ESTRUCTURA DEL DNA

Estructura del DNA

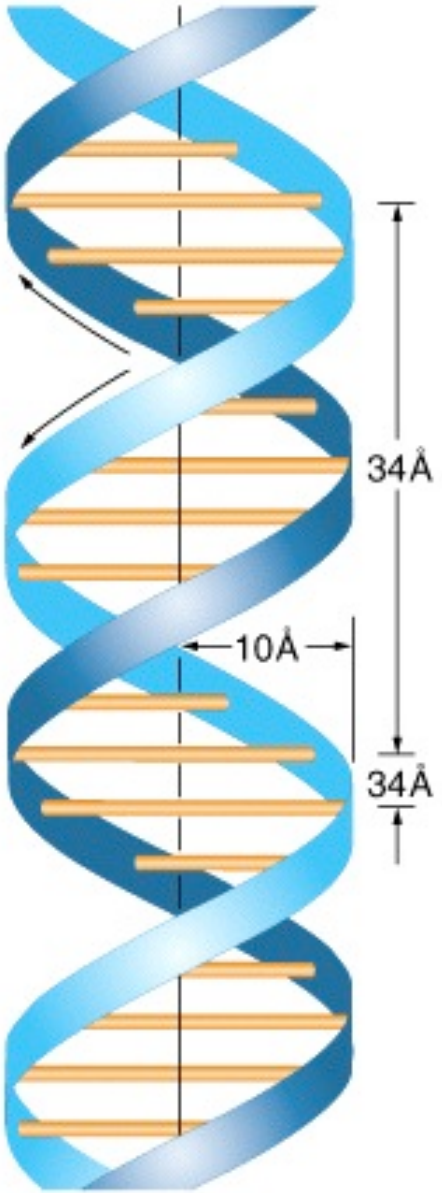
Surco mayor y menor

Radio de 10Å & diámetro de 20Å

3.4Å entre pares de nucleótidos

34Å / 360° por vuelta

10 nucleótidos por vuelta



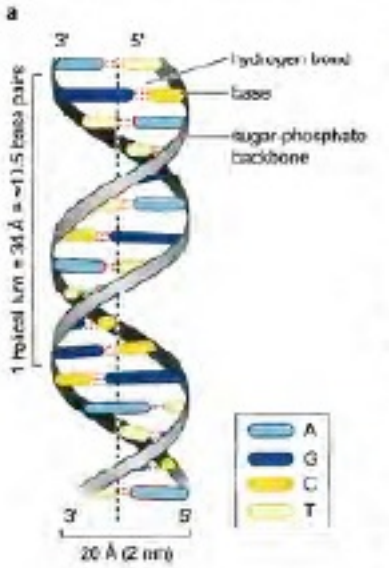
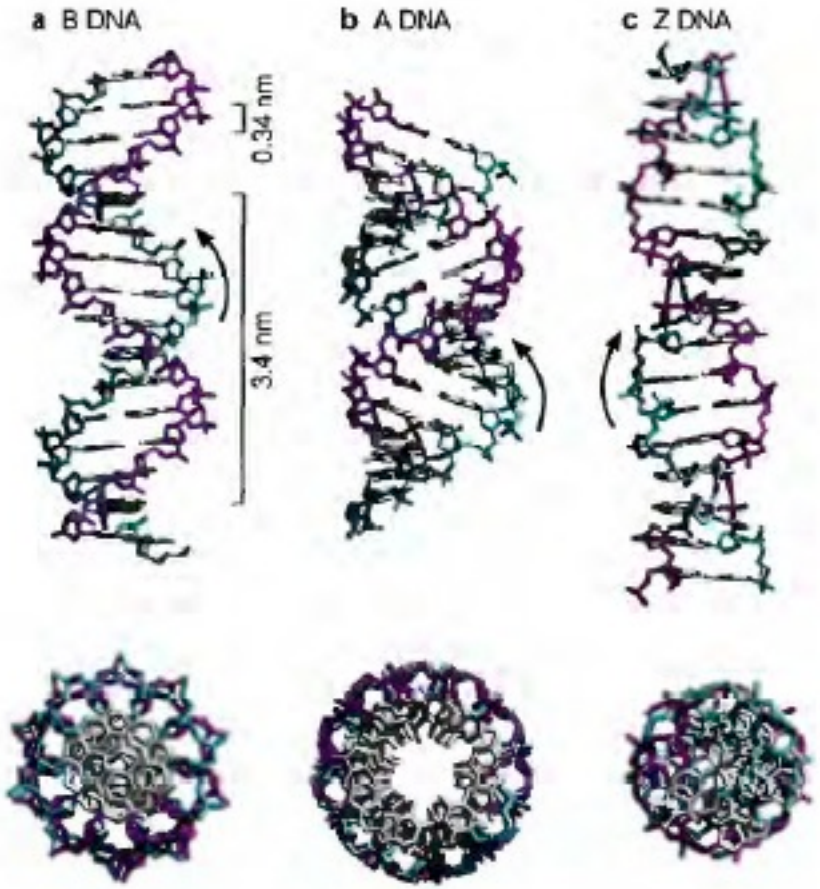
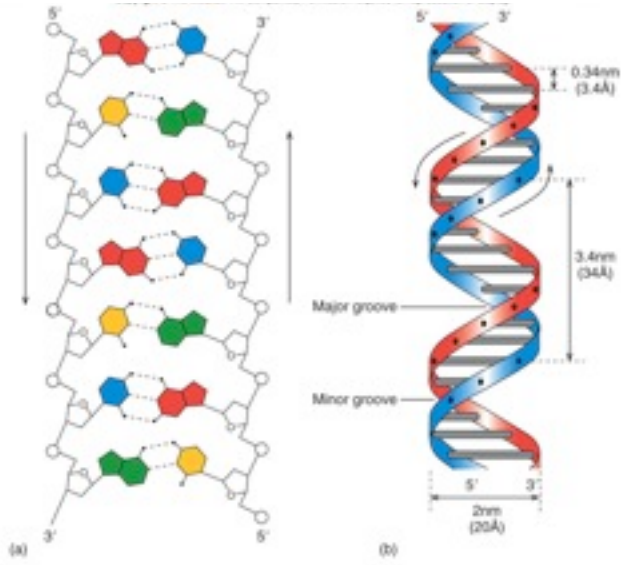
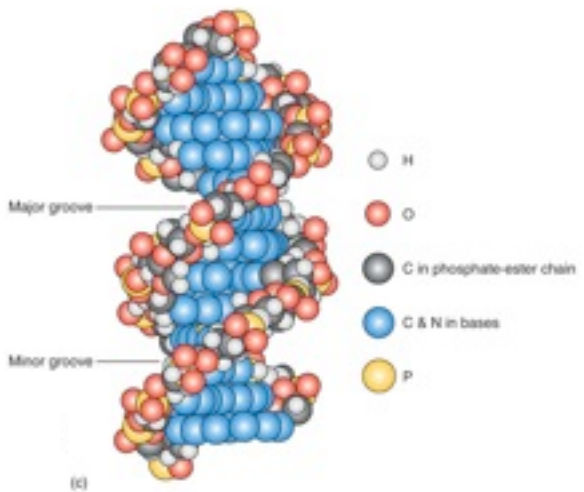


FIGURE 5-1 The helical structure of DNA. (a) Schematic model of the double helix. One turn of the helix (34 Å or 3.4 nm) spans



DNA





1. DNA de doble hebra
2. Hebras son antiparalelas
3. G-C 3 puentes de hidrógeno
4. A-T 2 puentes de hidrógeno
5. Hebras complementarias
6. Surcos mayor y menor presentan distintas superficies
7. DNA celular es casi exclusivamente tipo B-DNA
8. B-DNA tiene ~10.5 bp/vuelta helicoidal

