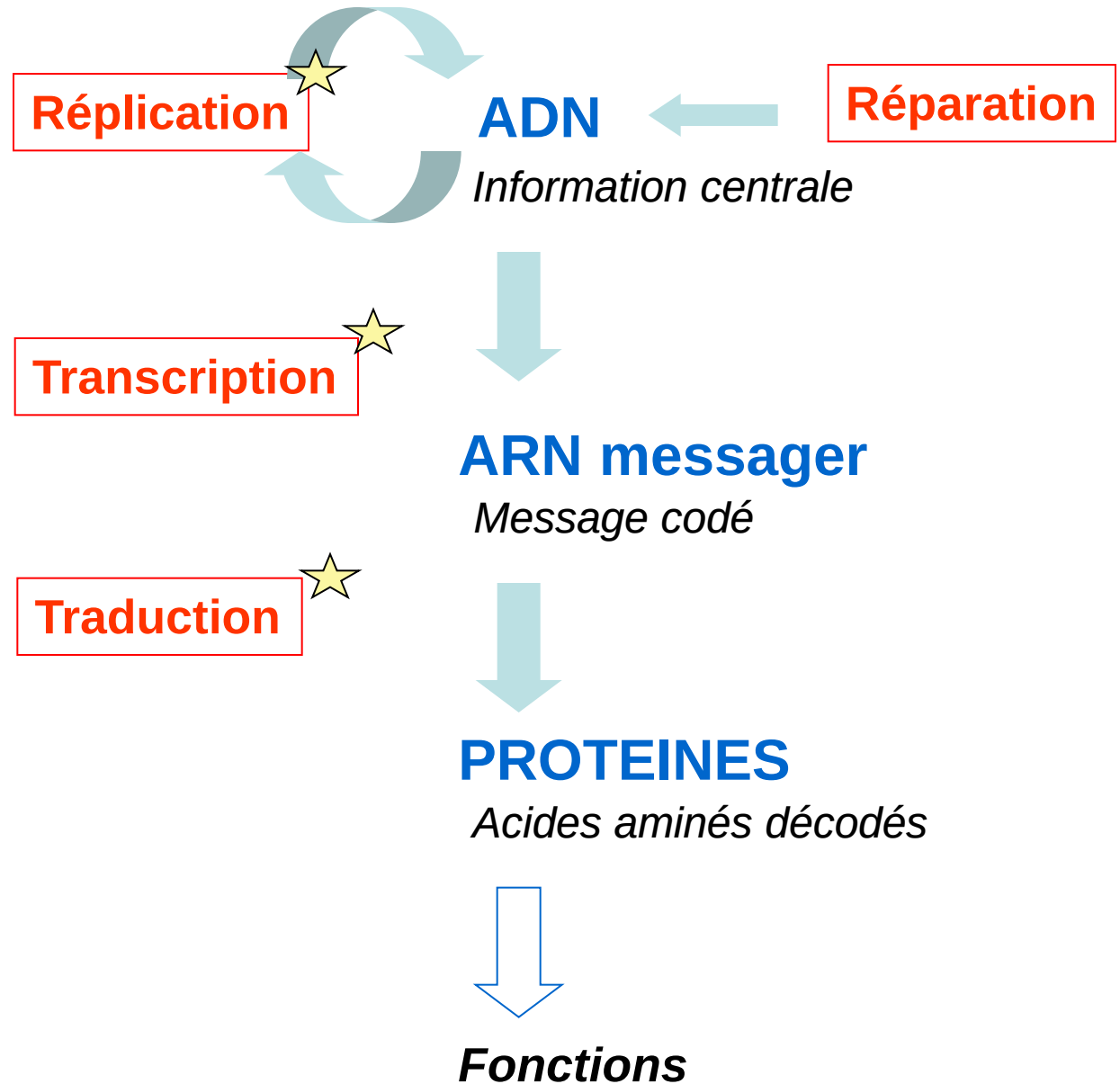


Biologie Moléculaire L2 (BIOL201)



Biologie Moléculaire L2

- **D. Gautheret**
 - ADN et génomes (2 cours)
 - Transcription (4 cours)
- **C. Regeard**
 - Réplication
 - Traduction
- **C. Pasternak, F Constantinesco**
 - Traduction

Cours 1: Structure de l'ADN

Poly : Dokeos > L2 Bio > Biol201

<http://rna.igmors.u-psud.fr/gautheret/cours/>

100% open access

Daniel GAUTHERET - Version 2012.0

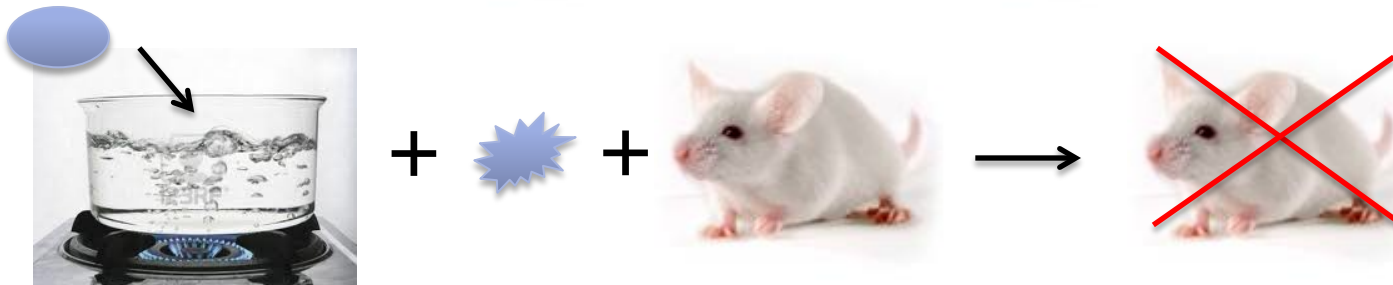
Historique

- **1869 Miescher**
 - Etude des constituants des cellules végétales: lipides, carbohydrates, acides nucléiques. Début de purification des constituants cellulaires.
- **1928 Griffith**
 - Existence d'un principe transformant
- **1944 Avery**
 - Premières expériences de transformation. Démontrent que le « principe transformant » est l'ADN.

Expérience de Griffith (1928)

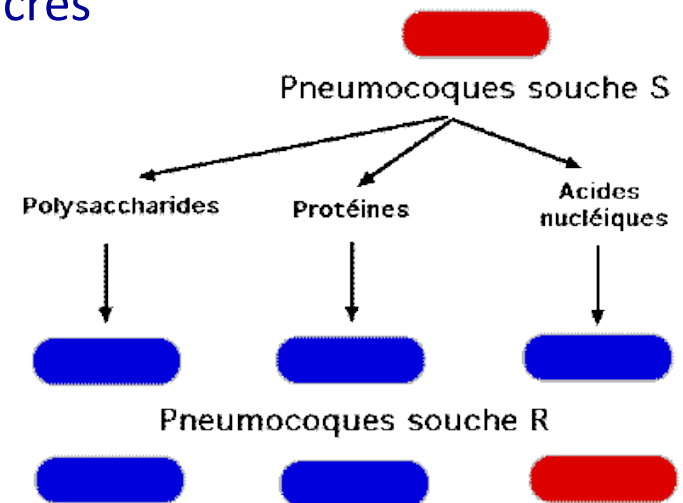
- *Pneumococcus pneumoniae* (responsable de pneumonies)

- Souche infectieuse: smooth (S) capsule gélatineuse
- Souche mutante: rough (R) dépourvue de la capsule gélatineuse



Expérience de Avery (1944)

- Isoler l'ADN des bactéries S et le mélanger avec des bactéries R
- Après culture sur boîte, on obtient des bactéries R et S.
 - Les bactéries S sont dites **transformées**
 - Le principe transformant est l'ADN
(L'expérience ne fonctionne ni avec les sucres ni avec les protéines)



