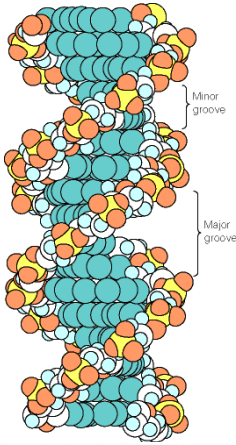
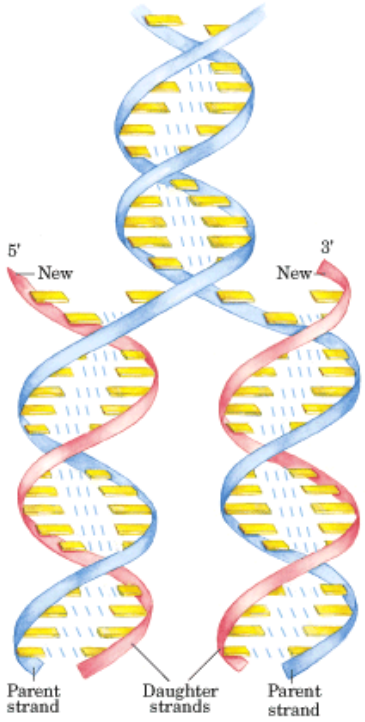
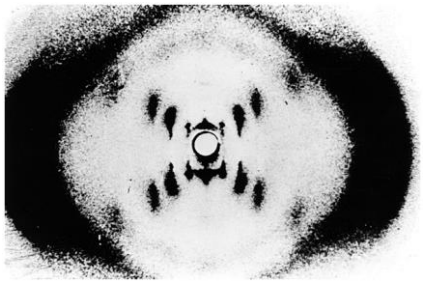
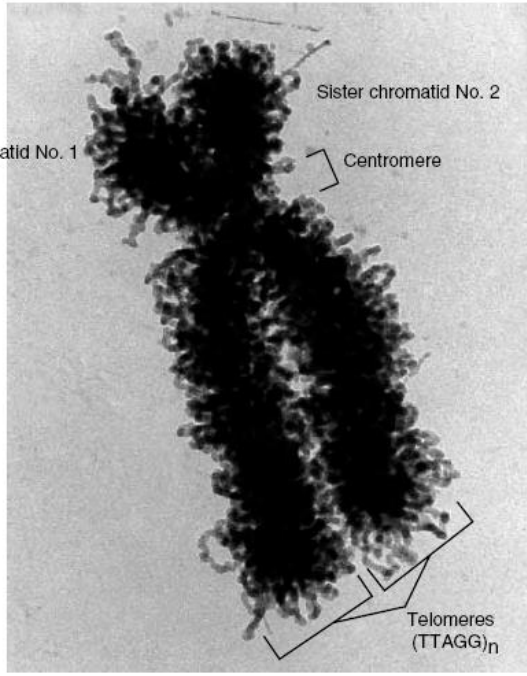
TEMA 6.
NUCLEÓSIDOS, NUCLEÓTIDOS Y ACIDOS NUCLEICOS

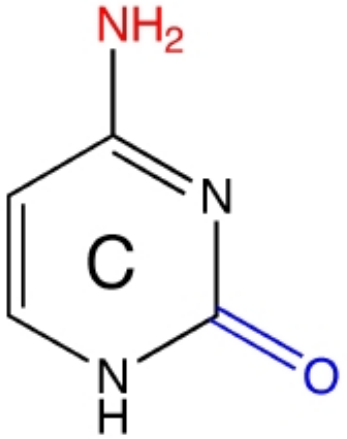




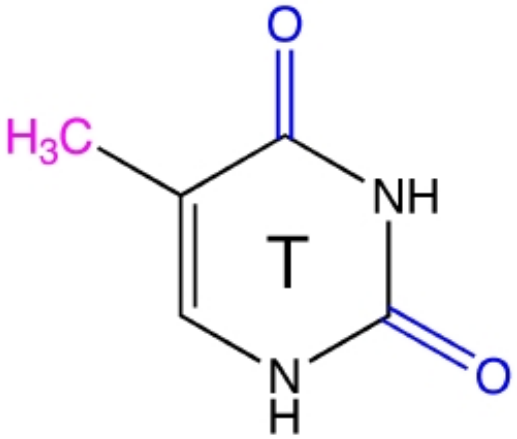




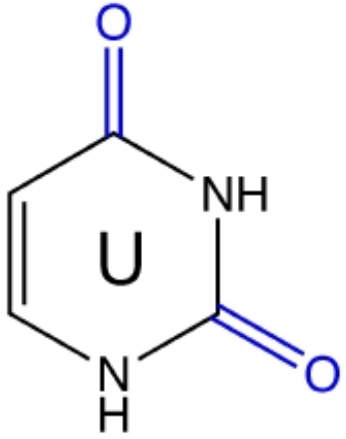
Las bases nitrogenadas presentes en los ácidos nucleicos



Citosina
2-0xo-4-aminopirimidina

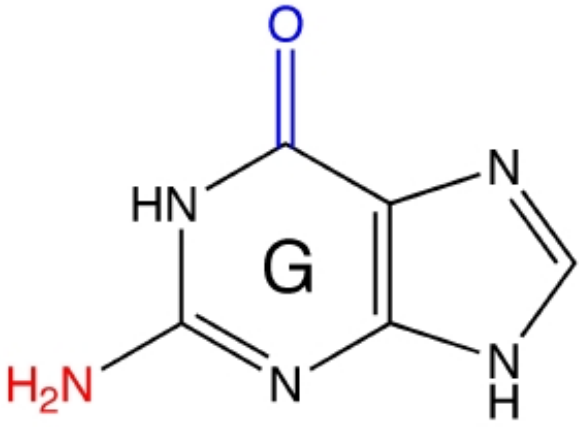


Timina
2,4-Dioxo-5-metilpirimidina



Uracilo
2,4-Dioxopirimidina

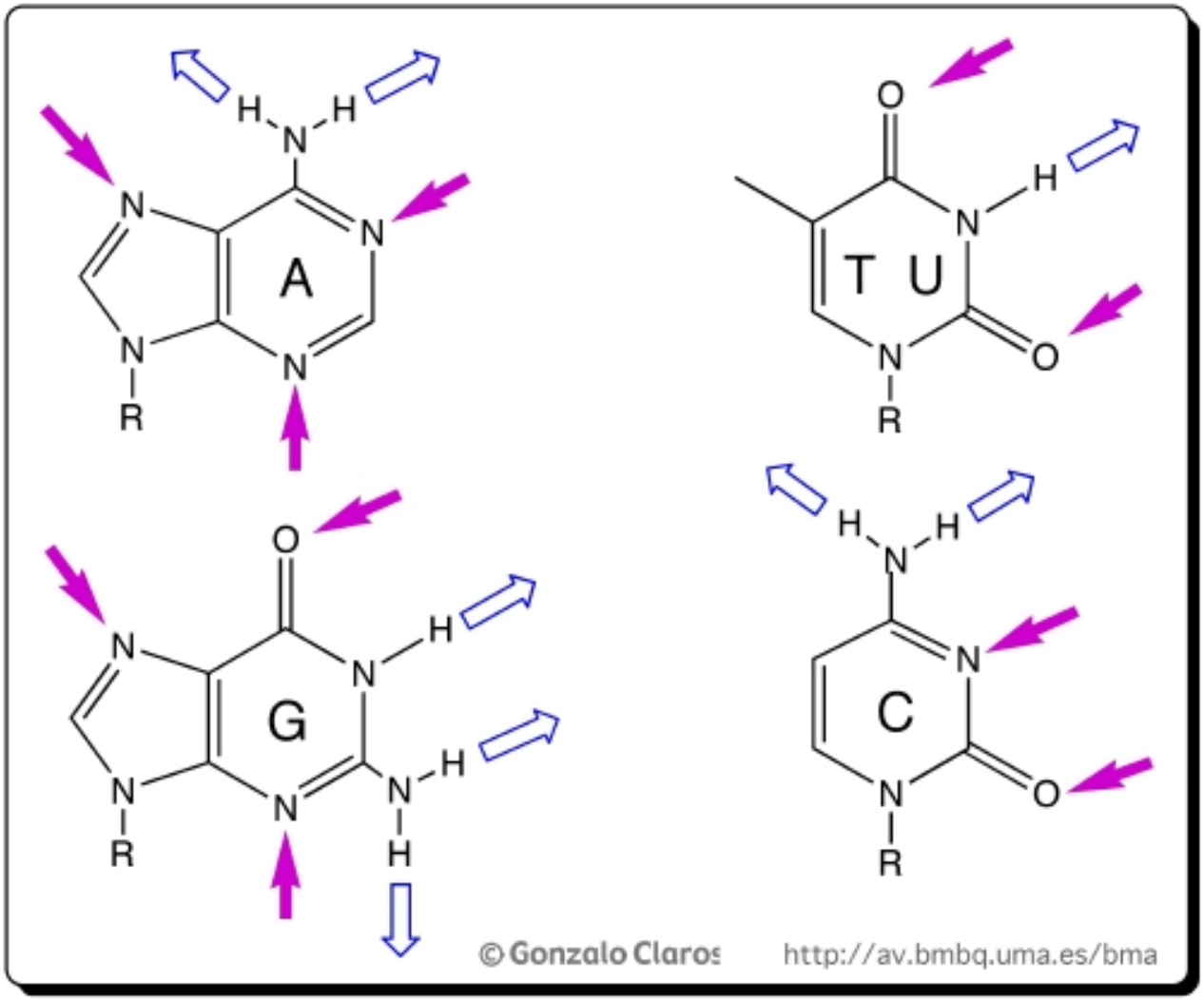
Adenina
6-Aminopurina



Guanina
2-Amino-6-oxopurina

- Dipolos

Las bases tienen átomos muy electronegativos (N y O) dentro y fuera del anillo aromático, por lo que existe una atracción asimétrica de los electrones de la molécula y, por tanto, se forman dipolos que permiten formar puentes de hidrógeno.



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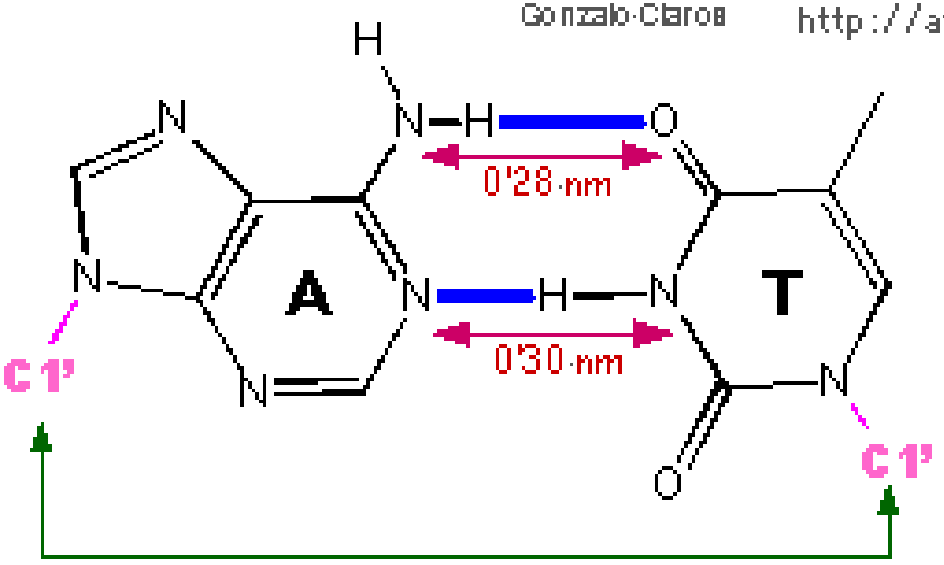
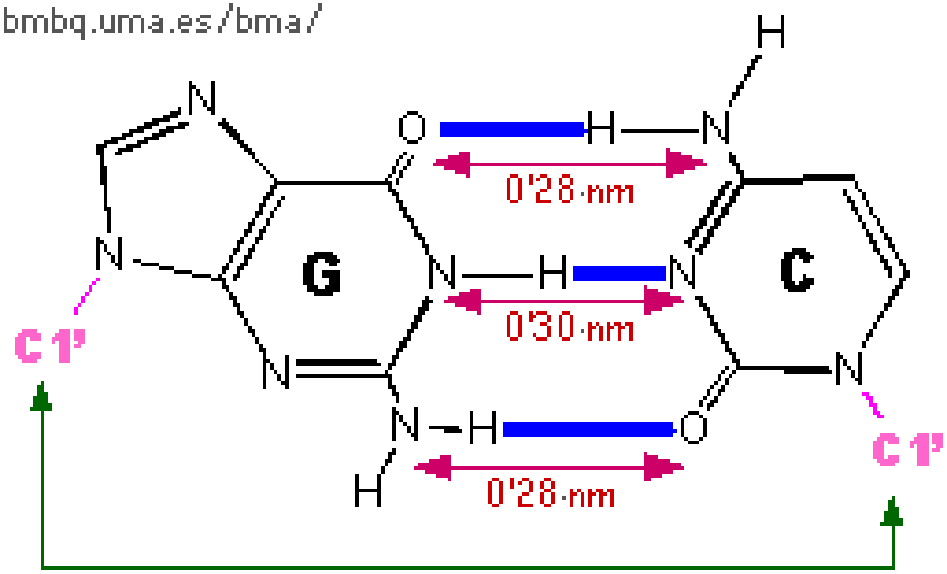


1'11 nm



1'08 nm

Tautomería

Watson y Crick postularon la tautomería de las bases para explicar que se puedan aparear de manera distinta a la que ellos proponían y generar mutaciones espontáneas.

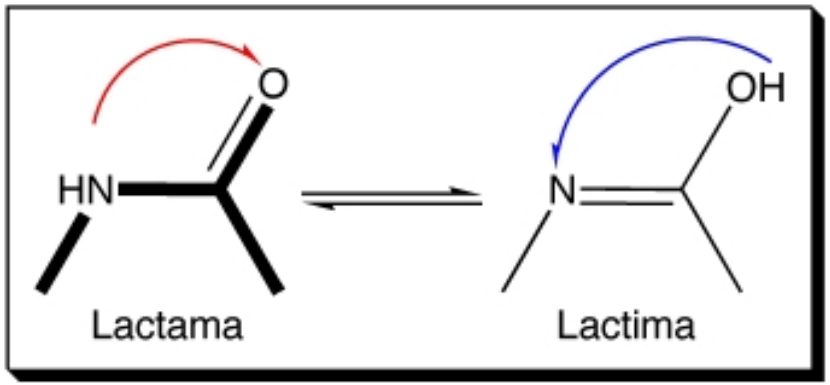
Hay dos tipos de tautomería:

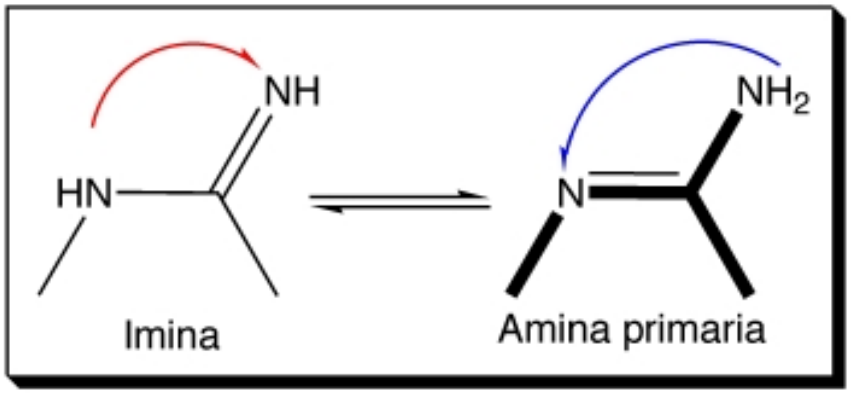
Tautomería ceto-enólica (lactama-lactima)

Interconvierte un grupo ceto (=O) y enol (—OH) extracíclicos cerca de un N cíclico. Se puede producir en la G, C, T y U.

Tautomería imina-amina

Interconvierte un amino (—NH) extracíclico en un imino (=NH) cerca de un N cíclico. Se puede dar en G, A y C.





