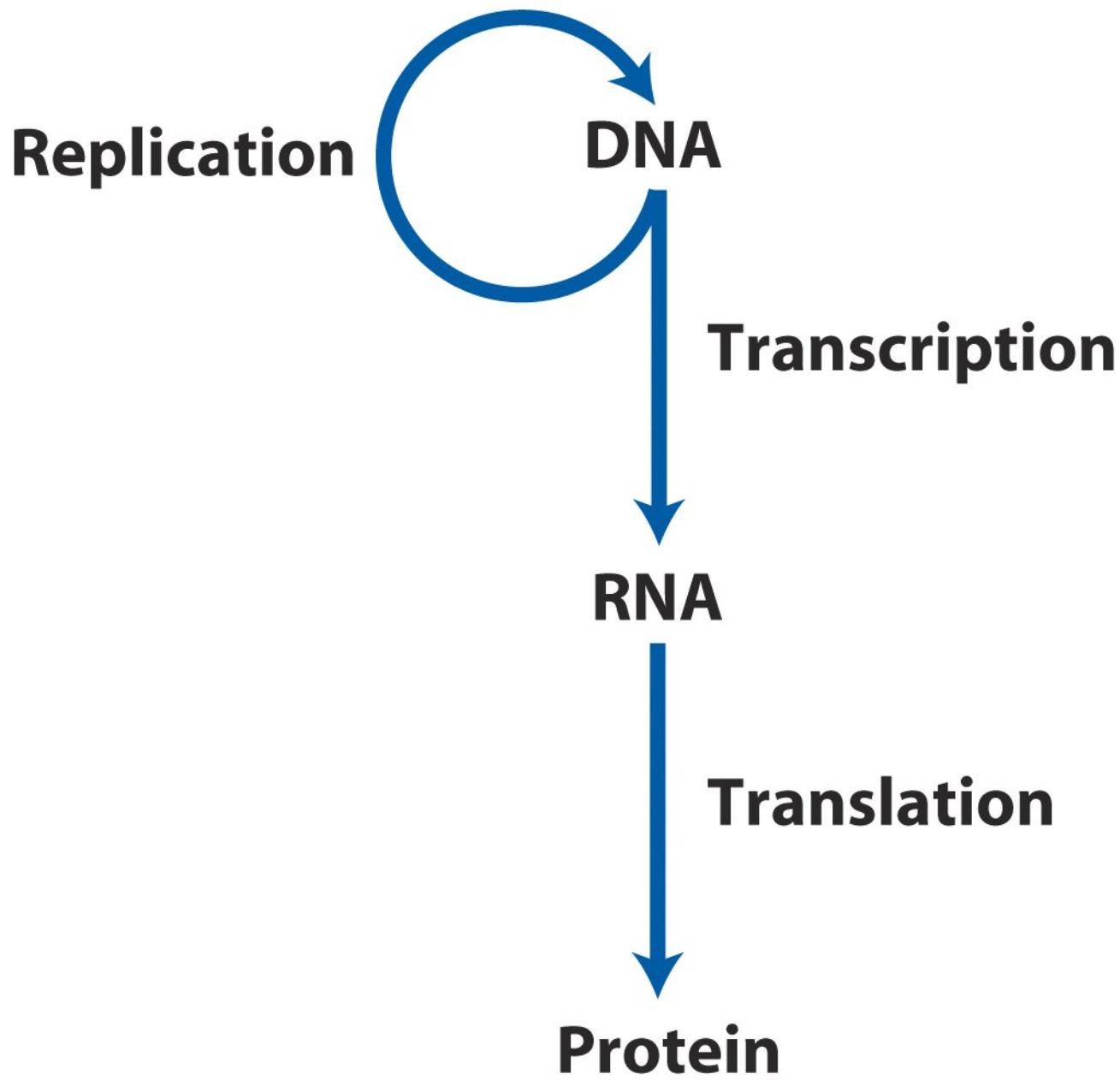
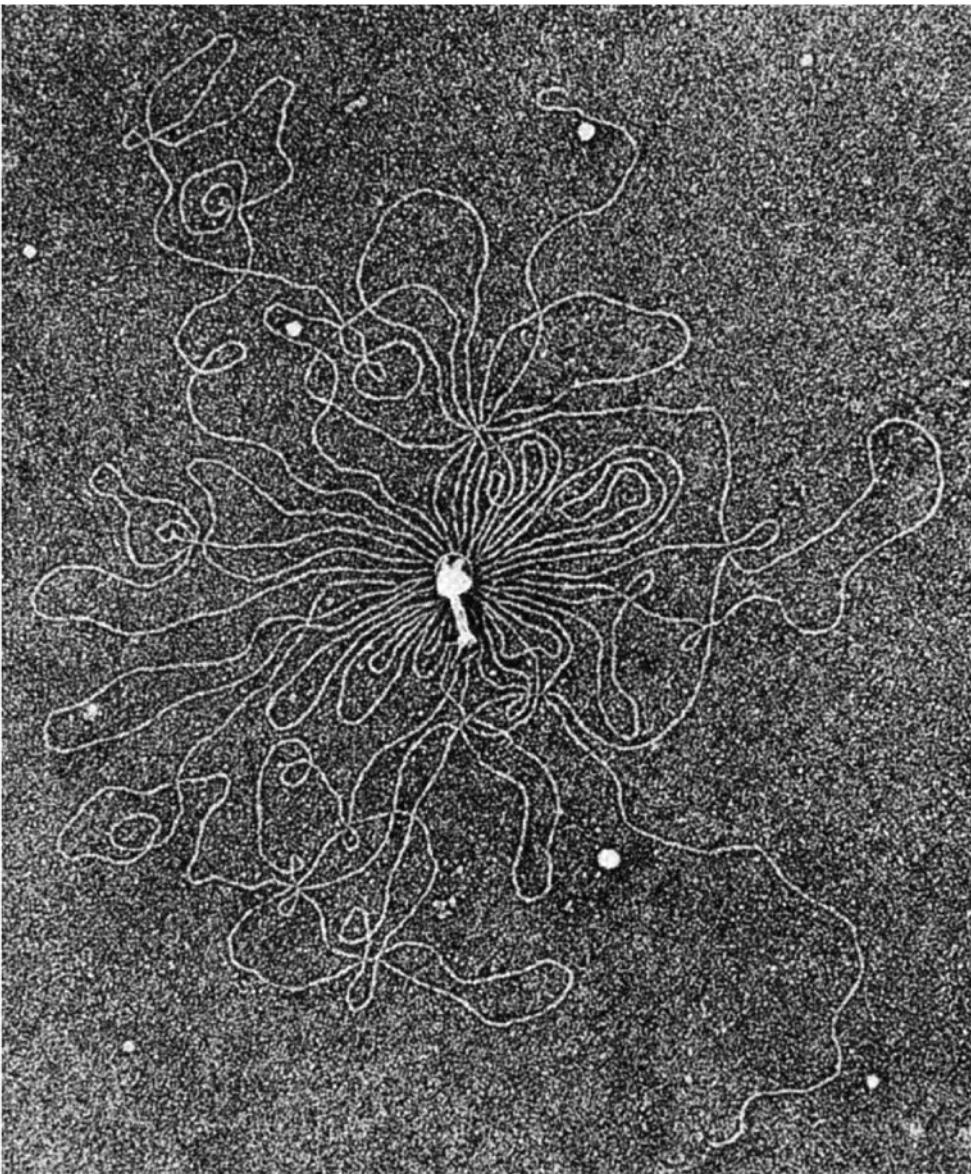


David L. Nelson and Michael M. Cox

# **Lehninger Principles of Biochemistry Fourth Edition**

## **Chapter 24: Genes and Chromosomes**

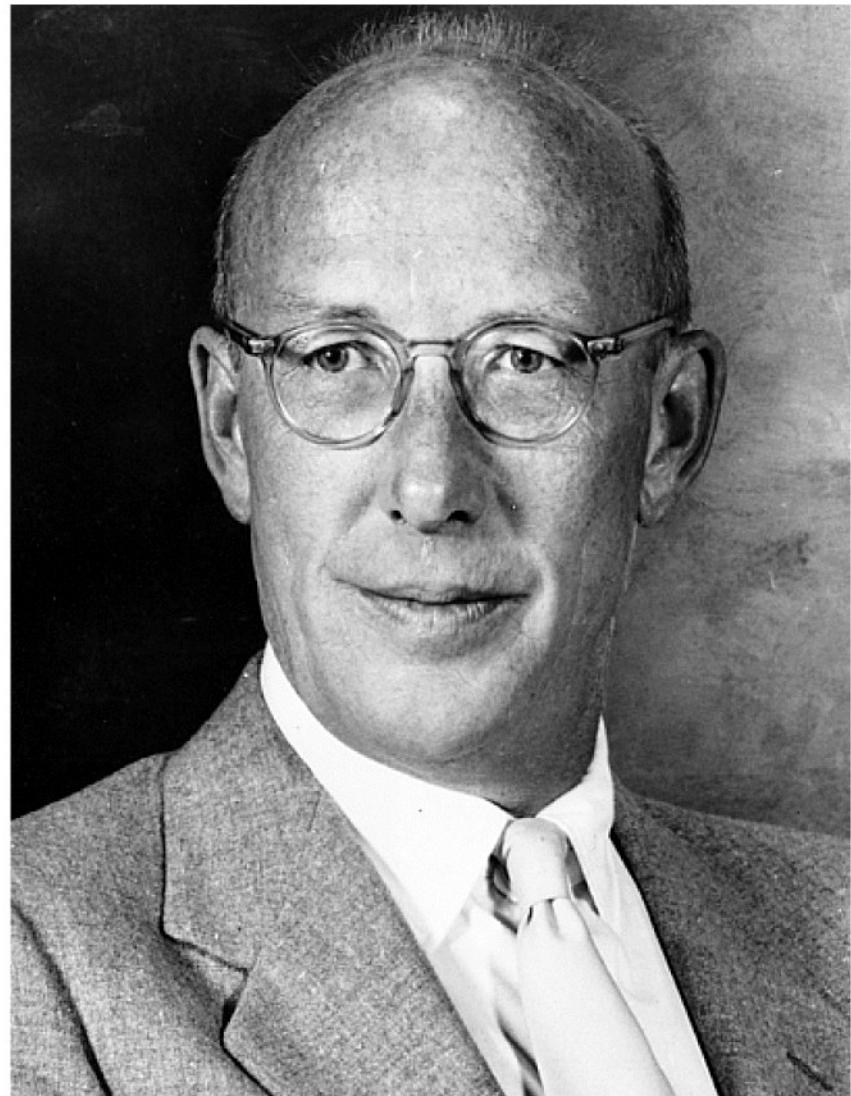




0.5  $\mu\text{m}$

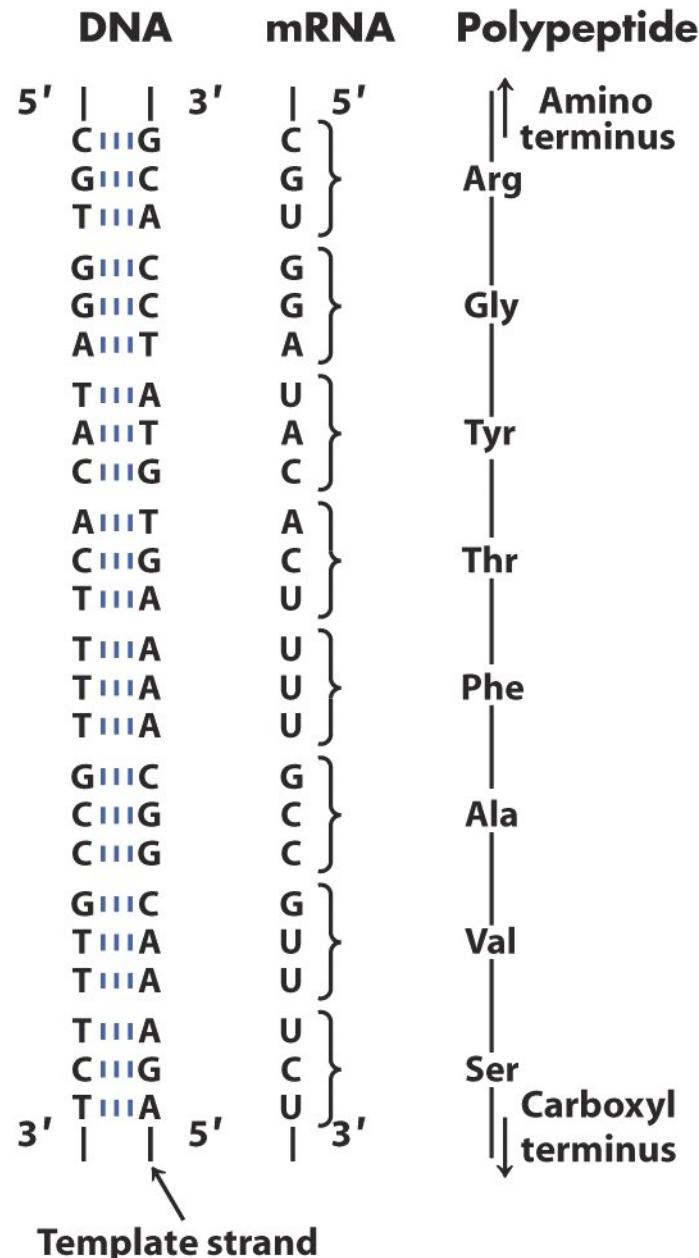


**George W. Beadle,  
1903–1989**



**Edward L. Tatum,  
1909–1975**

One gene - one enzyme



Yeast (16 chromosomes) masses from  $1,5 \times 10^8$   
to  $1 \times 10^9$  Daltons

From 230 000 to 1532 0000 bp

Human chromosomes up to  $279 \ 10^6$  bp

Each diploid cell 2 m of DNA

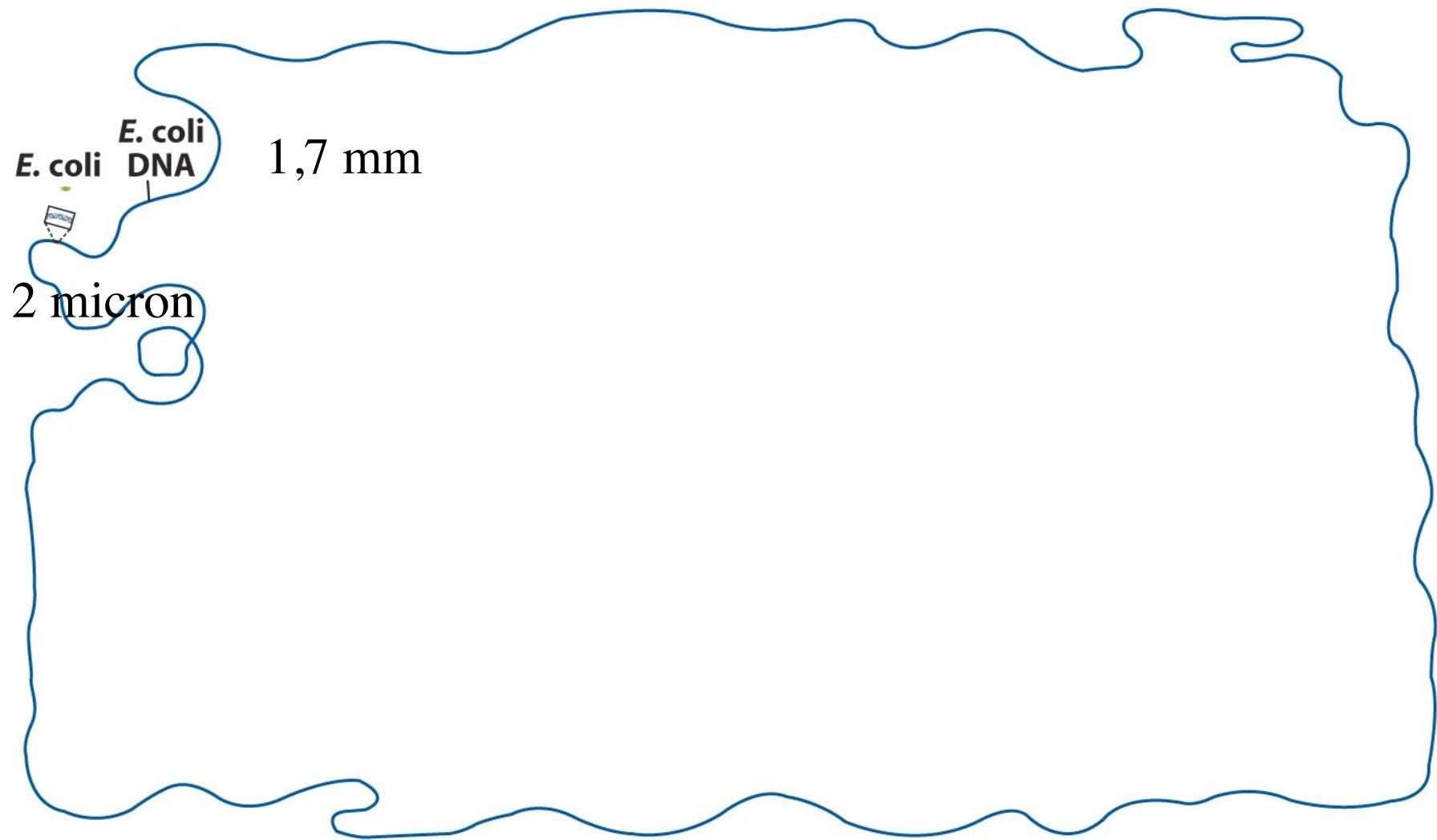
$10^{14}$  cells /body  $> 2 \times 10^{11}$  km

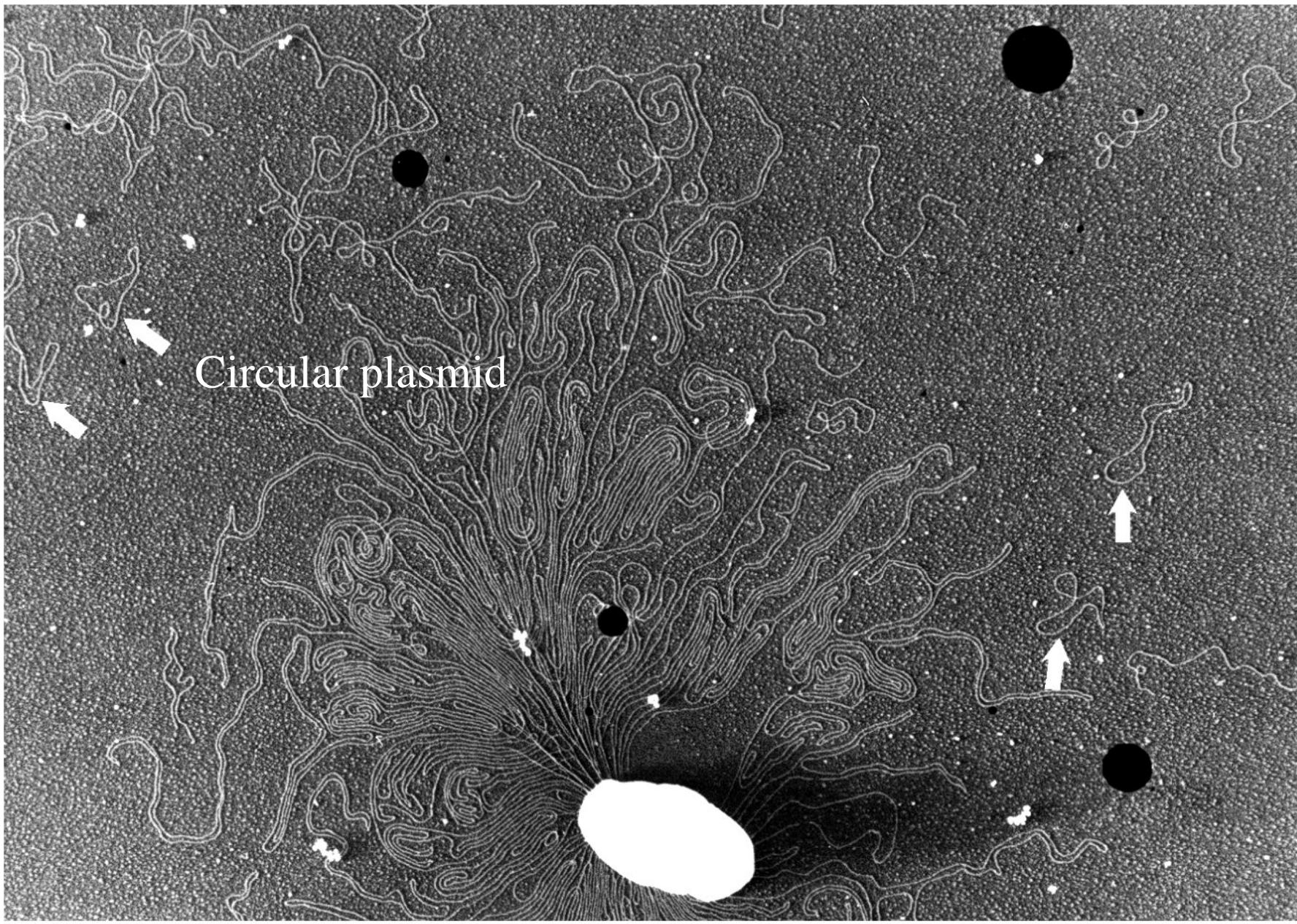
(earth circumference  $4 \times 10^4$  km  
Earth-sun  $1,5 \times 10^8$  km)

**TABLE 24-1** The Sizes of DNA and Viral Particles for Some Bacterial Viruses (Bacteriophages)

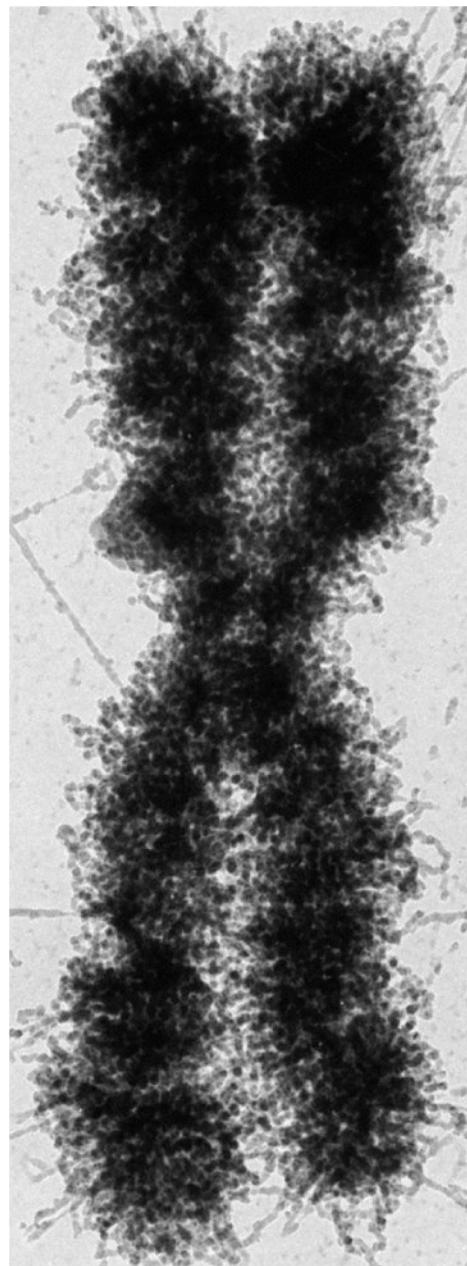
<i>Virus</i>	<i>Size of viral DNA (bp)</i>	<i>Length of viral DNA (nm)</i>	<i>Long dimension of viral particle (nm)</i>
φX174	5,386	1,939	25
T7	39,936	14,377	78
λ (lambda)	48,502	17,460	190
T4	168,889	60,800	210

**Note:** Data on size of DNA are for the replicative form (double-stranded). The contour length is calculated assuming that each base pair occupies a length of 3.4 Å (see Fig. 8-15).

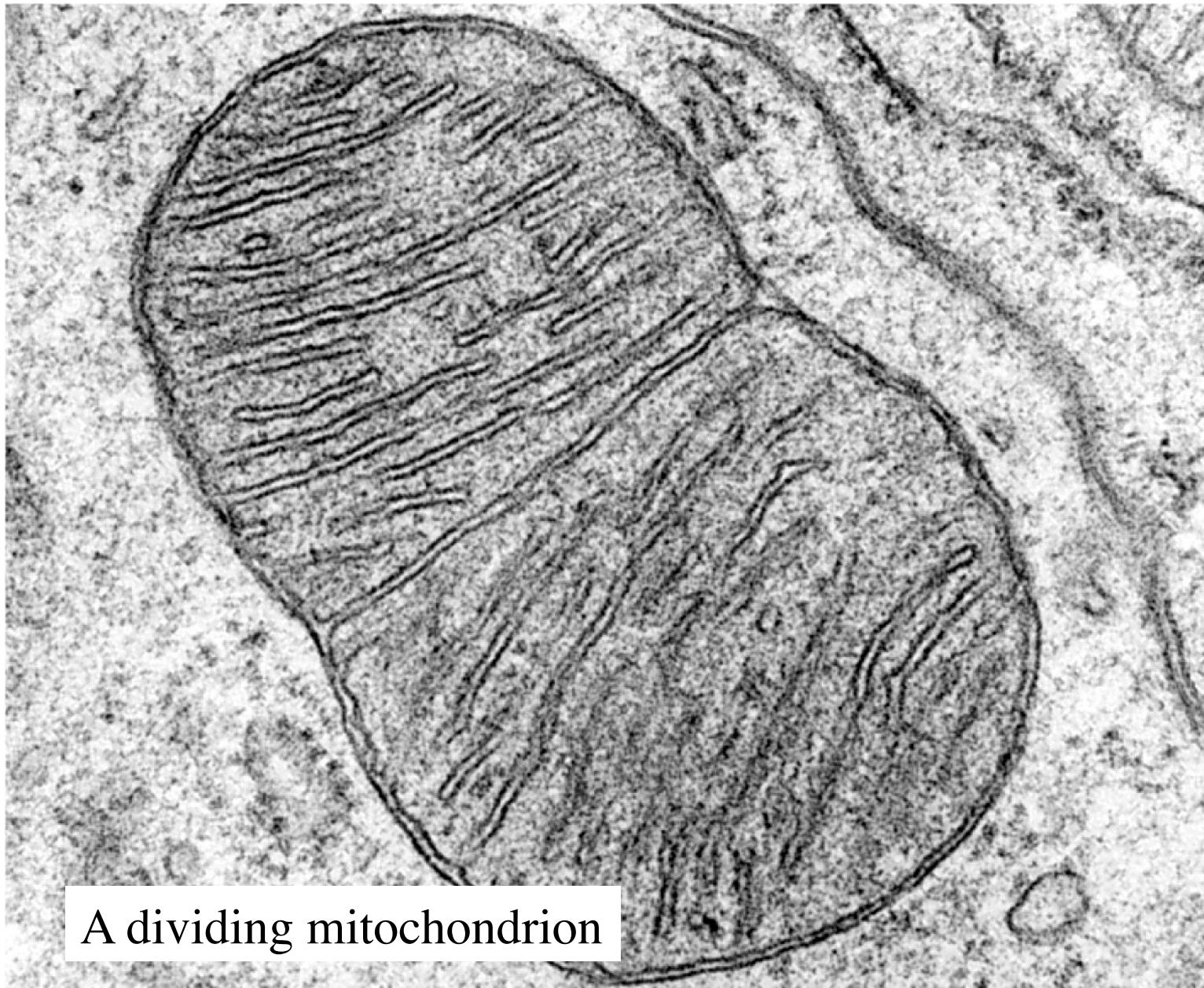




Circular plasmid







A dividing mitochondrion

**TABLE 24–2** DNA, Gene, and Chromosome Content in Some Genomes

	Total DNA (bp)	Number of chromosomes*	Approximate number of genes
Bacterium ( <i>Escherichia coli</i> )	4,639,221	1	4,405
Yeast ( <i>Saccharomyces cerevisiae</i> )	12,068,000	16 <sup>†</sup>	6,200
Nematode ( <i>Caenorhabditis elegans</i> )	97,000,000	12 <sup>‡</sup>	19,000
Plant ( <i>Arabidopsis thaliana</i> )	125,000,000	10	25,500
Fruit fly ( <i>Drosophila melanogaster</i> )	180,000,000	18	13,600
Plant ( <i>Oryza sativa</i> ; rice)	480,000,000	24	57,000
Mouse ( <i>Mus musculus</i> )	2,500,000,000	40	30,000–35,000
Human ( <i>Homo sapiens</i> )	3,200,000,000	46	30,000–35,000

**Note:** This information is constantly being refined. For the most current information, consult the websites for the individual genome projects.

\*The diploid chromosome number is given for all eukaryotes except yeast.

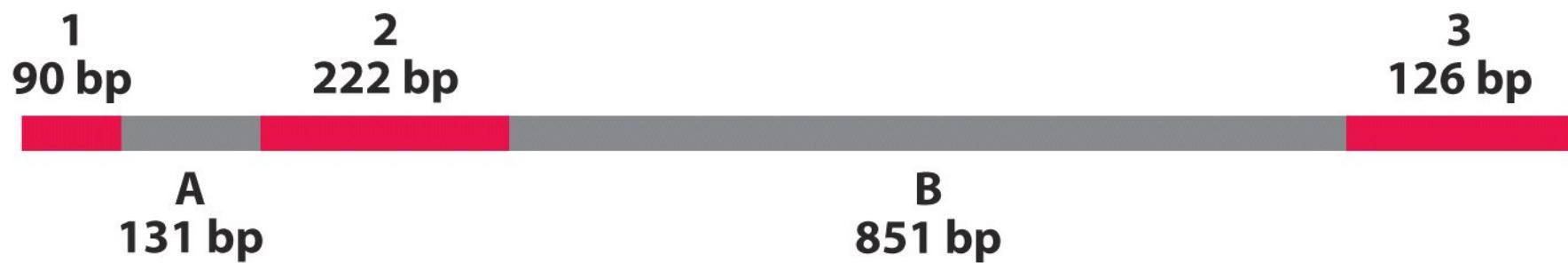
<sup>†</sup>Haploid chromosome number. Wild yeast strains generally have eight (octoploid) or more sets of these chromosomes.

