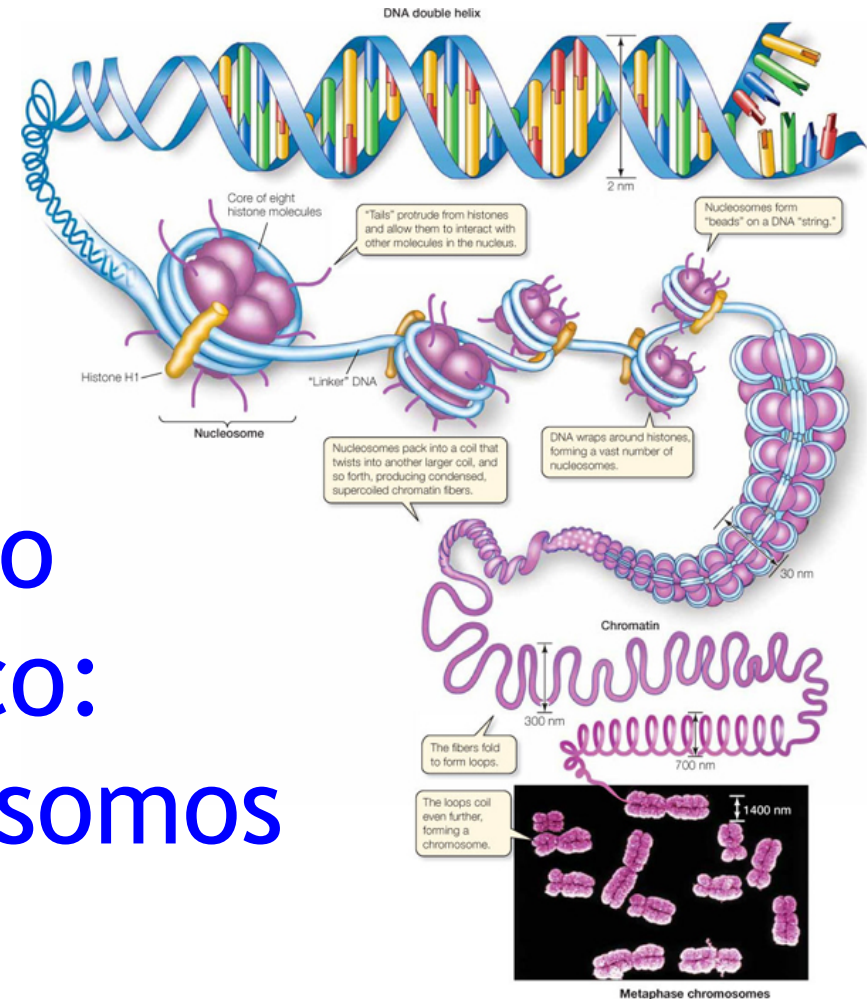


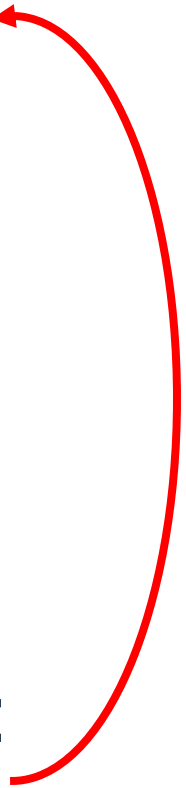
Aula 3

Compactação do Material Genético: Cromatina e cromossomos



Dimensões do DNA

- Diâmetro de 2 nm (0,002 μM)
- 1 par de bases = 0,34 nm
- Comprimento do DNA de *E. coli*:
1600 μM
- Comprimento da célula de *E. coli*:
2 μM

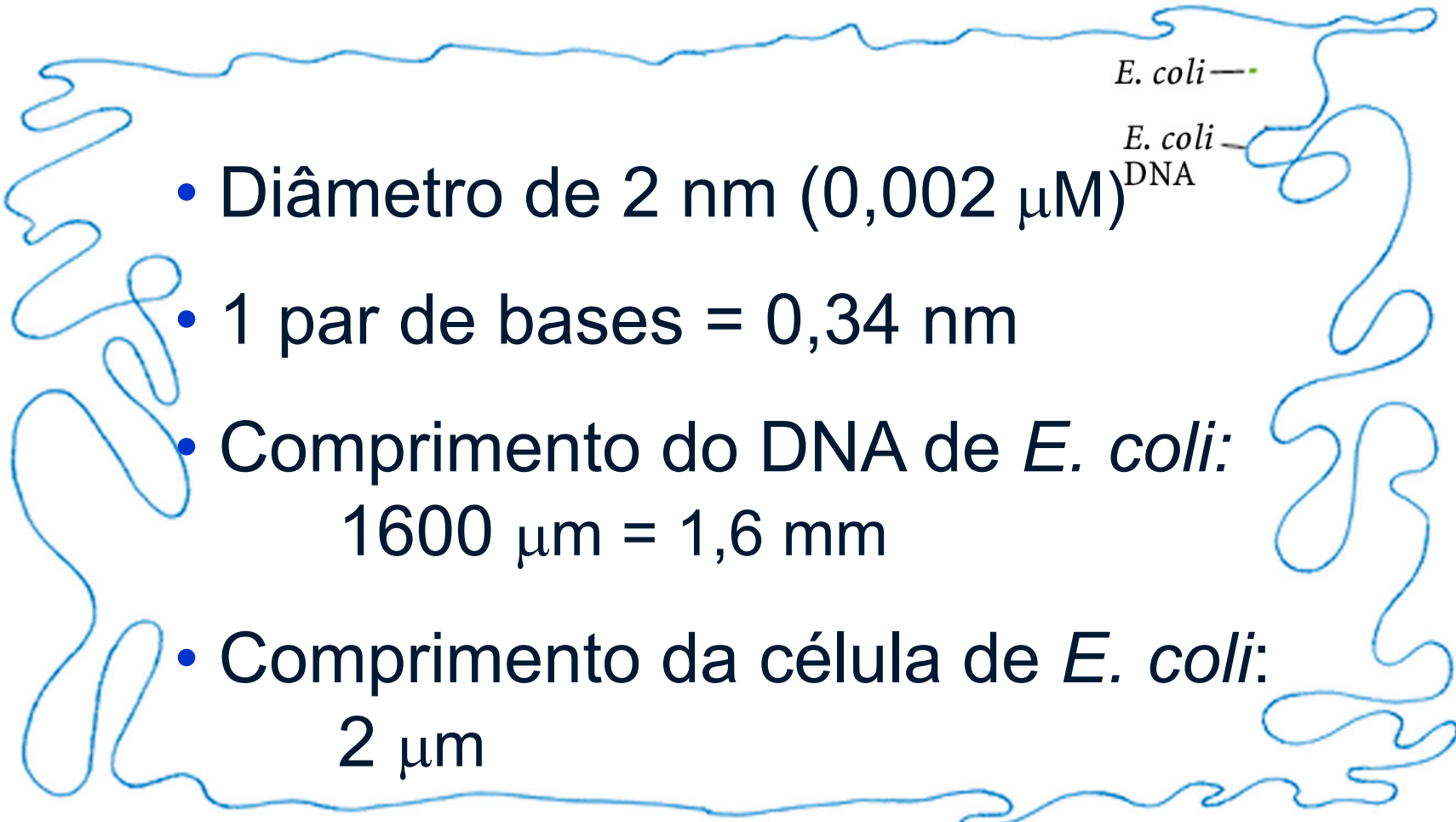
E. coli — 

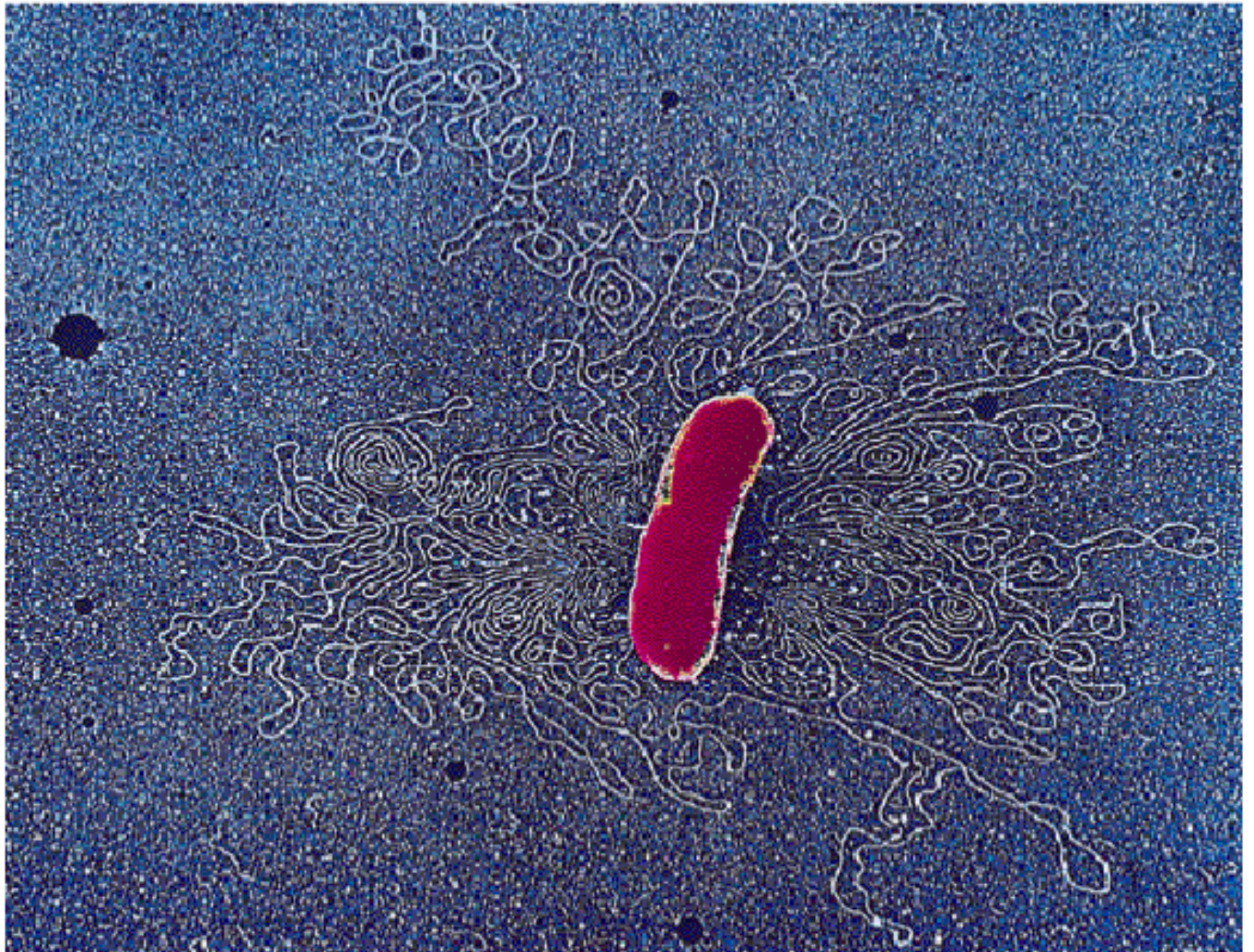
Dimensões do DNA

- Diâmetro de 2 nm (0,002 μm)
- 1 par de bases = 0,34 nm
- Comprimento do DNA de *E. coli*:
1600 μm = 1,6 mm
- Comprimento da célula de *E. coli*:
2 μm

E. coli —

E. coli
DNA —





Dimensões do material genético

E. coli

- 1 cromossomo circular
- $\sim 4,4 \times 10^6$ bp
- ~ 4.000 genes
- 1,6 mm comprimento
- 1 gene/1000 bp
- 2.500 genes/mm DNA
- plasmídeos

H. sapiens

- 46 cromossomos
- $\sim 3,2 \times 10^9$ bp
- ~ 20.000 genes
- 2 m comprimento/célula
- 1 gene/100.000 bp
- 50 genes/mm DNA
- DNA circular ($1,6 \times 10^3$ bp) nas mitocôndrias

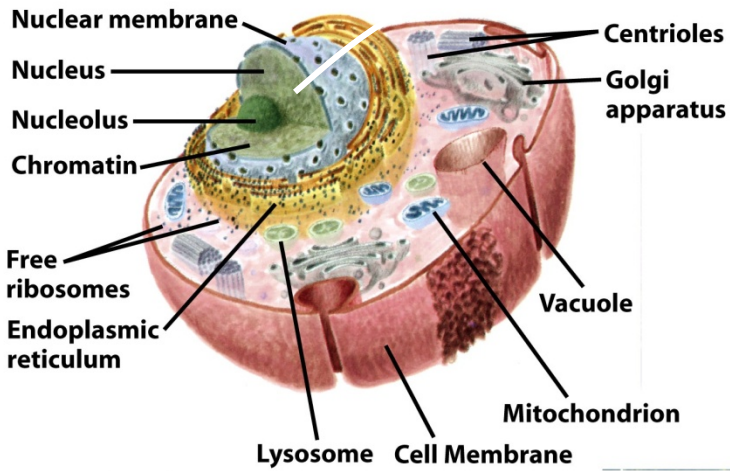
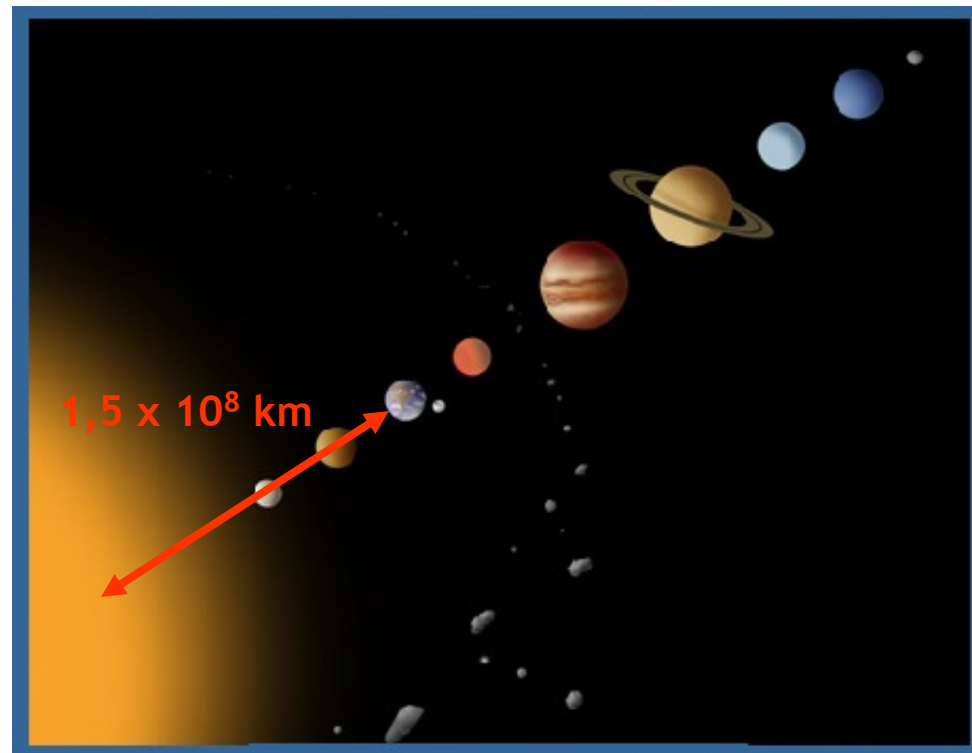