RNA


Coat initiation
A protein stop
Replicase initiation
Coat stop

Hibridación

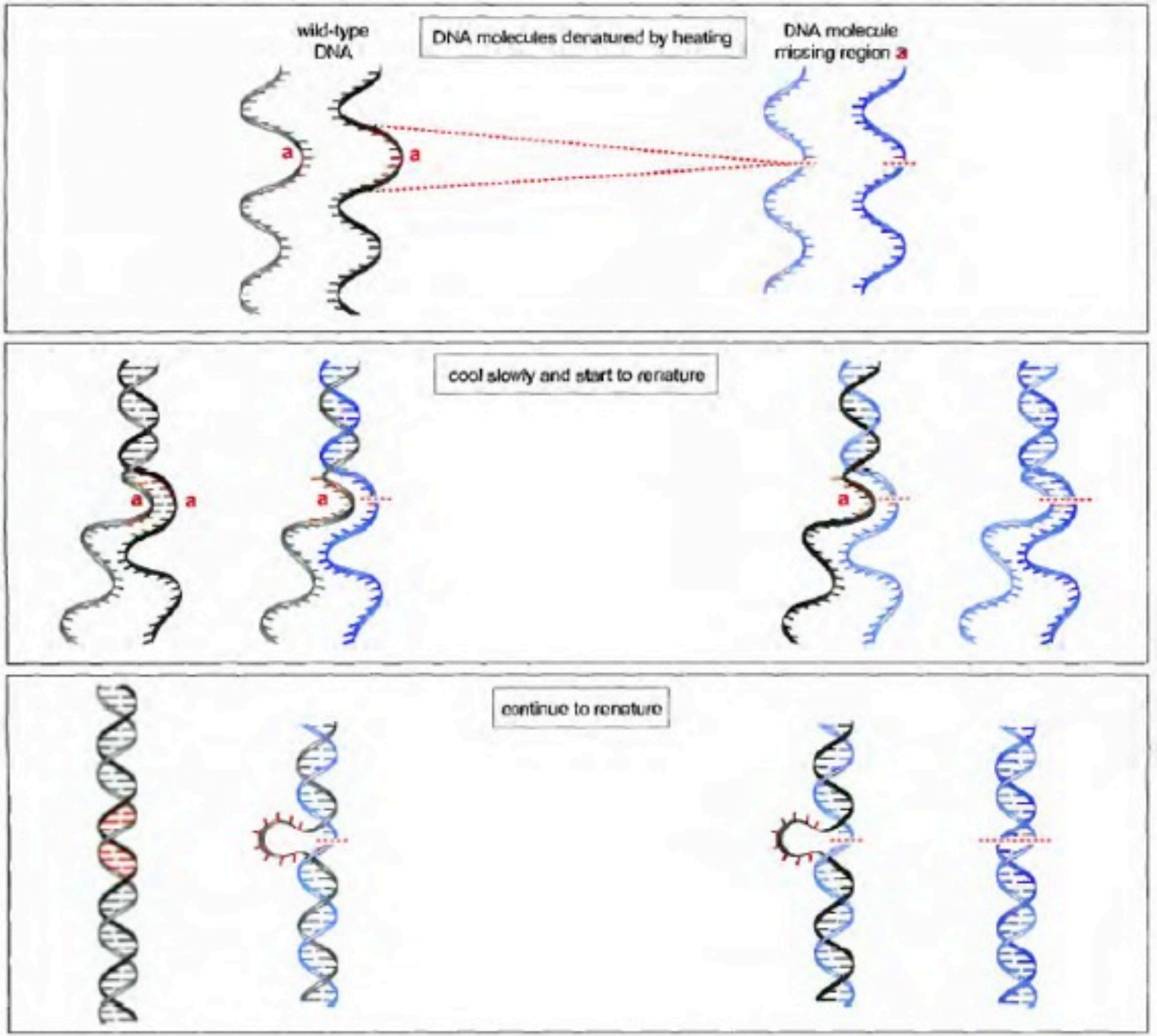
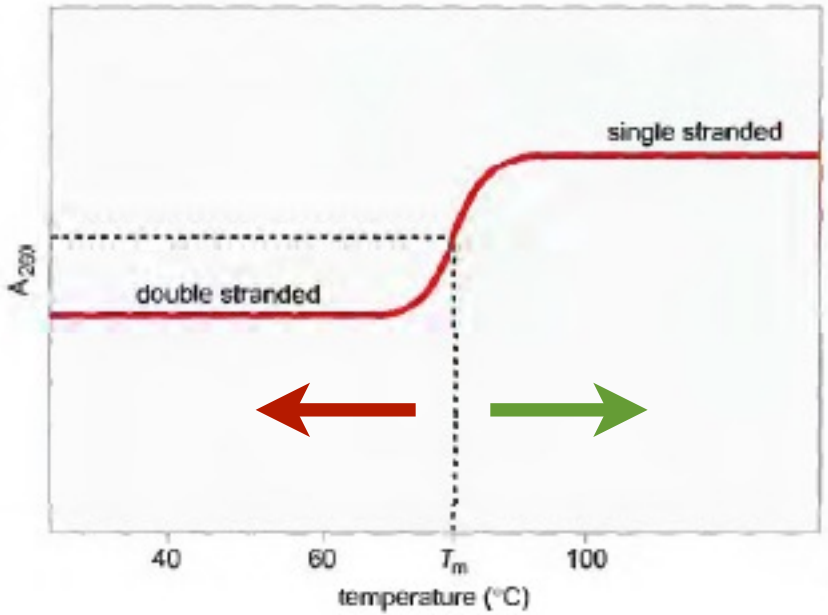


FIGURE 6-15 DNA denaturation curve.



Rojo: baja sal
Verde: alta sal

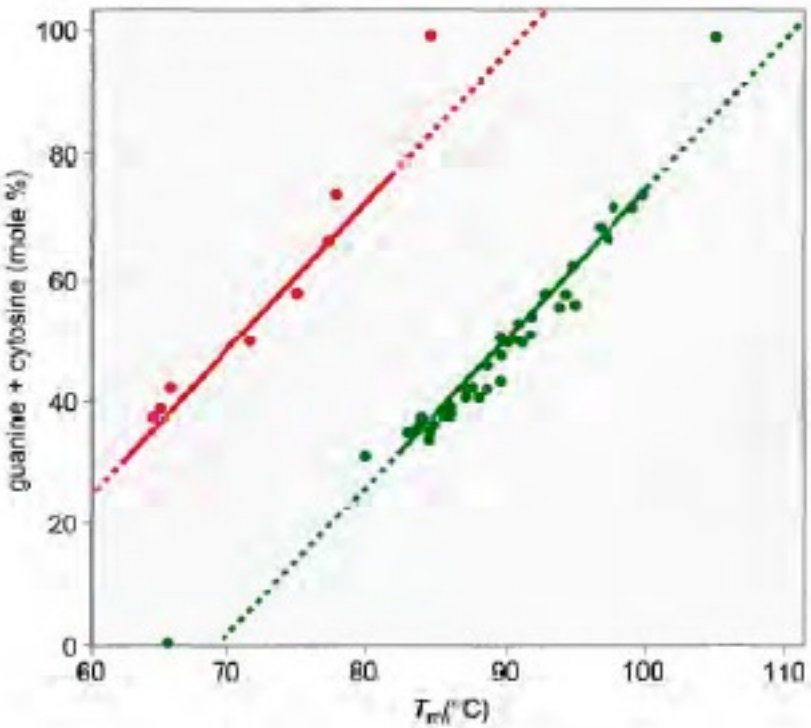


FIGURE 6-16 Dependence of DNA denaturation on C + C content and on salt concentration. The greater the G + C content, the higher the temperature must be to denature the DNA strand. DNA from different sources was dissolved in solutions of low (red line) and high (green line) concentrations of salt at pH 7.0. The points represent the temperature at which the DNA denatured, graphed against the G + C content. (Source: Data from Marmur J. and Doty P. 1962. *Journal of Molecular Biology* 5: 120. Copyright © 1962, with permission from Elsevier Science.)

Astringencia de Hibridación.

•Regulable por temperatura y concentración de sal.
•Esto permite controlar la hibridación selectiva entre la sonda y el blanco específico (secuencia homóloga). El grado de homología tolerada por las condiciones de hibridación permite detectar secuencias no idénticas.
•El resultado depende directamente de la temperatura por debajo del Tm calculado que se use.
•Para una solución acuosa (sin sal) la fórmula para calcular el Tm es:

$Tm = 69.3^{o}C + 0.41(\%\ G + C)^{o}C$

Notar que el contenido de GC tiene un efecto directo sobre el Tm.
Ejemplos:

$Tm = 69.3^{o}C + 0.41(40)^{o}C = 85.7^{o}C$
$Tm = 69.3^{o}C + 0.41(45)^{o}C = 87.5^{o}C$
$Tm = 69.3^{o}C + 0.41(60)^{o}C = 93.9^{o}C$

(Valores reales: E. coli: 51%, humano: 43%, algunos virus: 60%)

Efecto de la concentración de sal.