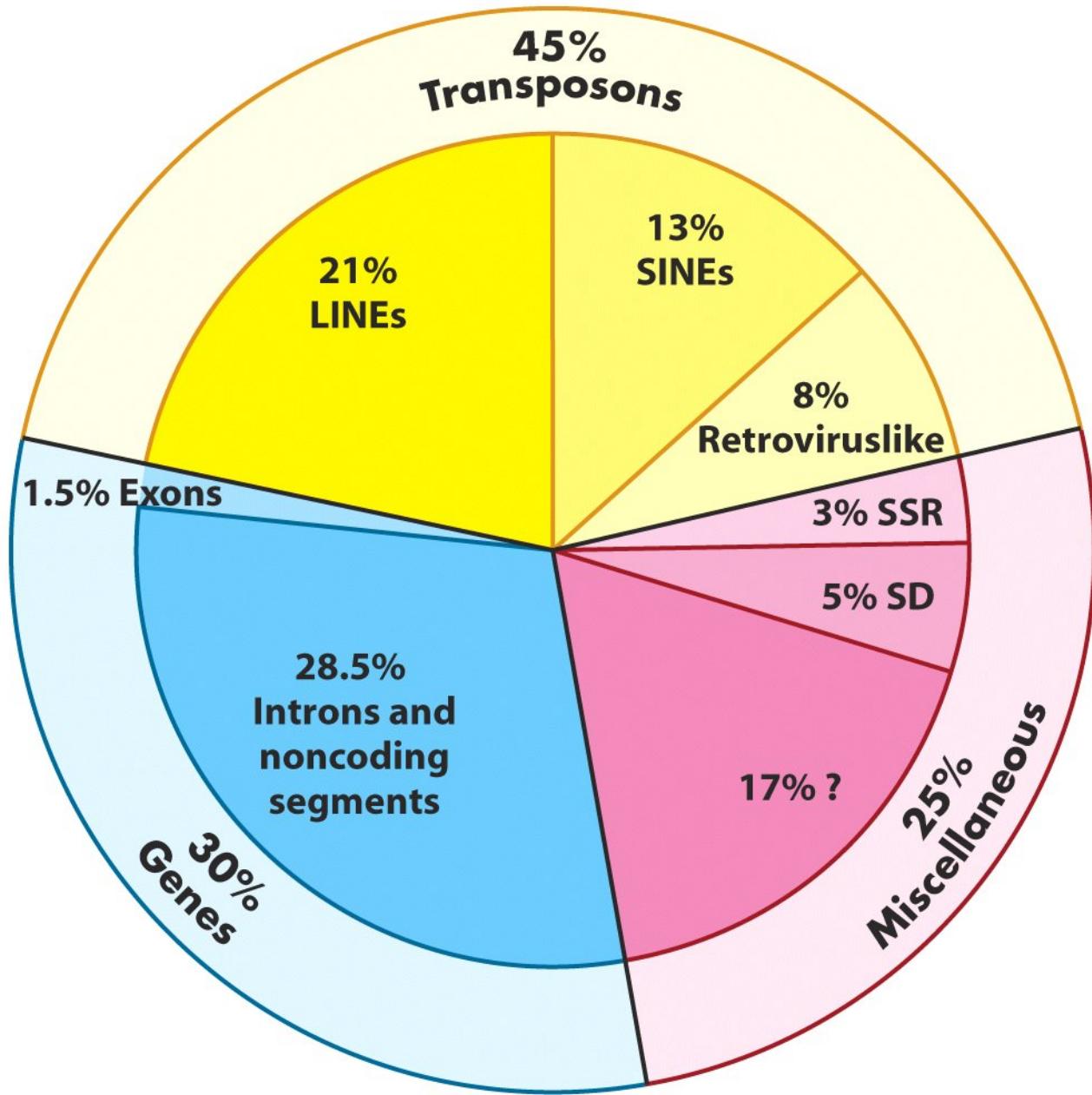
<sup>‡</sup>Number for females, with two X chromosomes. Males have an X but no Y, thus 11 chromosomes in all.

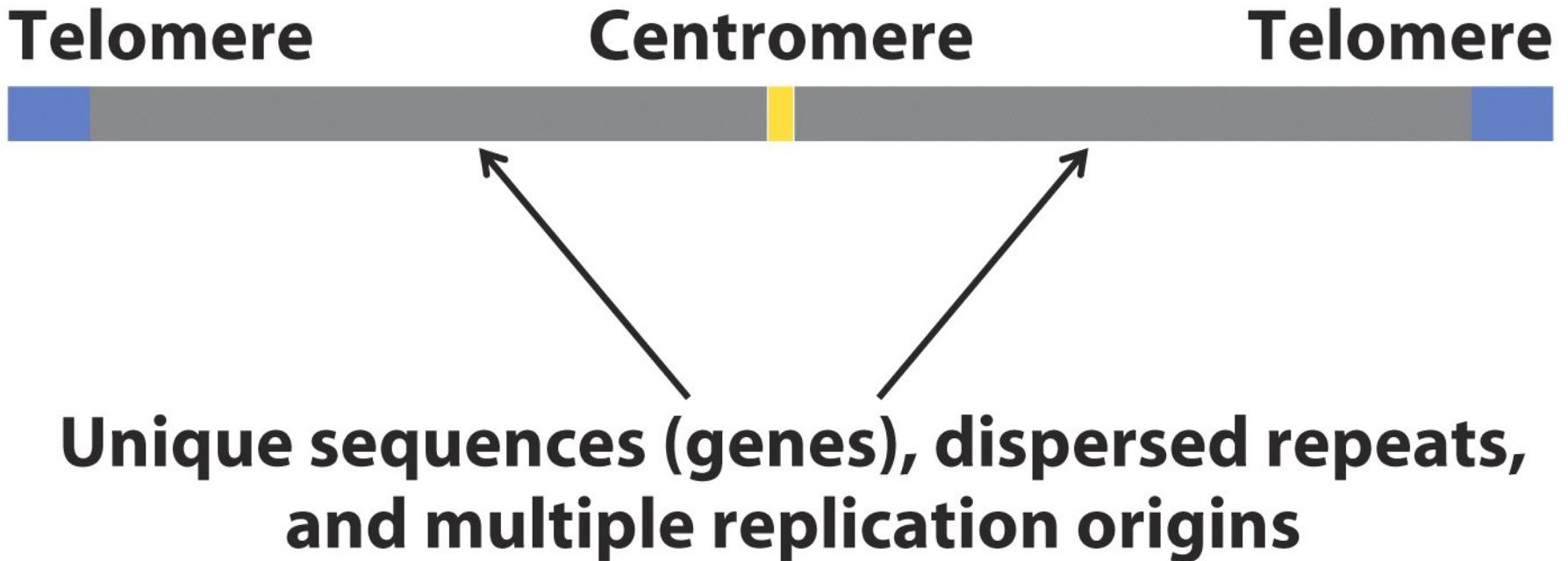
## Ovalbumin gene



## Hemoglobin $\beta$ subunit







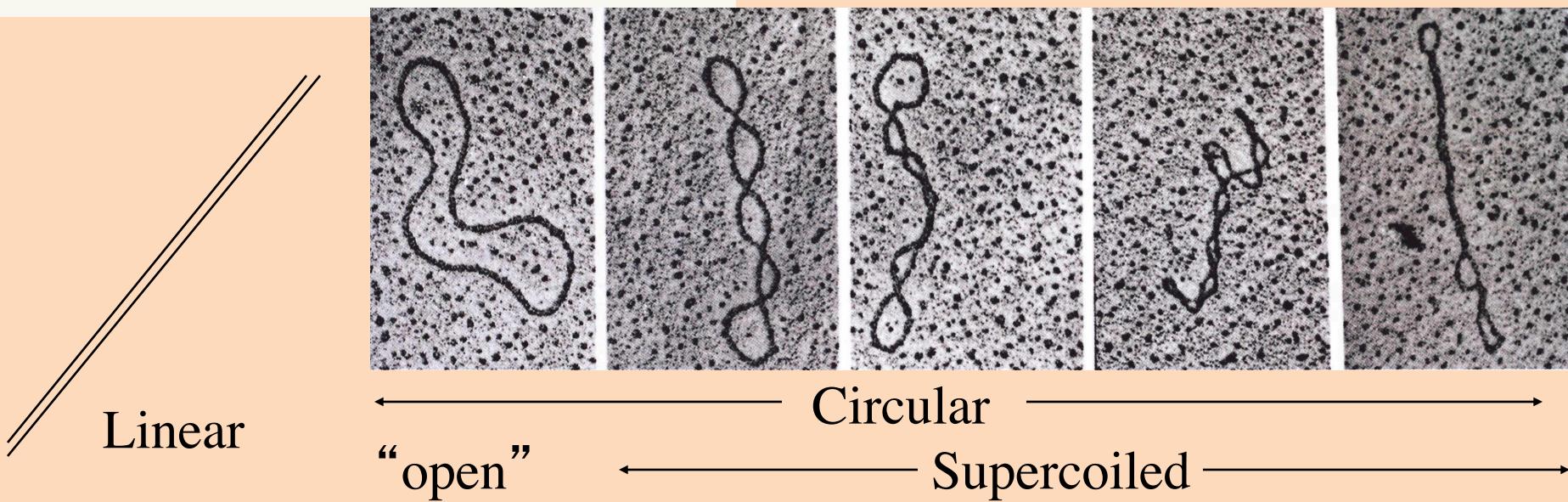
# DNA topology

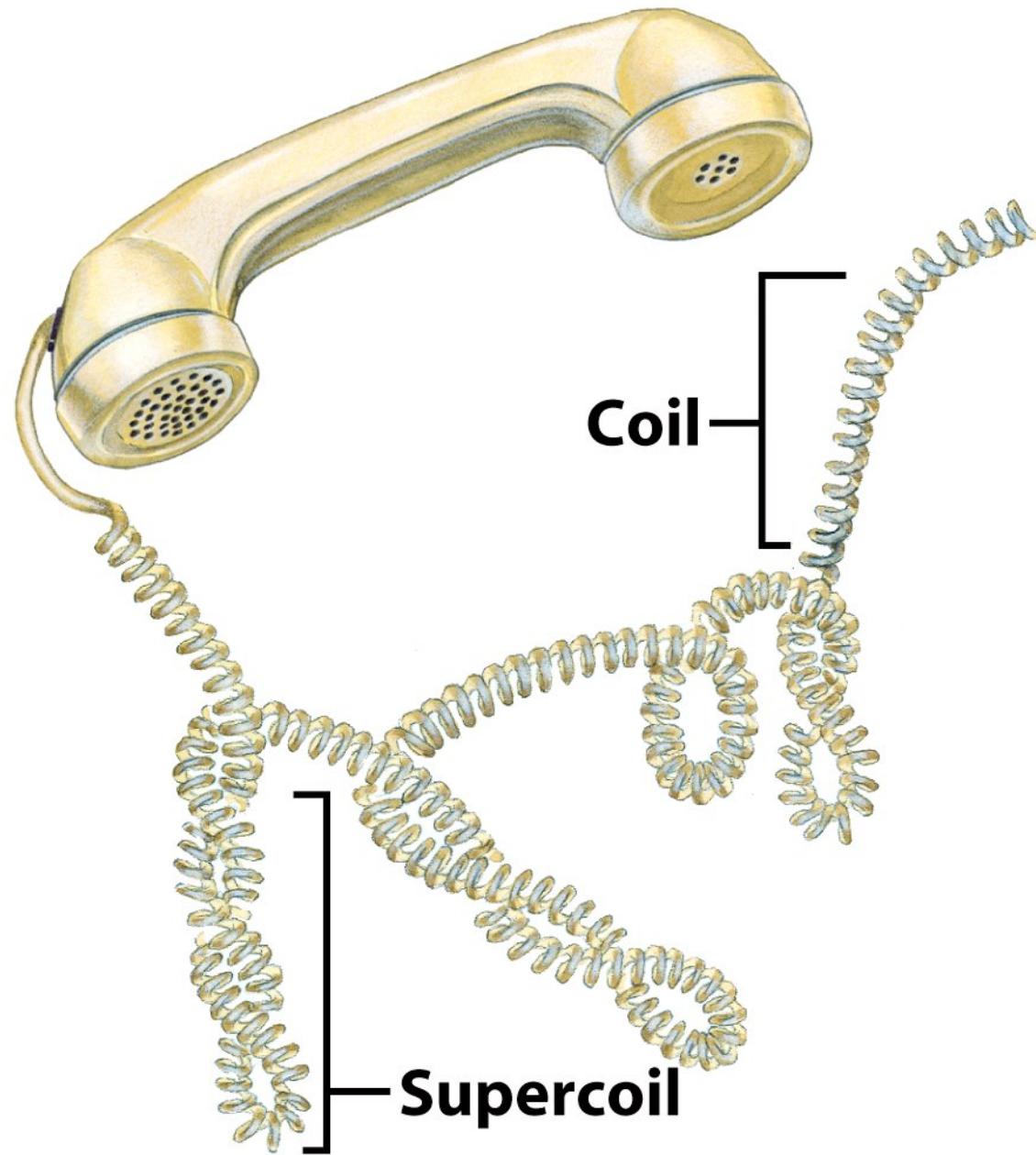
The same double-stranded DNA molecule can have different conformations

Relative radius of gyration

Linear > circles > supercoils

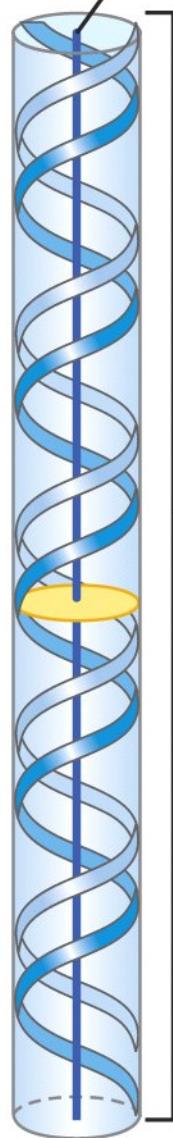
Frictional coefficient influences electrophoretic mobility and sedimentation rate.



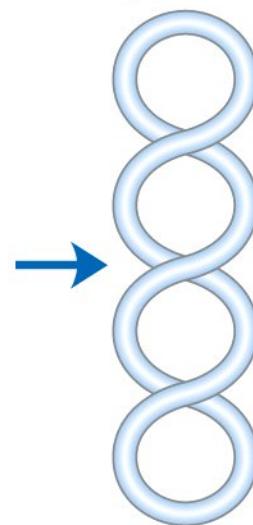


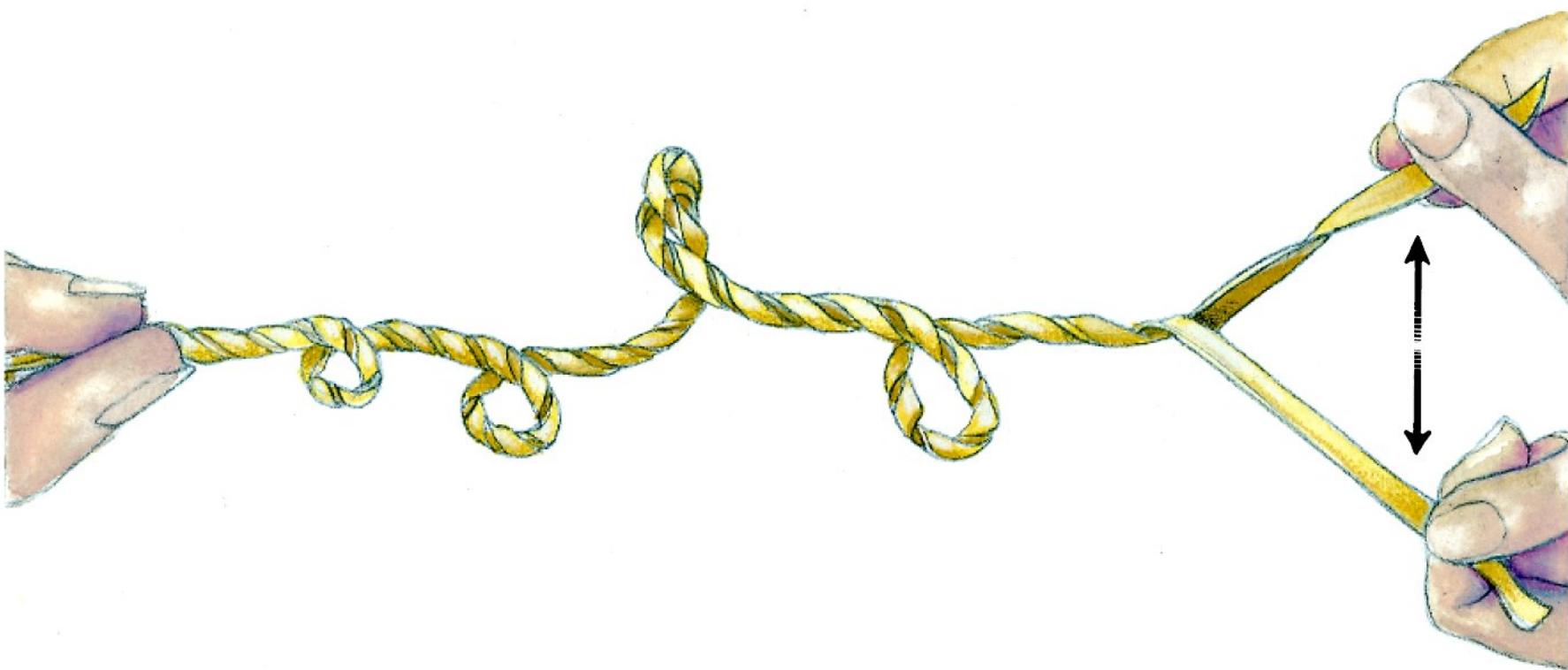
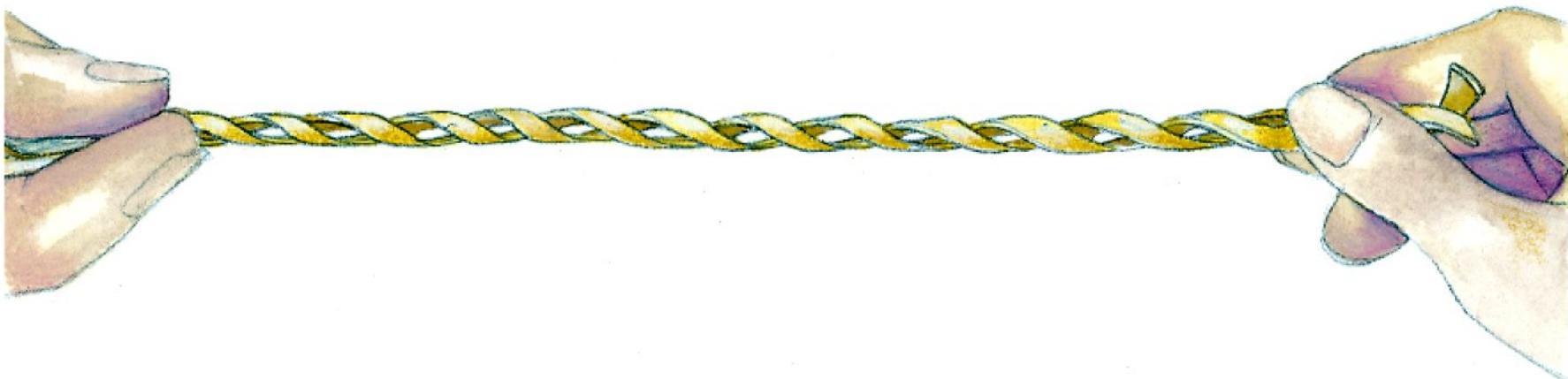
DNA double  
helix (coil)

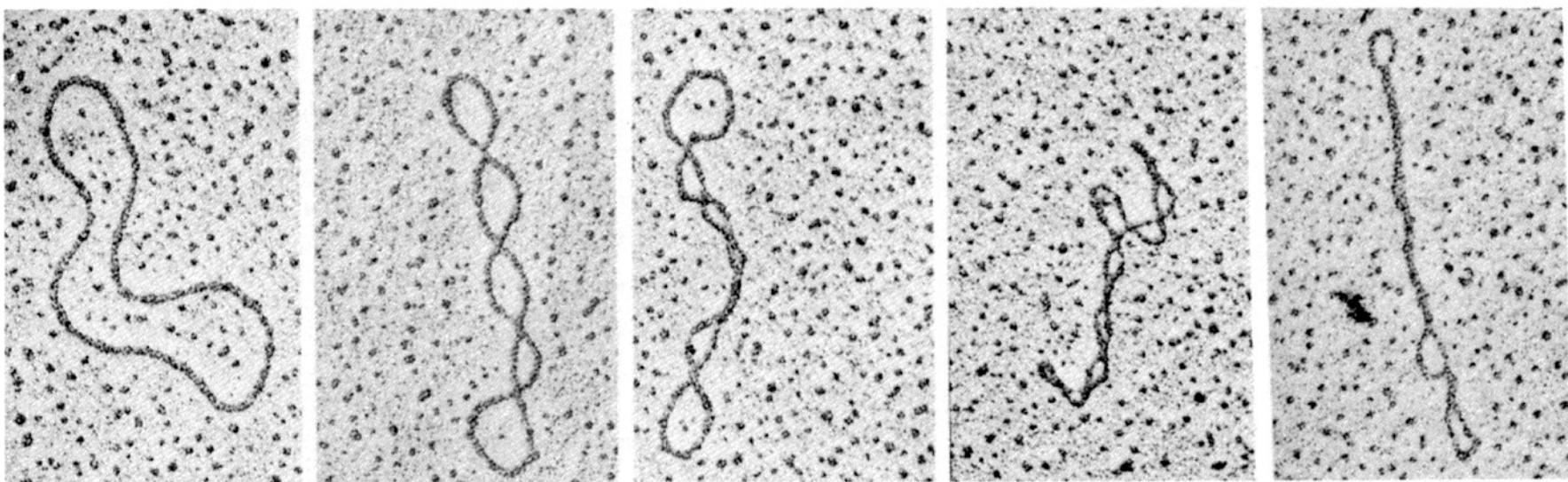
Axis



DNA  
supercoil

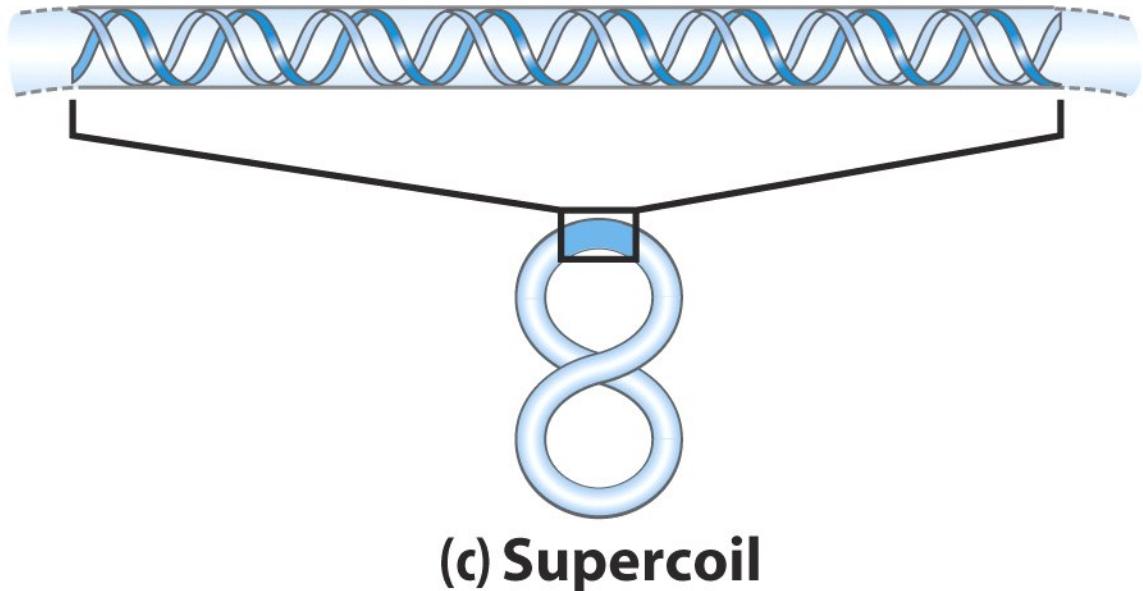
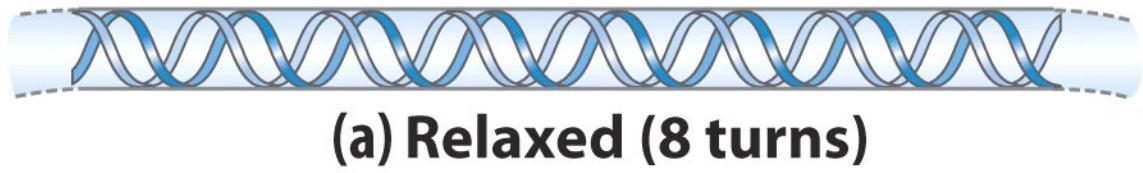


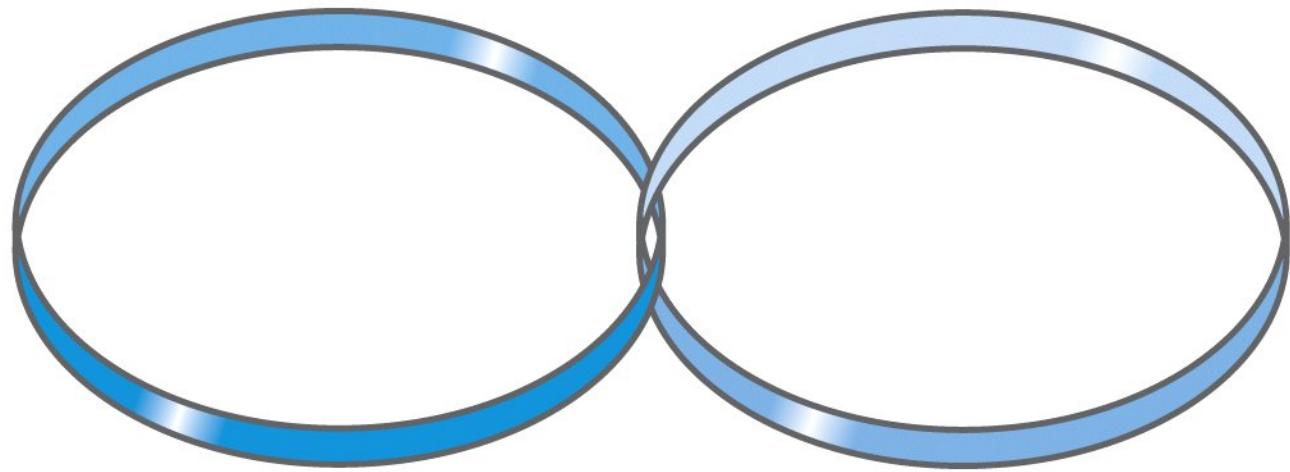




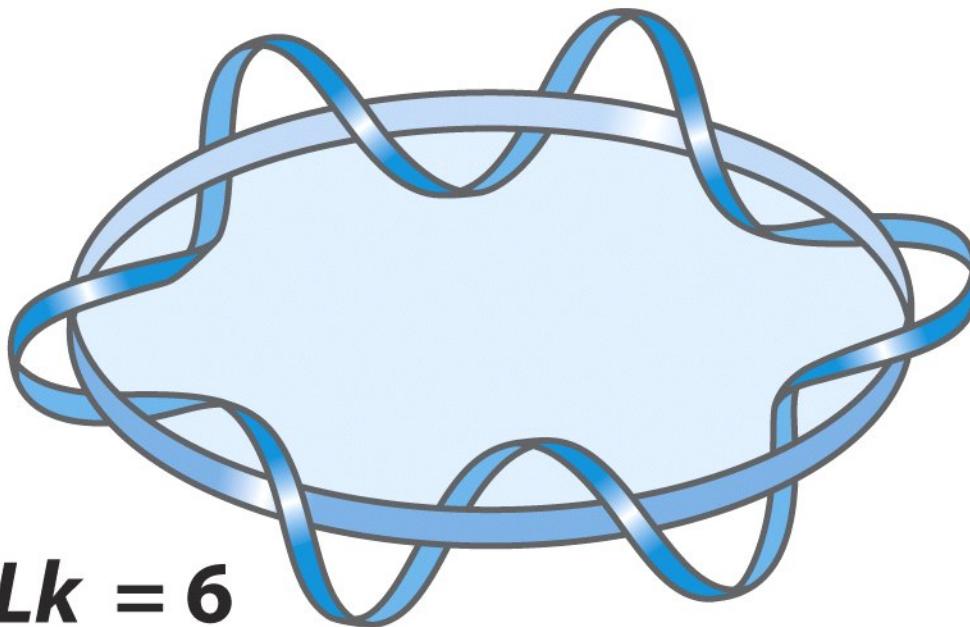
0.2  $\mu\text{m}$

84 bp at 10.5/turn

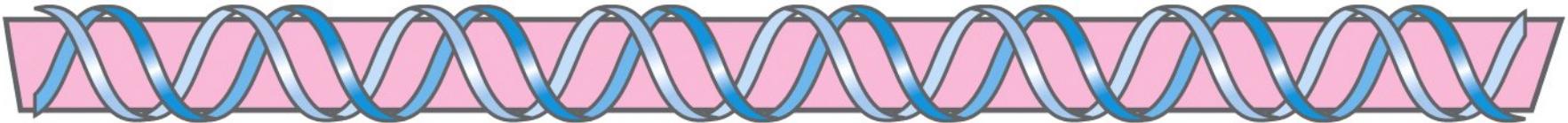




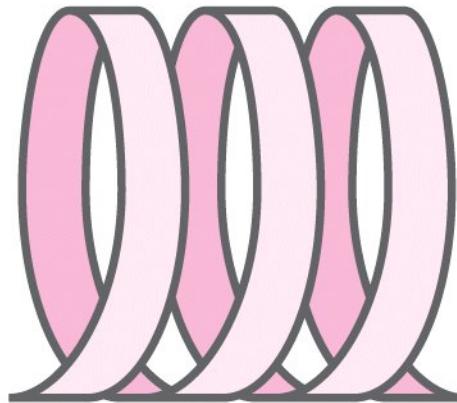
**(a)**  $Lk = 1$



**(b)**  $Lk = 6$



**Straight ribbon (relaxed DNA)**



**Large writhe, small change in twist**



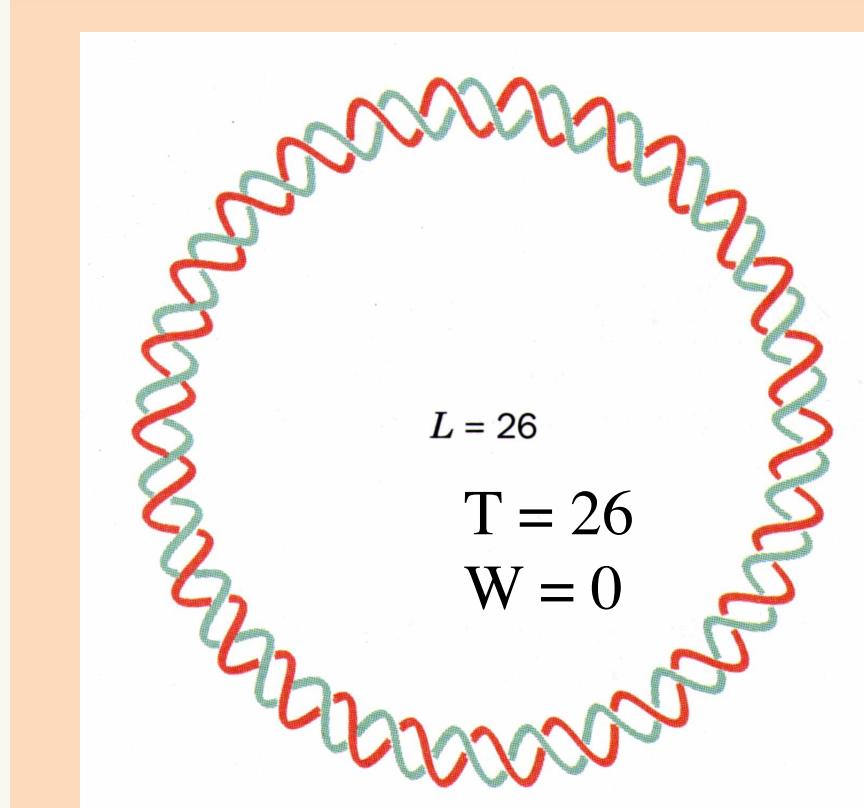
**Zero writhe, large change in twist**

# L, T, and W characterize superhelical DNA

L = *linking number* = number of times one strand wraps around the other. It is *an integer* for a closed circular DNA.