Figure 1-8 part 1 Fundamentals of Biochemistry, 2/e
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DNA de uma célula humana = 2 m

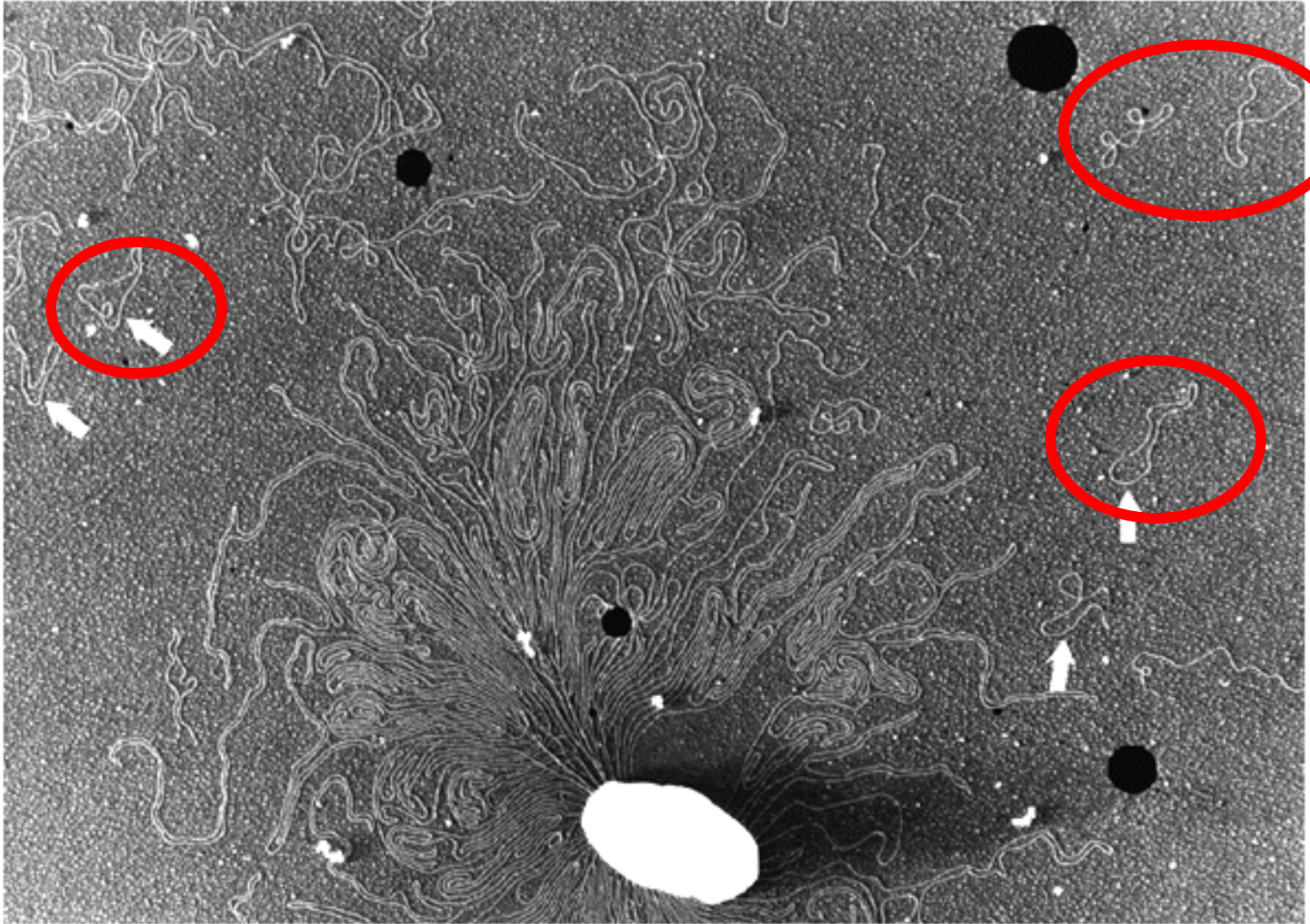
$\times 10^{14}$ células = **2×10^{11} km !**



Como o DNA é compactado para caber nas células?

- O mecanismo de compactação tem que permitir o acesso à informação e é um processo regulado.

Bactérias podem carregar plasmídeos

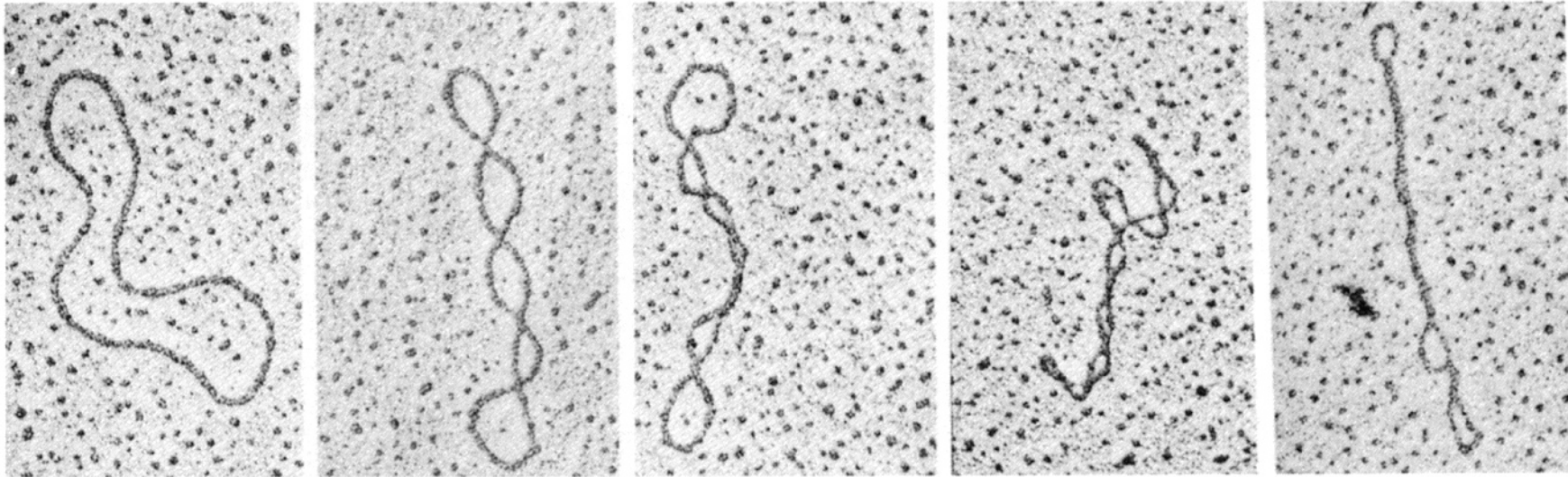


Plasmídeos: DNA circular extracromossômico

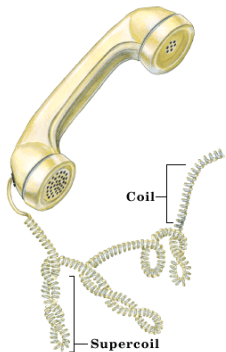
DNA é enovelado/superenrolado

Relaxado

Supercoil



0.2 μm

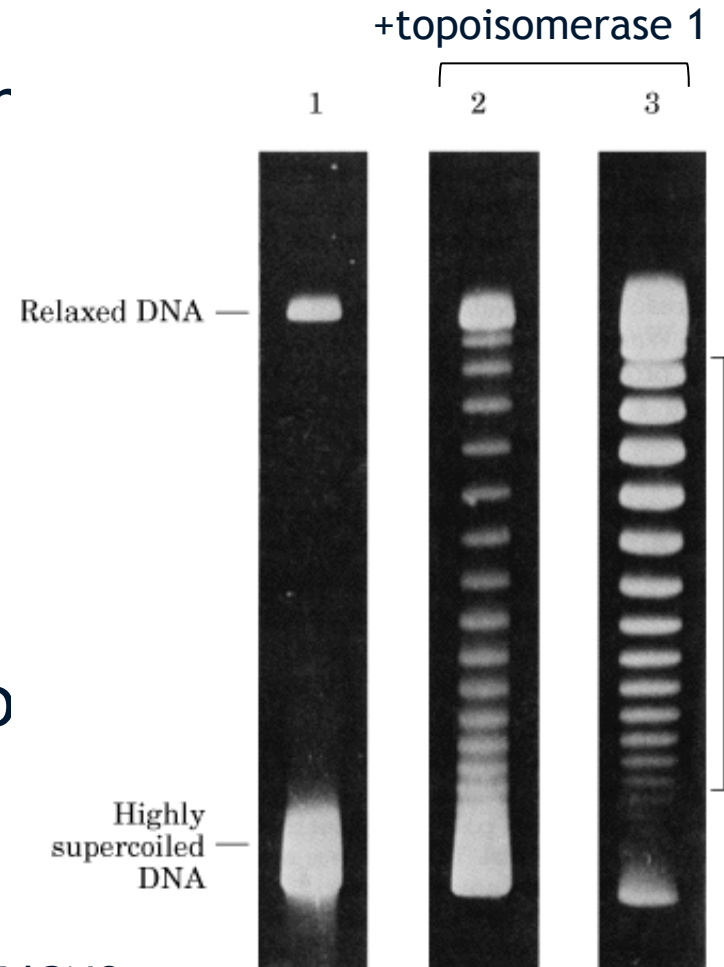


TOPOISOMERASES

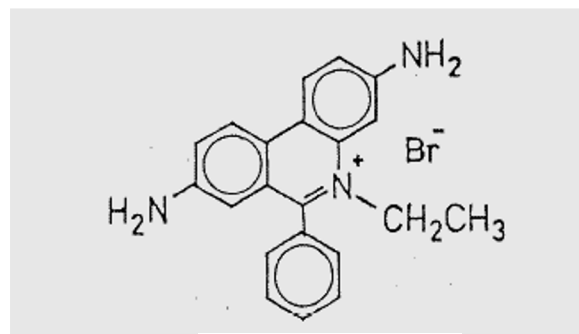
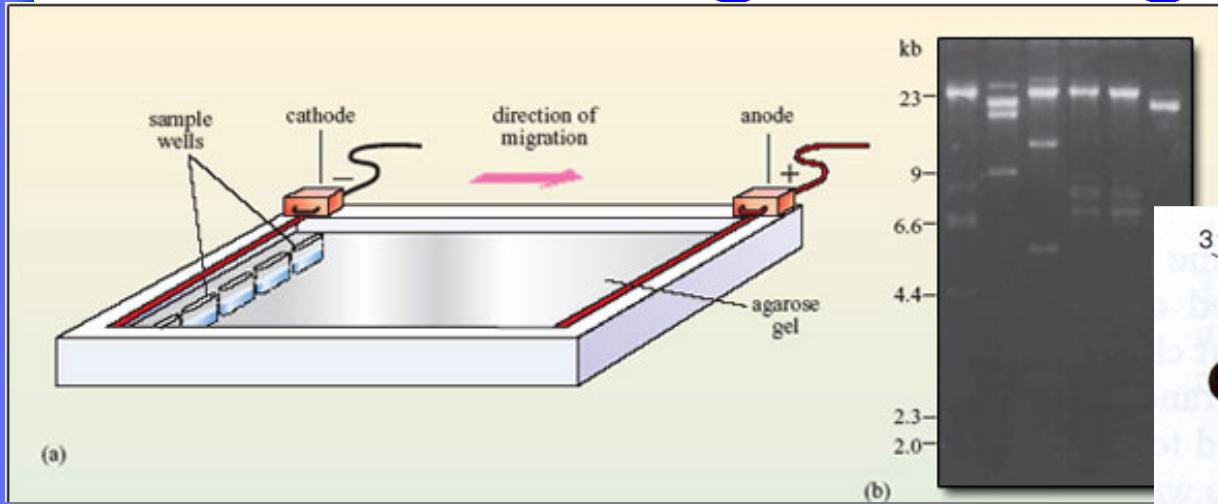
- enzimas que aumentam ou diminuem o enovelamento do DNA

- “supercoiling” de DNA é um processo regulado com precisão

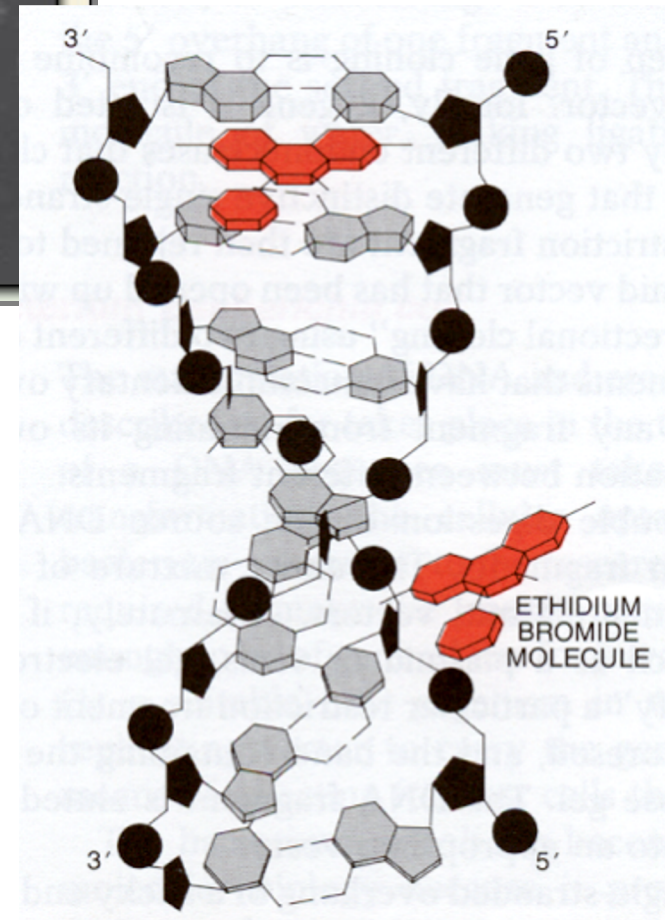
- influencia vários aspectos do metabolismo de DNA, como replicação, transcrição, recombinação e empacotamento.



