

Biologia evolutiva

Origem da variabilidade genética

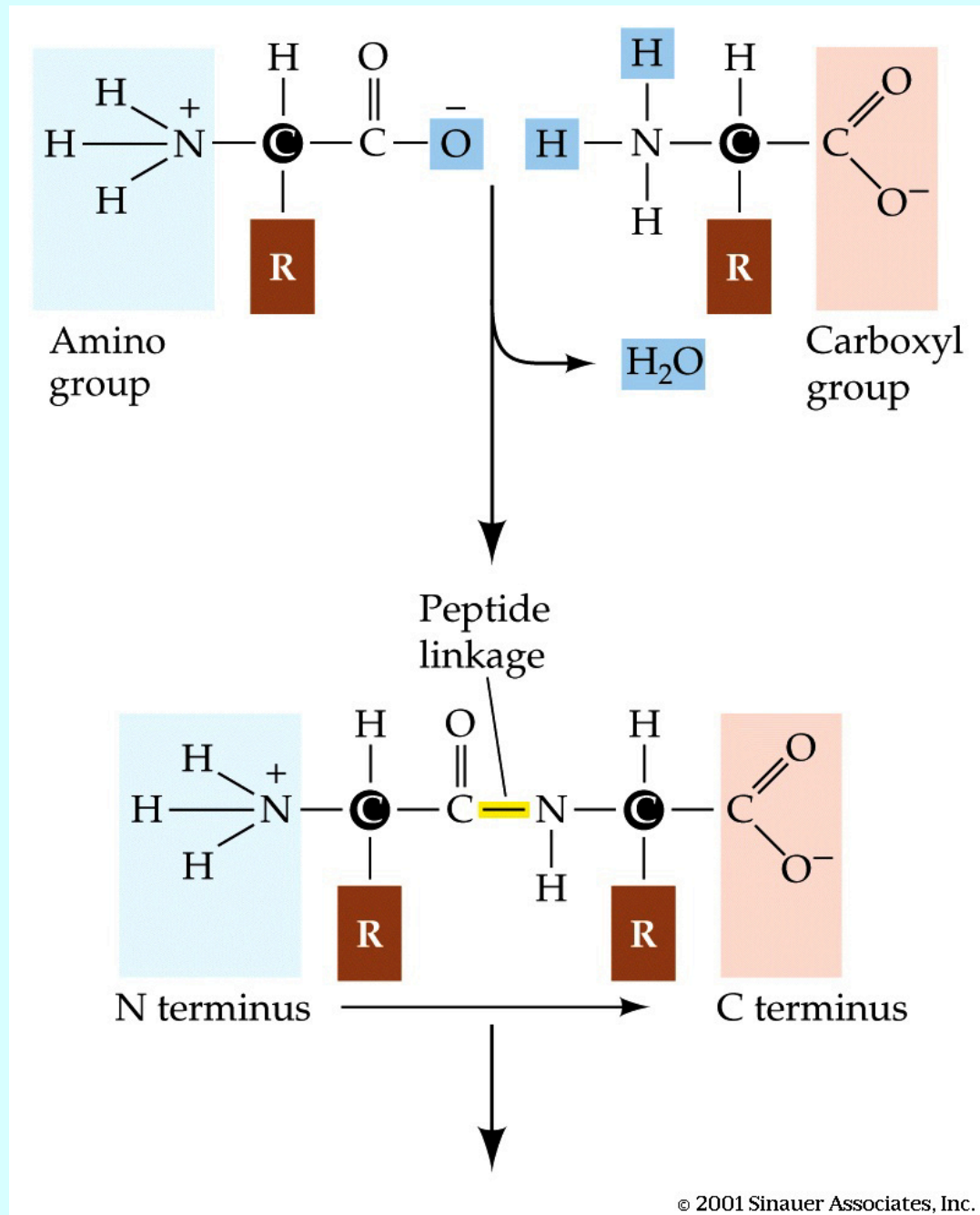
Sergio Russo Mاتيoli

Departamento de Genética e Biologia Evolutiva

Instituto de Biociências

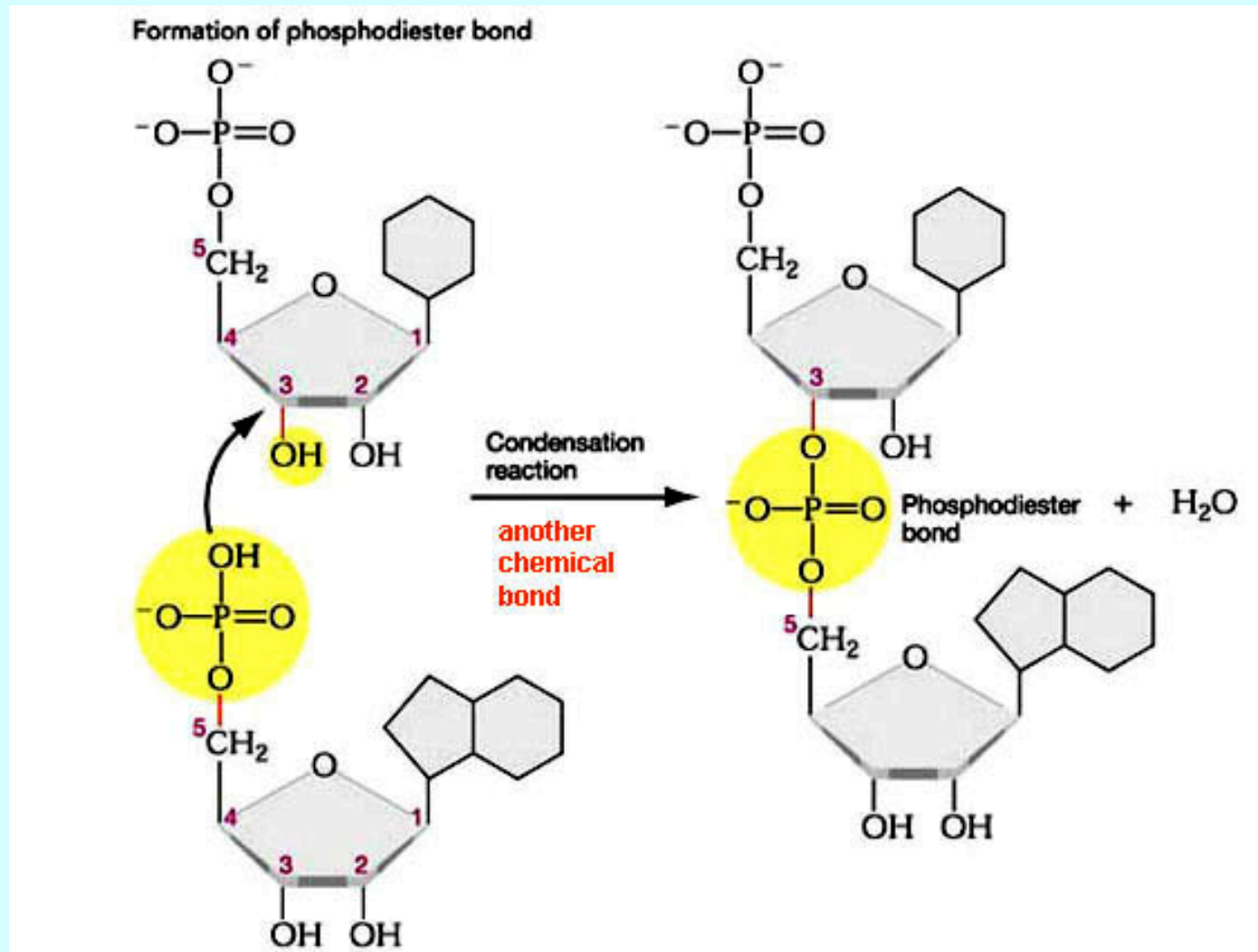
Universidade de São Paulo

Conceitos de Bioquímica básica



Ligação peptídica

Conceitos de Bioquímica básica



Ligação fosfodiéster

Friedrich Miescher (1844-1895)



Biólogo suíço, isolou, em 1869, vários compostos químicos ricos em fósforo de núcleos de células brancas do sangue (obtidas de pus), que ele denominou como nucleína.

Theodor Boveri (1862-1915)



Biólogo alemão, propôs, pouco após a redescoberta das leis de Mendel, conjuntamente com Walter Sutton, a teoria cromossômica da herança, na qual os fatores responsáveis pela hereditariedade estariam localizados nos cromossomos.

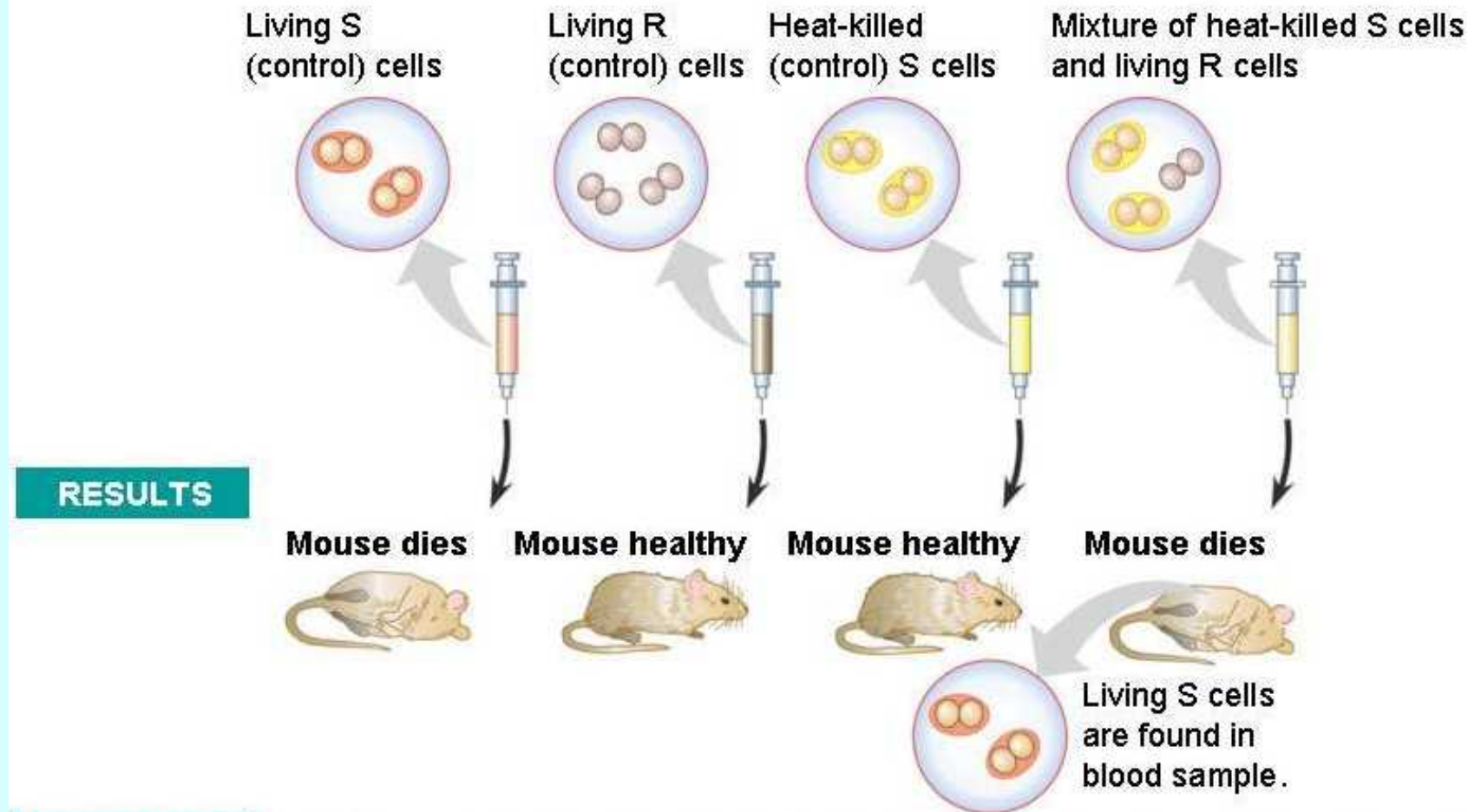
Frederick Griffith (1879-1928)



Médico inglês, ficou conhecido por caracterizar, em 1928 o “princípio transformante”, mostrando que o material genético constituía-se em uma substância química encontrada nas células mesmo que não vivas.

