Genética molecular

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- Página de la asignatura : **genmol.blog.unq.edu.ar**

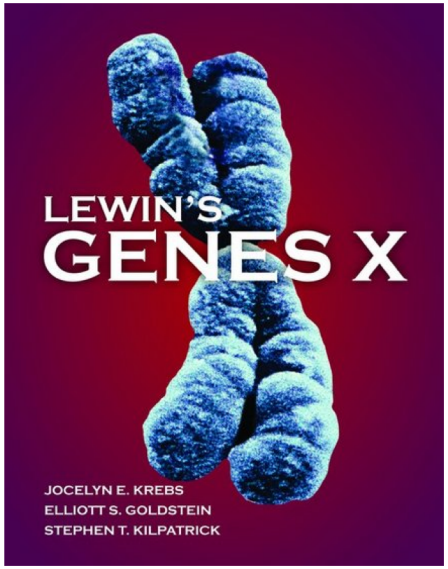
2 evaluaciones, 2 fechas de cada uno:
-Son equivalentes!, no hay distinción entre las fechas
-Se puede utilizar ambas fechas para mejorar la nota

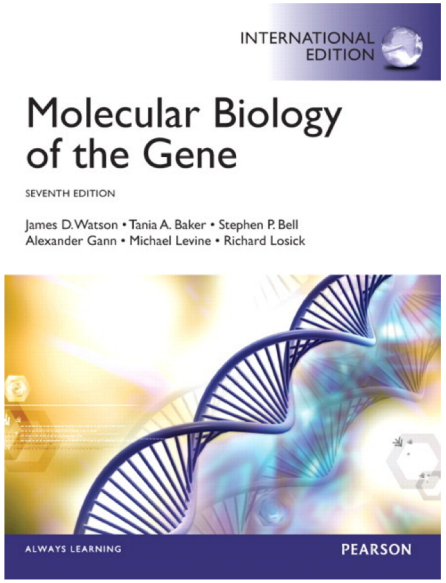
1 evaluación parcial de TPs

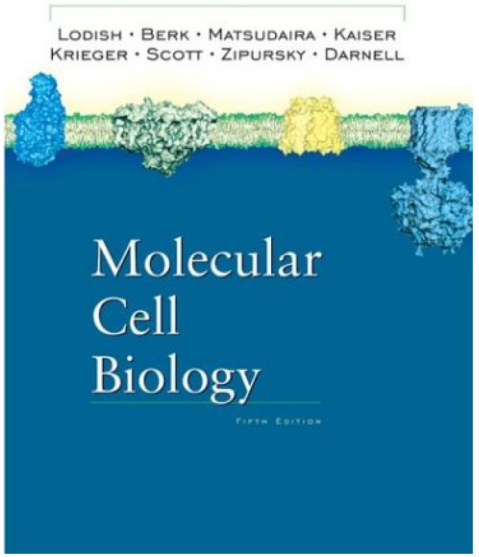
Régimen de promoción tal como lo establece la Universidad !

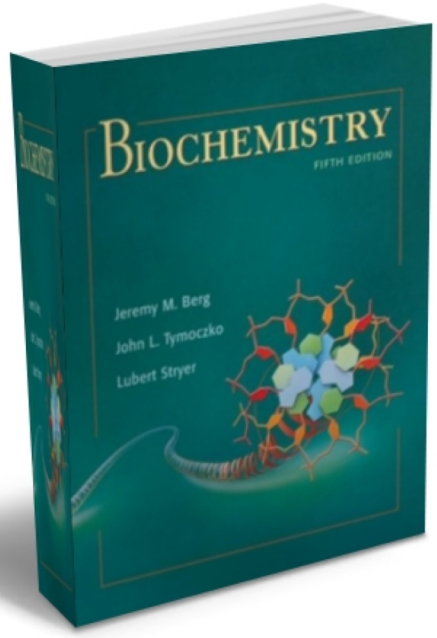
al menos 6 en cada evaluación y
el promedio de ambas debe dar al menos 7

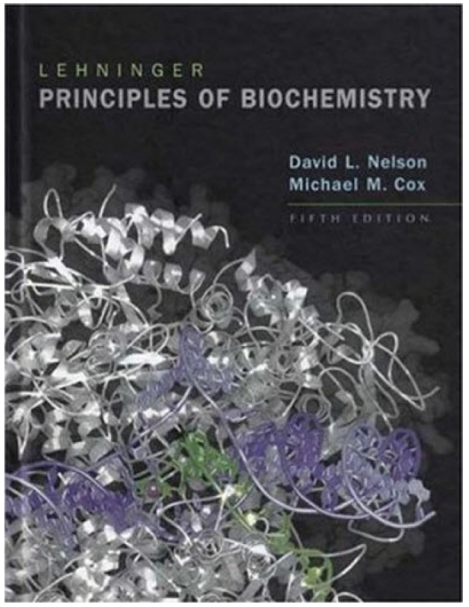
Bibliografía


LEWIN'S
GENES X
JOCELYN E. KREBS
ELLIOTT S. GOLDSTEIN
STEPHEN T. KILPATRICK


INTERNATIONAL EDITION
Molecular Biology of the Gene
SEVENTH EDITION
James D. Watson • Tania A. Baker • Stephen P. Bell
Alexander Gann • Michael Levine • Richard Losick
ALWAYS LEARNING
PEARSON


LODISH • BERK • MATSUDAIRA • KAISER
KRIEGER • SCOTT • ZIPURSKY • DARNELL
Molecular Cell Biology
FIFTH EDITION


BIOCHEMISTRY
FIFTH EDITION
Jeremy M. Berg
John L. Tymoczko
Lubert Stryer

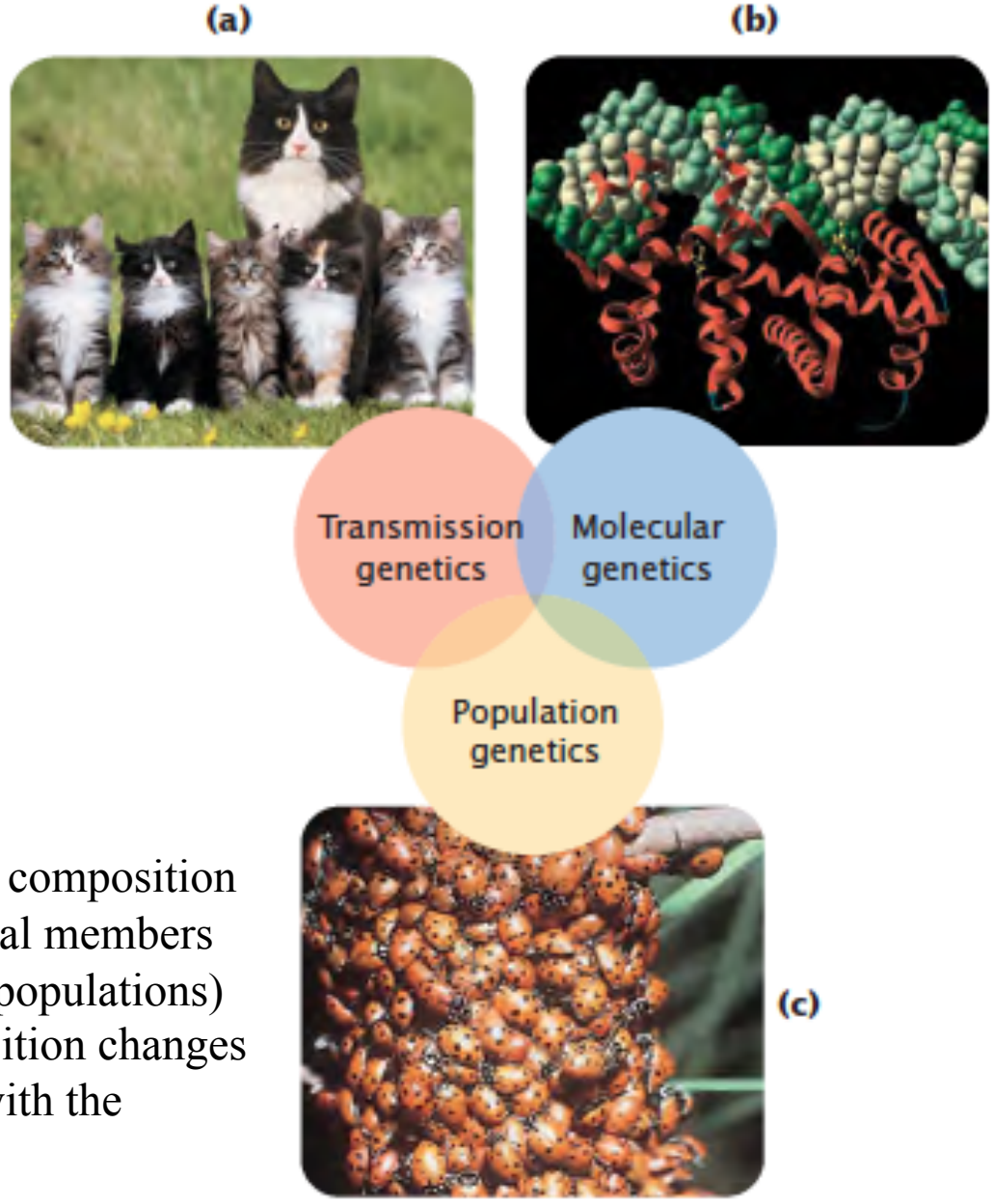

LEHNINGER
PRINCIPLES OF BIOCHEMISTRY
David L. Nelson
Michael M. Cox
FIFTH EDITION

Genetics

The science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment.

La ciencia de la herencia, que trata de las semejanzas y diferencias de organismos relacionados que resultan de la interacción de sus genes y el medio ambiente.

the basic principles of heredity and how traits are passed from one generation to the next.

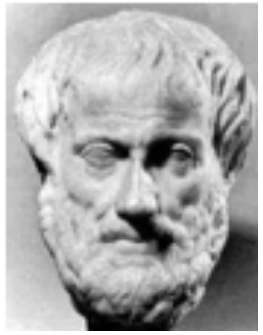


the chemical nature of the gene itself: how genetic information is encoded, replicated, and expressed. It includes the cellular processes of replication, transcription, and translation (by which genetic information is transferred from one molecule to another) and gene regulation (the processes that control the expression of genetic information).

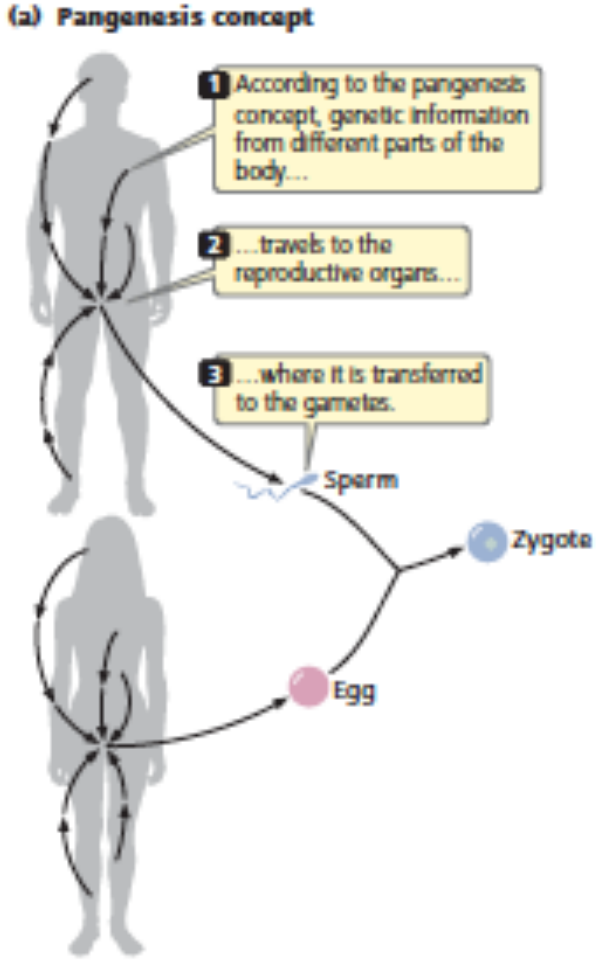
Explores the genetic composition of groups of individual members of the same species (populations) and how that composition changes geographically and with the passage of time

Bases moleculares de la Herencia

Las primeras preguntas de la herencia



Aristóteles (384-322 AC) Ambos padres contribuyen a la creación de los hijos a través de la mezcla de sangres o humores.

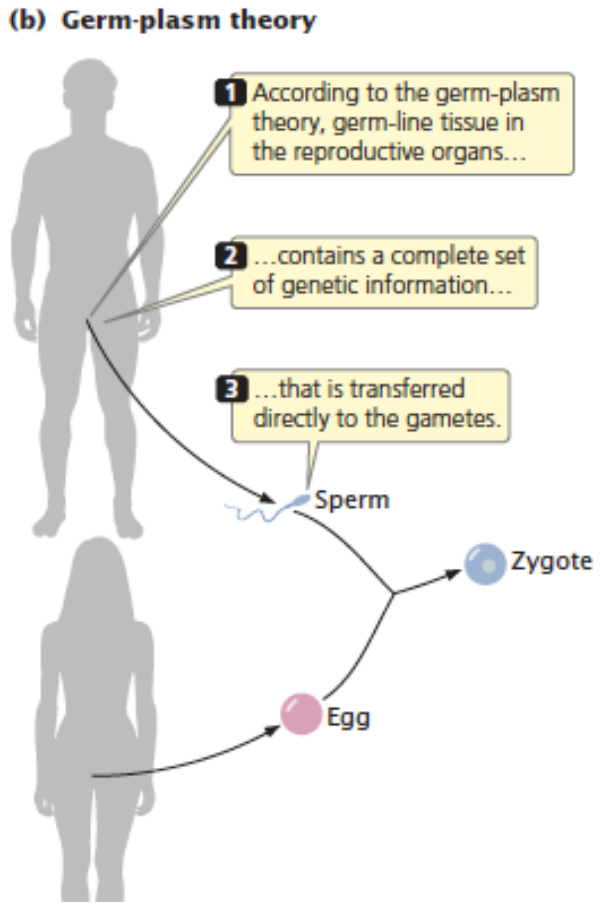


specific particles, later called gemmules, carry information from various parts of the body to the reproductive organs, from which they are passed to the embryo at the moment of conception

Preformationists in the seventeenth and eighteenth ries believed that sperm or eggs contained fully formed humans (the homunculus). Shown here is a drawing of a homunculus inside a sperm. [Science VU/Visuals Unlimited.]

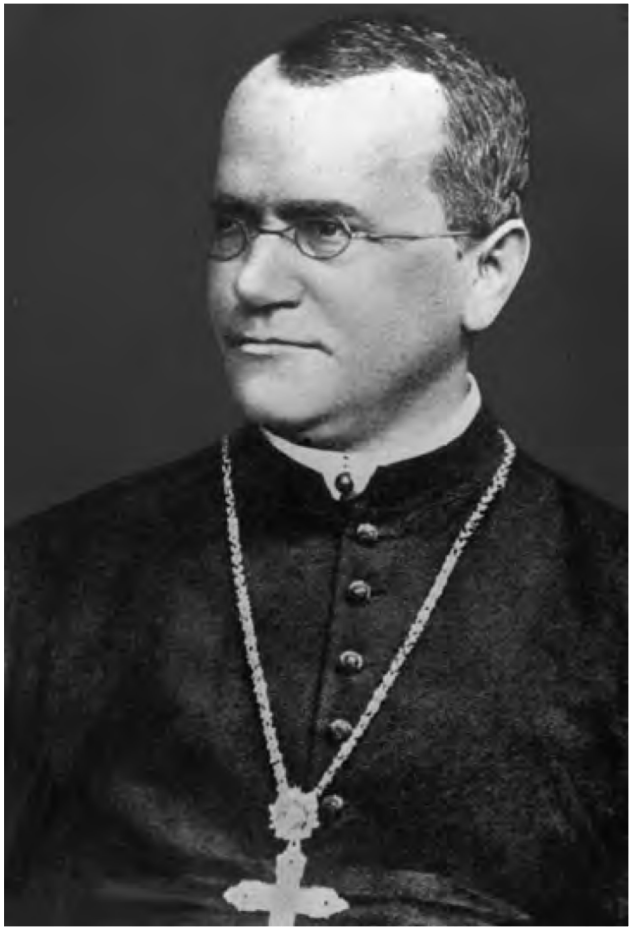
Another early notion of heredity was **blending inheritance**,which proposed that offspring are a blend, or mixture, of parental traits. This idea suggested that the genetic material itself blends, much as blue and yellow pigments blend to make green paint.