Se considera en la fórmula de Tm Efectivo:
TmEf = 81.5 + 16.6(log M [Na+]) + 0.41(%G+C) - 0.72(% formamida)

La sal más usada en hibridación es SSC (standard sodium citrate). Concentraciones distintas de esta solución se usan en distintas etapas del procedimiento de hibridación. Los siguientes son los valores de concentración de Na+ (en Molar) para distintas diluciones de SSC.
20X: 3.3000
10X: 1.6500
5X: 0.8250
2X: 0.3300
1X: 0.1650
0.1X: 0.0165

Ejemplos: con un DNA con contenido GC del 40% usando 50% formamida (valores típicos):

5XSSC, TmEf=60.513°C
1XSSC, TmEf=48.91°C

Otro dato importante: la presencia de 1% de mismatch (no-identidad) entre dos DNAs, la Tm disminuye en 1.4°C. Por ejemplo, si en una hibridación con dos DNAs se usa un temperatura de hibridación 20°C debajo del Tm, la identidad entre ambos debe ser al menos de 85.7% para que hibriden:
100% - (20°C/1.4°C) = 85.7% identidad.

Sobreenrollamiento

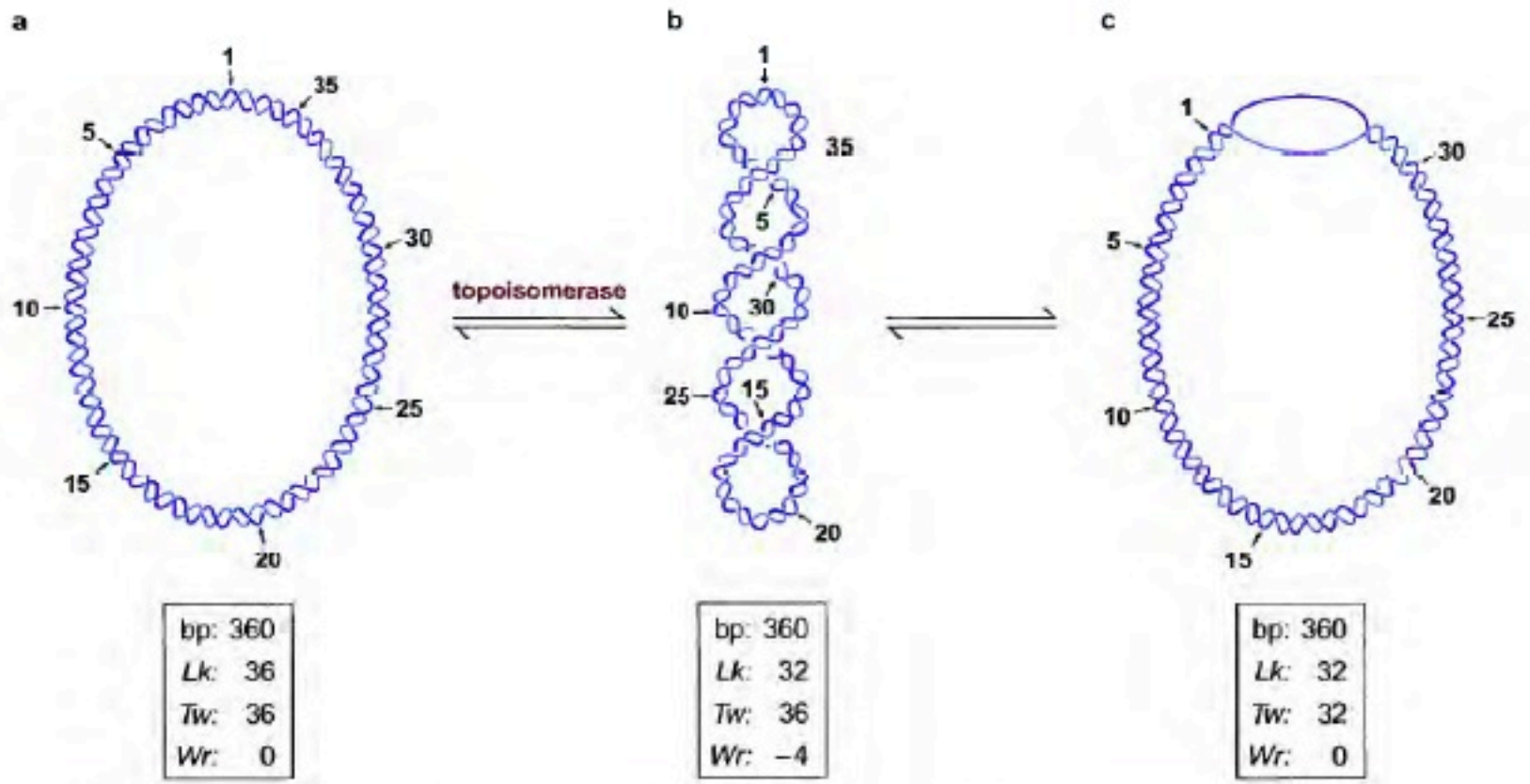


FIGURE 6-17 Topological states of covalently closed, circular (ccc) DNA. The figure shows conversion of the relaxed (a) to the negatively supercoiled (b) form of DNA. The strain in the supercoiled form may be taken up by supertwisting (b) or by local disruption of base pairing (c). [Adapted from a diagram provided by Dr. M. Gellert.] (Source: Modified from Kornberg A. and Baker T.A. 1992. *DNA replication*, 11–21, p. 32. © 1992 by W. H. Freeman and Company. Used with permission.)

DNA Genómico in vivo: 1 s.e. negativo/200pb

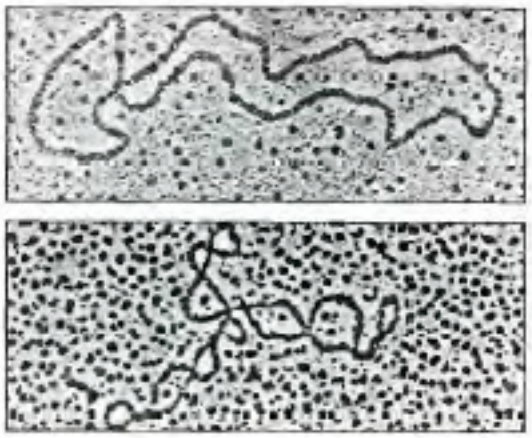
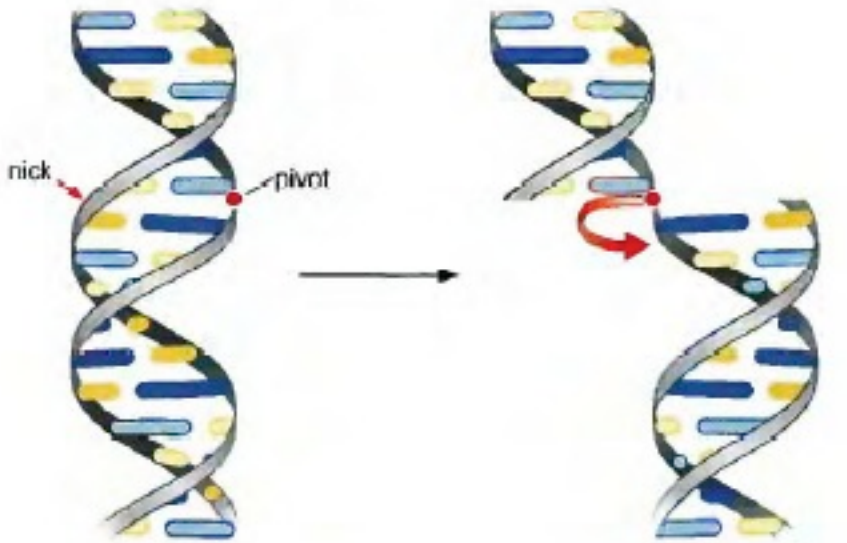
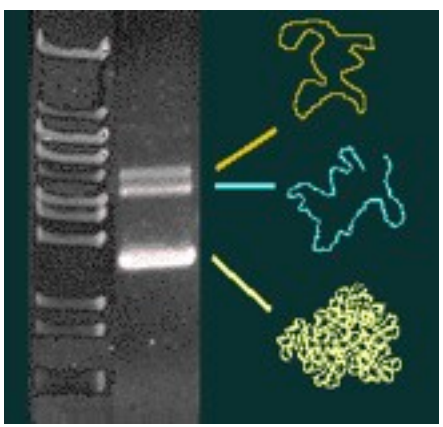


FIGURE 6-20 Electron micrograph of supercoiled DNA. The upper electron micrograph is a relaxed (nonsupercoiled) DNA molecule of bacteriophage PM2. The lower electron micrograph shows the phage in its supertwisted form. (Source: Electron micrographs courtesy of Wang J.C. 1982. *Scientific American* 247: 97.)





