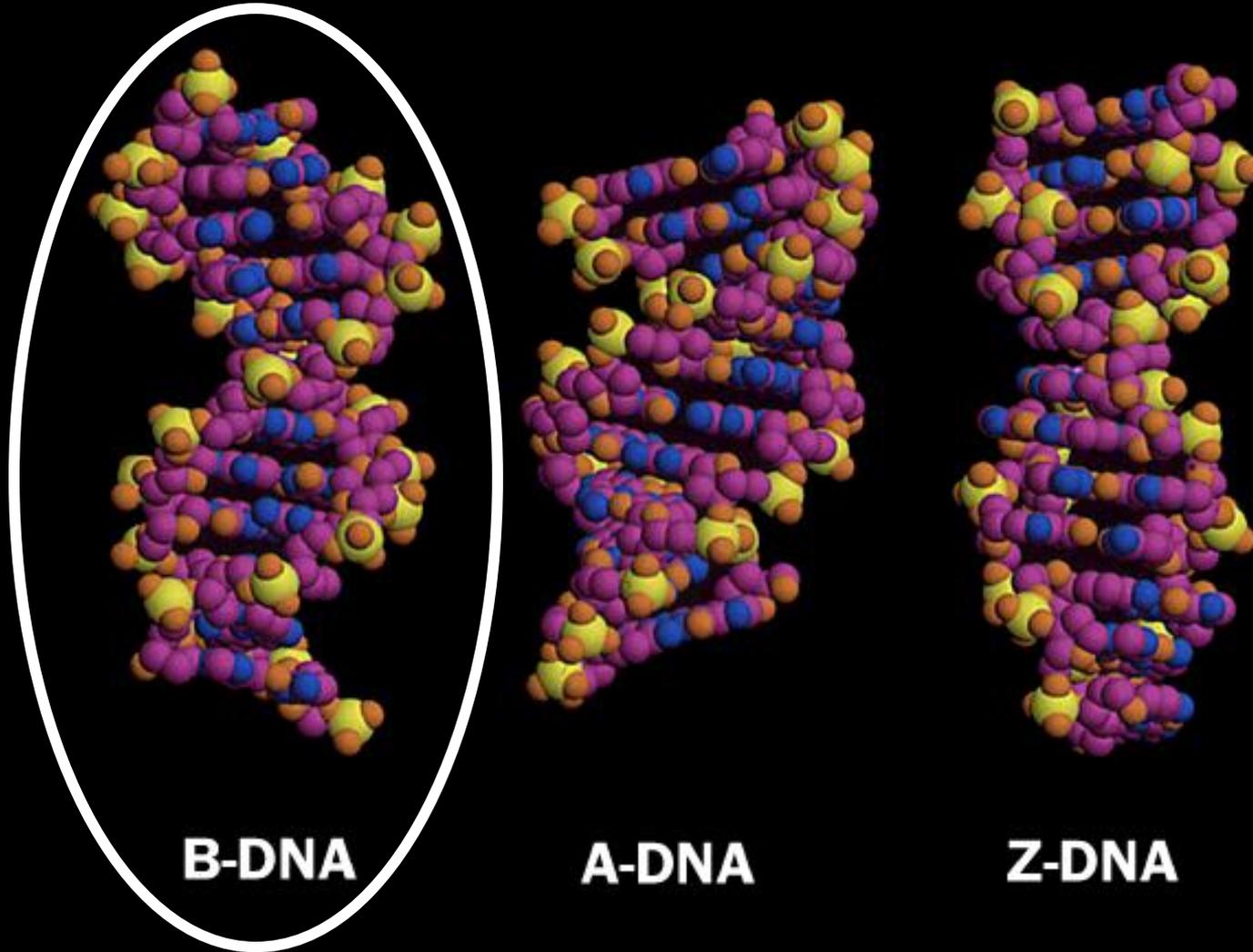
- Condições de umidade muito baixa
- Forma mais “compacta; curta”
- Encontrado nos híbridos DNA:RNA (transcrição)
- RNA:RNA (genoma de vírus)

- **DNA Z**

- Forma mais “estreita”
- Sequências GC repetidas
- A dupla hélice gira para a esquerda
- Altas concentrações de cátions
- Volta completa a cada 12 pb