Weismann proposed the germ-plasm theory , which holds that the cells in the reproductive organs carry a complete set of genetic information that is passed to the egg and sperm



Gregor Mendel Discovered the Basic Principles of Heredity

He conducted breeding experiments from 1856 to 1863 and presented his results publicly at meetings of the Brno Natural Science Society in 1865. Mendel's paper from these lectures was published in 1866.



Las claves del éxito

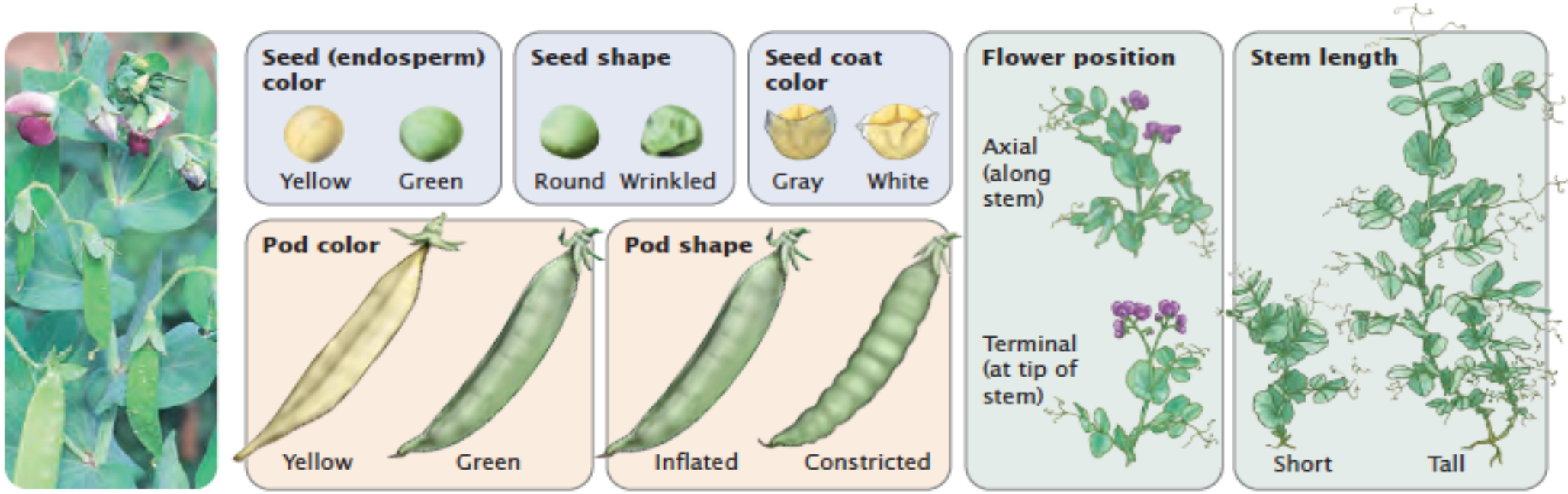
"El sistema"

- es una planta de crecimiento rápido

- produce mucha descendencia (semillas) lo que permite un análisis matemático con significancia estadística

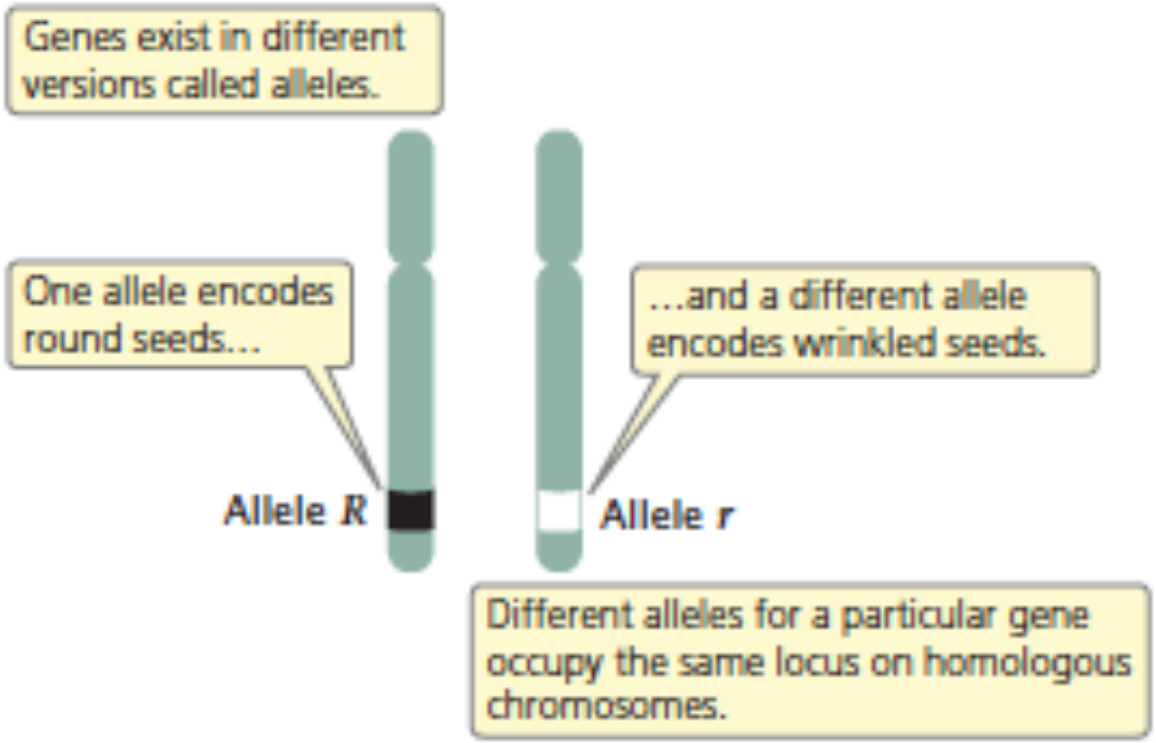
- disponibilidad de un gran número de variedades con diferentes carácterísticas y geneticamente puros

Las 7 características que eligió para el estudio



He avoided characteristics that display a range of variation; instead, he focused his attention on those ***<u>that exist in two easily differentiated forms</u>****, such as white versus gray seed coats, round versus wrinkled seeds, and inflated versus constricted pods.*

He adopted an experimental approach and interpreted his results by **using mathematics.**



¿Gen?

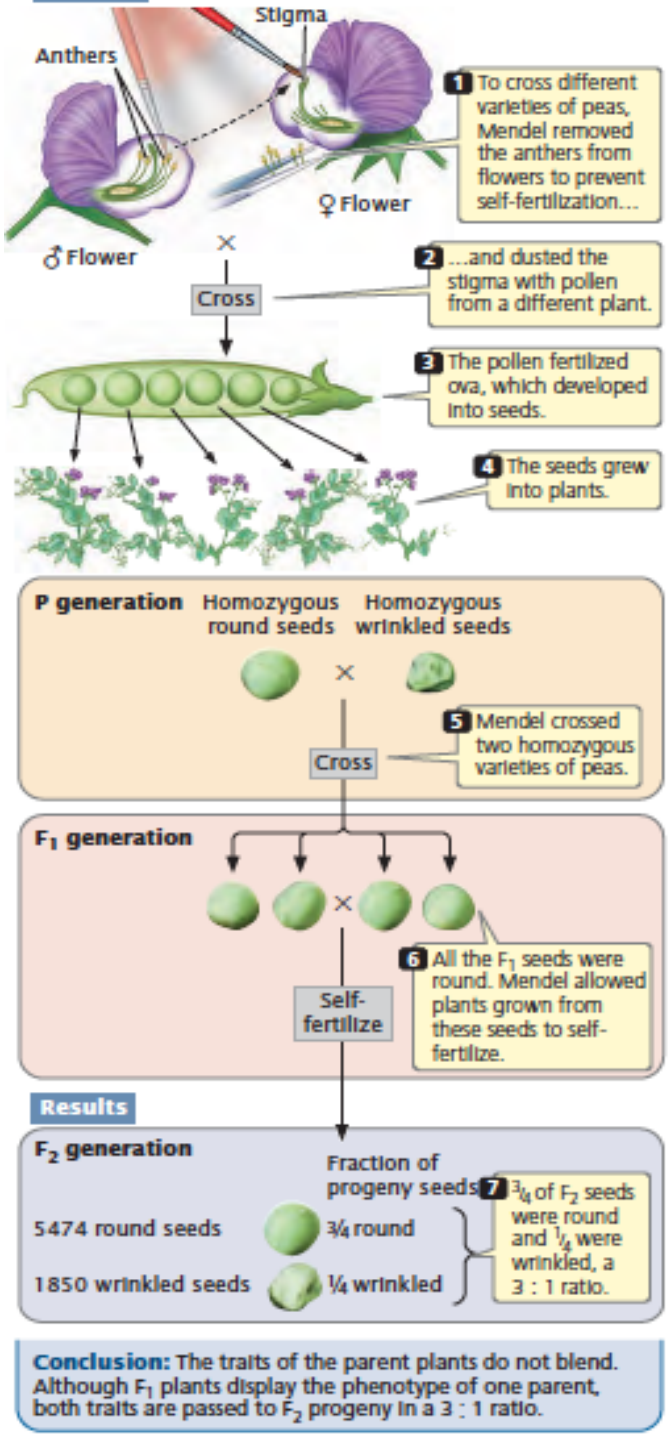
¿Alelo?

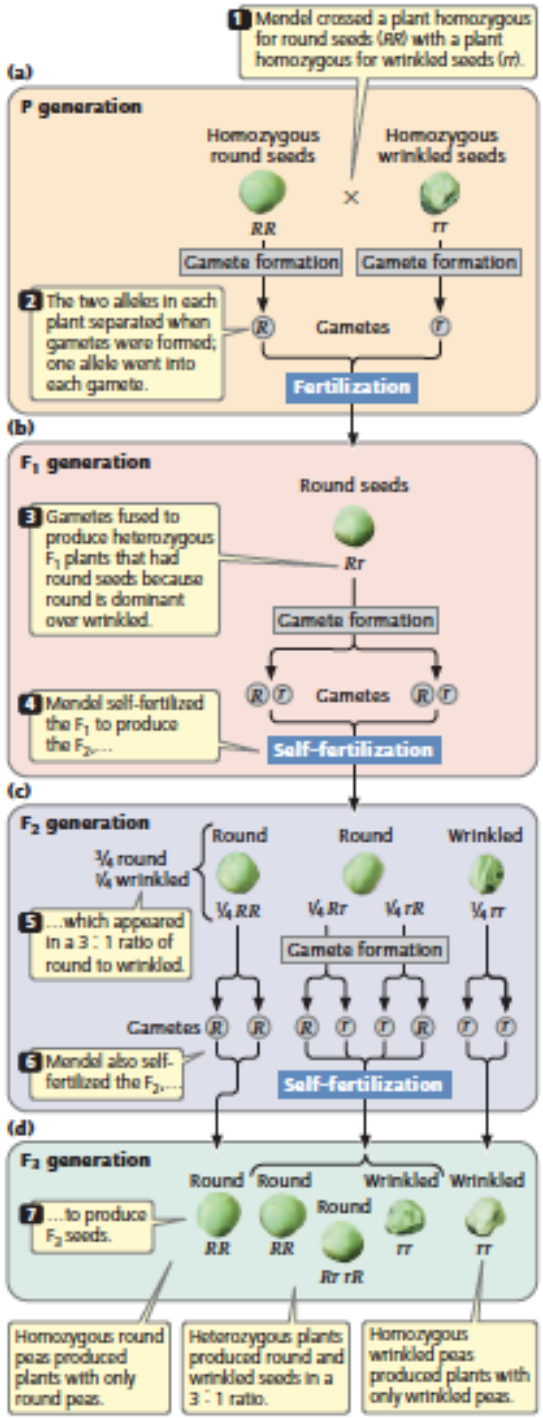
¿Locus?

¿Homocigota?

¿Loci?

Monohybrid Crosses Reveal the Principle of Segregation and the Concept of Dominance





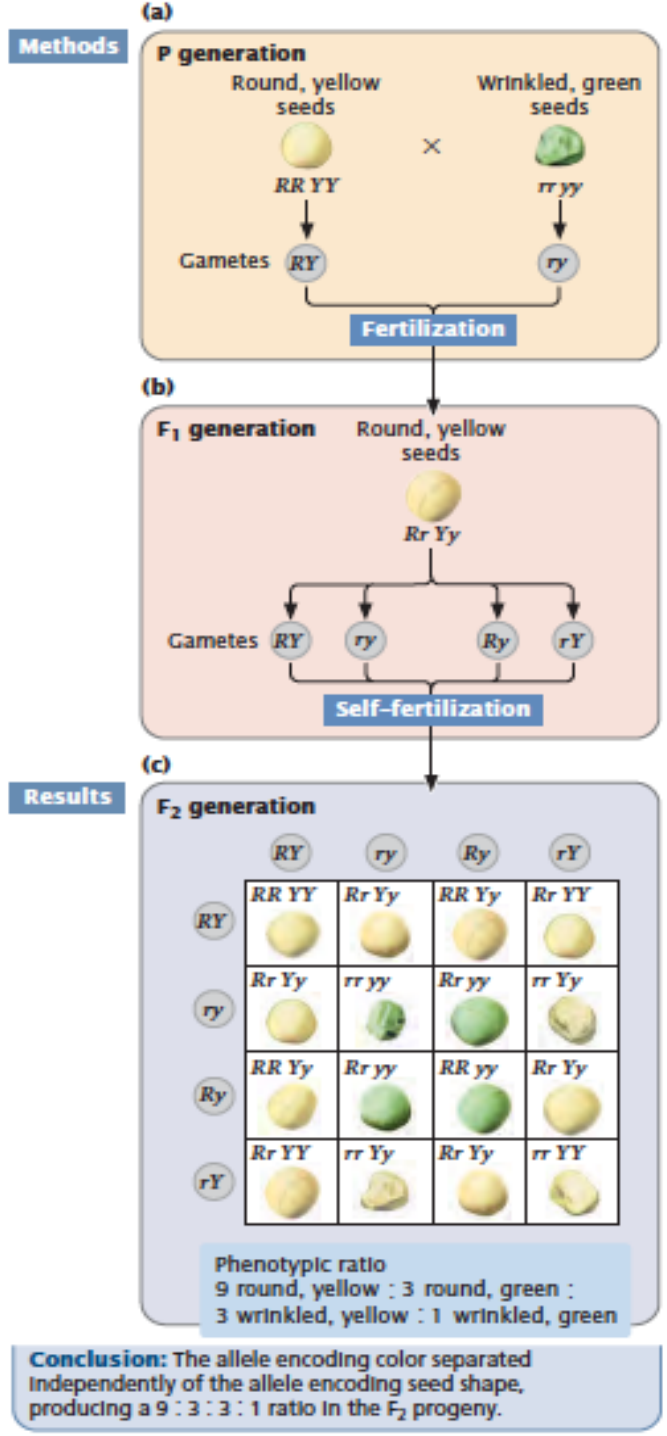
The presence of both round and wrinkled seeds in the F2 could be explained only if the F1 plants possessed both round and wrinkled genetic factors that they had inherited from the P generation. He concluded that **each plant must therefore possess two genetic factors encoding a character.**

The second conclusion that Mendel drew from his monohybrid crosses was that **the two alleles in each plant separate when gametes are formed, and one allele goes into each gamete.**

The concept of dominance was the third important conclusion that Mendel derived from his monohybrid crosses.