T = twists/turns in the DNA (No. bp/10.4; *positive* for right-handed DNA)

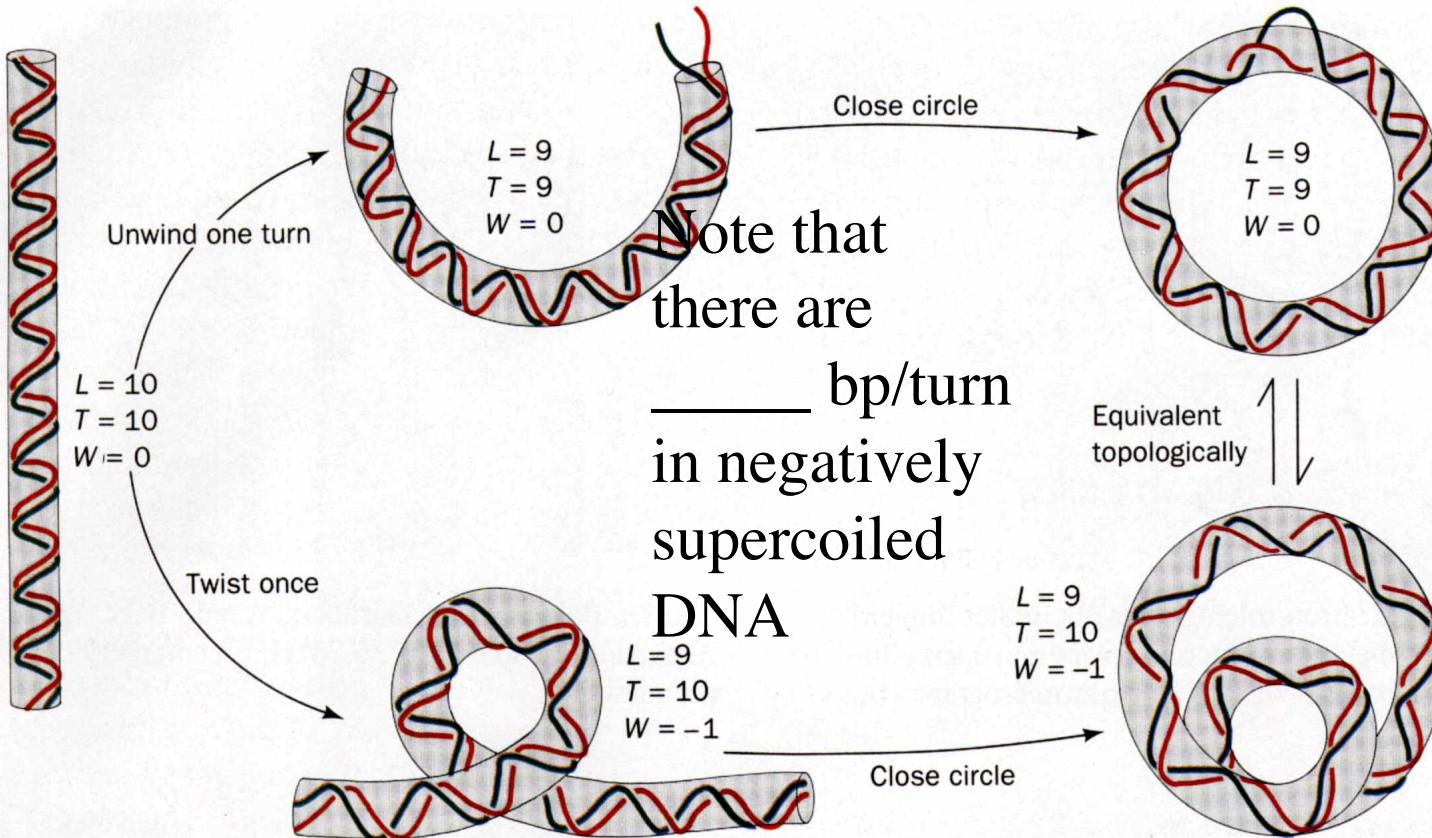
W = writhes = number of turns of the *helix* around the superhelical axis

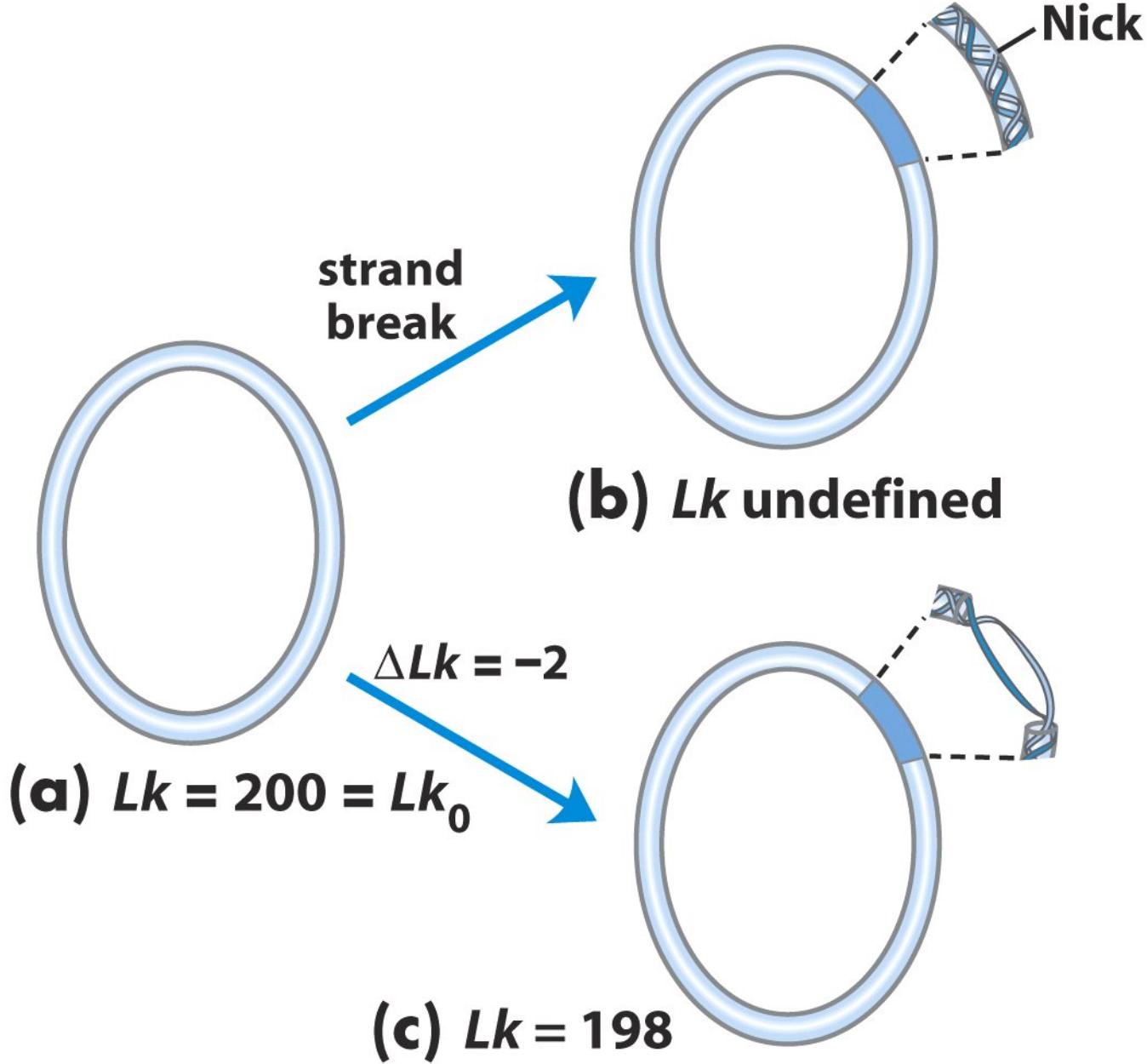


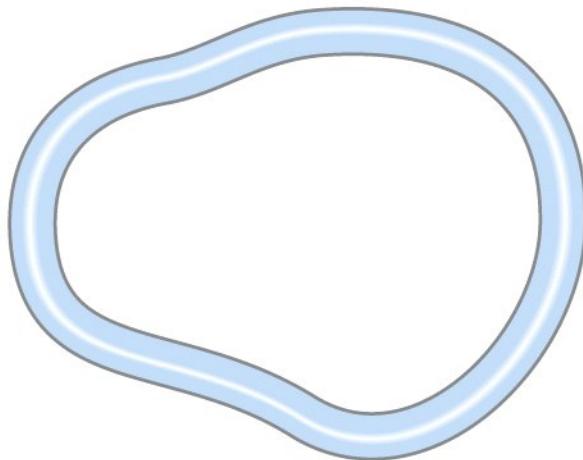
$$L = T + W$$

What kind of number is L??

# Naturally occurring superhelical DNA is underwound







**Relaxed DNA**

$$Lk = 200$$

$$\Delta Lk = -2$$



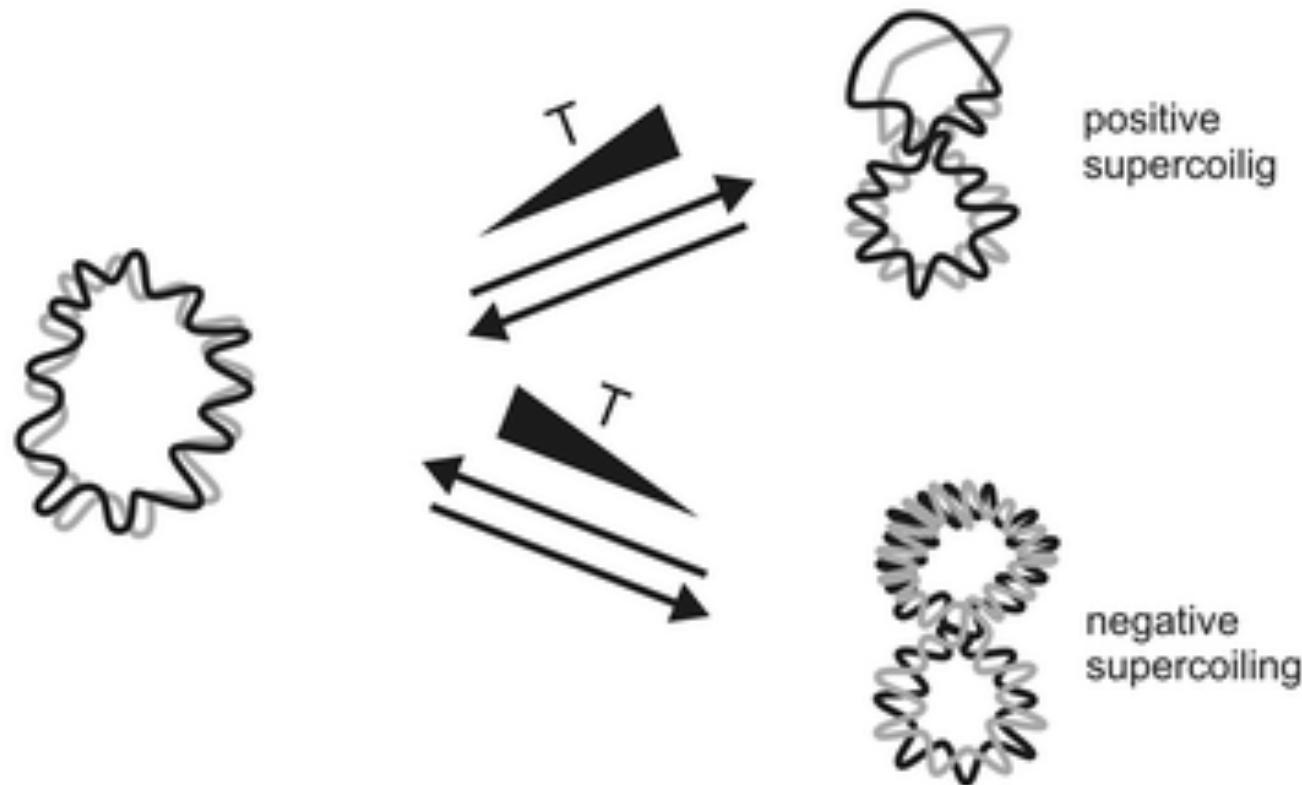
**Negative supercoils**  
 $Lk = 198$

$$\Delta Lk = +2$$



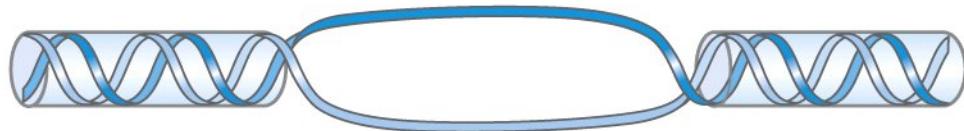
**Positive supercoils**  
 $Lk = 202$

## Temperature-dependent plasmid supercoiling

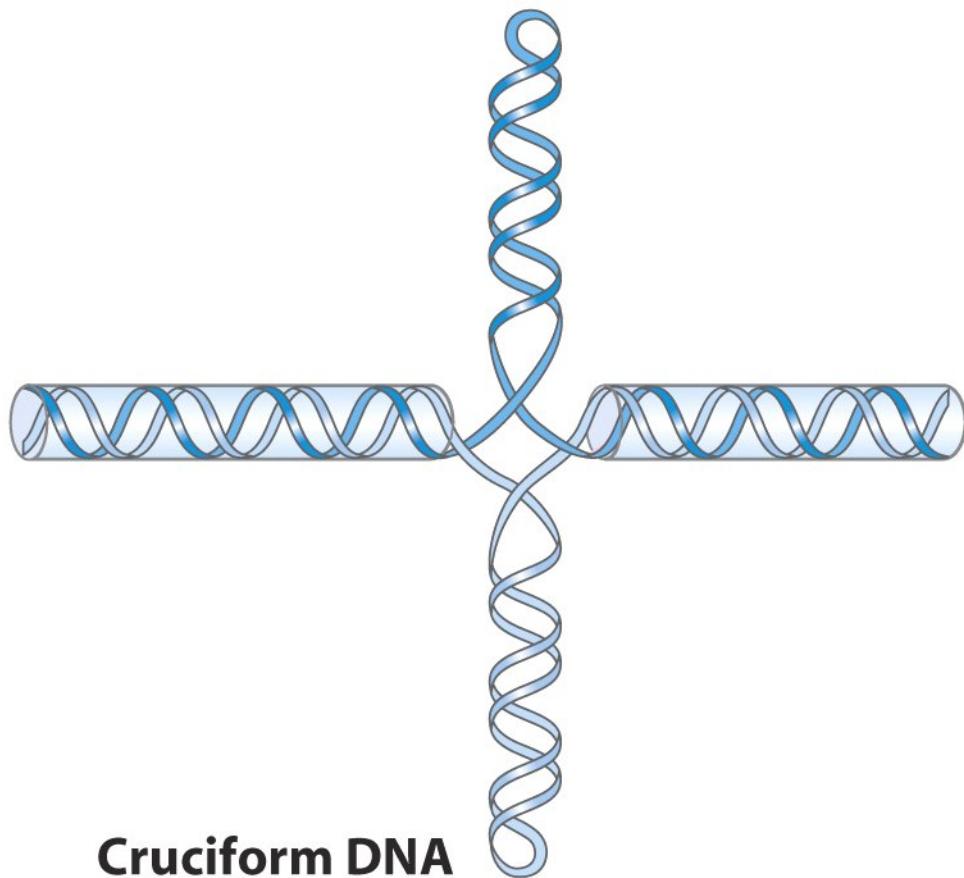




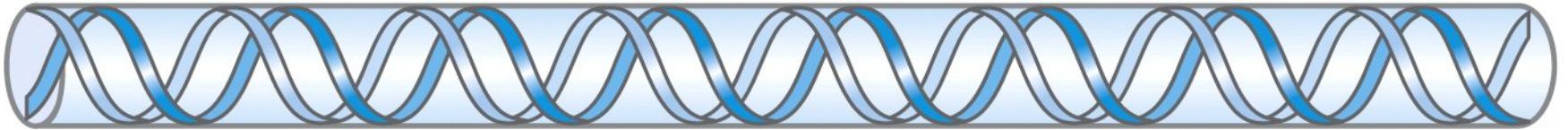
**Relaxed DNA**



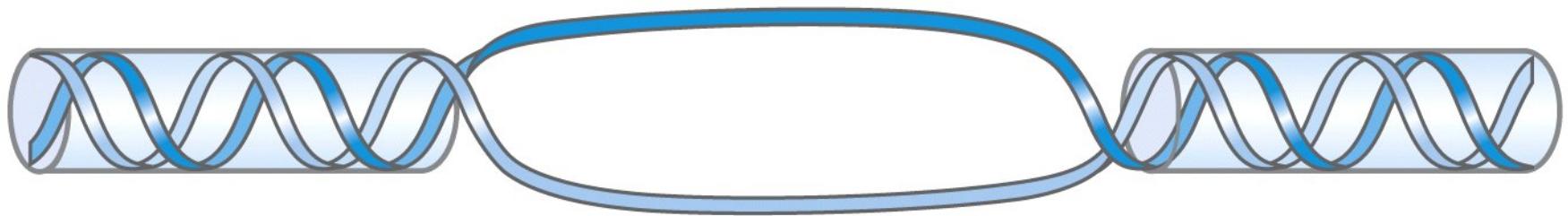
**Underwound DNA**



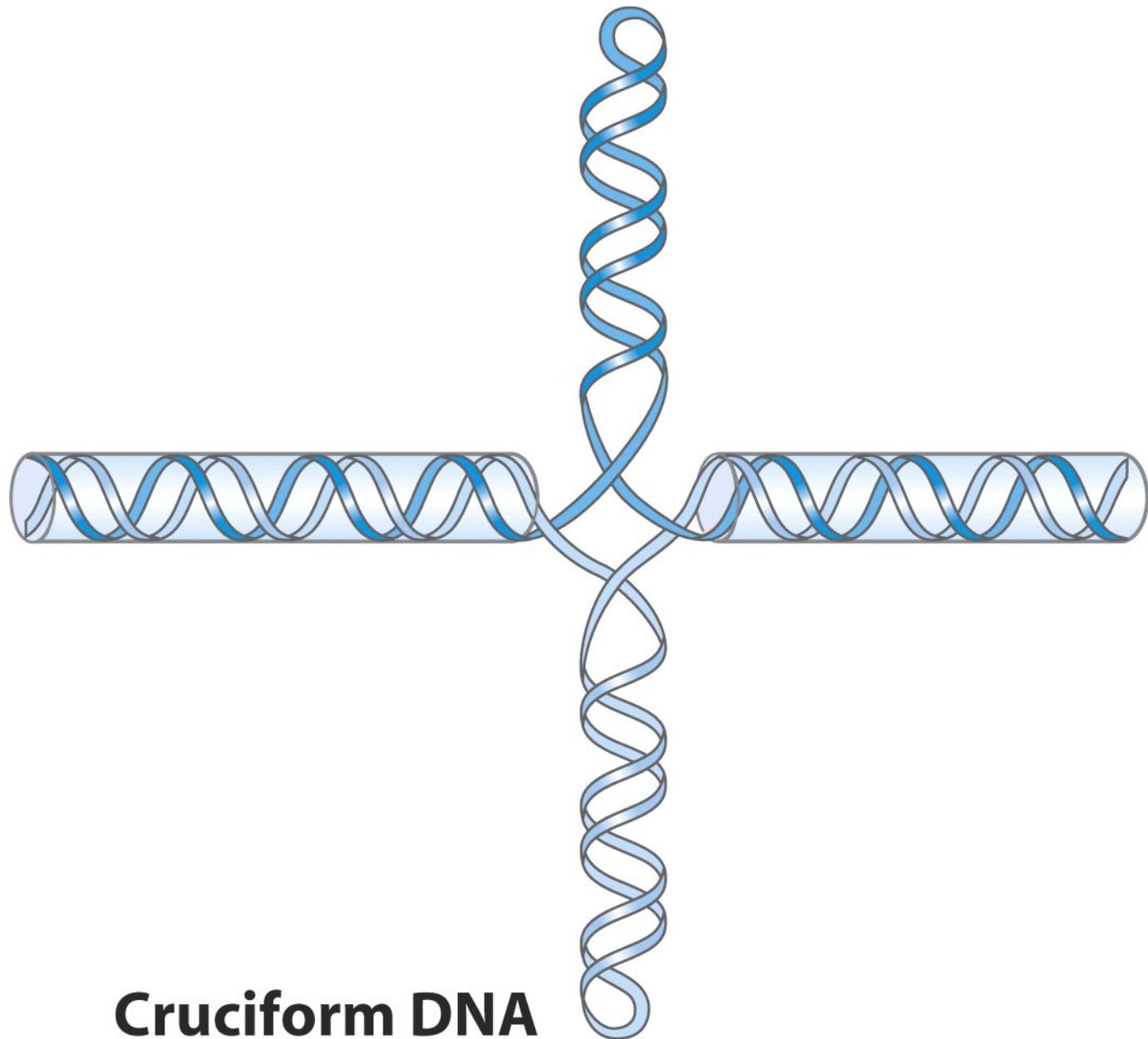
**Cruciform DNA**



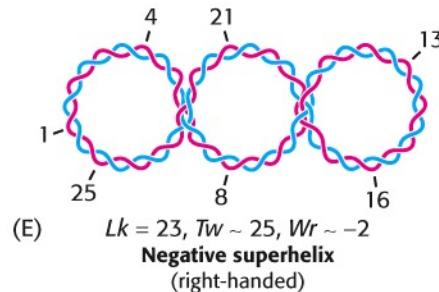
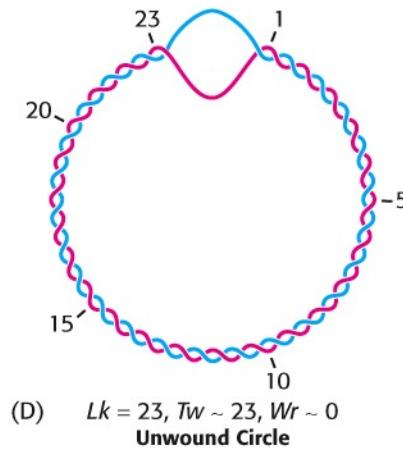
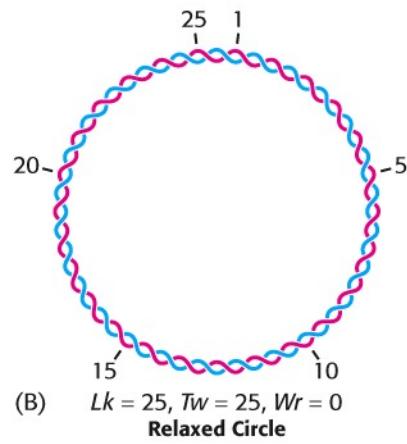
**Relaxed DNA**