A Dupla Hélice



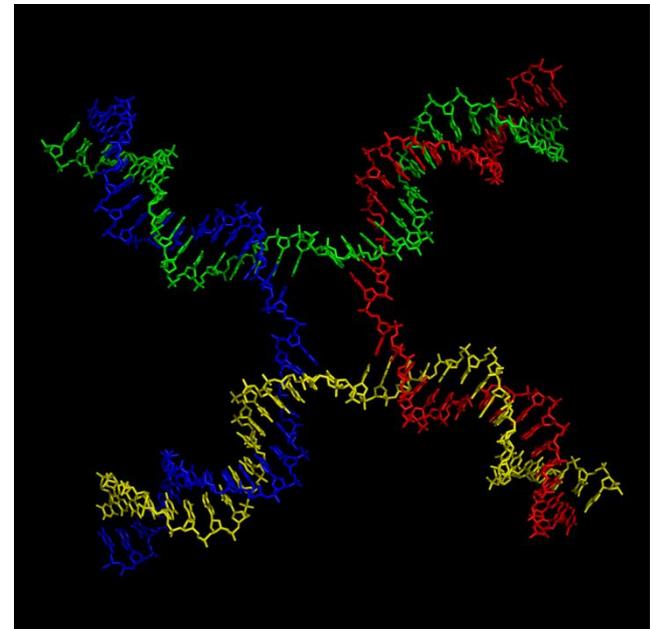
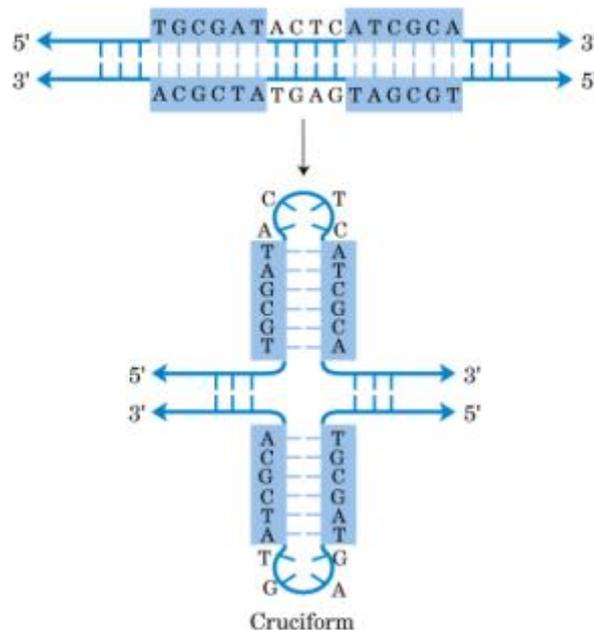
O B-DNA é o predominante em condições fisiológicas