O experimento de Frederick Griffith

EXPERIMENT Bacteria of the “S” (smooth) strain of *Streptococcus pneumoniae* are pathogenic because they have a capsule that protects them from an animal’s defense system. Bacteria of the “R” (rough) strain lack a capsule and are nonpathogenic. Frederick Griffith injected mice with the two strains as shown below:



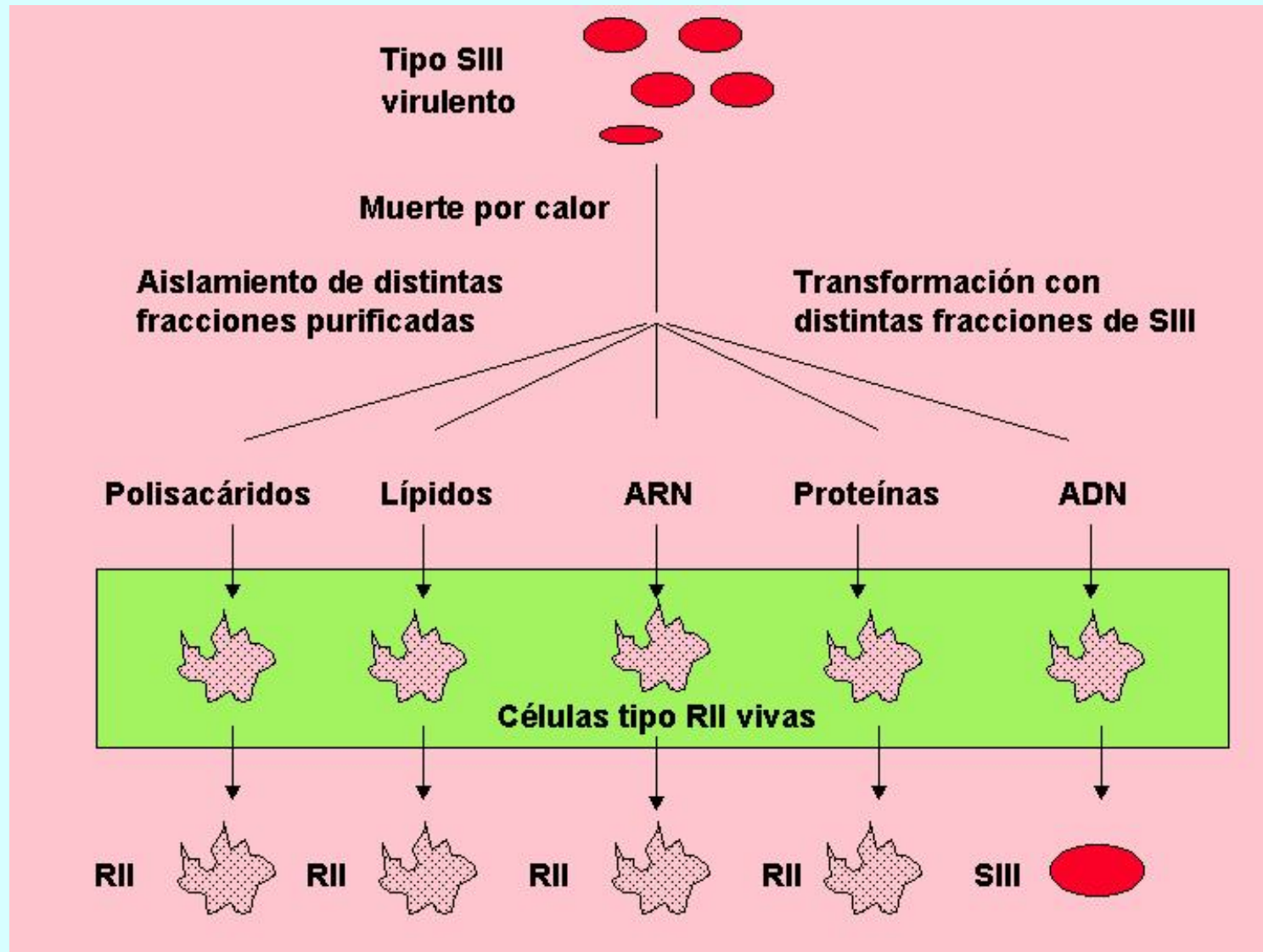
CONCLUSION Griffith concluded that the living R bacteria had been transformed into pathogenic S bacteria by an unknown, heritable substance from the dead S cells.

Oswald Theodore Avery (1877-1955)

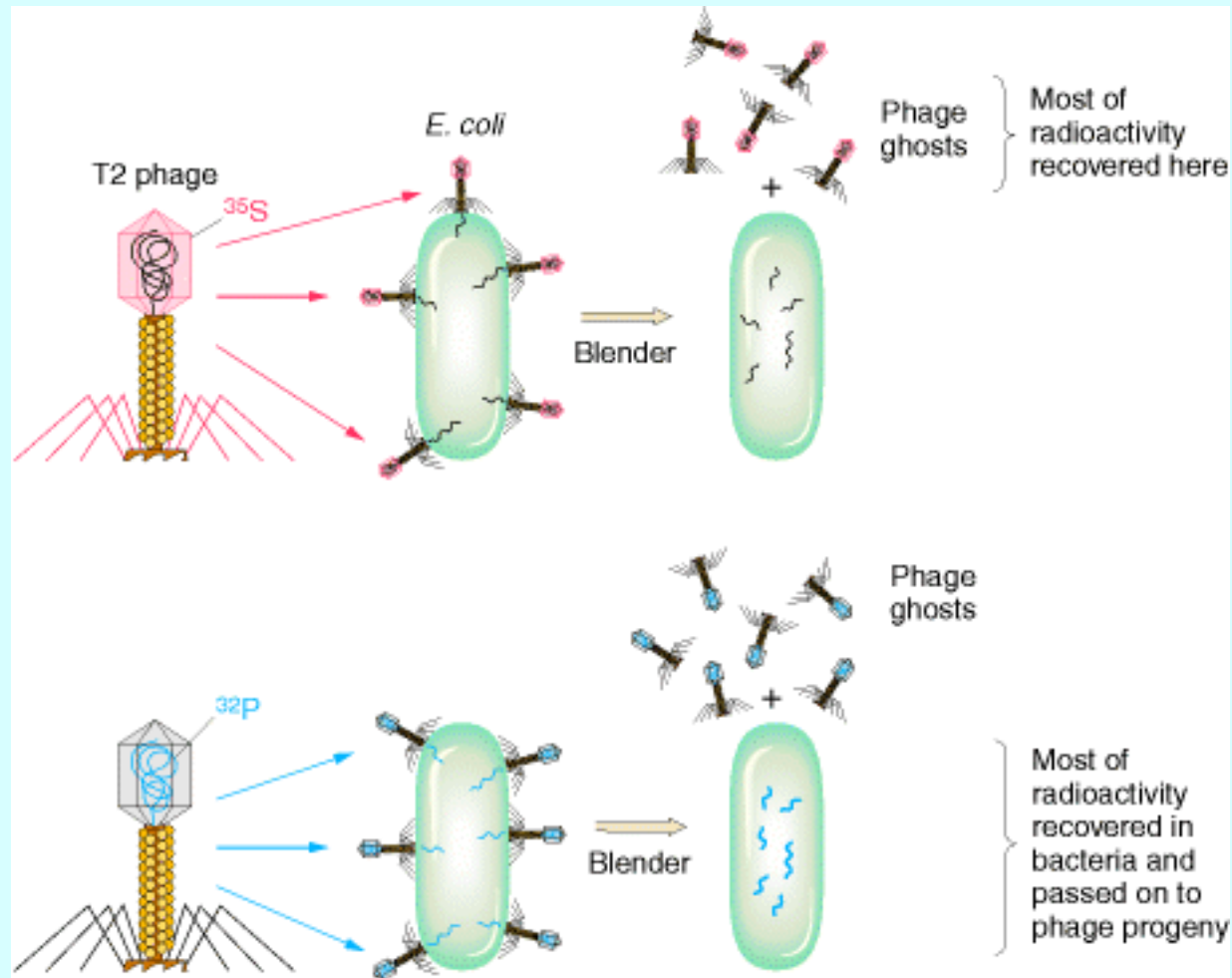


Médico canadense, juntamente com os americanos Colin MacLeod e Maclyn McCarty, demonstrou que o DNA é o material genético em 1944.

O experimento de Avery, MacLeod e MacCarthy (1944)

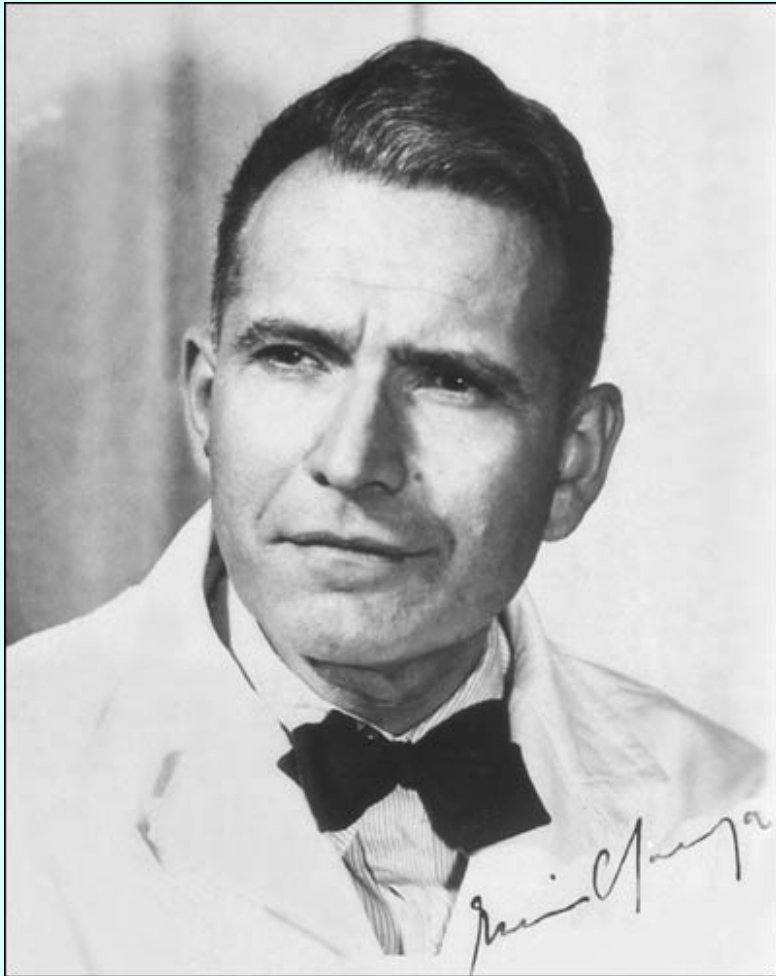


O experimento de Hershey e Chase



Hershey, Alfred D. and Chase, Martha (1952) Independent functions of viral protein and nucleic acid in growth of bacteriophage. J Gen Physiol. 1:39-56.