Cromatina eucariótica

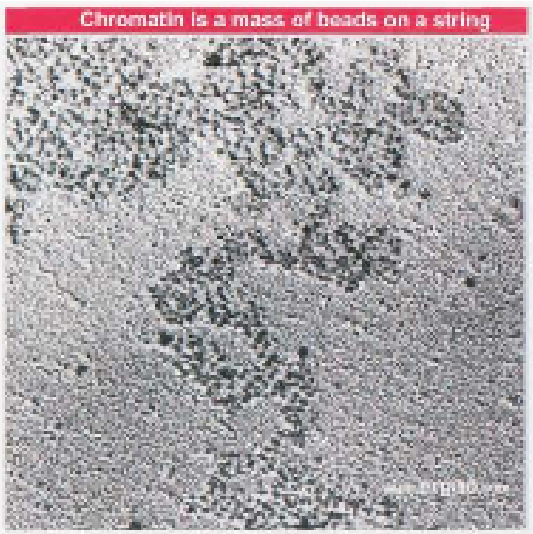


Figure 20.1 Chromatin spilling out of lysed nuclei consists of a compactly organized series of particles. Photograph kindly provided by Pierre Chambon.

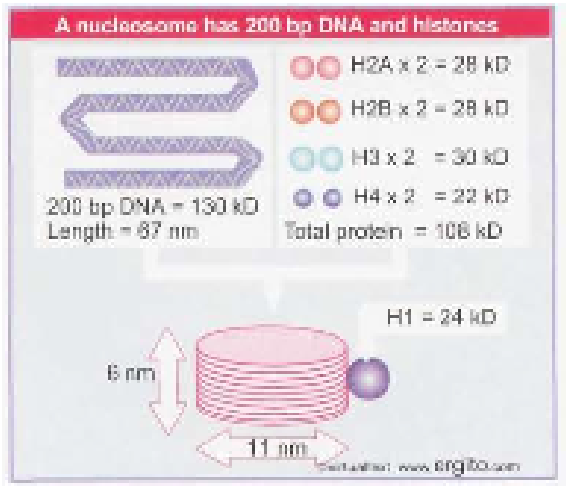
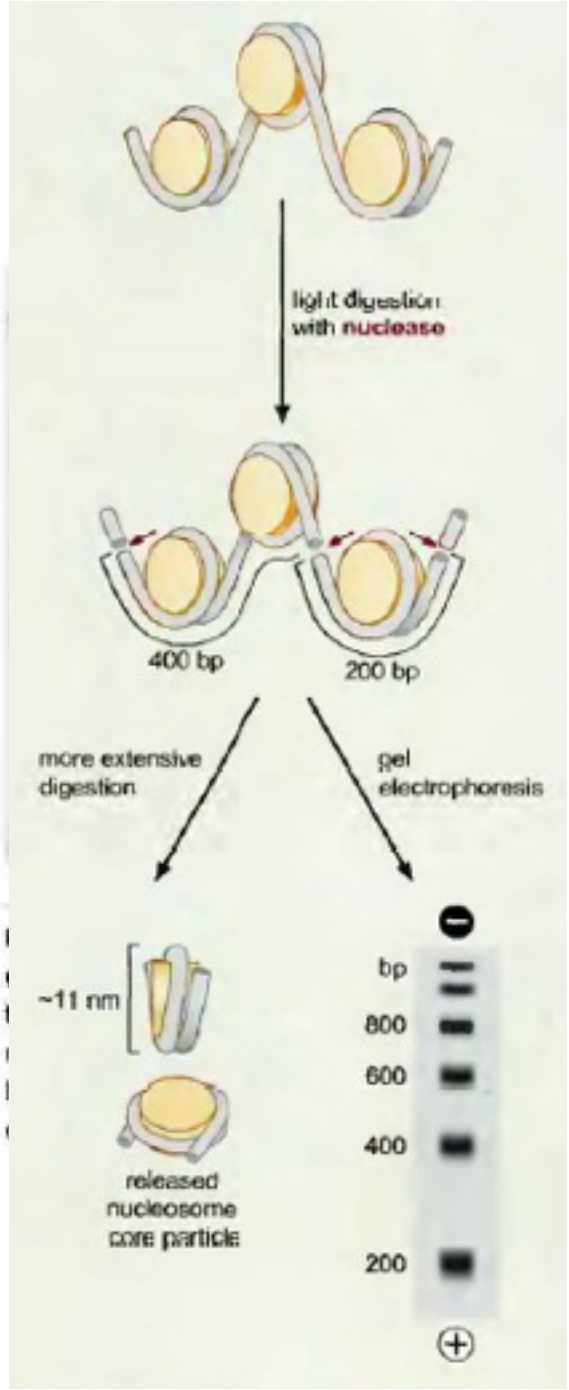


Figure 20.3 The nucleosome consists of approximately equal masses of DNA and histones (including H1). The predicted mass of the nucleosome is 262 kD.

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%

← ¿Carga?


light digestion
with nuclease
400 bp
200 bp
more extensive
digestion
gel
electrophoresis
~11 nm
released
nucleosome
core particle
bp
800
600
400
200

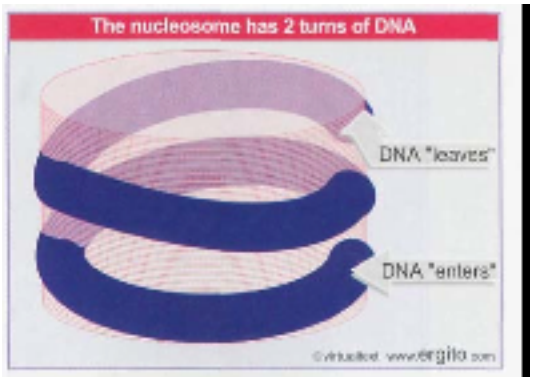


Figure 20.4 The nucleosome may be a cylinder with DNA organized into two turns around the surface.

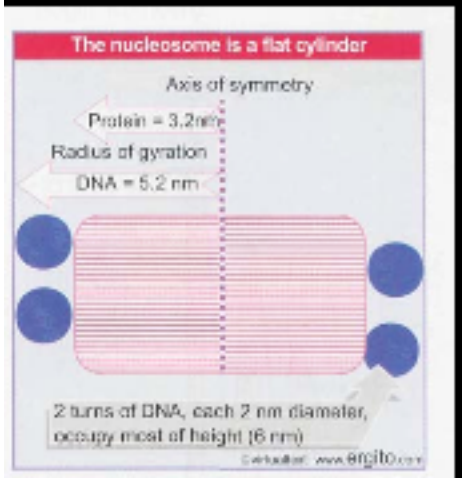


Figure 20.5 The two turns of DNA on the nucleosome lie close together.