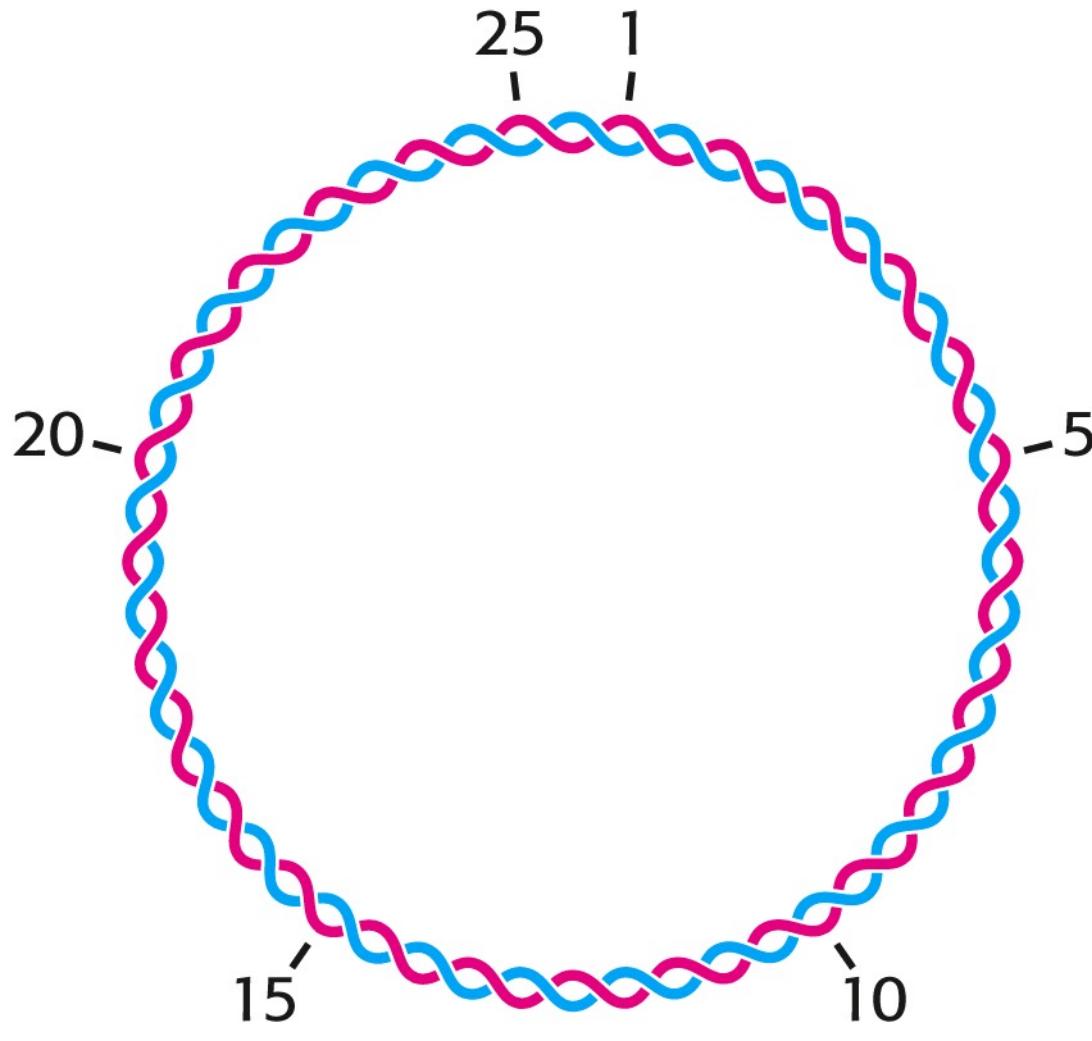
**Underwound DNA**



**Cruciform DNA**

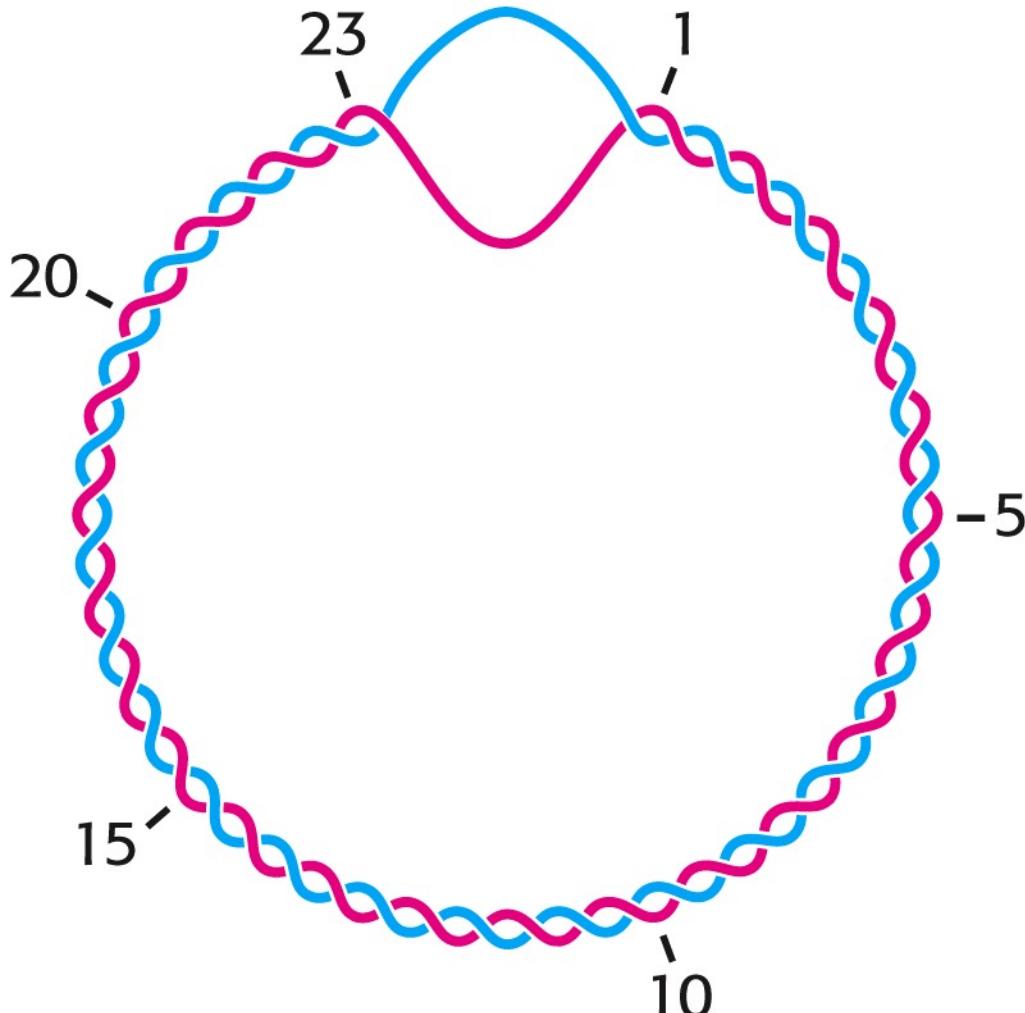




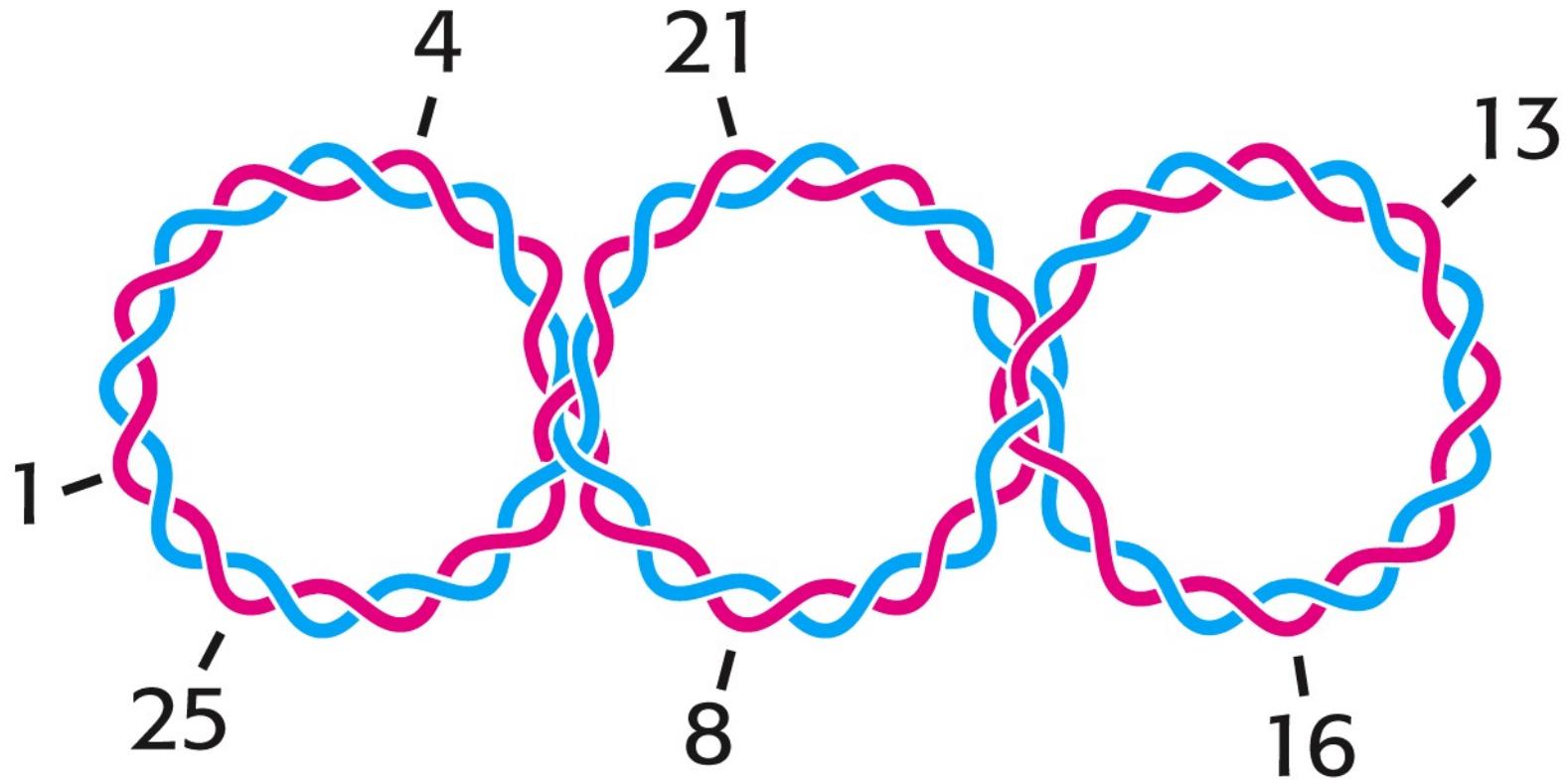


(B)  $Lk = 25$ ,  $Tw = 25$ ,  $Wr = 0$   
**Relaxed Circle**





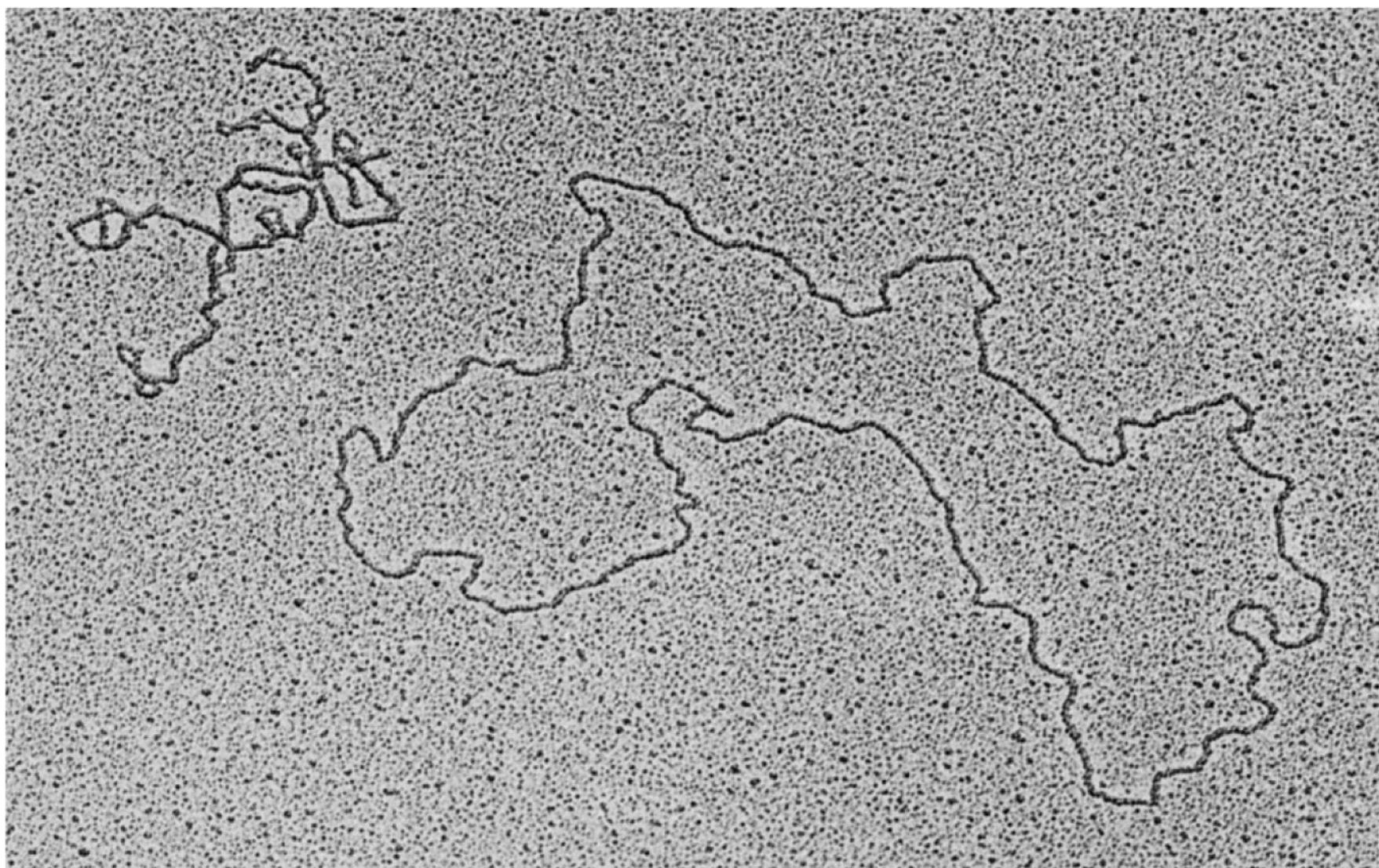
(D)       $Lk = 23$ ,  $Tw \sim 23$ ,  $Wr \sim 0$   
**Unwound Circle**



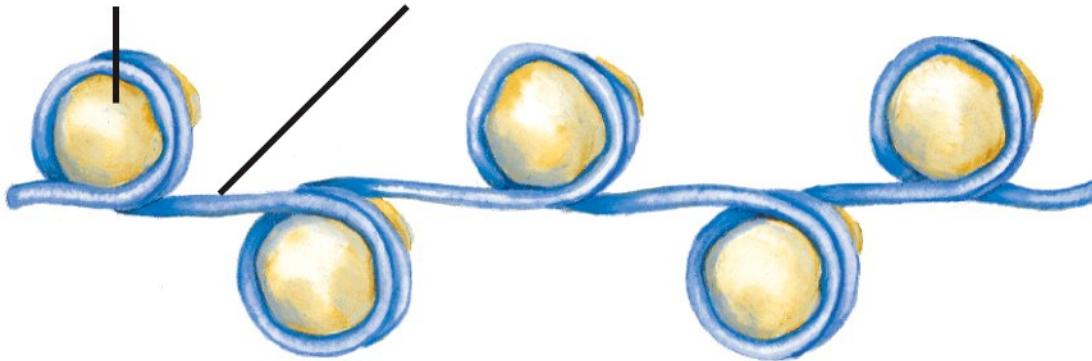
(E)

$$Lk = 23, Tw \sim 25, Wr \sim -2$$

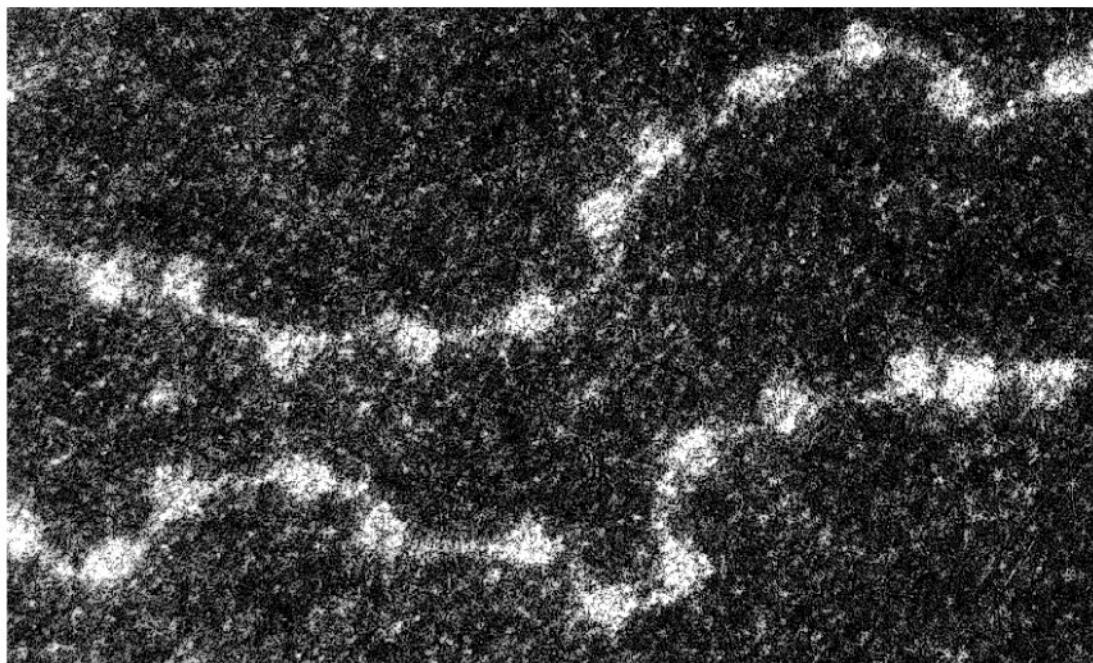
**Negative superhelix**  
(right-handed)