Mendel's fourth conclusion was that **the two alleles of an individual plant separate with equal probability into the gametes**

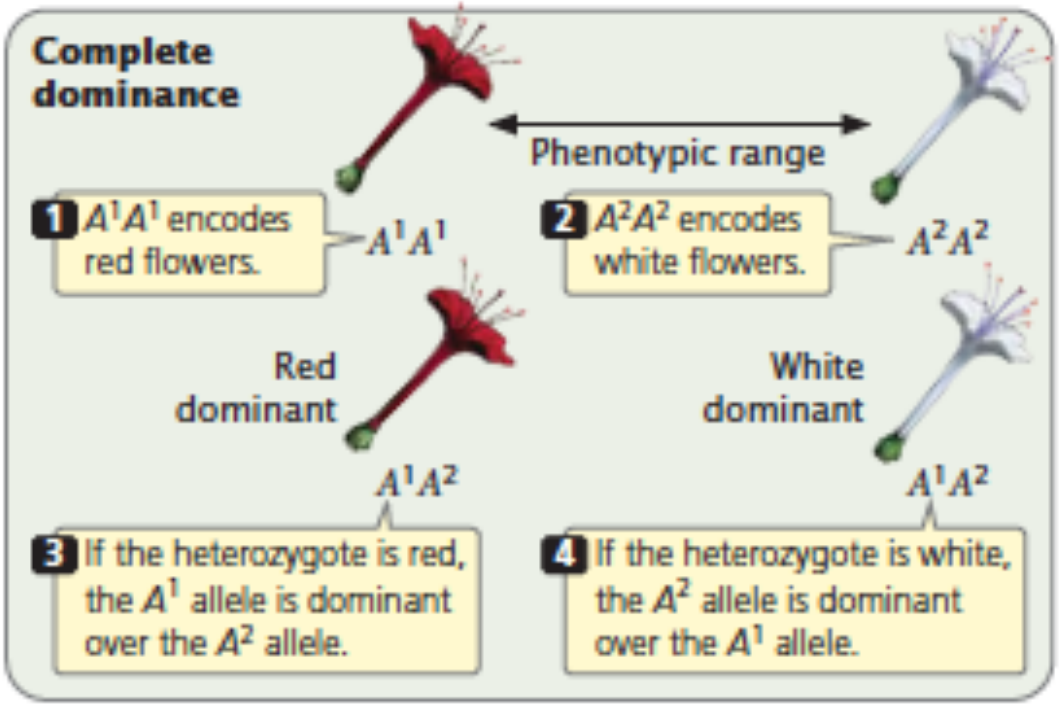
Dihybrid Crosses Reveal the Principle of Independent Assortment

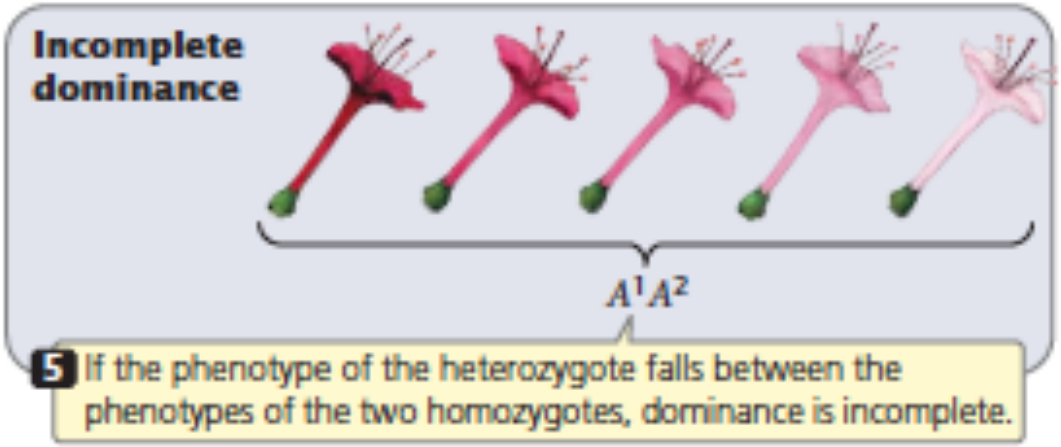


Observed Ratios of Progeny May Deviate from Expected Ratios by Chance

¿?

$$\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$





Differences between dominance, incomplete dominance, and codominance

Type of Dominance	Definition
Dominance	Phenotype of the heterozygote is the same as the phenotype of one of the homozygotes.
Incomplete dominance	Phenotype of the heterozygote is intermediate (falls within the range) between the phenotypes of the two homozygotes.
Codominance	Phenotype of the heterozygote includes the phenotypes of both homozygotes.

Penetrancia-expresividad

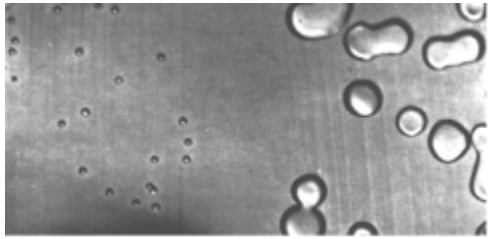
¿ Qué evidencias experimentales indican que los ácidos nucleicos están asociados a la información genética?

Herencia ? ADN

Las Primeras evidencias

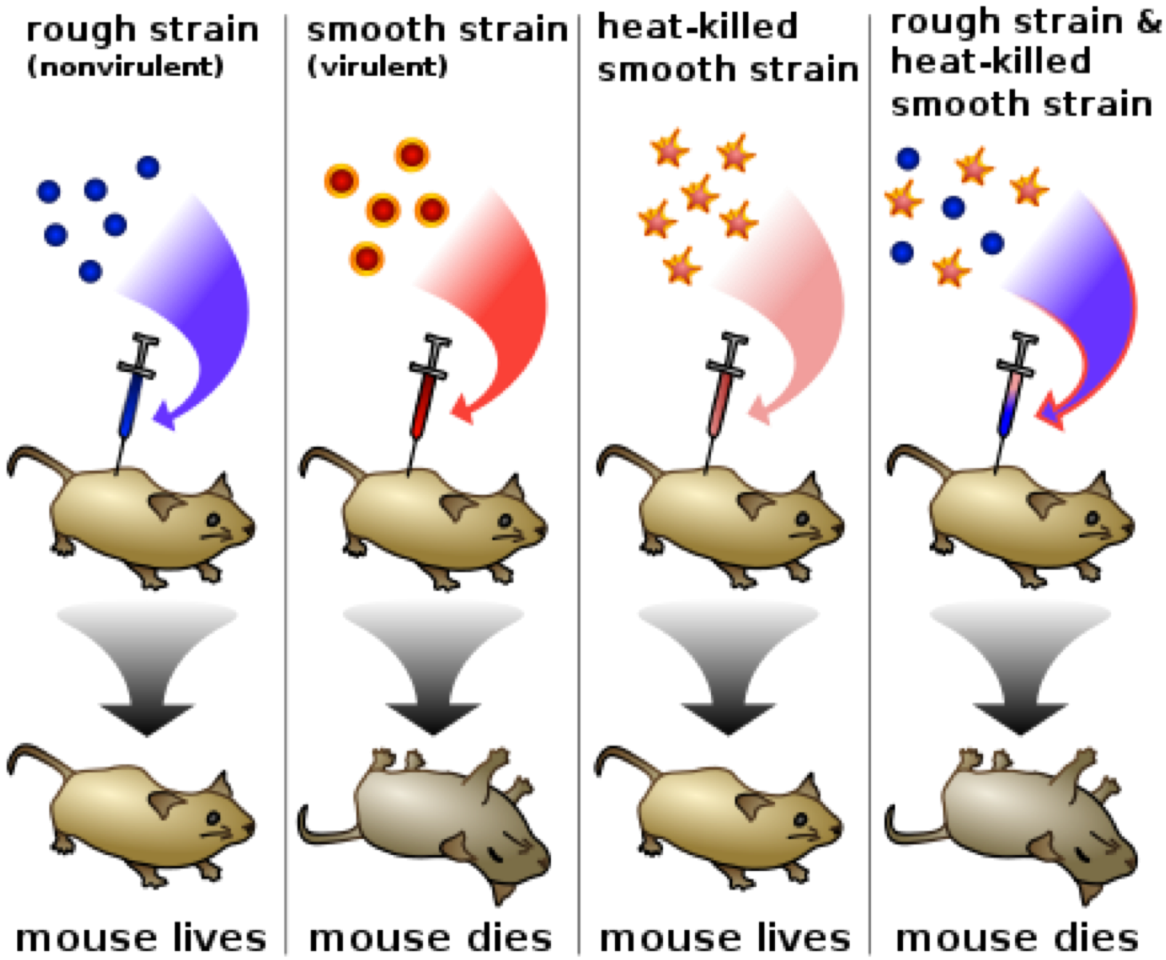
1928: Frederick Griffith

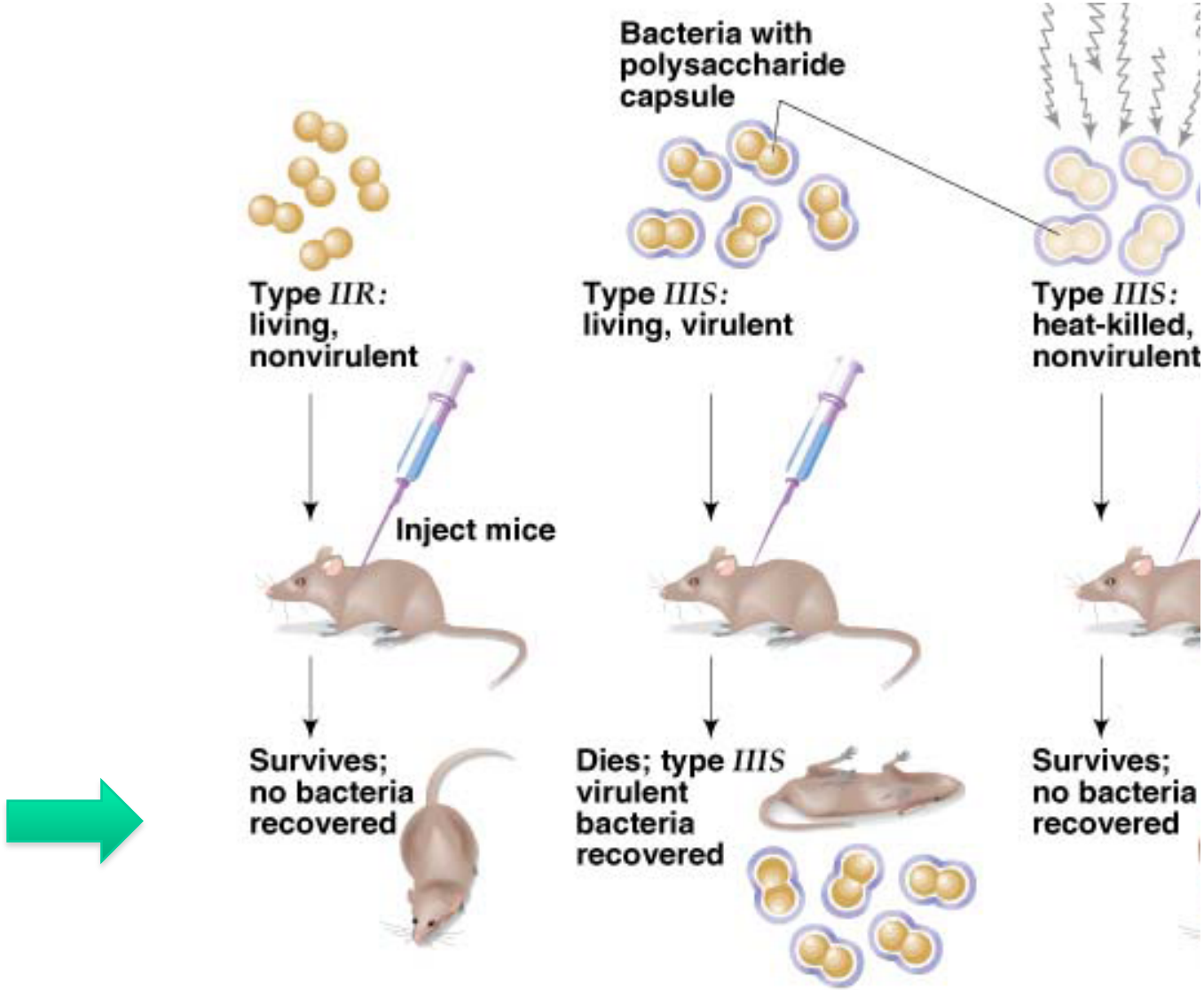
Infección con pneumococos



rugosas

Lisas virulentas




Bacteria with polysaccharide capsule
Type IIR: living, nonvirulent
Type IIIS: living, virulent
Type IIIS: heat-killed, nonvirulent
Inject mice
Survives; no bacteria recovered
Dies; type IIIS virulent bacteria recovered
Survives; no bacteria recovered

The Experiment That Transformed Biology

Discovering the Genetic Role of DNA

50th Anniversary →→→ 1944 – 1994

Oswald Avery in his Hospital laboratory
mid-1930s

Colin MacLeod
1936

Maclyn McCarty
1942

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

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

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND MACLYN MCCARTY,* M.D.

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

PLATE 1

(Received for publication, November 1, 1943)

Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microorganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

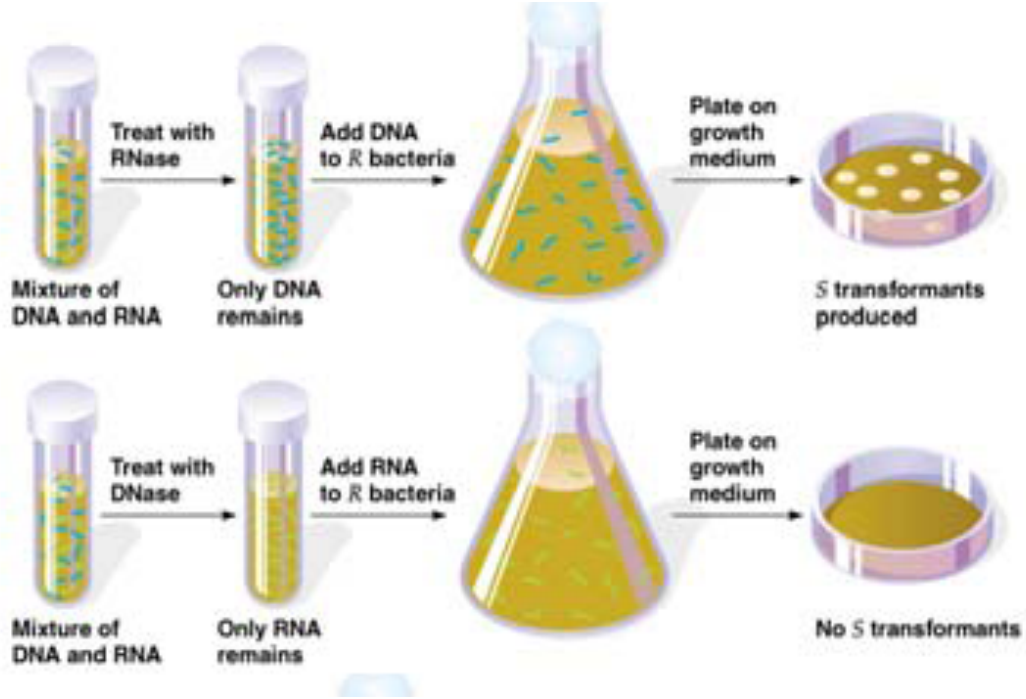
Griffith found that mice injected subcutaneously with a small amount of a living R culture derived from Pneumococcus Type II together with a large inoculum of heat-killed Type III (S) cells frequently succumbed to infection, and that the heart's blood of these animals yielded Type III pneumococci in pure culture. The fact that the R strain was avirulent and incapable by itself of causing fatal bacteremia and the additional fact that the heated suspension of Type III cells contained no viable organisms brought convincing evidence that the R forms growing under these conditions had newly acquired the capsular structure and biological specificity of Type III pneumococci.

The original observations of Griffith were later confirmed by Neufeld and Levinthal (2), and by Baurhenn (3) abroad, and by Dawson (4) in this laboratory. Subsequently Dawson and Sia (5) succeeded in inducing transformation *in vitro*. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system. Later, Alloway (6) was able to cause

* Work done in part as Fellow in the Medical Sciences of the National Research Council.

El ADN como principio transformante

Avery, MacLeod & McCarty (1944)



INDEPENDENT FUNCTIONS OF VIRAL PROTEIN AND NUCLEIC ACID IN GROWTH OF BACTERIOPHAGE*

By A. D. HERSHEY AND MARTHA CHASE

(From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island)

(Received for publication, April 9, 1952)

The work of Doermann (1948), Doermann and Dissoway (1949), and Anderson and Doermann (1952) has shown that bacteriophages T2, T3, and T4 multiply in the bacterial cell in a non-infective form. The same is true of the phage carried by certain lysogenic bacteria (Lwoff and Gutmann, 1950). Little else is known about the vegetative phase of these viruses. The experiments reported in this paper show that one of the first steps in the growth of T2 is the release from its protein coat of the nucleic acid of the virus particle, after which the bulk of the sulfur-containing protein has no further function.

Materials and Methods.—Phage T2 means in this paper the variety called T2H (Hershey, 1946); T2*h* means one of the host range mutants of T2; UV-phage means phage irradiated with ultraviolet light from a germicidal lamp (General Electric Co.) to a fractional survival of 10^{-6}.

Sensitive bacteria means a strain (H) of *Escherichia coli* sensitive to T2 and its h mutant; resistant bacteria B/2 means a strain resistant to T2 but sensitive to its h mutant; resistant bacteria B/2*h* means a strain resistant to both. These bacteria do not adsorb the phages to which they are resistant.

"Salt-poor" broth contains per liter 10 gm. bacto-peptone, 1 gm. glucose, and 1 gm. NaCl. "Broth" contains, in addition, 3 gm. bacto-beef extract and 4 gm. NaCl.