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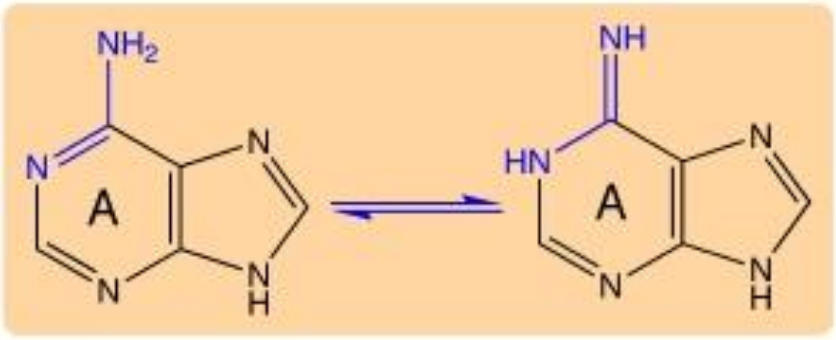

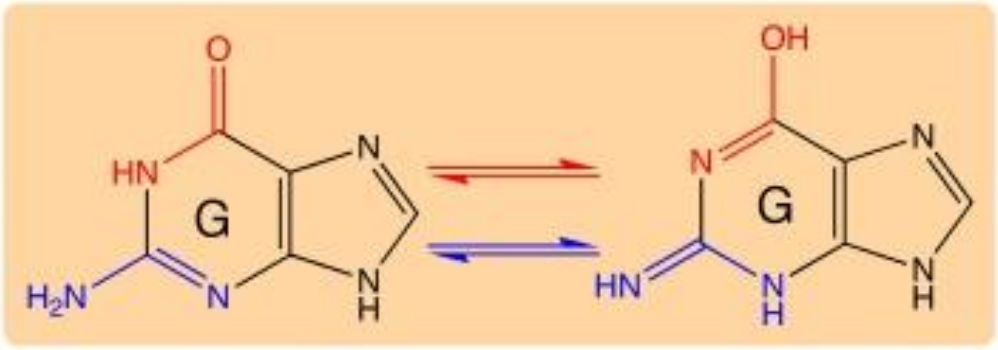

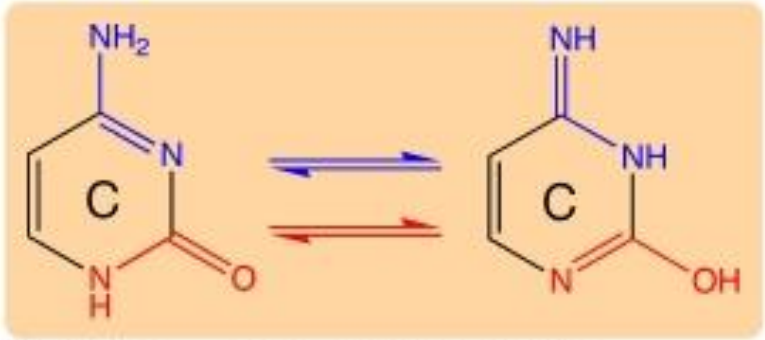

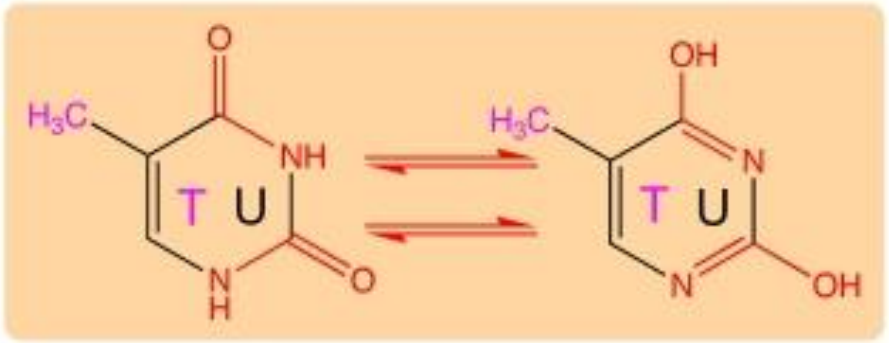

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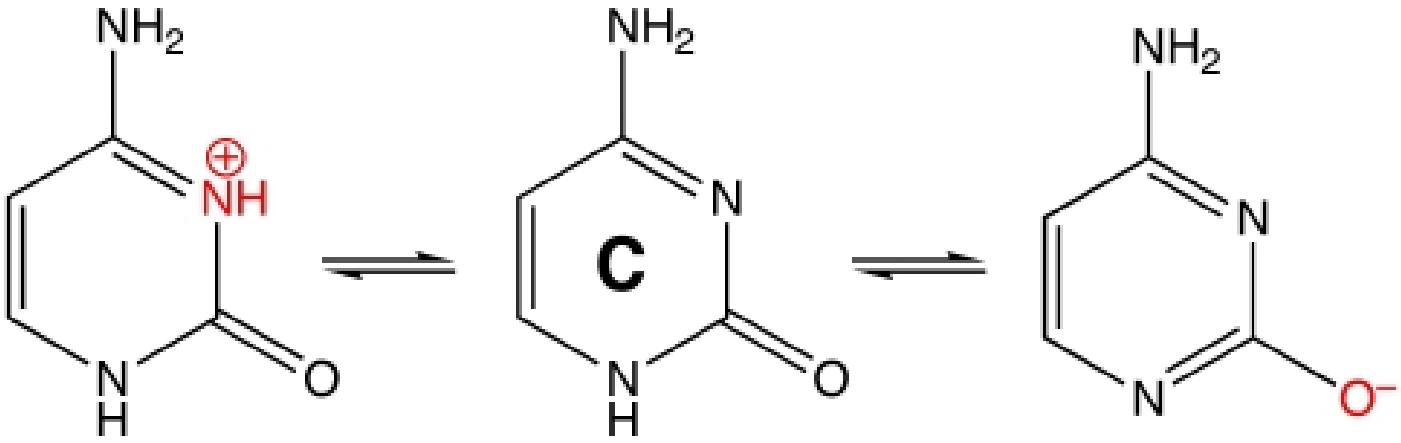
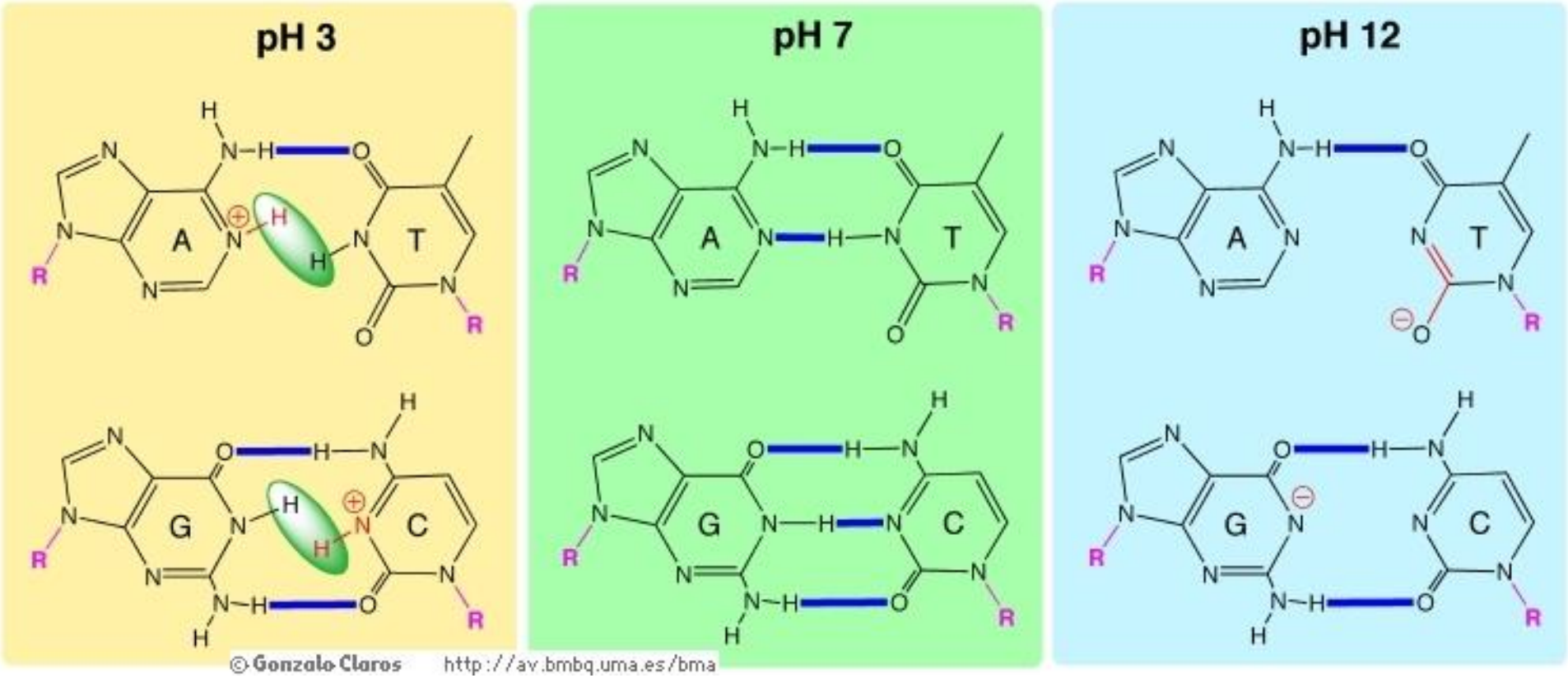
Naturaleza básica

Todas las bases nitrogenadas son bases débiles
aunque los grupos ceto (=O) pueden tautomerizar a enol (–OH), perder el proton y conferir cierta acidez (lactimas).



Al no tener ningún grupo ceto, Adenina es la más básica de todas.


pH 3
pH 7
pH 12
A
T
G
C
R
H
N
O
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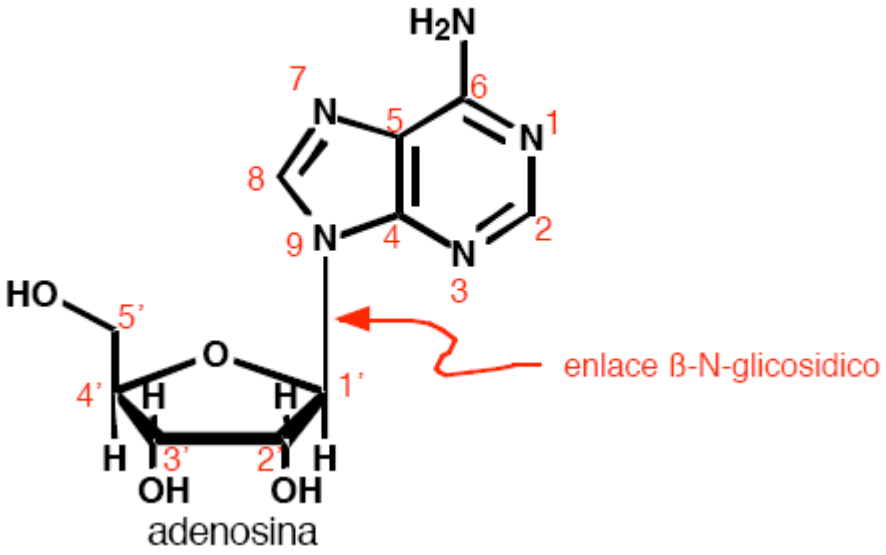
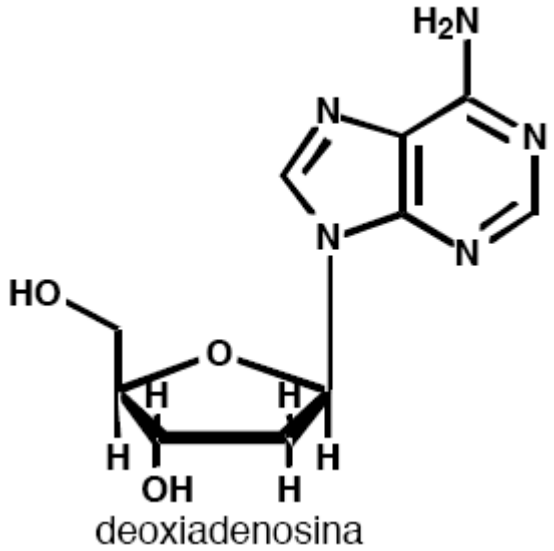


9–β-Ribofuranosiladenina



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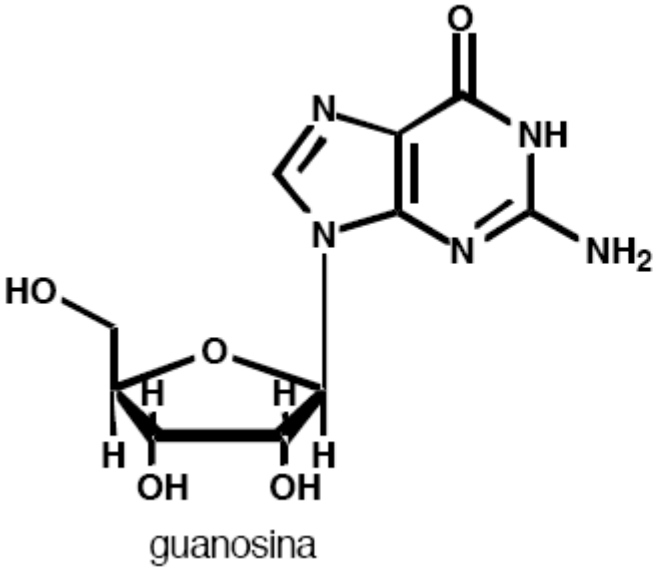
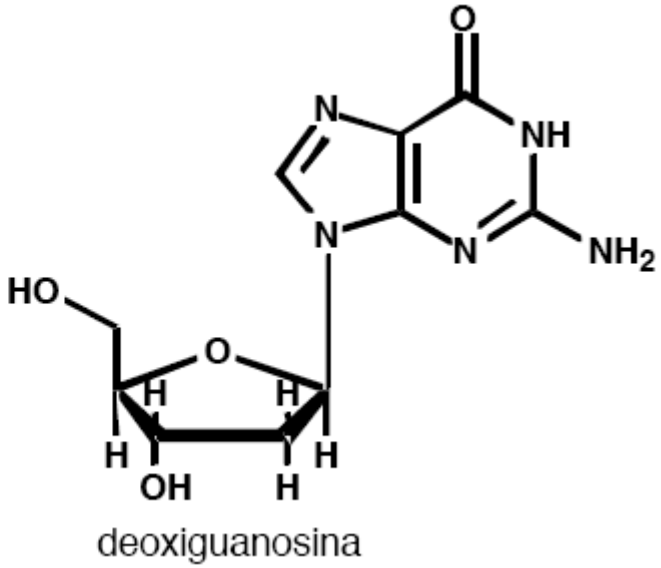
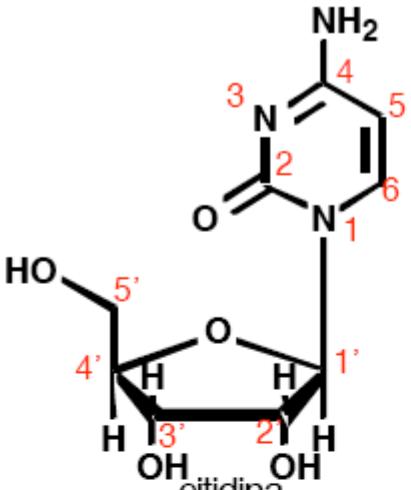


9–β-Ribofuranosilguanosina



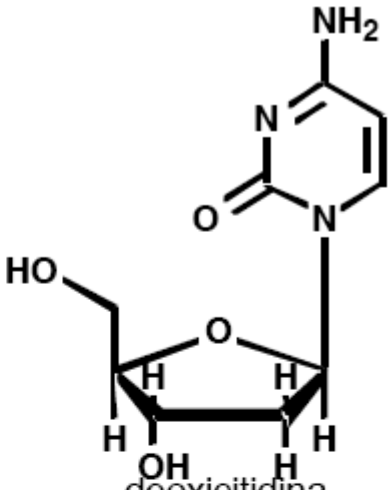
9–β-Desoxiribofuranosilguanosina

1–β-Ribofuranosilcitosina

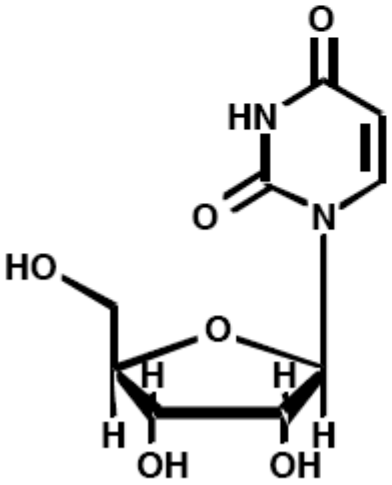


citidina

1–β-Desoxiribofuranosilcitosina

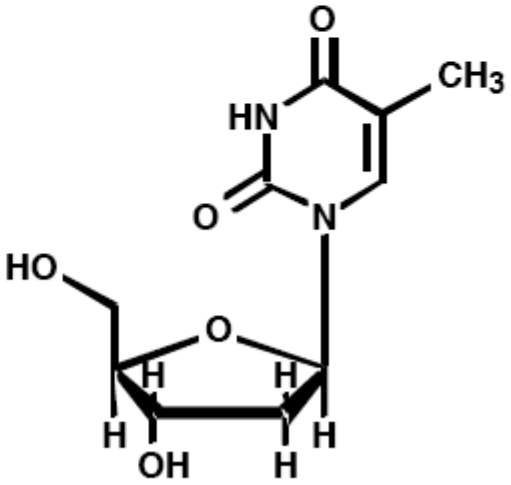


deoxicitidina



uridina

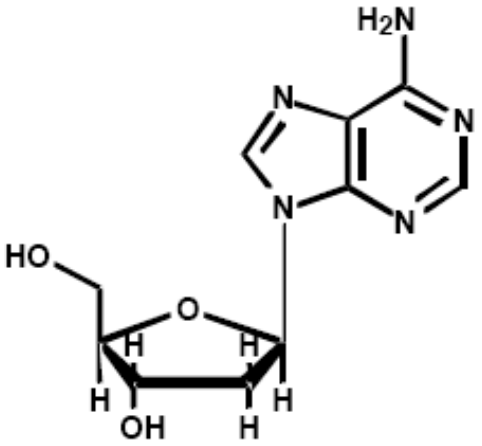
1–β-Ribofuranosiluracilo



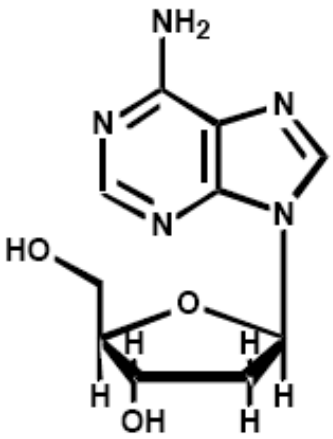
timidina
(deoxitimidina)

1–β-Desoxiribofuranosiltimina

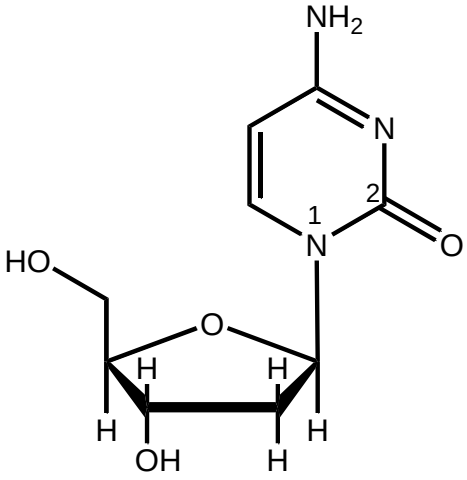
Conformaciones *sin* y *anti* de la deoxiadenosina



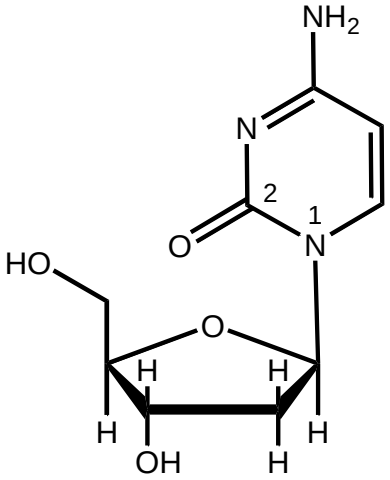
anti-deoxiadenosina



sin-deoxiadenosina



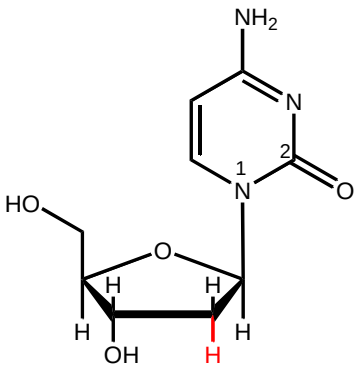
***anti*-deoxicitidina**



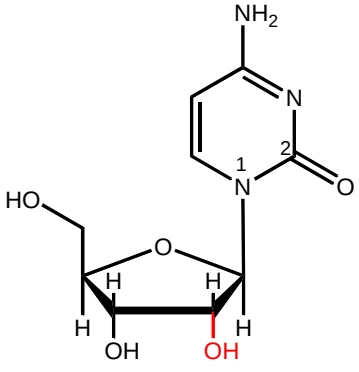
***sin*-deoxicitidina**

Hidrólisis química en medio ácido

Los mecanismos deben justificar las diferencias de velocidad observadas entre ribonucleósidos y desoxinucleósidos