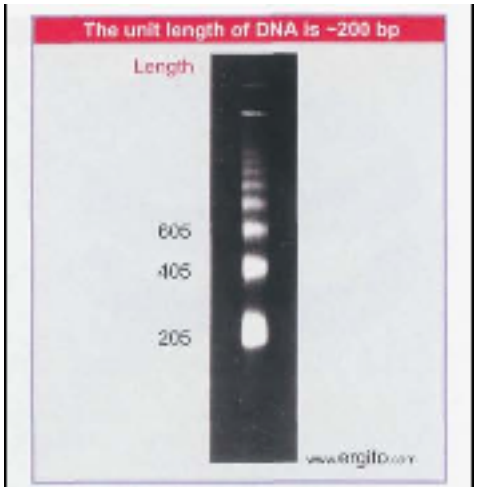


Figure 20.7 Micrococcal nuclease digests chromatin in nuclei into a multimeric series of DNA bends that can be separated by gel electrophoresis. Photograph kindly provided by Markus Noll.

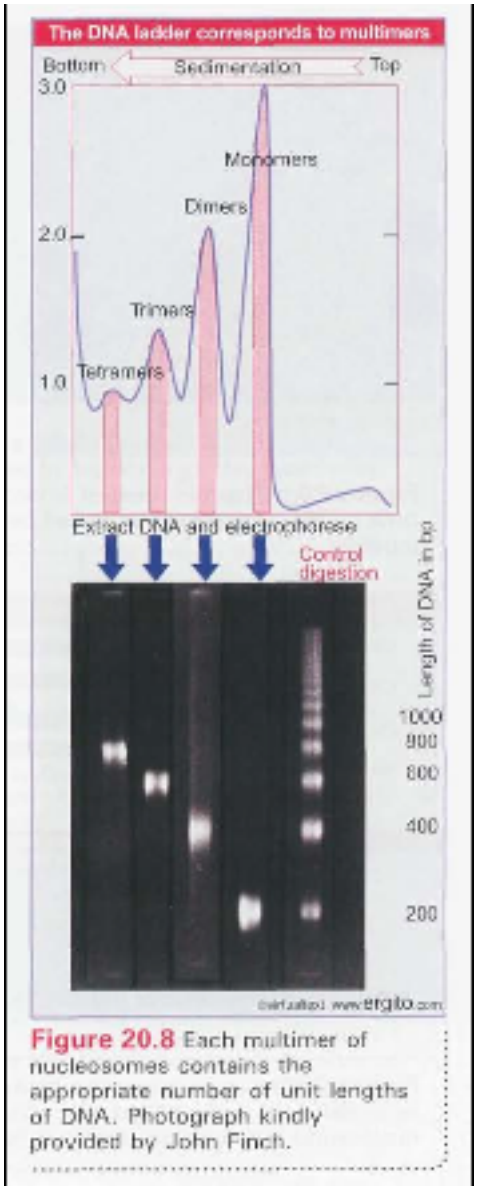


Figure 20.8 Each multimer of nucleosomes contains the appropriate number of unit lengths of DNA. Photograph kindly provided by John Finch.

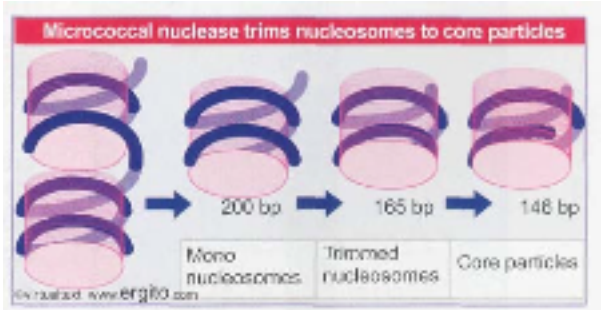


Figure 20.10 Micrococcal nuclease initially cleaves between nucleosomes. Mononucleosomes typically have ~200 bp DNA. End-trimming reduces the length of DNA first to ~165 bp, and then generates core particles with 146 bp.

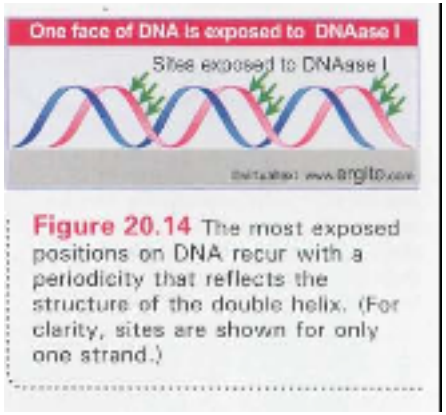


Figure 20.14 The most exposed positions on DNA recur with a periodicity that reflects the structure of the double helix. (For clarity, sites are shown for only one strand.)

Figure 20.9 Micrococcal nuclease reduces the length of nucleosome monomers in discrete steps. Photograph kindly provided by Roger Kornberg.

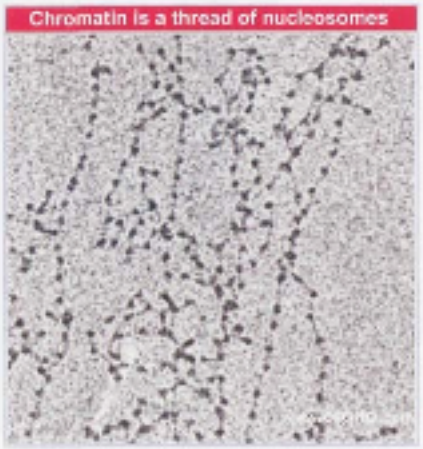


Figure 20.17 The 10 nm fiber in partially unwound state can be seen to consist of a string of nucleosomes. Photograph kindly provided by Barbara Hamkalo.

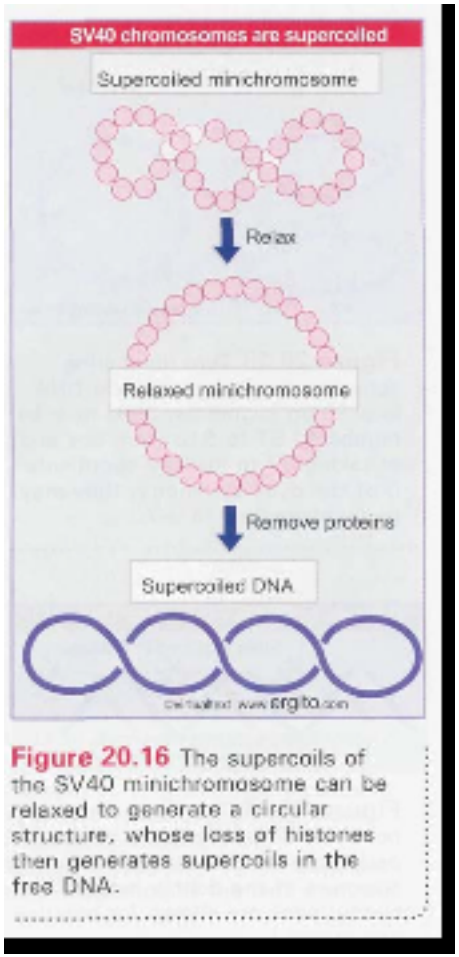


Figure 20.16 The supercoils of the SV40 minichromosome can be relaxed to generate a circular structure, whose loss of histones then generates supercoils in the free DNA.

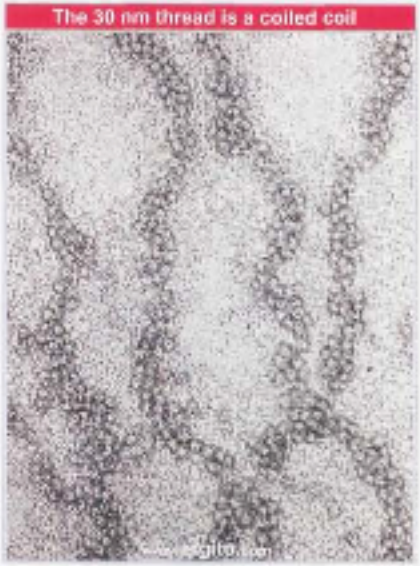


Figure 20.19 The 30 nm fiber has a coiled structure. Photograph kindly provided by Barbara Hamkalo.