Eletróforeses de DNA em gel de agarose



Brometo de etídeo



TOPOISOMERASES

<http://www.youtube.com/watch?v=3QWA-tFdGN8>

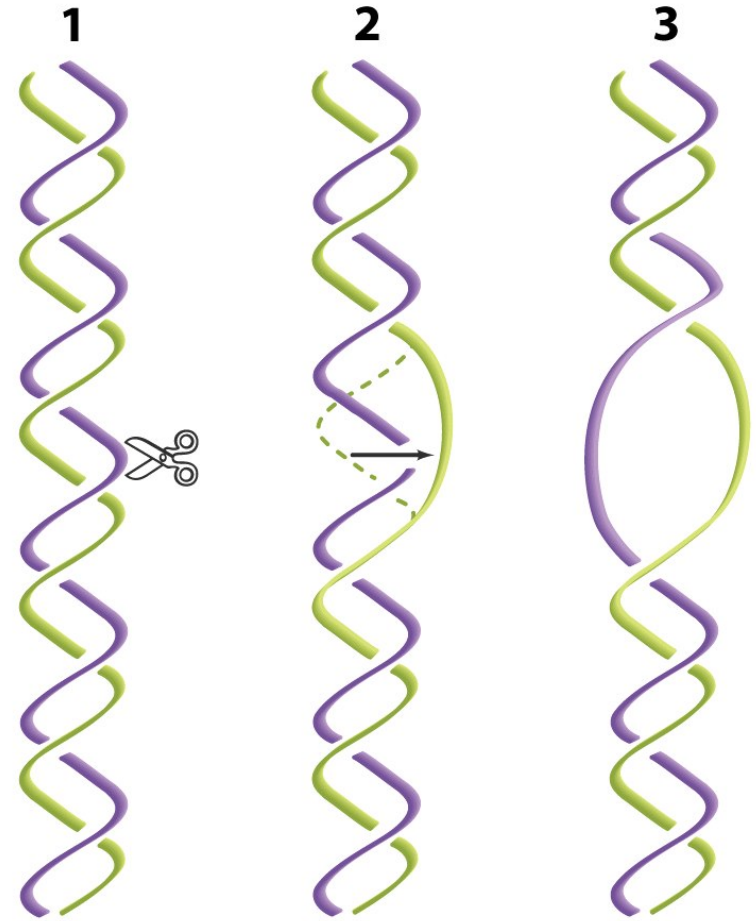


TOPOISOMERASES

2 tipos:

Tipo I:

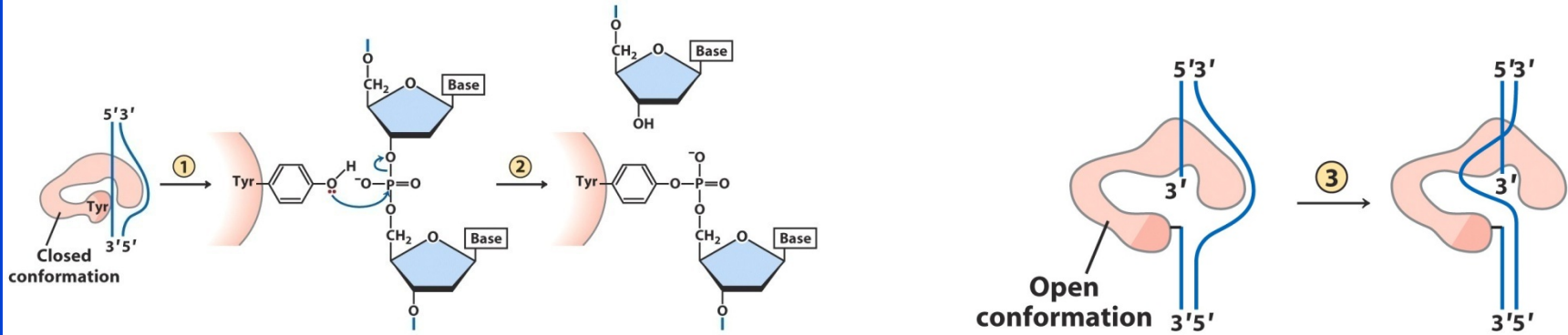
- ▶ quebra **uma** das fitas do DNA
- ▶ a fita intacta é passada através da quebra
- ▶ religação



Duplex DNA
(n turns)

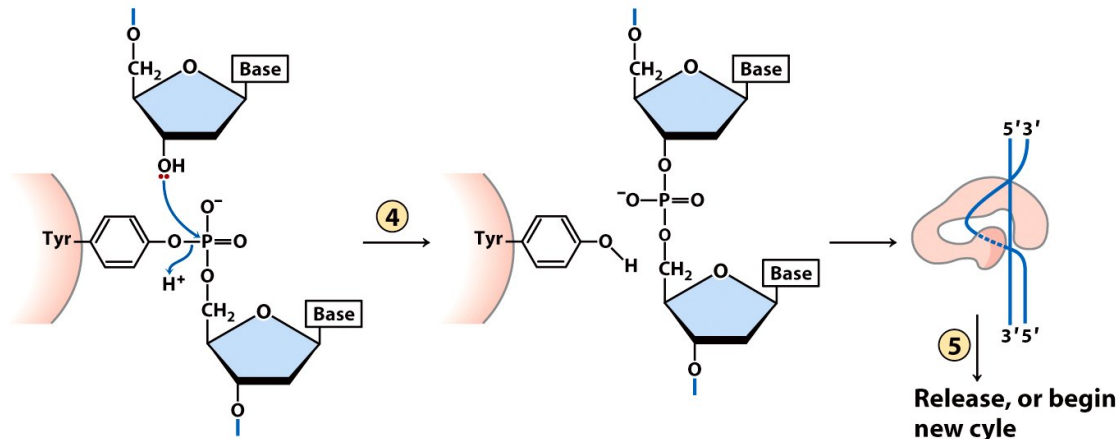
Duplex DNA
($n - 1$ turns)

Mecanismo de ação: Topoisomerases de tipo I



After DNA binds (step ①), an active-site Tyr attacks a phosphodiester bond on one DNA strand in step ②, cleaving it, creating a covalent 5'-P-Tyr protein-DNA linkage, and liberating the 3'-hydroxyl group of the adjacent nucleotide.

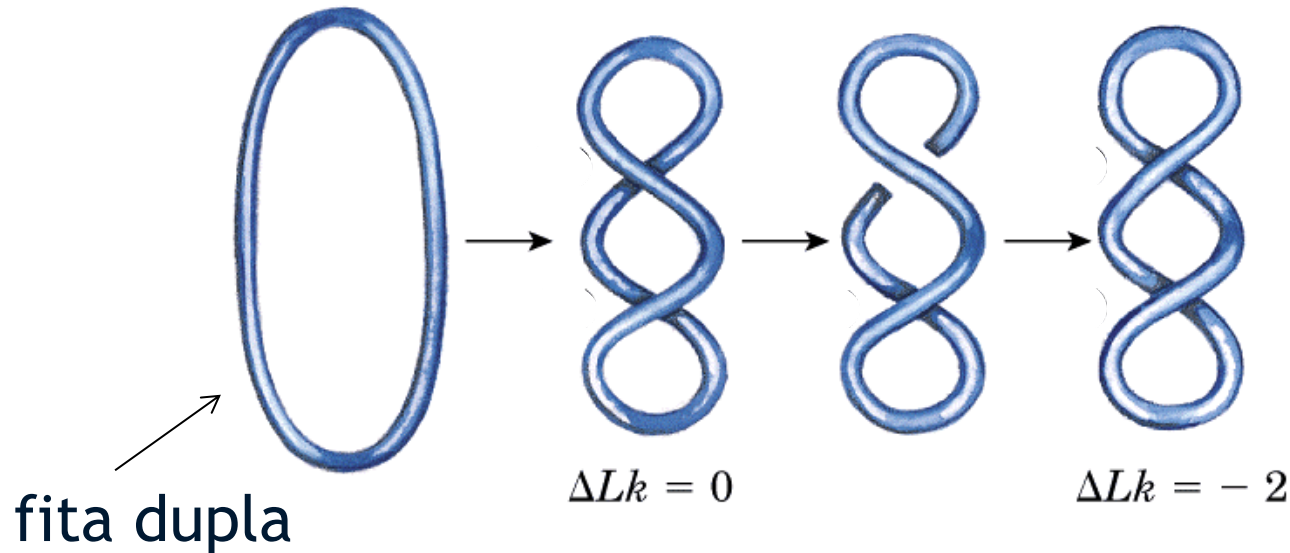
In step ③ the enzyme switches to its open conformation, and the unbroken DNA strand passes through the break in the first strand.



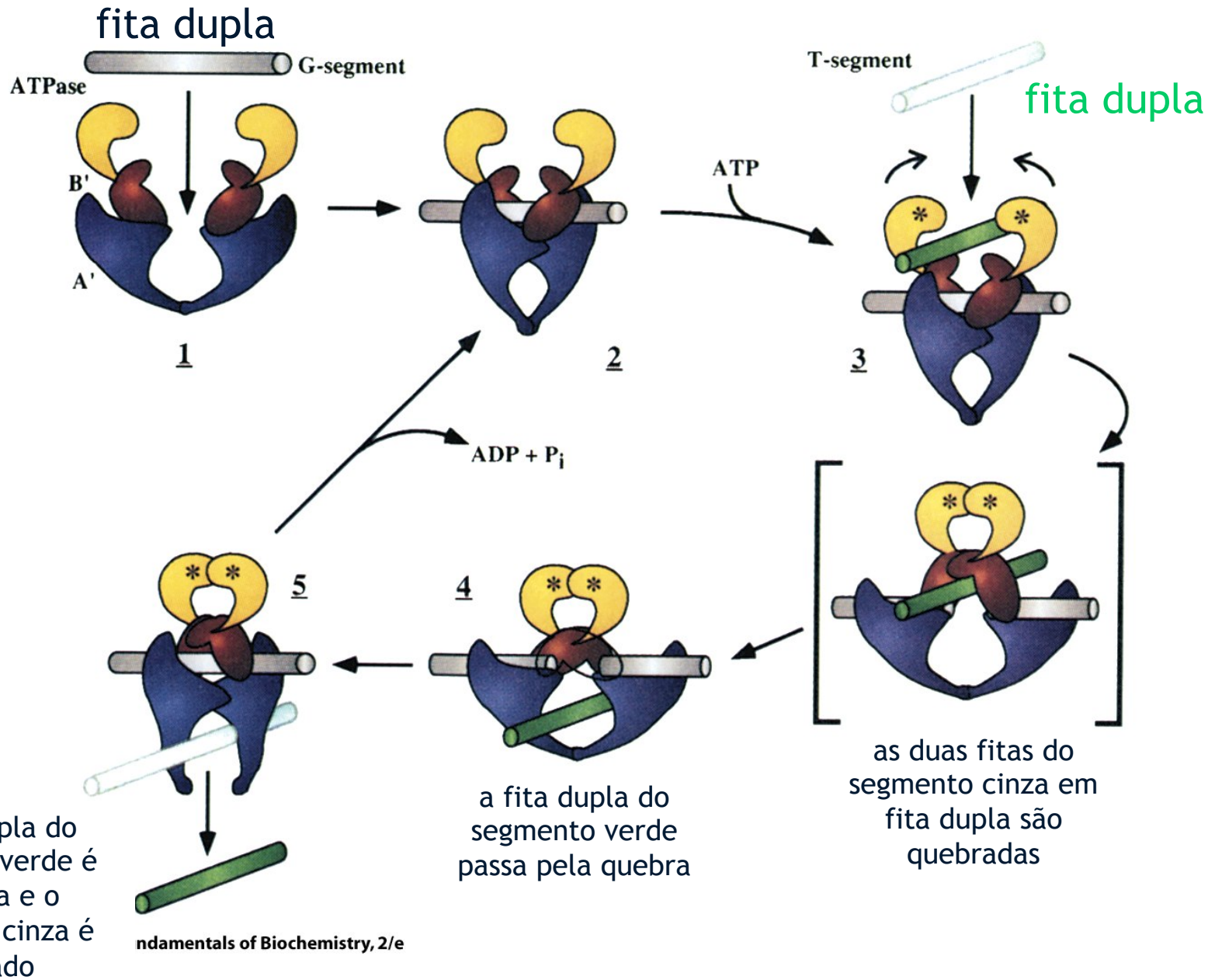
With the enzyme in the closed conformation, the liberated 3'-hydroxyl group attacks the 5'-P-Tyr protein-DNA linkage in step ④ to religate the cleaved DNA strand.