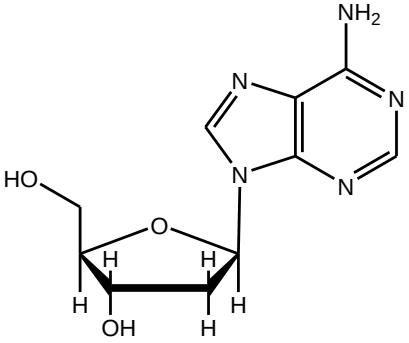


mayor velocidad

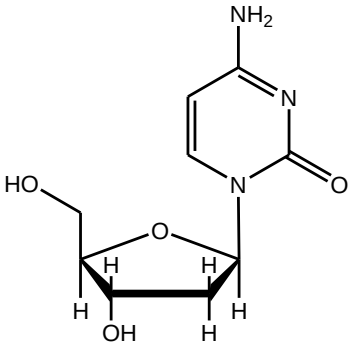


menor velocidad

Y las diferencias de velocidad observadas entre nucleósidos de bases púricas y de bases pirimídicas

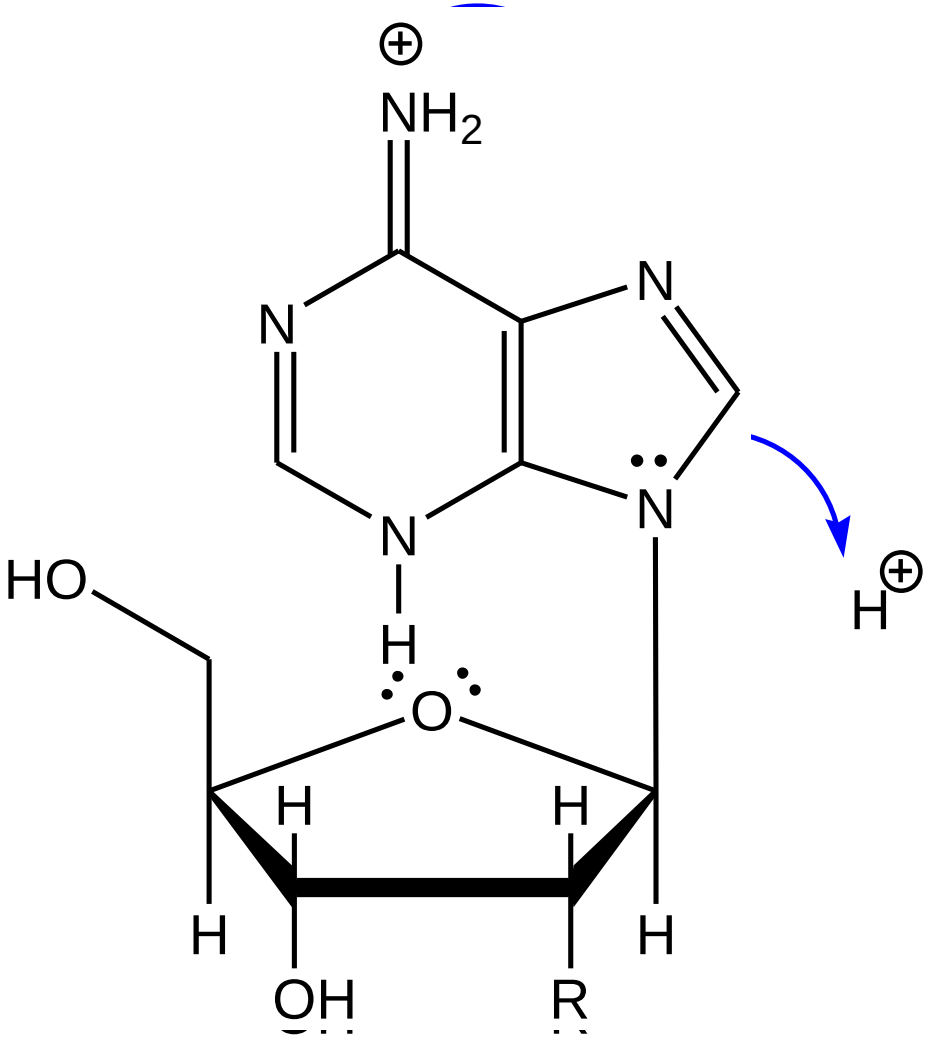


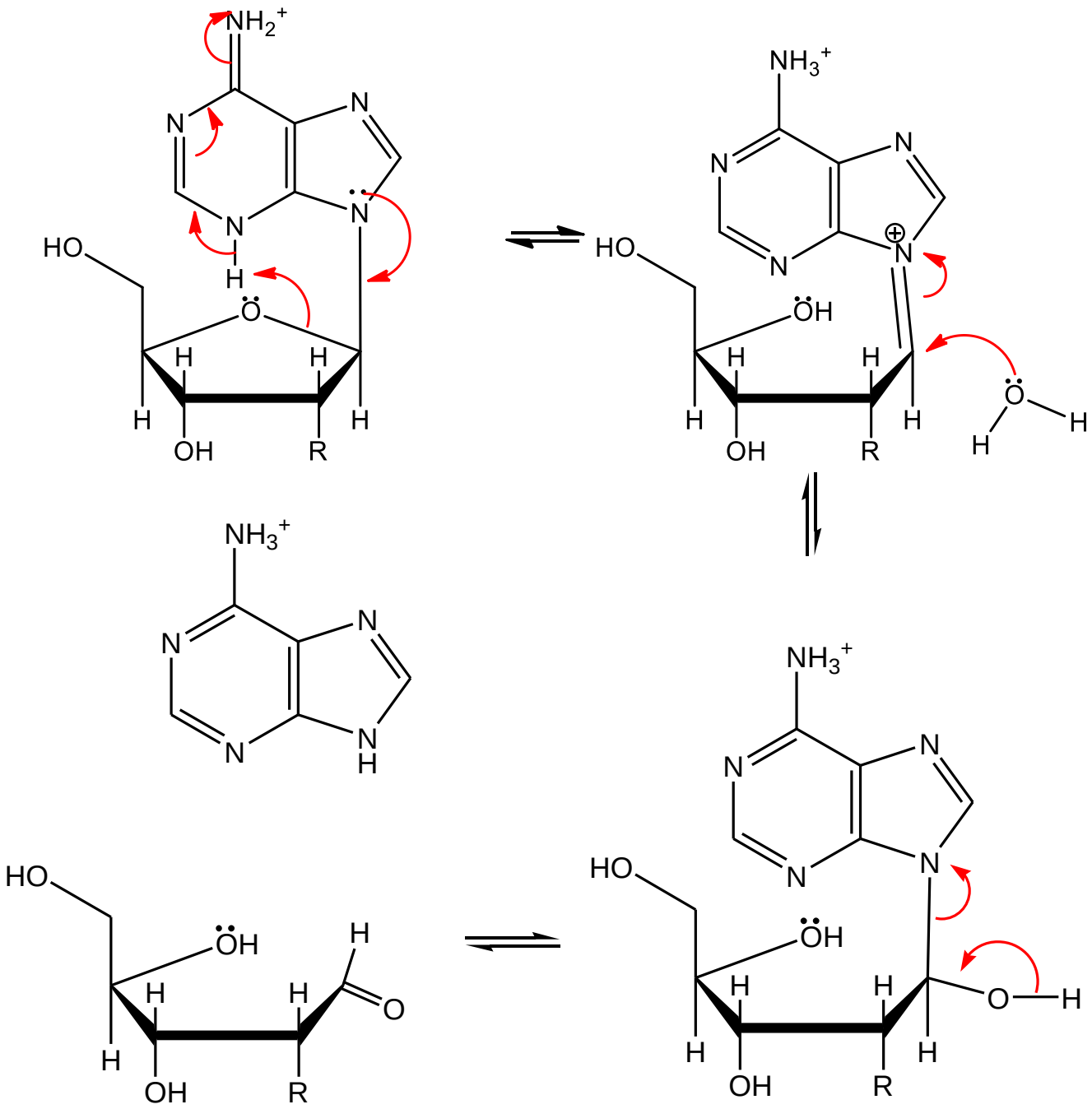
mayor velocidad



menor velocidad

Hidrólisis ácida de nucleósidos




NH2+
N
N
N
N
H
HO
O
H
H
H
H
OH
R
NH3+
N
N
N
N
H
HO
OH
H
H
H
H
OH
R
O
H
H
NH3+
N
N
N
N
H
HO
OH
H
H
H
H
OH
R
O
H
NH3+
N
N
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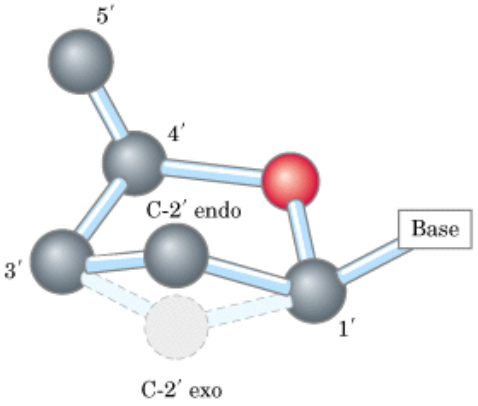
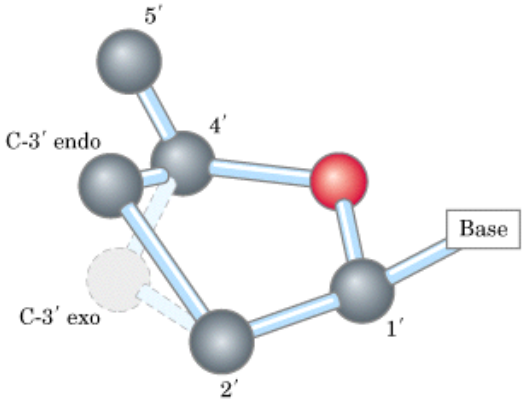
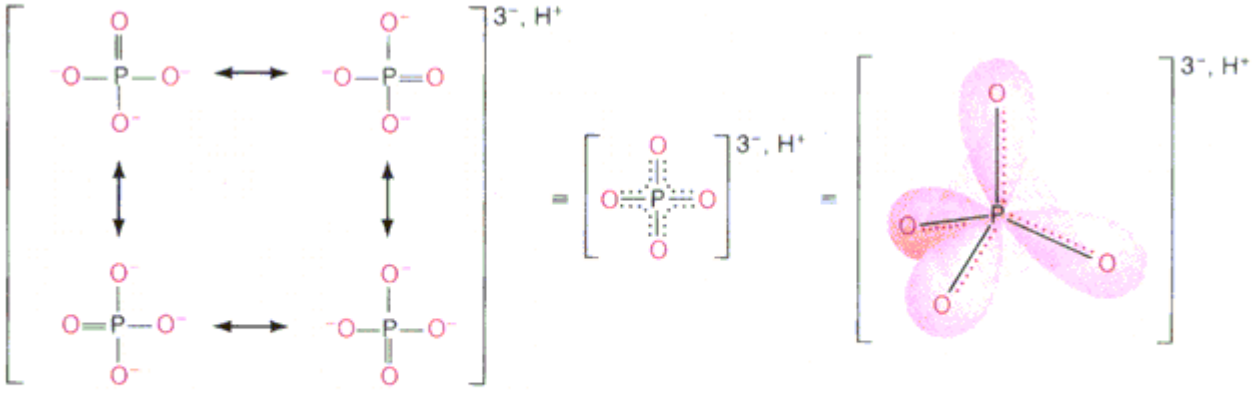
OH
H
H
H
H
OH
R
O
H





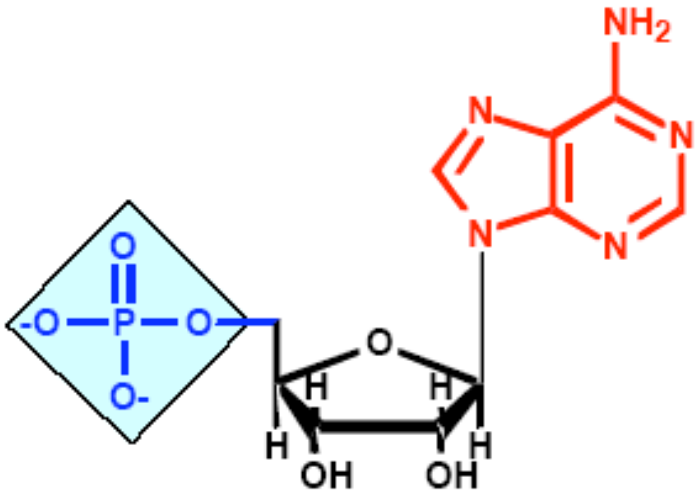


(a) Structures of phosphate ion contributing to resonance stabilization

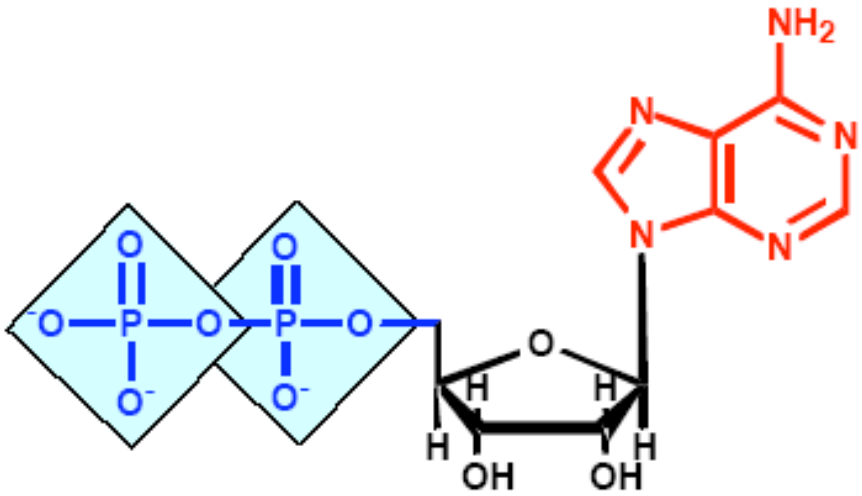
(b) Resonance hybrid

(c) Molecular orbitals of tetrahedral phosphate ion

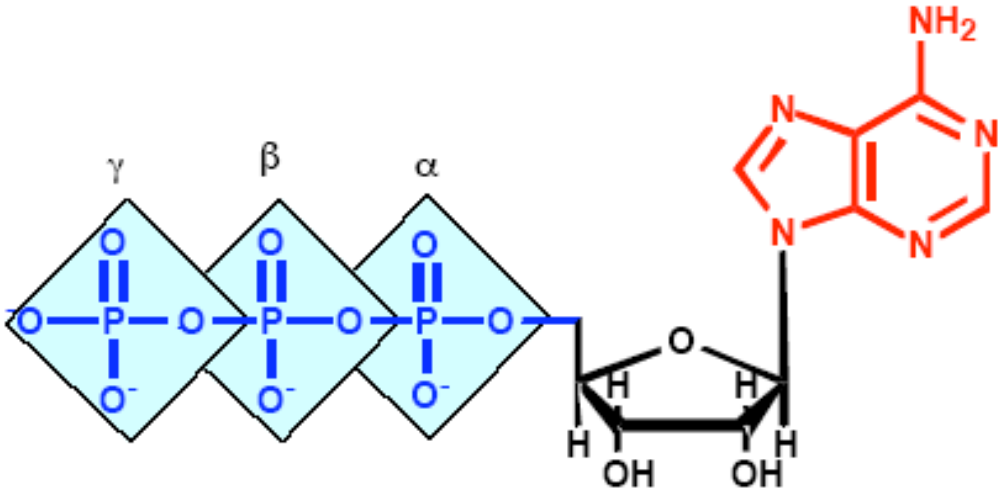
Nucleótidos



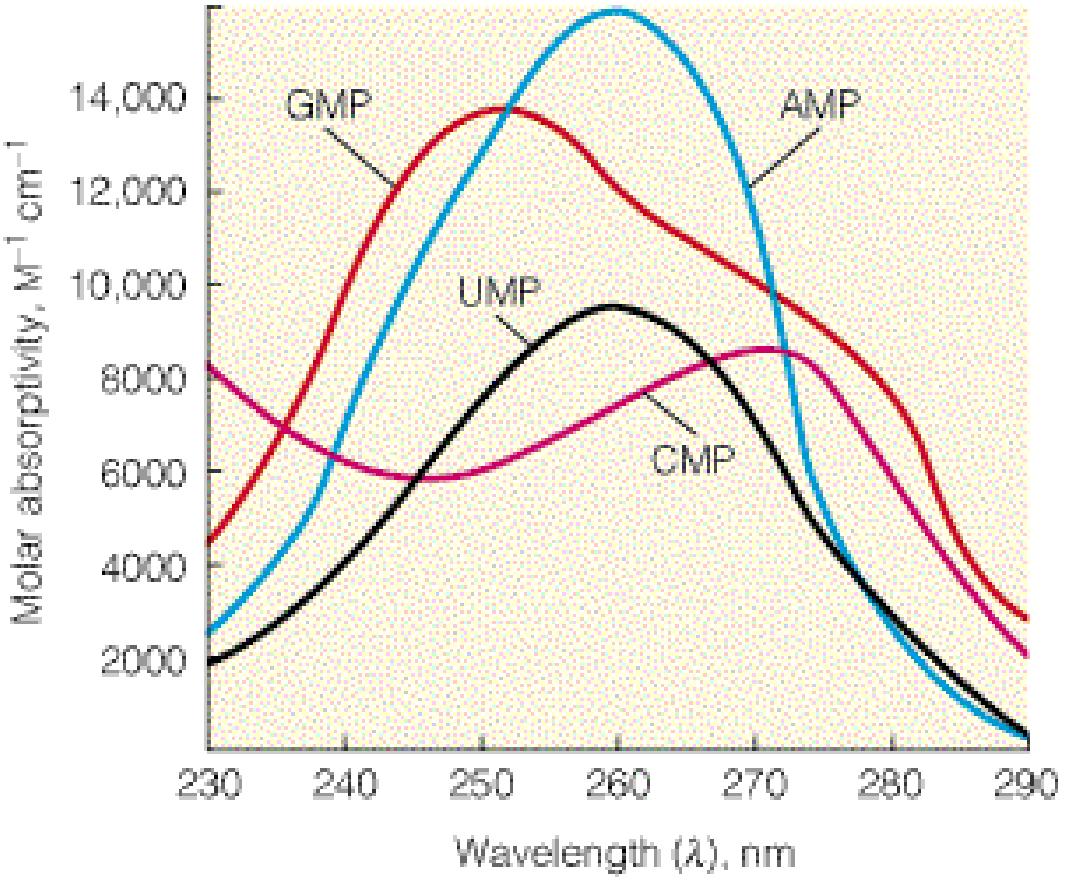
adenosina 5'-monofosfato
(AMP)