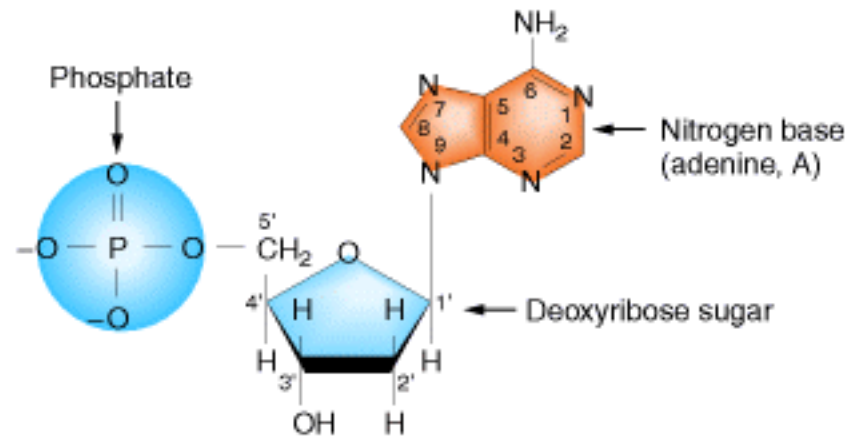
Erwin Chargaff (1905-2002)



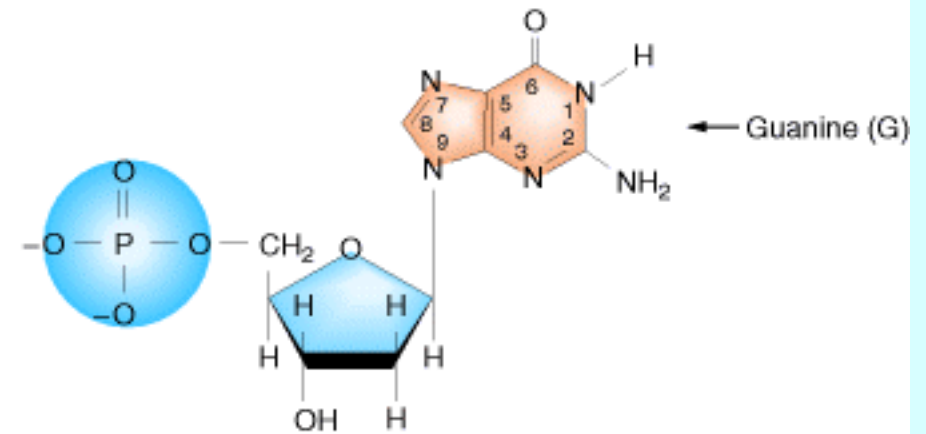
Bioquímico austríaco que emigrou para os E.U.A. durante o período nazista. Formulou as regras: $\%A = \%T$ e $\%C = \%G$, importante para se desvendar a estrutura do DNA.

Estrutura do DNA

Purine nucleotides

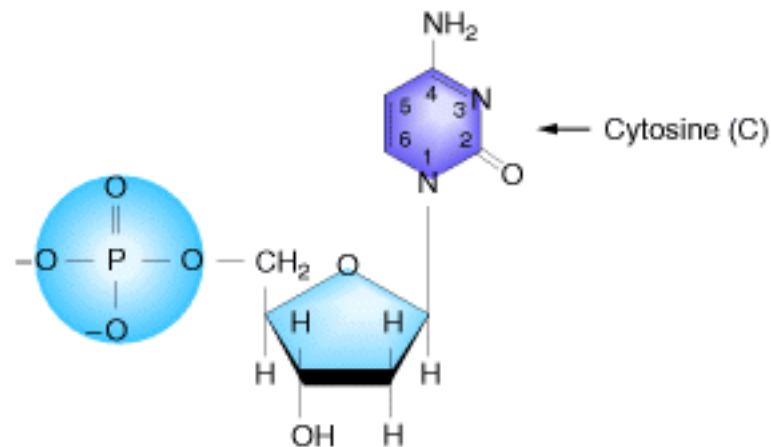


Deoxyadenosine 5'-phosphate (dAMP)

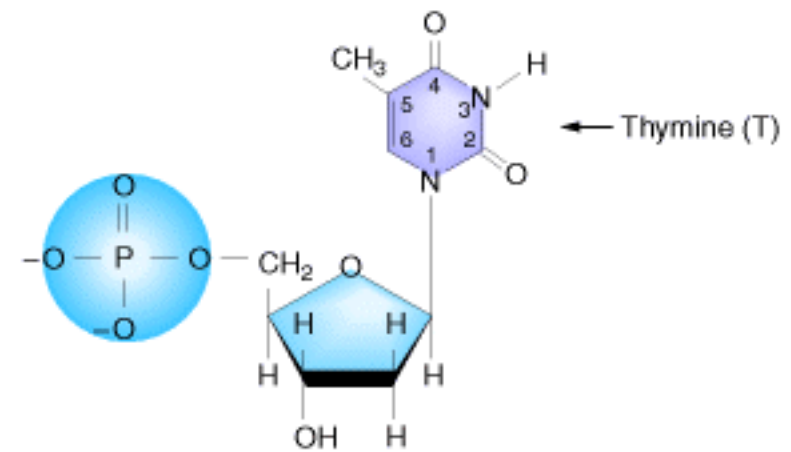


Deoxyguanosine 5'-phosphate (dGMP)

Pyrimidine nucleotides



Deoxycytidine 5'-phosphate (dCMP)



Deoxythymidine 5'-phosphate (dTMP)

Rosalind Franklin

(1920-1958)



Biofísica inglesa, foi pioneira no uso da difração de Raios-X em biomoléculas.

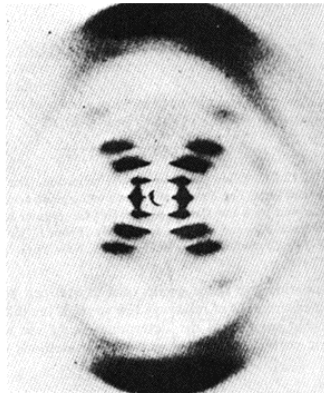
James Dewey Watson (1928-)

Francis Harry Compton Crick (1916-2004)



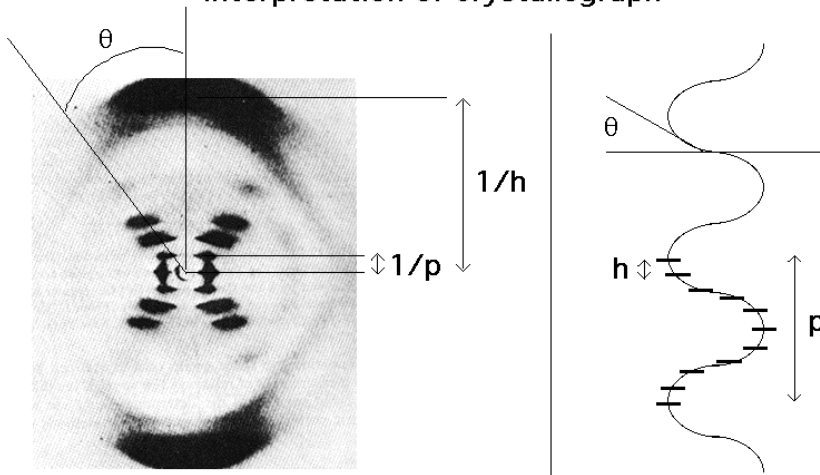
Zoólogo americano e físico inglês, propuseram um modelo de estrutura do DNA baseados em difração de Raios X de cristais da molécula obtidos por R. Fanklin. Receberam o prêmio Nobel de 1963 por isso, Franklin já havia falecido.

Interpretação estrutural da difração de Raios X por cristais de DNA



X-ray
diffraction
pattern from
B form of
DNA

Interpretation of crystallograph



θ - tilt of helix (angle from perpendicular to long axis)

$h = 3.4 \text{ \AA}$ (Distance between bases)

$p = 34 \text{ \AA}$ (Distance for one complete turn of helix; Repeat unit of the helix)

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribonucleic 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 we described in rather (discussed), and for this reason we shall not comment on it.

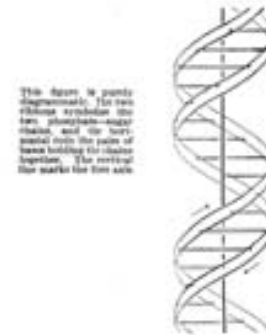
We wish to put forward a radically different structure for the salt of deoxyribonucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining 3'-n-deoxyribose residues with 2',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 Farberg's 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 Farberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There 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 the same assumption the other member must be thymine; similarly for



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

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^{2,3} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribonucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atoms would make too close a van der Waals contact.

The previously published X-ray data^{4,5} on deoxyribonucleic 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 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*, **157**, 336 (1945); *Proc. U.S. Nat. Acad. Sci.*, **30**, 36 (1945).

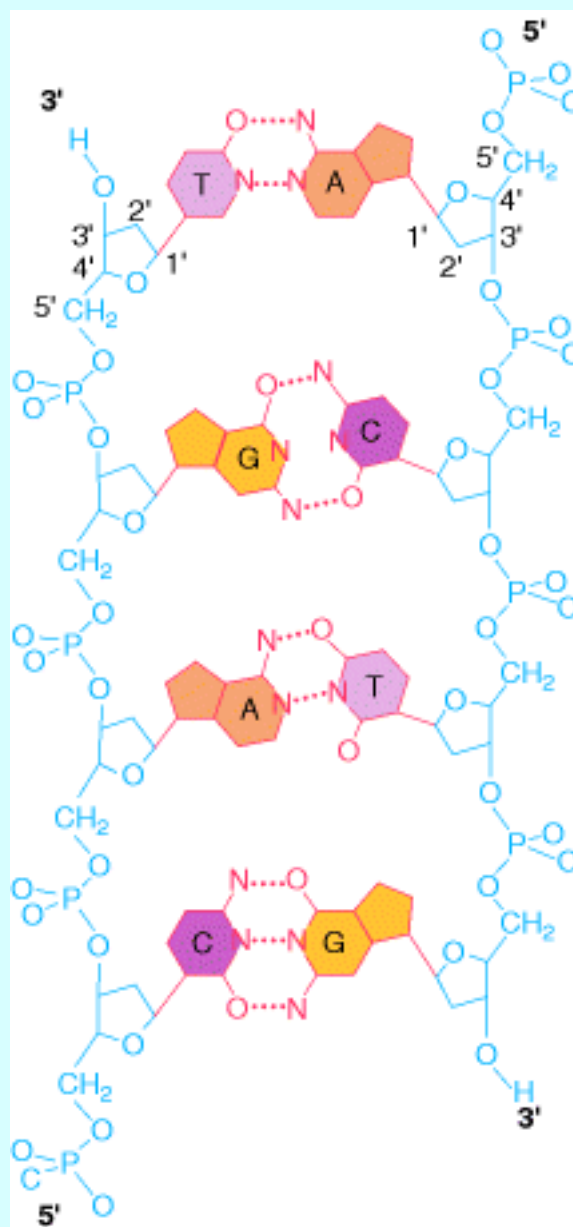
² Chargaff, E., *Ann. Chem. Phys.*, **6**, 554 (1935).

³ Chargaff, E., for references see Chargaff, E., *Dissemination*, C. and Chargaff, E., *Biophys. J.*, **1**, 10 (1952).