Topoisomerases do tipo II:

- DNA girase (*E. coli*)
 - quebram **ambas as fitas** de DNA
 - requerem ATP
 - faz parte do replissomo

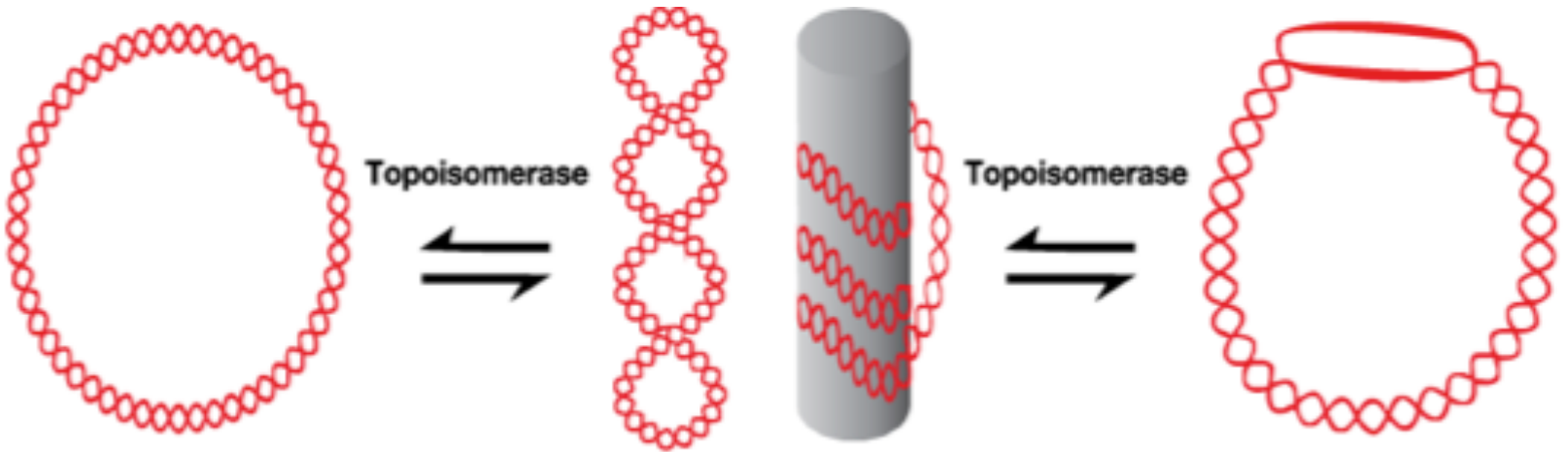


DNA girase (Topoisomerase II)



DNA em
solução

DNA enrolado
em proteínas



Plectonêmica Toroidal

Superenrolado

Estrutura
terciária

Relaxado
Forma Plana

Tenso Plana
Fitas abertas

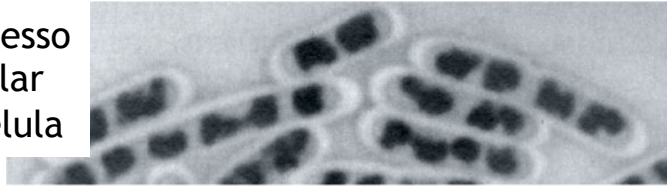
Inibidores de DNA girase

- Inibem replicação e transcrição bacteriana
- Antibióticos:
 - novobiocina: previne ligação de ATP
 - ácido oxolínico – impede a quebra e religação do DNA
 - quinolonas: ciprofloxacina, levofloxacina – mantém DNA ligado à girase

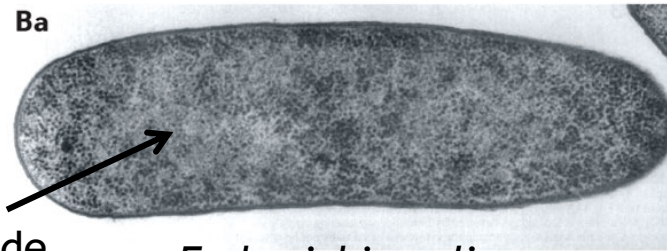


DNA de bactérias está organizado em nucleóides

Bacillus em processo de divisão celular
>1 nucleóide/célula



Ba



nucleóide

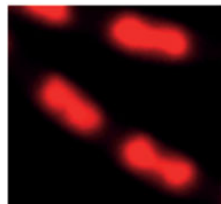
Escherichia coli

Bb

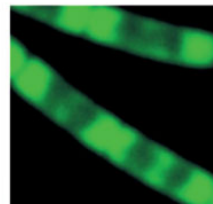


ribossomos

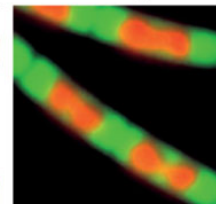
Ca Nucleoid



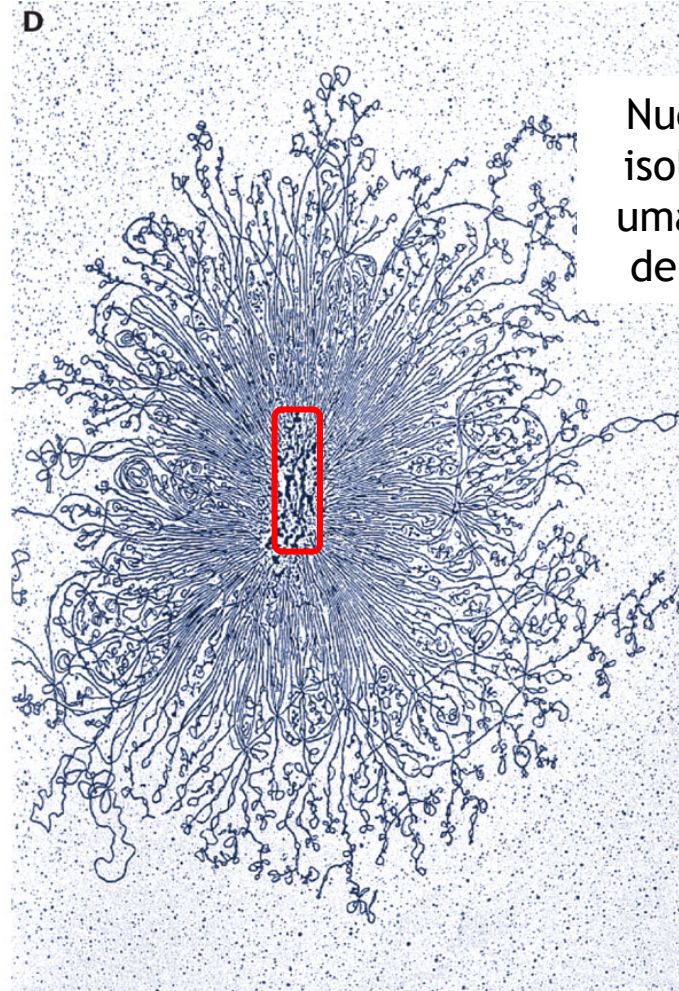
Cb Ribosomes



Cc Overlay



Bacillus subtilis

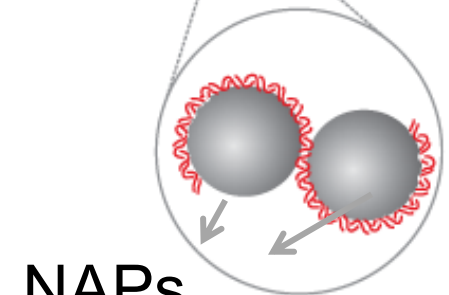
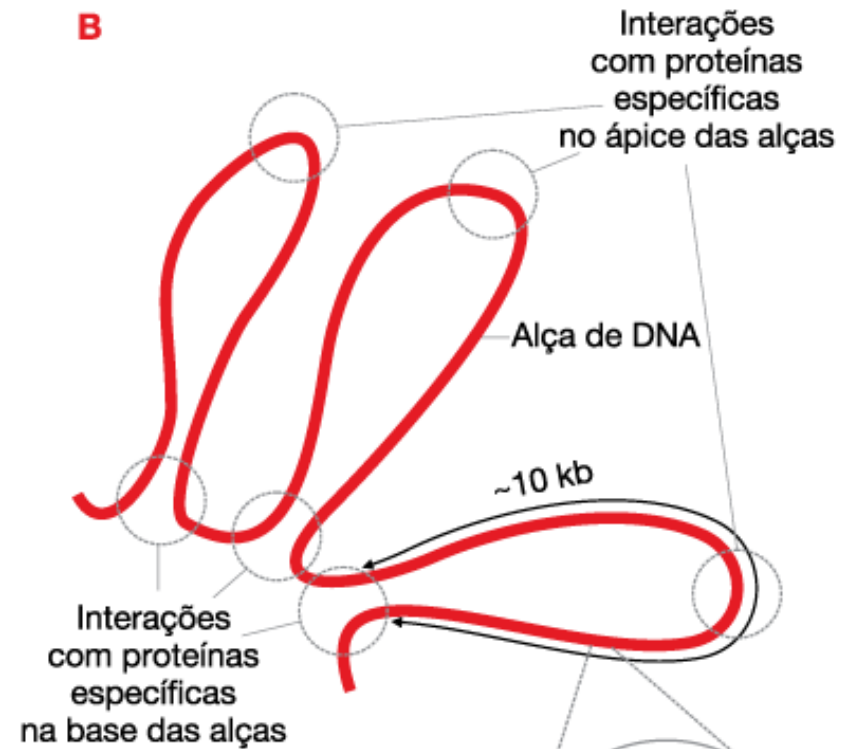
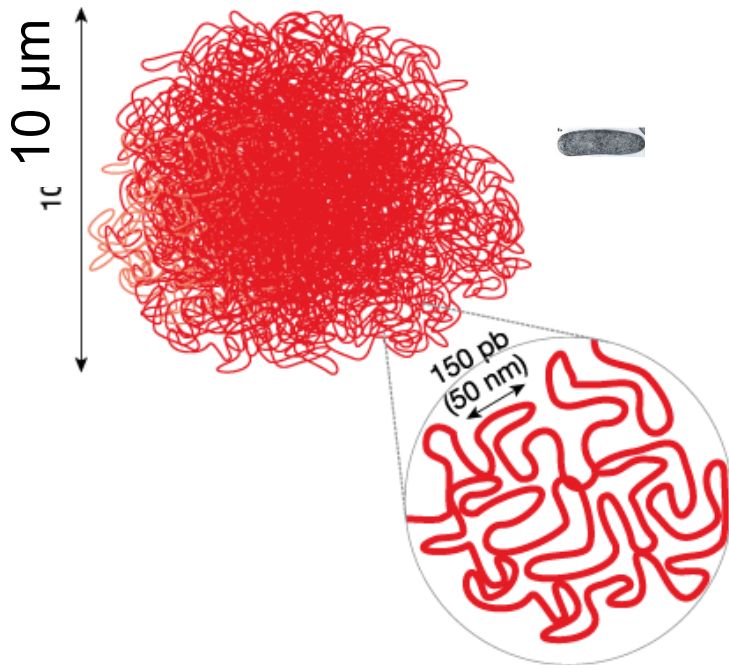


Nucleóide isolado de uma célula de *E. coli*

Empacotamento do cromossomo bacteriano

B

Cromossomo em solução ocupa espaço muito maior que a célula bacteriana ($\sim 1-2 \mu\text{m}$)



NAPs

(proteínas associadas ao nucleóide)

Cromatina: DNA + proteínas estruturais

Dimensões do material genético

E. coli

- 1 cromossomo circular
- ~ $4,4 \times 10^6$ bp
- ~ 4.000 genes
- 1,6 mm comprimento
- 1 gene/1000 bp
- 2.500 genes/mm DNA
- Densidade: 50-100 mg/mL

H. sapiens

- 46 cromossomos
- ~ $3,2 \times 10^9$ bp
- ~ 30.000 genes
- 2 m comprimento
- 1 gene/100.000 bp
- 50 genes/mm DNA
- DNA circular ($1,6 \times 10^3$ bp) nas mitocôndrias

DNA de eucariotos está organizado em cromossomos

1 cromossomo = 1 molécula de DNA em G1 e intérfase
Cromossomo metafásico: 2 moléculas de DNA