**Histone core  
of nucleosome**      **Linker DNA  
of nucleosome**



**(a)**

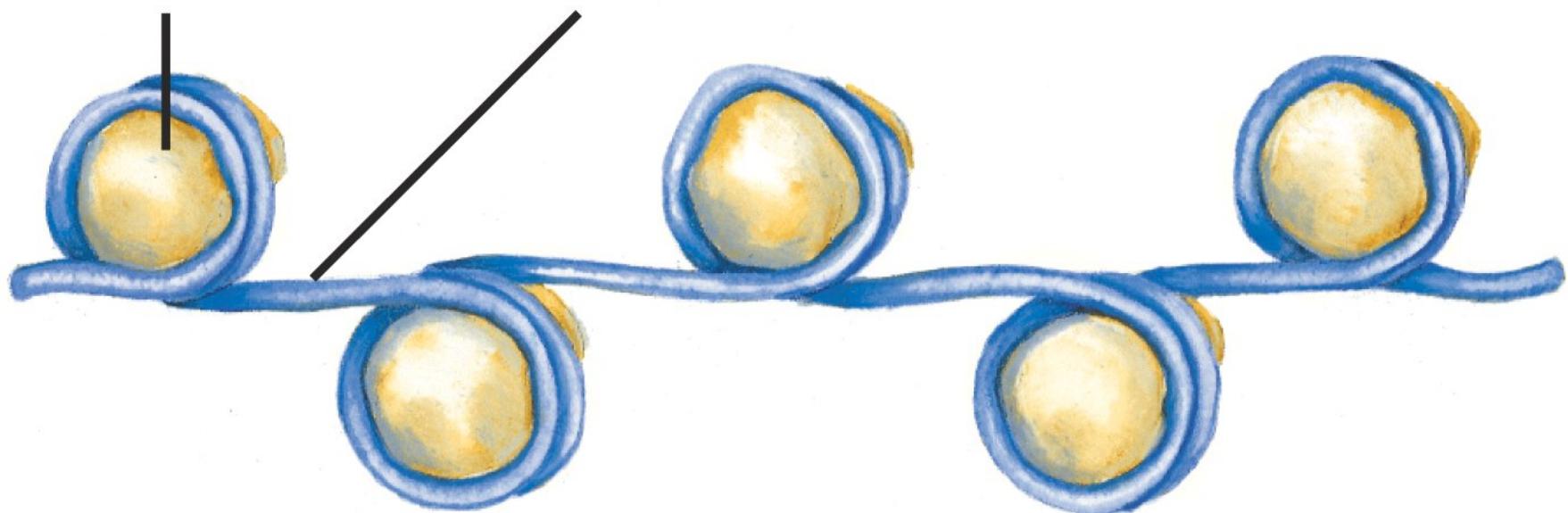


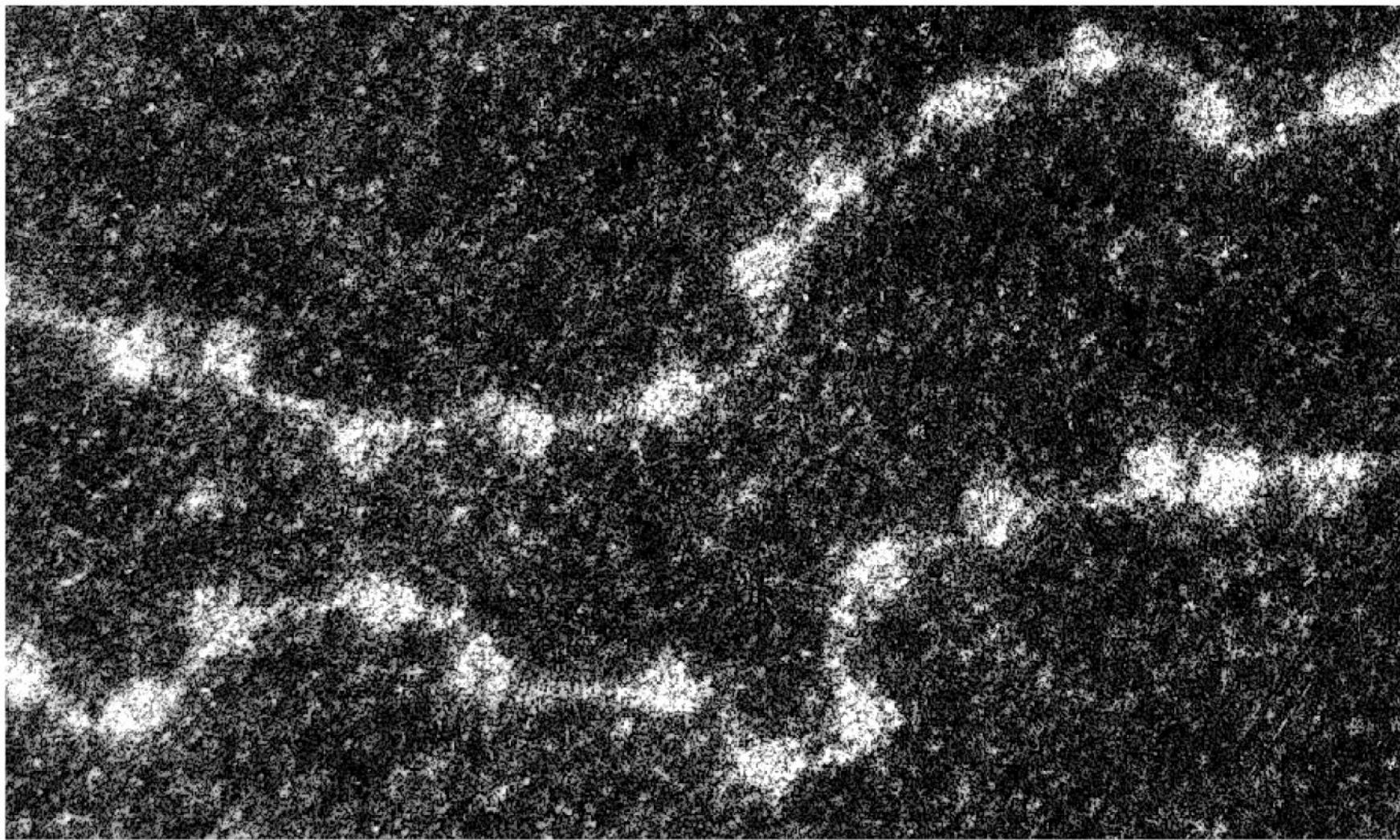
**(b)**

50 nm

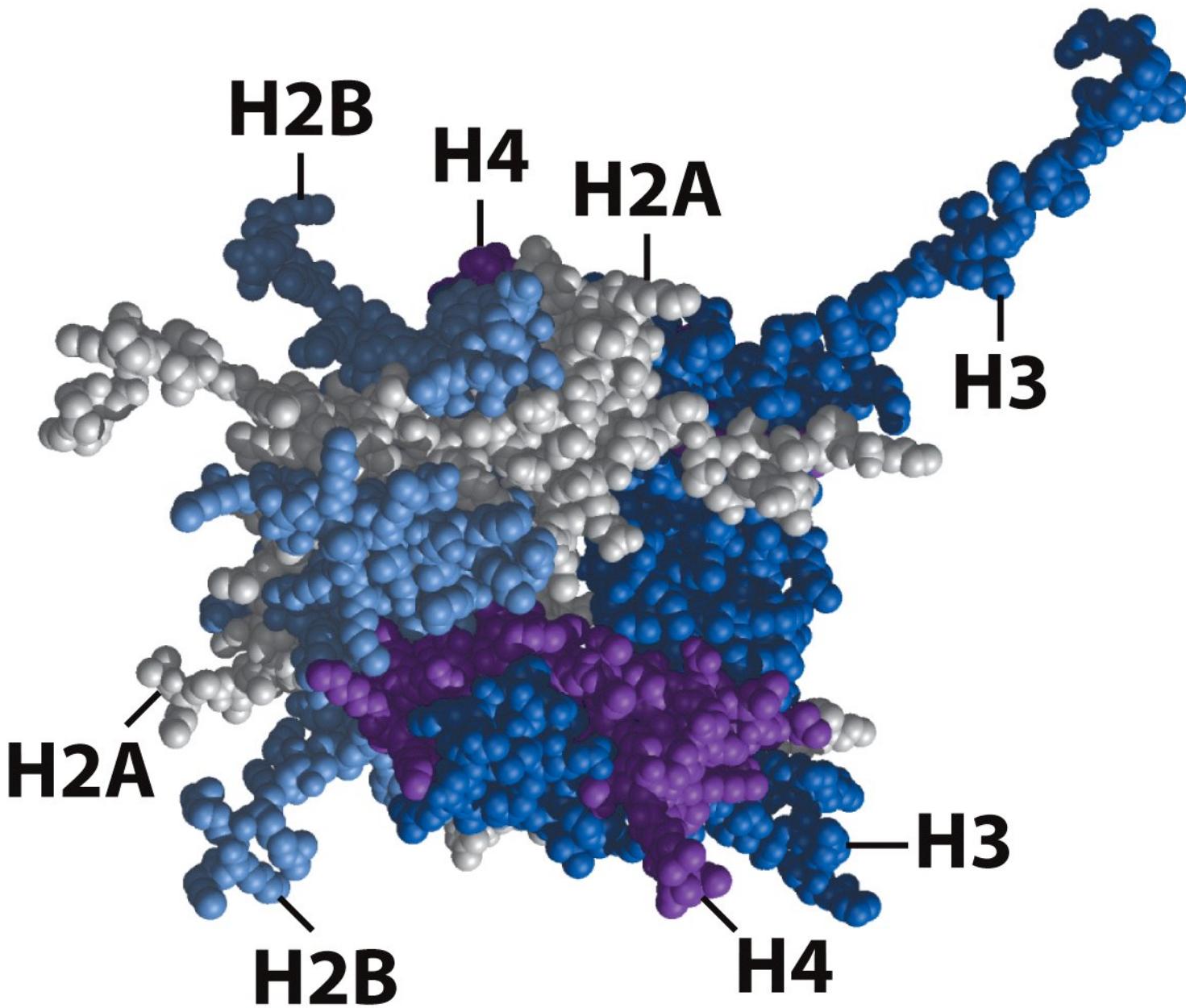
**Histone core  
of nucleosome**

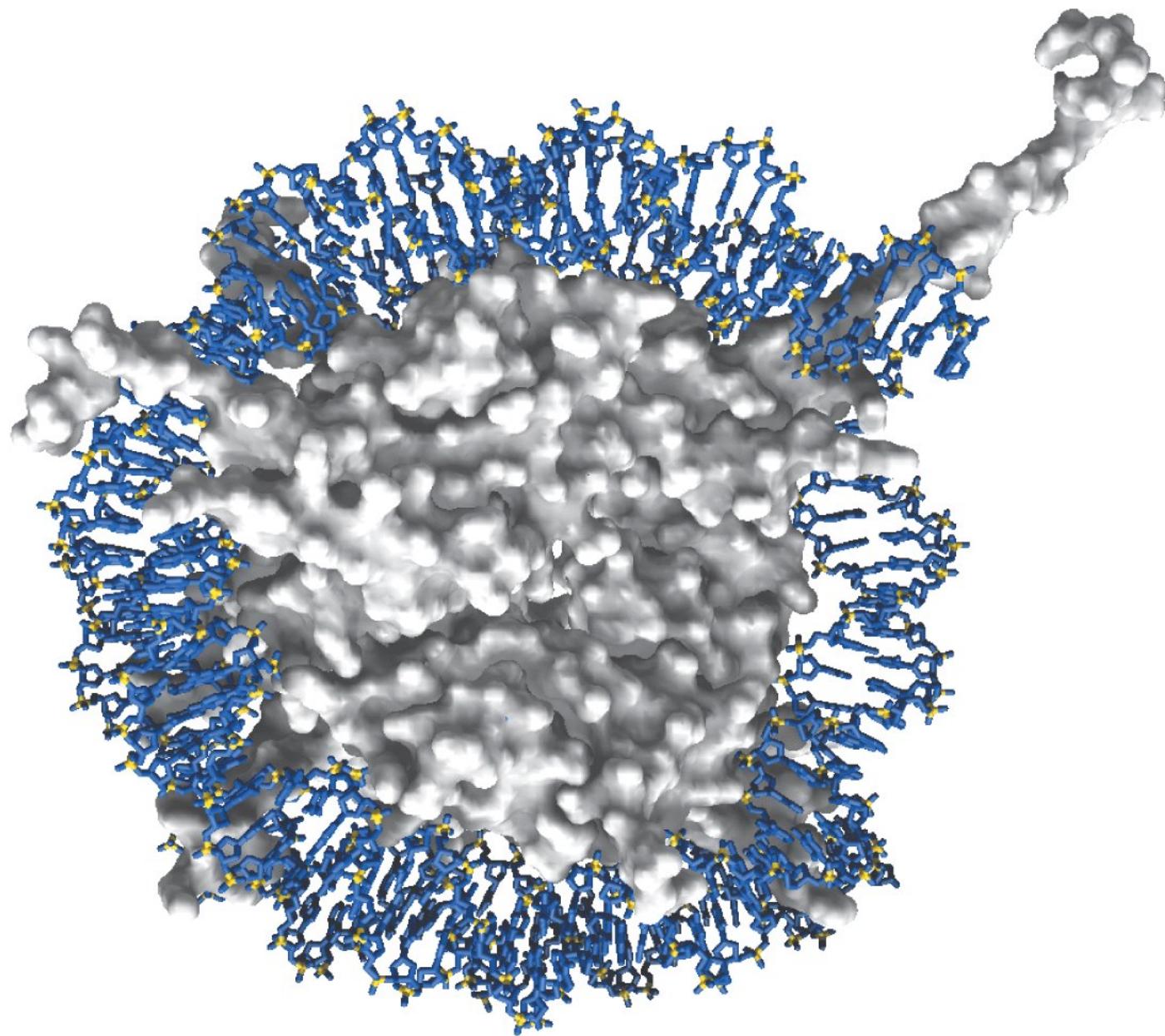
**Linker DNA  
of nucleosome**

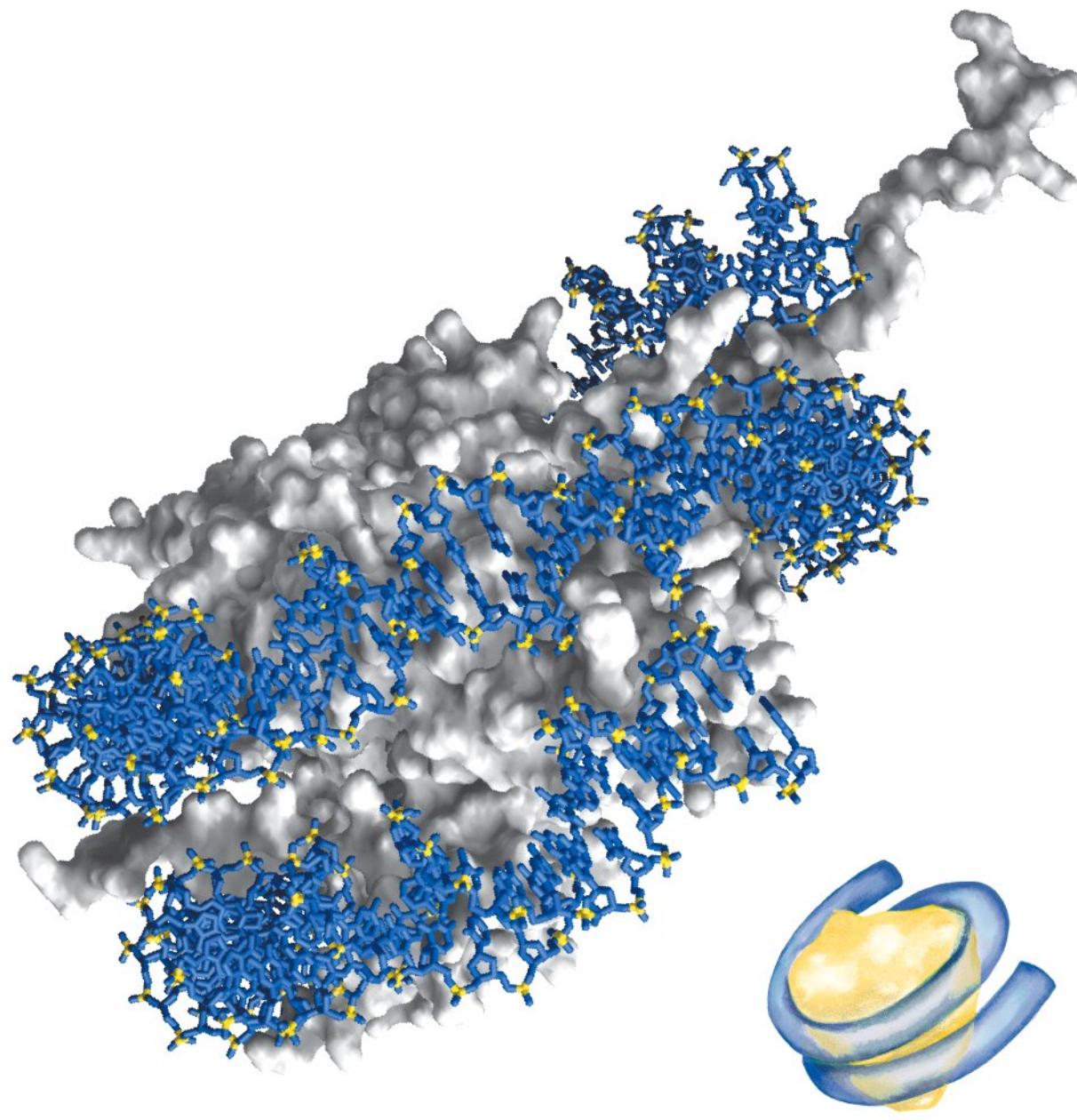




50 nm



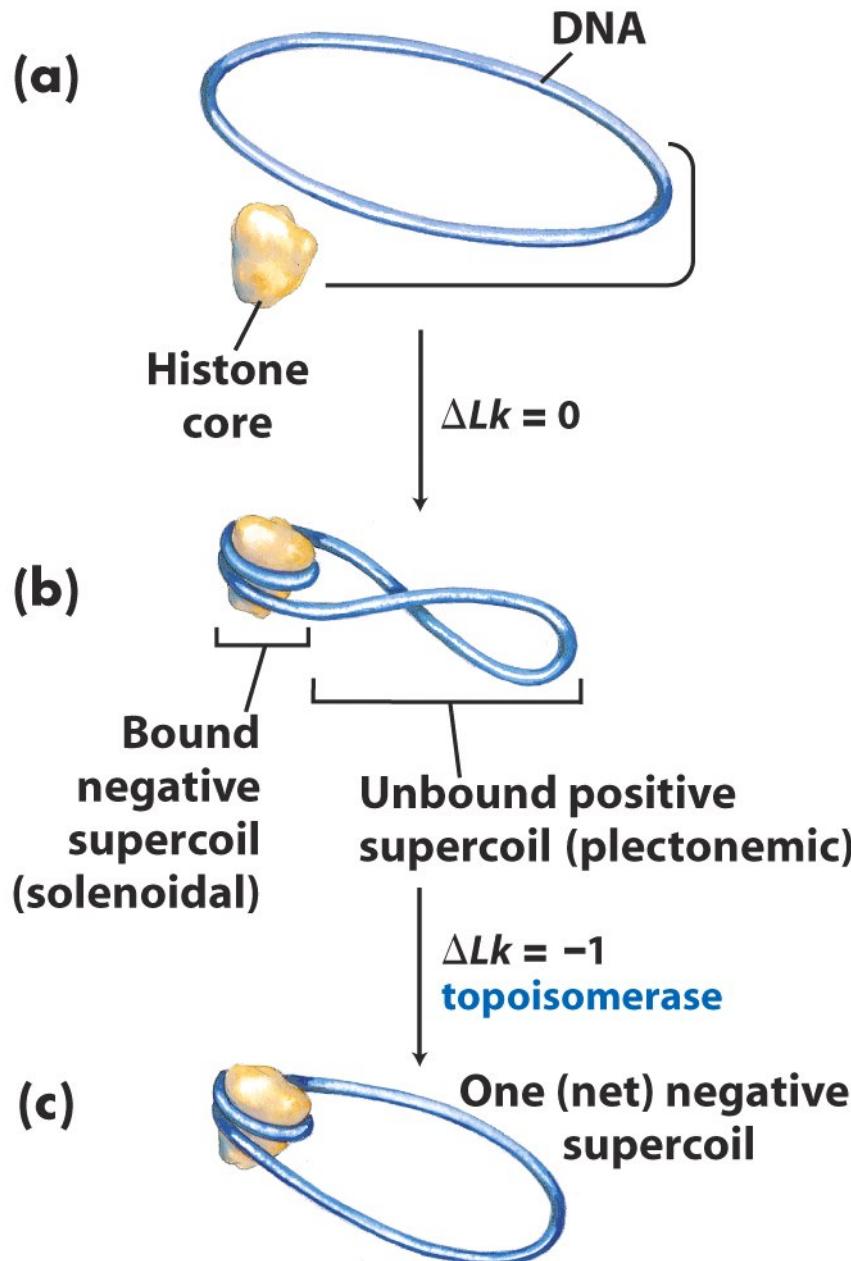


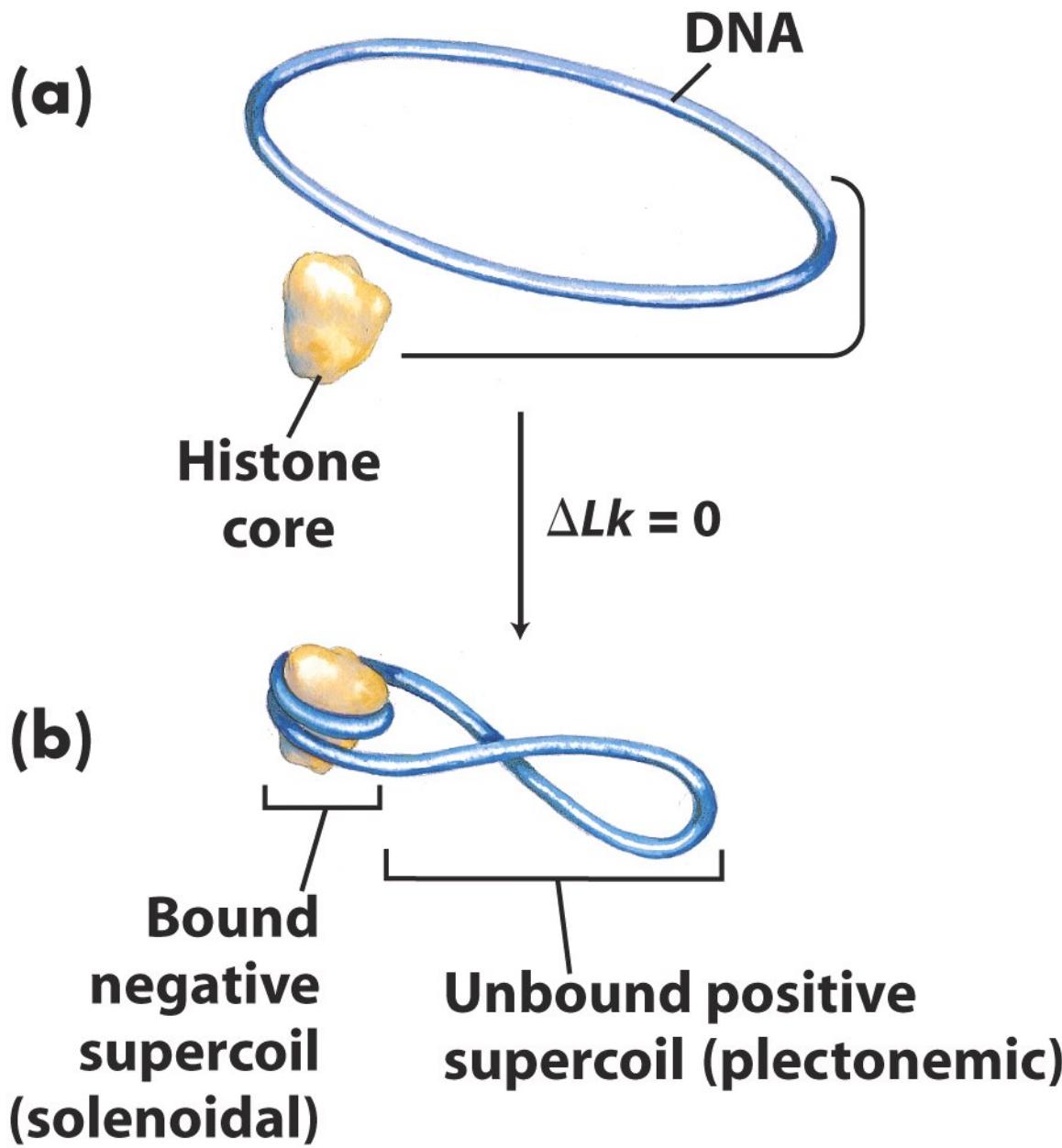


**TABLE 24–3** Types and Properties of Histones

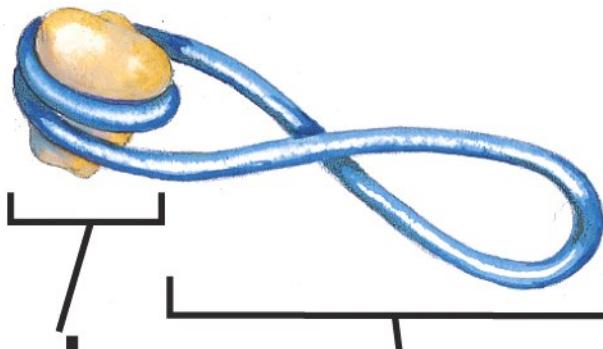
Histone	Molecular weight	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1*	21,130	223	29.5	11.3
H2A*	13,960	129	10.9	19.3
H2B*	13,774	125	16.0	16.4
H3	15,273	135	19.6	13.3
H4	11,236	102	10.8	13.7

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





(b)

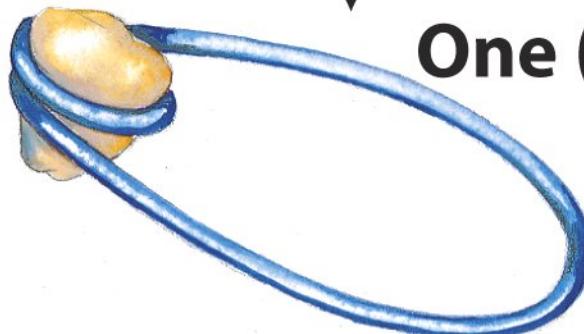


**Bound  
negative  
supercoil  
(solenoidal)**

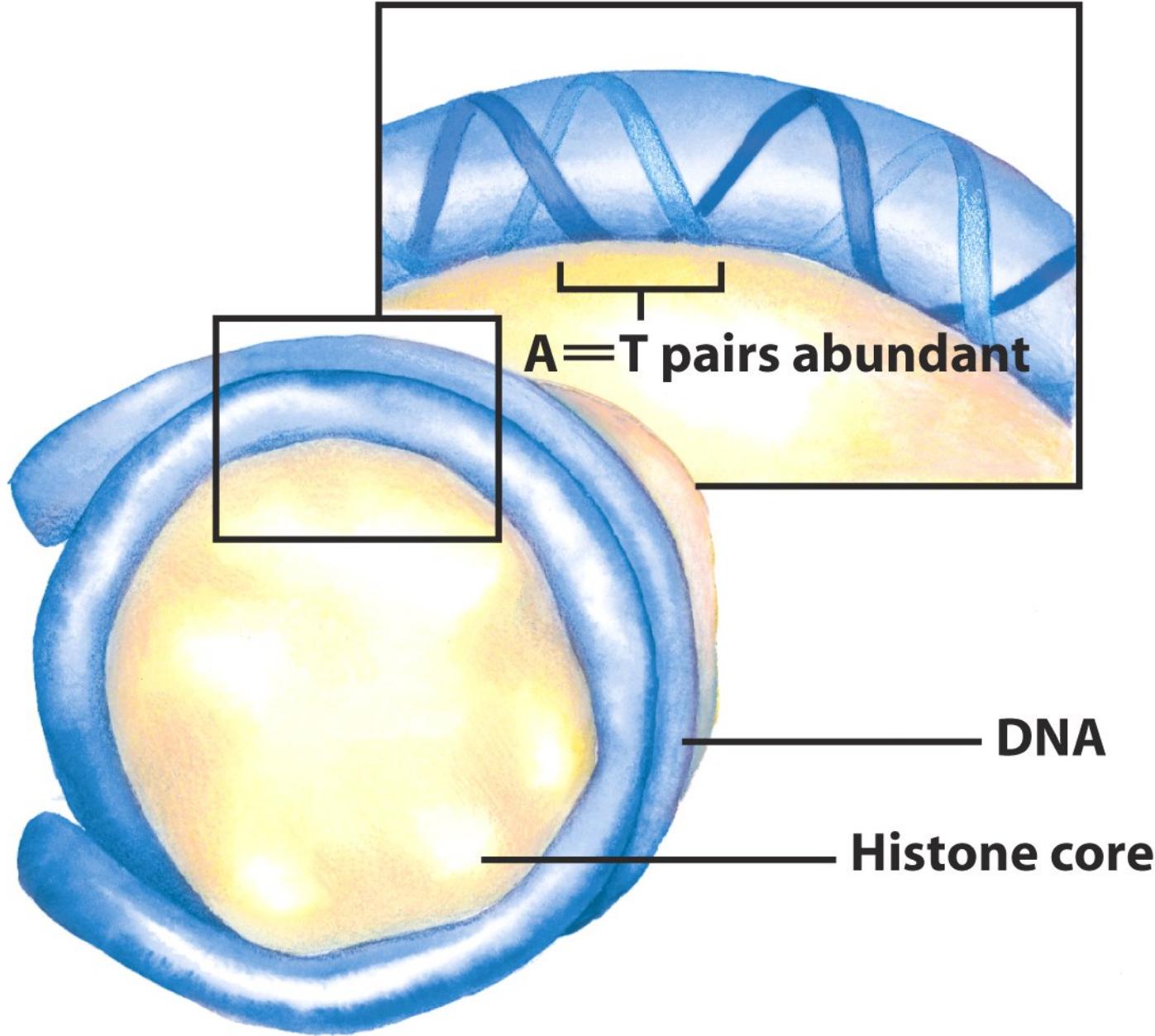
**Unbound positive  
supercoil (plectonemic)**

$\Delta Lk = -1$   
**topoisomerase**

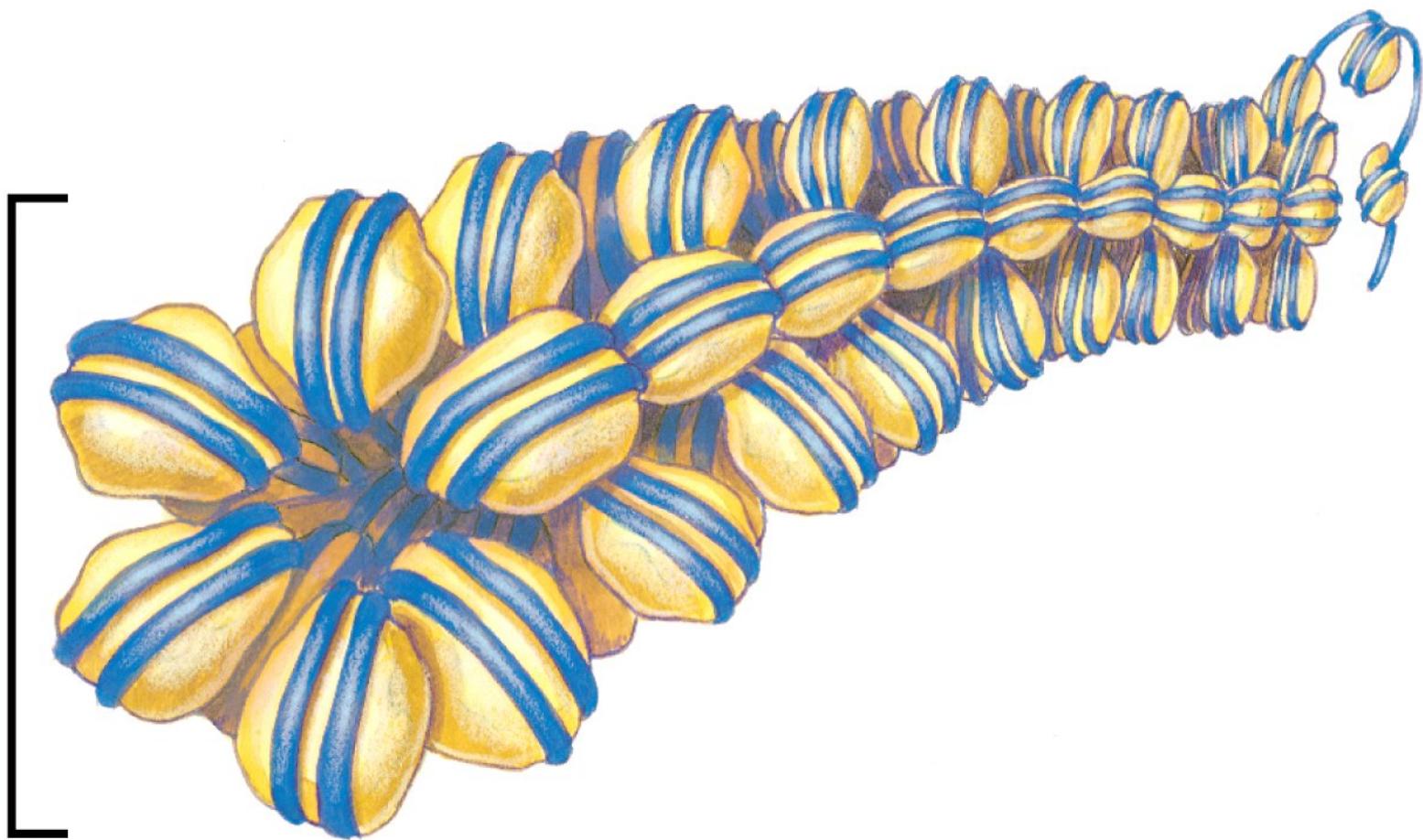
(c)