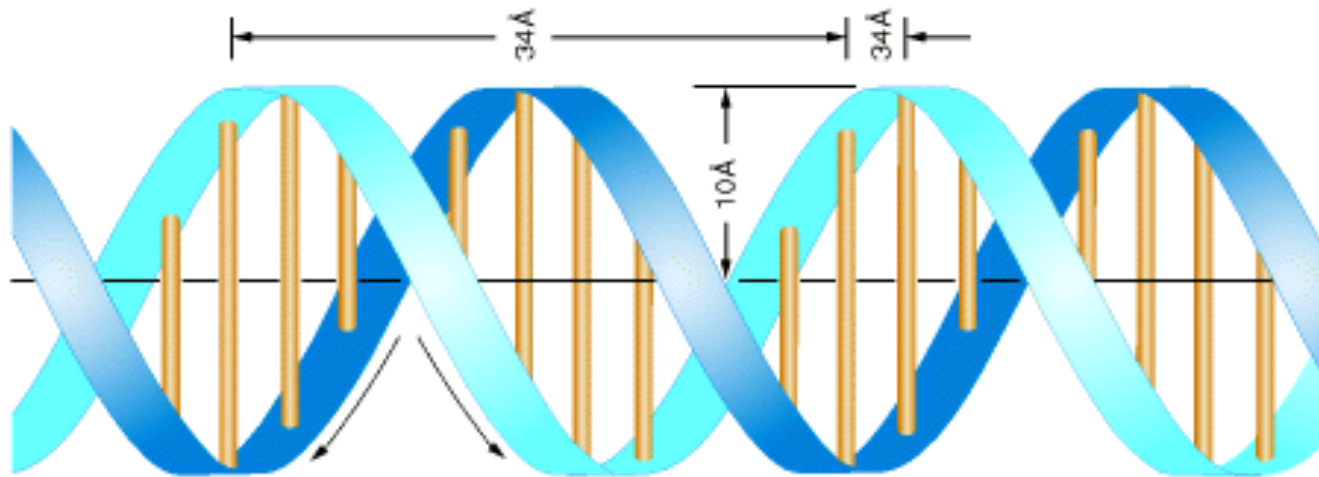
⁴ Wilkins, M. H. F., *Proc. Roy. Soc. (London)*, **18**, 202 (1952).

⁵ Wilkins, M. H. F., and Donohue, J. T., *Biophys. J.*, **18**, 192 (1952).

Estrutura antiparalela do DNA

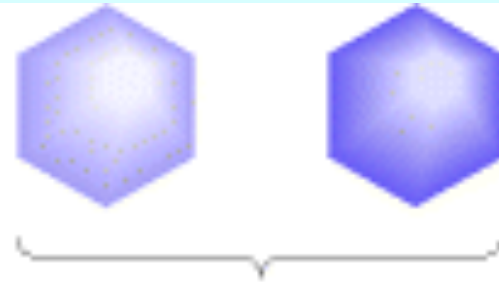


Estrutura do DNA: dimensões

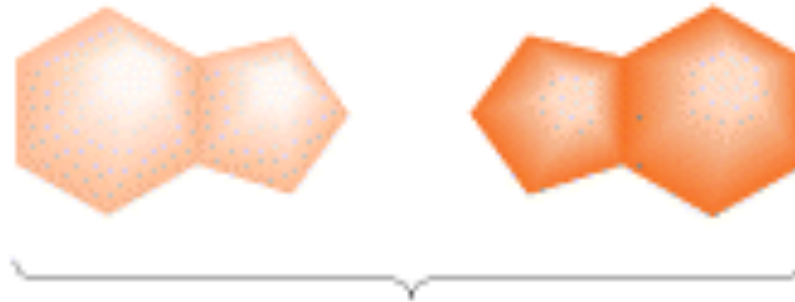


Estrutura do DNA: bases púricas e pirimídicas

Pyrimidine + pyrimidine: DNA too thin



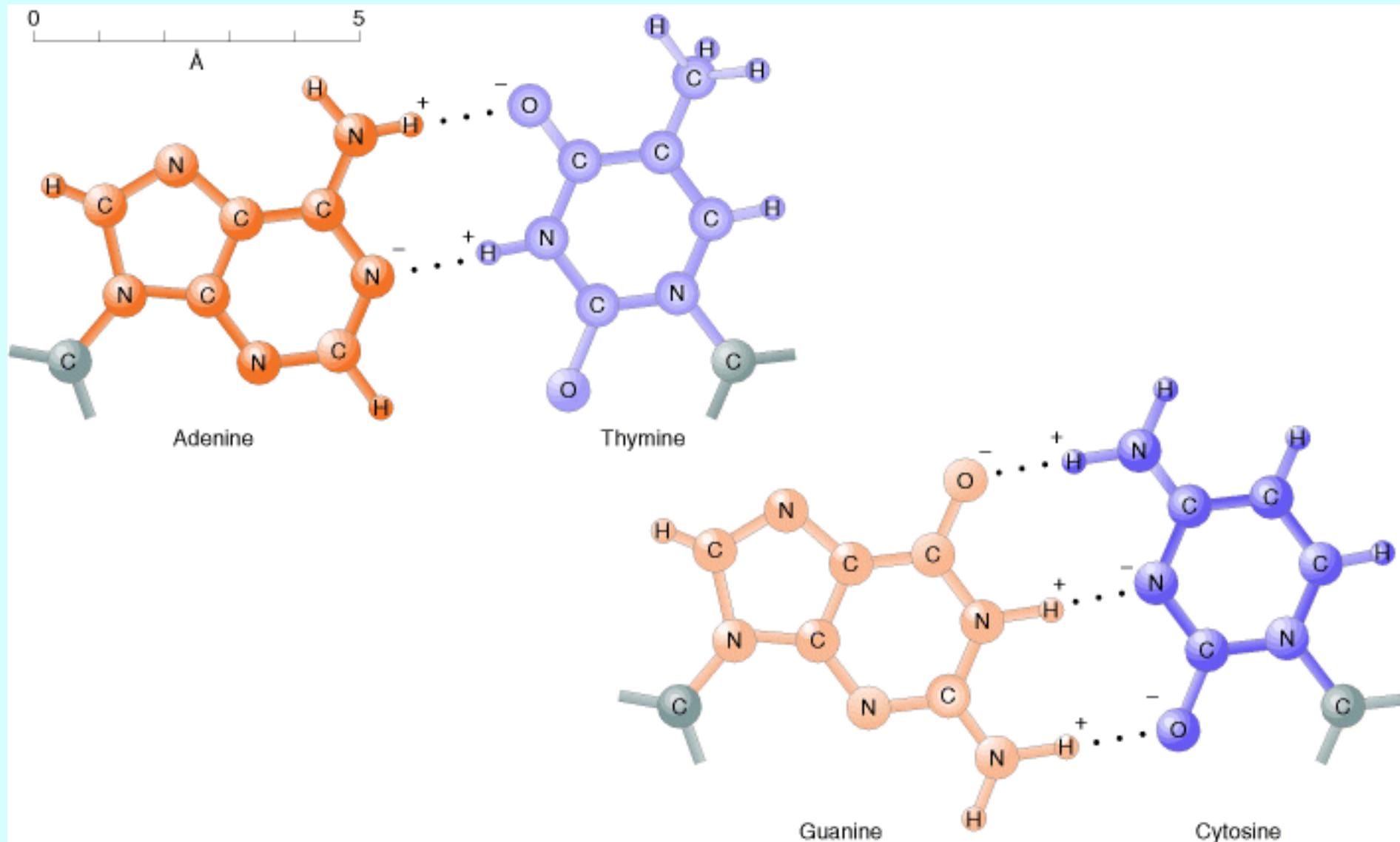
Purine + purine: DNA too thick



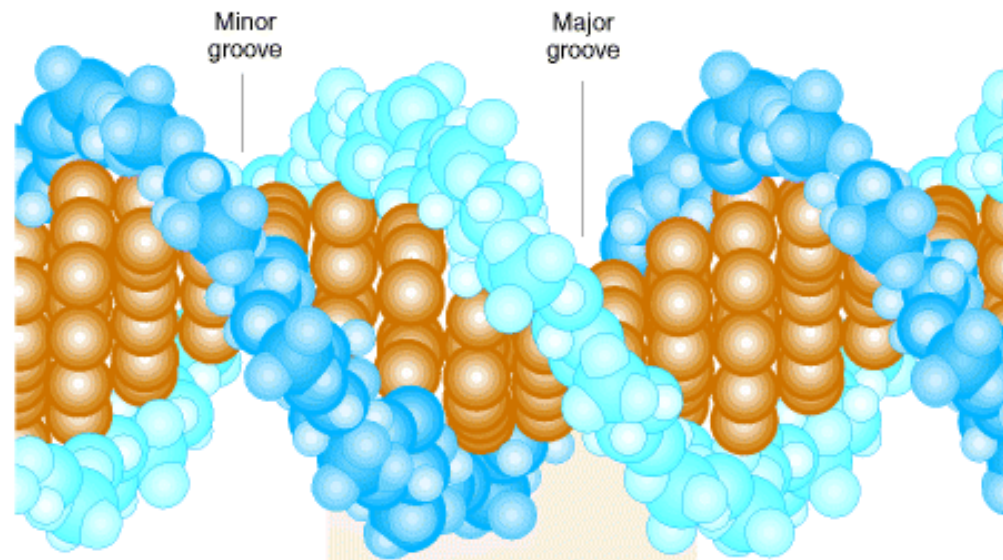
Purine + pyrimidine: thickness compatible with X-ray data