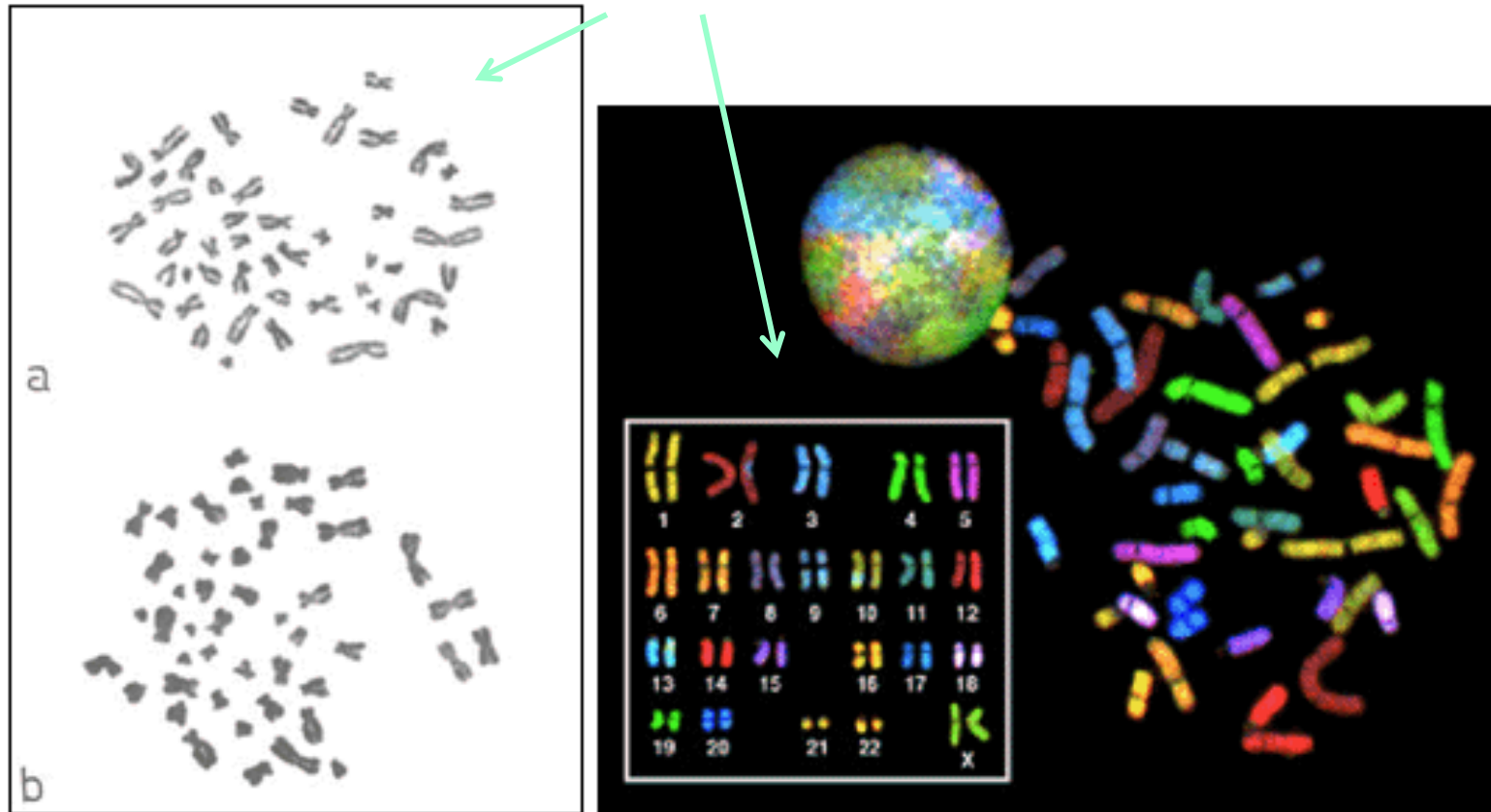
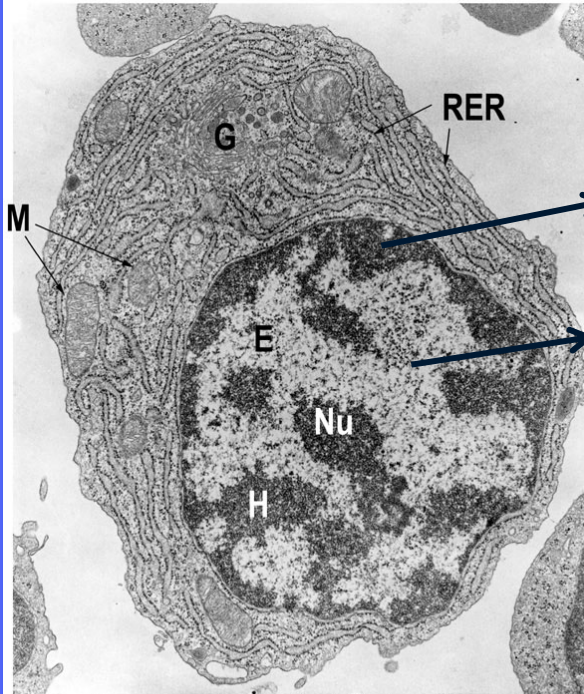


Image of painted (SKY) chromosomes – where each of the 23 pairs of chromosomes comes in a distinct color. Courtesy of DeWitt Stetten, Jr., Museum of Medical Research.

Compactação de Genomas Eucarióticos

Cromatina: Proteínas nucleares específicas + DNA



Nu-nucleus, E-euchromatin, H-heterochromatin, M-mitochondria, RER-rough endoplasmic reticulum, G-golgi complex

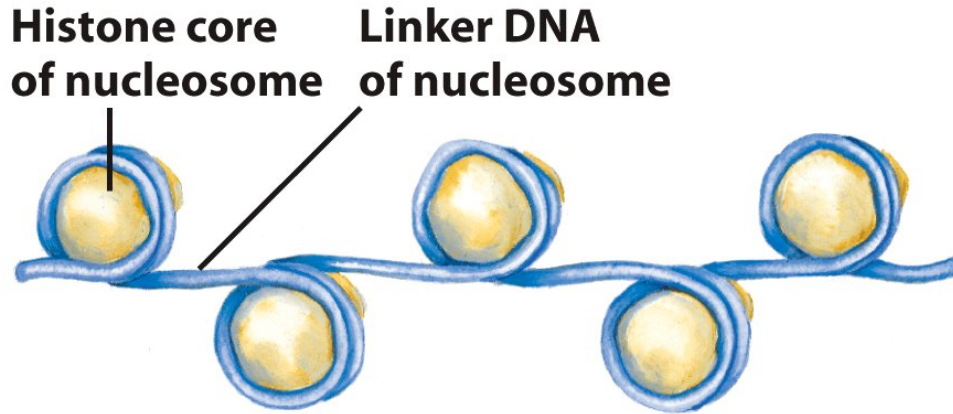
Heterocromatina: mais compactada

Eucromatina: menos compactada

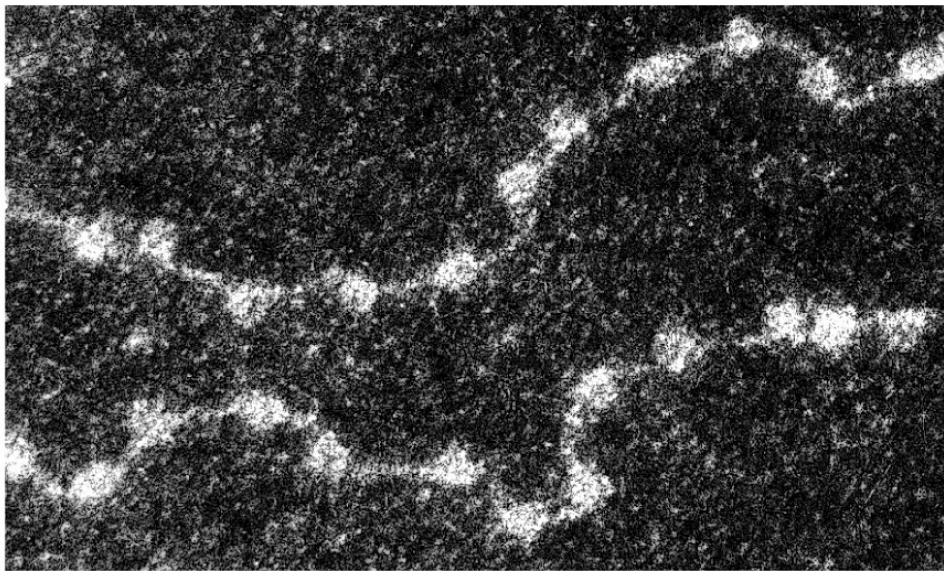
Cromossomo metafásico:
Estado mais condensado e já duplicado (mitose ou meiose)



Nucleossomos



(a)



(b)

50 nm

- unidades fundamentais de organização da cromatina.

- complexos de **histonas** ligados ao DNA

- espaçados regularmente, como contas em um colar

Tipos e propriedades das histonas

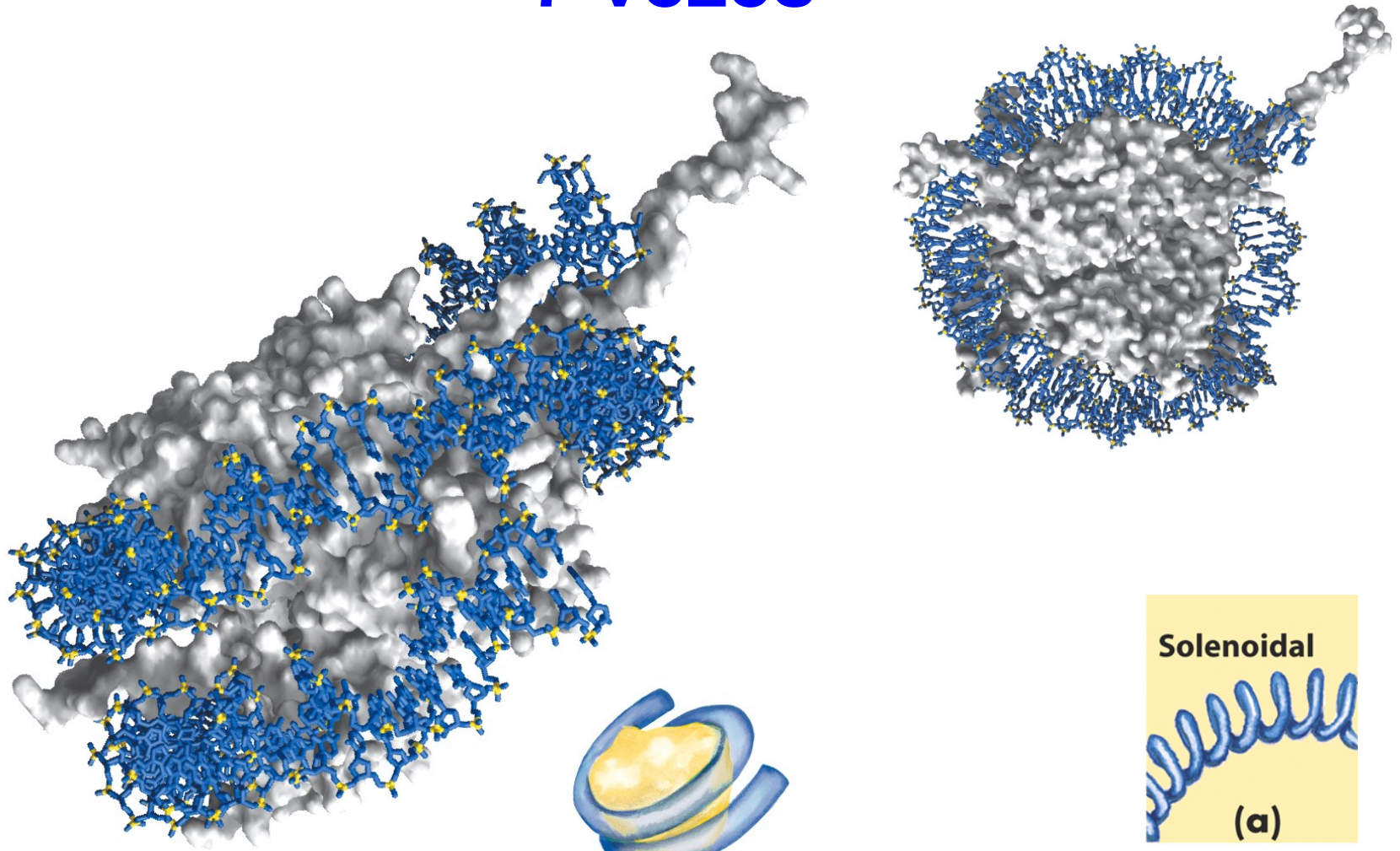
table 24-3

Types and Properties of Histones				
Histone	Molecular weight	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1*	21,130	223	29.5	1.3
H2A*	13,960	129	10.9	9.3
H2B*	13,774	125	16.0	6.4
H3	15,273	135	9.6	13.3
H4	11,236	102	10.8	13.7

*The sizes of these histones vary somewhat from species to species. The numbers given here are for bovine histones.

Proteínas relativamente pequenas,
com carga líquida positiva

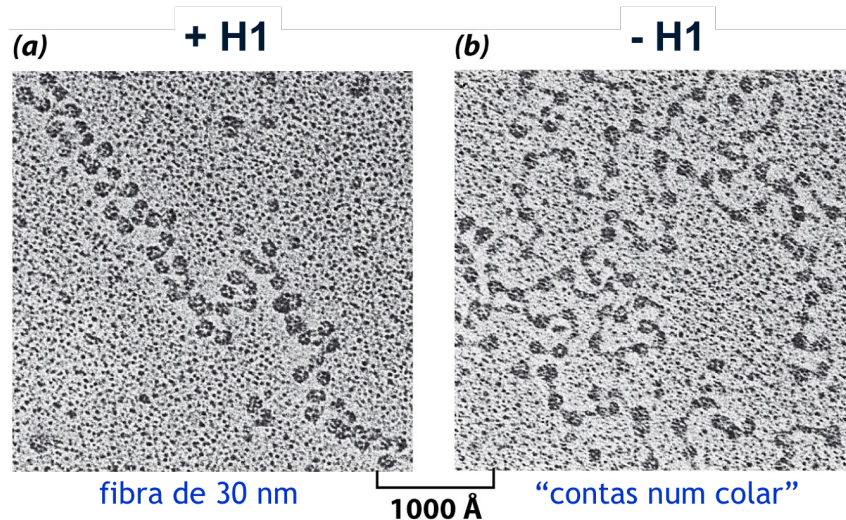
Compactação do DNA em nucleossomos: ~ 7 vezes