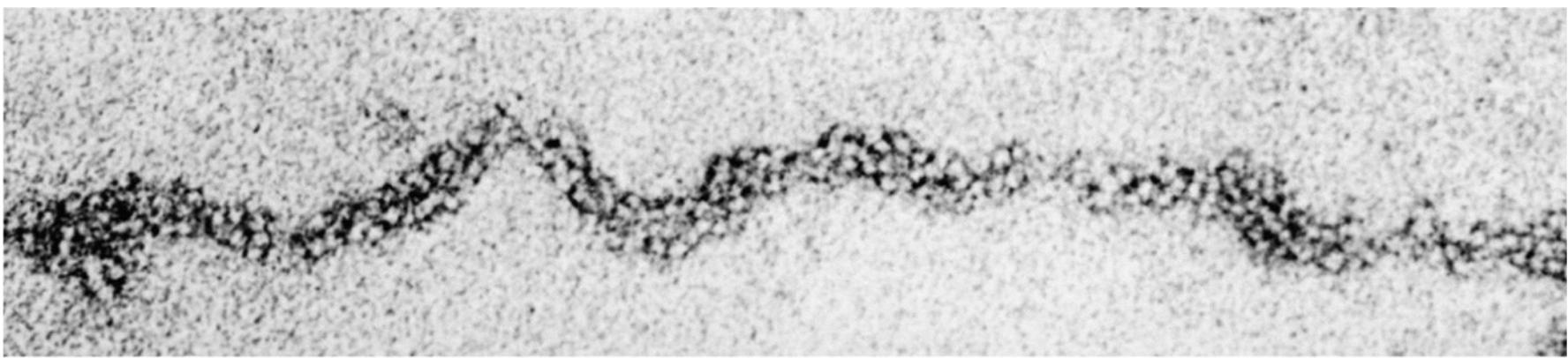


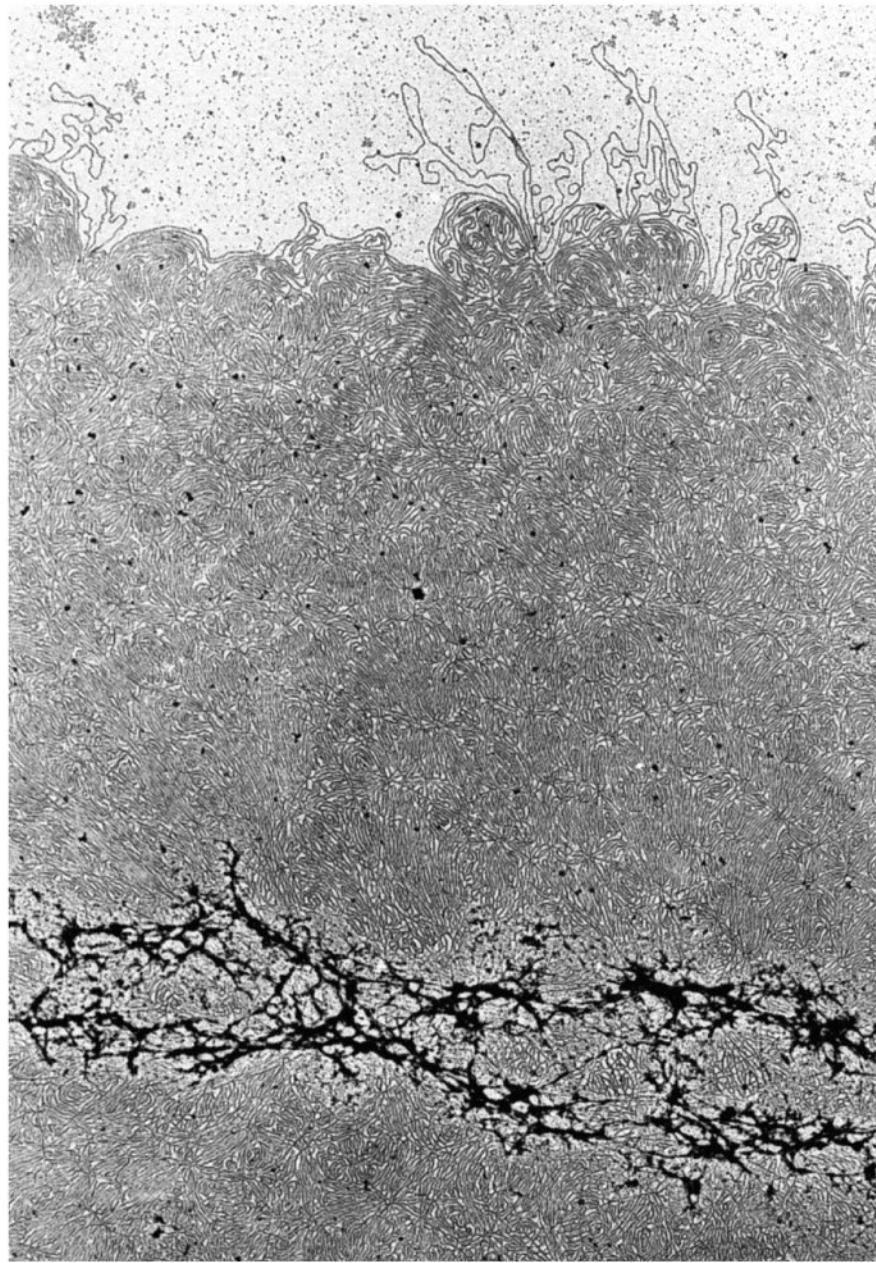
**One (net) negative  
supercoil**

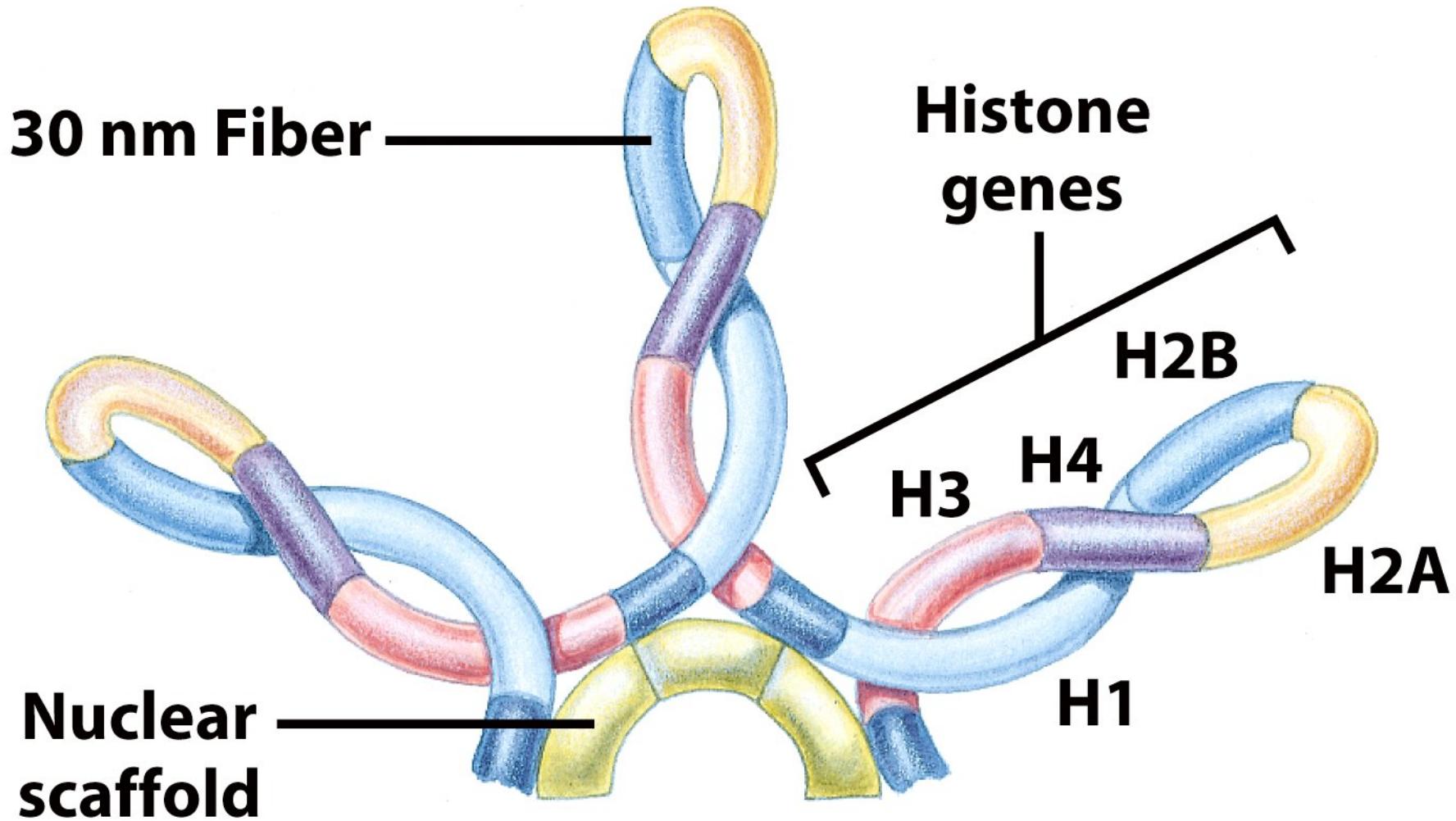


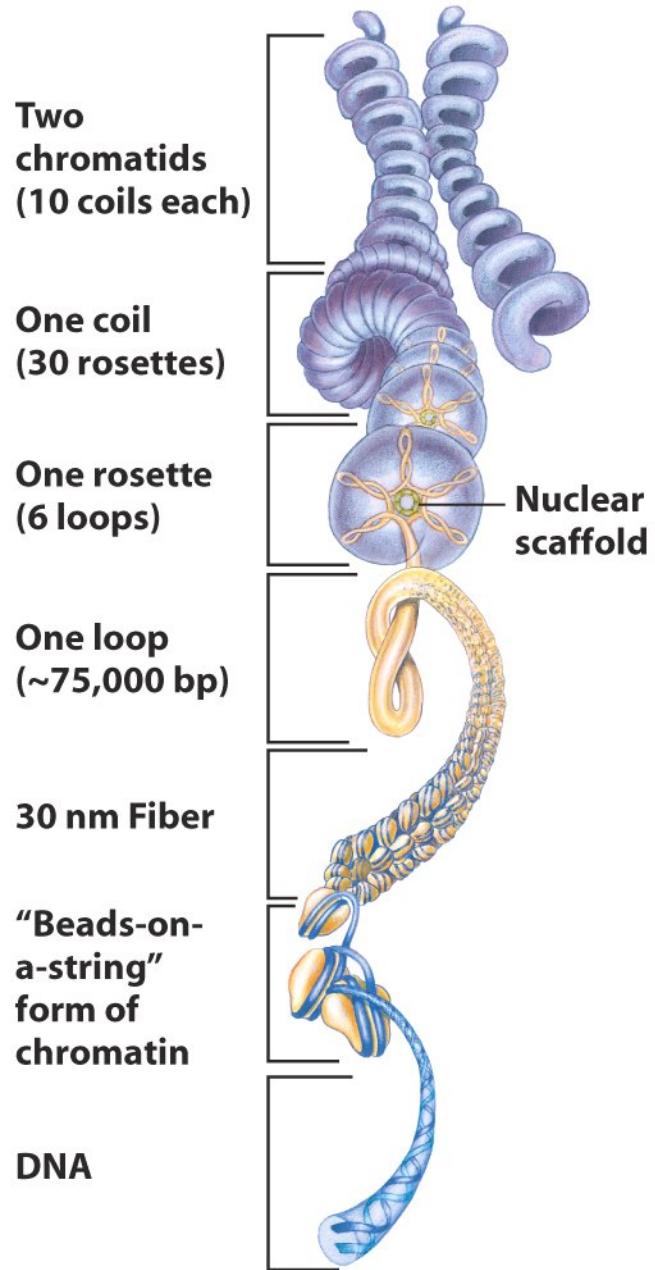
**30  
nm**

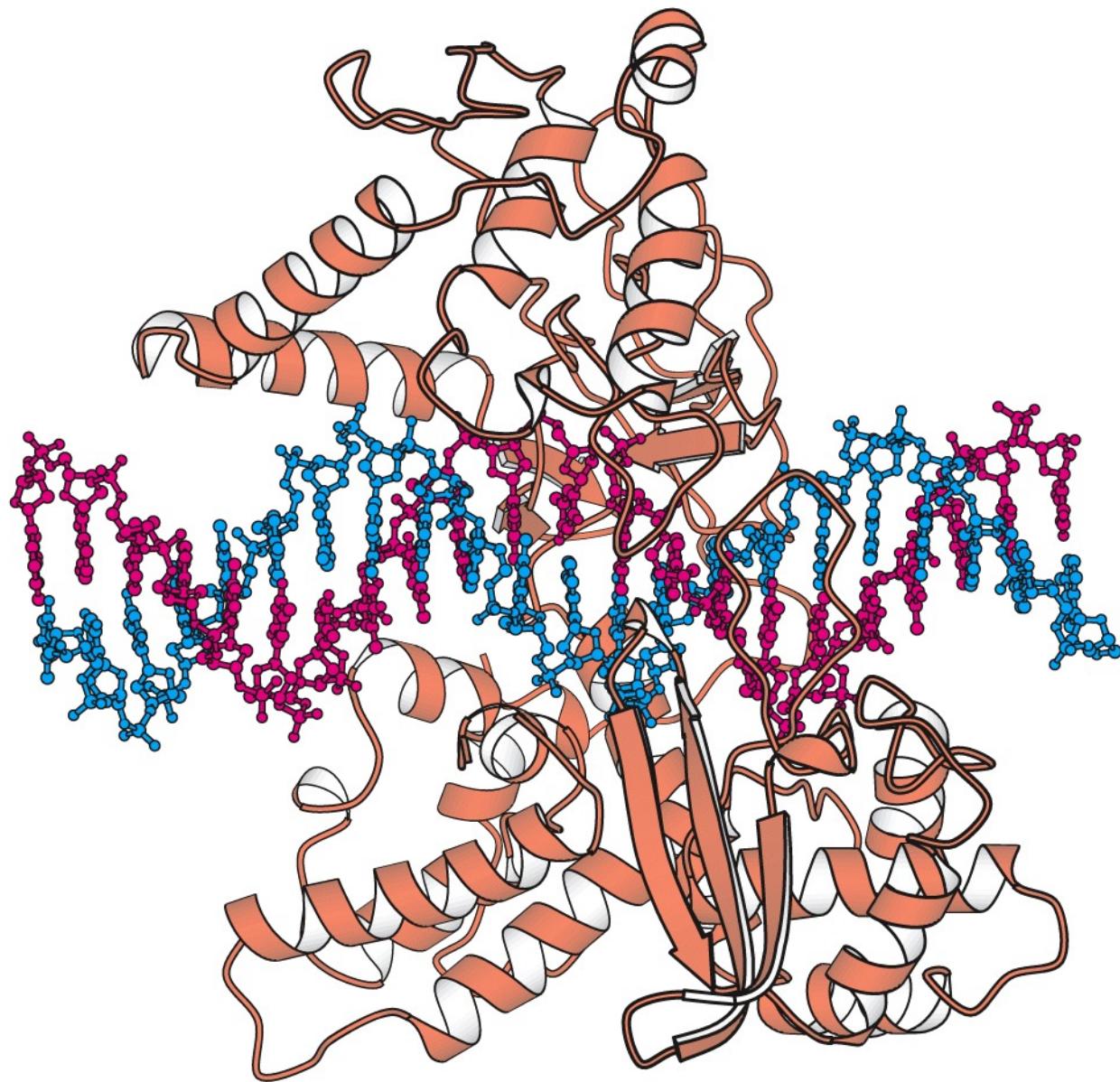






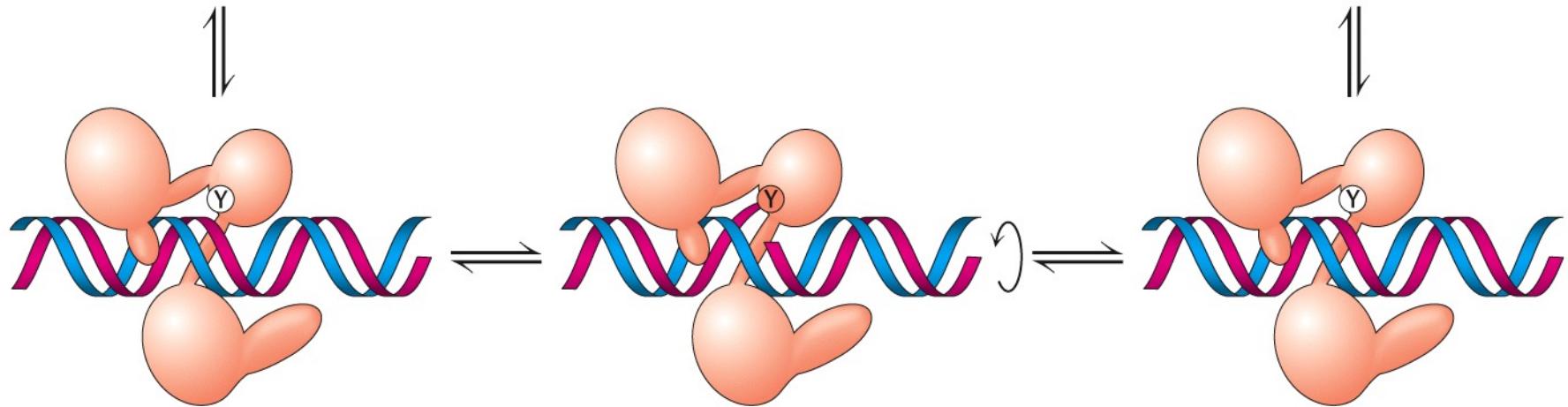






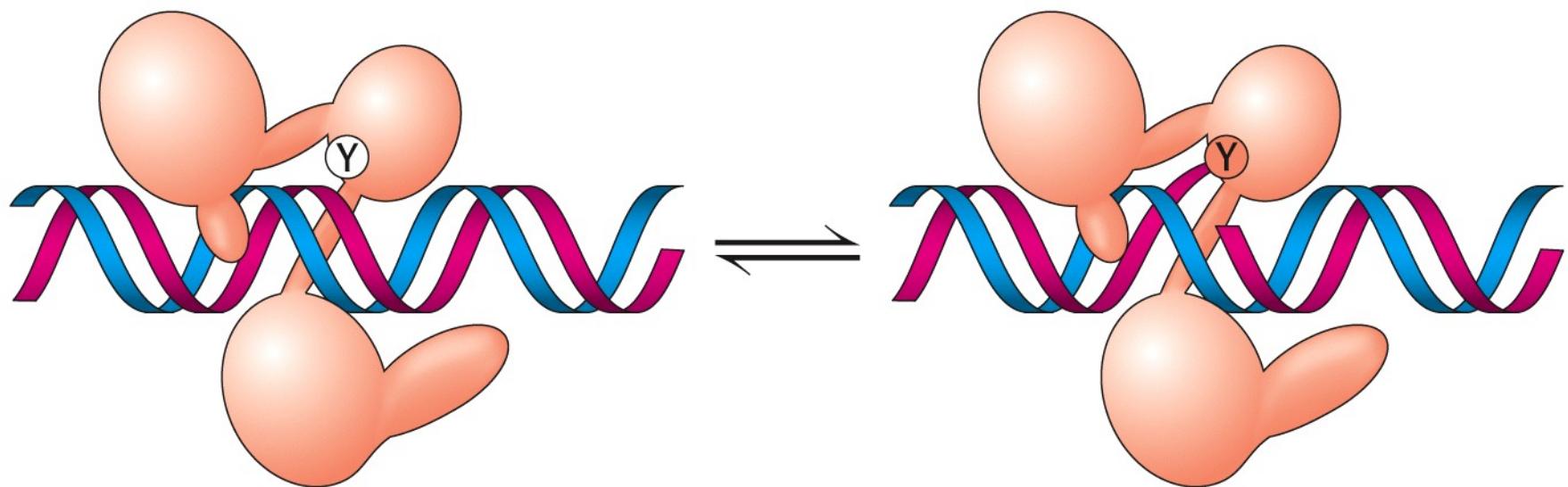


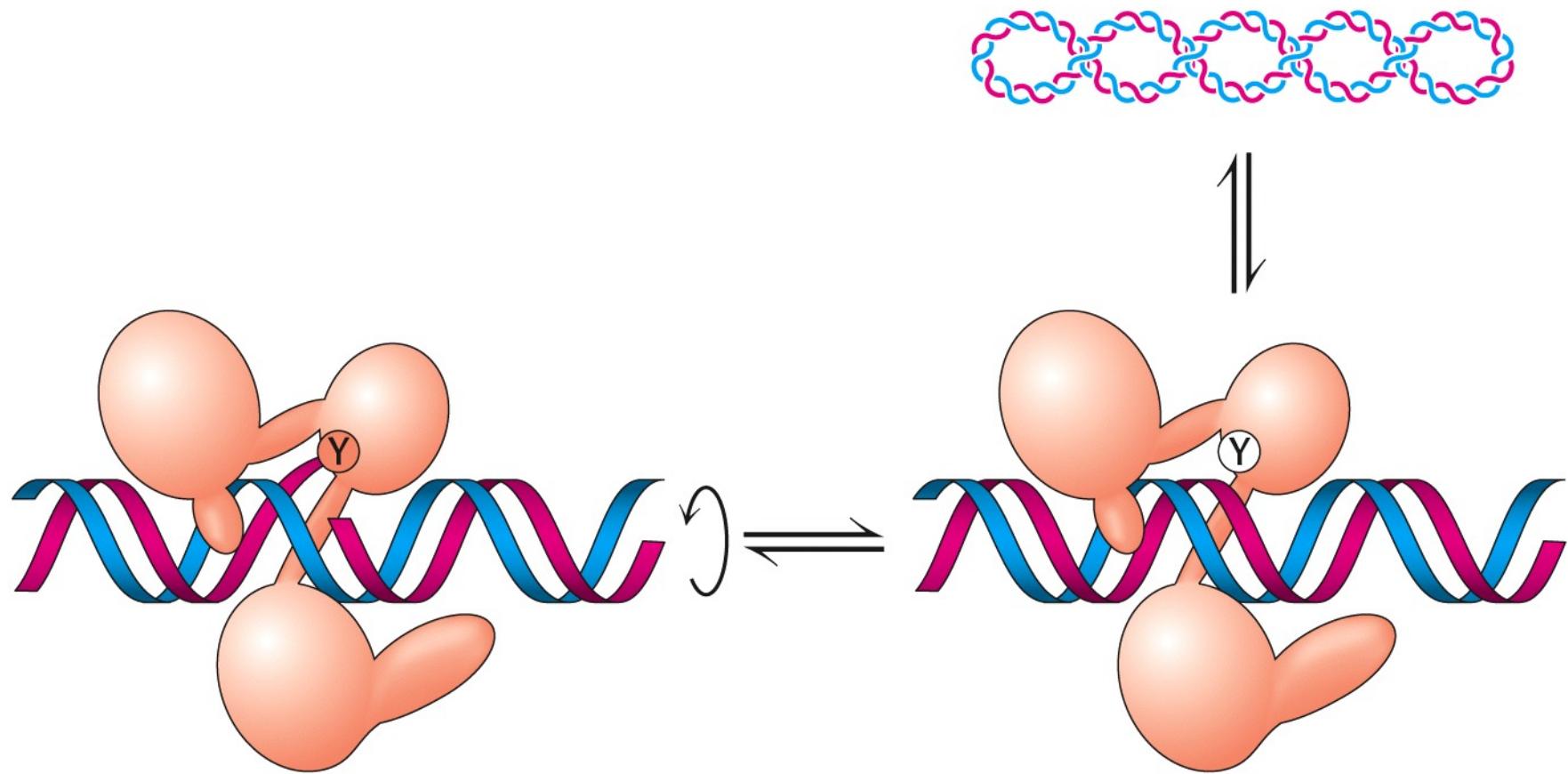
Negatively supercoiled DNA

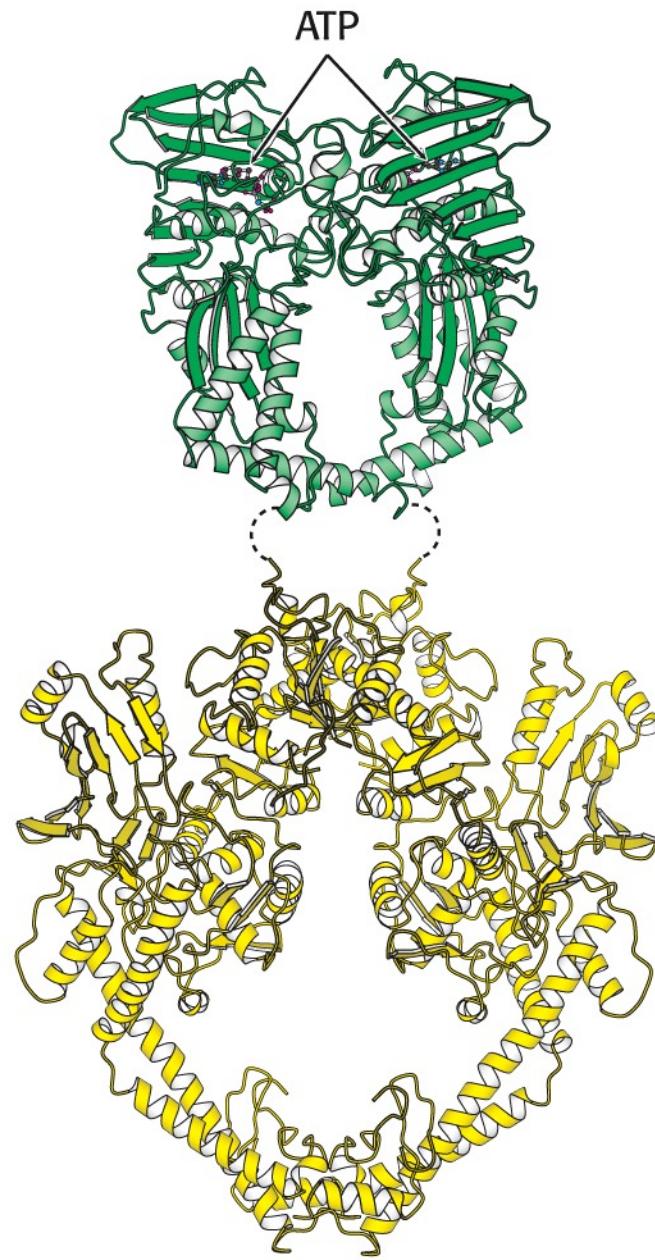


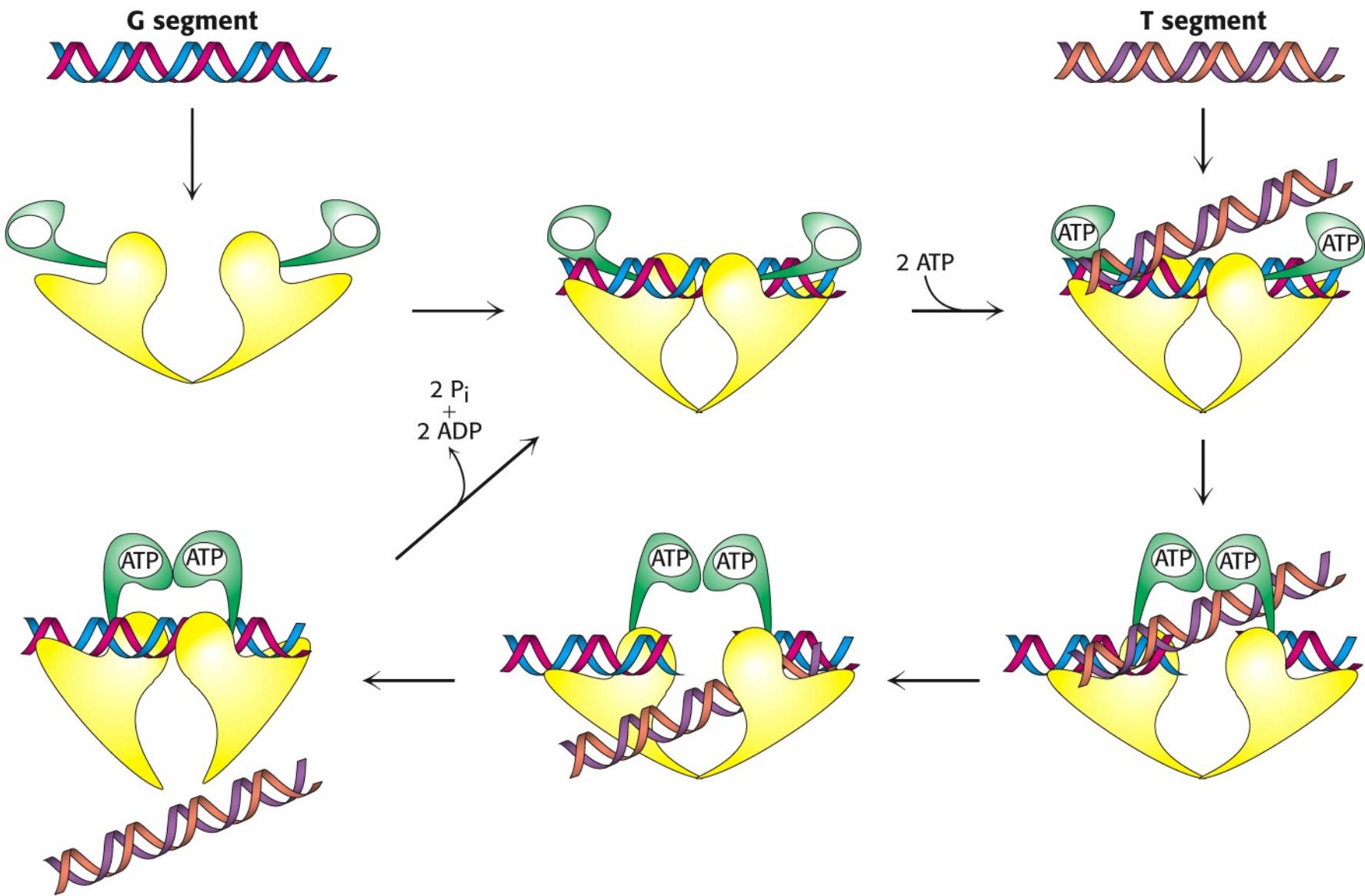


**Negatively supercoiled DNA**



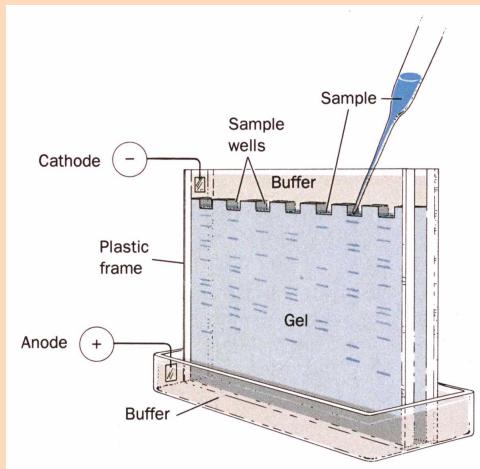




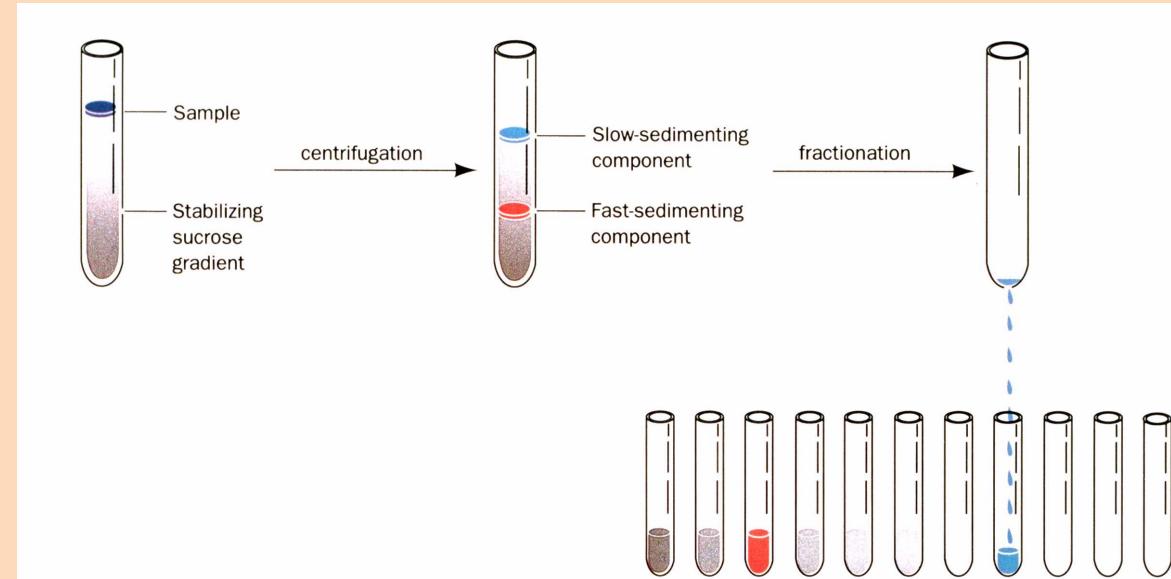


# Detecting different DNA topoisomers

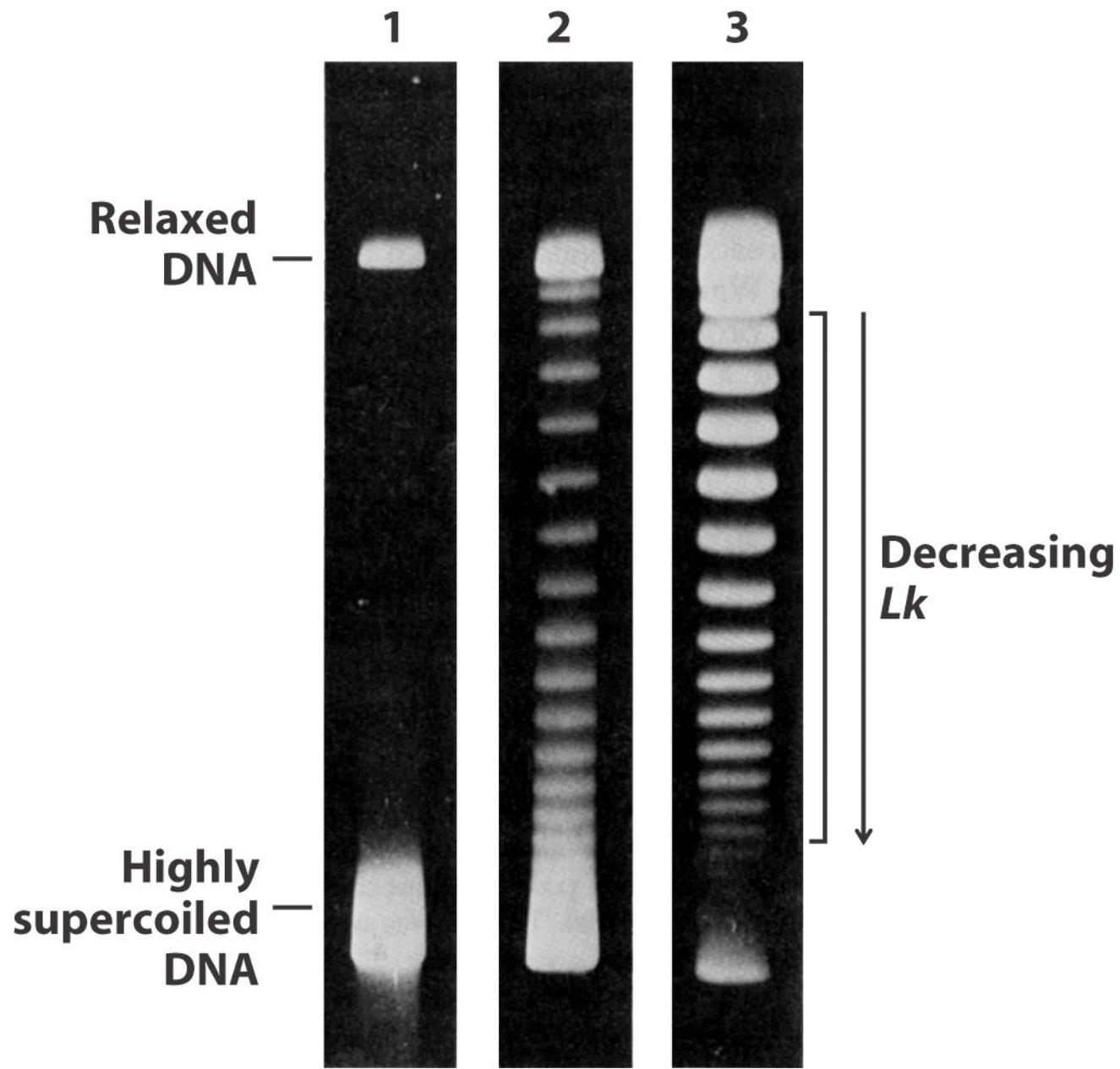
## A. Electrophoretic mobility



## B. Sedimentation rate

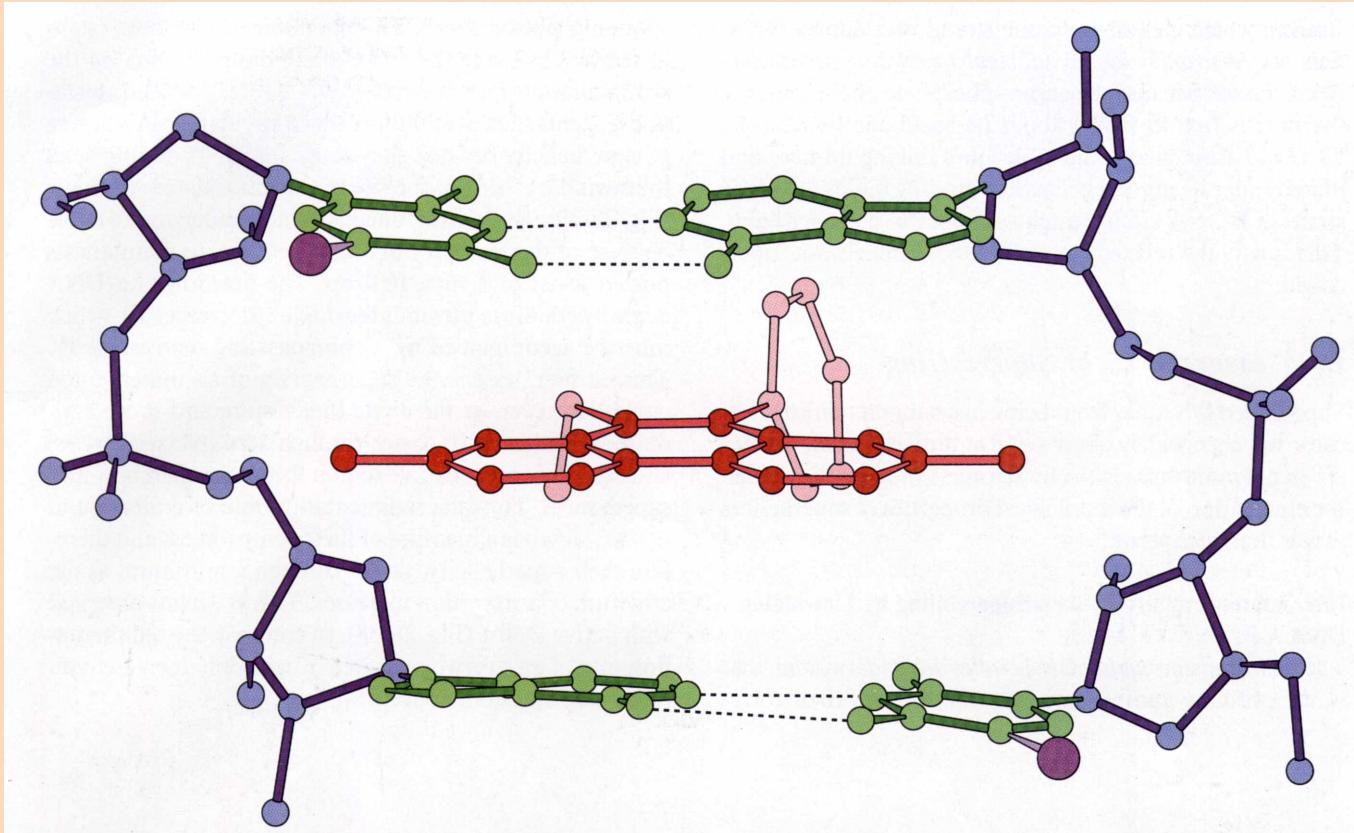


Which form goes fastest in each technique?

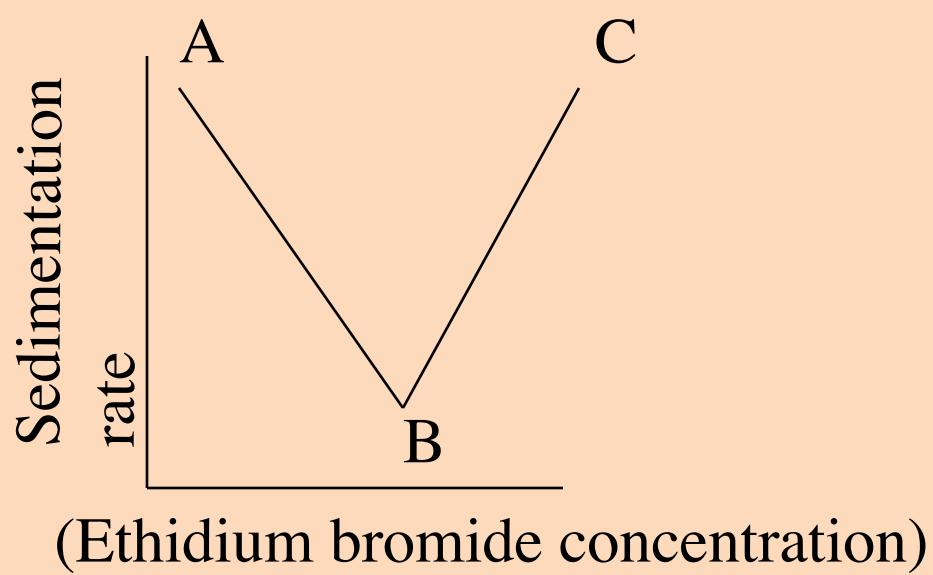


# Detecting different DNA topoisomers

C. Binding of dyes: ethidium bromide in UpA: an example of an agent.



# Detecting different DNA topoisomers



Gel electrophoresis of a mixture of linear, open, and supercoiled DNA treated with EB

Top (-)  
— Linear/open

Supercoiled > open > supercoiled  
(negative)                (\_\_\_\_\_)

≡ topoisomers

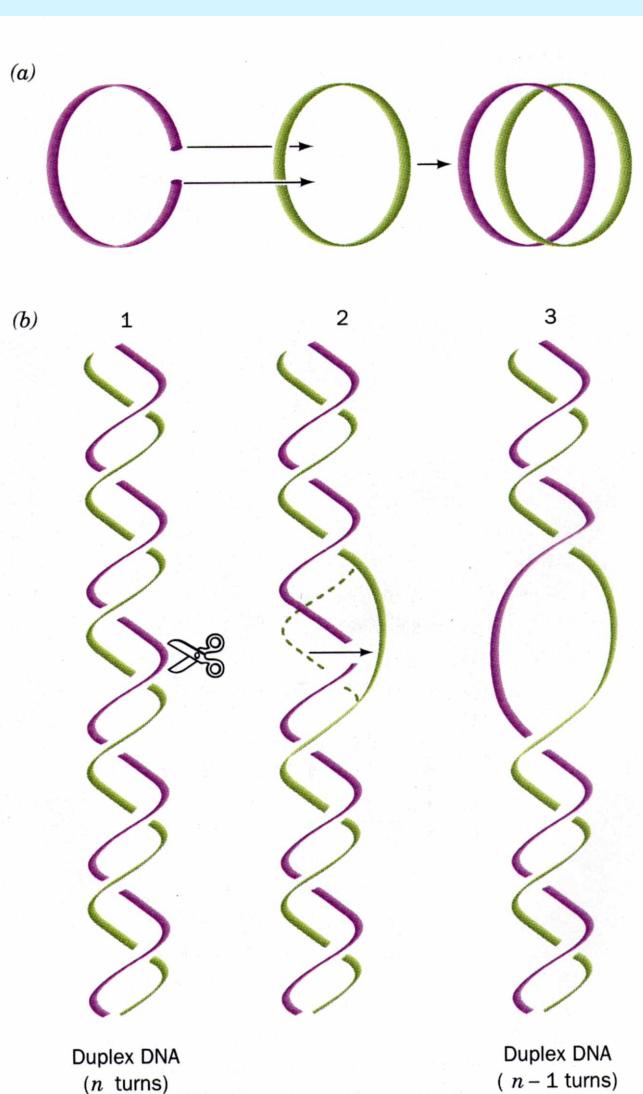
Bottom (+)

# Topoisomerases change the linking number of superhelical DNA

Type I topos change L in units of *one* by breaking a single strand of DNA and allowing the duplex to unwind.

Type II topos change L in units of *two* by breaking both strands and allowing a pass-through of both strands of the double helix.

# Type I topoisomerases (nicking-closing enzymes)

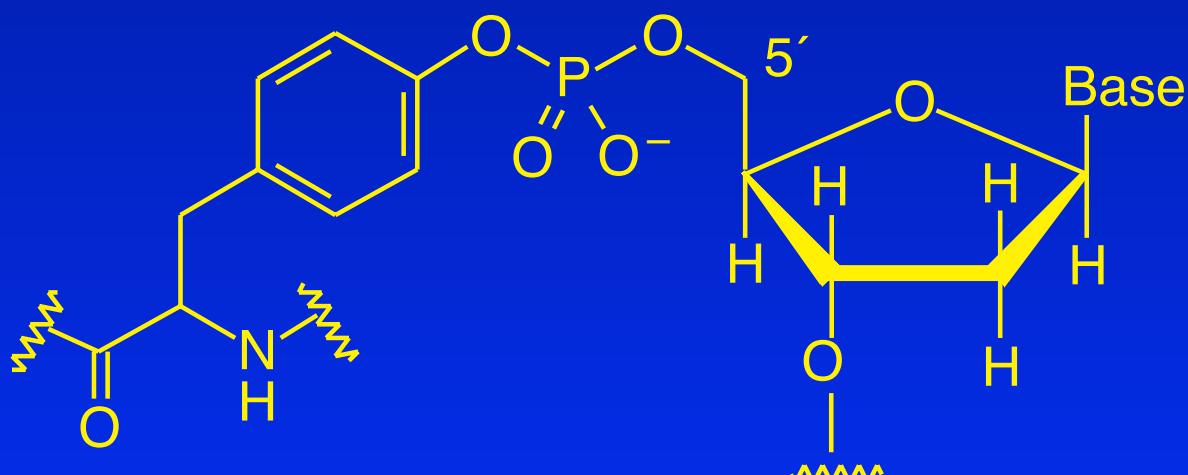


DNA ( $n$  turns) + topoisomerase  
>covalent ***DNA-enzyme intermediate***>  
dsDNA ( $n-1$  turns) + topoisomerase

The formation of a covalent DNA-enzyme complex preserves the free energy of the ***phosphodiester bind*** in DNA as a phosphodiester bond between DNA and ***Tyrosine***.  
(What other amino acid might function instead of ***tyrosine***?)