Glycerol-lactate medium contains per liter 70 mM sodium lactate, 4 gm. glycerol, 5 gm. NaCl, 2 gm. KCl, 1 gm. NH_4Cl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 gm. gelatin, 10 mg. P (as orthophosphate), and 10 mg. S (as $MgSO_4$), at pH 7.0.

Adsorption medium contains per liter 4 gm. NaCl, 5 gm. K_2SO_4, 1.5 gm. KH_2PO_4, 3.0 gm. Na_2HPO_4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.01 gm. gelatin, at pH 7.0.

Veronal buffer contains per liter 1 gm. sodium diethylbarbiturate, 3 mM $MgSO_4$, and 1 gm. gelatin, at pH 8.0.

The HCN referred to in this paper consists of molar sodium cyanide solution neutralized when needed with phosphoric acid.

* This investigation was supported in part by a research grant from the National Microbiological Institute of the National Institutes of Health, Public Health Service. Radioactive isotopes were supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, United States Atomic Energy Commission.

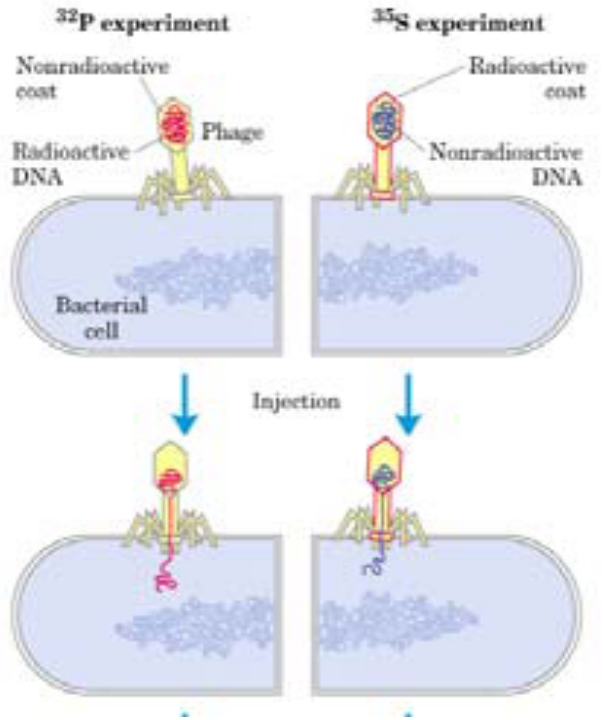
DISCUSSION

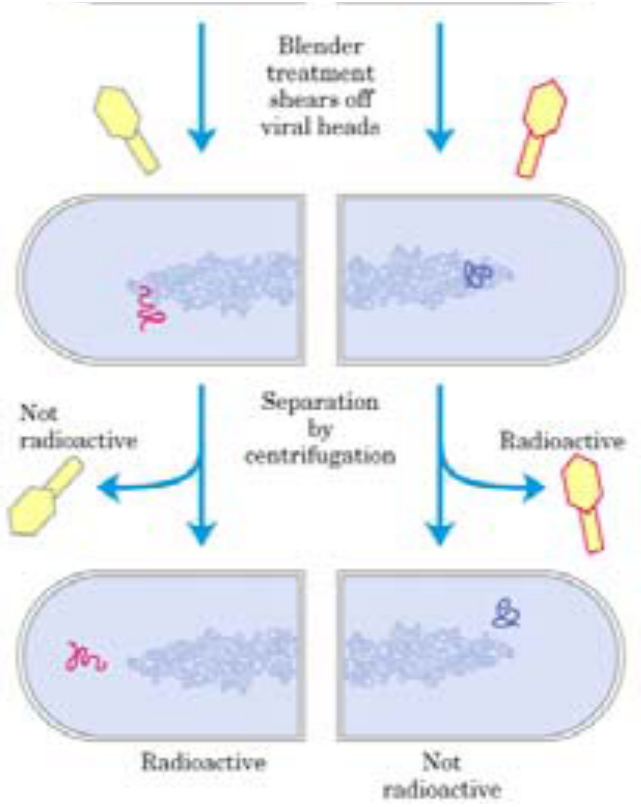
We have shown that when a particle of bacteriophage T2 attaches to a bacterial cell, most of the phage DNA enters the cell, and a residue containing at least 80 per cent of the sulfur-containing protein of the phage remains at the cell surface. This residue consists of the material forming the protective membrane of the resting phage particle, and it plays no further role in infection after the attachment of phage to bacterium.

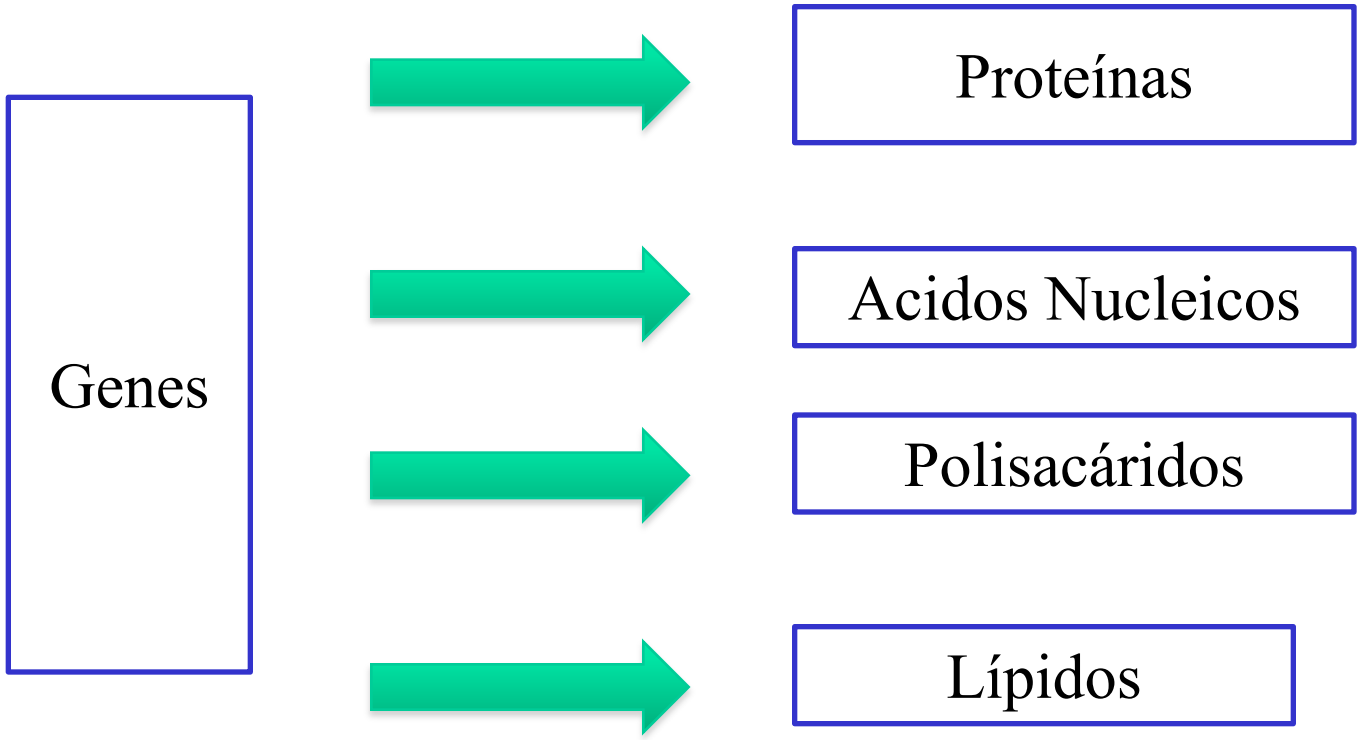
These facts leave in question the possible function of the 20 per cent of sulfur-containing protein that may or may not enter the cell. We find that little or none of it is incorporated into the progeny of the infecting particle, and that at least part of it consists of additional material resembling the residue that can be shown to remain extracellular. Phosphorus and adenine (Watson and Maaløe, 1952) derived from the DNA of the infecting particle, on the other hand, are transferred to the phage progeny to a considerable and equal extent. We infer that sulfur-containing protein has no function in phage multiplication, and that DNA has some function.

Alfred Hershey y Martha Chase (1952)

Determinaron que el ADN es el material genético en el bacteriófago T2






Genes
Proteínas
Acidos Nucleicos
Polisacáridos
Lípidos

Proteínas

Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues.

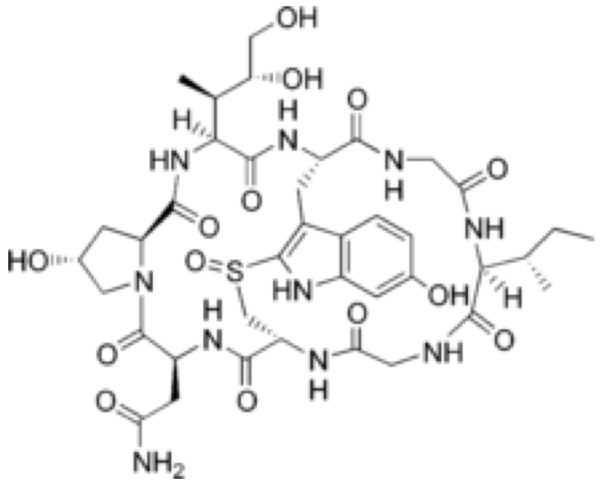
Amino ácidos

α-amino acids (generic formula H2NCHRCOOH in most cases, where R is an organic substituent known as a "side-chain”) often the term "amino acid" is used to refer specifically to these. They include **the 23 proteinogenic** ("protein-building") amino acids,

A **linear chain of amino acid** residues is called a polypeptide.

A protein contains at least one long polypeptide.

Short polypeptides, containing less than 20–30 residues, are rarely considered to be proteins and are commonly called peptides, or sometimes oligopeptides.



Nonribosomal peptides (NRP)

- lineales
- 23 aa proteinogénicos?

¿Porqué?

La estructura del ADN

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

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

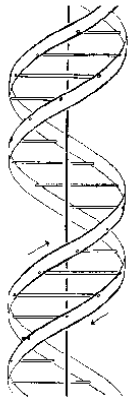
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

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 ac
proposed by Pauling and Corey
their manuscript available t
publication. Their model co
twined chains, with the phos
axis, and the bases on the out
this structure is unsatisfacto
(1) We believe that the mat
X-ray diagrams is the salt, not
the acidic hydrogen atoms it is
would hold the structure toget
negatively charged phosphate
repel each other. (2) Some
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 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 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

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

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*,

re of Deoxypentose c Acids

roperties of deoxypentose molecular structure con- X-ray diffraction studies [1]) show the basic molecular mplicity. The purpose of describe, in a preliminary ental evidence for the poly- ration being helical, and n in the natural state. A will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1-3], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid ('structure *B*' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the inter-nucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[5] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the *n*th layer line being proportional to the square of J_n, the *n*th order Bessel function. A straight line may be drawn approximately through

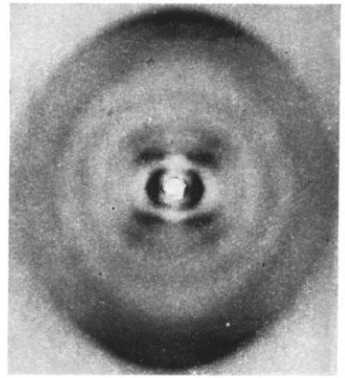


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats *n* times along the helix there will be a meridional reflexion (J_n[2]) on the *n*th layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[5] being to reproduce the intensity distribution about the origin around the new origin, on the *n*th layer line, corresponding to *C* in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

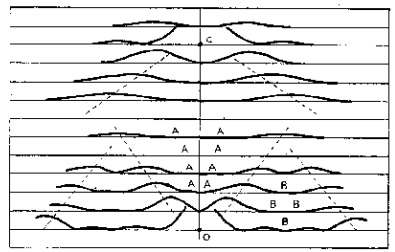


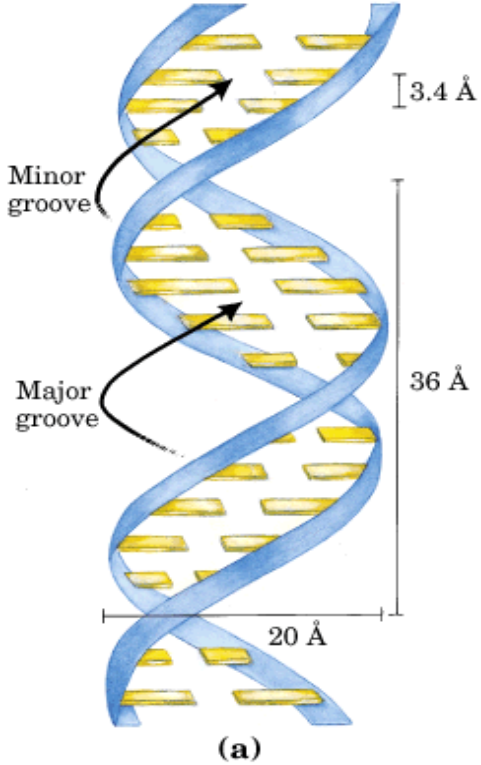
Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about O on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About *C* on the tenth layer line similar functions are plotted for an outer diameter of 12 A.

REGLAS DE CHARGAFF PARA ADN DE DOBLE HÉLICE

•La proporción de Adenina (A) es igual a la de Timina (T). A = T . La relación entre Adenina y Timina es igual a la unidad (A/T = 1).
•La proporción de Guanina (G) es igual a la de Citosina (C). G= C. La relación entre Guanina y Citosina es igual a la unidad (G/C=1).
•La proporción de bases púricas (A+G) es igual a la de las bases pirimidínicas (T+C). (A+G) = (T + C). La relación entre (A+G) y (T+C) es igual a la unidad (A+G)/(T+C)=1.
•Sin embargo, la proporción entre (A+T) y (G+C) era característica de cada organismo, pudiendo tomar por tanto, diferentes valores según la especie estudiada. Este resultado indicaba que los ácidos nucleicos no eran la repetición monótona de un tetranucleótido. Existía variabilidad en la composición de bases nitrogenadas

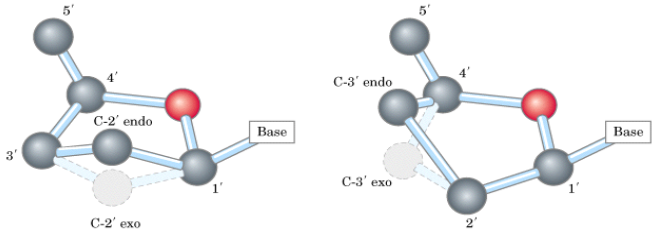
Procedencia del ADN	A	G	C	T	5-Me-C
Timo de Bovino	28,2	21,5	21,2	27,8	1,3
Esperma de bovino	28,7	22,2	20,7	27,3	1,3
Germen de trigo	27,3	22,7	16,8	27,1	6,0
Saccharomyces	31,3	18,7	17,1	32,9	-
Escherichia coli	26,0	24,9	25,2	23,9	-
Mycobacterium tuberculosis	15,1	34,9	35,4	14,6	-
ØX174	24,3	24,5	18,2	32,3	-
T3	23,7	26,2	27,7	23,5	-
T5	30,3	19,5	19,5	30,8	-
T7	32,4	18,3		32,4	17,0 HMC
Virus ARN	**A**	**G**	**C**	**U**	
Mosaico del tabaco (TMV)	29,8	25,4	18,5	26,3	
Mosaico amarillo nabo	22,6	17,2	38,0	22,2	
Poliomielitis	28,6	24,0	22,0	25,4	
Encéfalo miocarditis del ratón	27,3	23,5	23,2	25,9	
Reovirus Tipo 3	28,0	22,3	22,0	27,9	
Tumor de las heridas	31,1	18,6	19,1	31,3	

2 Cadenas enrolladas sobre el mismo eje formando una doble hélice a la derecha

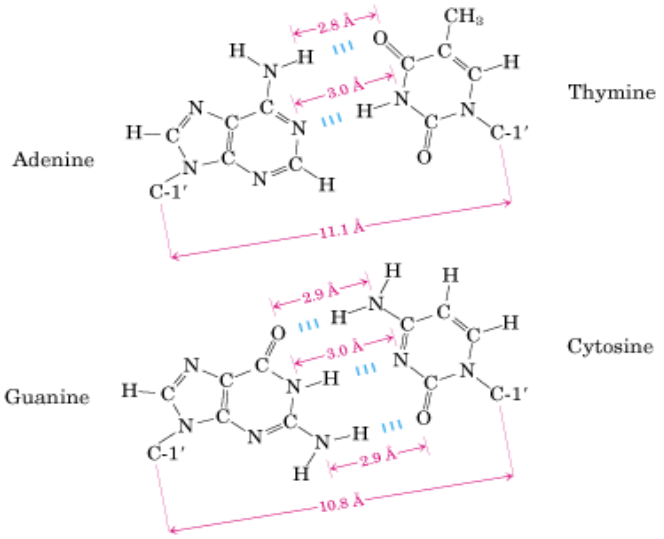


• El esqueleto hidrofilico de grupos fosfato y deoxiribosa alternantes está expuesto al agua del ambiente