146 nt

Histona H1

- Histona de ligação
- Conecta os nucleossomos entre si
- Aumenta o grau de compactação



configuração fechada

configuração aberta

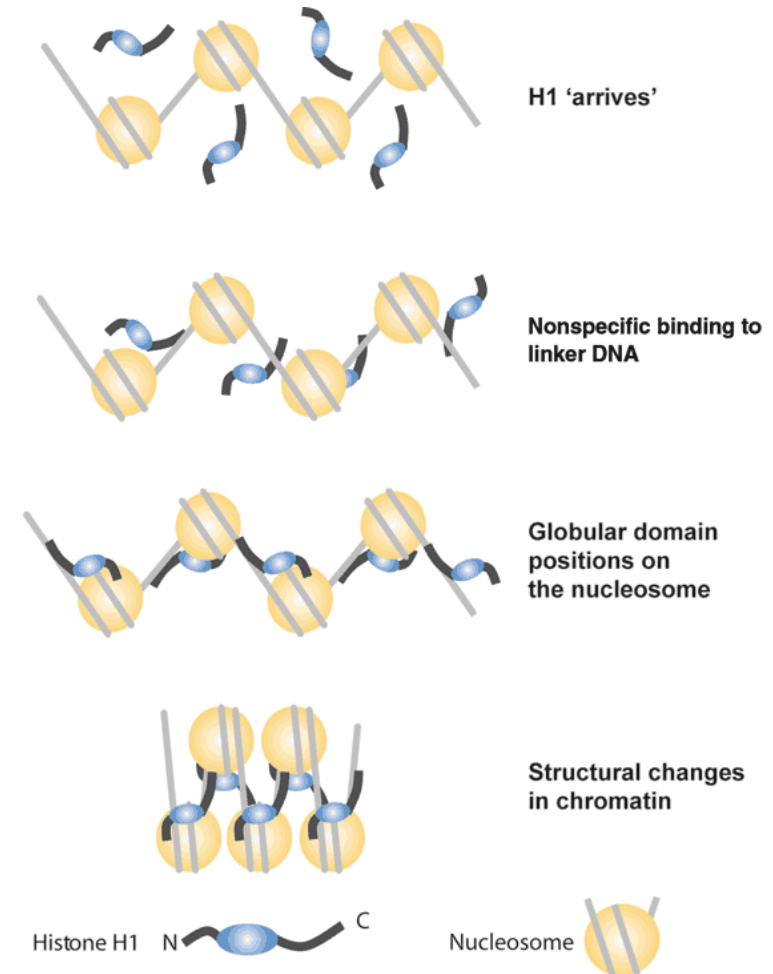


Figure 23-48 Fundamentals of Biochemistry, 2/e

(H2A, H2B, H3, H4)₂

**Histone
octamer**

DNA

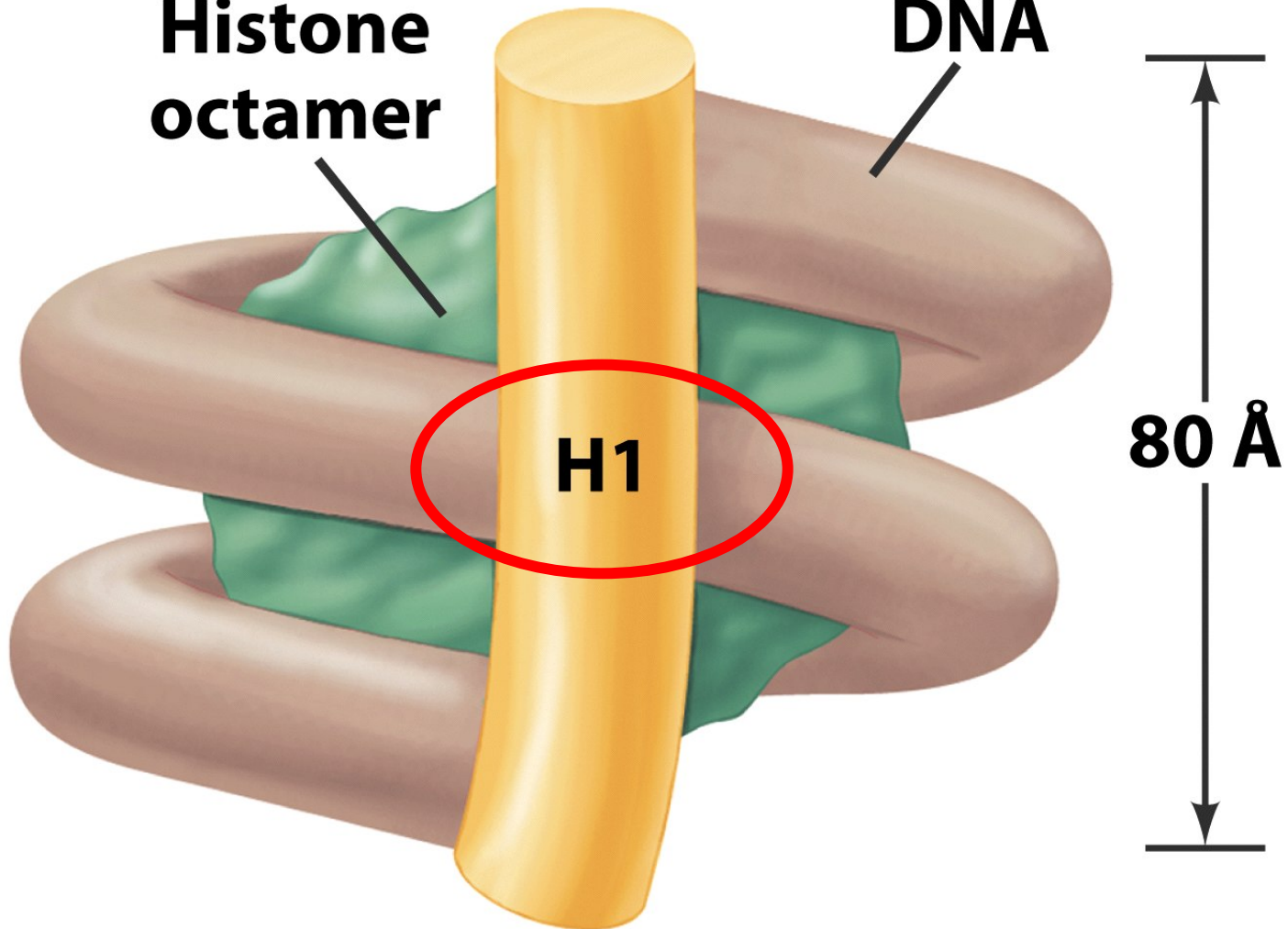


Figure 23-47 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

Histona H1 fica no interior da fibra de 30 nm

solenóide

1 nm = 10 Å

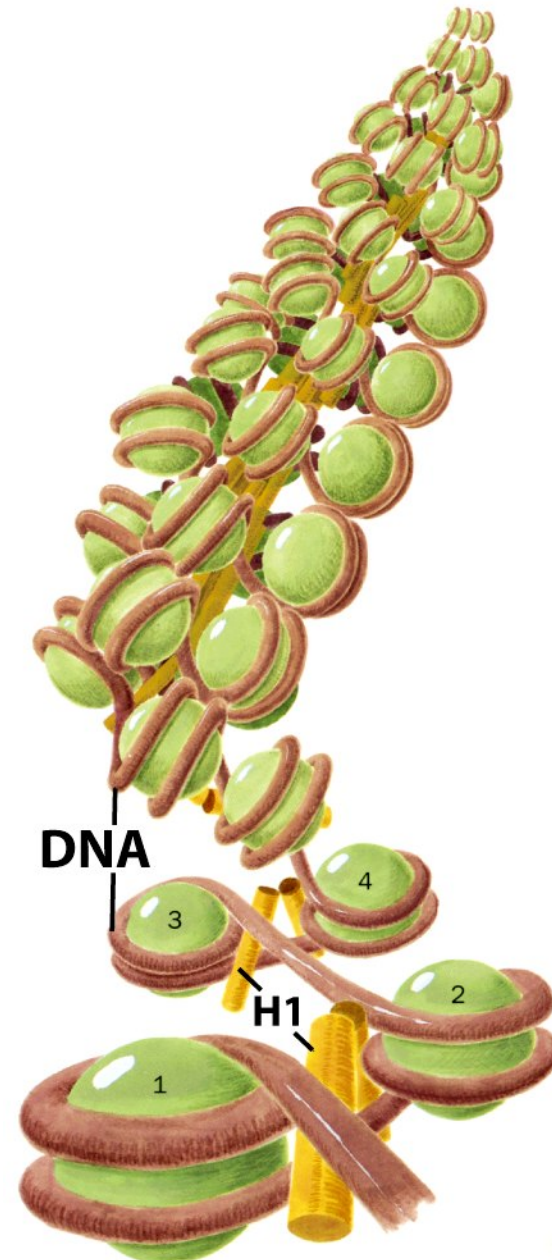
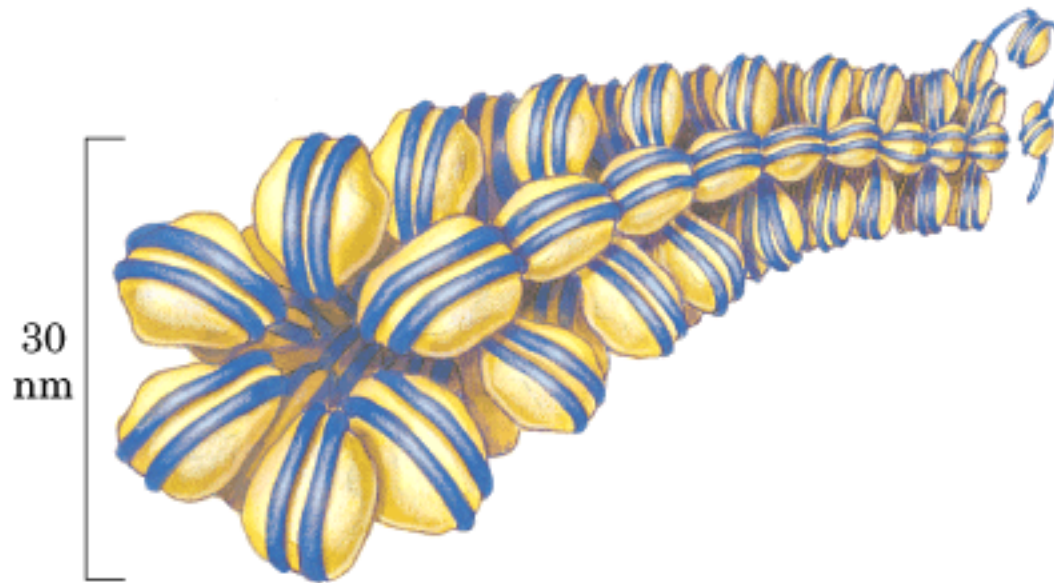


Figure 23-50 Fundamentals of Biochemistry, 2/e
© 2006 John Wiley & Sons

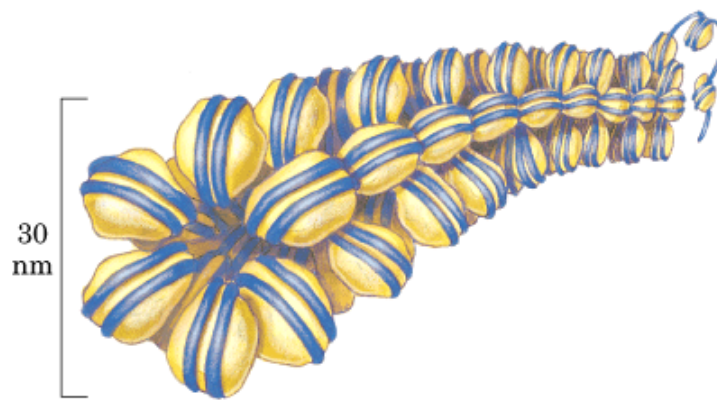
Compactação do DNA em fibras de 30 nm = ~100 vezes



(a)

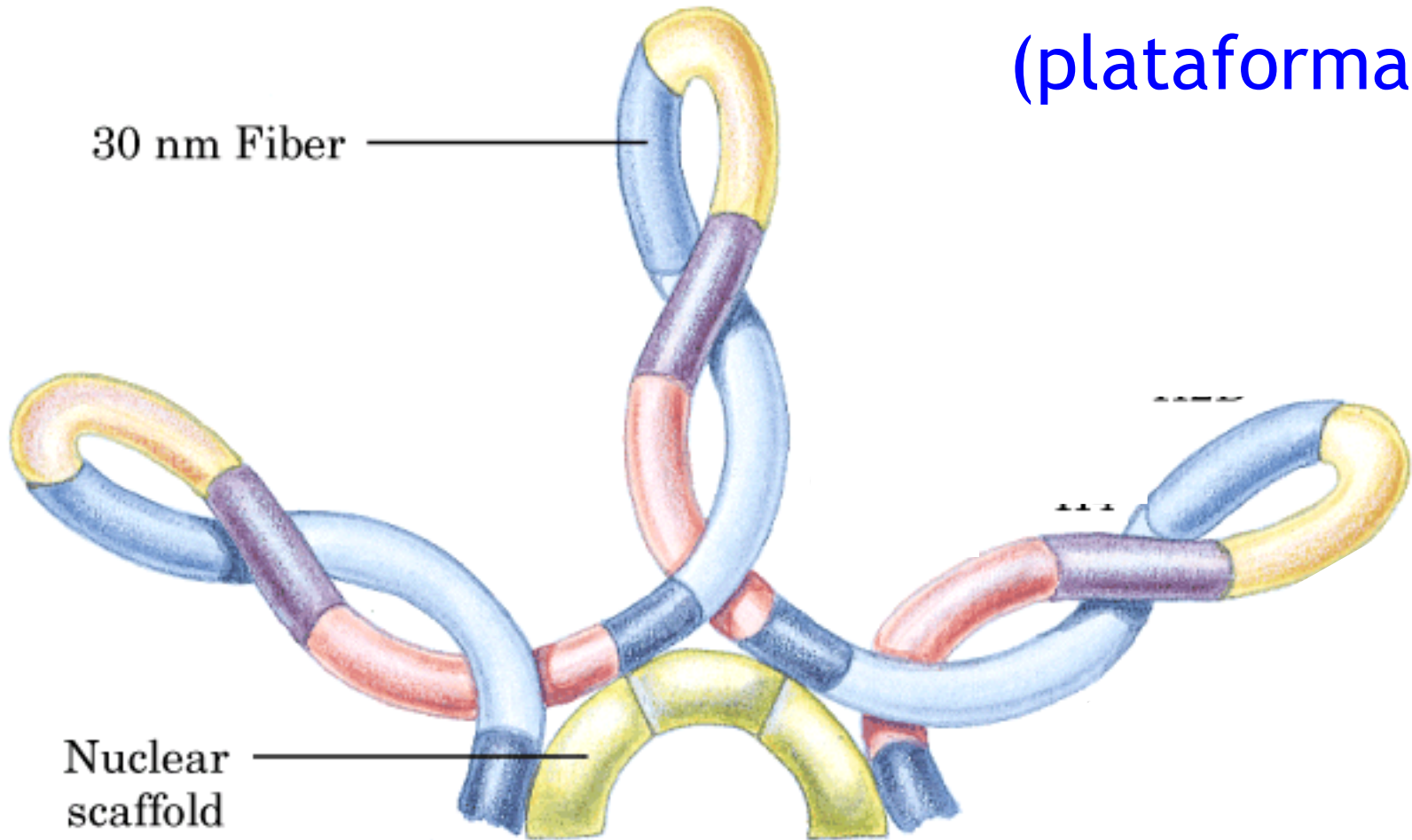


(b)