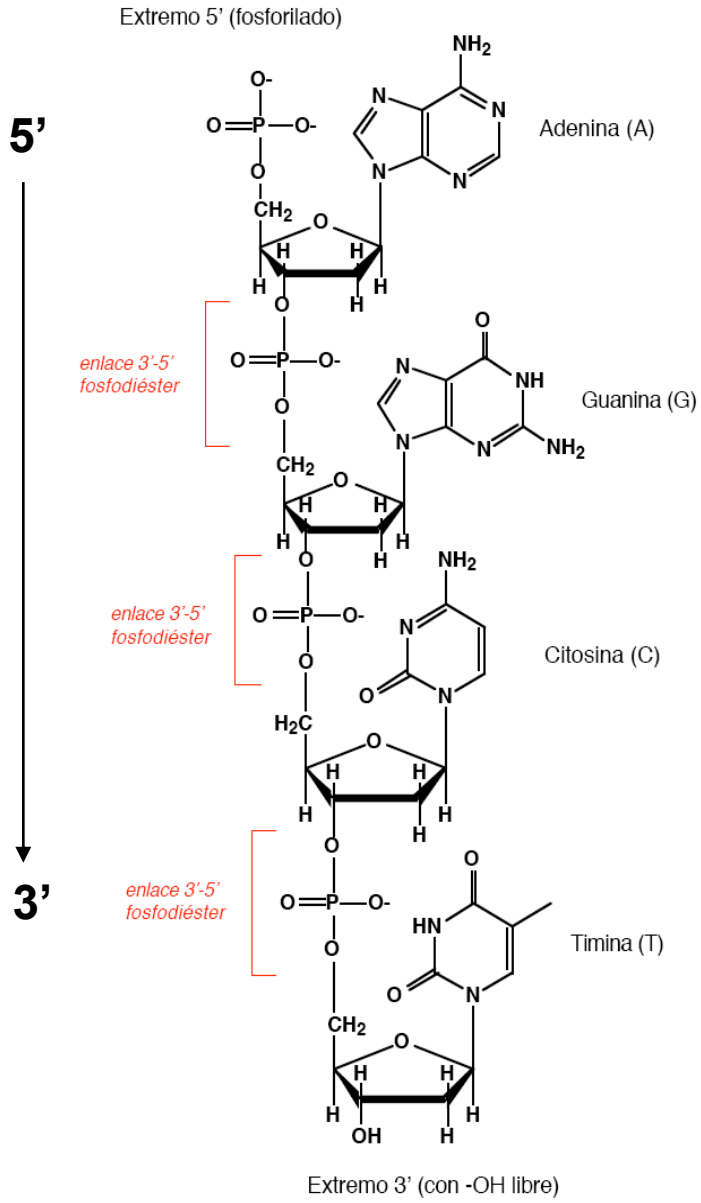
adenosina 5'-difosfato
(ADP)

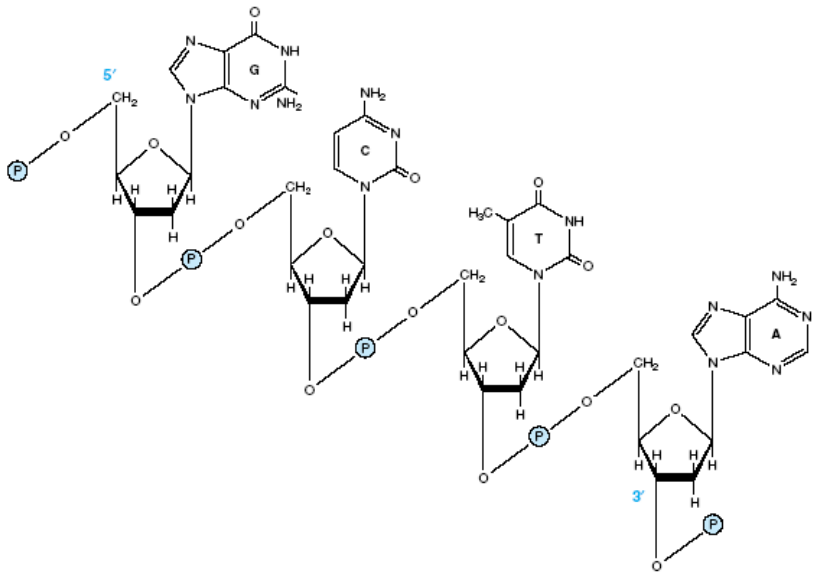


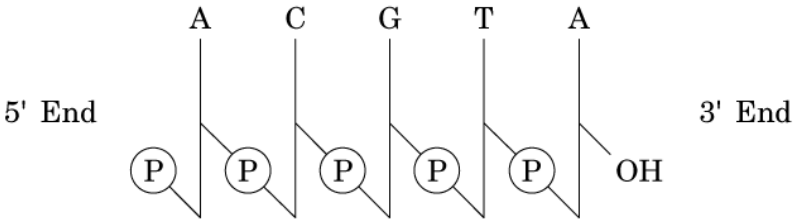
adenosina 5'-trifosfato
(ATP)


14,000
12,000
10,000
8000
6000
4000
2000
Molar absorptivity, M^{-1} cm^{-1}
GMP
AMP
UMP
CMP
230
240
250
260
270
280
290
Wavelength (λ), nm

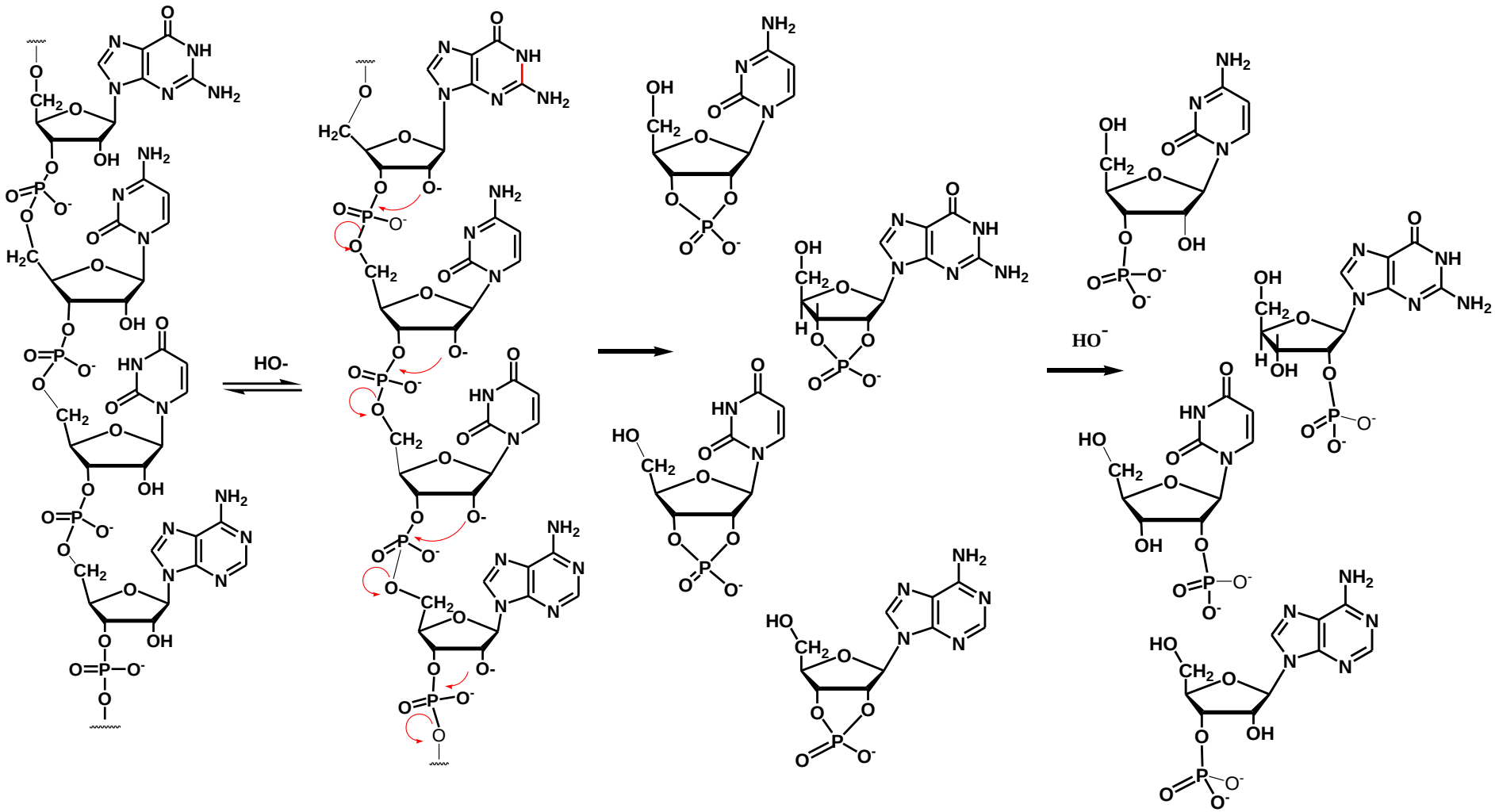
Estructura primaria del los ácidos nucleicos



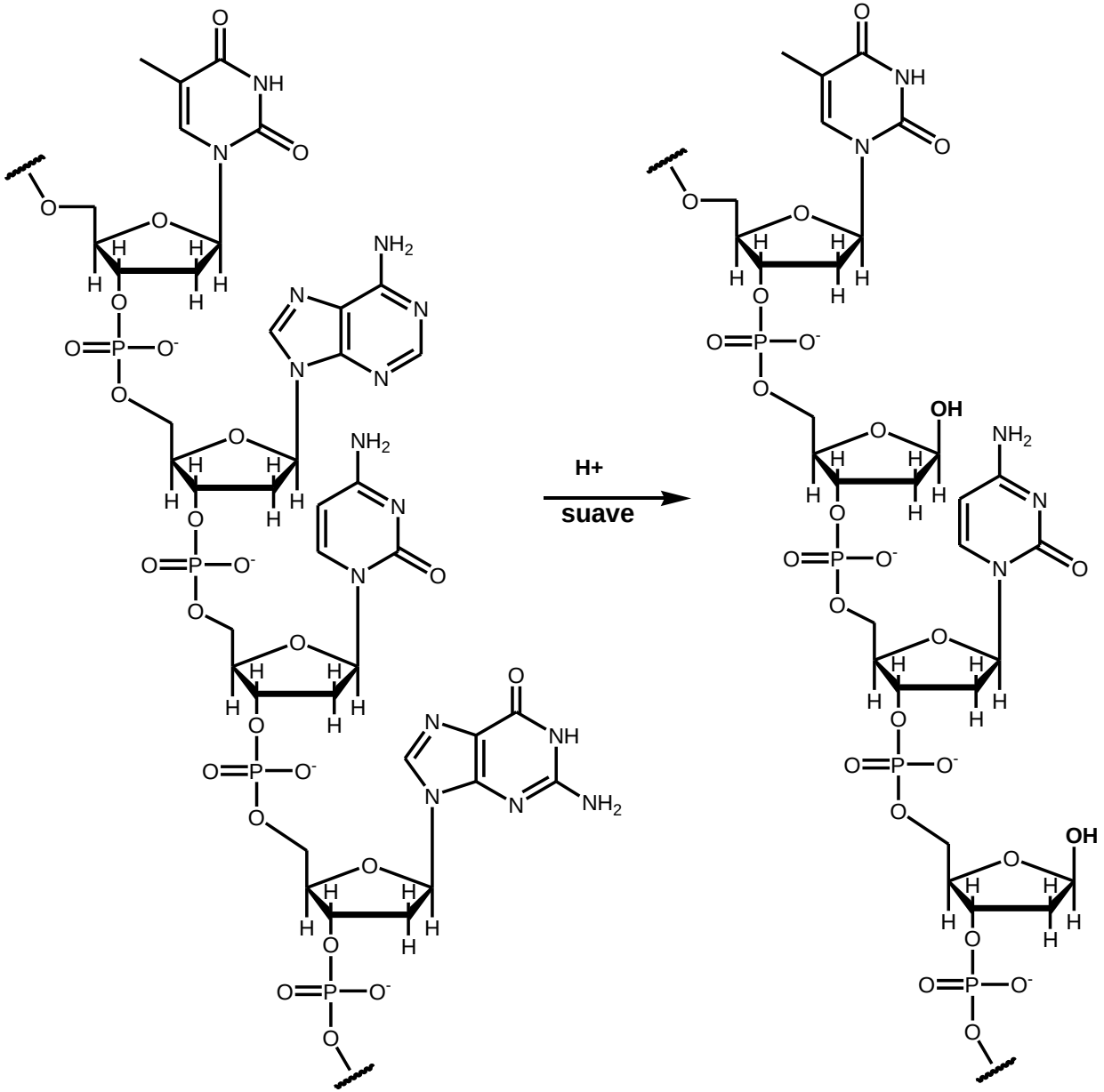




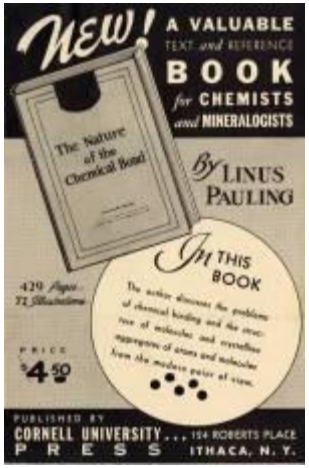
Hidrólisis alcalina suave de ARN



Hidrólisis ácida suave de un polidesoxiribonucleótido



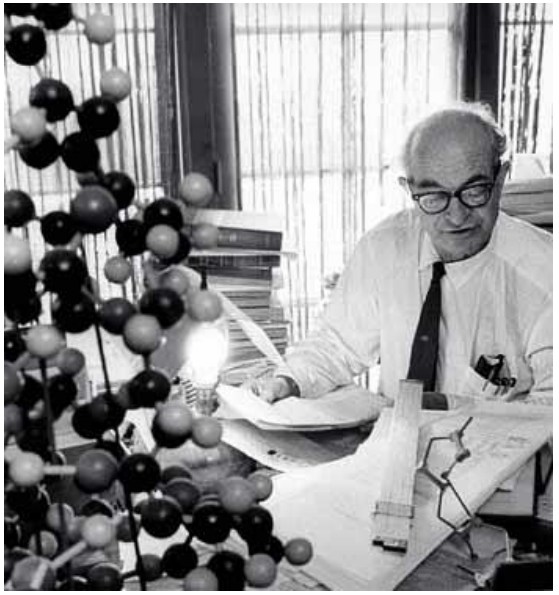
Linus Pauling (March 1932). "The nature of the chemical bond. III. The transition from one extreme bond type to another". *Journal of the American Chemical Society.*



1939

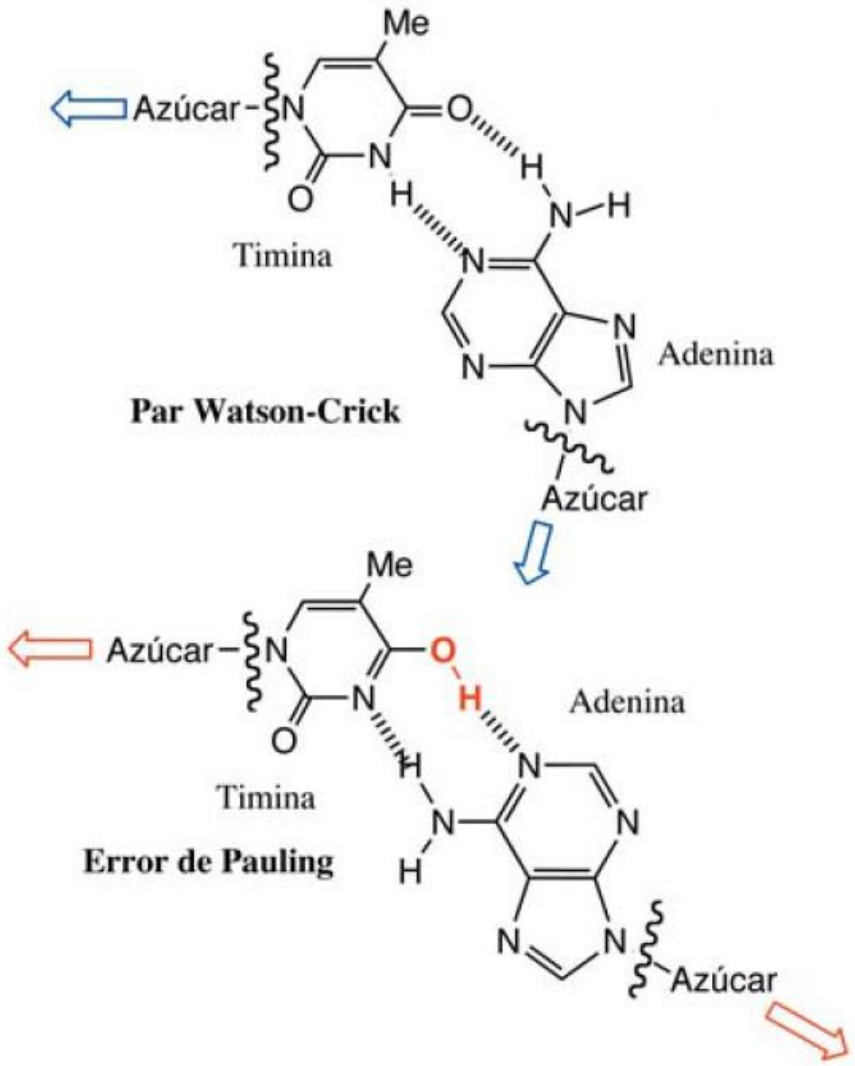
He received the Nobel Prize in Chemistry in 1954 "for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances".

Linus Pauling



Pauling, L; Corey, RB (1951). "Configurations of Polypeptide Chains With Favored Orientations Around Single Bonds: Two New Pleated Sheets". Proceedings of the National Academy of Sciences of the United States of America 37 (11): 729–40.

Pauling L, Corey RB (February 1953). "A Proposed Structure For The Nucleic Acids". Proc Natl Acad Sci U S A. 39 (2): 84–97.