• El anillo de furanosa está en la conformación C-2´endo

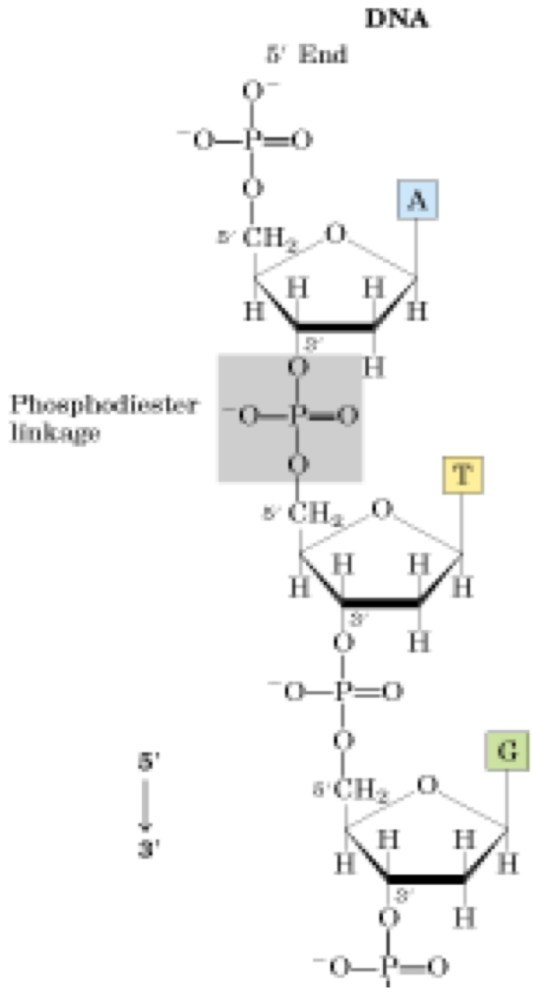


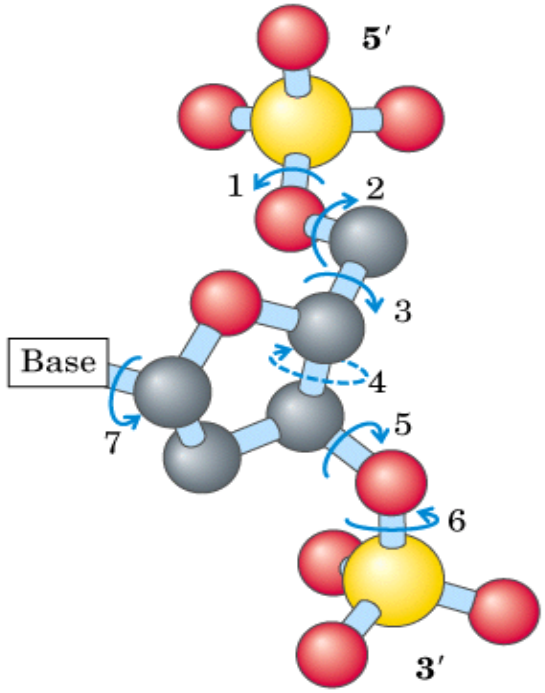
• Las bases están apiladas en el interior de la doble helice, con sus planos perpendiculares al eje de la doble hélice



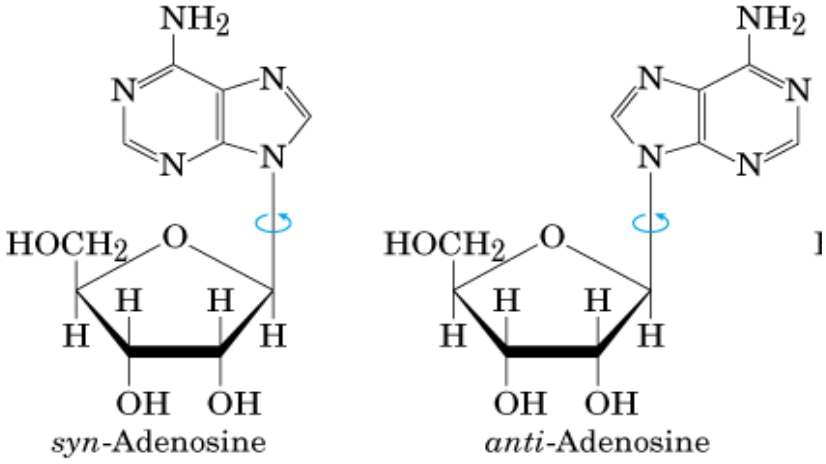
• El apareamiento de las dos cadenas genera un surco mayor y un surco menor en la superficie de la doble hèlice

G ≡ C A = T

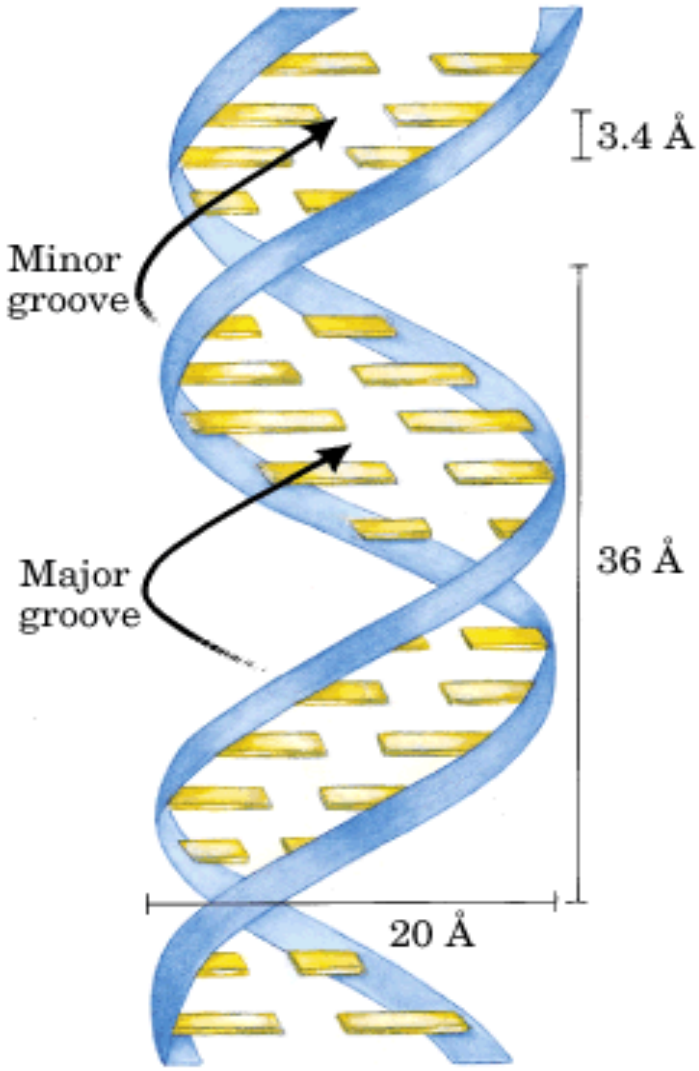

DNA
5′ End
A
T
G
Phosphodiester linkage
5′
3′


5′
1
2
3
4
5
6
7
Base
3′

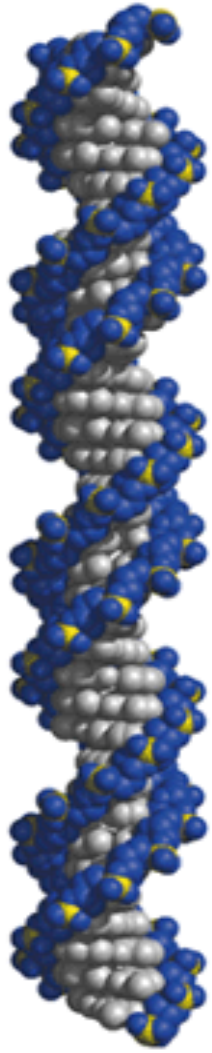
(a)


NH2
HOCH2
OH OH
syn-Adenosine
anti-Adenosine

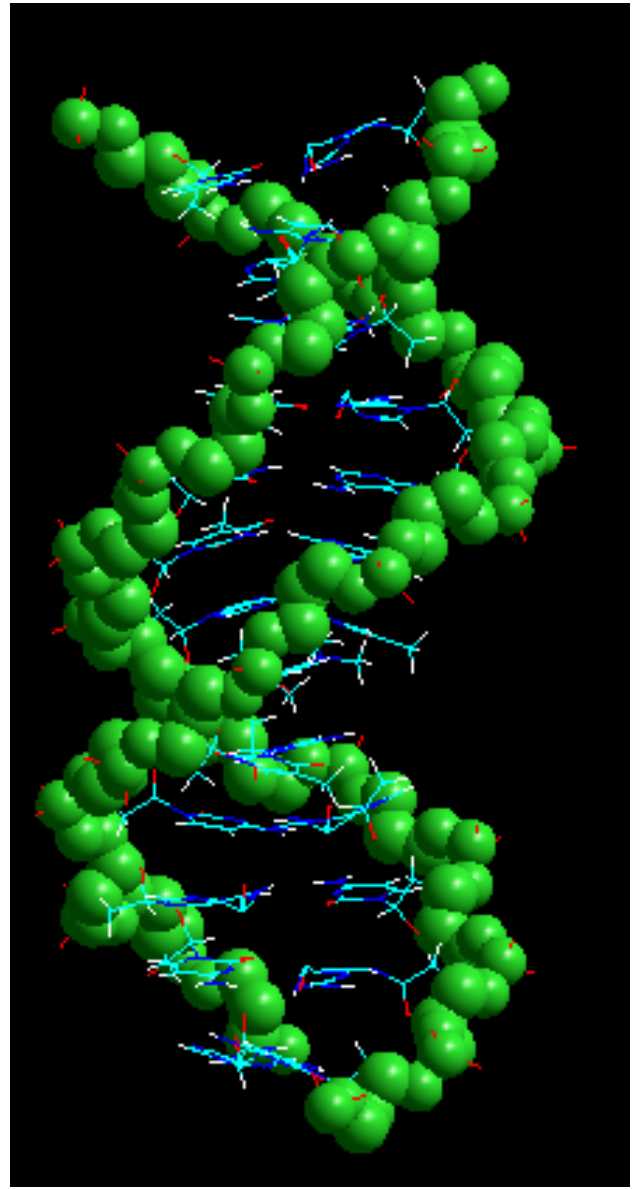
(b)

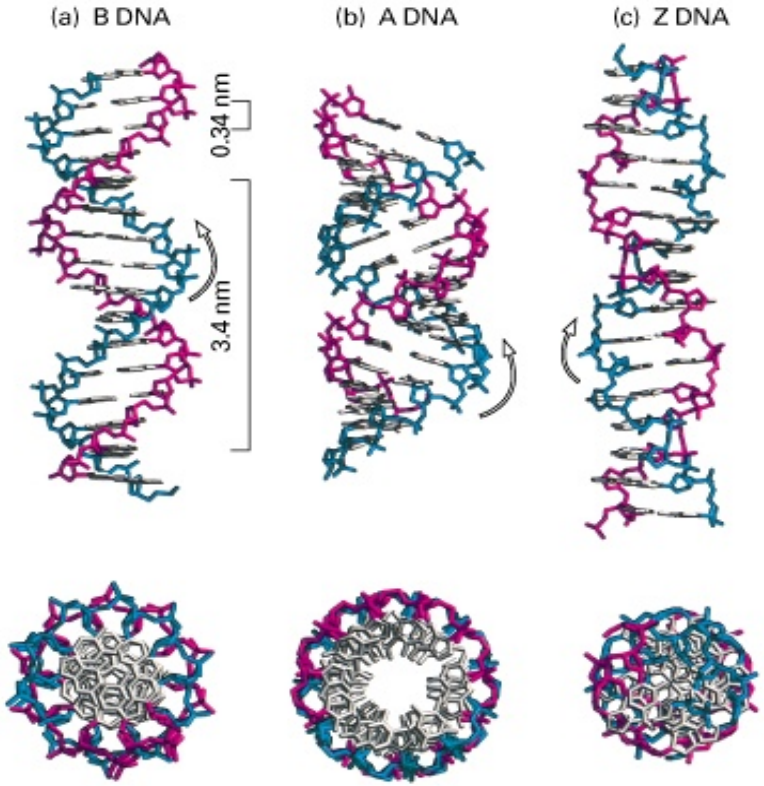


(a)



(c)



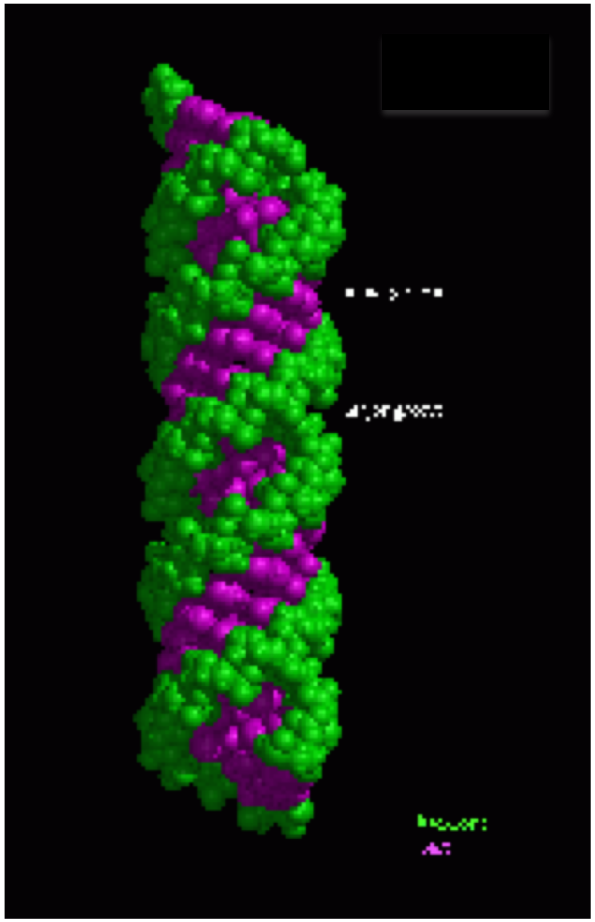


ADN-B: ADN en disolución, 92% de humedad relativa, se encuentra en soluciones con baja fuerza iónica se corresponde con el modelo de la Doble Hélice.

ADN-A: ADN con 75% de humedad, requiere Na, K o Cs como contraiones, presenta 11 pares de bases por giro completo y 26 A de diámetro. Es interesante por presentar una estructura parecida a la de los híbridos ADN-ARN y a las regiones de autoapareamiento ARN-ARN.

ADN-Z: doble hélice sinistrosa (enrollamiento a izquierda), 12 pares de bases por giro completo, 18 A de diámetro, se observa en segmentos de ADN con secuencia alternante de bases púricas y pirimidínicas (GCGCGC), debido a la conformación alternante de los residuos azúcar-fosfato sigue un curso en zig-zag. Requiere una concentración de cationes superior a la del ADN-B, y teniendo en cuenta que las proteínas que interaccionan con el ADN tienen gran cantidad de residuos básicos sería posible que algunas convirtieran segmentos de ADN-B en ADN-Z.