Historique

- **1950 Chargaff & Todd**

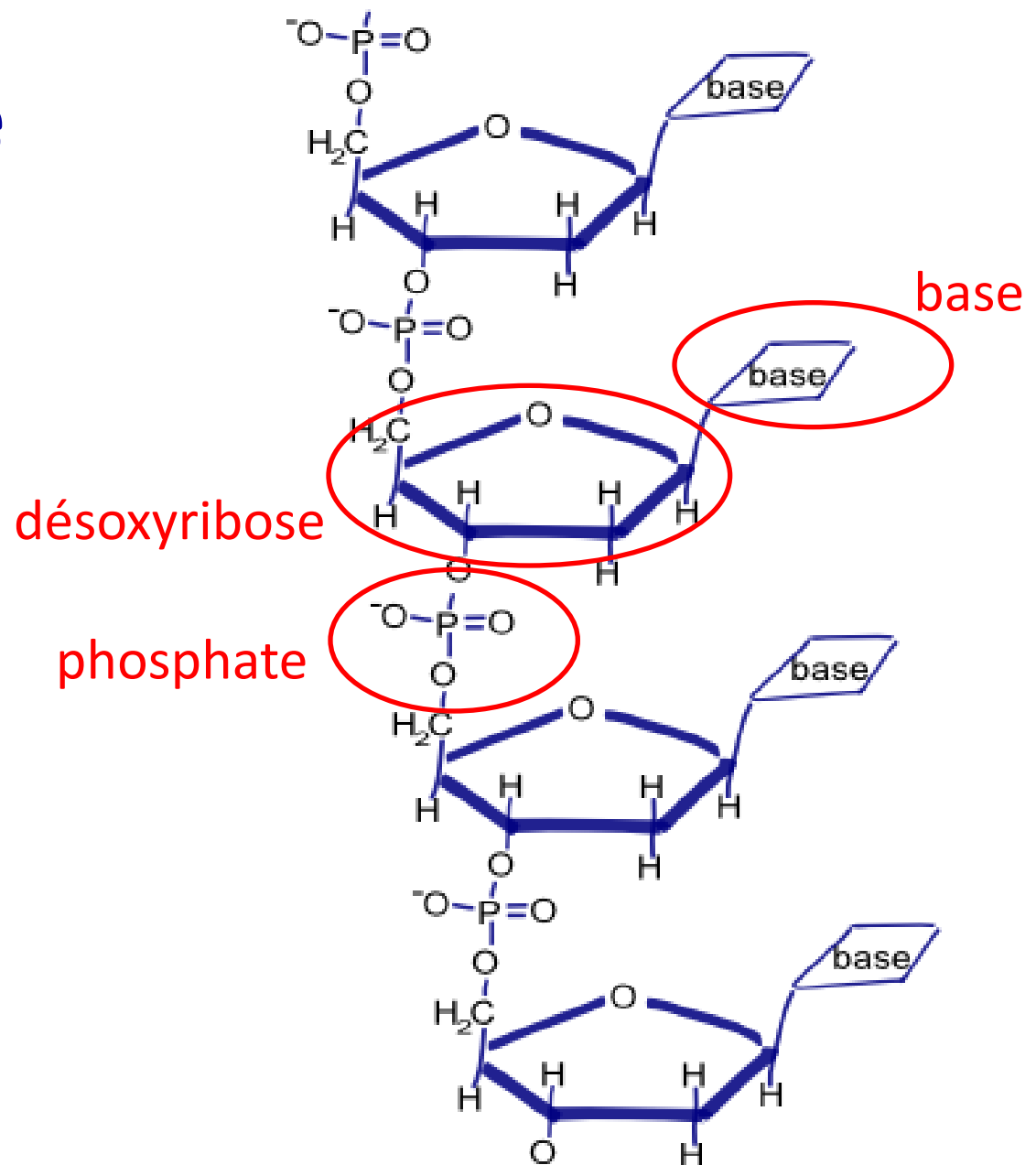
- Chimie de l'ADN. Les « règles de Chargaff »: quantité identique de A et T d'une part, G et C d'autre part. -> notion d'appariement.

- **1953 Watson & Crick**

- Structure en double hélice de l'ADN. Franklin/Wilkins: étude aux rayons X de fibres d'ADN.

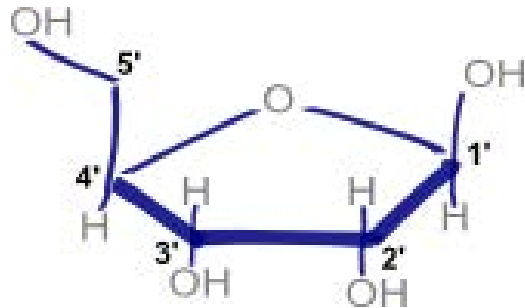
Structure chimique de l'ADN

- Le squelette
- Les bases

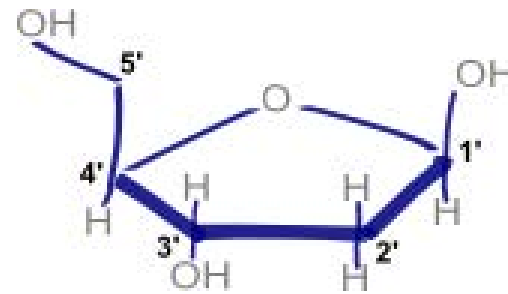


Les sucres de l'ADN et de l'ARN

- Connaître la numérotation des carbones (1' à 5')!
- Conformation β sur le carbone anomérique (1'), énantiomère D
- Dans l'ADN l'évolution a sélectionné le **déoxyribose** pour transmettre l'information
 - 2' H moins attaquant que OH.
 - En 3' la position sera bloquée par la liaison phosphodiester



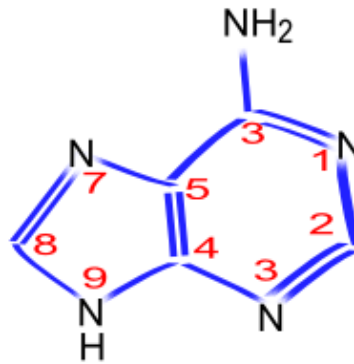
β D ribose (ARN)



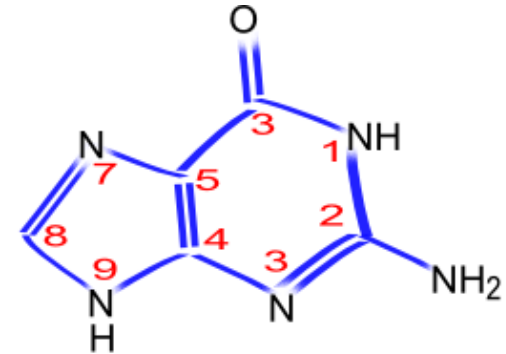
β D désoxyribose (ADN)

Les bases

Base puriques

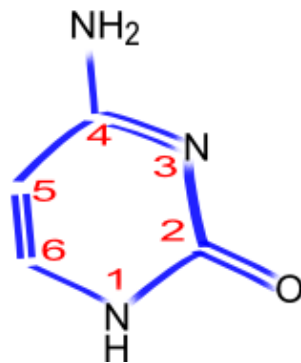


adenine

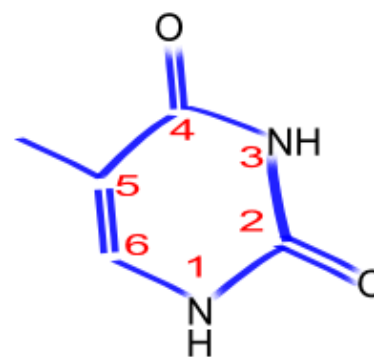


guanine

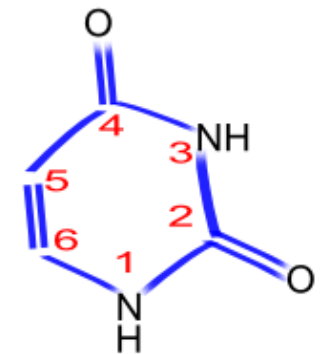
Base pyrimidiques



cytosine

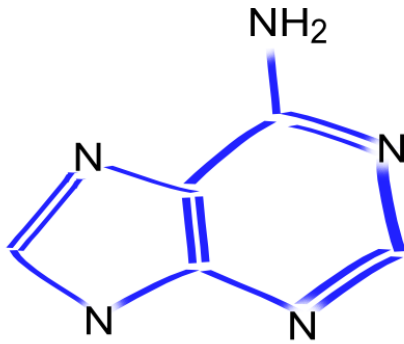


thymine



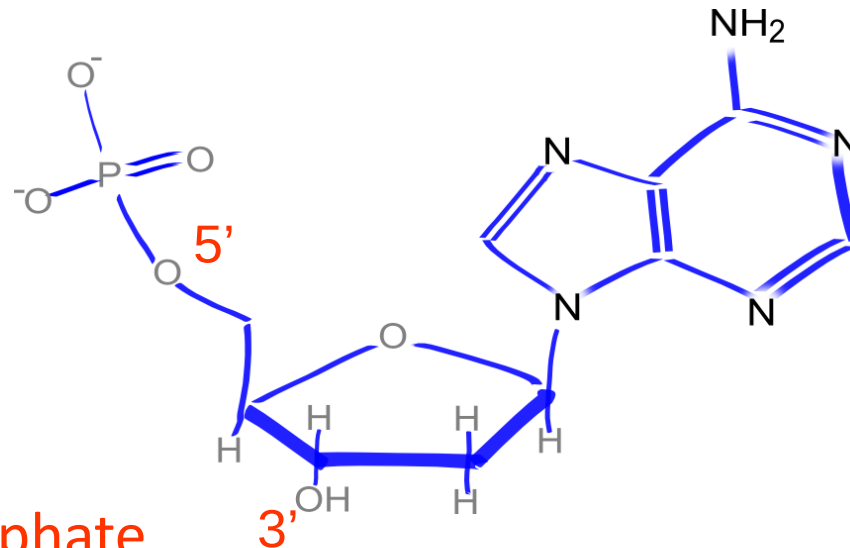
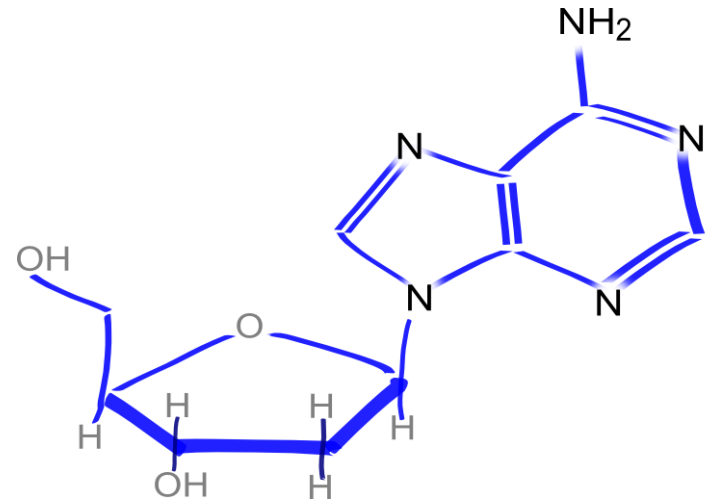
uracil

De la base au nucléotide



Base

Nucléoside =
sucre + base



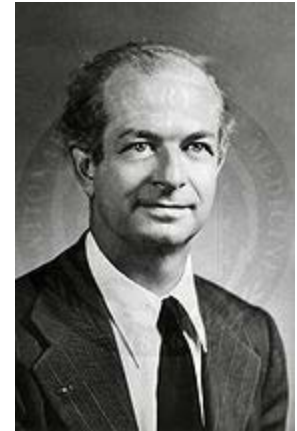
Nucléotide =
sucre + base + phosphate

Et la Structure?