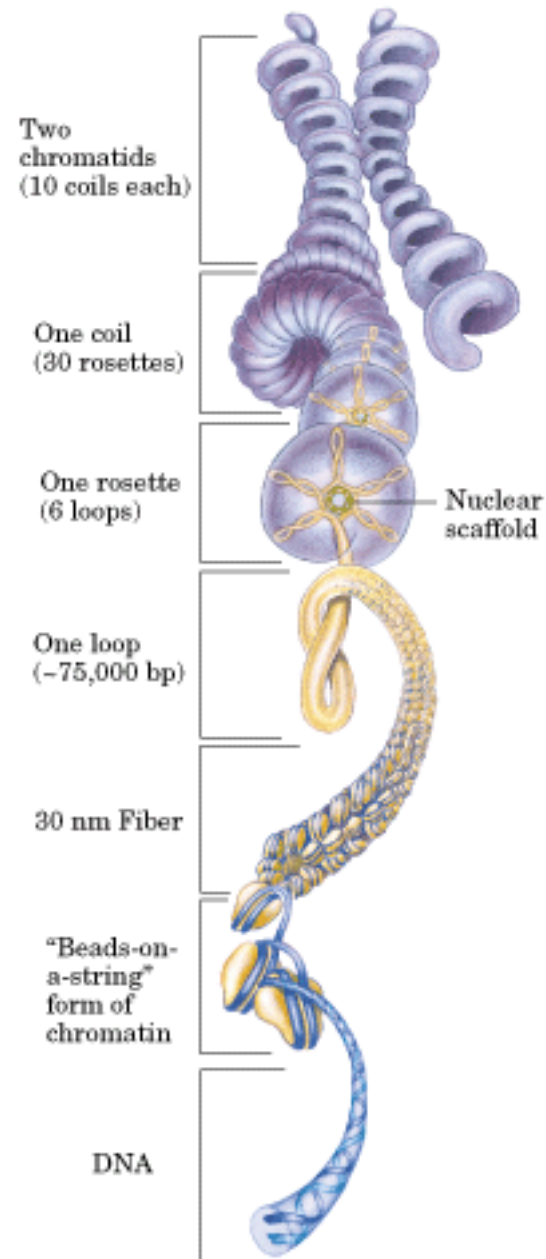
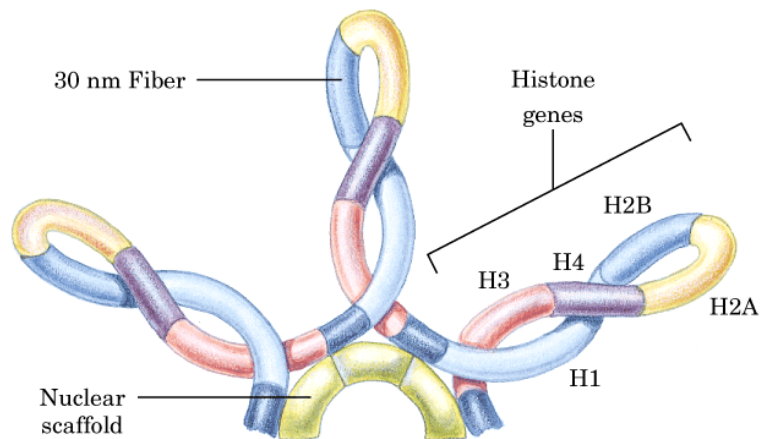
A fibra de 30 nm
forma alças ligadas
a proteínas da
matriz nuclear
(plataforma)

30 nm Fiber



Nuclear
scaffold
plataforma

**Compactação
do DNA em um
cromossomo
eucarioto =
~10.000 vezes**



Cromossomo depletado de histonas.

DNA



Plataforma

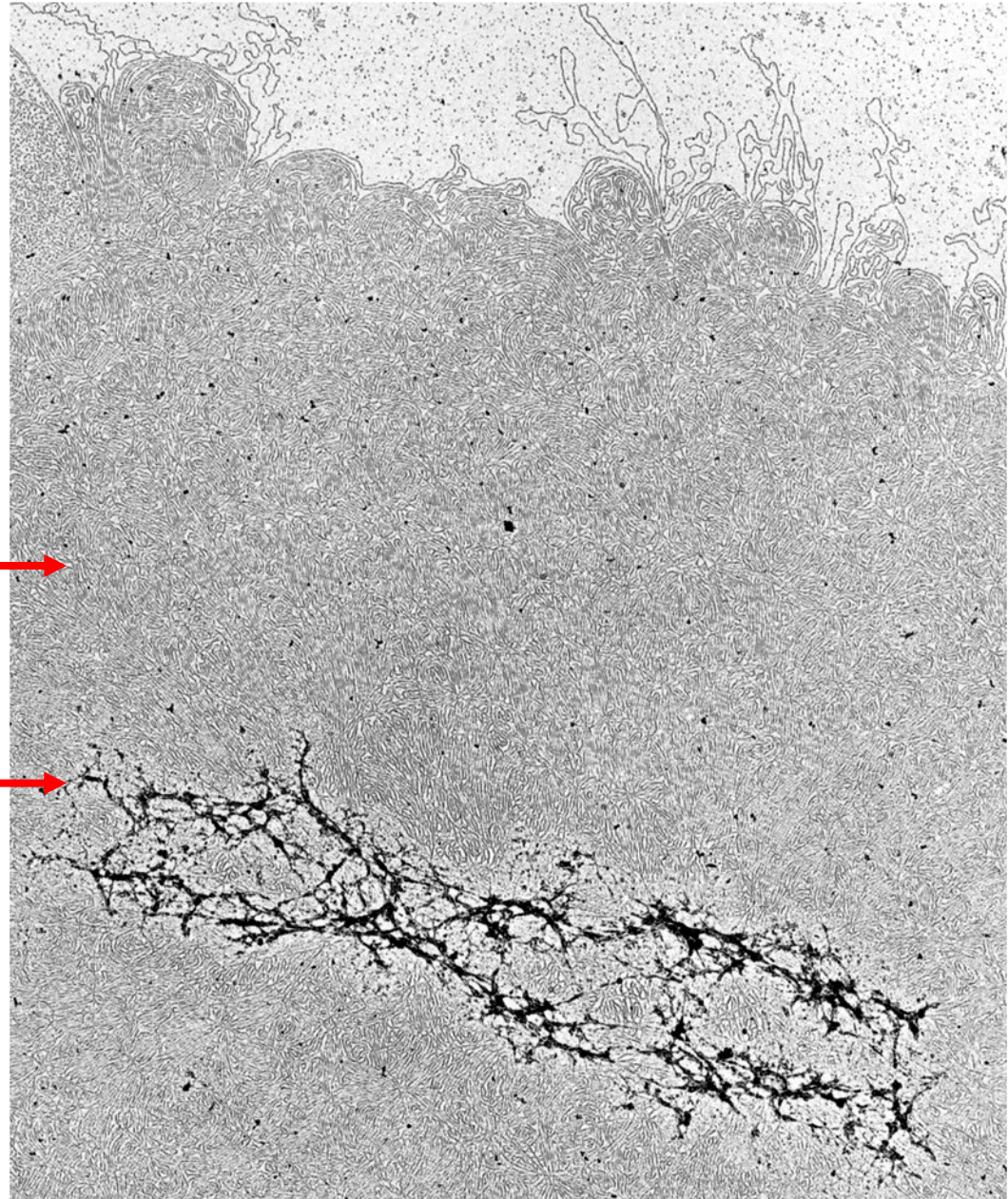


Figure 23-51a Fundamentals of Biochemistry, 2/e

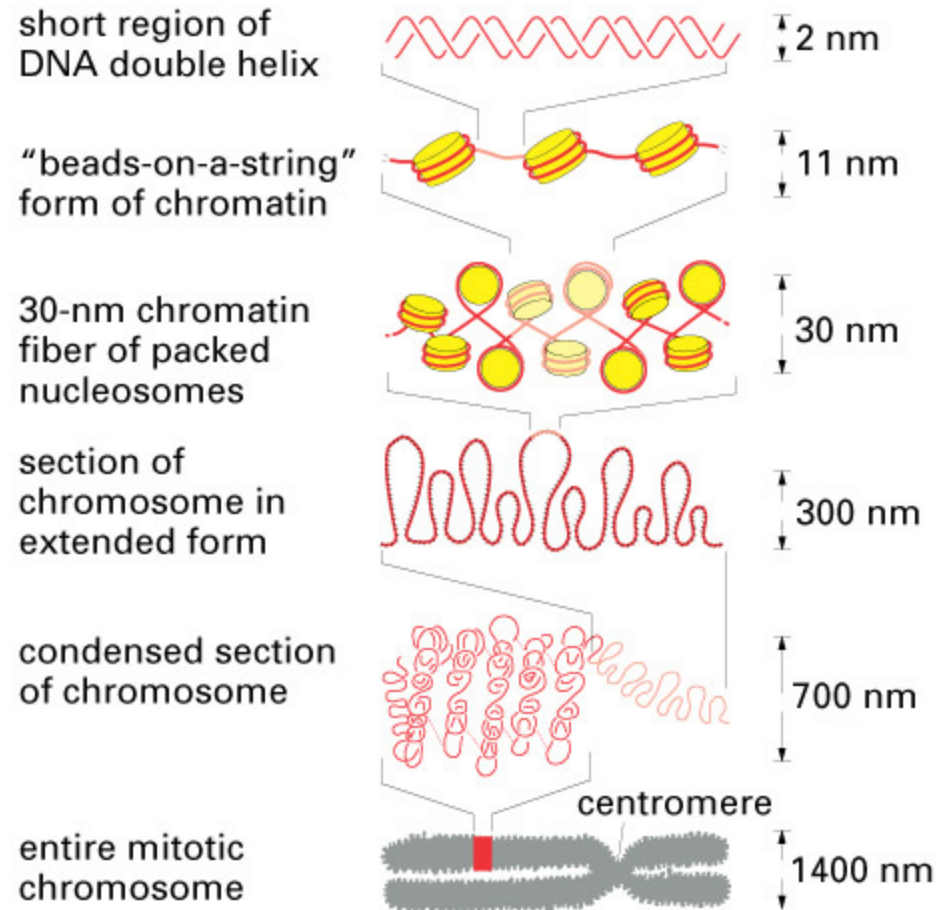
Empacotamento do DNA em cromossomos

Animações

<http://www.youtube.com/watch?v=gbSIBhFwQ4s>

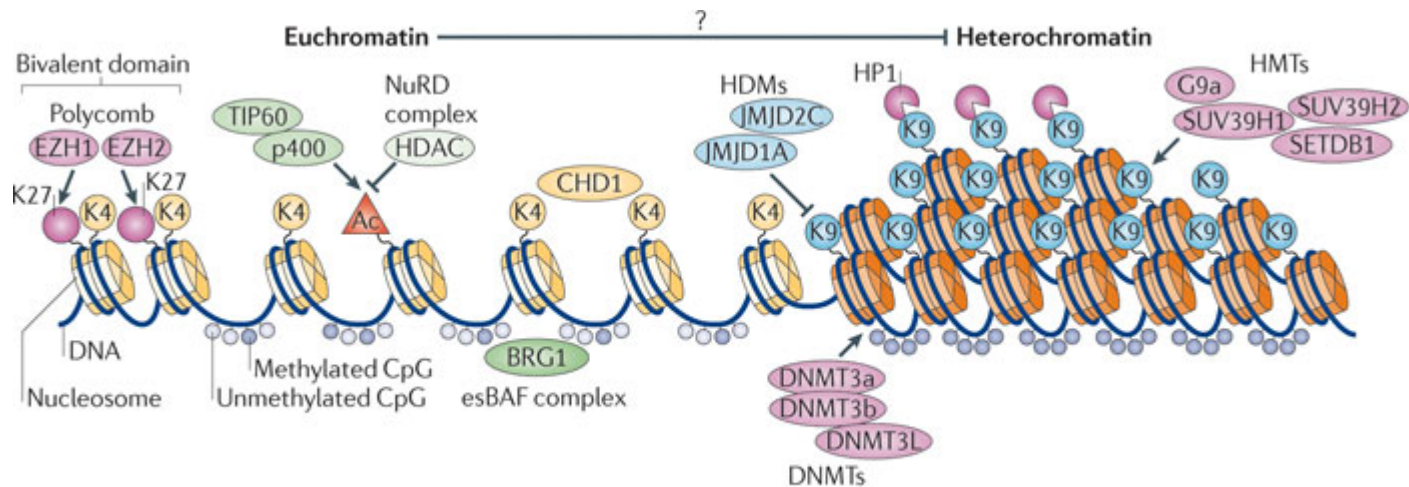
<http://www.dnalc.org/view/16627-Animation-29-DNA-is-packaged-in-a-chromosome-.html>