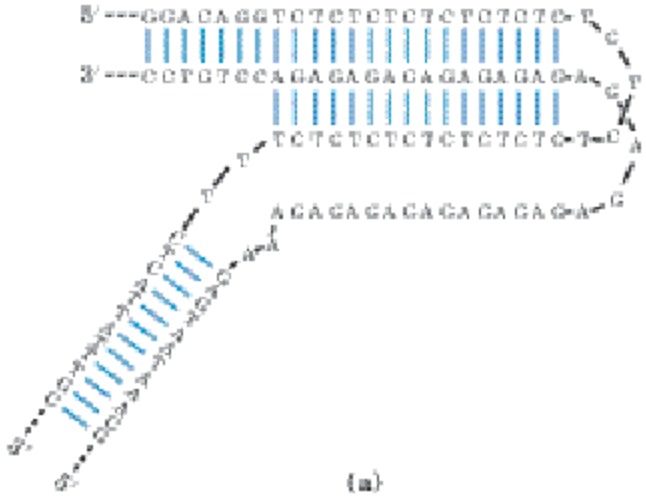
	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′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

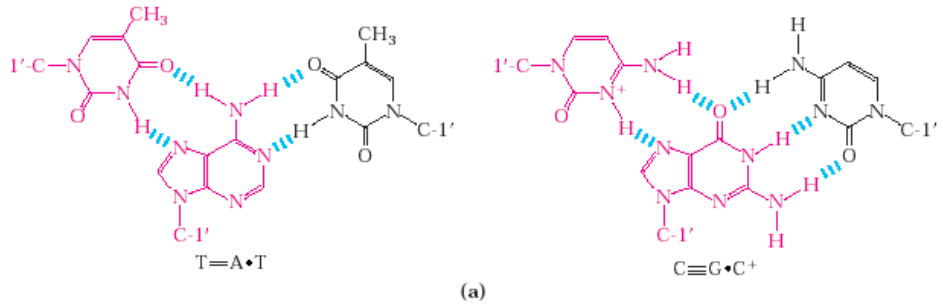


La estructura del híbrido DNA-RNA es la de una doble hélice con las características generales de un A-RNA, o del A'-RNA. Típicamente se detectan 11-12 pares de bases por vuelta de hélice.

ADN triple hélice o ADN-H: "In vitro" es posible obtener tramos de triple hélice intercalando oligonucleótidos cortos constituidos solamente por pirimidinas (timinas y citosinas) en el surco mayor de una doble hélice. Este oligonucleótido se une a pares de bases A-T y G-C mediante enlaces de hidrógeno tipo Hoogsteen que se establecen entre la T o la C del oligonucleótido y los pares A-T y G-C de la doble hélice. No se sabe la función biológica del ADN-H aunque se ha detectado en cromosomas eucarióticos.

Apareamiento tipo Hoogsteen

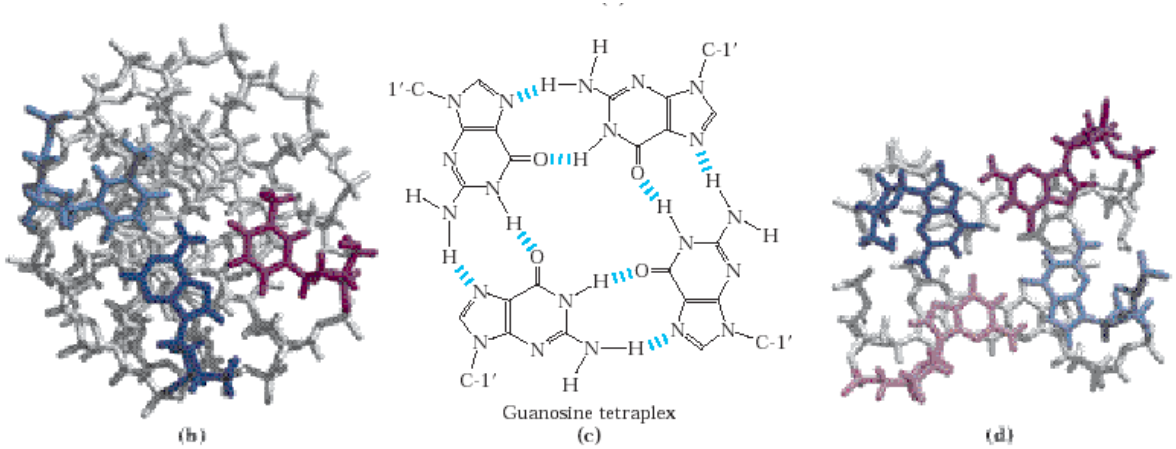




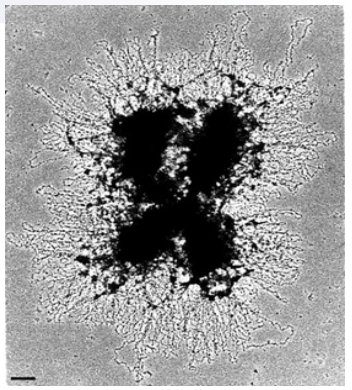
Estables a pH bajos (C^+,pKa= 7.5)

ADN cuadruplexo: "In vitro" se han obtenido cuartetos de Guanina (ADN cuadruplexo) unidas mediante enlaces tipo Hoogsteen, empleando polinucleótidos que solamente contienen Guanina (G). Los extremos de los cromosomas eucarióticos (telómeros) tienen una estructura especial con un extremo 3' OH de cadena sencilla (monocatenario) en el que se repite muchas veces en tandem una secuencia rica en Guaninas. Se piensa que el ADN cuadruplexo telomérico serviría para proteger los extremos cromosómicos de la degradación enzimática. Ejemplo de secuencia telomérica rica en guaninas (G):

5´P TTGGGTTGGGGTTGGGG.....TTGGGG 3'OH



Empaquetamiento del ADN Eucariota



En el genoma humano tenemos 3 x 10^9 bp distribuidos en 23 cromosomas

La forma B-DNA ocupa 3.4 A/bp

La longitud total del ADN celular humano es de 2 metros!!!

Debemos empaquetarlo en un núcleo con un diámetro de 5 μm
(10.000 veces)

El DNA durante la interfase se encuentra condensdo formando un complejo nucleoproteico denominado **cromatina**

Chromatin Proteins

Chromatin proteins

1. Histone Proteins (small, positively charged– rich in lysine and arginine residues)

    Core histones: H2A, H2B, H3, H4

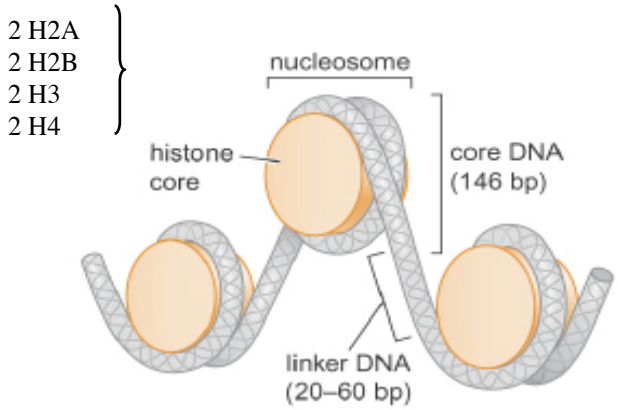
    Linker histone: H1

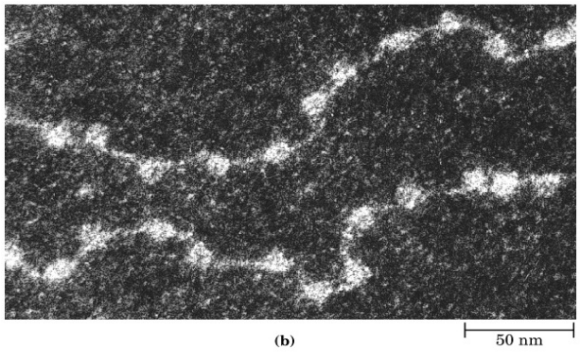
2. Nonhistone chromosomal proteins

TABLE 7-5 General Properties of the Histones

Histone type	Histone	Molecular weight (M_r)	% of Lysine and Arginine
Core histones	H2A	14,000	20%
	H2B	13,900	22%
	H3	15,400	23%
	H4	11,400	24%
Linker histone	H1	20,800	32%

El ADN se enrolla alrededor del núcleo histónico: Nucleosomas





"Beads on a String"

<u>Nucleosomes</u>

-Contain a histone core octomer + 146 bp core DNA

-Spaced ~200 bp apart

(146 bp core DNA + 20-60 bp linker DNA)

-Core DNA is protected DNases

The Histone Amino-Terminal Tails Stabilize DNA Wrapping around the Octamer

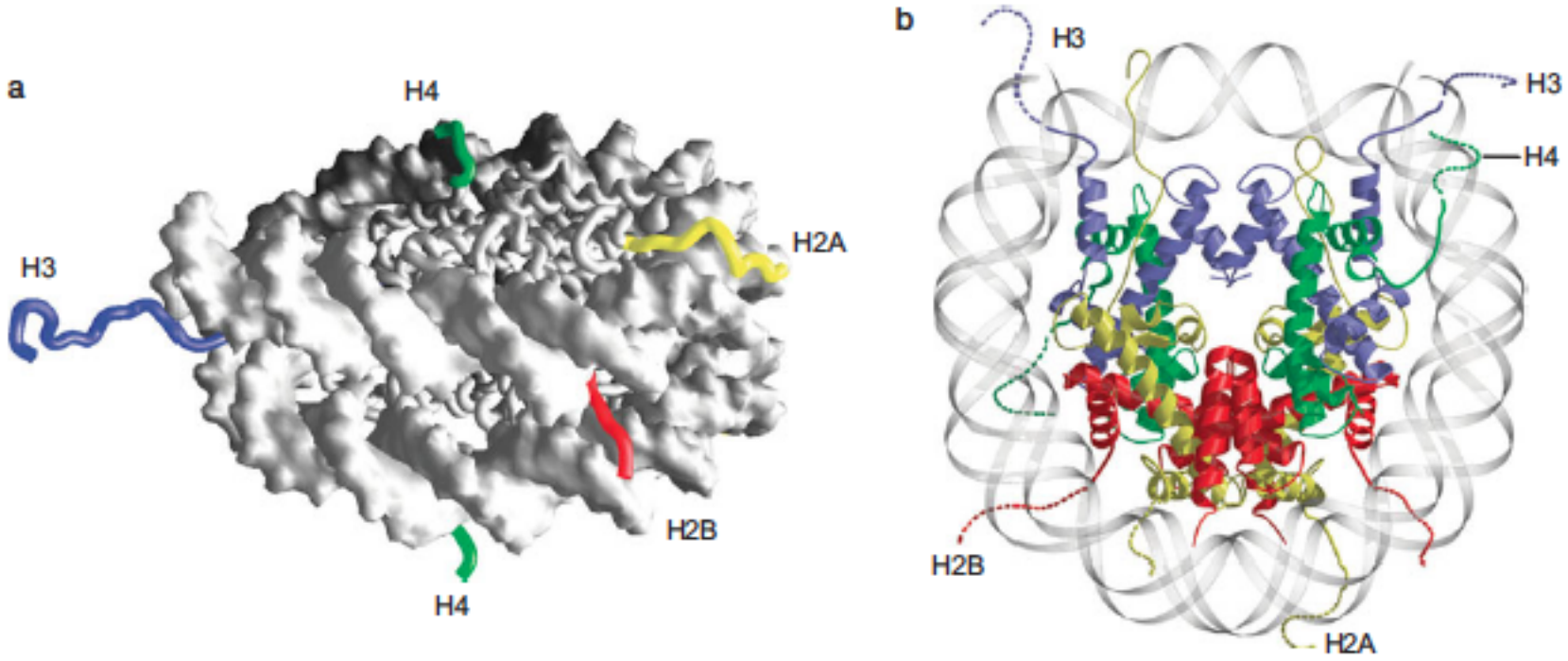
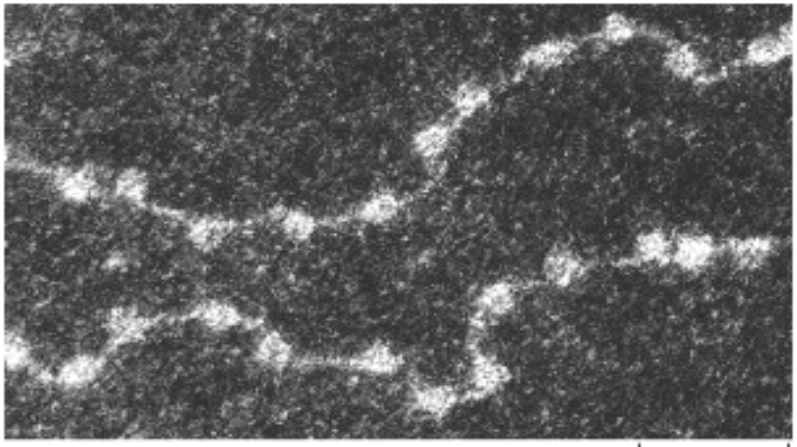


FIGURE 8-26 Histone tails emerge from the core of the nucleosome at specific positions. (a) The side view illustrates that the H3 and H2B tails emerge from between the two DNA helices. In contrast, the H4 and H2A tails emerge either above or below both DNA helices. (Luger K. et al. 1997. *Nature* **389:** 251–260.) Image prepared with GRASP. (b) The position of the tails relative to the entry and exit of the DNA. This view reveals that the histone tails emerge at numerous positions relative to the DNA. (Davey C.A. et al. 2002. *J. Mol. Biol.* **319:** 1097–1113.) Image prepared with MolScript, BobScript, and Raster3D.

La Histona H1 une 2 hélices de ADN



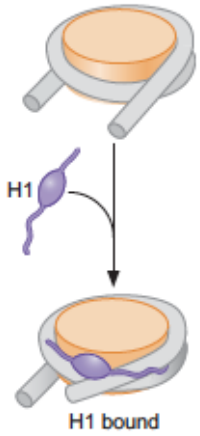
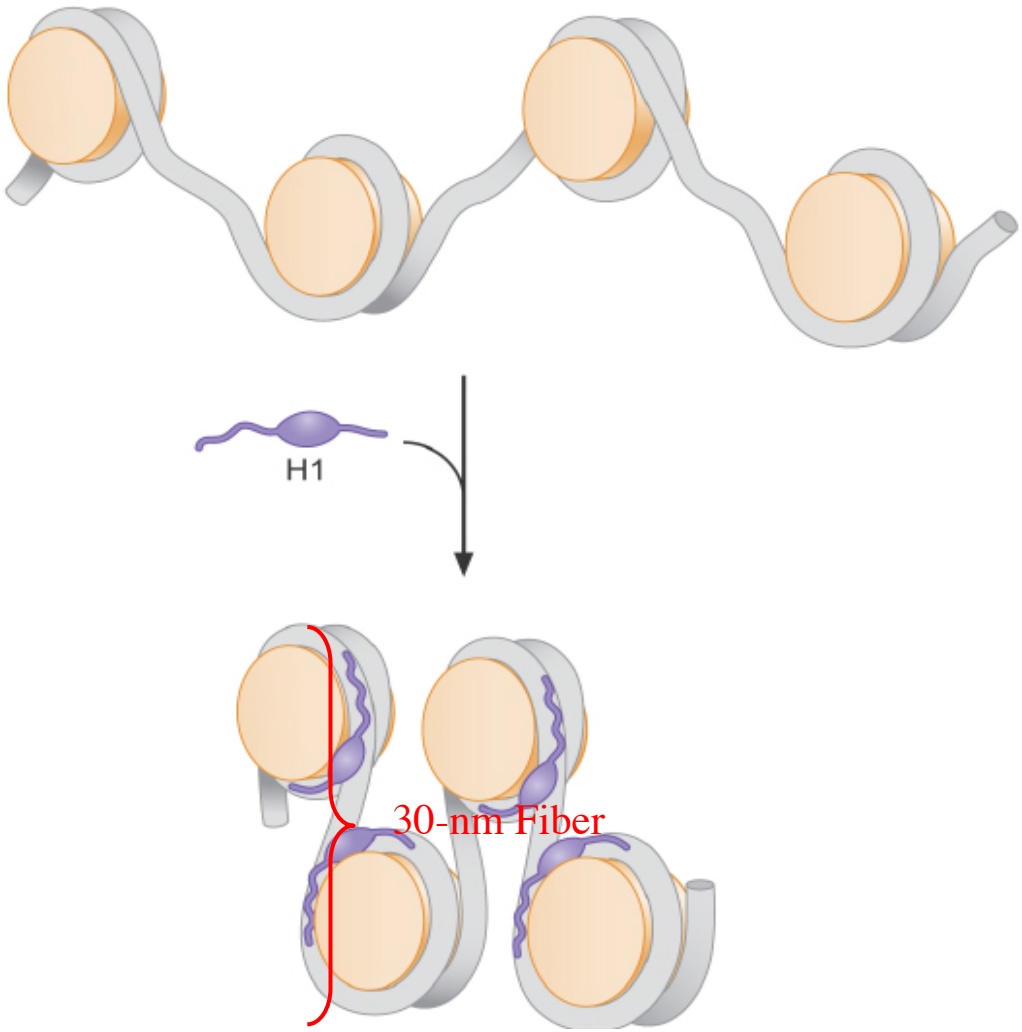
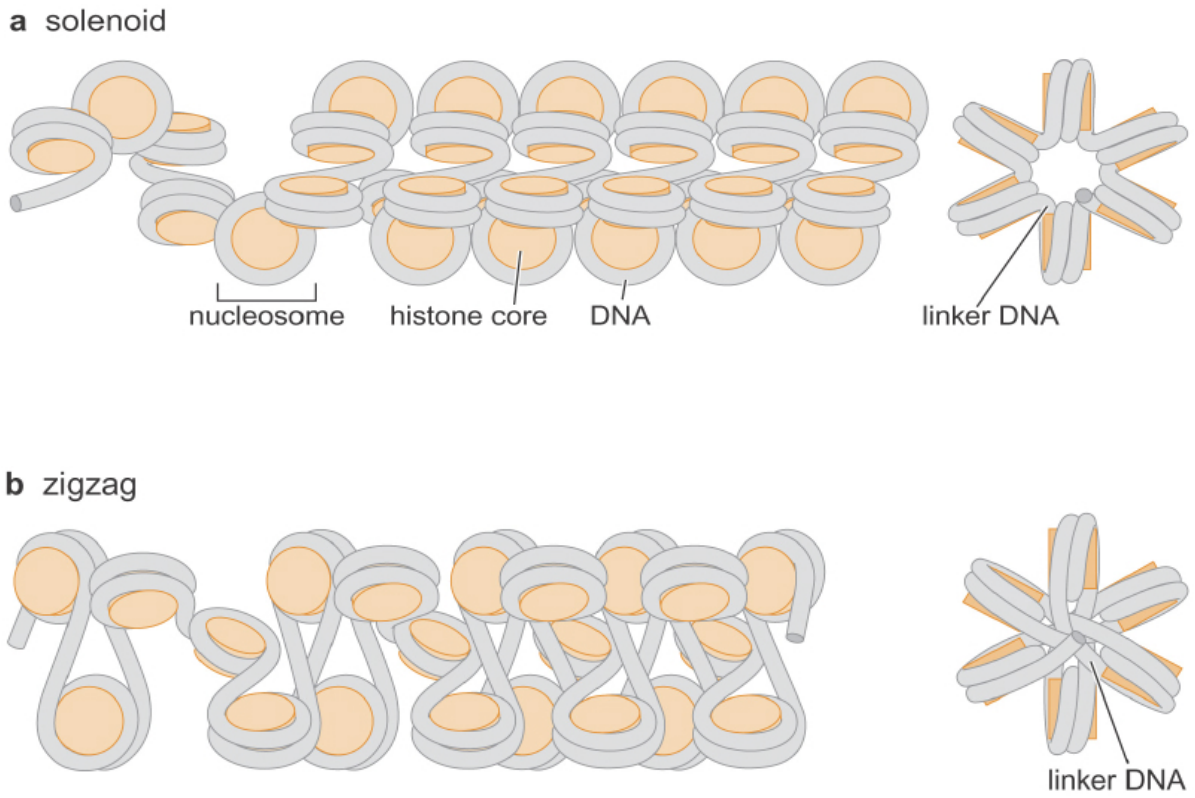