Le modèle de Pauling
publié en 1953, avec les
phosphates au centre
d'une triple hélice.

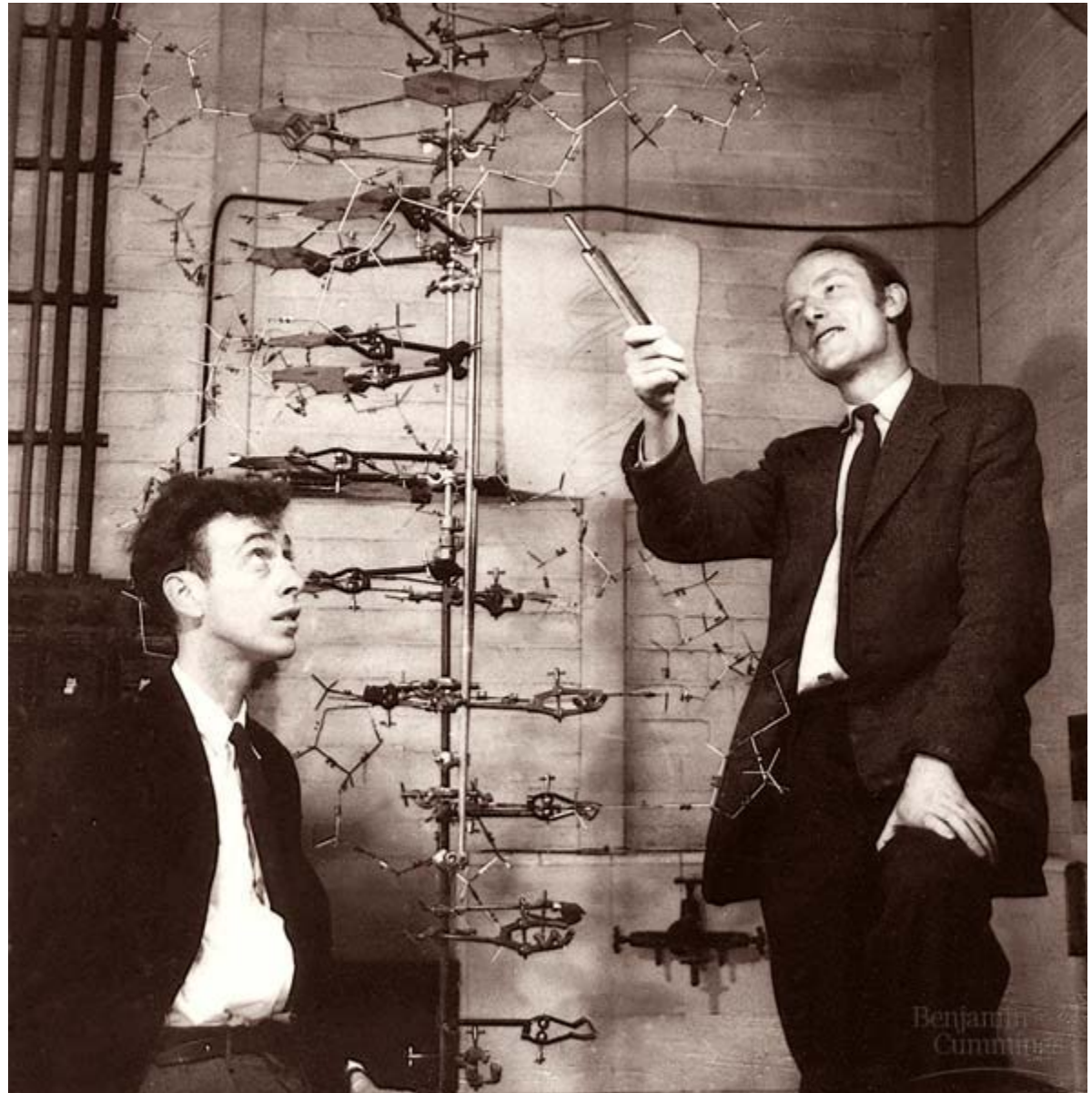
Farooq Hussain, Wikipedia



Linus Pauling

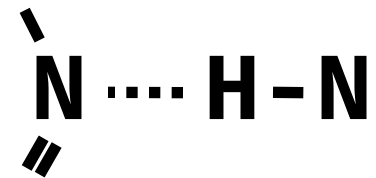
La double hélice

James Watson et
Francis Crick



La liaison hydrogène

- **Liaison électrostatique entre:**
 - Atome riche en électrons (nucléophile)
 - Atome n'ayant que des protons (électrophile)
- **Par exemple:**
 - N et O possèdent des électrons libres
 - Ils sont nucléophiles
- **Liaison faible**
 - Différent de liaison covalente

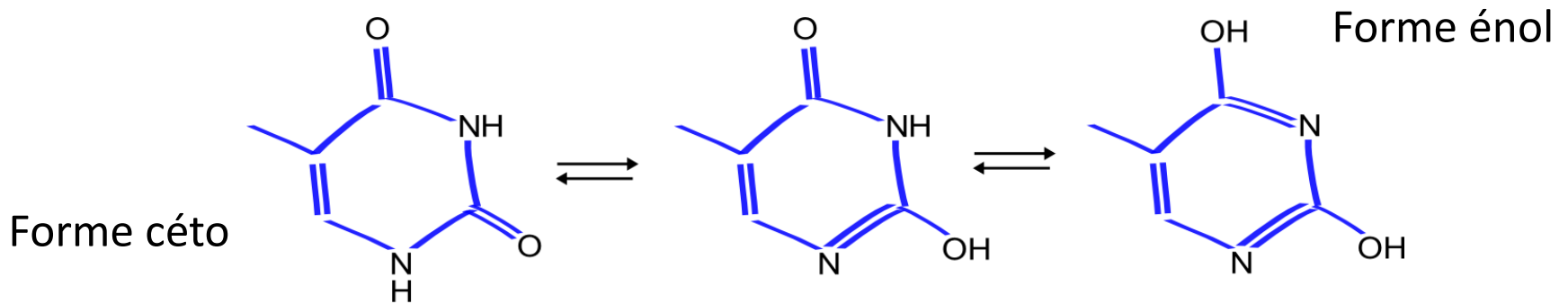


Accepteur

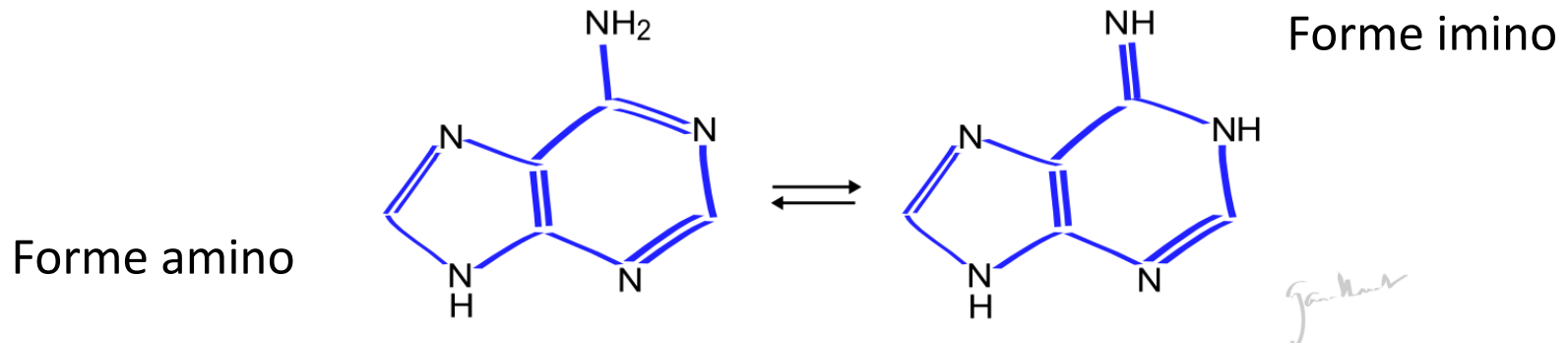
Donneur

Les tautomères

Tautomérie céto-énolique (thymine)