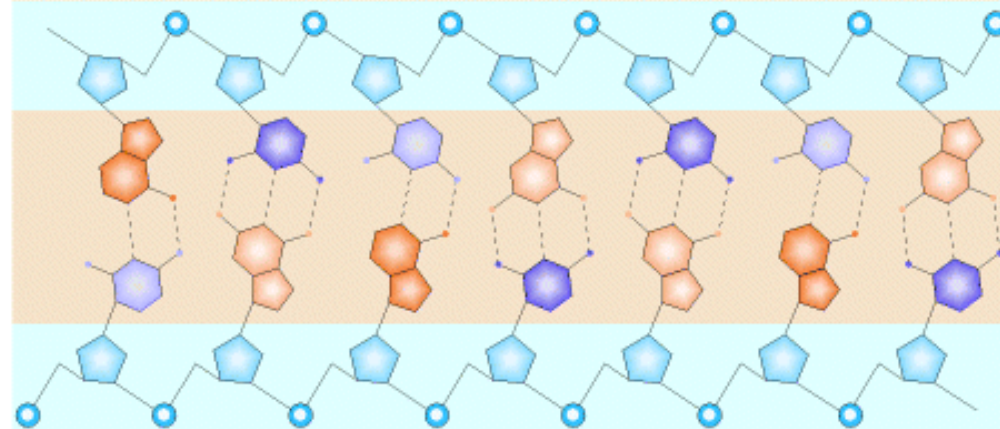
Estrutura do DNA: pontes de hidrogênio



Estrutura do DNA: sulcos

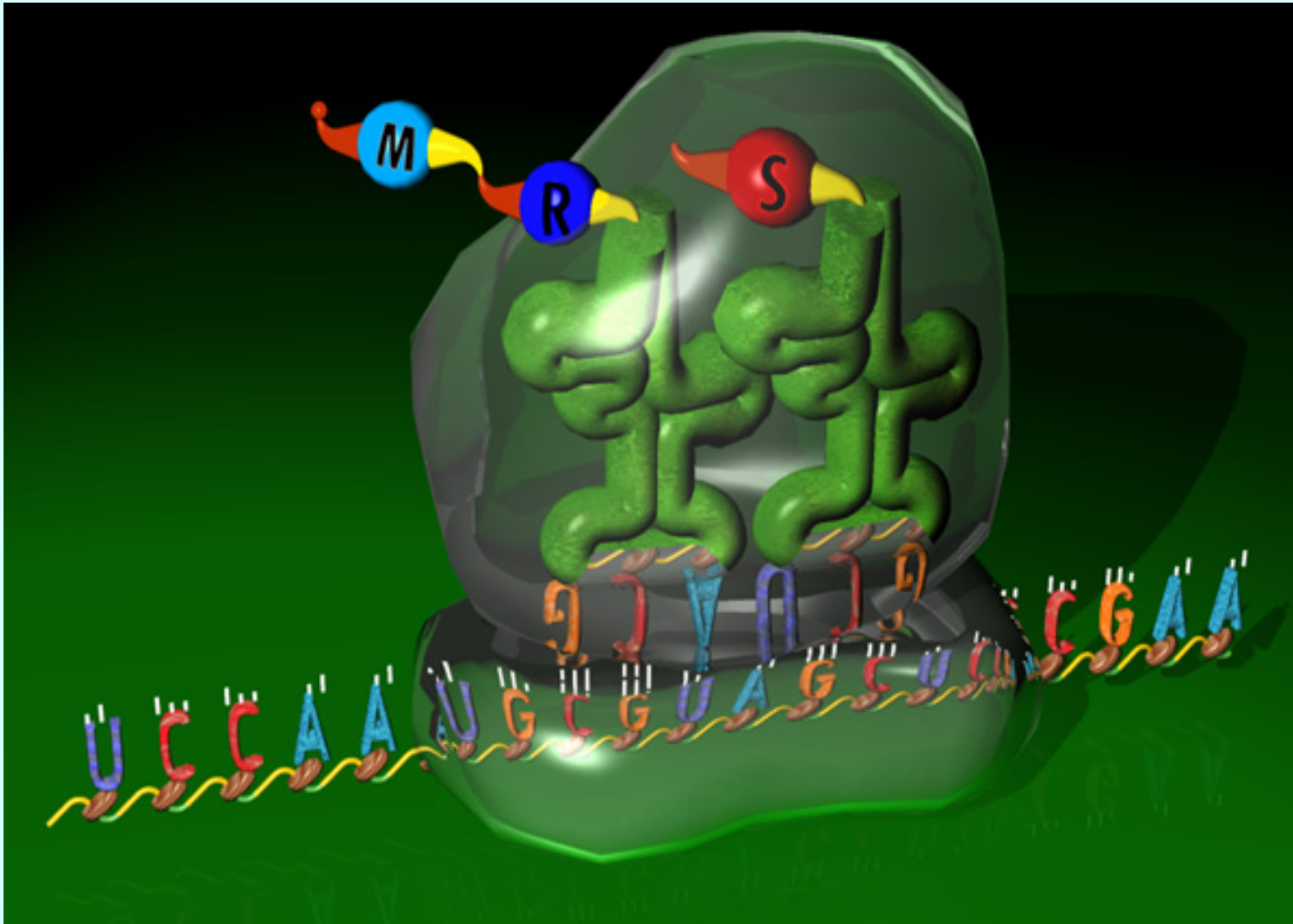


(a)

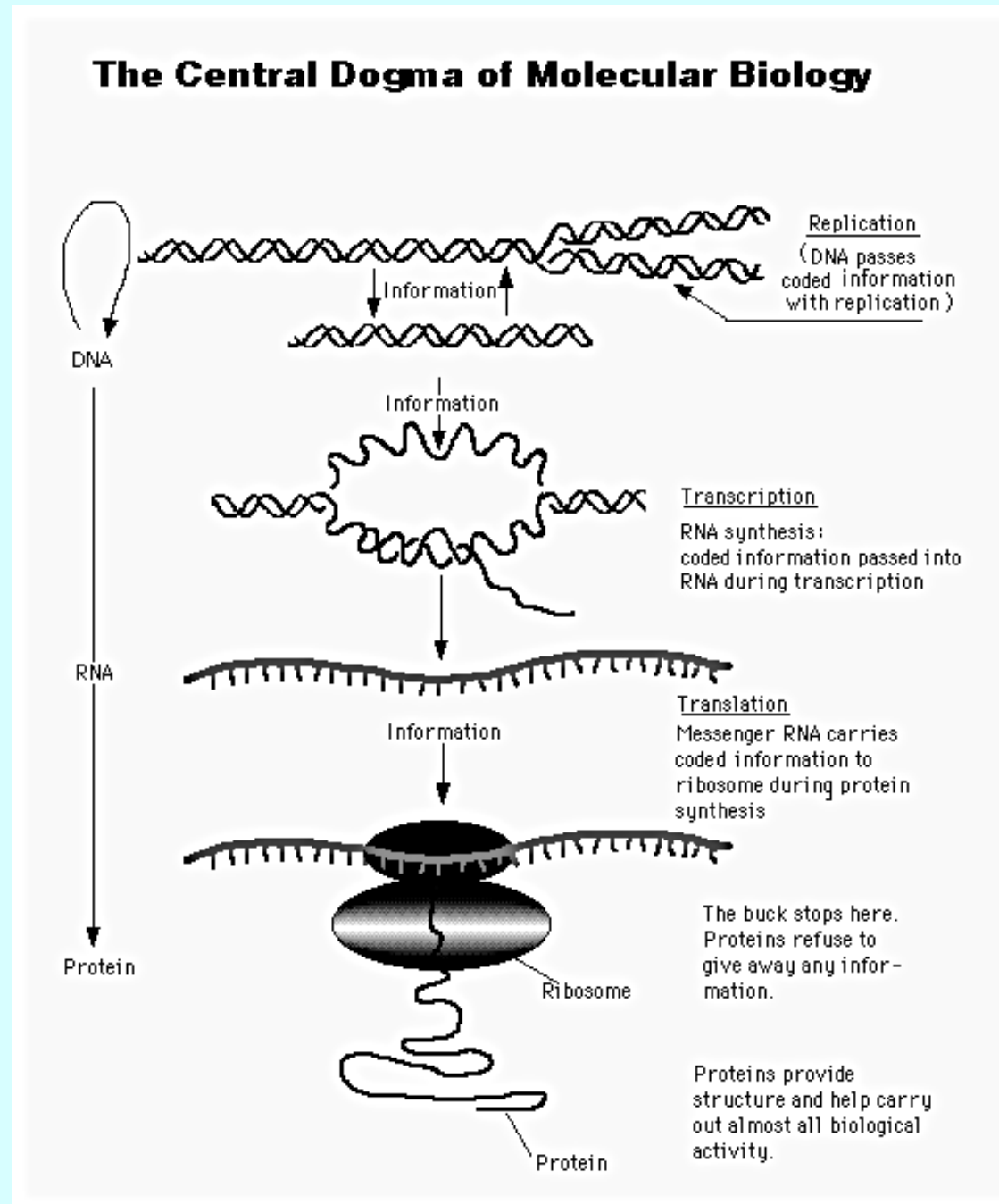


(b)