# Covalent intermediate

- Active-site tyrosine attacks DNA phosphate
- Forms transient phosphotyrosine



# Classification of topois

## ■ Type IA

Cleaves one strand

5'-phosphotyrosine linkage

$$\Delta L_k = \pm 1$$

## ■ Type IB

Cleaves one strand

3'-phosphotyrosine linkage

$$\Delta L_k = \pm 1$$



# Structure and mechanism

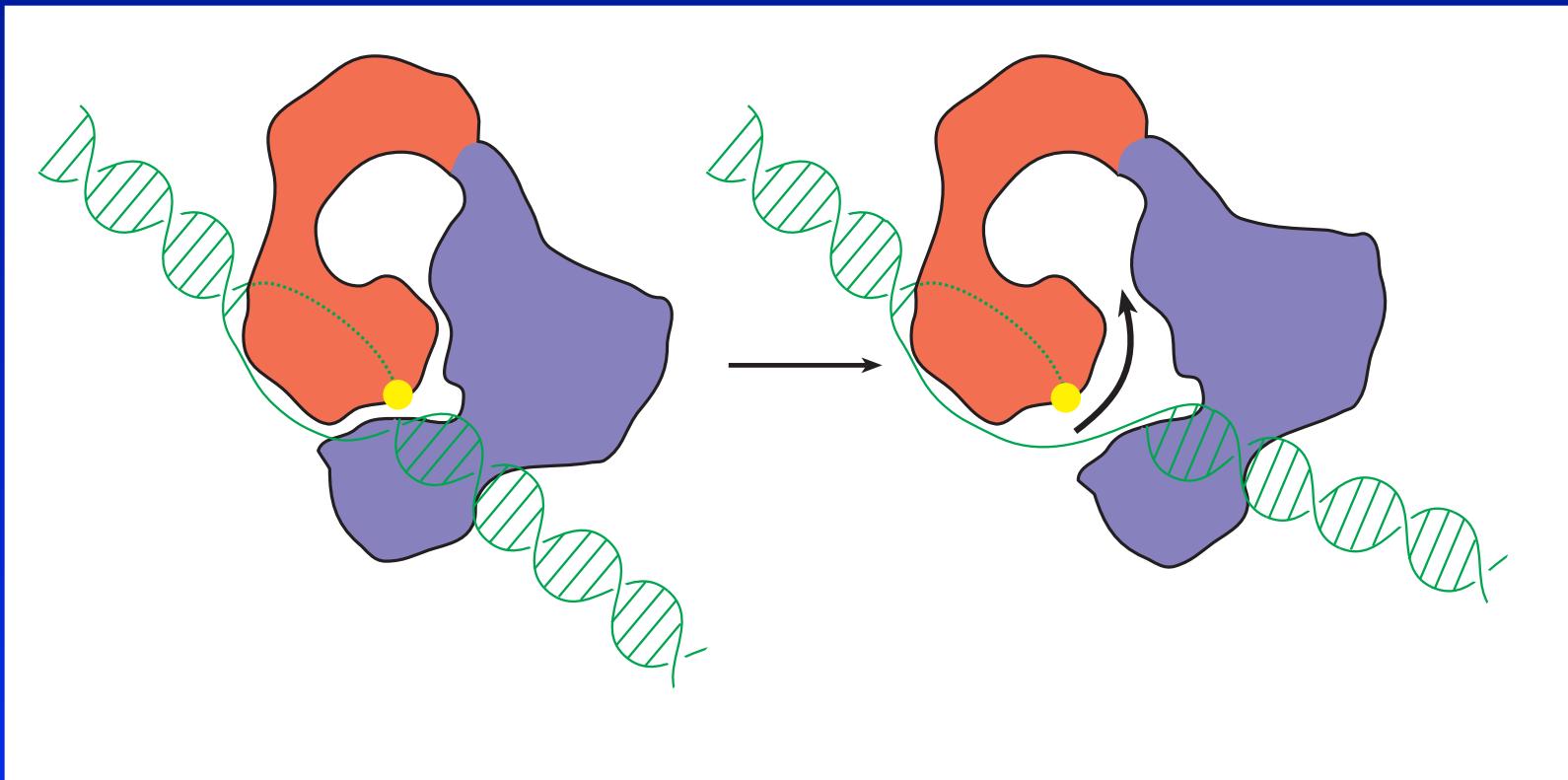
## ■ Type IA

Monomeric (97 kDa; *E. coli*)

Only binds to -ve supercoils

Works by strand-passage (decatenation)

# Type IA mechanism



# Structure and mechanism

## ■ Type IB

Approx 90 kDa

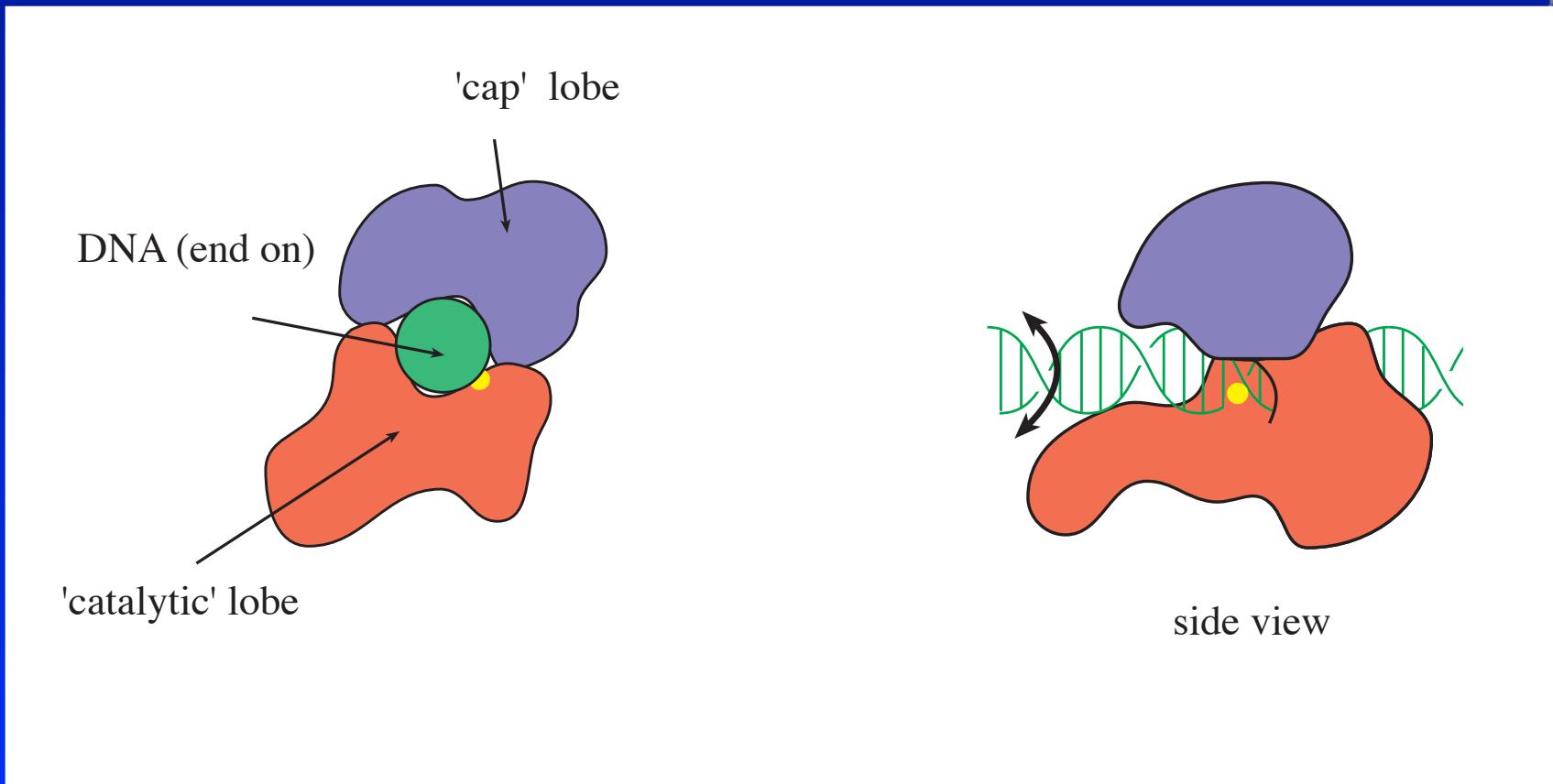
Monomeric

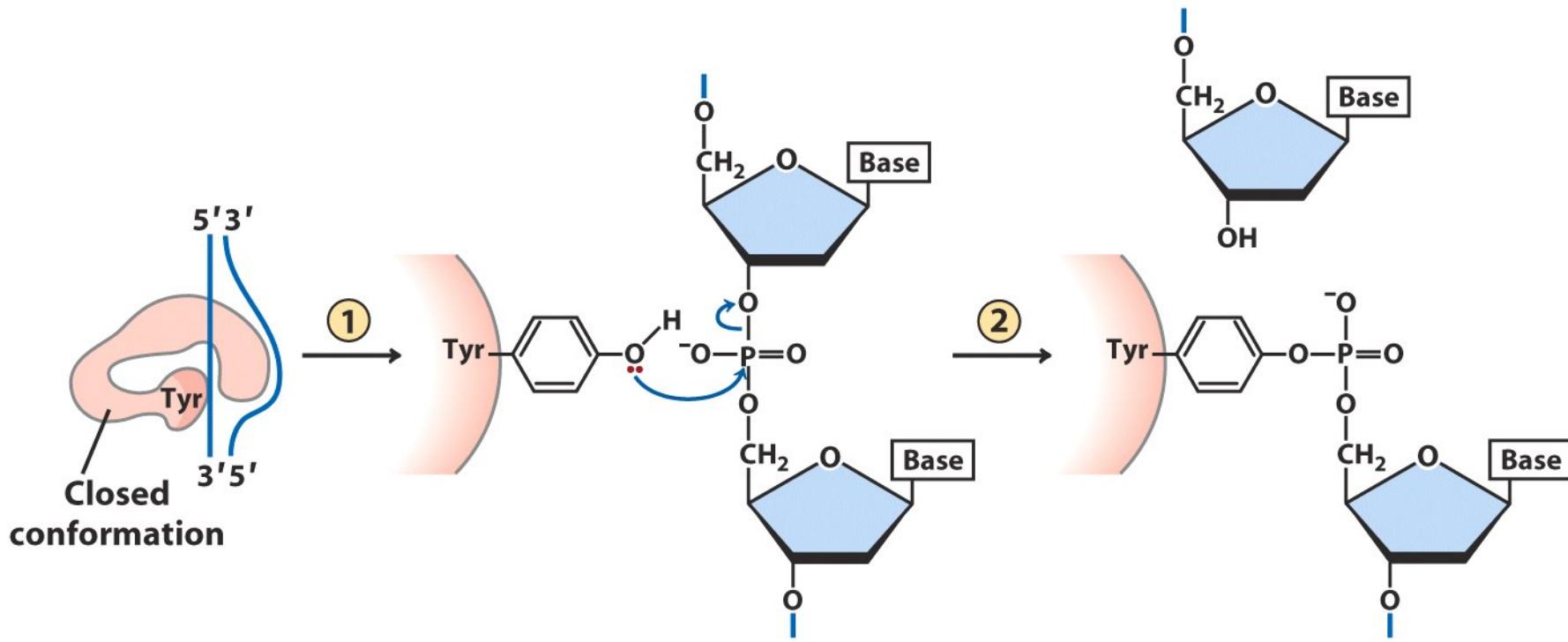
Binds to +ve and -ve supercoils

no DNA distortion

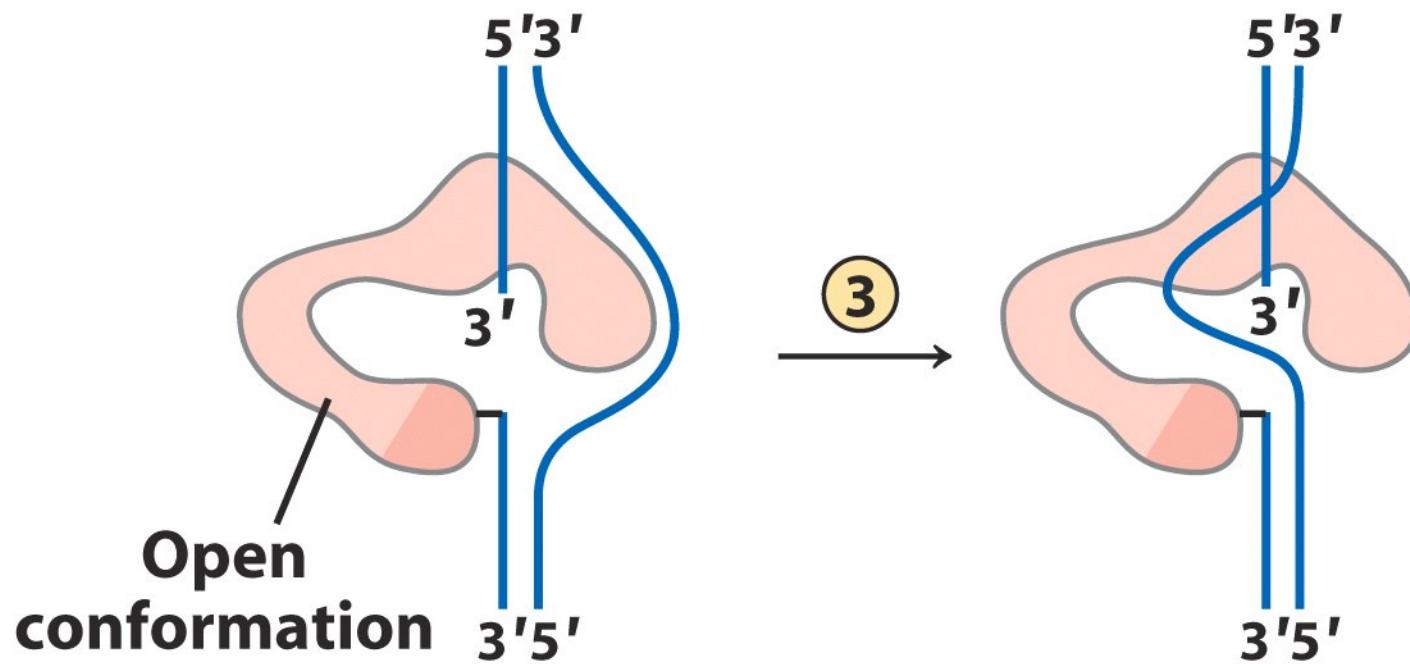


# Type IB mechanism

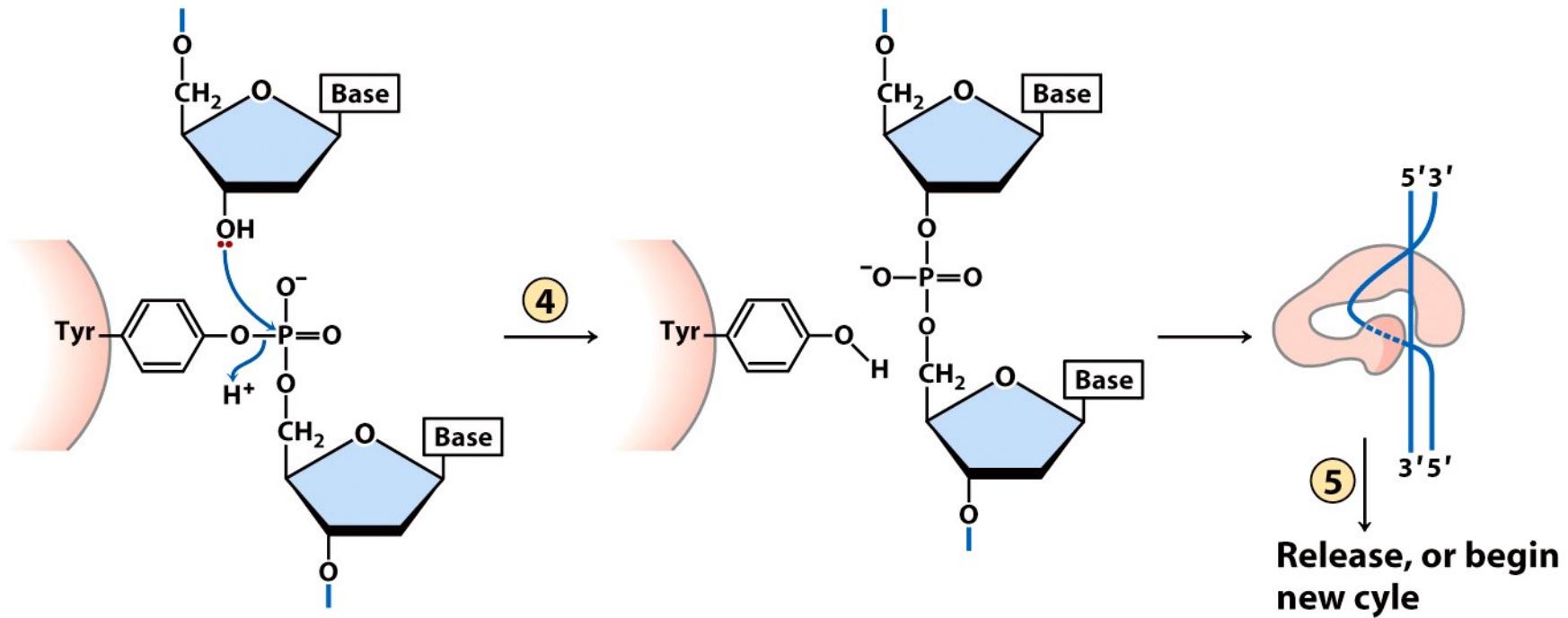




After DNA binds (step ①), an active-site Tyr attacks a phosphodiester bond on one DNA strand in step ②, cleaving it, creating a covalent 5'-P-Tyr protein-DNA linkage, and liberating the 3'-hydroxyl group of the adjacent nucleotide.

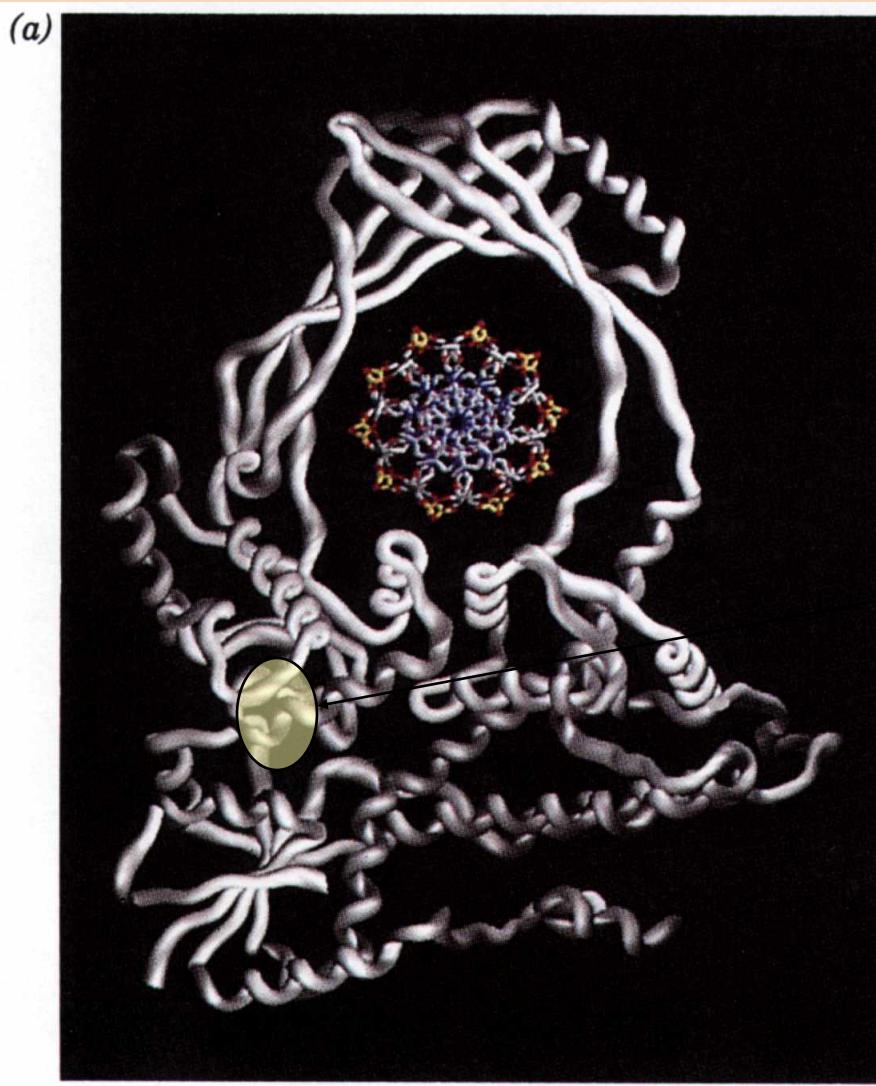


In step ③ the enzyme switches to its open conformation, and the unbroken DNA strand passes through the break in the first strand.



**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.**

# Model of DNA topo I (Ec N-terminus)



Note relative size of the enzyme compared to the cross section of the DNA helix, and how the enzyme encircles and holds the DNA.

Tyrosine (Y)

Position of active site tyr.  
How does it contact DNA?  
How do we know it is this amino acid?

# Classification of topois

## ■ Type II

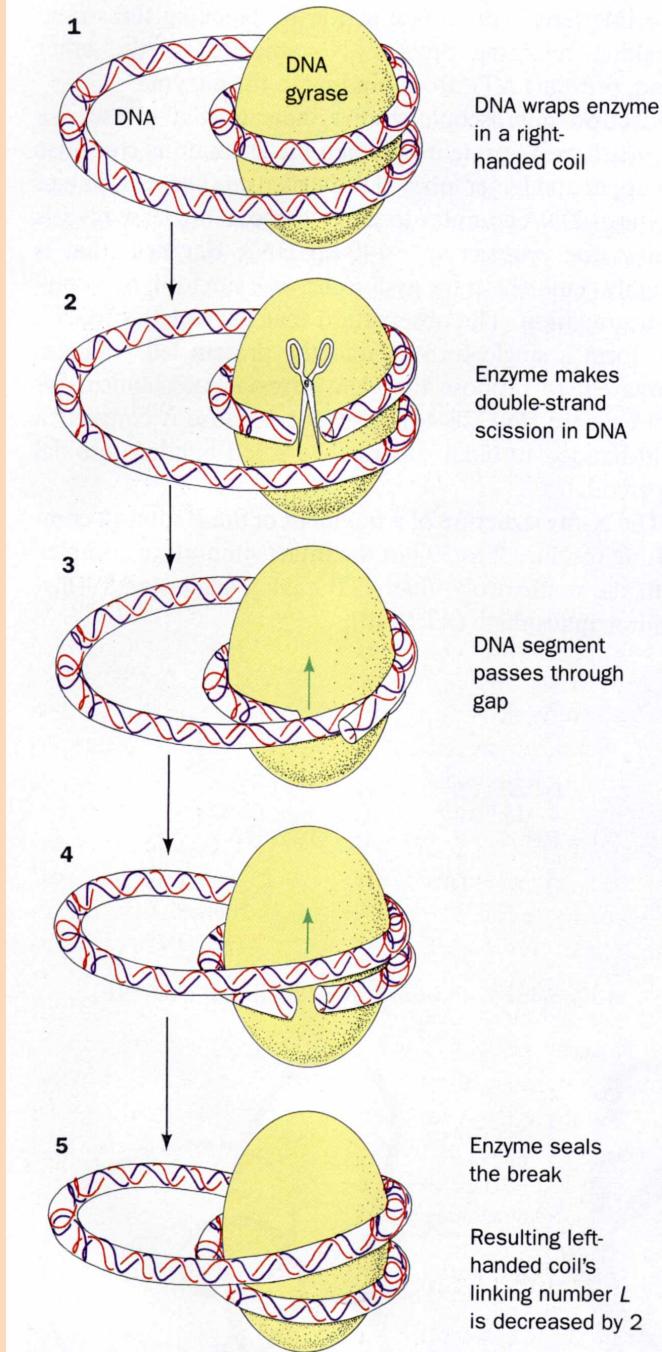
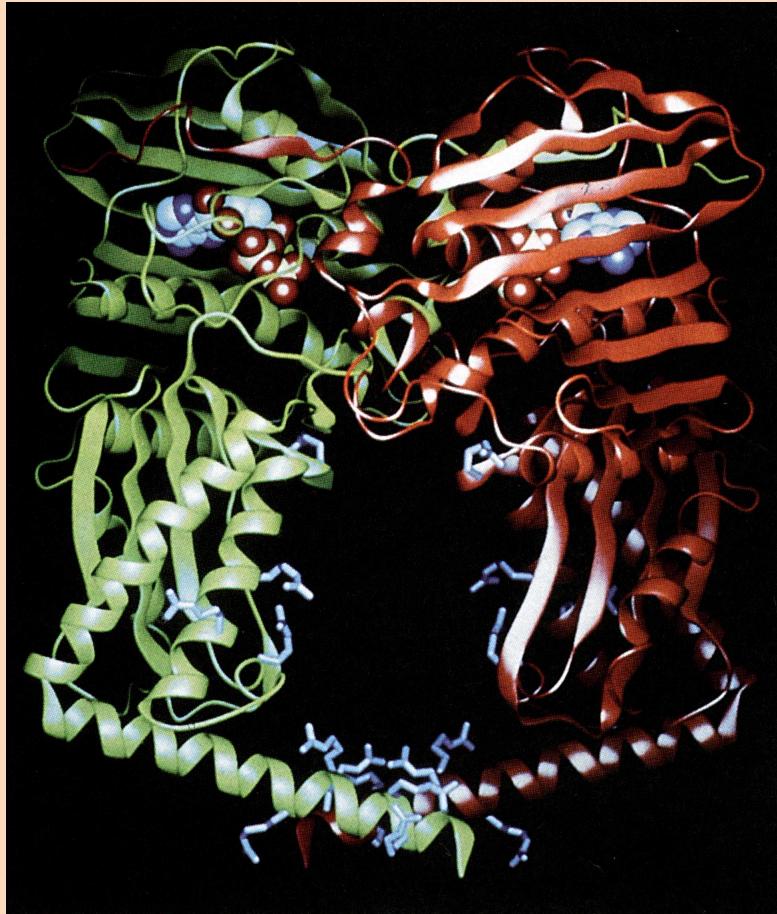
Cleaves both strands (4 bp stagger)

5'-phosphotyrosine

$\Delta Lk = \pm 2$

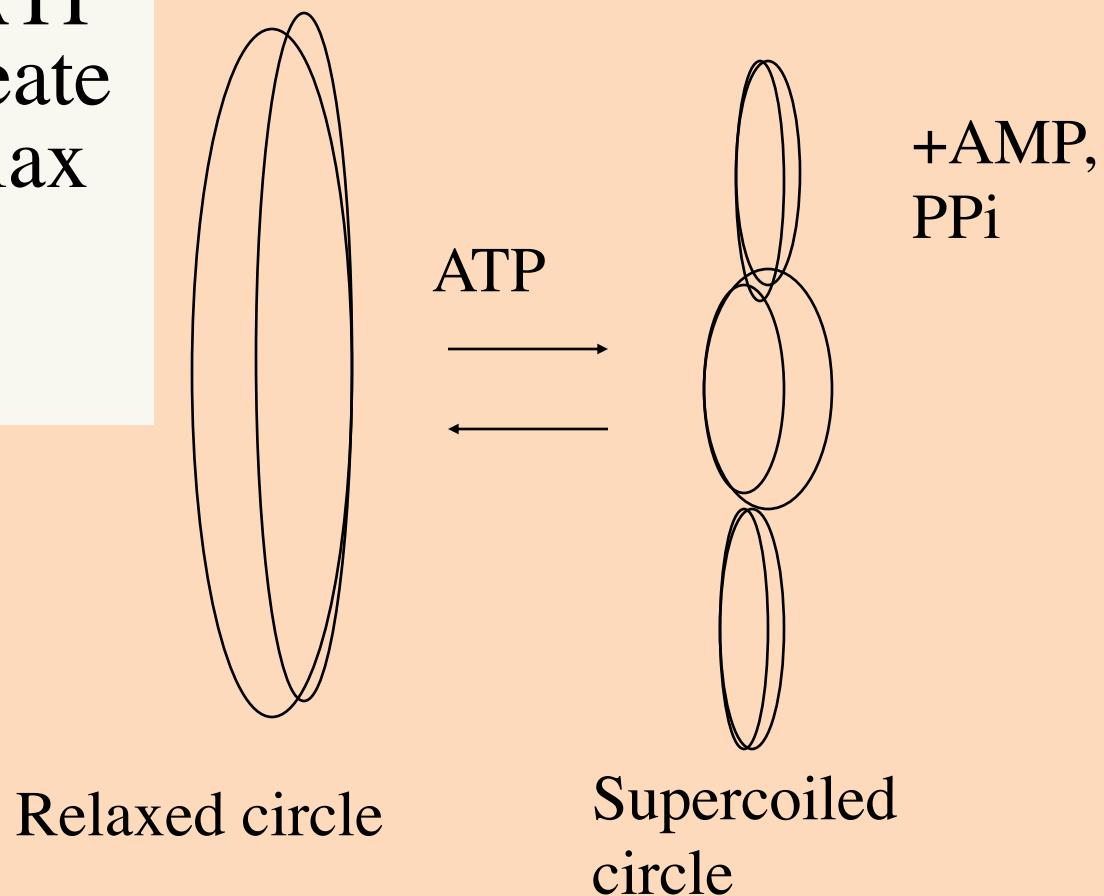
ATP-dependent

# Proposed mechanism of topo II



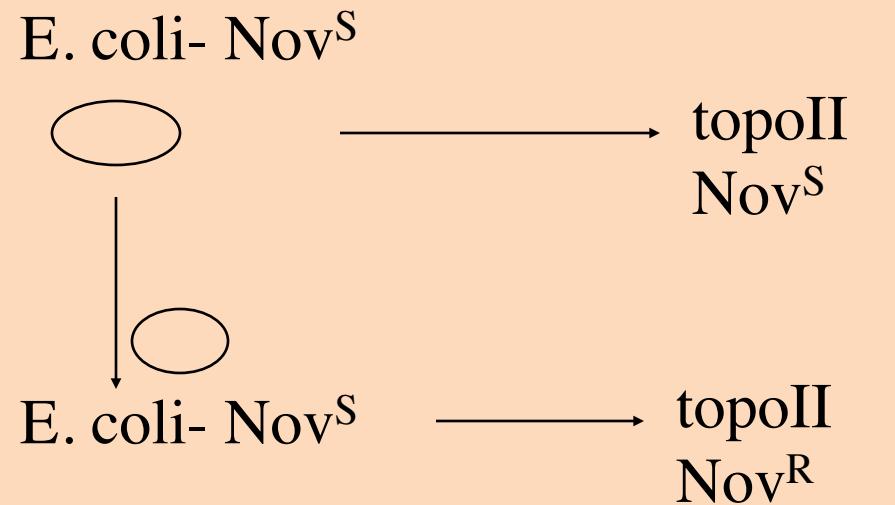
# Prokaryotic Topoisomerase II is a DNA gyrase

In the presence of ATP DNA gyrase can create supercoils; it can relax supercoils in the absence of ATP



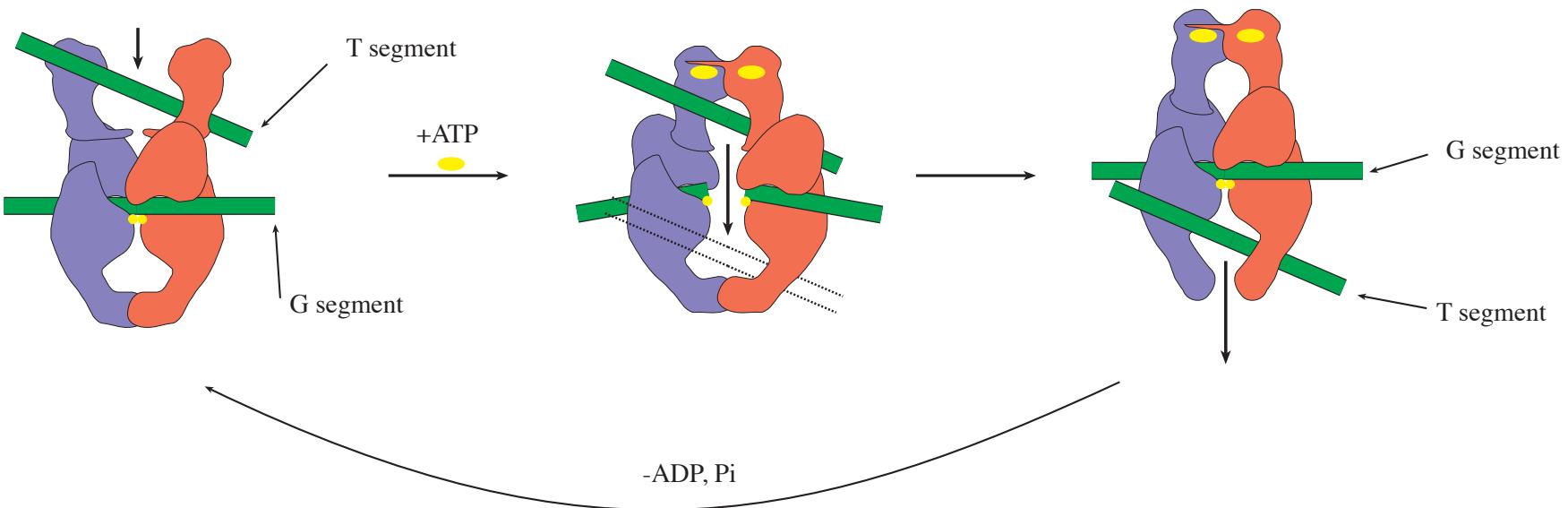
# Inhibitors of DNA gyrase inhibit DNA replication

Two antibiotics, oxolinic acid and novobiocin inhibit replication.