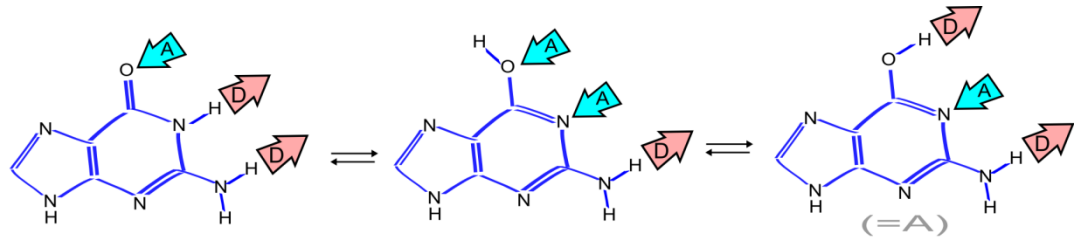


Tautomérie amino-imino (adenine)



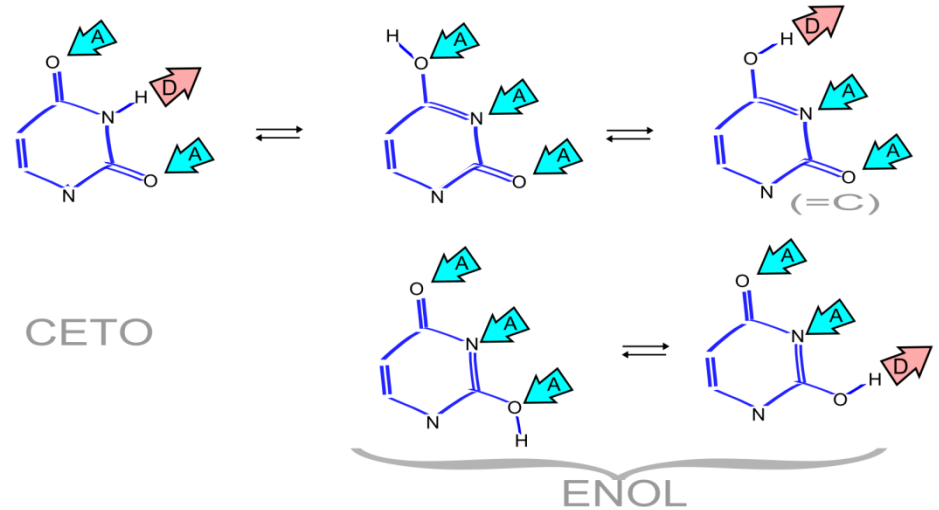
Selon le
tautomère, les
bases peuvent
former des
liaisons
hydrogènes
différentes

G

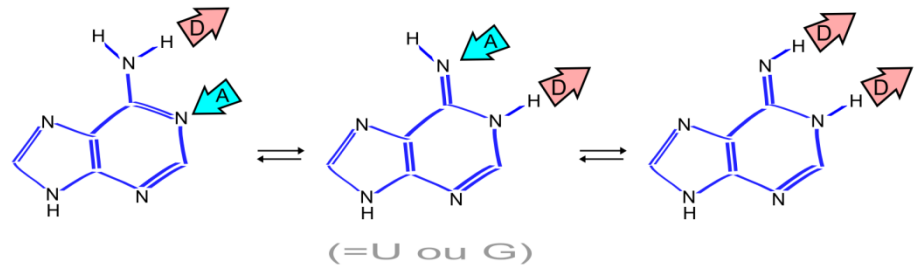


U

CETO

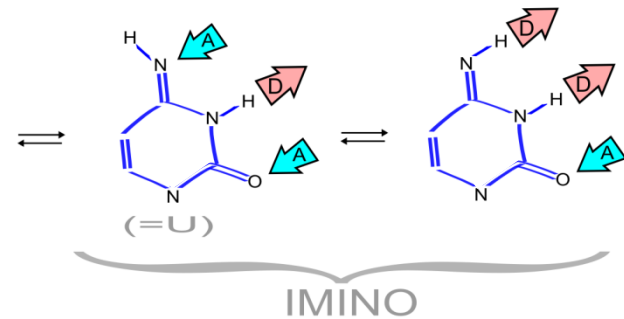


A

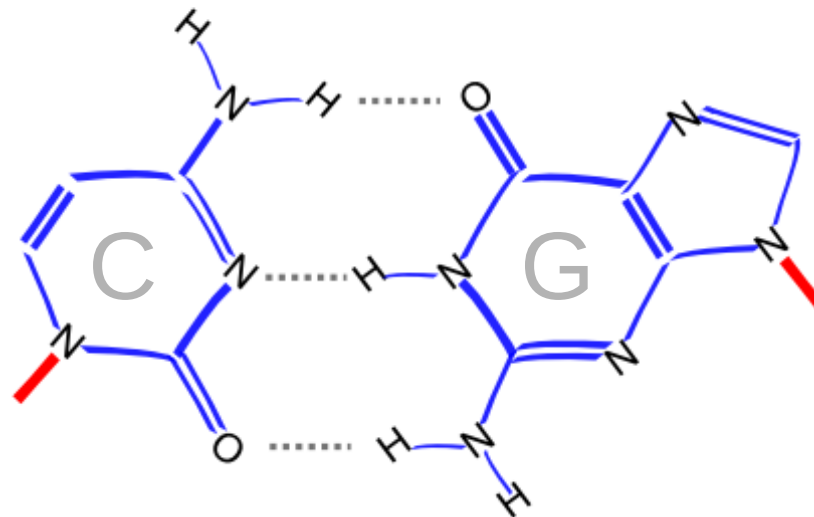
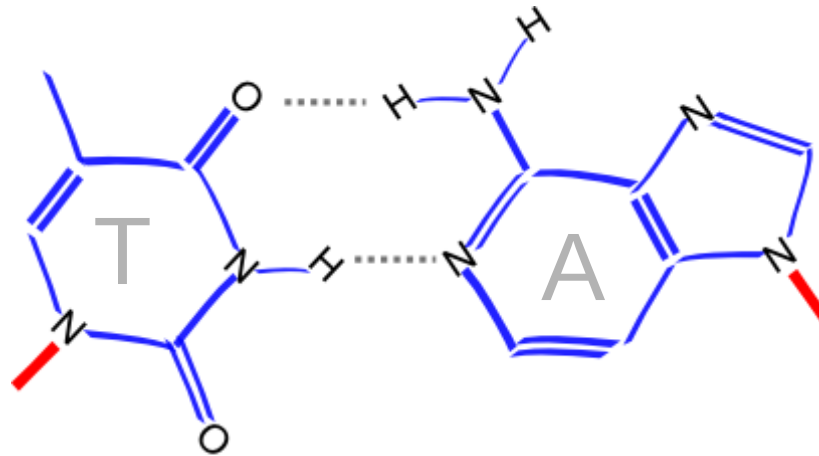


C

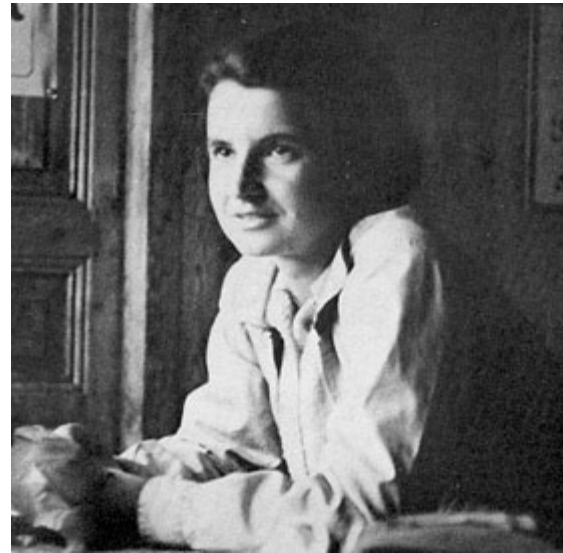
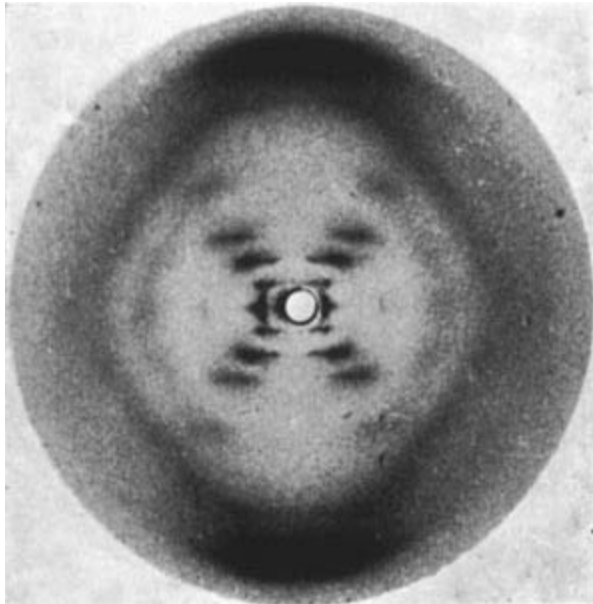
AMINO