Me
Azúcar
N
O
N
O
H
H
N
H
Timina
N
N
N
Adenina
N
Par Watson-Crick
Azúcar
Me
Azúcar
N
O
H
N
O
Adenina
H
N
Timina
N
N
H
Error de Pauling
N
N
Azúcar

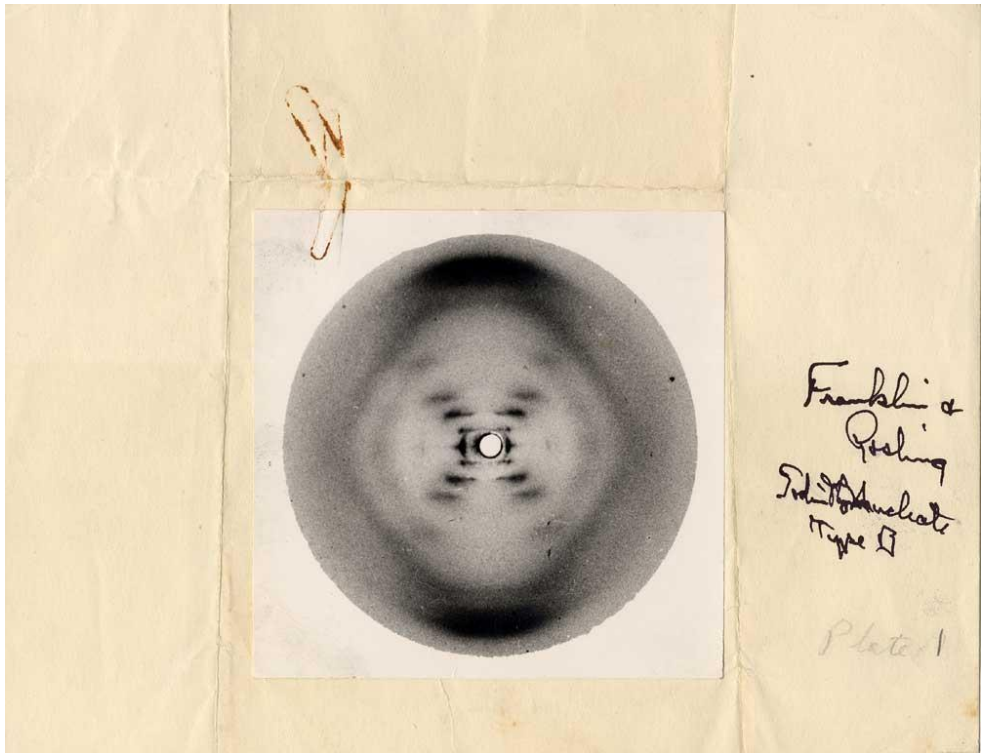
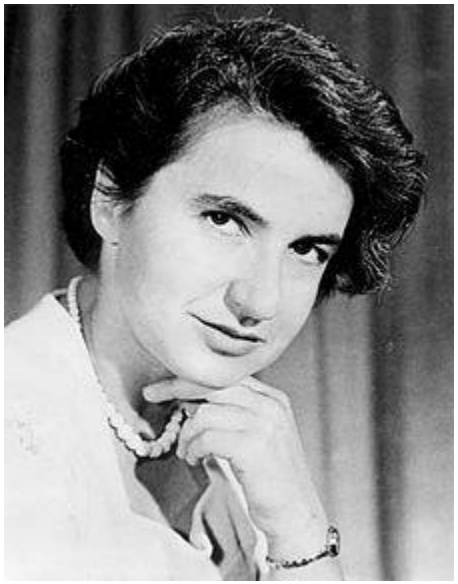
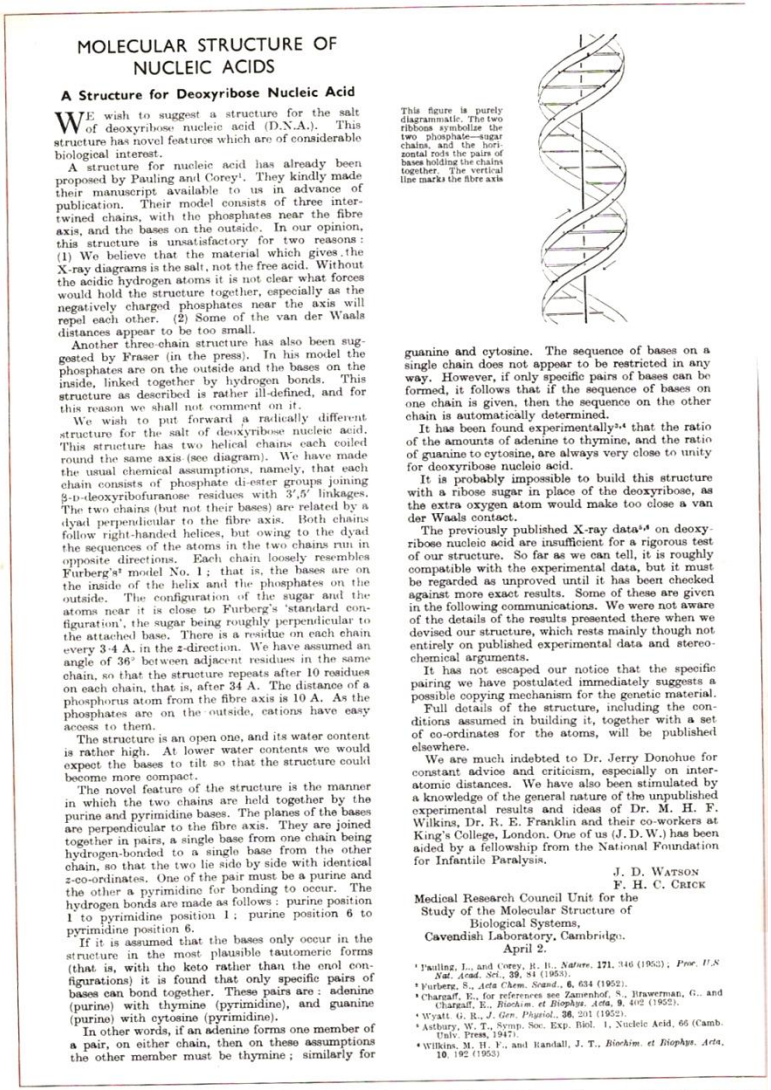


Foto 51



Rosalind Elsie Franklin

Las notas manuscritas que deja Rosalind para sus conferencias no dejan lugar a duda: tenía muy claro lo que significaban las imágenes que había obtenido y sabía interpretarlas; tenía las claves del retrato de la molécula de ADN, incluidas sus medidas. Dicen las anotaciones de Rosalind: «Conclusión. Una gran hélice en muchas de las cadenas, los fosfatos en el exterior, puentes fosfato-fosfato entre las hélices, interrumpidos por moléculas de agua. Hay enlaces disponibles para proteínas» [...]
http://www.poramoralaciencia.com/2013/04/16/rosalind-franklin-descubridora-del-adn/



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 inter-twined 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 di-ester 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 inter-atomic 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.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

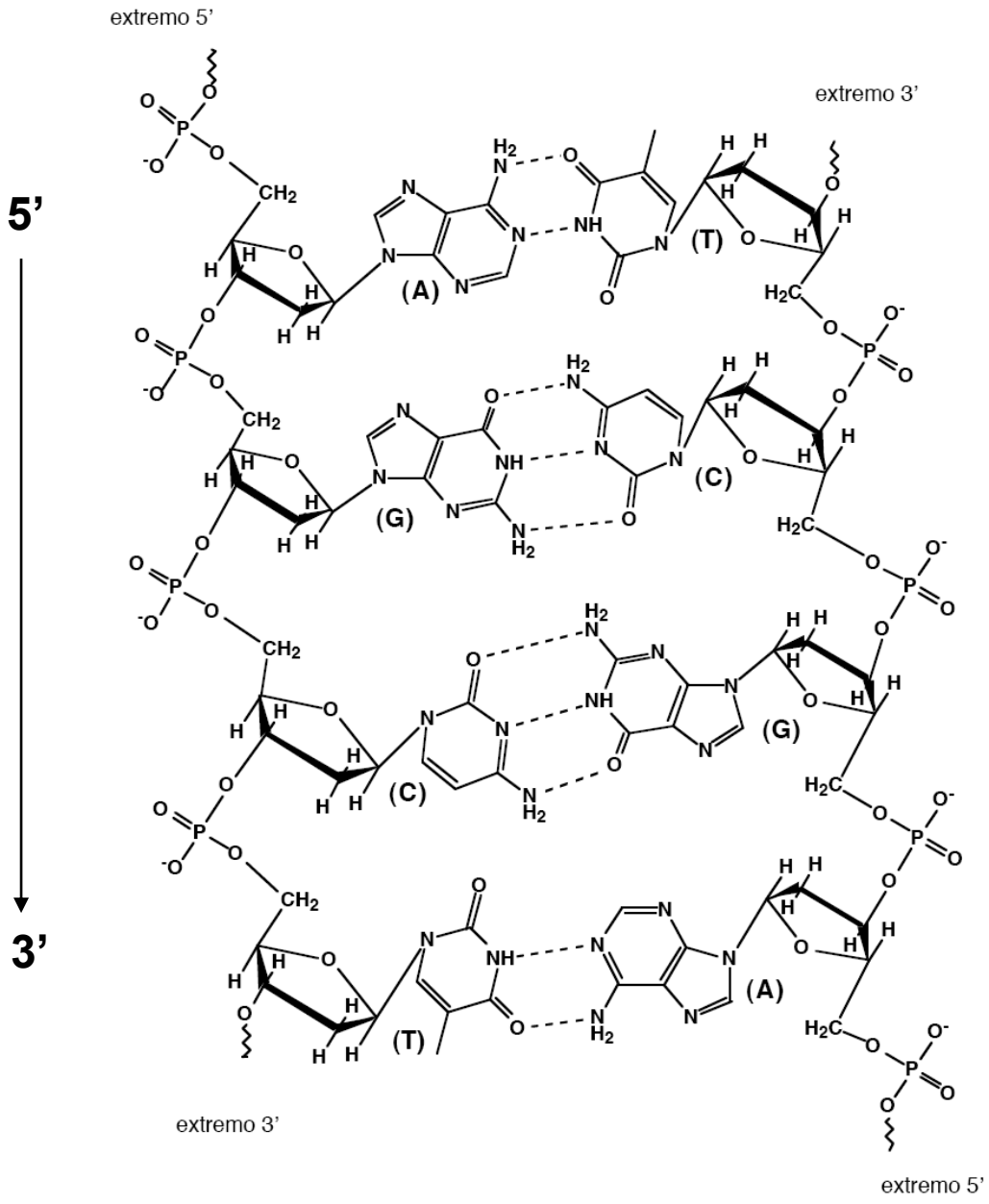
James Watson, Francis Crick, Maurice Wilkins y Rosalind Franklin. Fuente: undsci.berkeley.edu

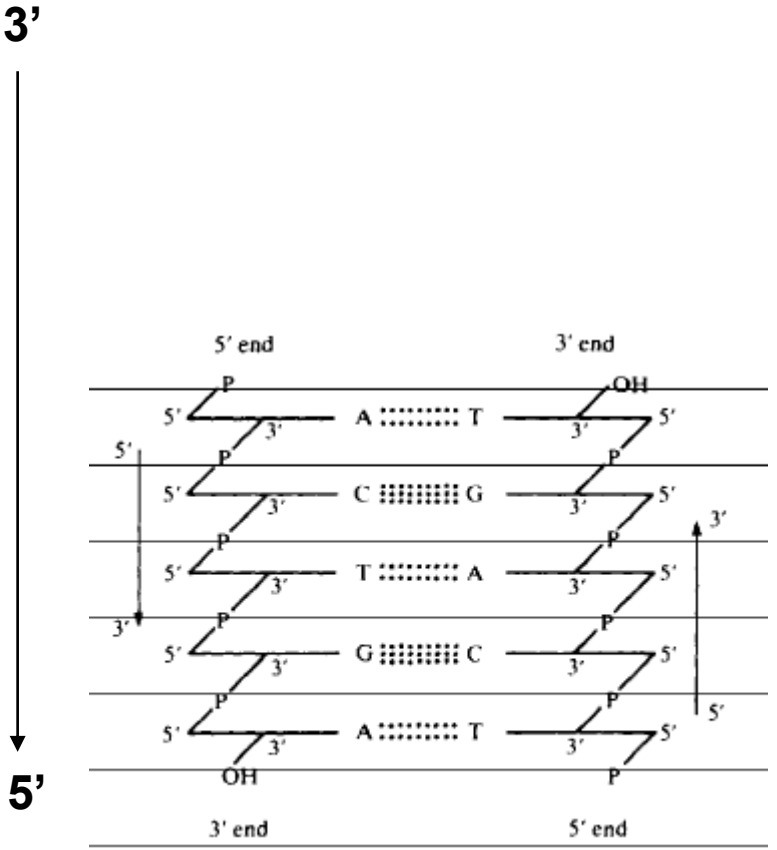
Este artículo reproducido aquí arriba, escrito por J. D. Watson y F. H. C. Crick, constituye un hito de la bioquímica moderna. (Reimpreso en su totalidad, con permiso especial de Nature, *Abril 25, 1953, p. 737). Unas semanas después, fue seguido por un segundo artículo, en el cual, se describió en forma más explícita, el proceso de la replicación.*

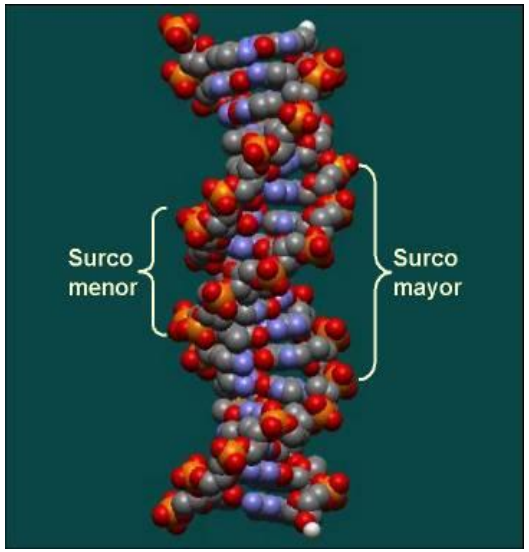
Watson, Crick y Wilkins consiguieron en 1962 el Premio Nobel de Fisiología y Medicina por su trabajo en el descubrimiento de la estructura del ADN, y aunque el nombre de Rosalind Franklin no se mencionó, ni se reconoció su contribución al descubrimiento de la estructura del ADN, ésta fue al menos comparable a la que tuvieron los galardonados.

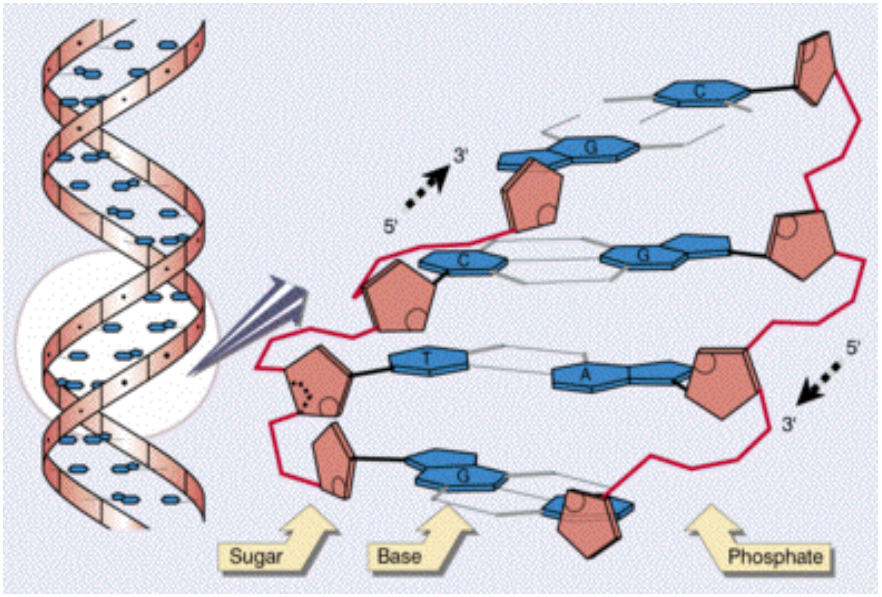
http://www.nature.com/nature/dna50/watsoncrick.pdf