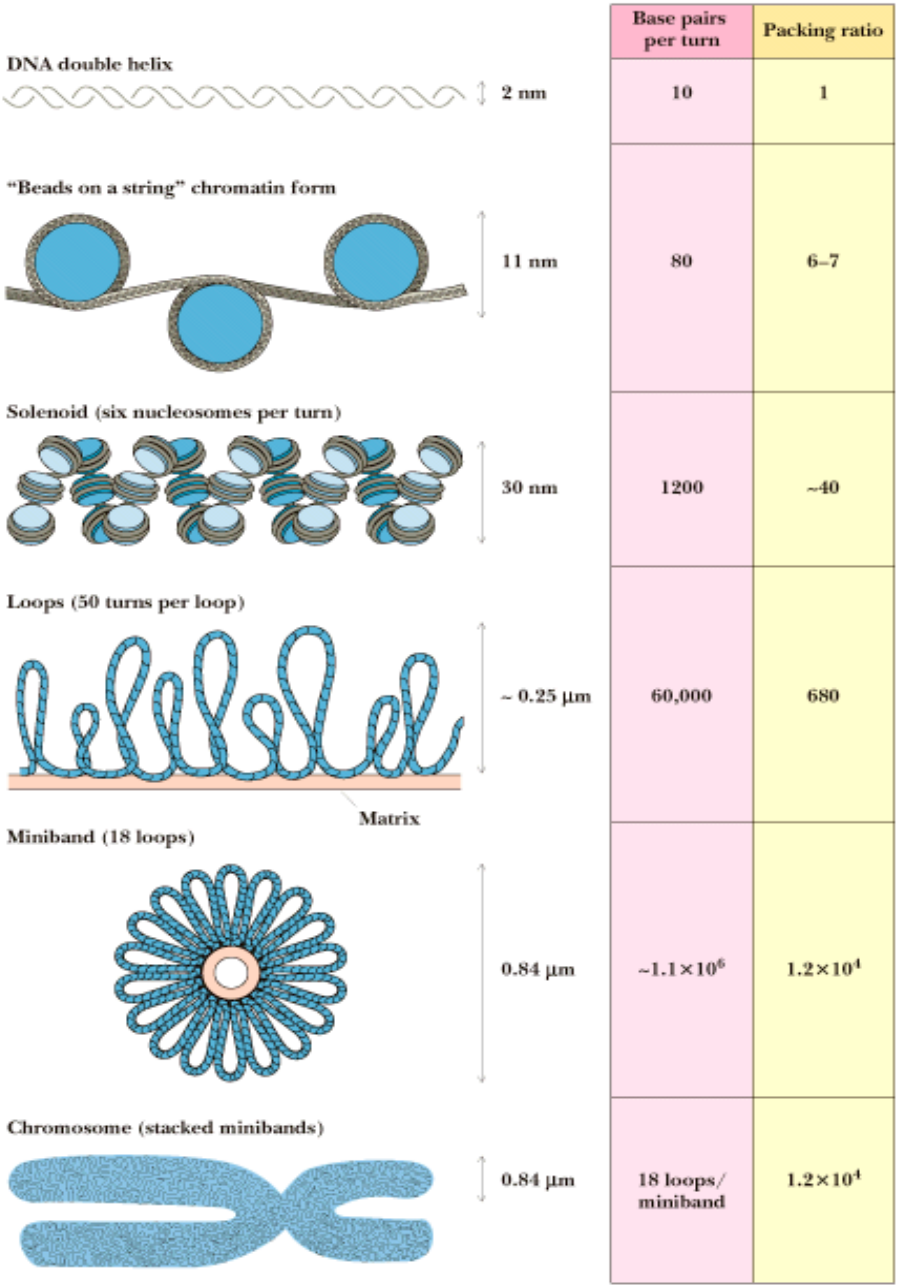
FIGURE 8-27 Histone H1 binds two DNA helices. Upon interacting with a nucleosome, histone H1 binds to the linker DNA at one end of the nucleosome and the central DNA helix of the nucleosome bound DNA (the middle of the 147 bp bound by the core histone octamer).



2 Modelos para la fibra de cromatina de 30-nm



Un modelo de la estructura del cromosoma



DNA exists in chromatin form during **interphase**

DNA in most compact form (chromosomes) during **metaphase** of mitosis

The Amino-Terminal Tails of the Histones Are Frequently Modified

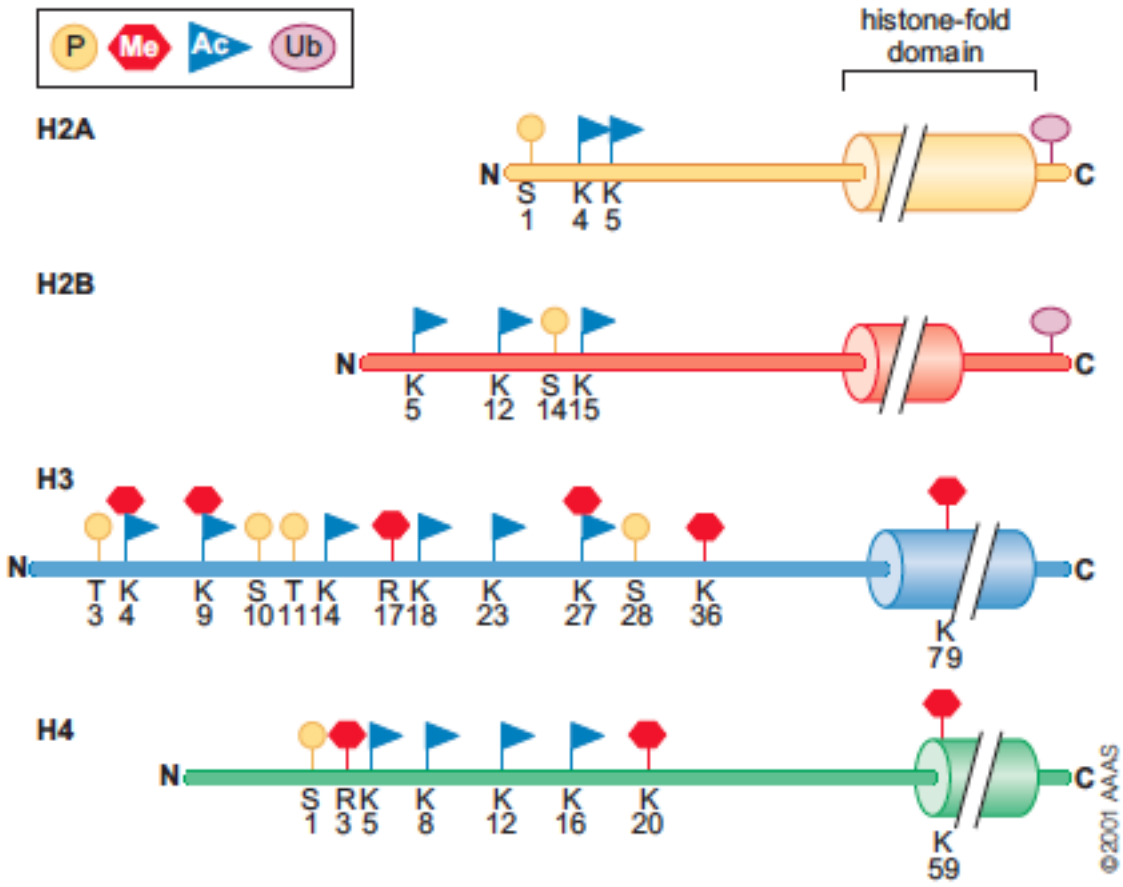


FIGURE 8-39 Modifications of the histone amino-terminal tails alters the function of chromatin. The sites of known histone modifications are illustrated on each histone. Although the types of histone modifications continue to grow, for simplicity, only sites of acetylation, methylation, phosphorylation, and ubiquitinylation are shown. The majority of these modifications occur on the tail regions, but there are occasional modifications within the histone fold (e.g., methylation of lysine 79 of histone H3). (Adapted, with permission, from Alberts B. et al. 2002. *Molecular biology of the cell*, 4th ed., Fig. 4-35. © Garland Science/Taylor & Francis LLC; and, with permission, from Jenuwein T. and Allis C.D. 2001. *Science* **293**: 1074–1080, Figs. 2 and 3. © AAAS.)

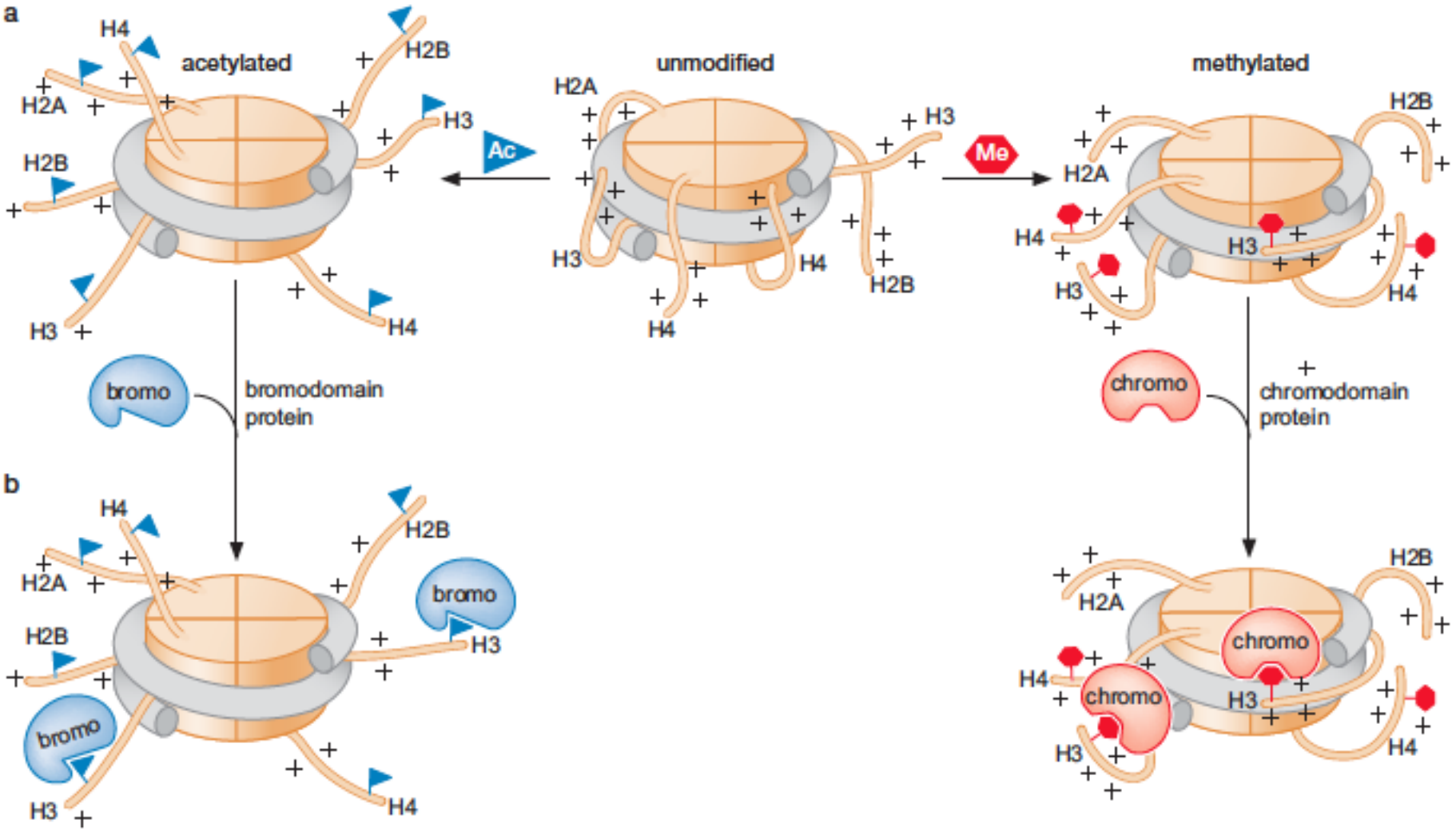


FIGURE 8-41 Effects of histone tail modifications. (a) The effect on the association with nucleosome-bound DNA. Unmodified and methylated histone tails are thought to associate more tightly with nucleosomal DNA than acetylated histone tails. (b) Modification of histone tails creates binding sites for chromatin-modifying enzymes.