Les paires de bases de Watson et Crick



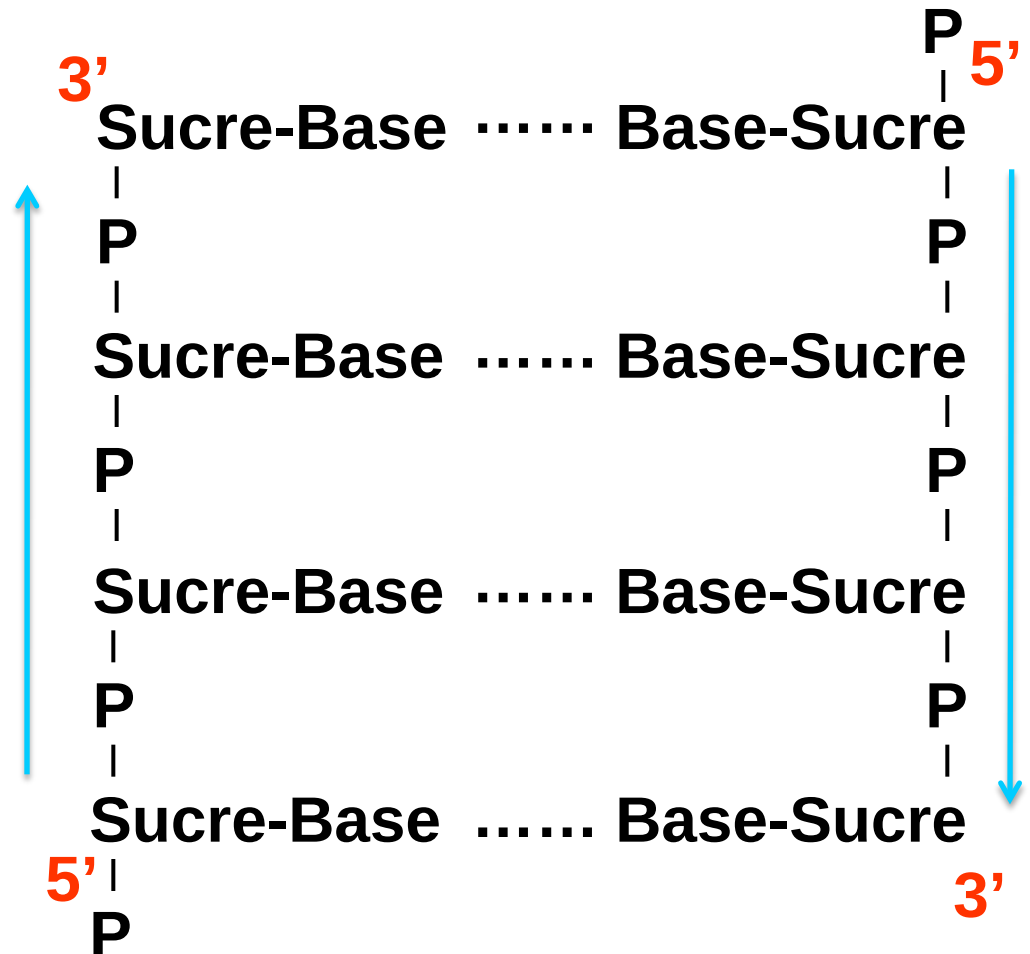
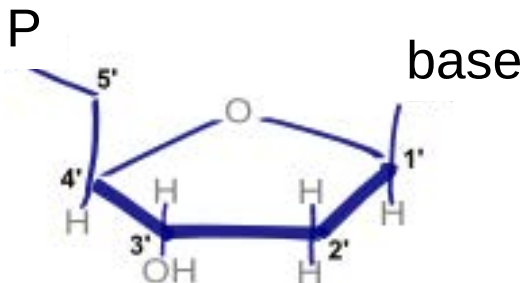
Les données de diffraction de rayons X



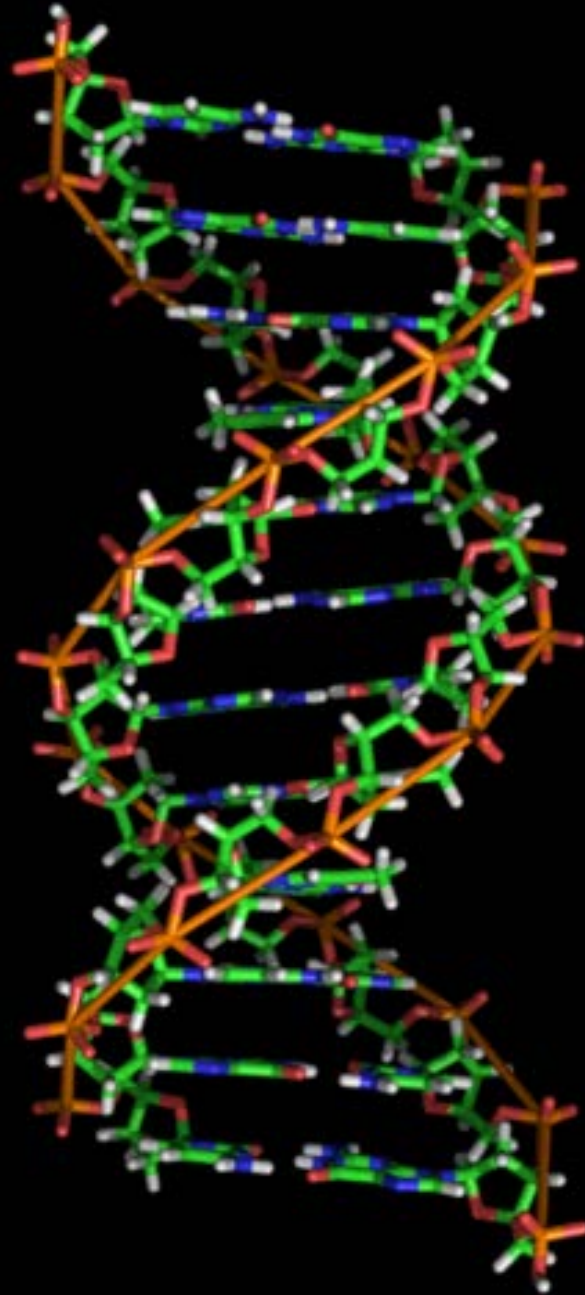
Rosalind Franklin

Structure de l'ADN

- Bases au centre
- 2 chaines antiparallèles



La double hélice



La publication de 1953

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and 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.



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

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles 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

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.

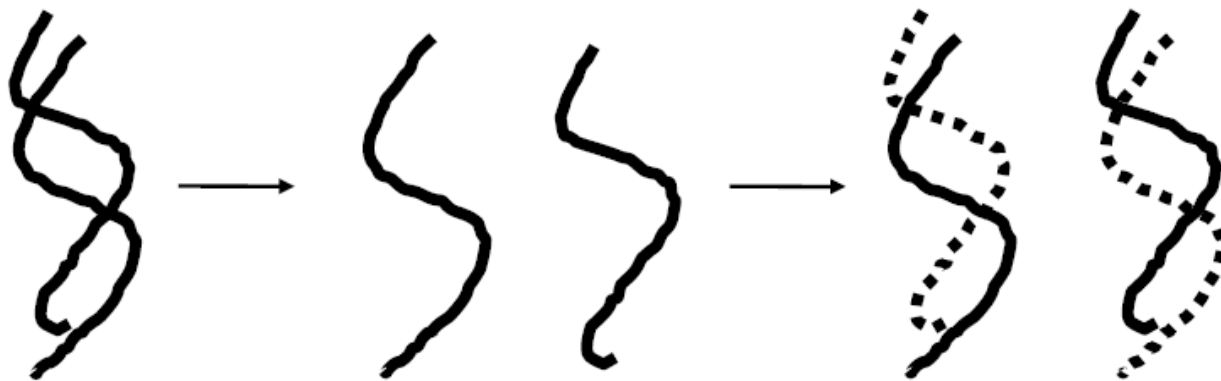
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

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

Pourquoi la découverte est-elle si importante?