Outras estruturas do DNA

Moléculas de DNA de fita simples – *vírus e bacteriófagos*

Estruturas cruciformes – regiões simétricas

Junções de Holliday – 4 fitas de DNA - *recombinação*



DIFERENÇAS ENTRE DNA E RNA

DNA

•Bases Nitrogenadas

– Adenina

– Guanina

– Citosina

– **Timina**

Purinas

Pirimidinas

RNA

– Adenina

– Guanina

– Citosina

– **Uracila**

•Açúcar

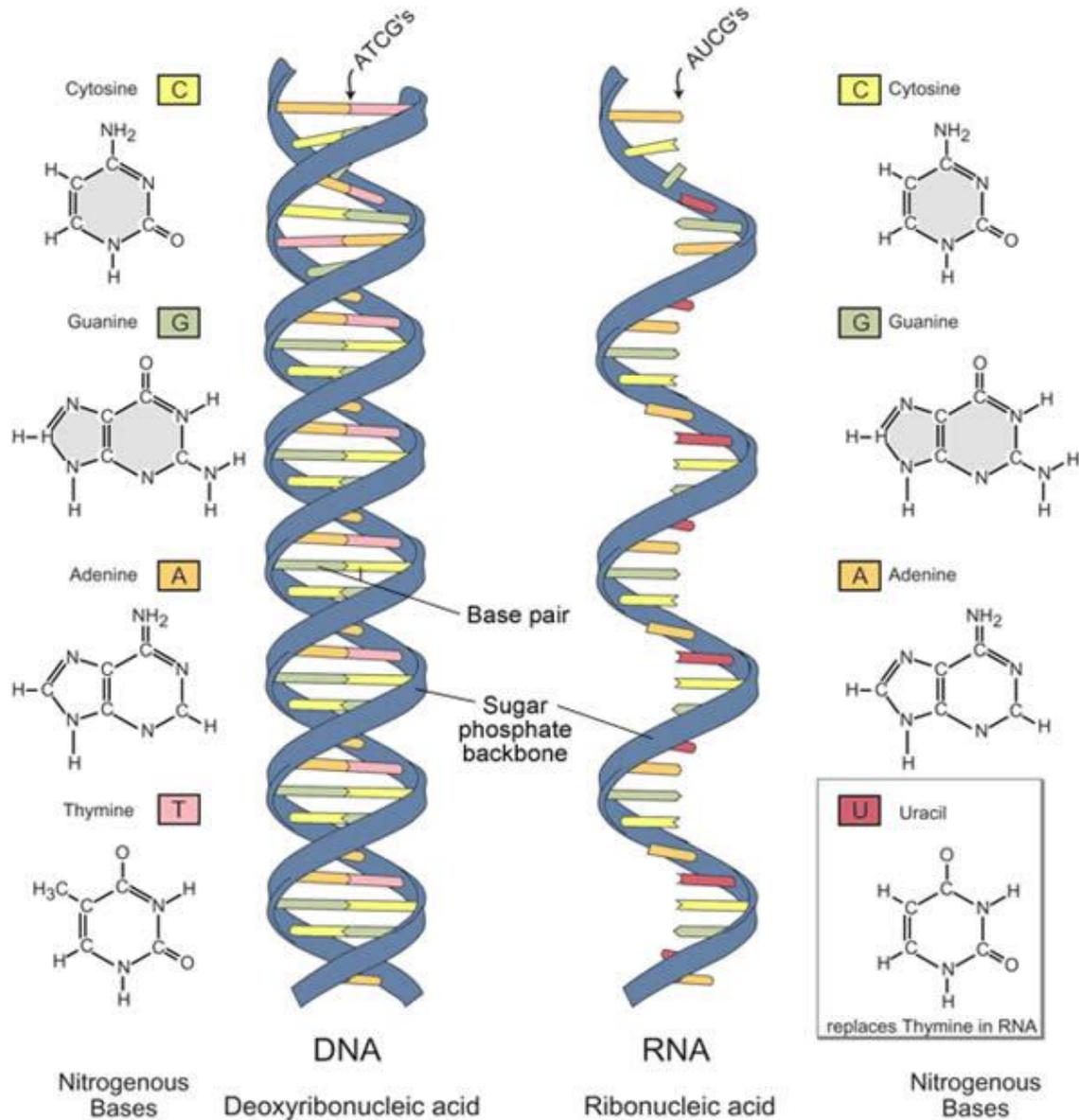
Desoxirribose

Ribose

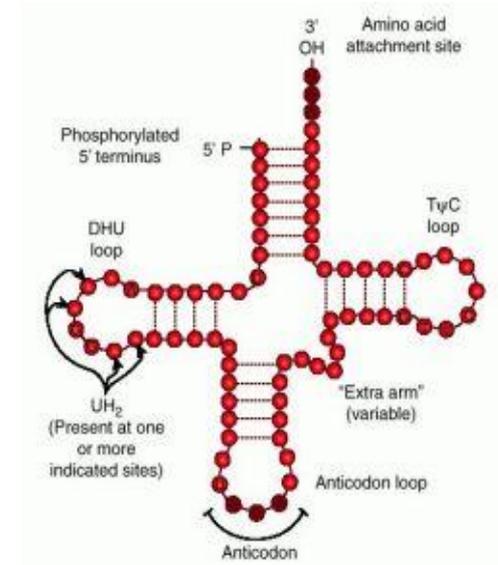
•Fita **dupla**

•Fita **simples**

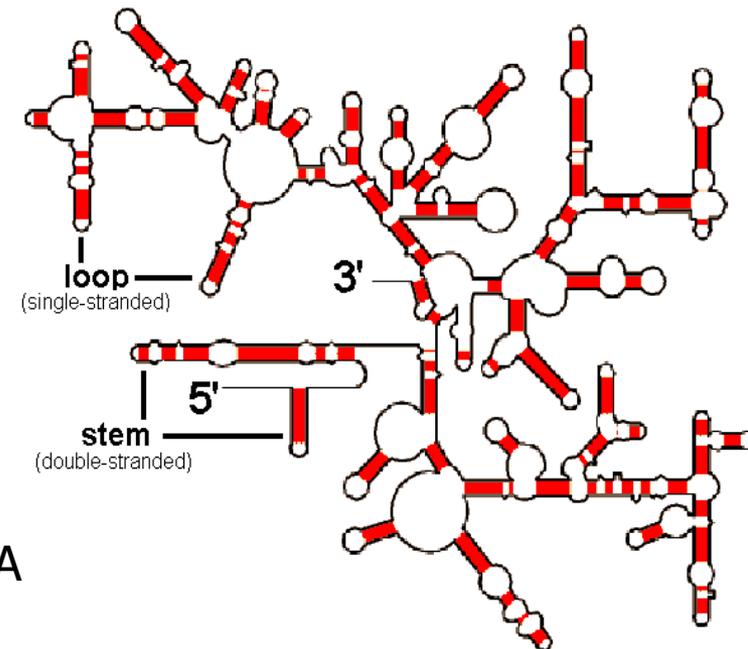
DIFERENÇAS ENTRE DNA E RNA



Estrutura do RNA



tRNA



rRNA

- **mRNA:** transfere a informação genética aos ribossomos (1 a 5 % do RNA total)
- **rRNA:** componente dos ribossomos – síntese de proteínas (75 % do RNA total)
- **tRNA:** transporta os aa até os ribossomos para a síntese de proteínas (10 a 15 % do total)

- snRNAs: RNAs nucleares pequenos
- scRNAs: RNAs citoplasmáticos pequenos
- snoRNAs: RNAs nucleolares pequenos



Manutenção básica
do metabolismo da
célula

RNAs não codificadores curtos:

- miRNAs: microRNAs
- siRNAs: RNAs de interferência
- piRNAs

RNAs não codificadores longos: funções
regulatórias ainda não elucidadas



Envolvidos na
regulação da
expressão dos genes

Dogma Central da Biologia Molecular