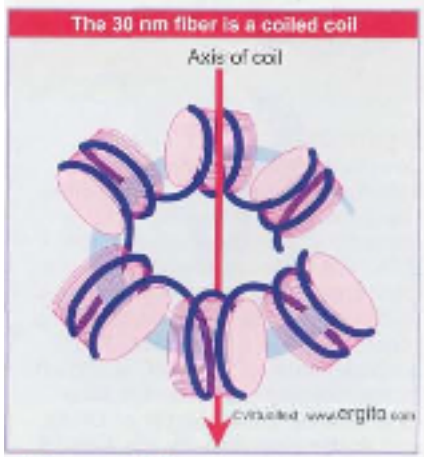


Figure 20.20 The 30 nm fiber may have a helical coil of 6 nucleosomes per turn, organized radially.

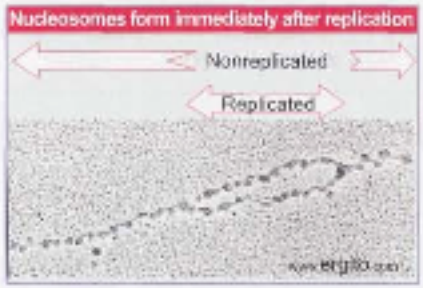


Figure 20.27 Replicated DNA is immediately incorporated into nucleosomes. Photograph kindly provided by S. MacKnight.

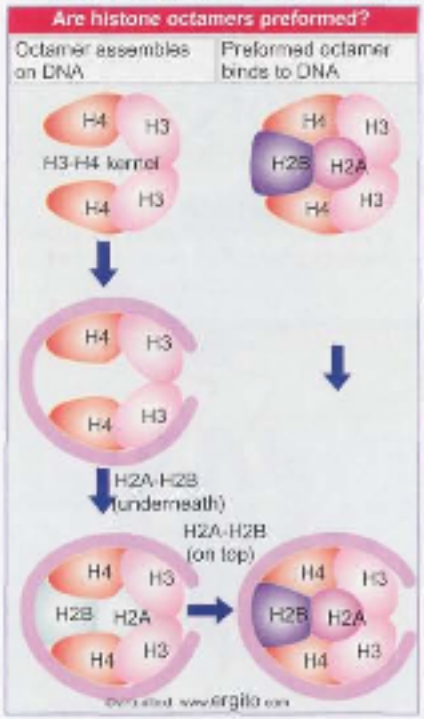


Figure 20.28 *In vitro*, DNA can either interact directly with an intact (crosslinked) histone octamer or can assemble with the $H3_2$-$H4_2$ tetramer, after which two H2A-H2B dimers are added.

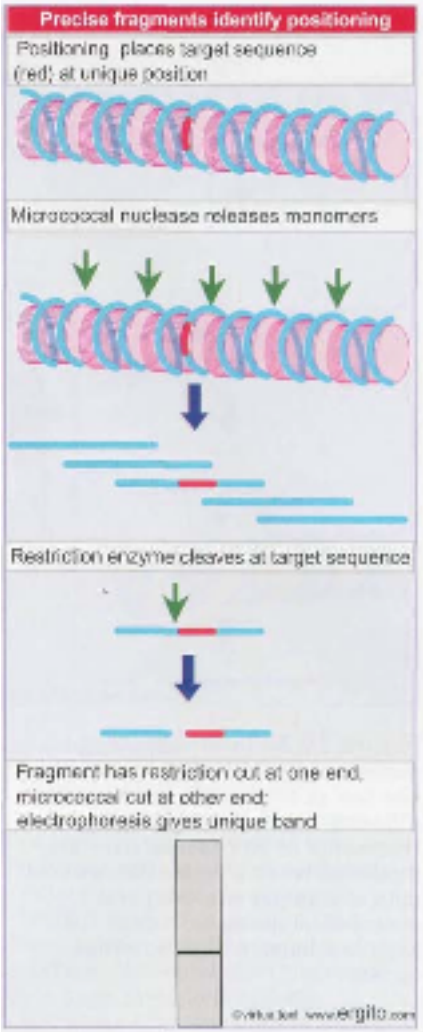
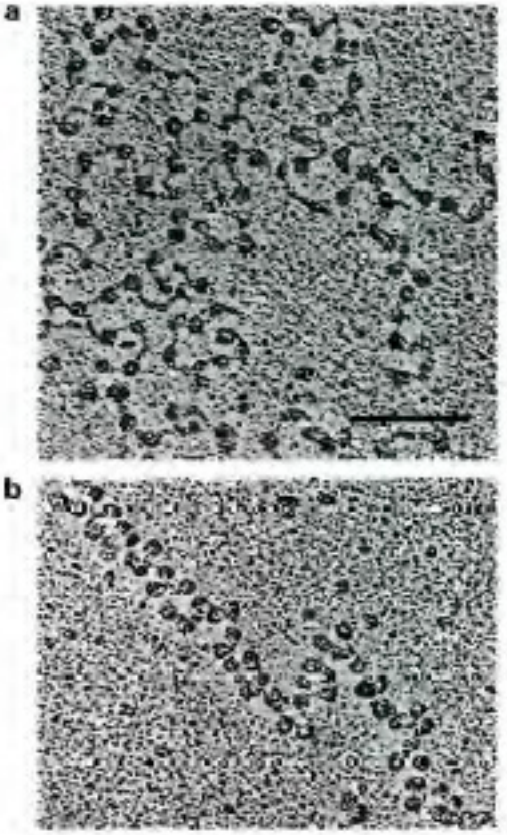


Figure 20.32 Nucleosome positioning places restriction sites at unique positions relative to the linker sites cleaved by micrococcal nuclease.

FIGURE 7-28 The addition of H1 leads to more compact nucleosomal DNA. The two images show an electron micrograph of nucleosomal DNA in the presence (a) and absence (b) of histone H1. Note the more compact and defined structure of the DNA in the presence of histone H1. (Source: Thoma et al. Involvement of histone H1 in the organization of the nucleosome. *J. Cell Biology*. 83: 410, figs 4 & 6.)



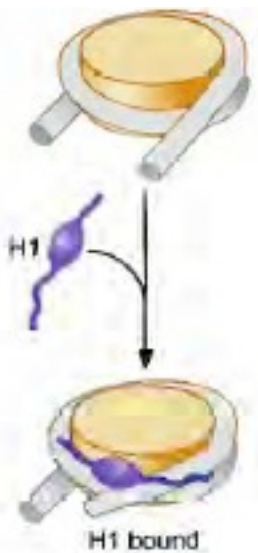
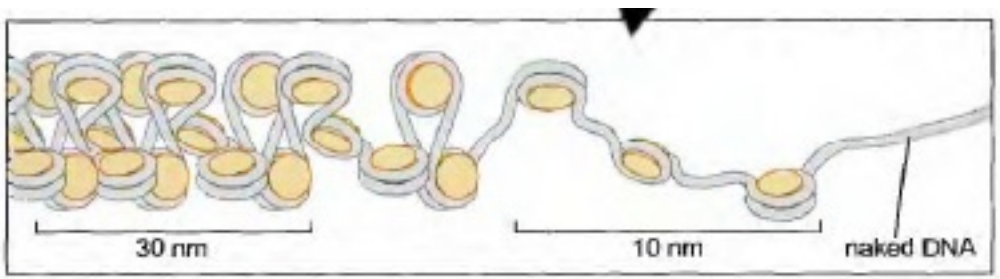
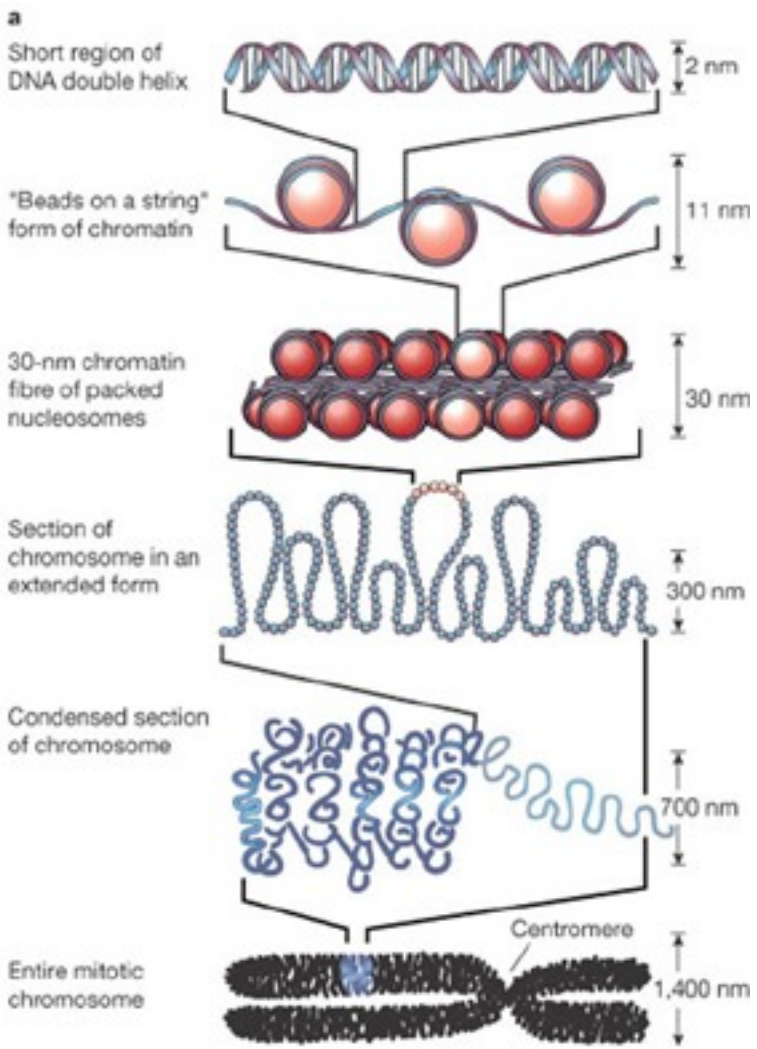