- Un modèle qui « explique la vie »

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



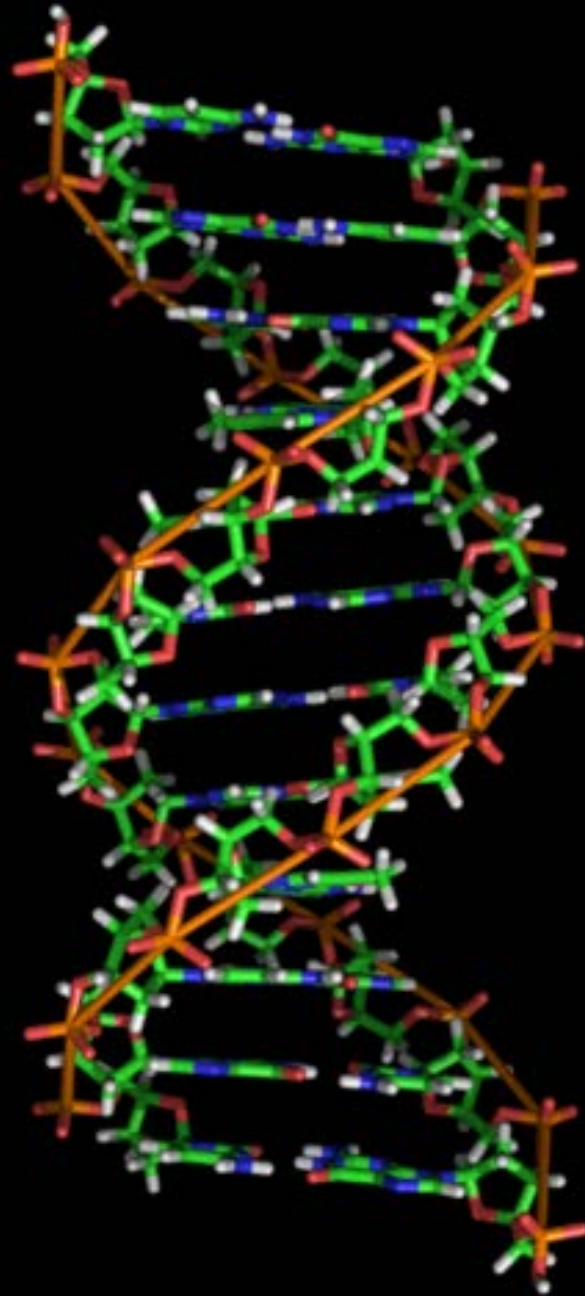
Historique (suite)

- **1953 Watson & Crick**
 - Rayons X sur fibres d'ADN.
- **1970 Dickerson & Rich**
 - Premiers cristaux d'ADN (oligonucléotide). Rayons X sur cristaux: meilleure résolution.

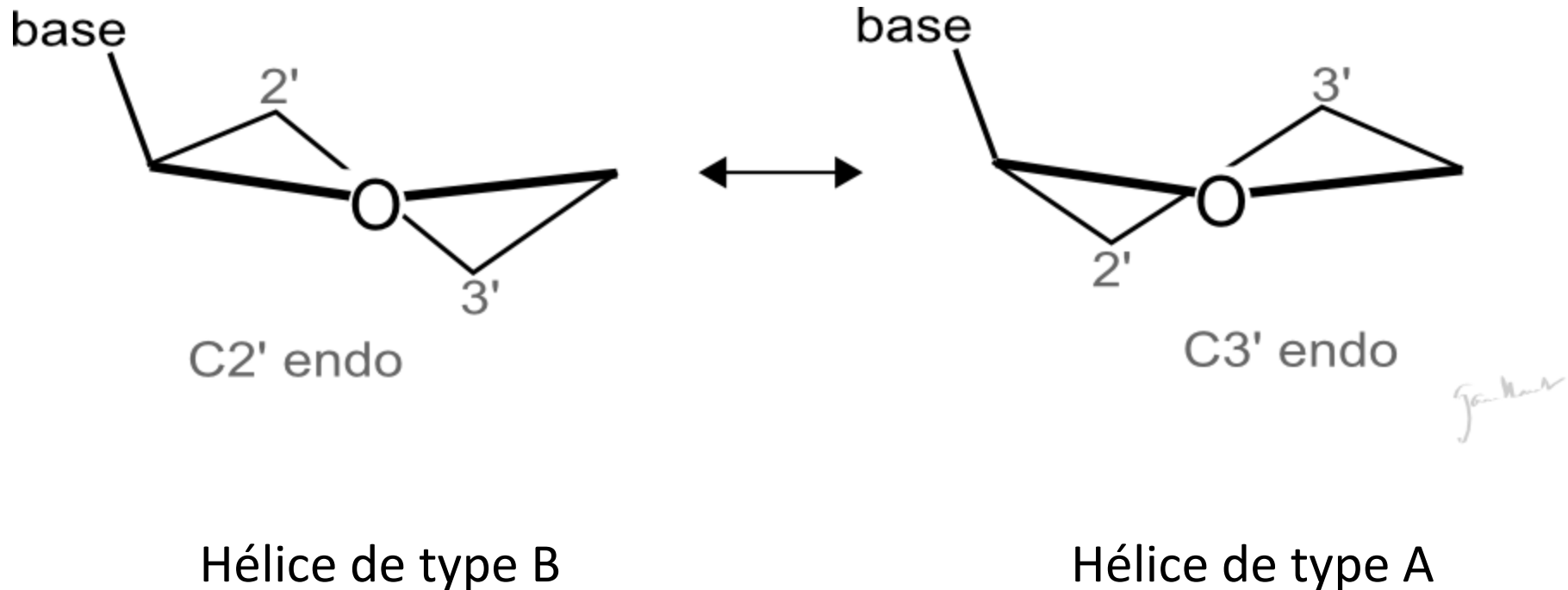
Les différentes formes d'hélices

- Des changements de conformation des sucres et de la base produisent différentes formes d'hélices
 - B
 - A
 - Z

La double hélice de forme B

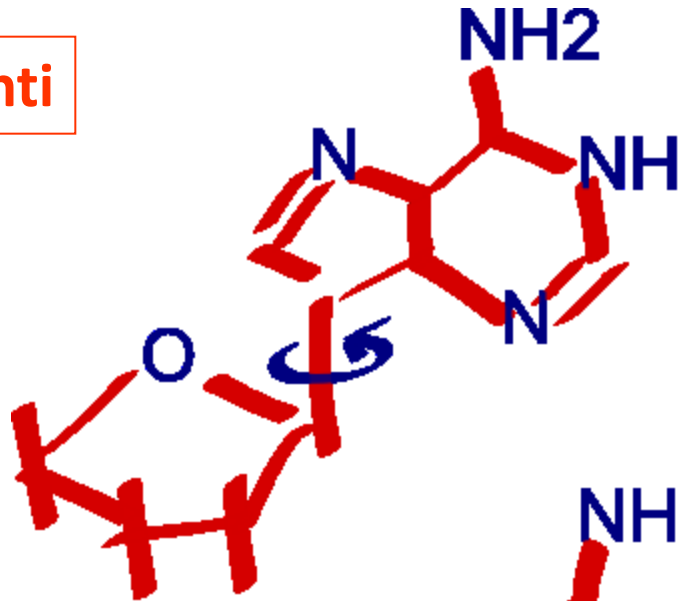


Conformation du sucre dans les hélices de type A et B



Conformation de la base par rapport au sucre

Anti



Base éloignée du sucre

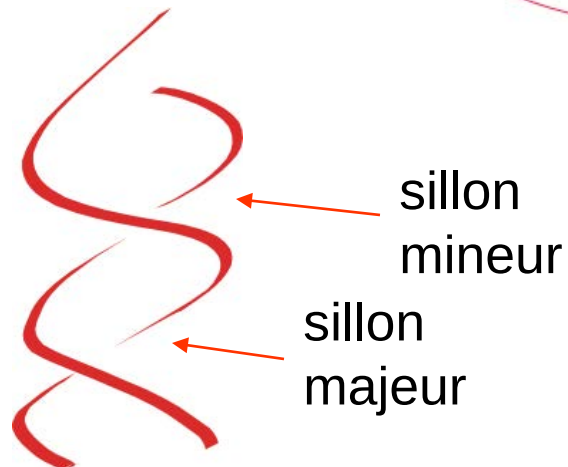
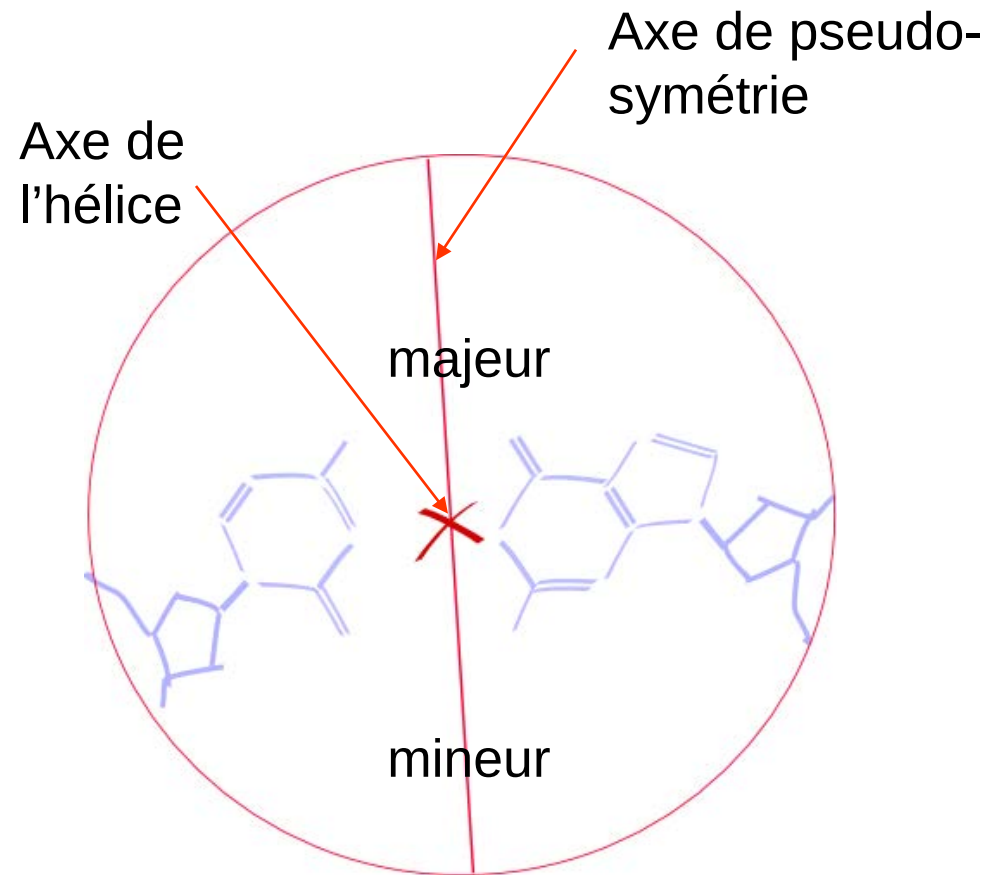
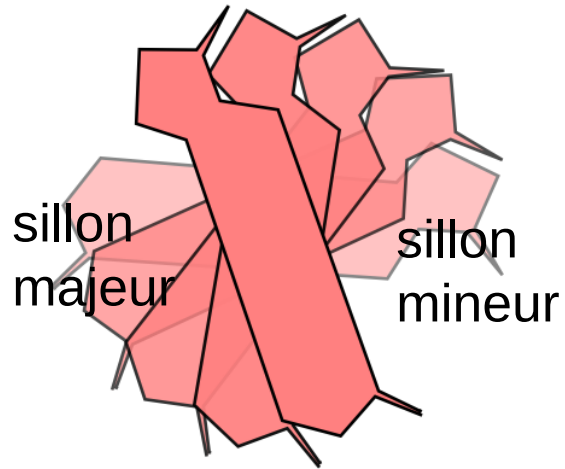
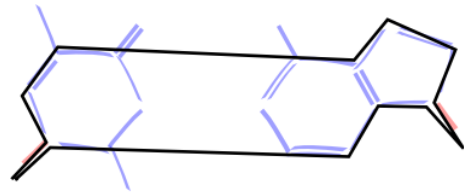
Rotation autour de la liaison C1'-N9