Visão geral do empacotamento do DNA eucarioto



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

A cromatina é dinâmica



Nature Reviews | Molecular Cell Biology

- Vários fatores modificam o grau de compactação do DNA

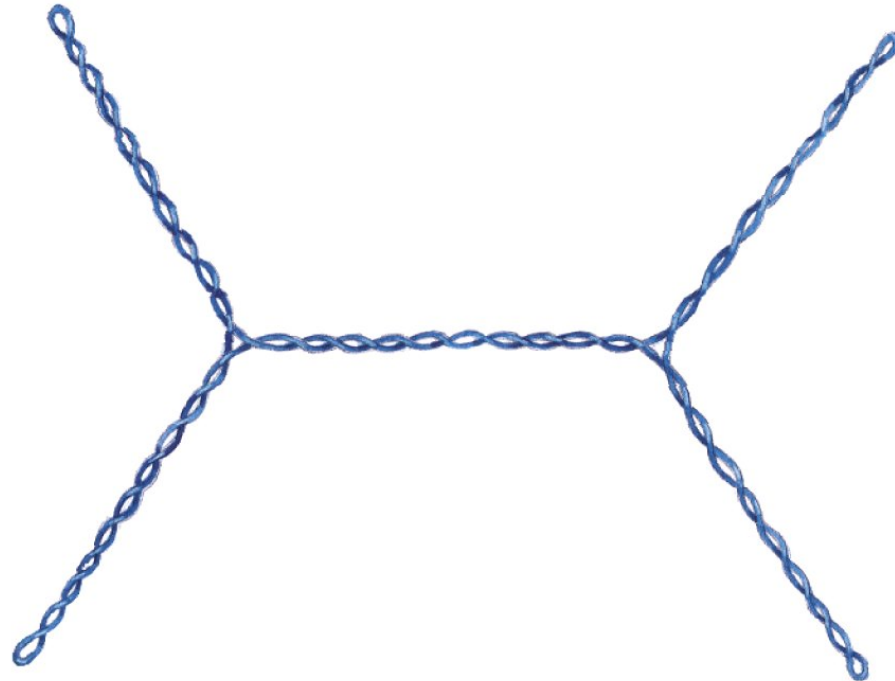
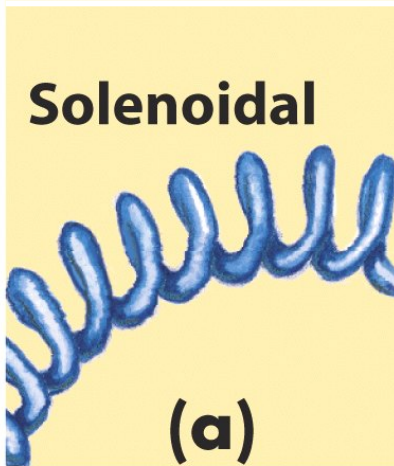
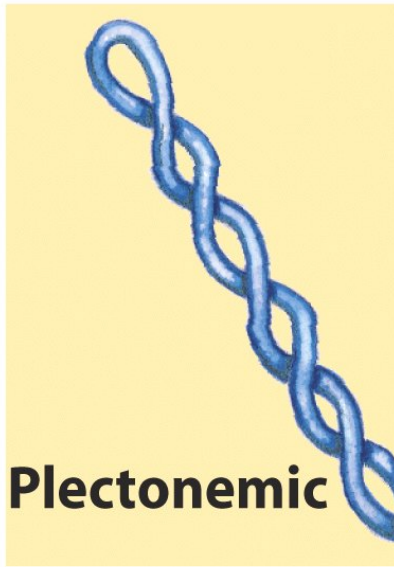
- regulação da expressão gênica/replicação/reparo
- epigenética

Assunto de aulas futuras!

Material Extra

- Compactação do DNA
- Cromatina e cromossomos

Tipos de superenrolamento



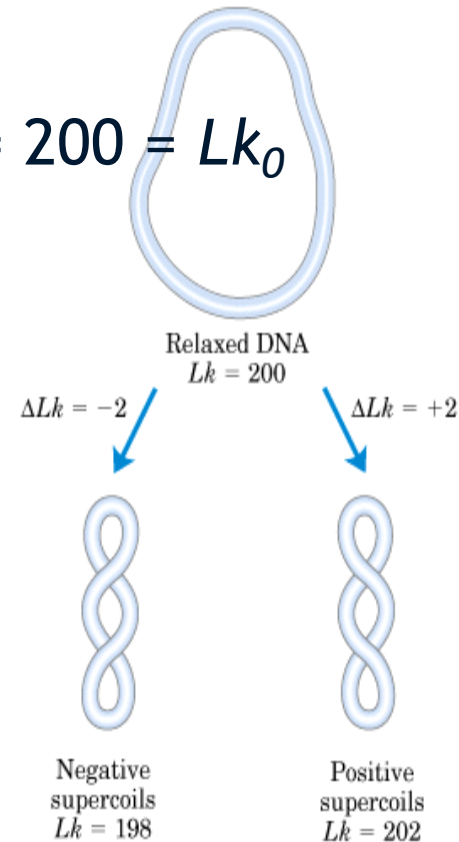
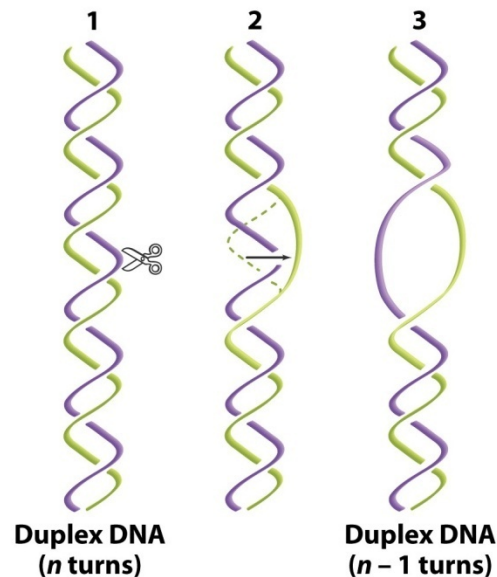
Número de ligação (Lk)

DNA relaxado: 10,5 nt/volta da hélice

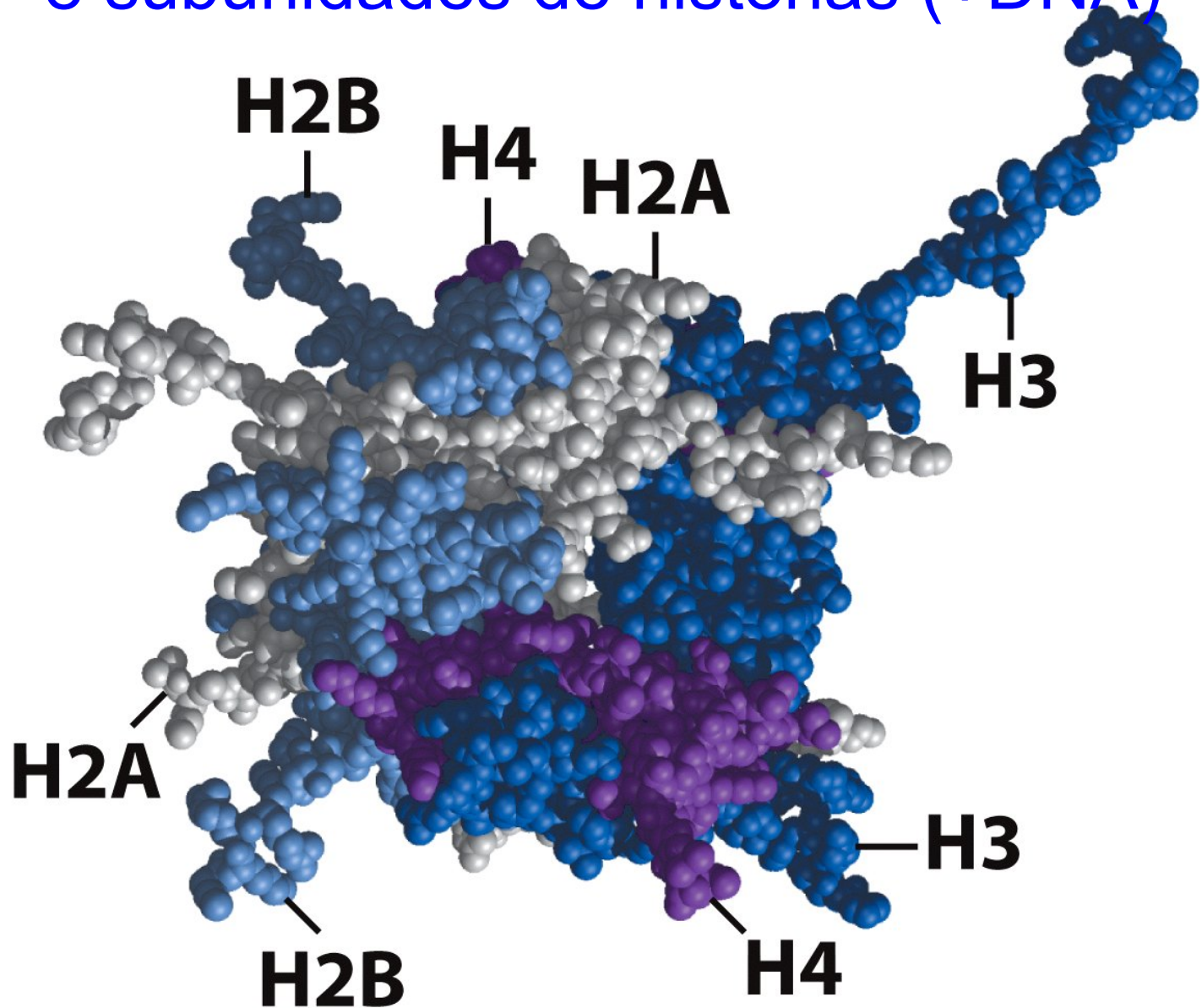
➤ 1 fita passa sobre o plano 1 vez a cada volta:

$$Lk = \text{bp} / 10,5 \text{ (linkage number)}$$

Exemplo: DNA de 2100 bp relaxado $\rightarrow Lk = 200 = Lk_0$



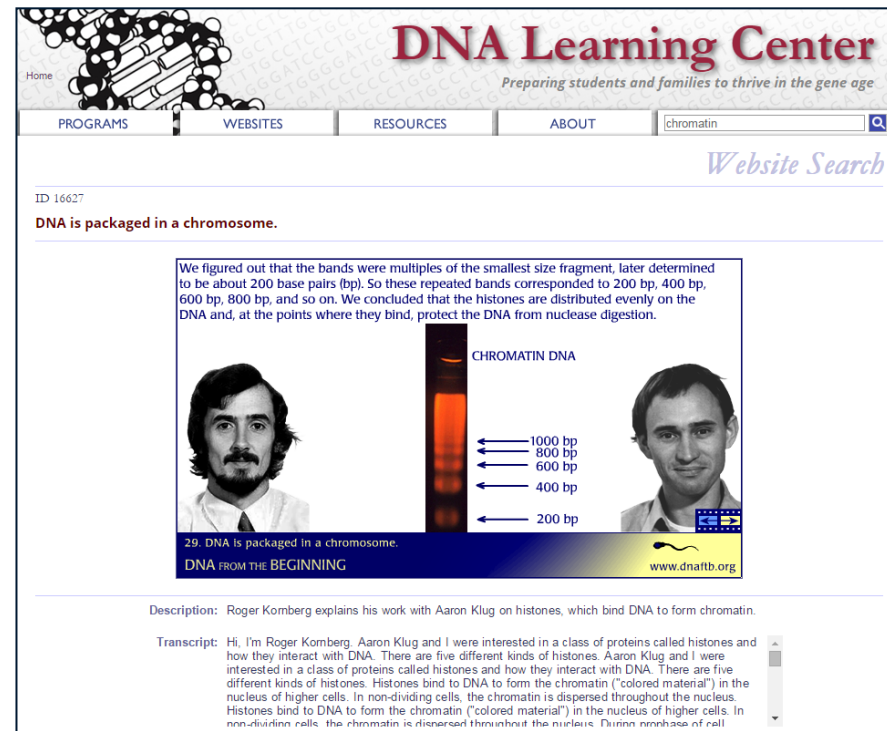
O nucleossomo é formado por 8 subunidades de histonas (+DNA)



Experimentos que demonstraram a compactação do DNA

- DNA learning center:

<http://www.dnalc.org/view/16627-Animation-29-DNA-is-packaged-in-a-chromosome-.html>



The screenshot shows the DNA Learning Center website interface. At the top, there is a logo for the DNA Learning Center with the tagline "Preparing students and families to thrive in the gene age". Below the logo are navigation tabs for "PROGRAMS", "WEBSITES", "RESOURCES", and "ABOUT". A search bar is visible on the right side of the navigation bar. The main content area displays the video player for the video "DNA is packaged in a chromosome". The video player includes a description of the experiment, a transcript, and a description of the video content.

DNA Learning Center
Preparing students and families to thrive in the gene age

Home

PROGRAMS WEBSITES RESOURCES ABOUT

chromatin

Website Search

ID 16627

DNA is packaged in a chromosome.

We figured out that the bands were multiples of the smallest size fragment, later determined to be about 200 base pairs (bp). So these repeated bands corresponded to 200 bp, 400 bp, 600 bp, 800 bp, and so on. We concluded that the histones are distributed evenly on the DNA and, at the points where they bind, protect the DNA from nuclease digestion.

CHROMATIN DNA

1000 bp
800 bp
600 bp
400 bp
200 bp

29. DNA is packaged in a chromosome.
DNA FROM THE BEGINNING

www.dnaltb.org

Description: Roger Kornberg explains his work with Aaron Klug on histones, which bind DNA to form chromatin.

Transcript: Hi, I'm Roger Kornberg, Aaron Klug and I were interested in a class of proteins called histones and how they interact with DNA. There are five different kinds of histones. Aaron Klug and I were interested in a class of proteins called histones and how they interact with DNA. There are five different kinds of histones. Histones bind to DNA to form the chromatin ("colored material") in the nucleus of higher cells. In non-dividing cells, the chromatin is dispersed throughout the nucleus. Histones bind to DNA to form the chromatin ("colored material") in the nucleus of higher cells. In non-dividing cells, the chromatin is dispersed throughout the nucleus. During mitosis of cell

Mudanças Estruturais dos Cromossomos durante o Ciclo Celular Eucarioto

- Outras proteínas envolvidas na condensação dos cromossomos
 - Coesinas
 - Condensinas