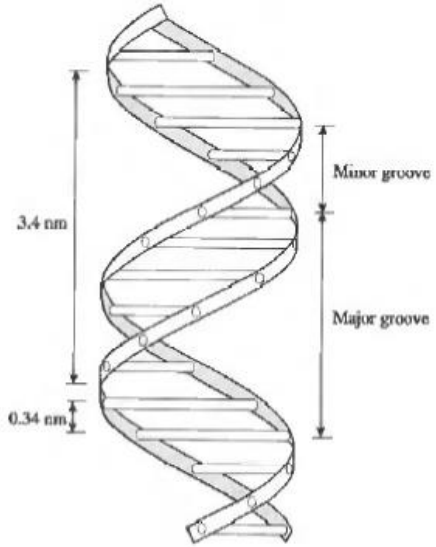
Estructura de una doble hebra de DNA

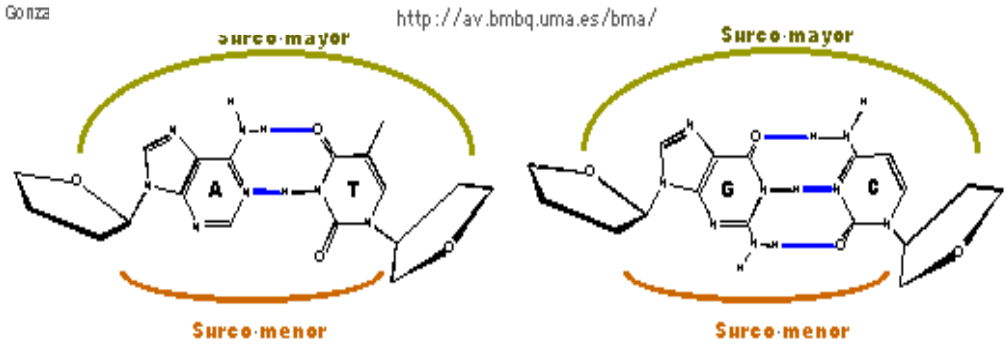


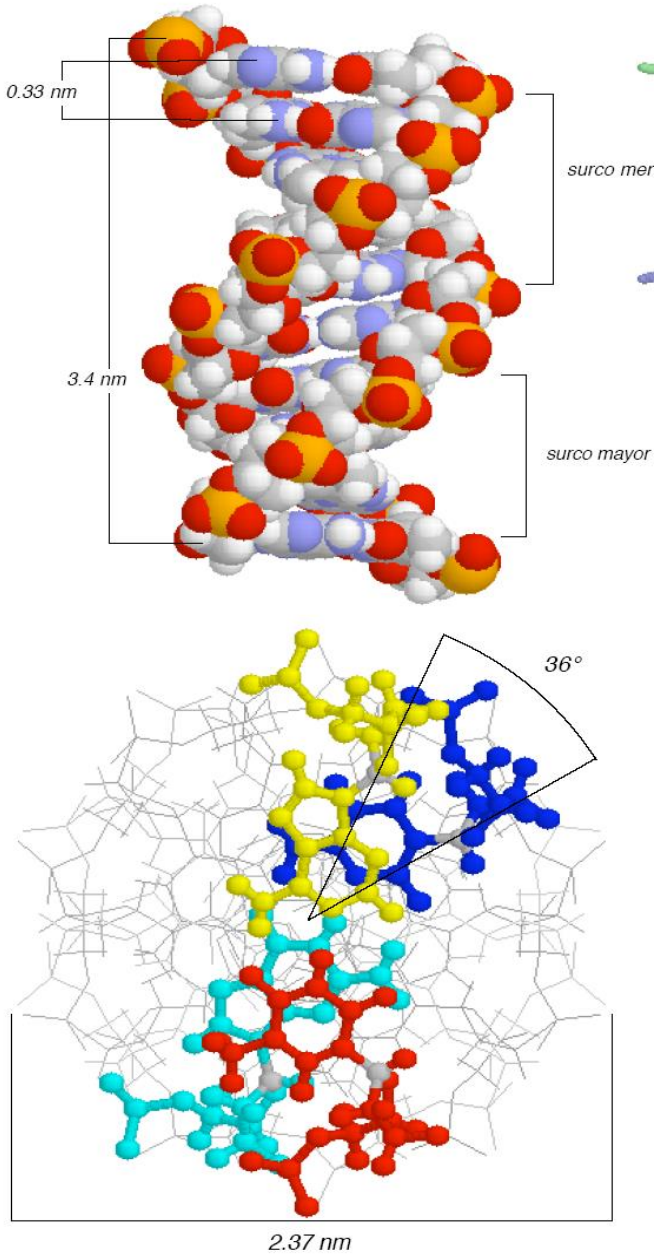


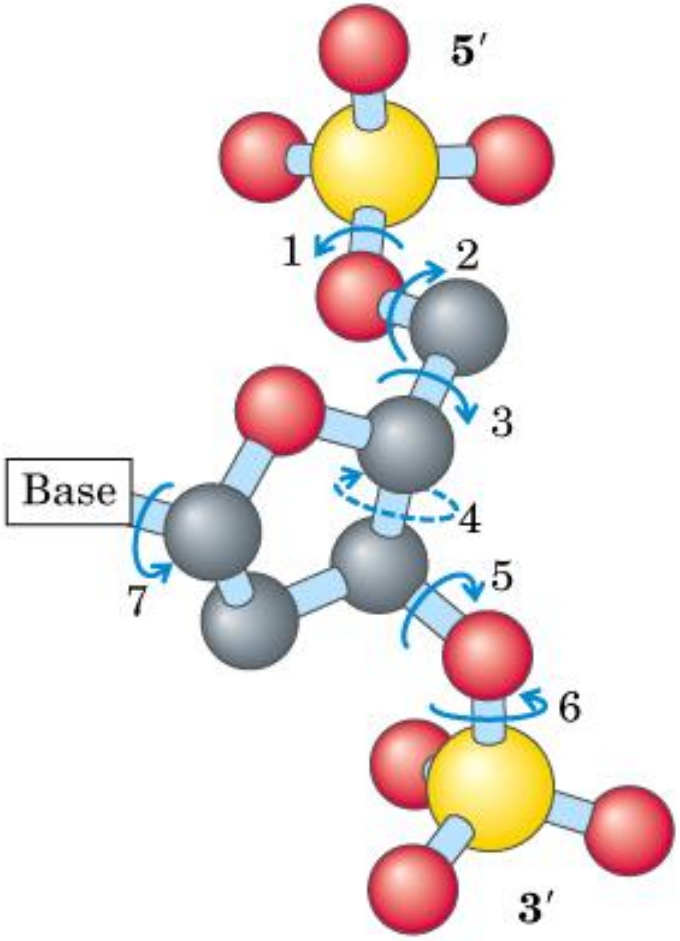

Surco
menor
Surco
mayor

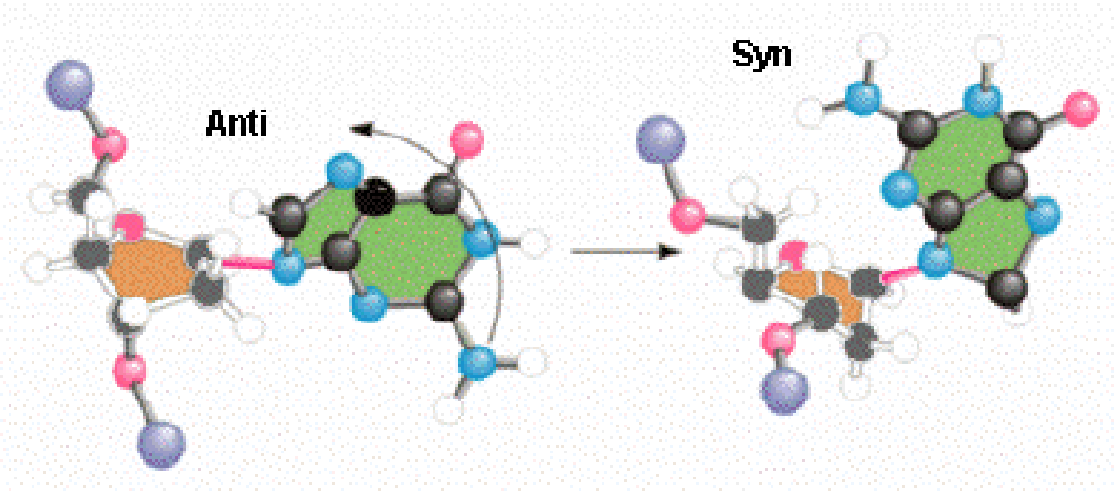

5'
3'
C
G
C
G
T
A
G
5'
3'
Sugar
Base
Phosphate

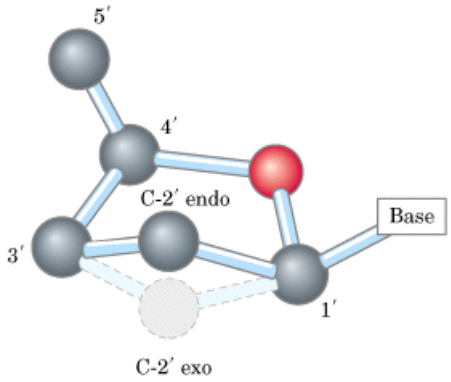

3.4 nm
0.34 nm
Minor groove
Major groove

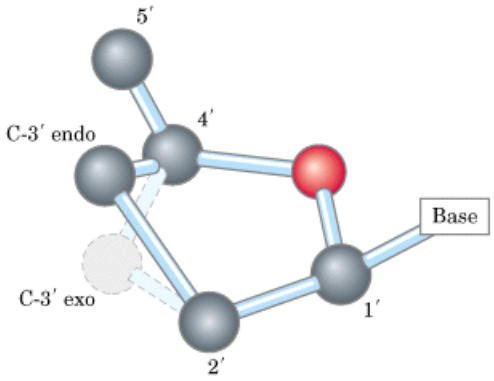

Gonza
http://av.bmbq.uma.es/bma/
Surco·mayor
A
T
Surco·menor
Surco·mayor
G
C
Surco·menor


0.33 nm
3.4 nm
surco mer
surco mayor
36°
2.37 nm

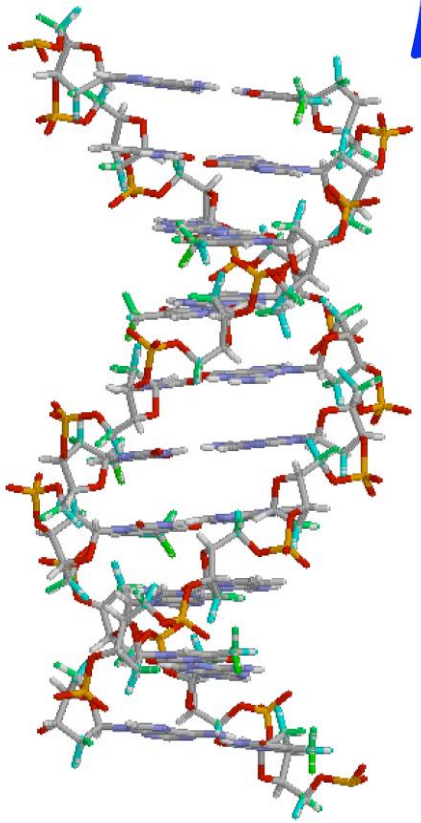

5′
1
2
3
Base
4
7
5
6
3′

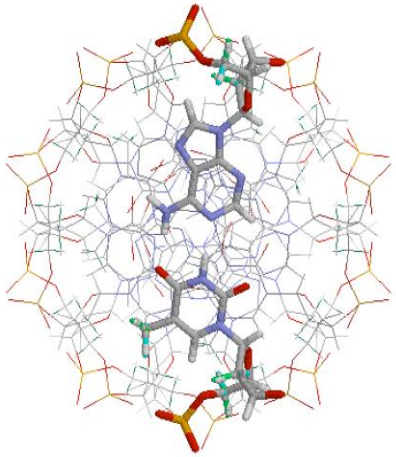

Anti
Syn


5′
4′
C-2′ endo
Base
3′
1′
C-2′ exo


5′
4′
C-3′ endo
Base
C-3′ exo
1′
2′

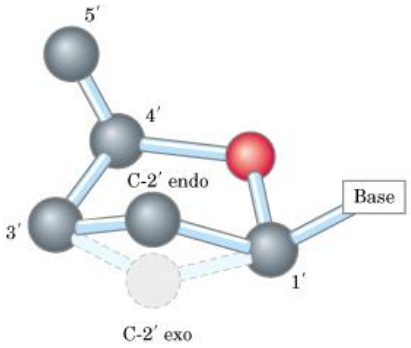
B-DNA



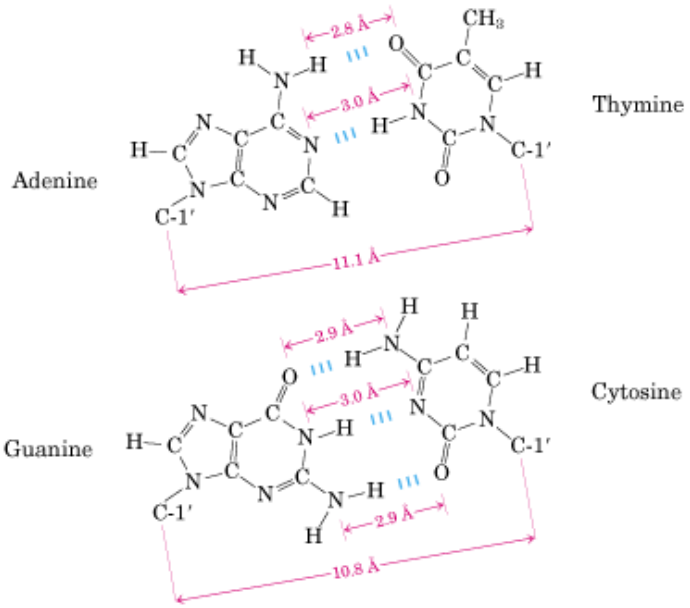


Giro a derechas

Conformación de desoxirribosa

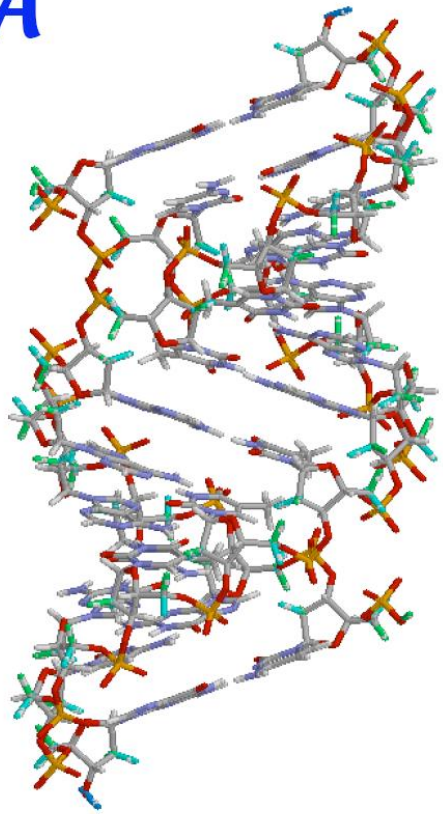


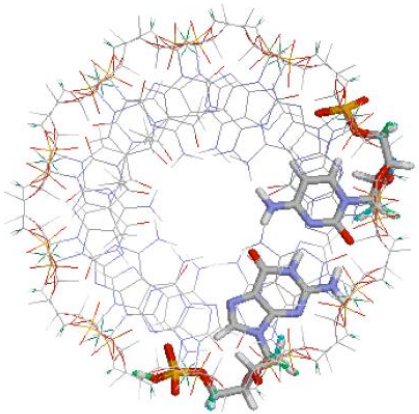
Apareamiento de bases



Conformación del enlace glicosídico *anti*

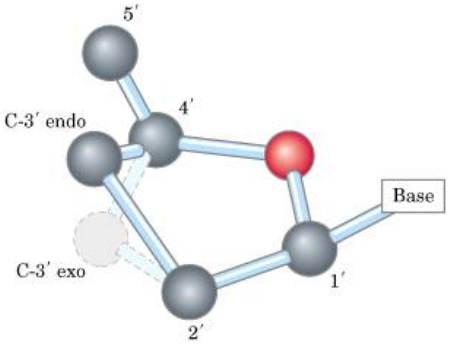
A-DNA



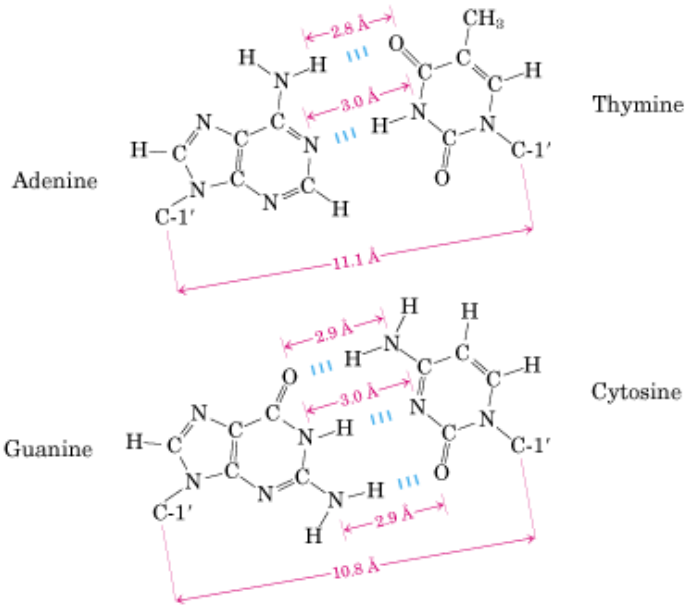


Giro a derechas

Conformación de desoxirribosa

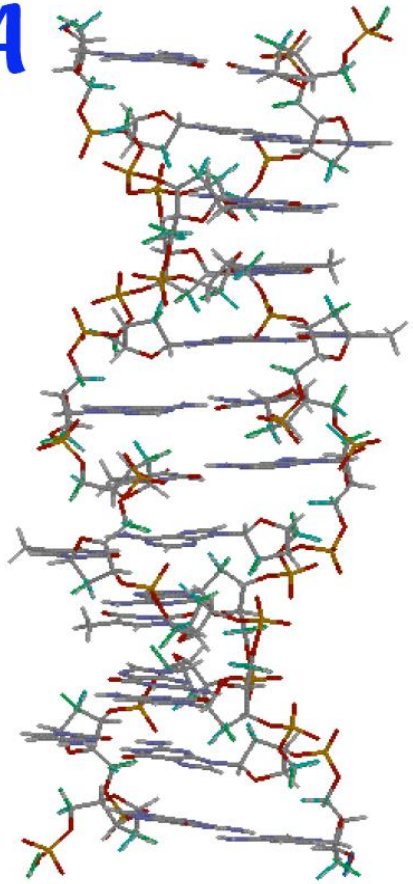


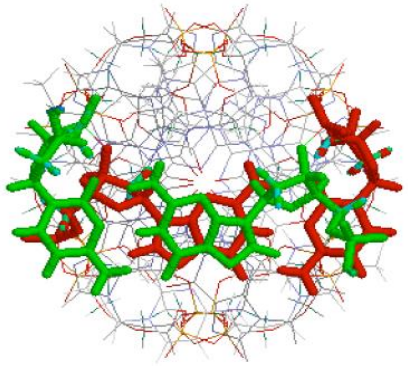
Apareamiento de bases



Conformación del enlace glicosídico *anti*

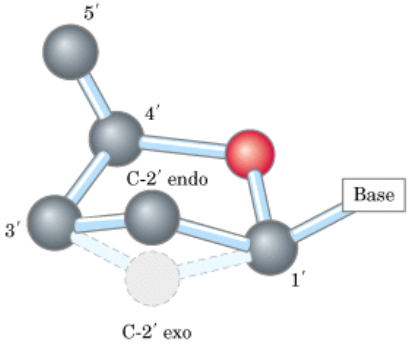
Z-DNA



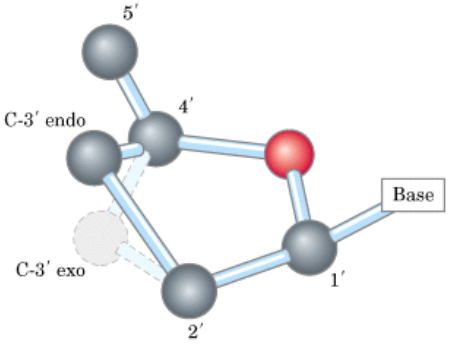


Giro a izquierdas

Conformación de desoxirribosa



Pirimidinas



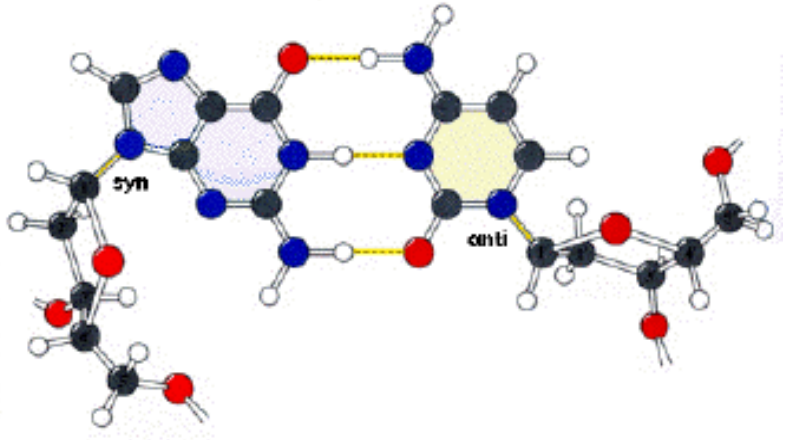
Purinas

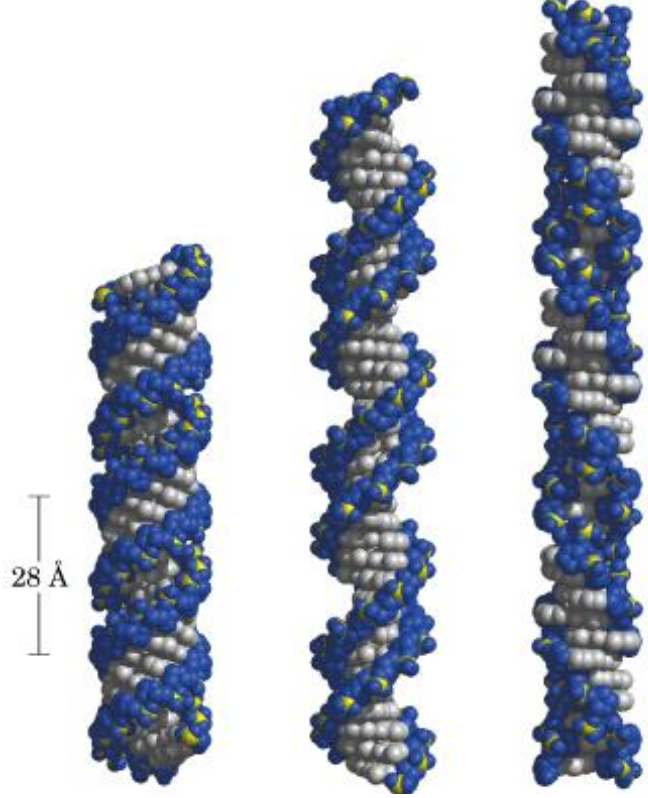
Conformación del enlace glicosídico:

sym **purinas**

anti **pirimidinas**

Apareamiento de bases

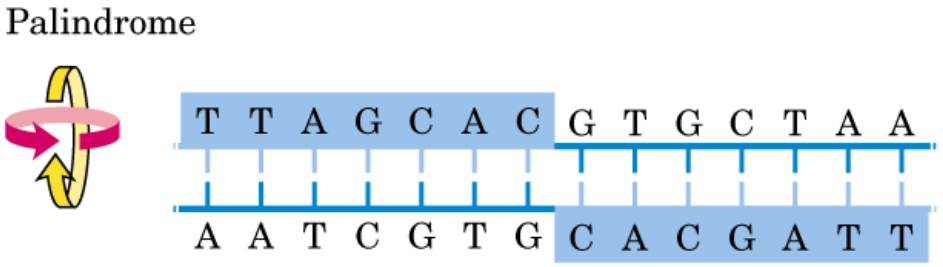




	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

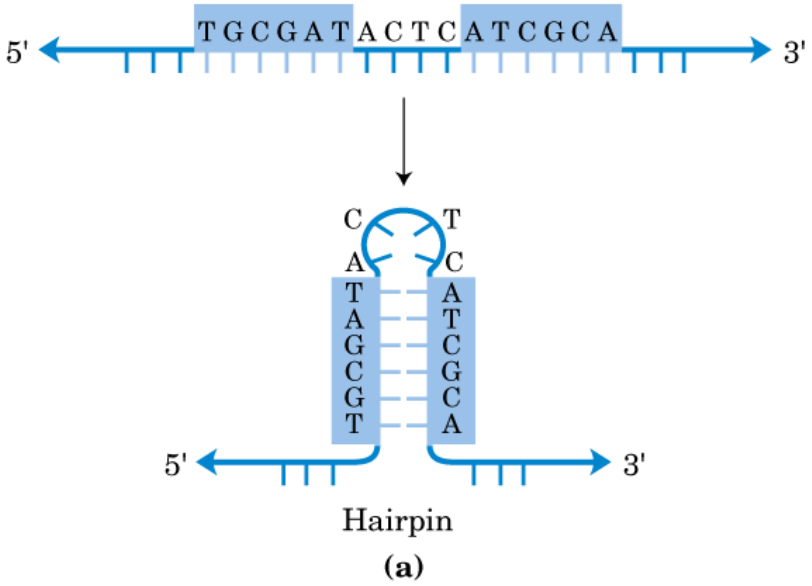
Secuencias que adoptan estructuras inusuales

Secuencias palindrómicas: regiones de ADN de doble hebra con secuencias de bases que se repiten en forma invertida entre ambas cadenas presentando doble simetría

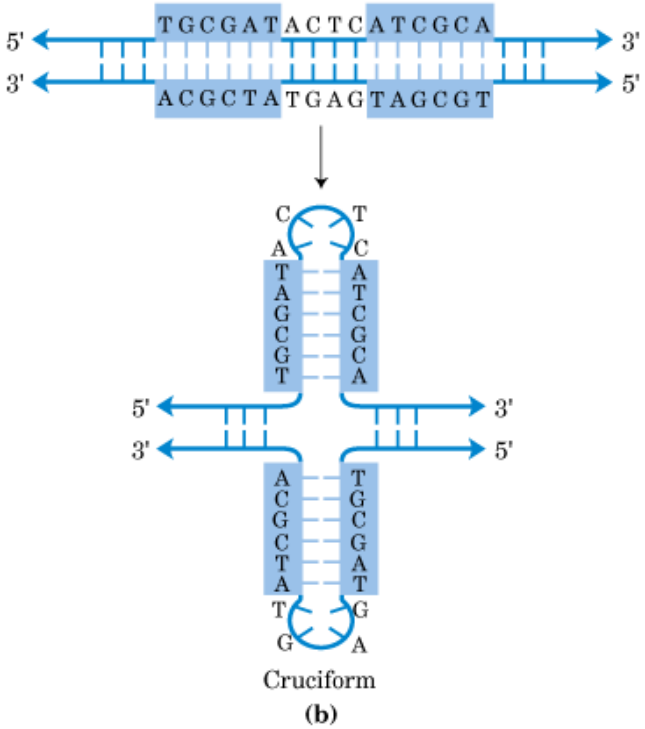


Son potenciales formadoras de:

Horquillas (ADN simple hebra y ARN)

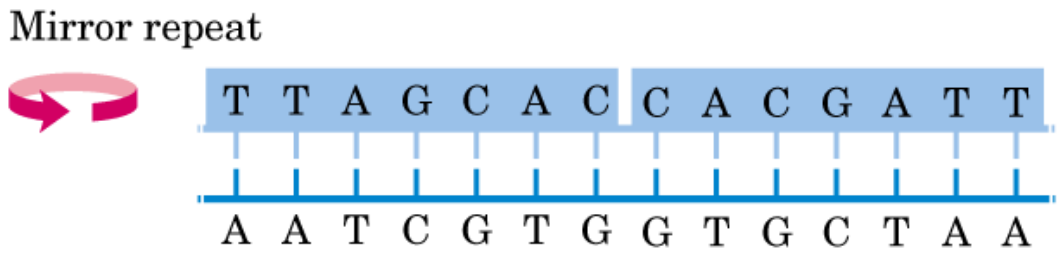


Estructuras cruciformes (ADN duplex)



Secuencias que adoptan estructuras inusuales

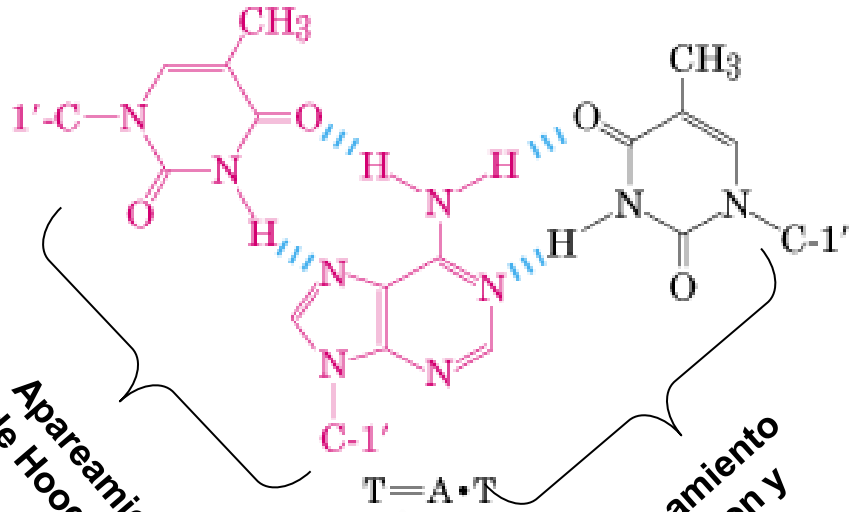
Repeticiones espejadas: regiones de ADN de doble hebra que no tienen secuencias complementarias en la misma cadena. Se encuentran en moléculas de ADN largas (varios miles e pares de bases)

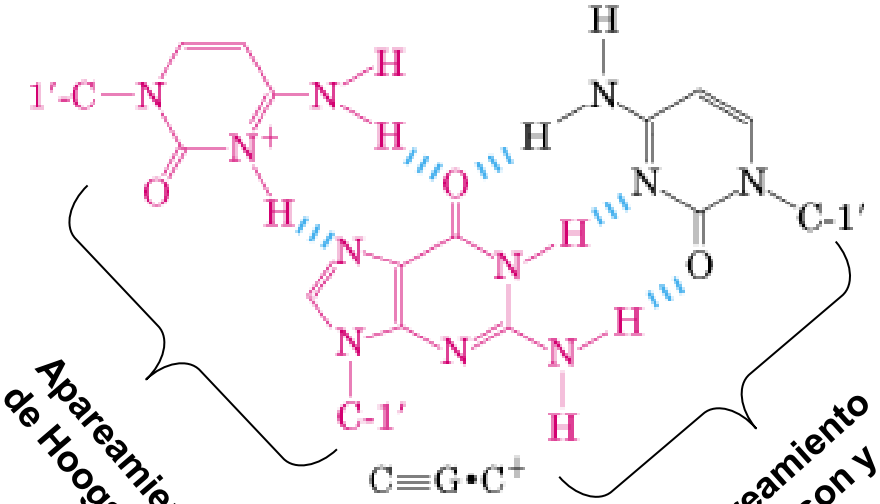


Estructuras de 3 o 4 hebras: ADN triplex y ADN cuadruplex

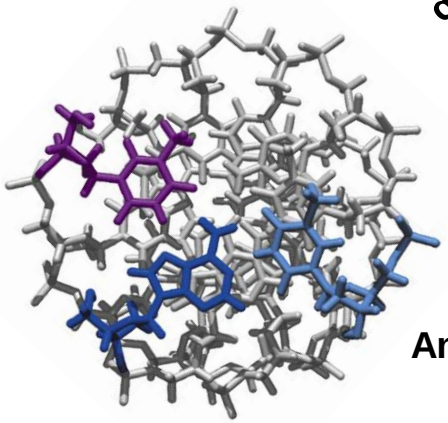
ADN triplex: se presenta en segmentos polipirimidinicos y polipurunicos

Apareamientos de bases





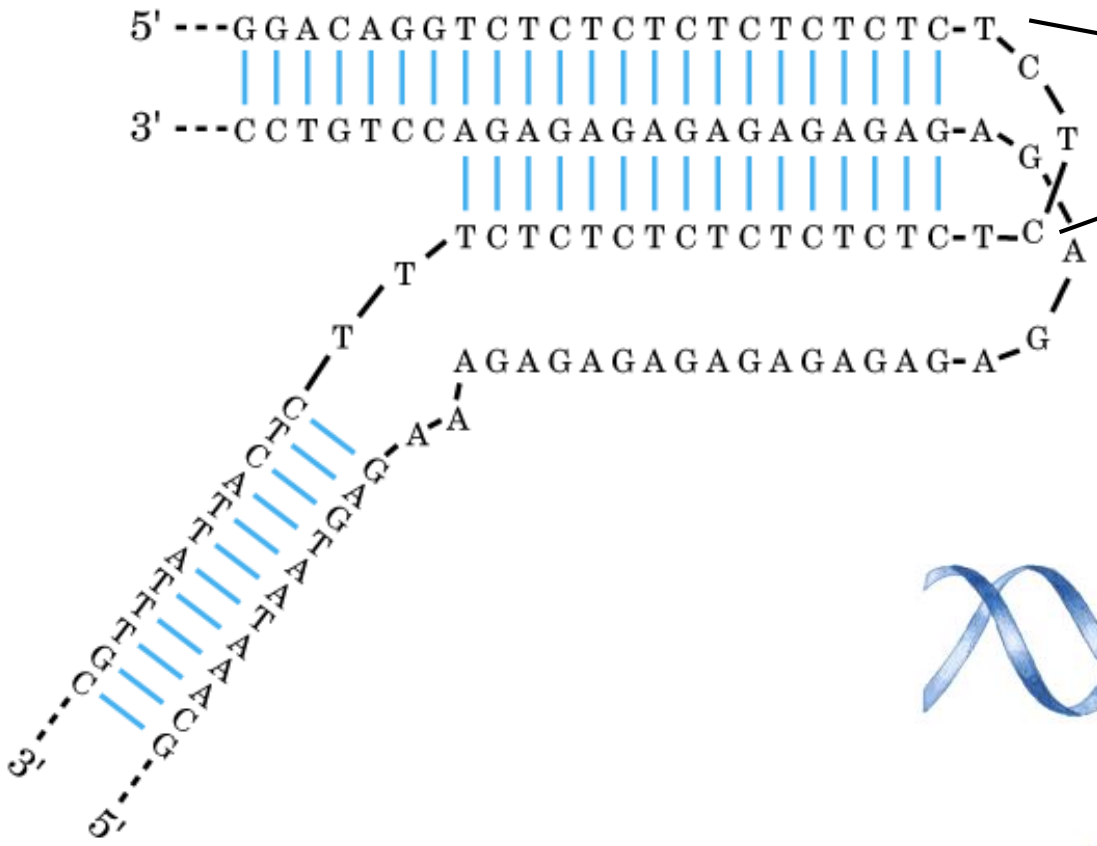
Las hélices triplex son más estables a pH ácido porque C tiene que estar protonada C^+ (pKa en la hebra 7,5, pKa normal 4,2)



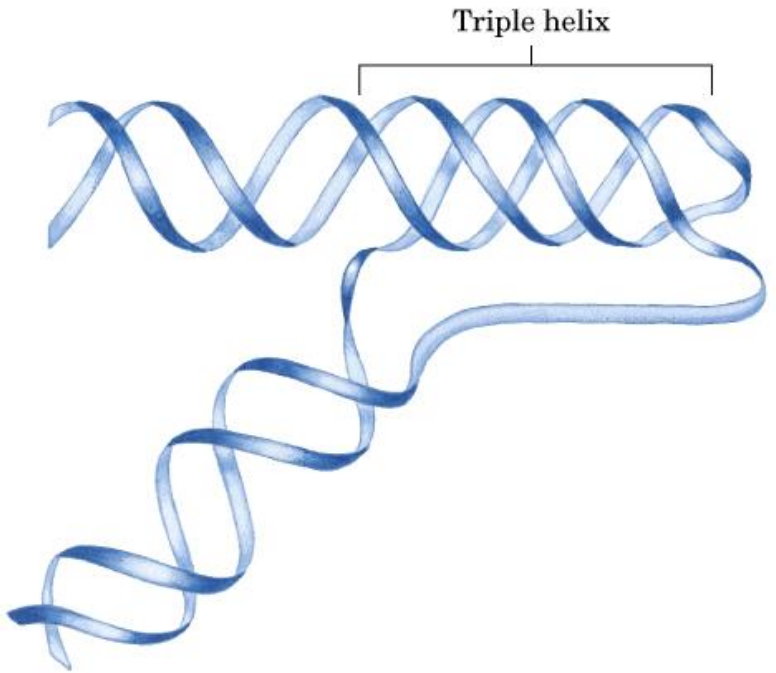
Paralelas

Antiparalelas

H-ADN: Hélice triplex en trayectos polipirimidina o polipurina
Con repeticiones espejadas

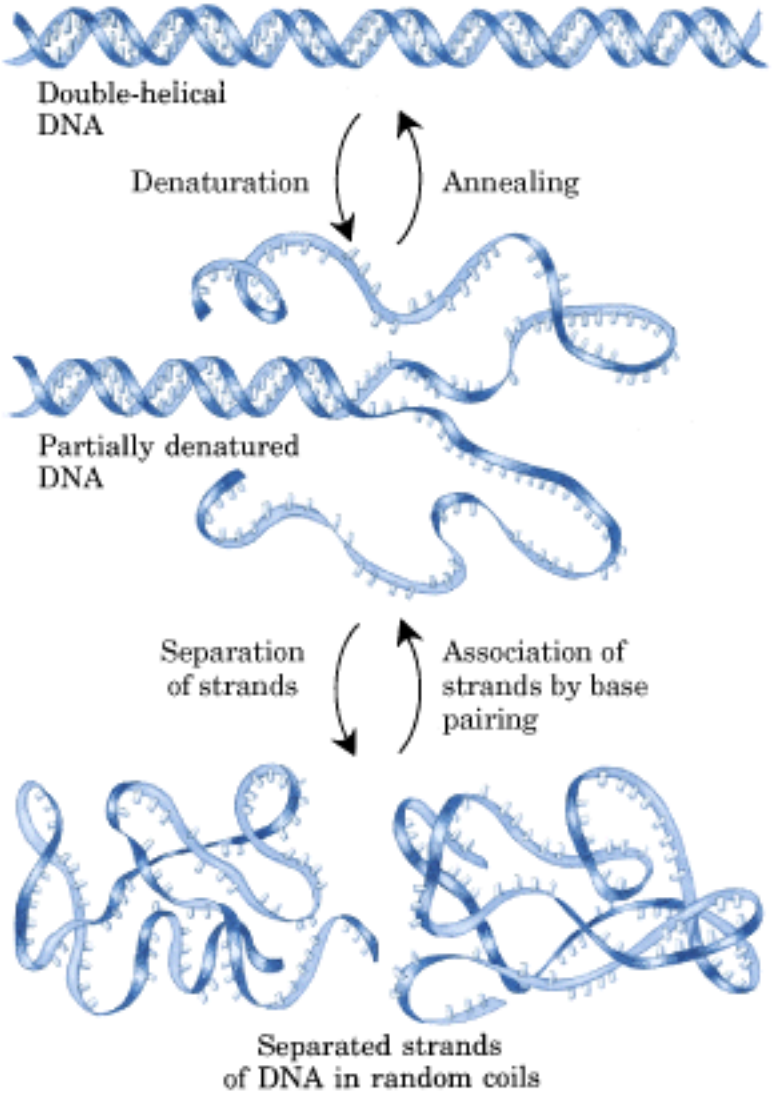


Repetición espejada
(pertenece a la misma hebra)