Base proche du sucre



syn

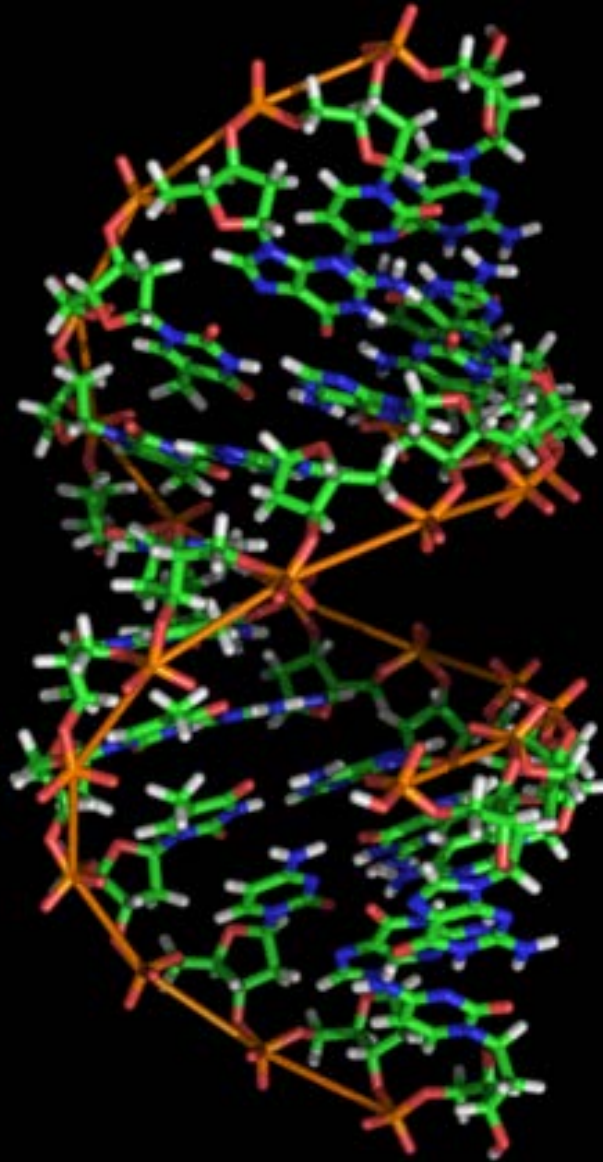
Les sillons



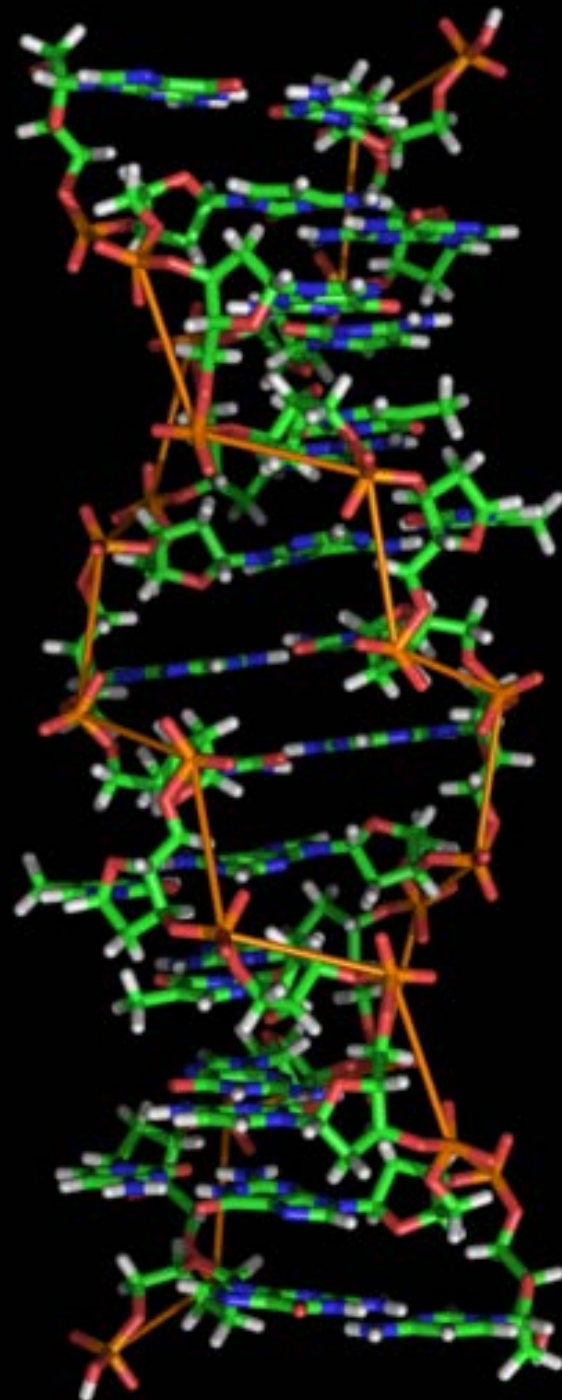
Les configurations de la double hélice: la forme B

- Double hélice droite
- Groupes phosphate à l'extérieur
- Chaînes antiparallèles
- Diamètre : 20 Å
- Nombre de paires de base par tour: 10,5
- Angle entre 2 plateaux de pb successifs: 36°
- Conformation des sucres :twist, C2' endo
- Liaison base-sucres: anti
- Distance entre 2 plateaux: 3,4 Å
- Axe de la double hélice centré sur le plateau de paire de bases
- Les sillons majeur et mineur ont la même profondeur
- Vue transversale: les groupes phosphate sont autour, les bases en anneau au milieu