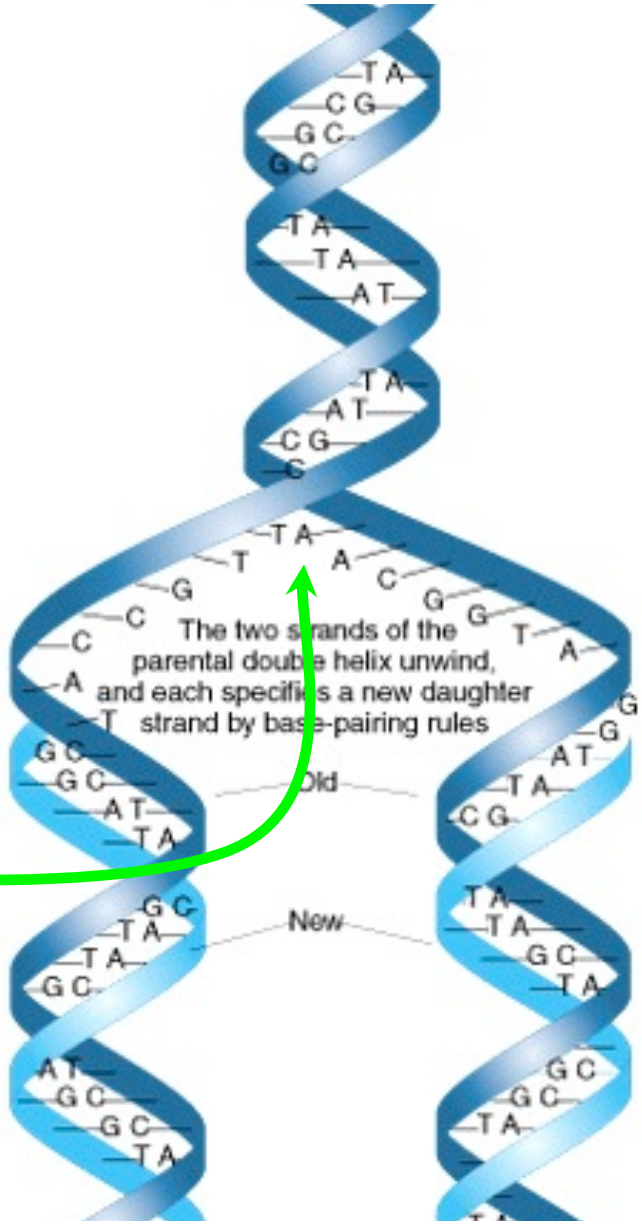
FIGURE 7-27 Histone H1 binds two DNA helices. Upon interacting with a nucleosome, histone H1 binds to the linker DNA at one end of the nucleosome and the central DNA helix of the nucleosome bound DNA (the middle of the 147 bp bound by the core histone octamer).


30 nm
10 nm
naked DNA


a
Short region of DNA double helix
2 nm
"Beads on a string" form of chromatin
11 nm
30-nm chromatin fibre of packed nucleosomes
30 nm
Section of chromosome in an extended form
300 nm
Condensed section of chromosome
700 nm
Centromere
Entire mitotic chromosome
1,400 nm

DNA REPLICATION

- model of replication proposed by Watson & Crick (1953)
- parental strand = template
- semiconservative model (new double helix has 1 template + 1 new daughter strand)
- replication fork



REPLICACION.
Propiedades de DNA polimerasa: i) 5'-3'; ii) requiere partidor

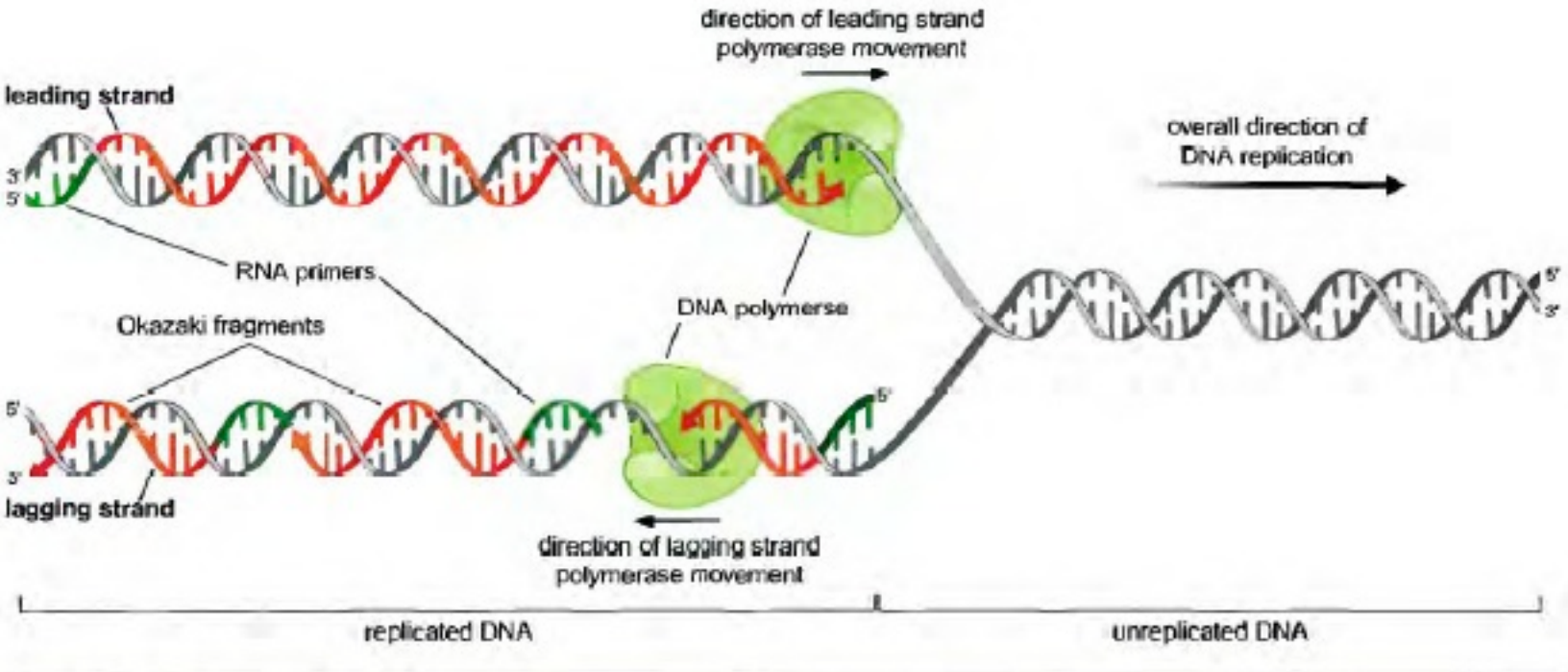


FIGURE 8-11 The replication fork. Newly synthesized DNA is indicated in red and RNA primers are indicated in green. The Okazaki fragments shown are artificially short for illustrative purposes. In the cell, Okazaki fragments can vary between 100 to greater than 1,000 bases.