Nucleosome Modification and Remodeling Work Together to Increase DNA Accessibility

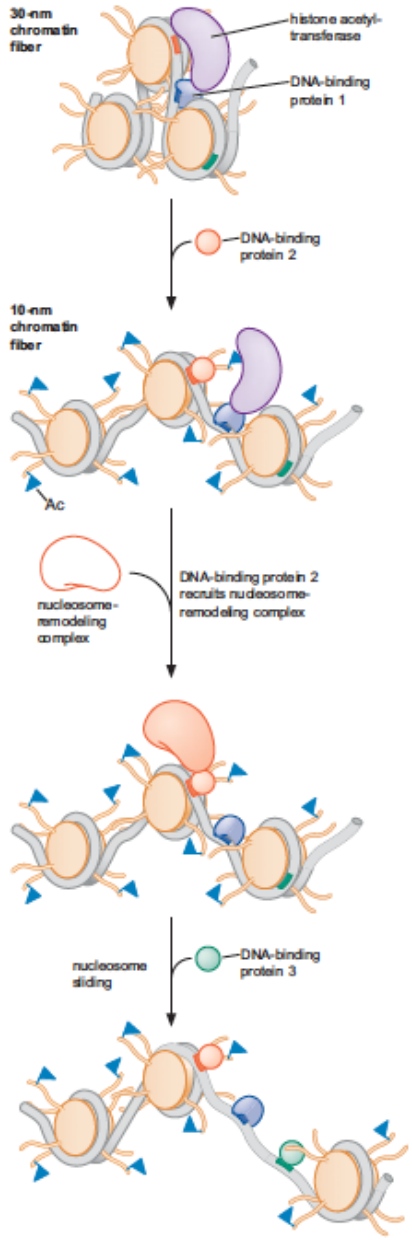
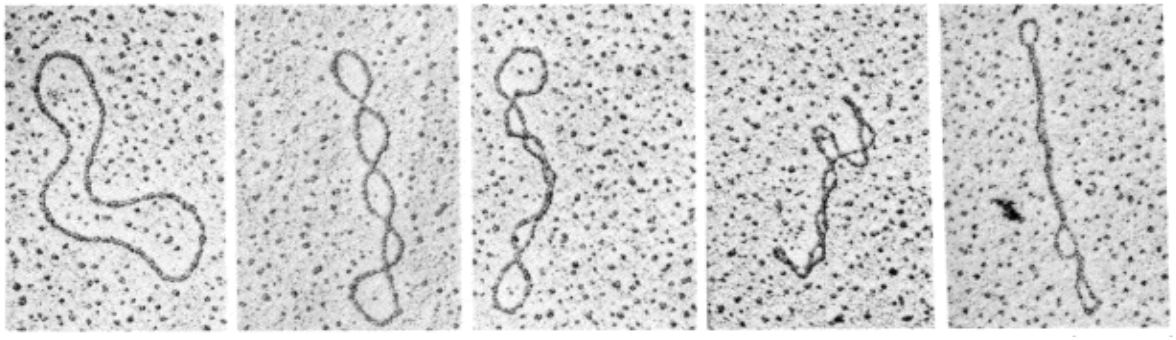


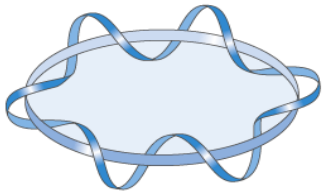
FIGURE 8-42 Chromatin-remodeling and histone-modifying complexes work together to alter chromatin structure. Sequence-specific DNA-binding proteins typically recruit these enzymes to specific regions of a chromosome. In the illustration, the blue DNA-binding protein first recruits a histone acetyltransferase that modifies the adjacent nucleosomes, increasing the accessibility of the associated DNA by locally converting the chromatin fiber from the 30-nm fiber to the more accessible 10-nm form. This increased accessibility allows the binding of a second DNA-binding protein (orange) that recruits a nucleosome-remodeling complex. Localization of the nucleosome-remodeling complex facilitates the sliding of the adjacent nucleosomes, which allows the binding site for a third DNA-binding protein (green) to be exposed. For example, this could be the binding site for the TATA-binding protein at a start site of transcription. Although we show the order of association as histone acetylation complex and then nucleosome-remodeling complex, both orders are observed and can be equally effective. It is also true that recruitment of a different histone-modifying complex could result in the formation of more compact and inaccessible chromatin.

¿Qué es el superenrollamiento?



$$L = T + W$$

Linking Number (L or L_k) = número de veces que dos cadenas están entrelazadas



Lk = 6

Twists (T or T_w) = número de vueltas de hélice

Relaxed (8 turns)

Writhes (W or W_r) = número de veces que el dúplex se entrecruza consigo mismo

Negative
supercoils

Qué hacen las topoisomerasas?

1. Cambian el linking number de la molécula de ADN mediante:

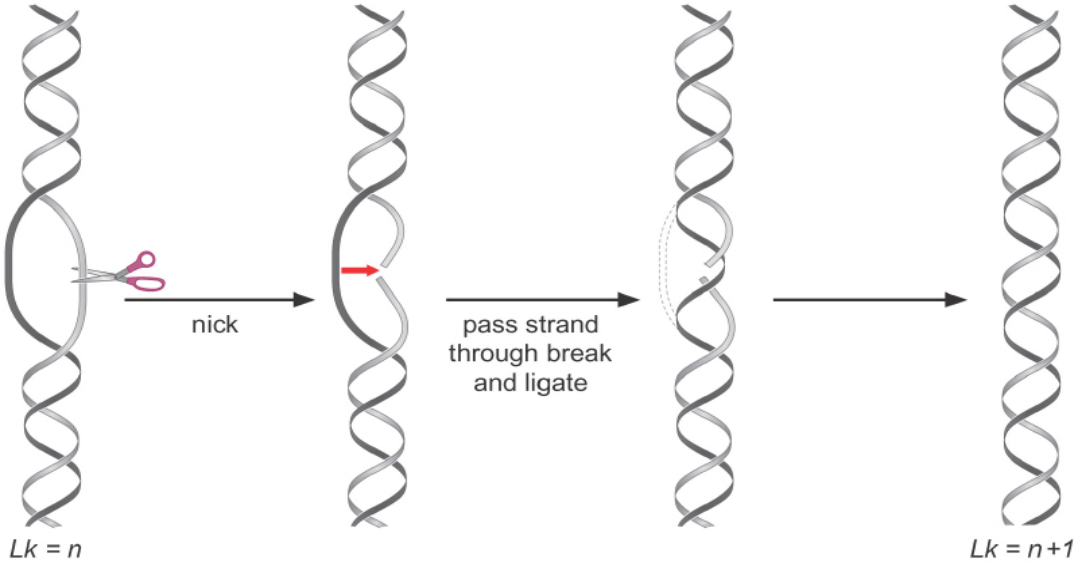
A) Cortando una o ambas cadenas y luego,

B) Enrollarlas mas o menos y uniendo nuevamente los extremos.

2. Usualmente relajan el ADN superenrollado

Type I Topoisomerases

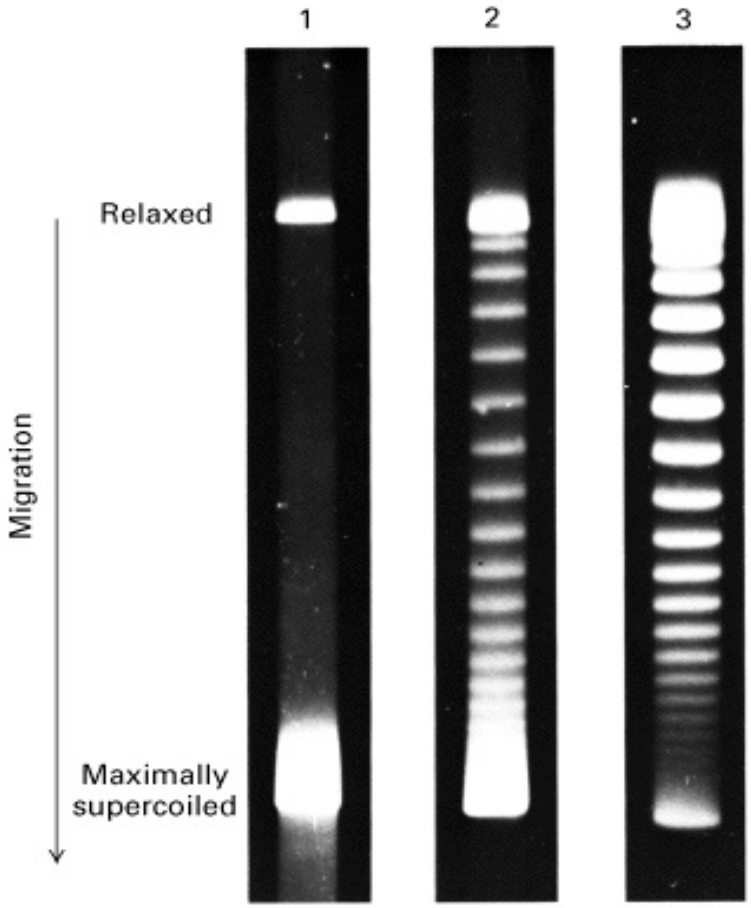
They relax DNA by nicking then closing one strand of duplex DNA. They **cut one strand** of the double helix, pass the other strand through, then rejoin the cut ends. They change the linking number by increments of +1 or –1.



Topo I from E. coli
1) acts to relax only negative supercoils
2) increases linking number by +1 increments

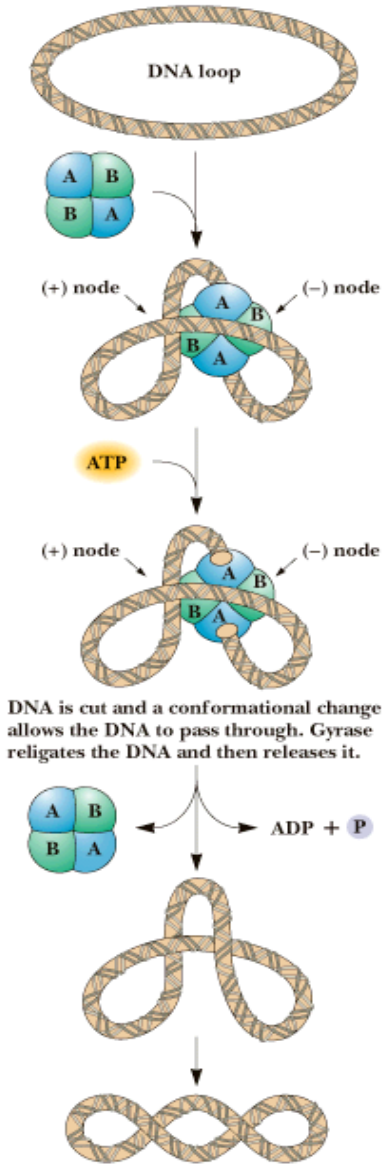
Topo I from eukaryotes
1) acts to relax positive or negative supercoils
2) changes linking number by –1 or +1 increments

Relaxation of SV40 DNA by Topo I



An E. coli Type II Topoisomerase: DNA Gyrase

Topo II (DNA Gyrase) from E. coli
1) Acts on both neg. and pos. supercoiled DNA
2) Increases the # of neg. supercoils by increments of 2
3) Requires ATP



Type II Topoisomerases

They relax or underwind DNA by cutting then closing both strands. They change the linking number by increments of +2 or –2.

All Type II Topoisomerases Can Catenate and Decatenate cccDNA molecules

Circular DNA molecules that use type II topoisomerases:

E. coli
-plasmids
-E. coli chromosome

Eukaryotes
-mitochondrial DNA
-circular dsDNA viruses (SV40)

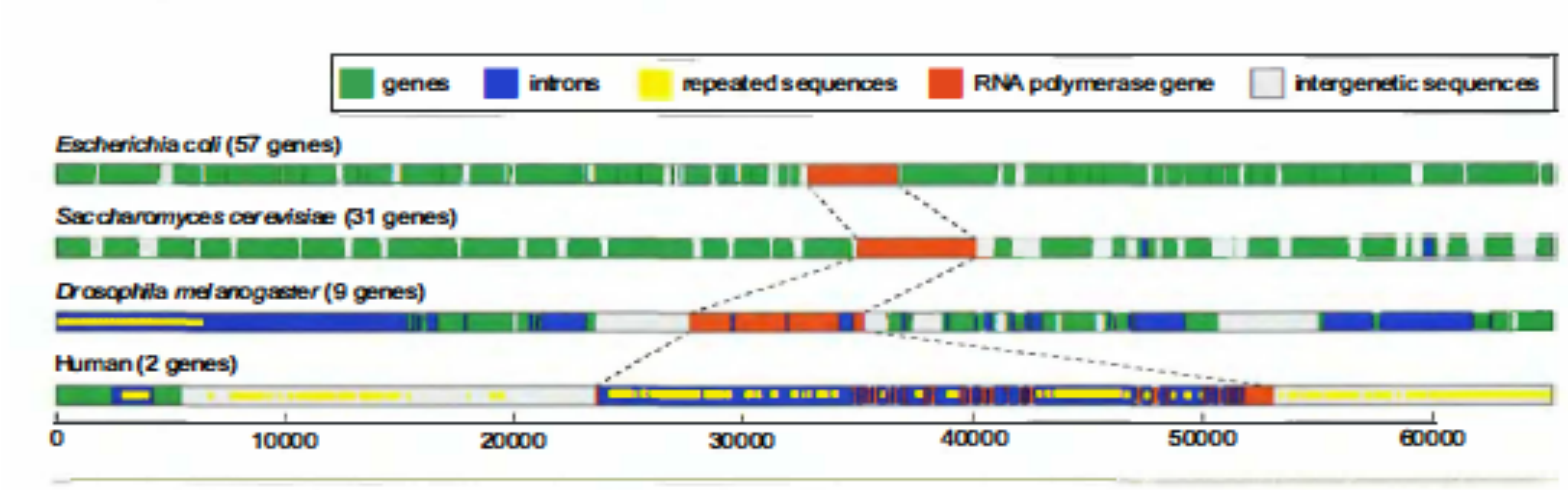
Genomas

TABLE 7-1 Variation in Chromosome Makeup in Different Organisms

Species	Number of chromosomes	Chromosome copy number	Form of chromosome(s)	Genome size (Mb)
PROKARYOTES				
Mycoplasma genitalium	1	1	Circular	0.58
***Escherichia coli* K-12**	1	1	Circular	4.6
Agrobacterium tumefaciens	4	1	3 Circular 1 Linear	5.67
Sinorhhizobium meliloti	3	1	Circular	6.7
EUKARYOTES				
***Saccharomyces cerevisiae* (budding yeast)**	16	1 or 2	Linear	12.1
Schizosaccharomyces pombe (fission yeast)	3	1 or 2	Linear	12.5
C. elegans (roundworm)	6	2	Linear	97
Arabidopsis thaliana (weed)	5	2	Linear	125
Drosophila melanogaster (fruit fly)	4	2	Linear	180
Tetrahymena thermophilus (protozoa)	Micronucleus 5 Macronucleus 225	Micronucleus 2 Macronucleus 10–10,000	Linear	220 (Micronucleus)
Fugu rubripes (fish)	22	2	Linear	365
Mus musculus (mouse)	19 + X and Y	2	Linear	2,500
Homo sapqiens	22 + X and Y	2	Linear	2,900

TABLE 7-2 Comparison of the Gene Density in Different Organisms' Genomes

Species	Genome size (Mb)	Approximate number of genes*	Gene density (genes/Mb)*
PROKARYOTES (bacteria)			
Mycoplasma genitalium	0.58	500	860
Streptococcus pneumonia	2.2	2,300	1,060
Escherichia coli K-12	4.6	4,400	950
Agrobacterium tumefaciens	5.7	5,400	960
Sinorhizobium meliloti	6.7	6,200	930
EUKARYOTES (animals)			
Fungi			
Saccharomyces cerevisiae	12	5,800	480
Schizosaccharomyces pombe	12	4,900	410
Protozoa			
Tetrahymena thermophila	220	> 20,000	> 90
Invertebrates			
Caenorhabditis elegans	97	19,000	200
Drosophila melanogaster	180	13,700	80
Strongylocentrotus purpuratus	845	~22,000	~26
Locusta migratoria	5,000	nd	nd
Vertebrates			
Fugu rubripes	365	> 31,000	> 85
Homo sapiens	2,900	27,000	9.3
Mus musculus	2,500	29,000	12
Plants			
Arabidopsis thaliana	125	25,500	200
Oryza sativa (rice)	430	> 45,000	> 100
Zea mays	2,200	> 45,000	> 20
Fritillaria assyriaca 1(tulip)	120,000	nd	nd


genes
introns
repeated sequences
RNA polymerase gene
intergenetic sequences
Escherichia coli (57 genes)
Saccharomyces cerevisiae (31 genes)
Drosophila melanogaster (9 genes)
Human (2 genes)
0
10000
20000
30000
40000
50000
60000

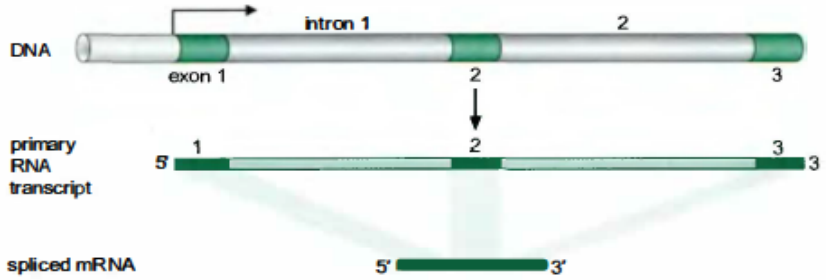


TABLE 7-3 Contribution of Introns and Repeated Sequences to Different Genomes

Species	Gene density (genes/Mb)	Average number of introns per gene*	Percentage of DNA that is repetitive*
PROKARYOTES (bacteria)			
Escherichia coli K-12	950	0	<1
EUKARYOTES (animals)			
Fungi			
Saccharomyces cerevisiae	480	0.04	3.4
Invertebrates			
Caenorhabditis elegans	200	5	6.3
Drosophila melanogaster	80	3	12
Vertebrates			
Fugu rubripes	75	5	2.7
Homo sapiens	8.5	6	46
Plants			
Arabidopsis thaliana	125	3	nd
Oryza sativa (rice)	470	nd	42

The Majority of Human Intergenic Sequences Are Composed of Repetitive DNA