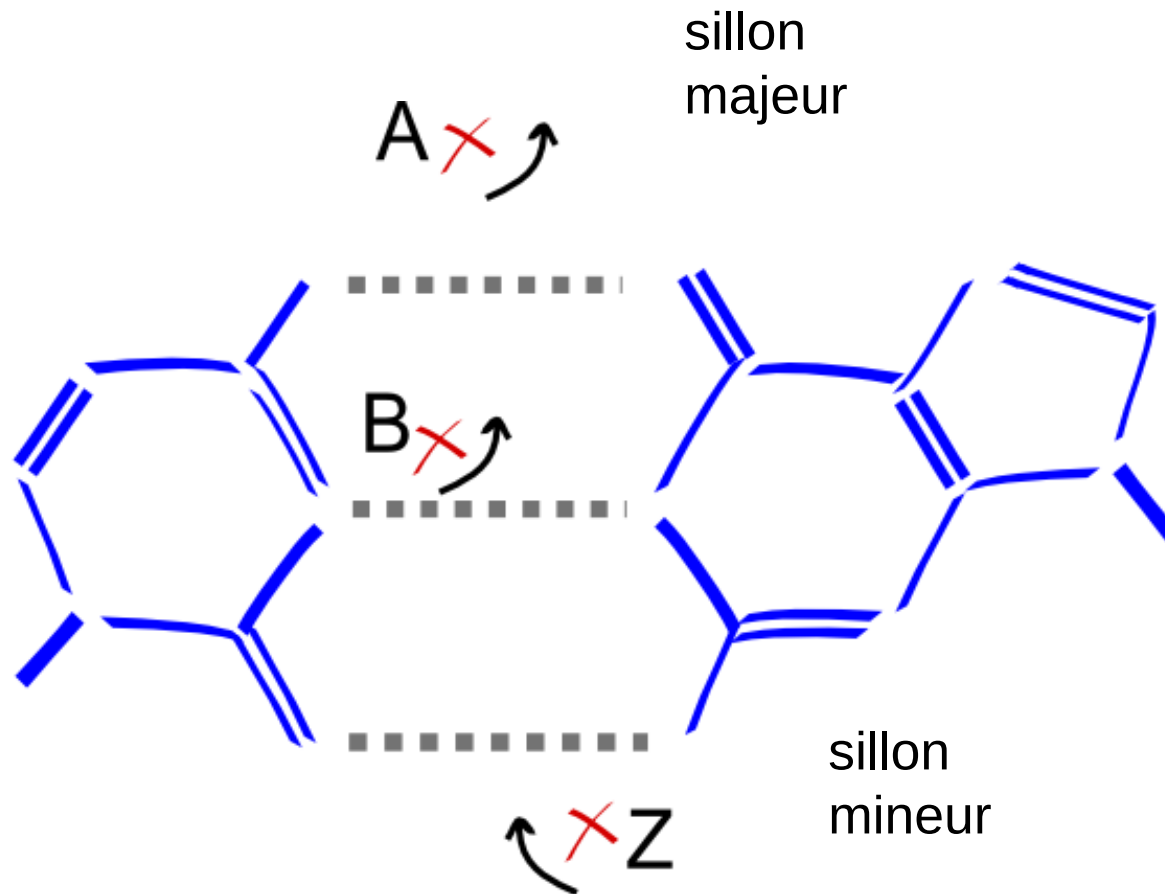
Hélice A



Hélice Z



Position de l'axe dans les 3 types d'hélice



Caractéristiques des hélices

A, B et Z

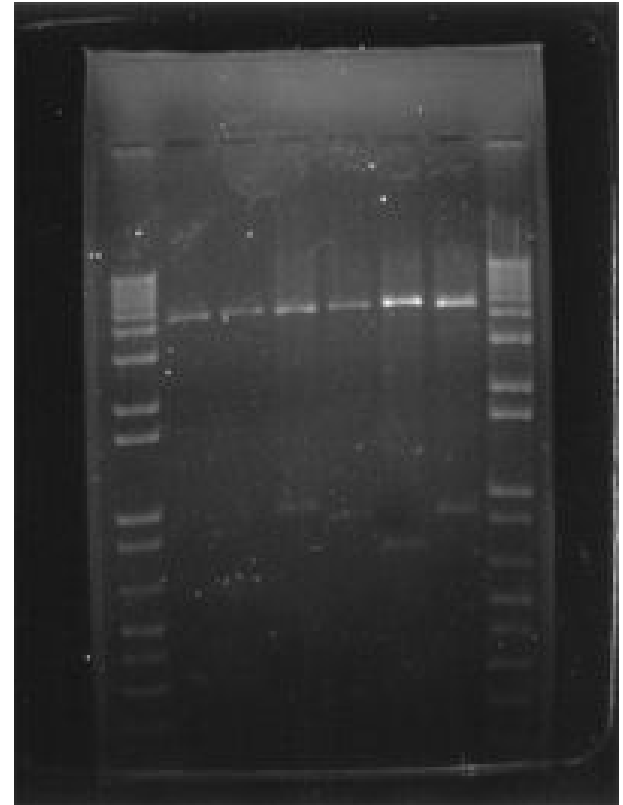
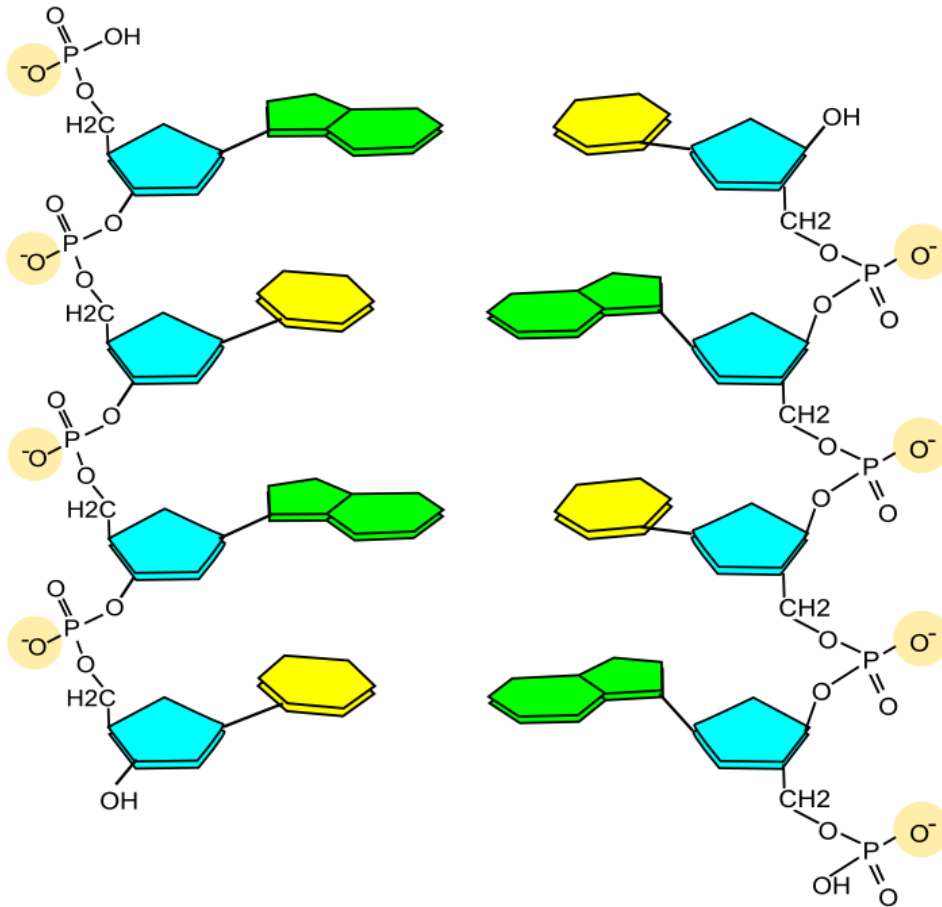
A	B	Z
ADN/ARN	ADN	ADN/ARN

Nt/tour		11	10	12
Sens hélice		droit	droit	gauche
Confo sucre		3' endo	2' endo	2' endo (py) 3' endo (pu)
Diamètre		26 Å	20 Å	18 Å
Liaison glycosidique		anti	anti	Anti (py) Syn (pu)
Déplacement bp/axe		4 Å	aucun	
Sillon majeur	largeur	3 Å	12 Å	plat
	prof	13,5 Å	9 Å	
Sillon mineur	largeur	11 Å	6 Å	étroit
	prof	3 Å	7,5 Å	profond

Propriétés physiques de l'ADN

L'ADN est chargé négativement

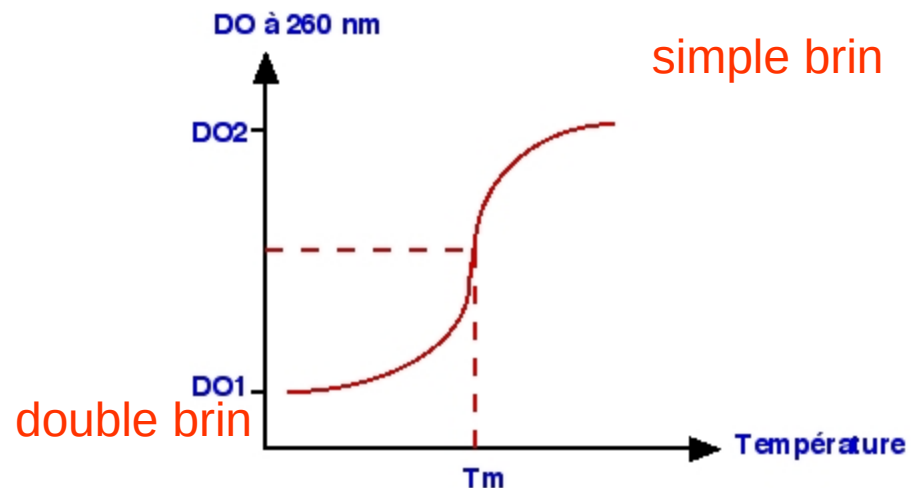
- Electrophorèse sur gel d'acrylamide:



L'ADN absorbe la lumière à 260nm (UV)

Le chauffage des solutions d'ADN produit une augmentation d'absorbance à 260 nm. Ce phénomène correspond à la dénaturation de l'ADN bicaténaire en 2 brins d'ADN monocaténares, d'où le doublement de densité optique.

On peut caractériser la température de fusion de l'ADN notée T_m qui correspond à la température à la moitié du phénomène.



Les brins d'ADN s'hybrident

- Hybridation = Association spontanée, spécifique et réversible de deux brins d'ADN complémentaires (formant des paires WC)
- L'hybridation est :
 - **spécifique** : une séquence d'ADN monobrin s'apparie à la séquence qui lui est complémentaire.
 - **réversible** : En jouant sur les conditions expérimentales (température) on peut entraîner ou briser (dissociation) l'hybridation de deux molécules d'ADN.
- Ce passage se visualise en mesurant la DO à 260nm.