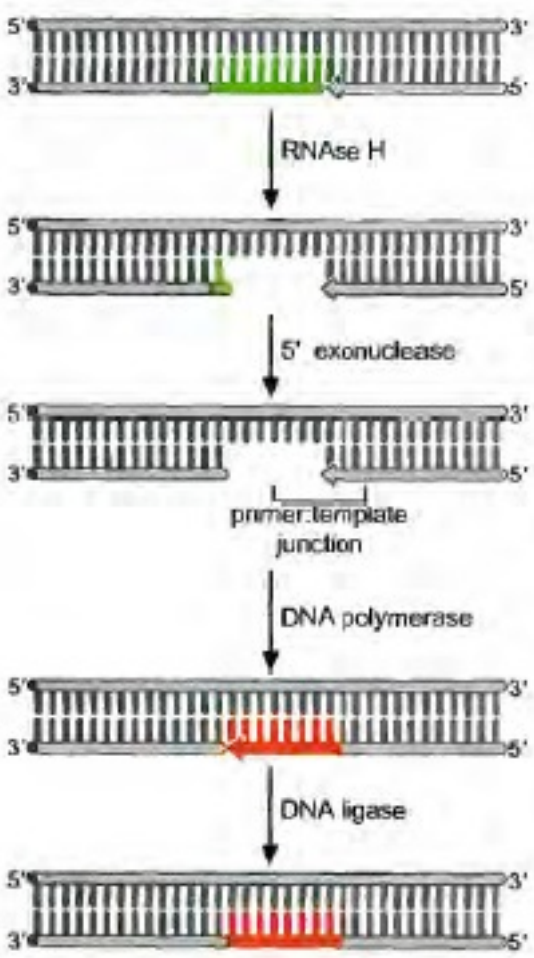


FIGURE 8-12 Removal of RNA primers from newly synthesized DNA. The sequential function of RNAse H, 5' exonuclease, DNA polymerase, and DNA ligase during the removal of RNA primers is illustrated. DNA present prior to RNA primer removal is shown in gray, the RNA primer is shown in green, and the newly synthesized DNA that replaces the RNA primer is shown in red.

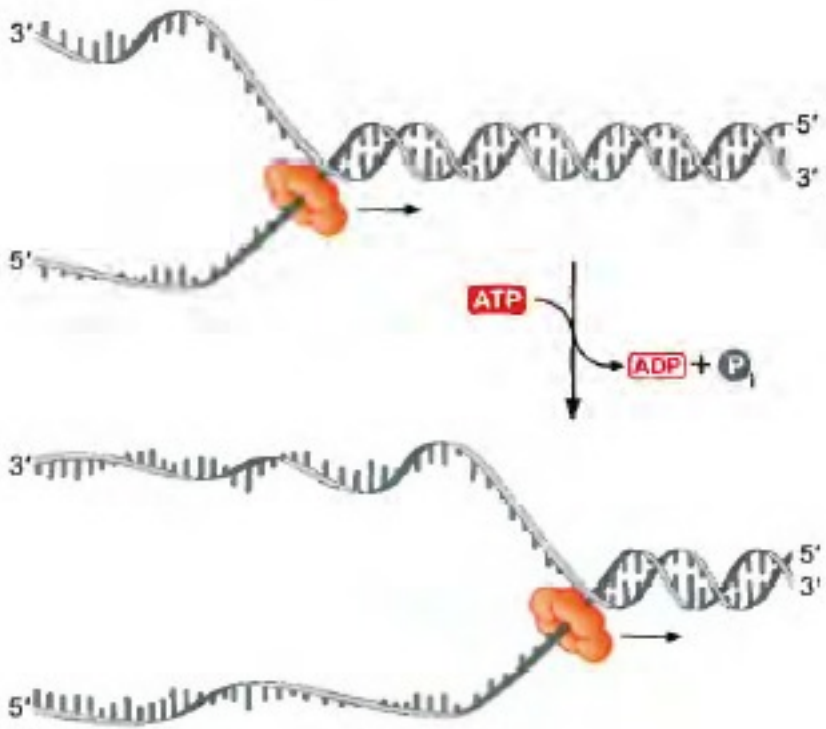
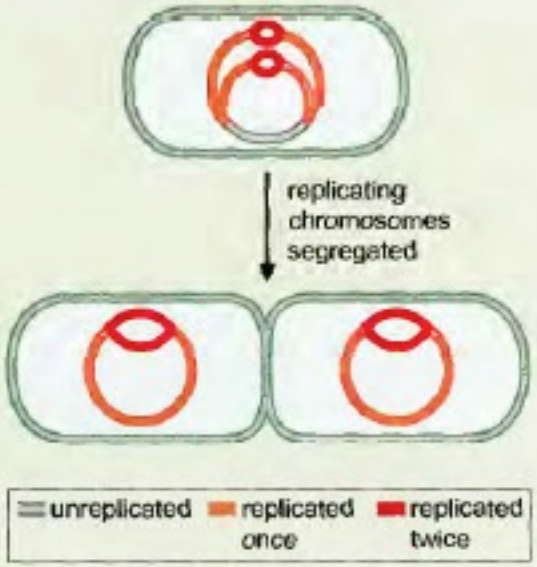


FIGURE 8-13 DNA helicases separate the two strands of the double helix. When ATP is added to a DNA helicase bound to ssDNA, the helicase moves with a defined polarity on the ssDNA. In the instance illustrated, the DNA helicase has a 5′→3′ polarity. This polarity means that the DNA helicase would be bound to the lagging strand template at the replication fork.



BOX 8-4 FIGURE 2 Origins of replication re-in
cation prior to cell division in rapidly growing cells.
the genome to be fully replicated prior to each round of
bacterial cells frequently have to initiate DNA replication
single origin prior to the completion of cell division. This
the chromosomes that are segregated into the daughter
being actively replicated. This is in contrast to eukaryotic
do not start chromosome segregation prior to the compl
DNA replication.

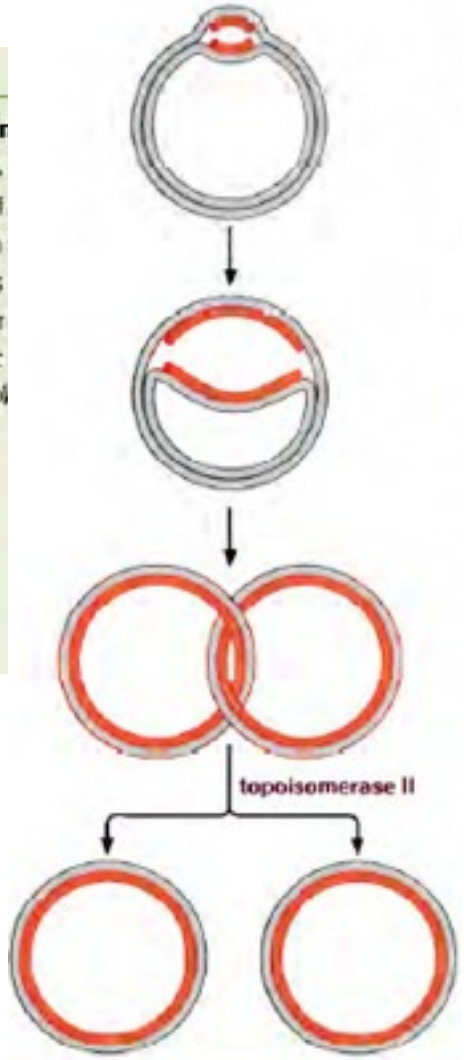


FIGURE 8-33 Topoisomerase II catalyzes the decatenation of replication products. After a circular DNA molecule is replicated, the resulting complete daughter DNA molecules remain linked to one another. Type II DNA topoisomerases can efficiently separate (or decatenate) these DNA circles.