Desnaturalización

Las soluciones de ADN nativo son muy viscosas a pH 7 y 25ºC



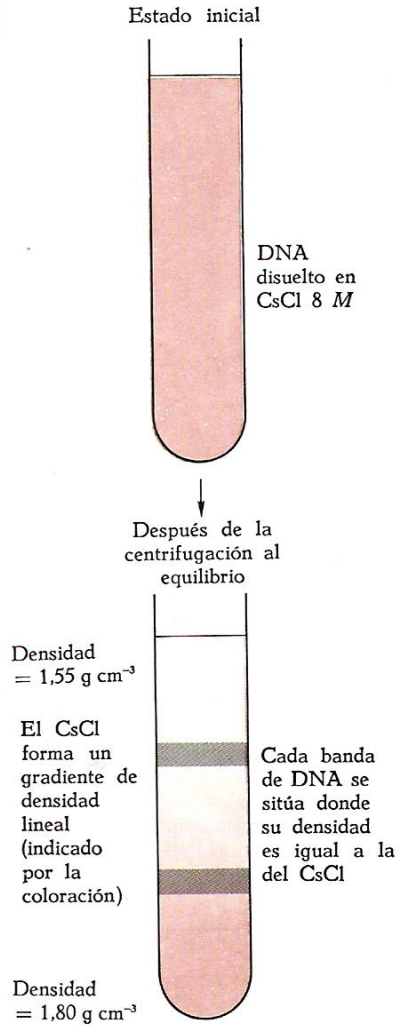
rápido

lento

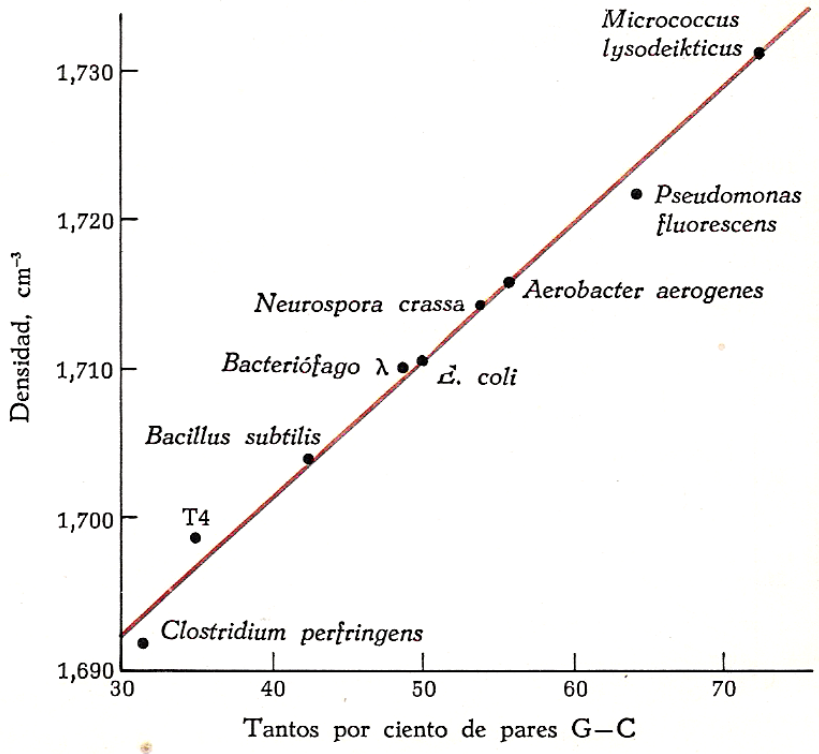
Al aumentar temperatura o variar el pH a situaciones extremas la viscosidad decrece abruptamente

Densidad de flotación

Determinación de la densidad de flotación.

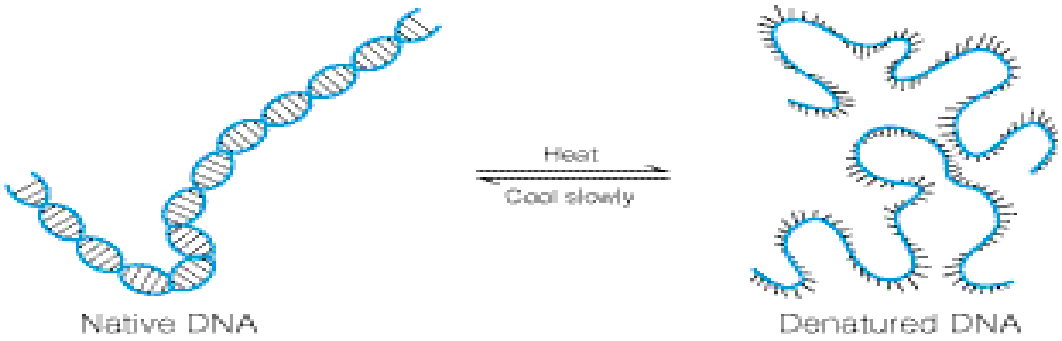


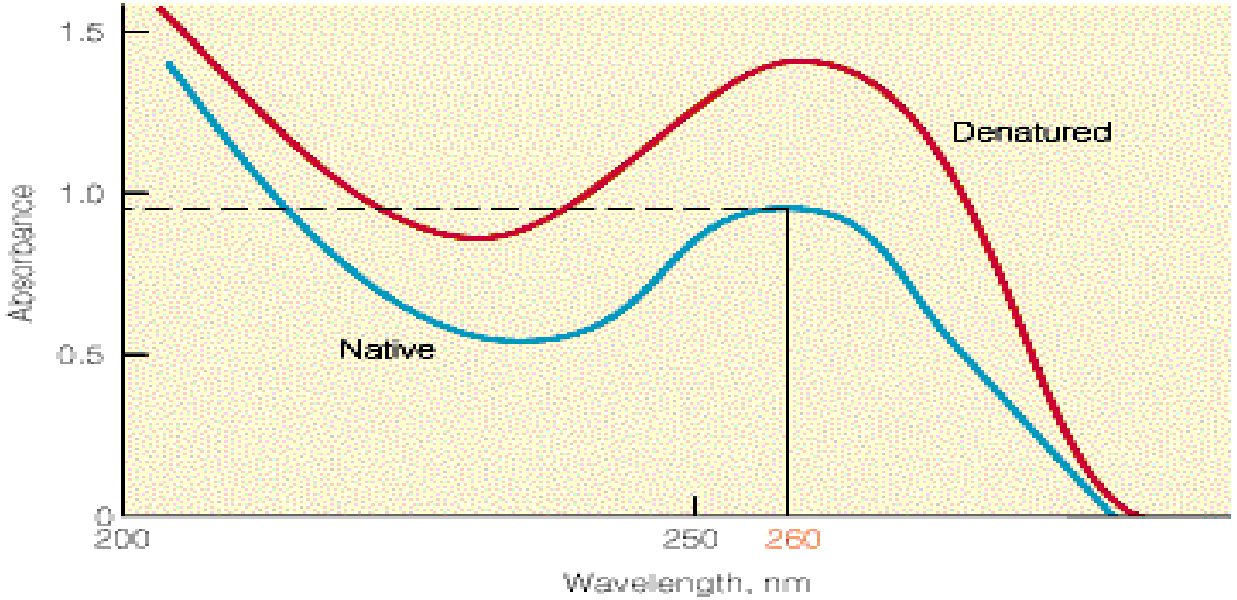
Relación entre la densidad de flotación del DNA *de doble hebra y su composición en bases.* [*Datos tomados de C. L. Schildkraut, J. Marmur y P. Doty,* J. Mol. Biol., 4: 430 (1962)].



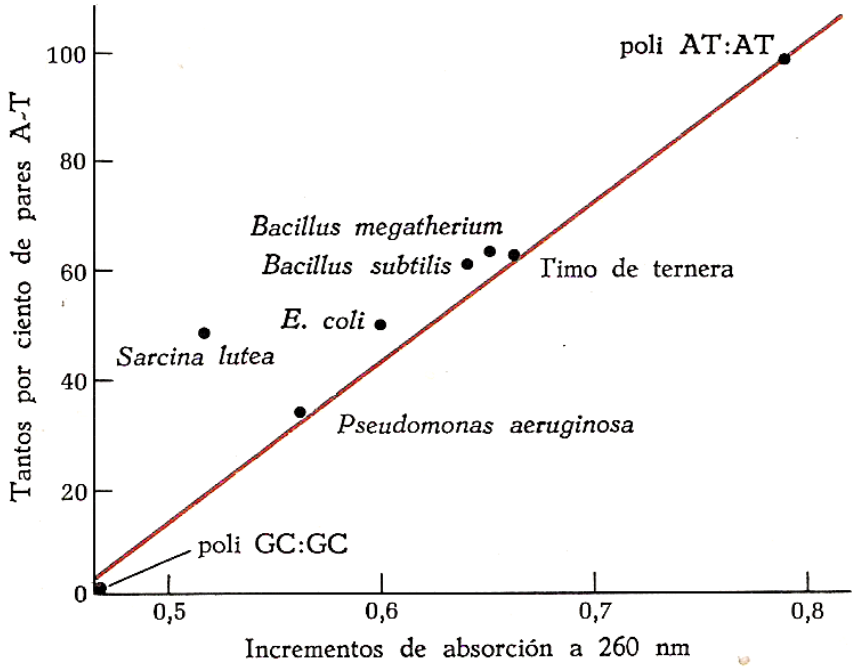
Efecto hipercrómico:

Aumento de absorbancia a 260 nm que manifiesta una estructura bicatenaria al perder su estructura nativa o plectonémica



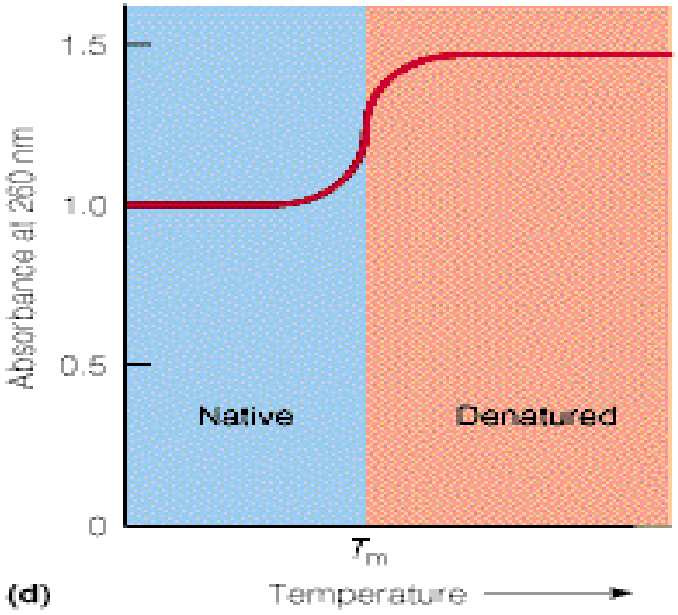


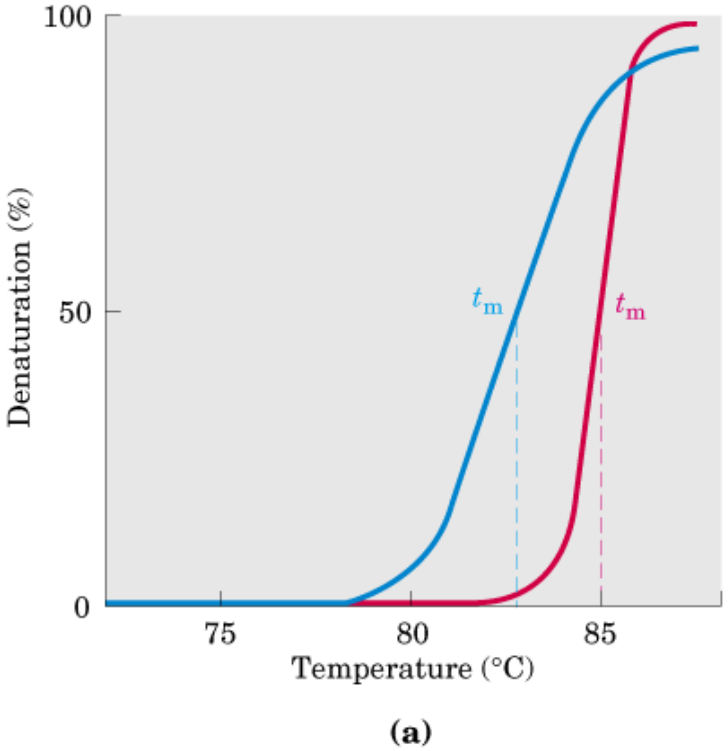
Estimación del porcentaje de pares de bases en función de la magnitud del efecto hipercrómico



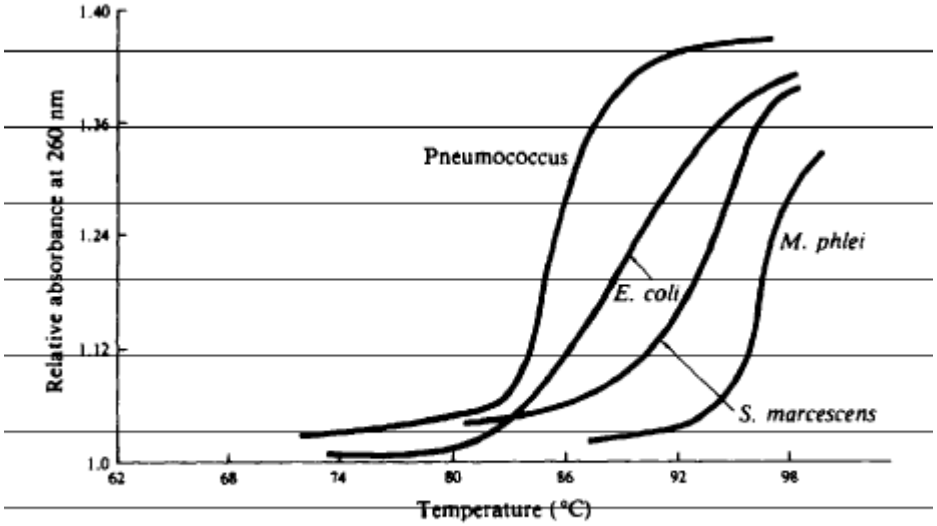
Temperatura media de fusión:

Temperatura a la cual se alcanza el 50 % de desnaturalización

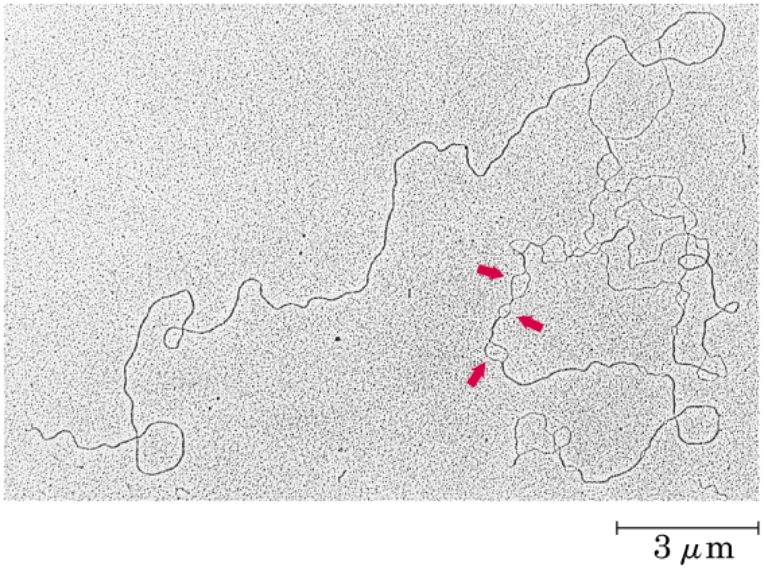




Tm y “complejidad” del ADN

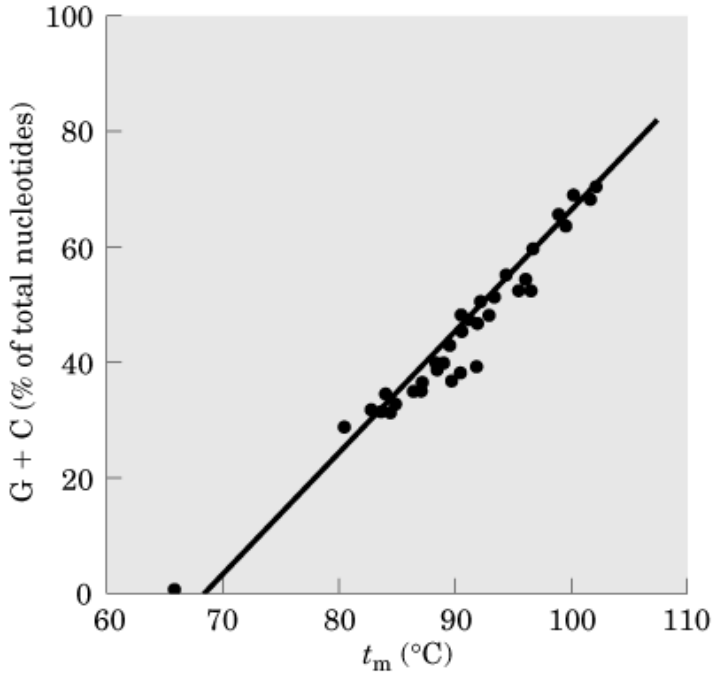


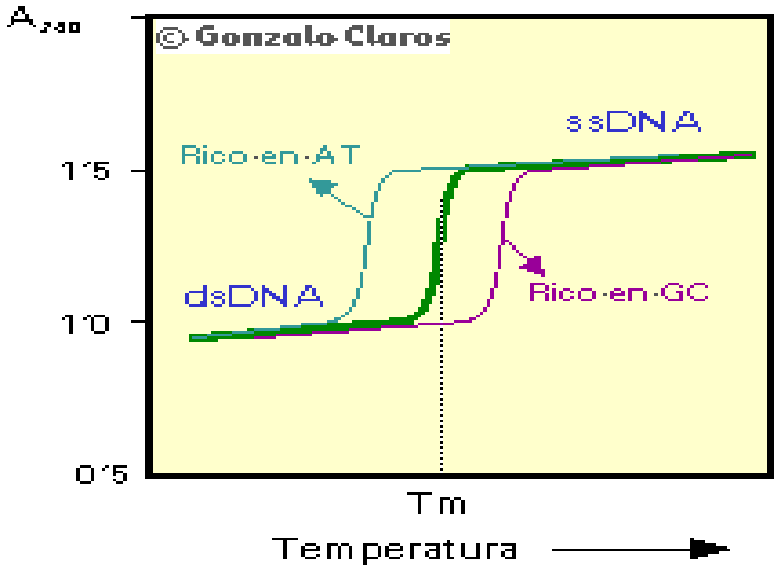
Melting curves for DNA from different species.

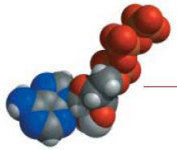


ADN parcialmente desnaturalizado
Las flechas indican regiones ricas en A=T

Estimación del porcentaje de pares de bases en función de Tm







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