Código genético



O Dogma central da Biologia molecular

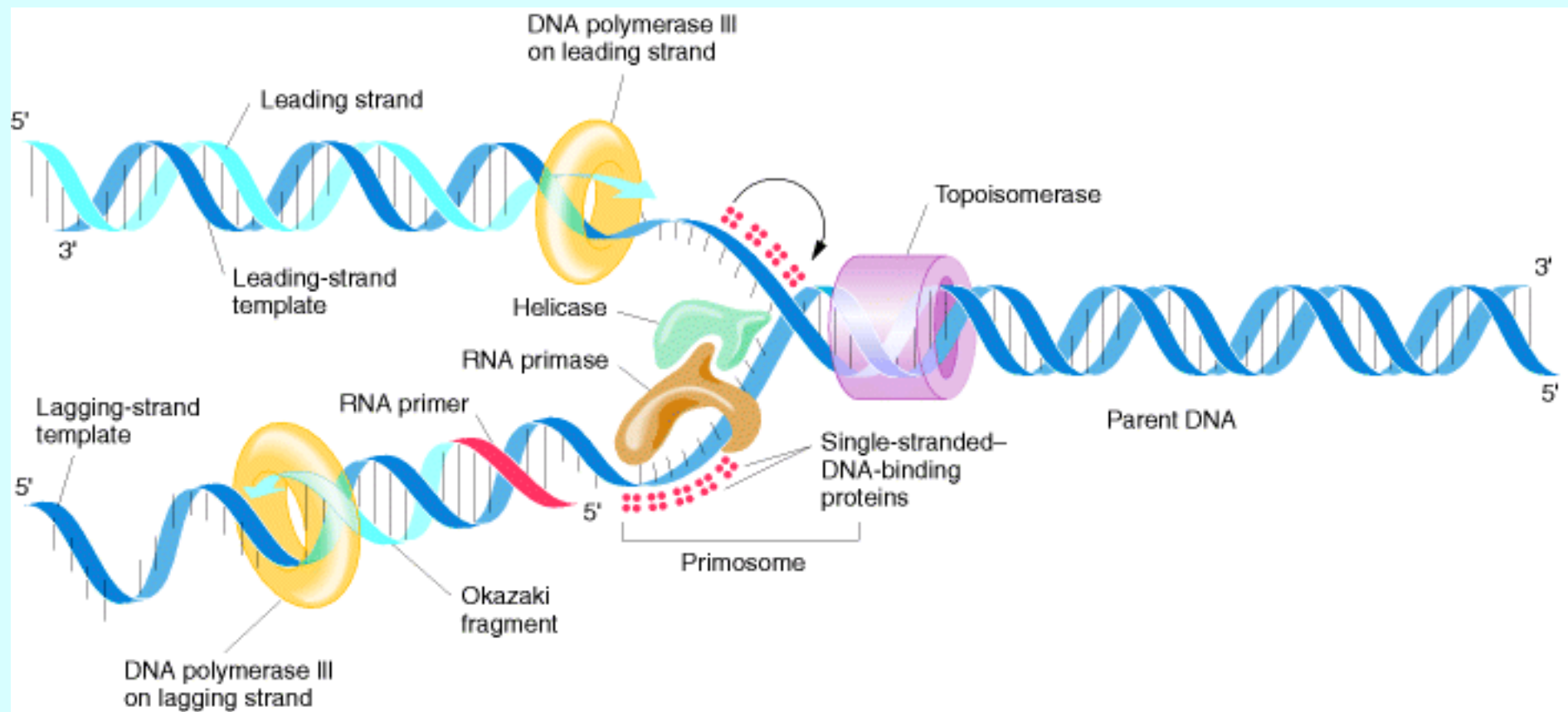


Código genético

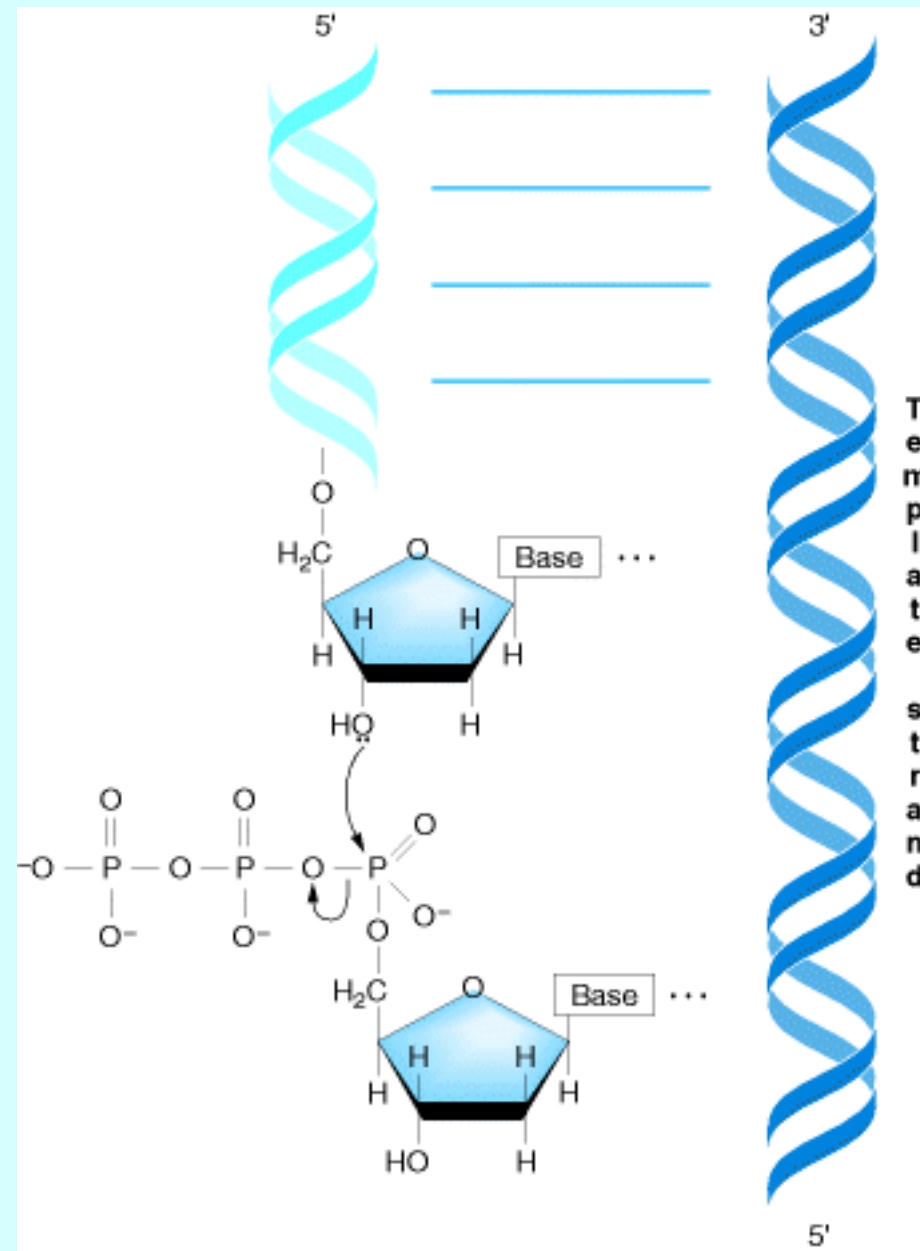
		Segunda base do códon							
		U	C	A	G				
Primeira base do códon	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } SER UCA } UCG }	UAU } Tyr UAC } UAA } UAG }	UGU } Cys UGC } UGA } UGG } Trp	U	C	A	G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U	C	A	G
	A	AUU } AUC } Ile AUA } AUG } Met	ACU } ACC } Thy ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U	C	A	G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U	C	A	G
						Terceira base do códon			

O código genético, escrito por convenção na forma na qual os códons aparecem no mRNA. Os três códons de terminação, UAA, UAG e UGA, estão no quadro em vermelho; o códon iniciador, AUG, está mostrado em verde.

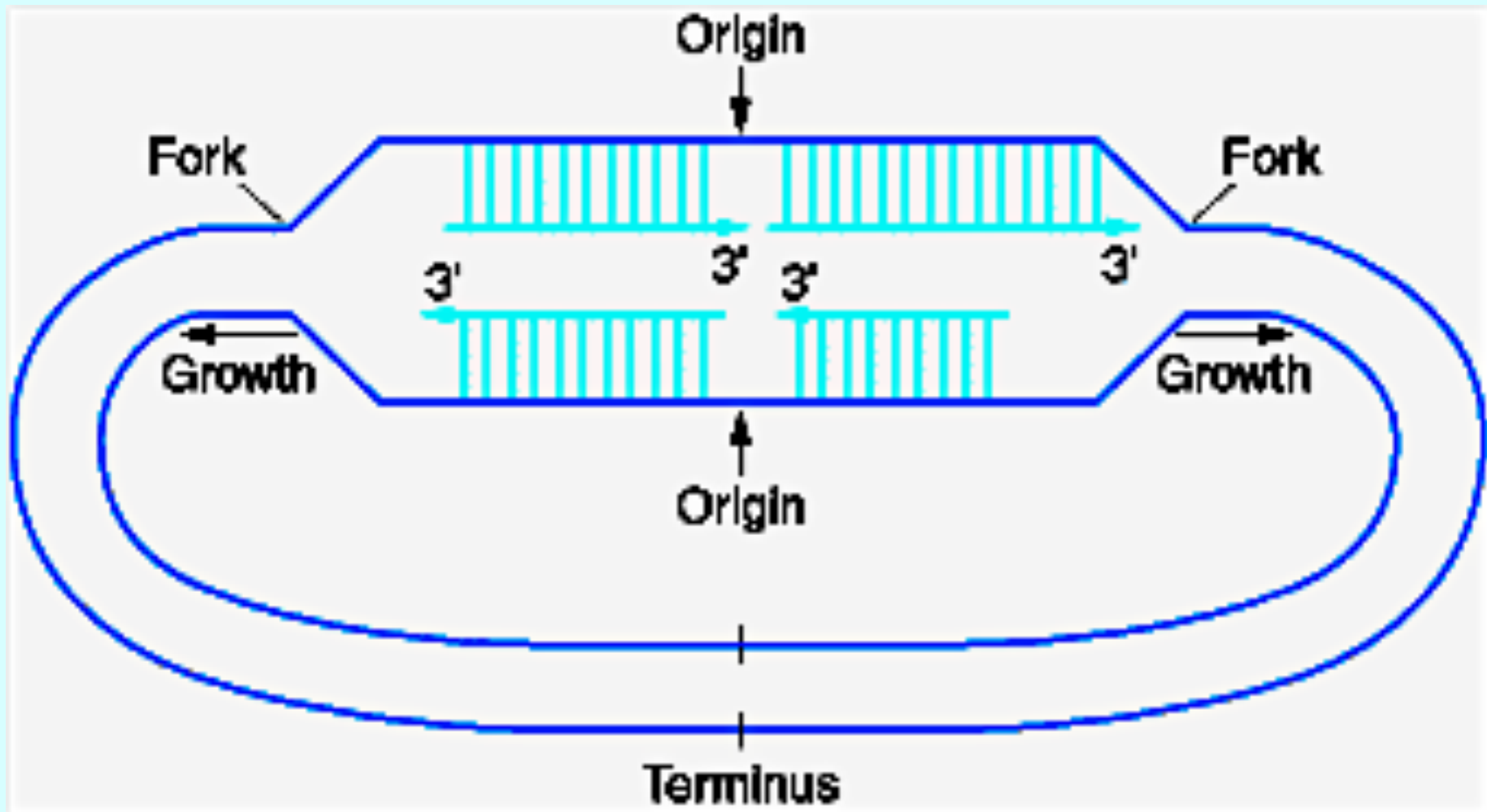
Replicação do DNA



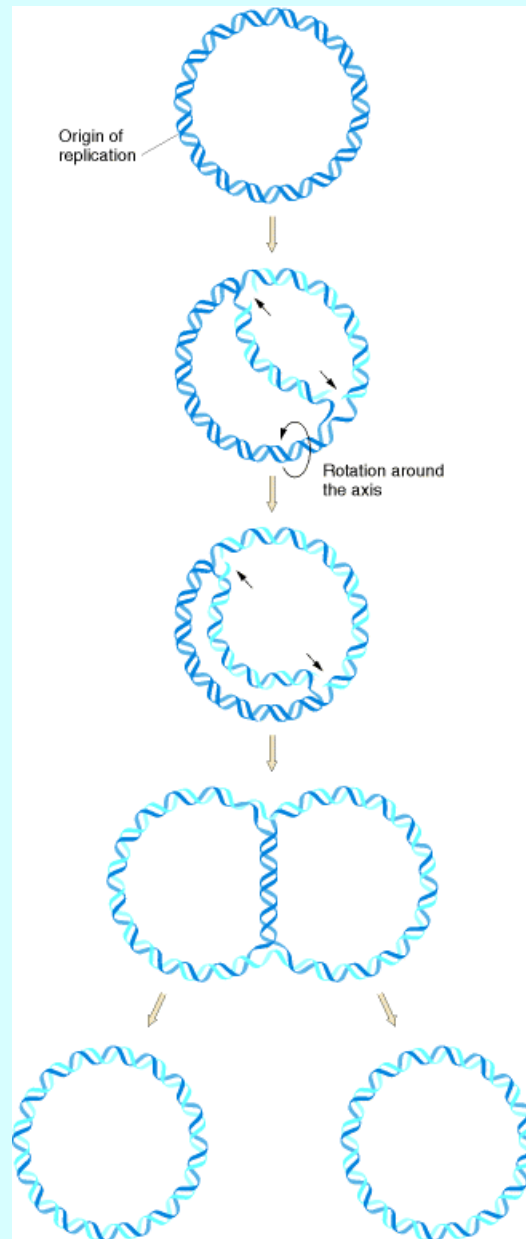
Replicação do DNA



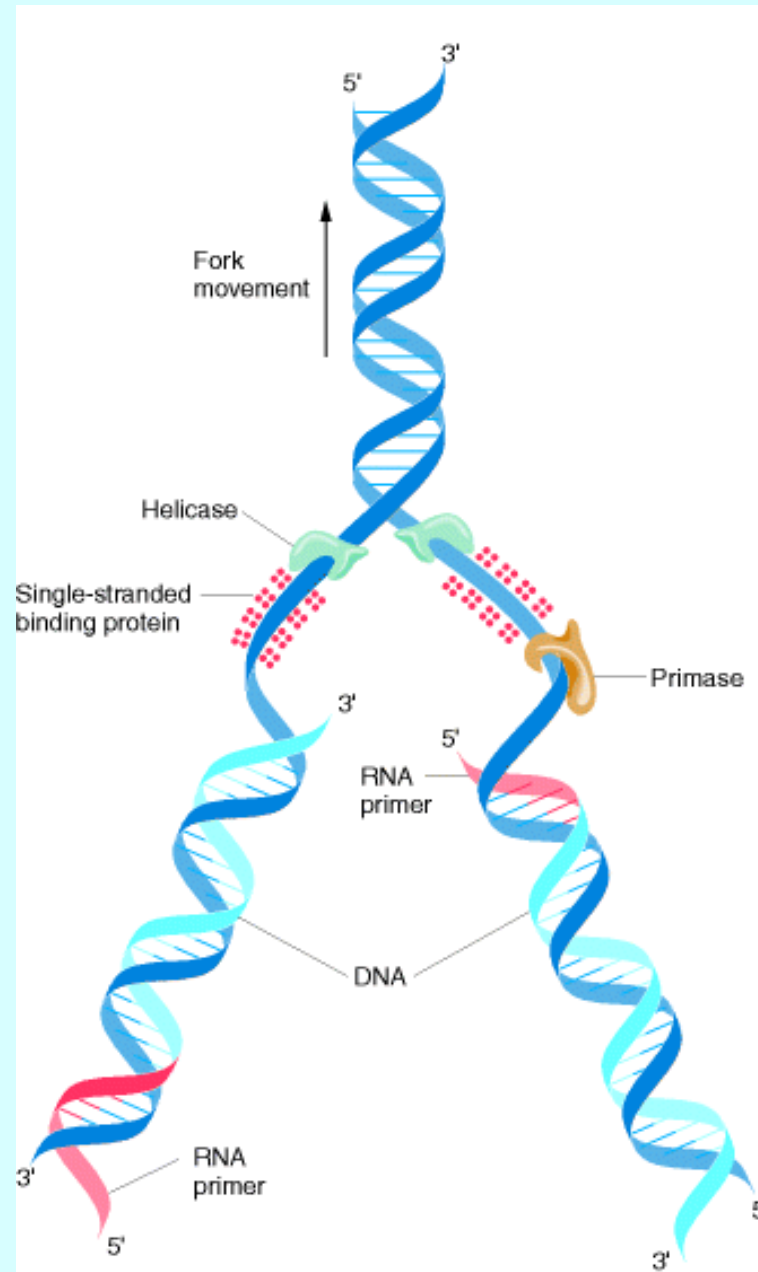
Replicação do DNA circular



Replicação do DNA circular



Enzimas da replicação do DNA



Filmes de replicação do DNA

Filmes de animação mostrando a replicação do DNA

Ver em:

https://www.youtube.com/watch?v=vNXFk_d6y80

http://www.dnatube.com/search/?search_id=dna+replication

Mutação e recombinação



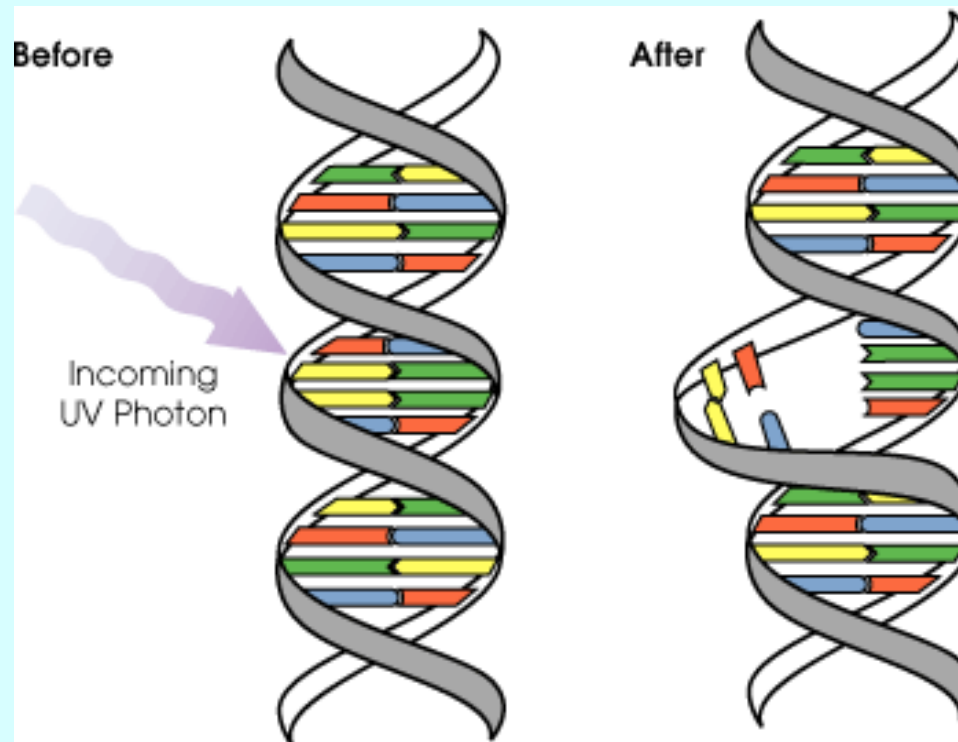
Wild-Type



White

Mutações

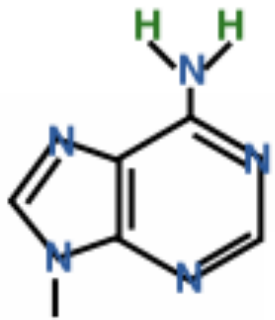
Hermann Müller, em 1943, recebe o prêmio Nobel por seus trabalhos com radiações ionizantes provocando mutações gênicas



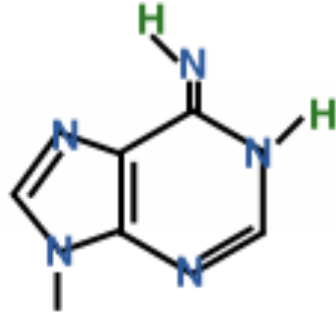
Mutações espontâneas

Tautômeros de bases nitrogenadas

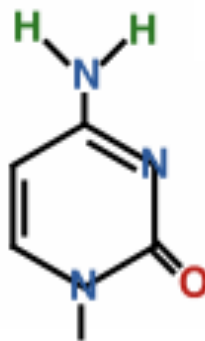
forma comum



forma rara

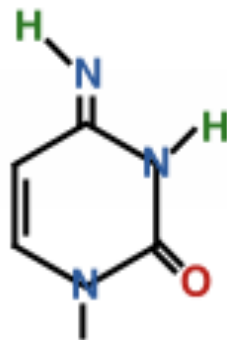


adenina



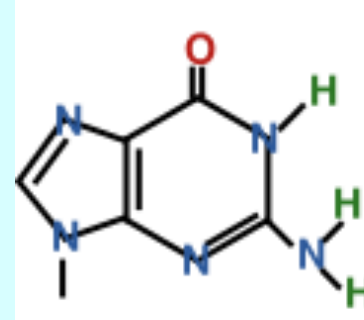
amino

citosina



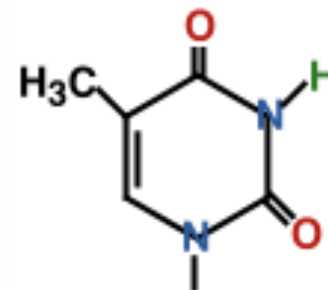
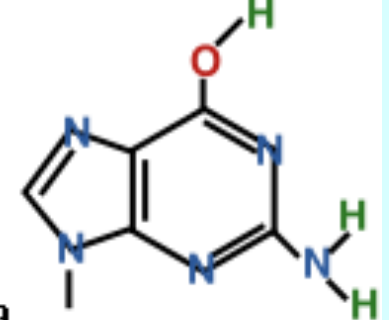
imino

forma comum



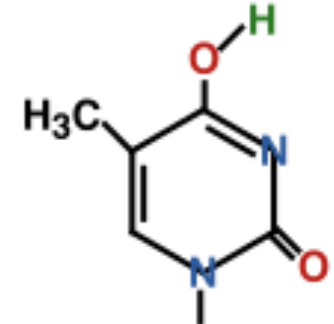
Guanina

forma rara



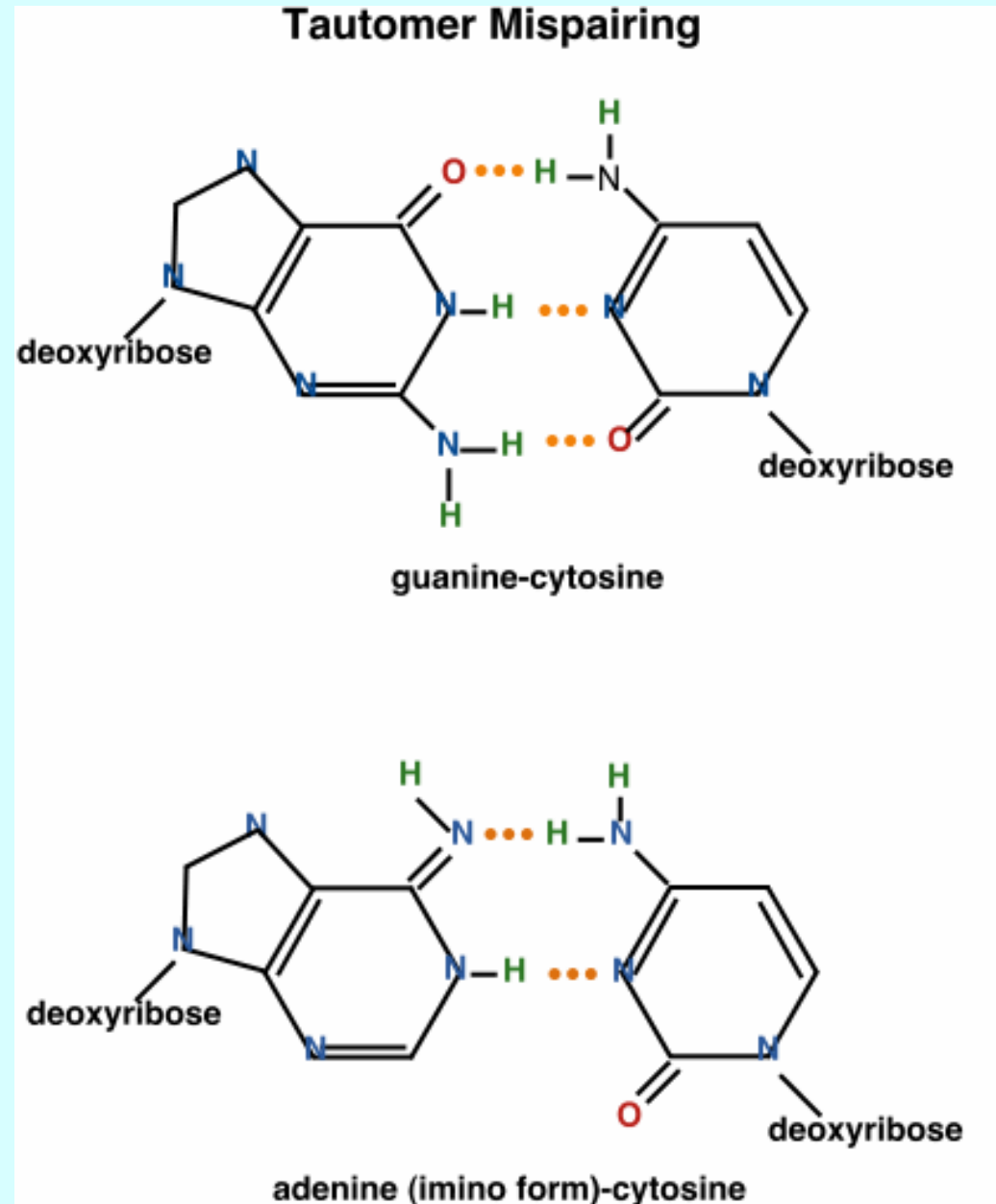
cetônica

timina



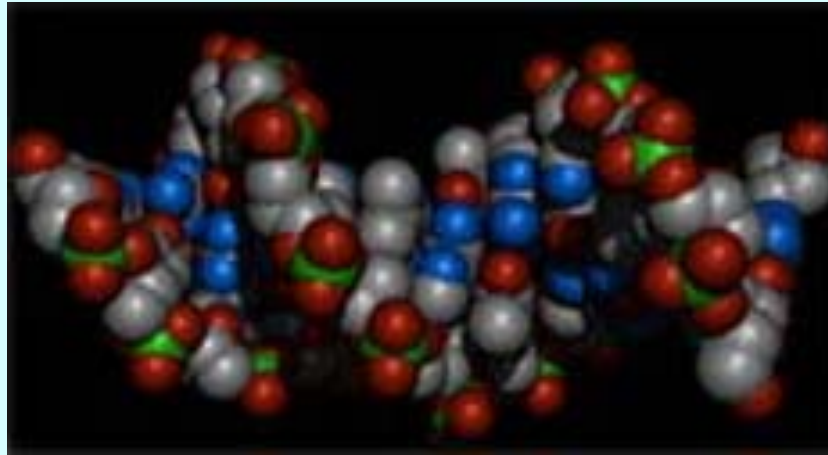
enólica

Mutações espontâneas



Mutações espontâneas

Radicais livres



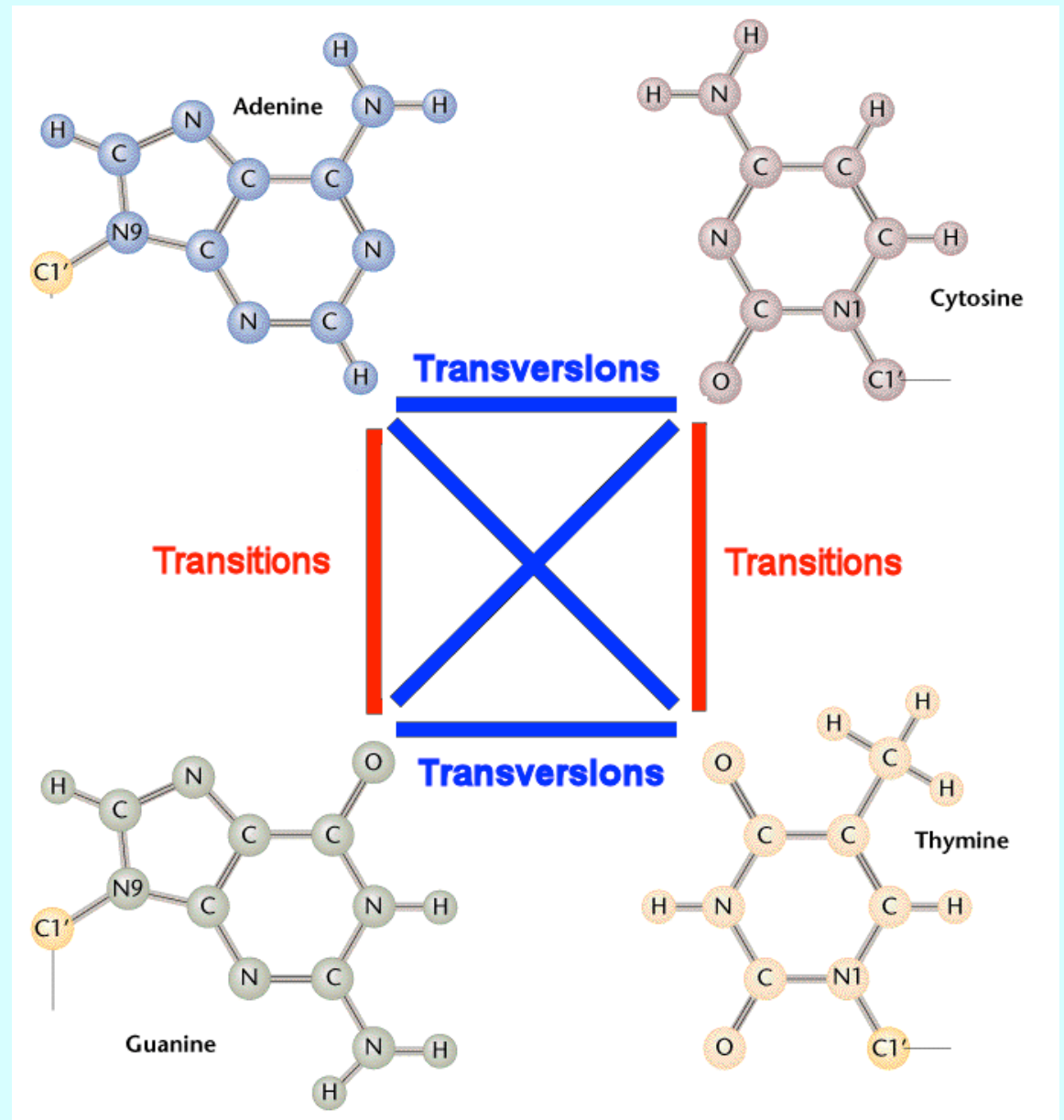
Origem: oxidação intracelular, radiações ionizantes

Efeito: alterações várias da estrutura do DNA

Tipos de mutação

Transições: mesmo tipo de base

Transversões: tipo de base diferente



Tipos de mutação (quanto ao efeito)

Sinônimas: mesmo
aminoácido
(sem troca de sentido)

GGU
glicina



GG**A**
glicina

Não sinônimas:
aminoácido diferente
(com troca de sentido,
missense)

CCU
prolina



CA**A**
histidina

Sem sentido:
aminoácido -> parada
("nonsense")

GGA
glicina



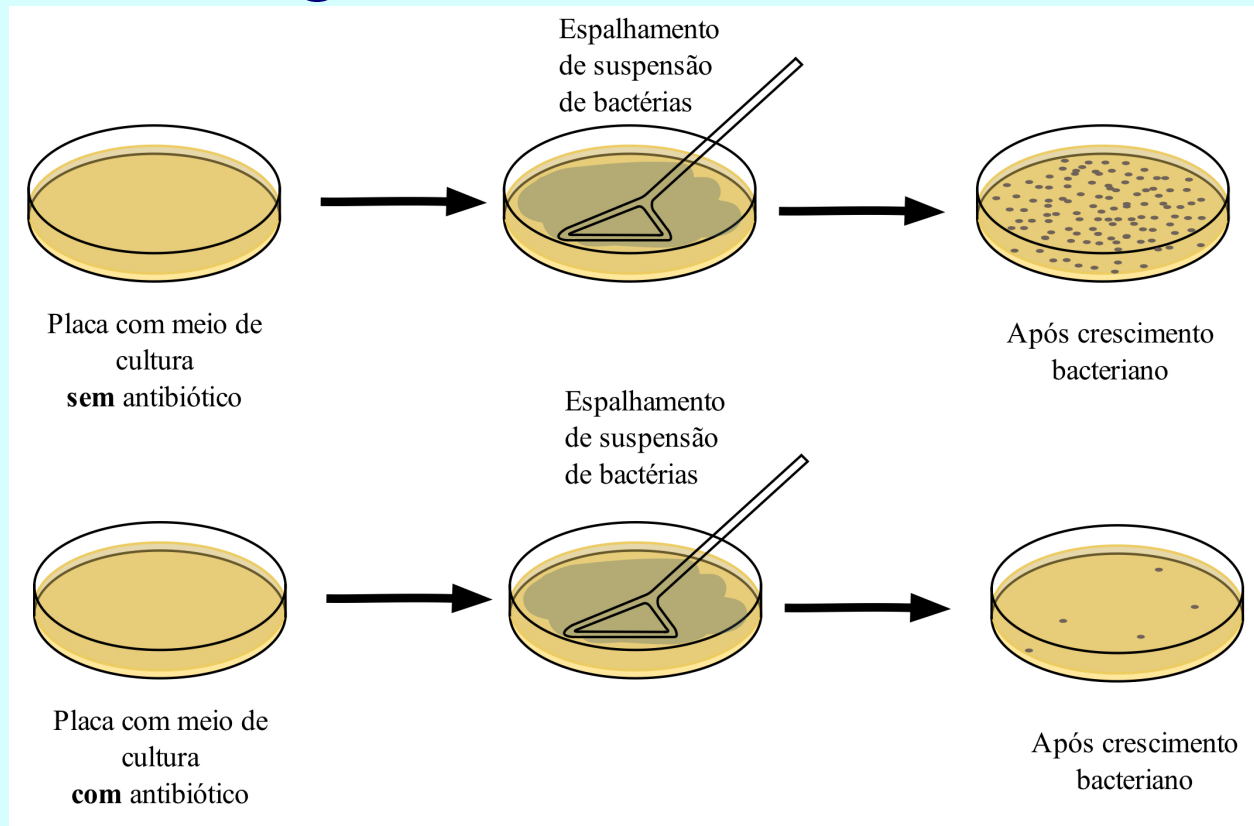
UGGA
Parada

Mutações e evolução

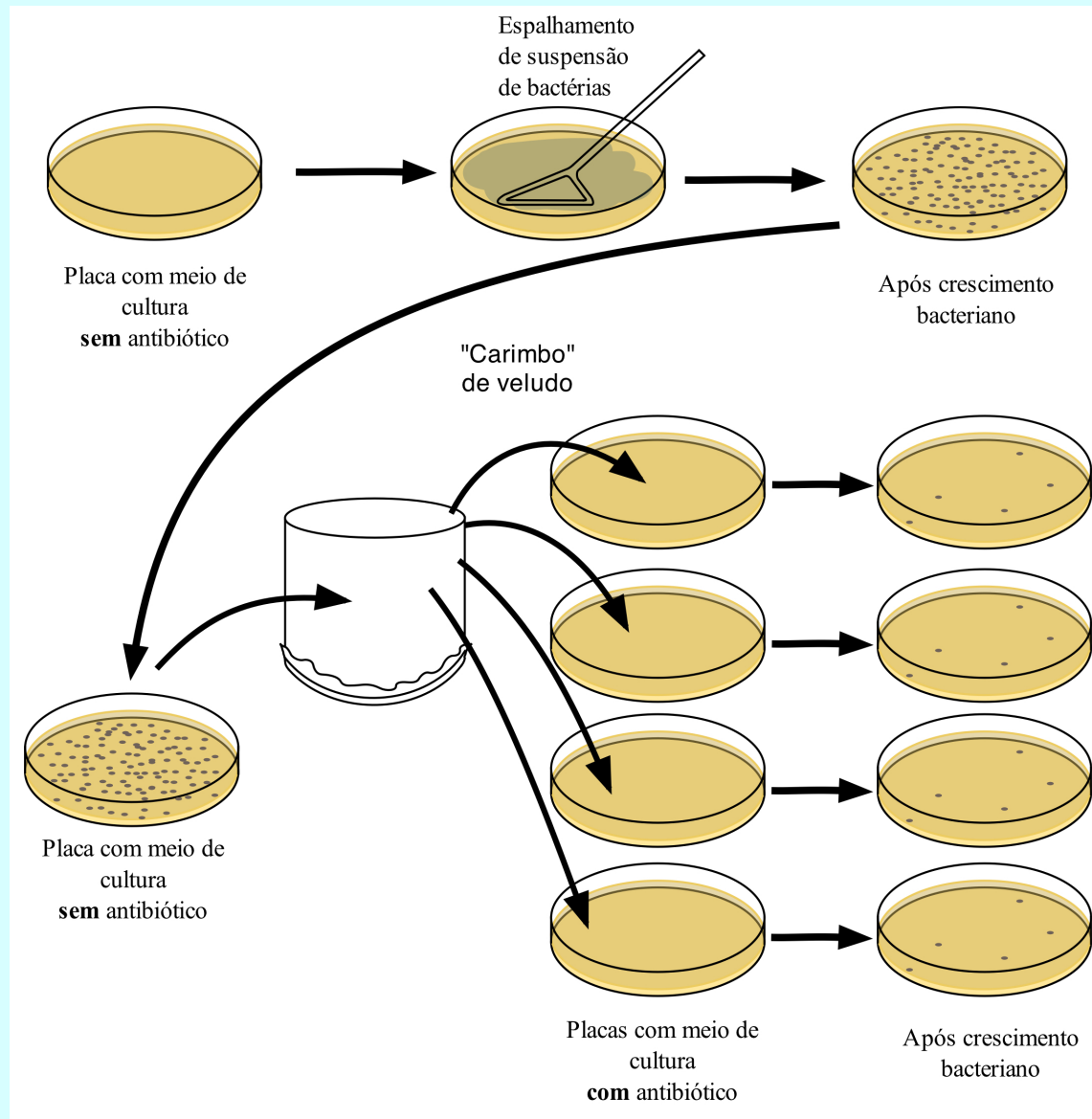
As mutações surgem por causa das mudanças ambientais ou ocorrem independentemente delas?

Mutações e evolução

Experimento: Se colocarmos bactérias que são sensíveis a um determinado antibiótico em um meio de cultura com antibiótico, algumas colônias crescerão:



Mutações e evolução



Experimento do
“carimbo de
veludo”
(Lederberg e
Lederberg, 1952)

Alterações estruturais