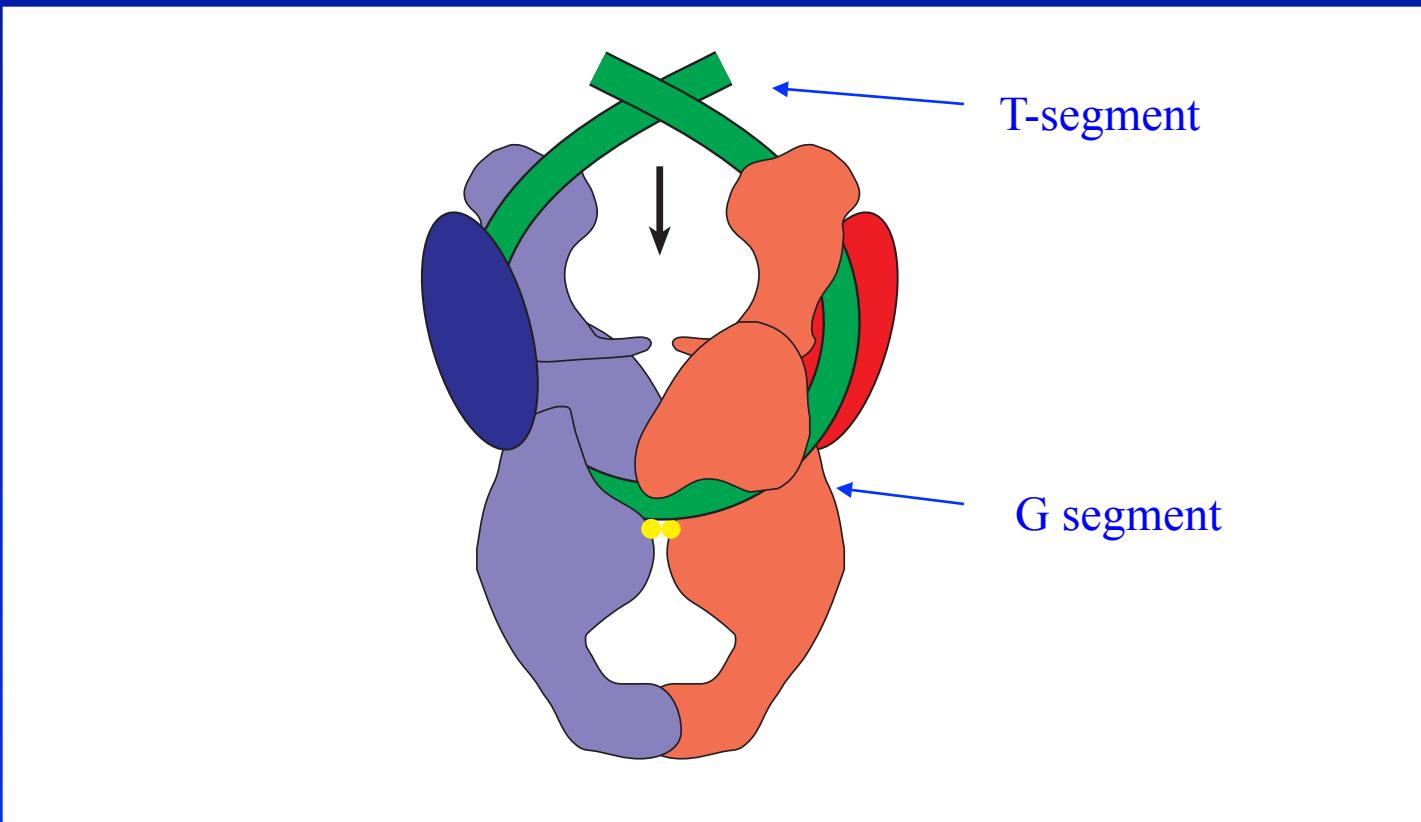
Mutants resistant to novobiocin have a novobiocin-resistant topo II activity in vitro, thus proving that the lethal activity of the drug is its inhibition of DNA topo II activity in vivo. This demonstrates that topo II is an (essential/non-essential) enzyme for cell viability.

# Type II mechanism



# How gyrase supercoils

- Gyrase wraps DNA around complex



# Structure and mechanism

## ■ Type II

Dimeric (approx. 170-190 kDa)

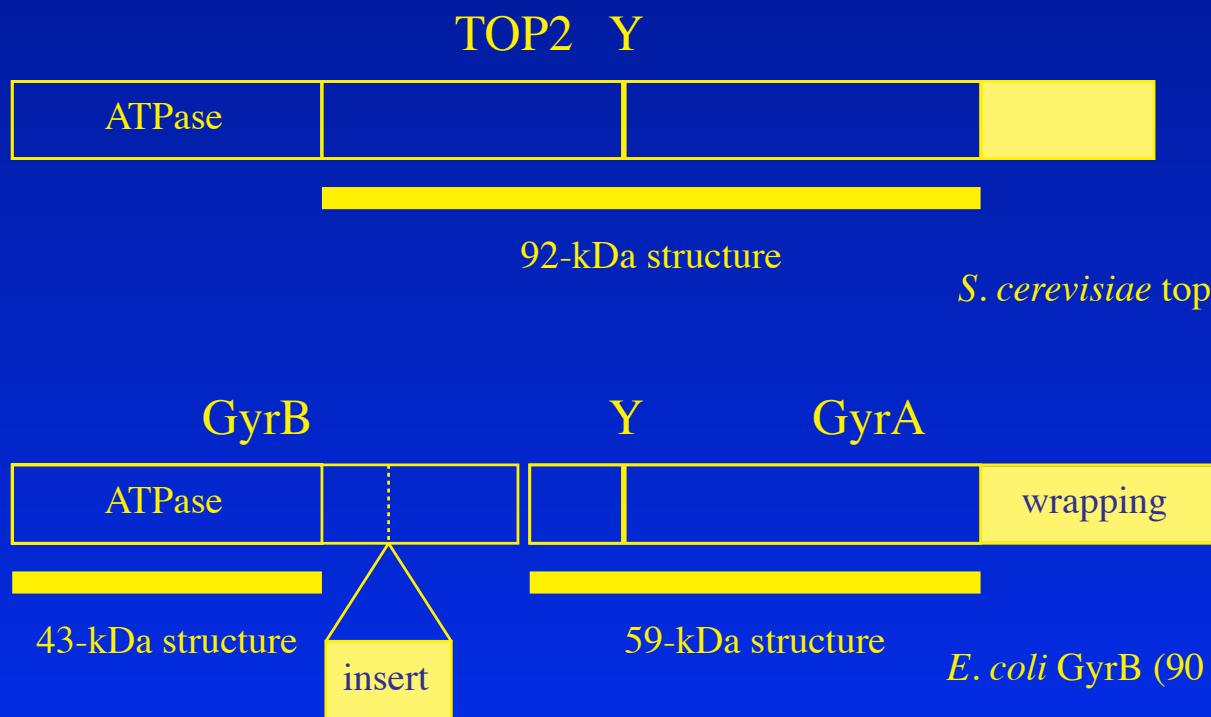
Tetrameric in bacteria

Interacts with 2 DNA segments

ATP-dependent clamp



# Gyrase and Type IIIs



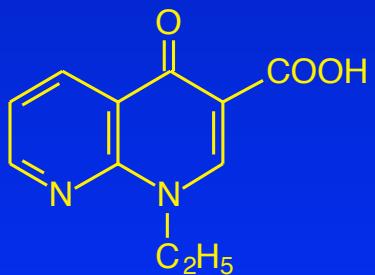
## Structures

# Drug interactions

## ■ Bacterial type IIs (gyrase and topo IV)



novobiocin

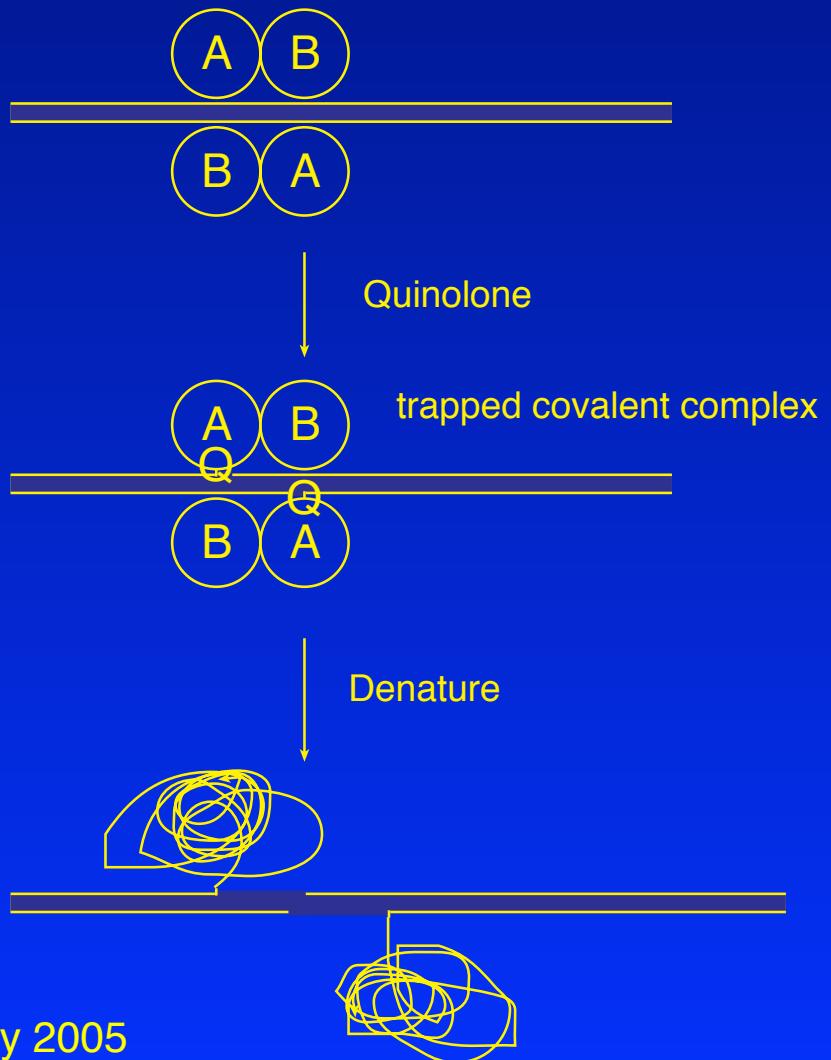


nalidixic acid

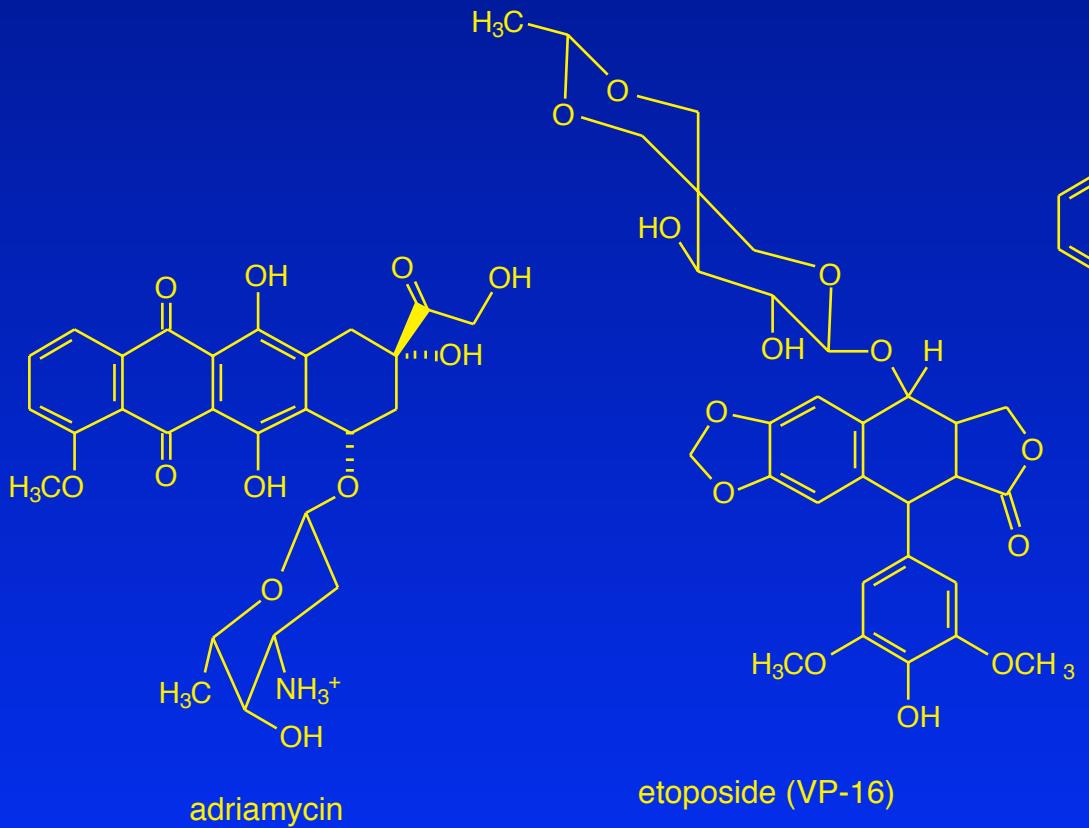


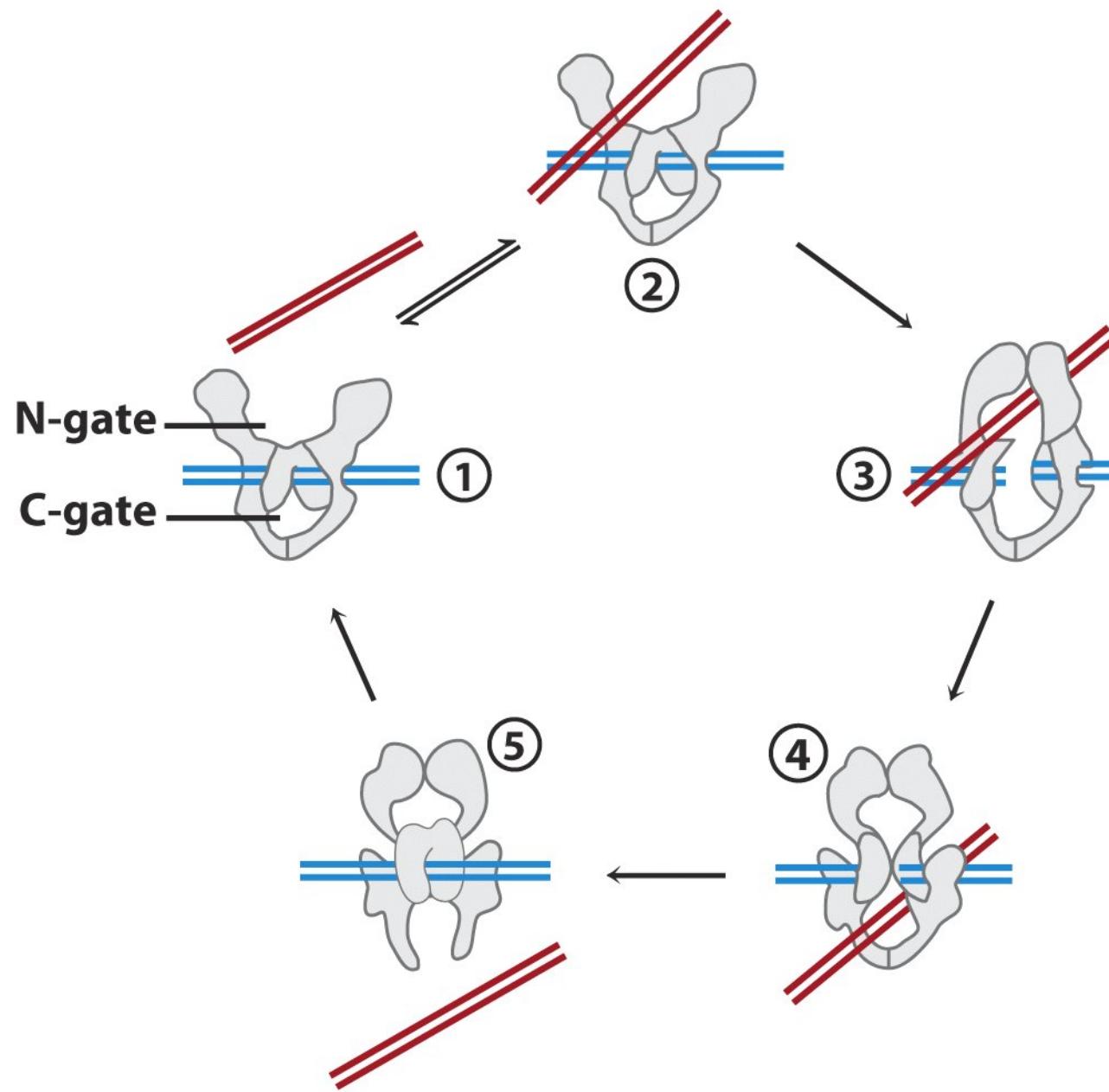
ciprofloxacin

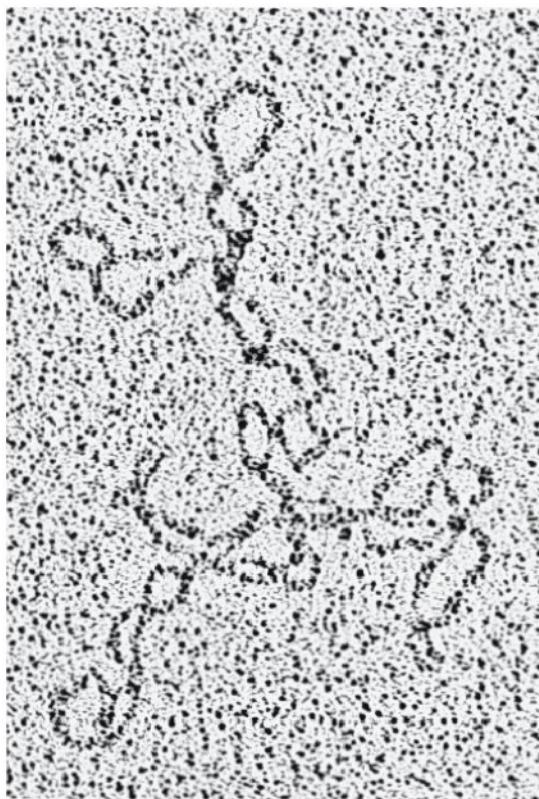
# Effect of quinolone drugs



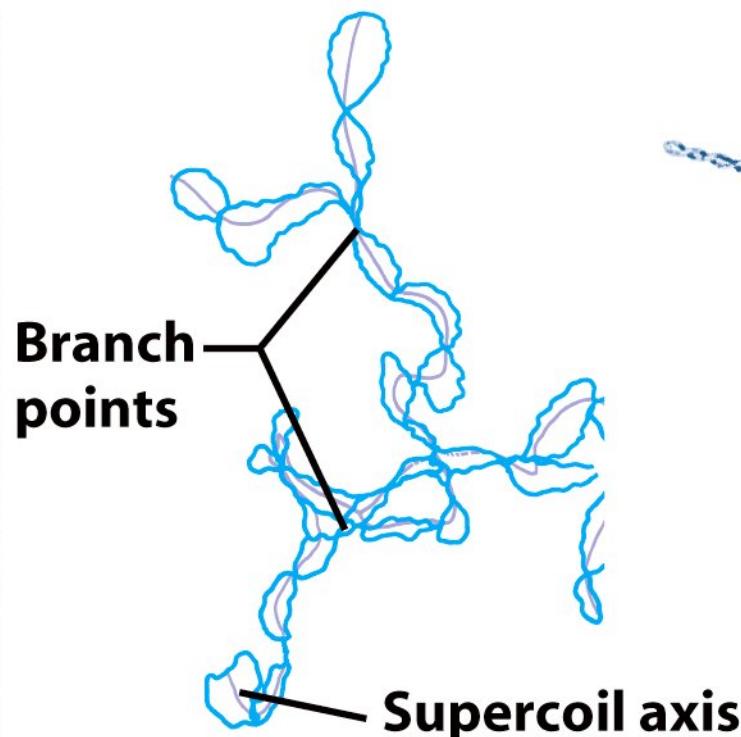
# Anti-tumour agents



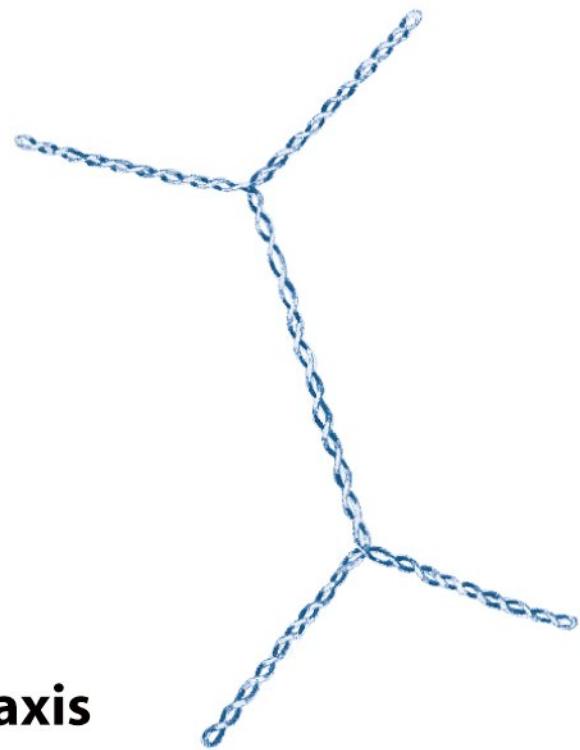




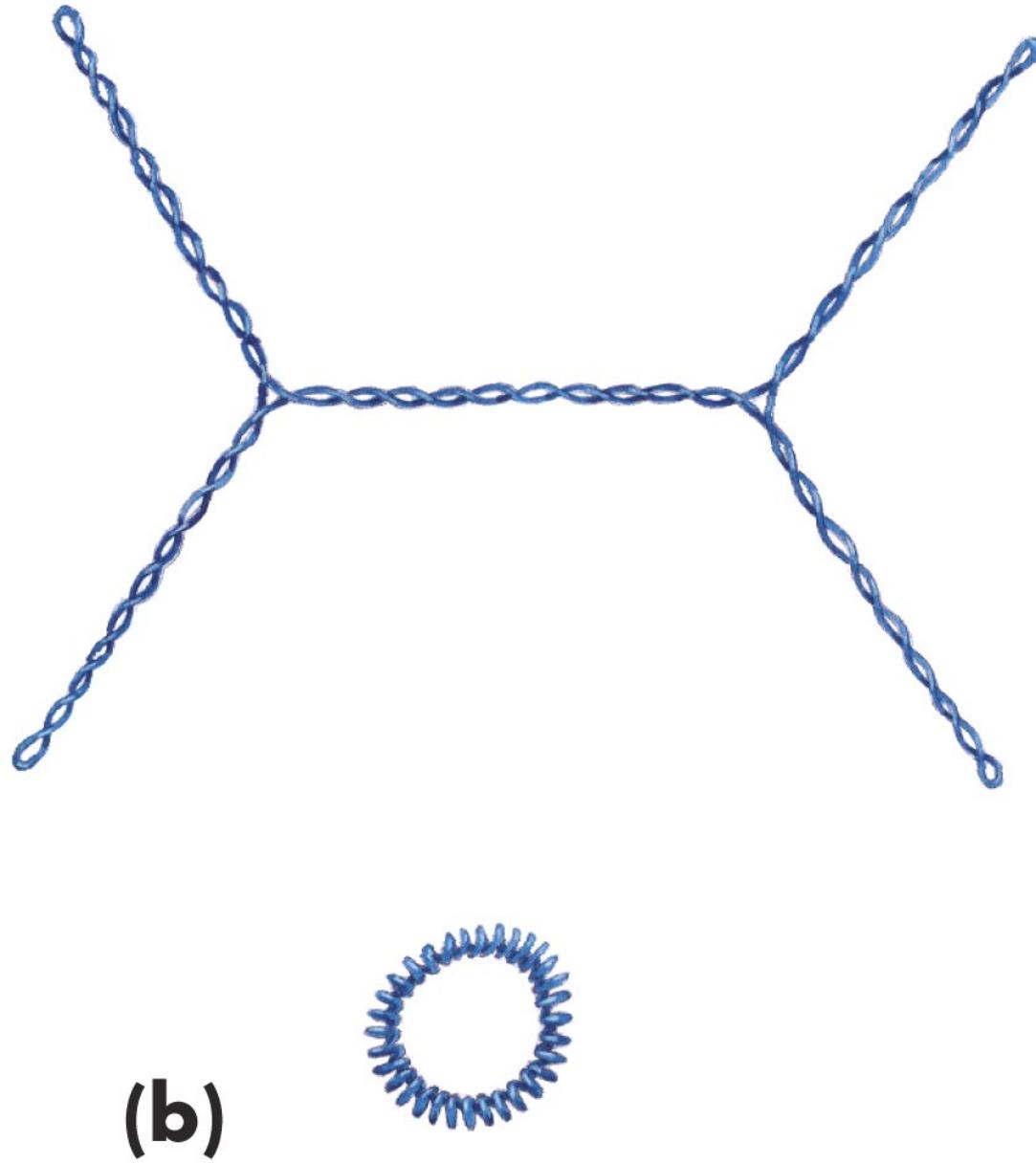
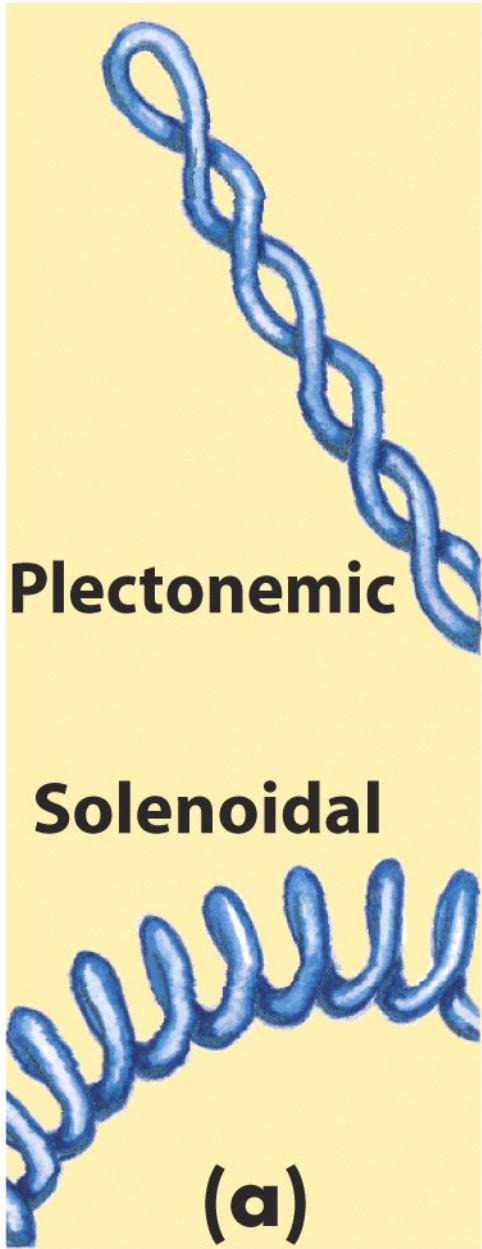
(a)

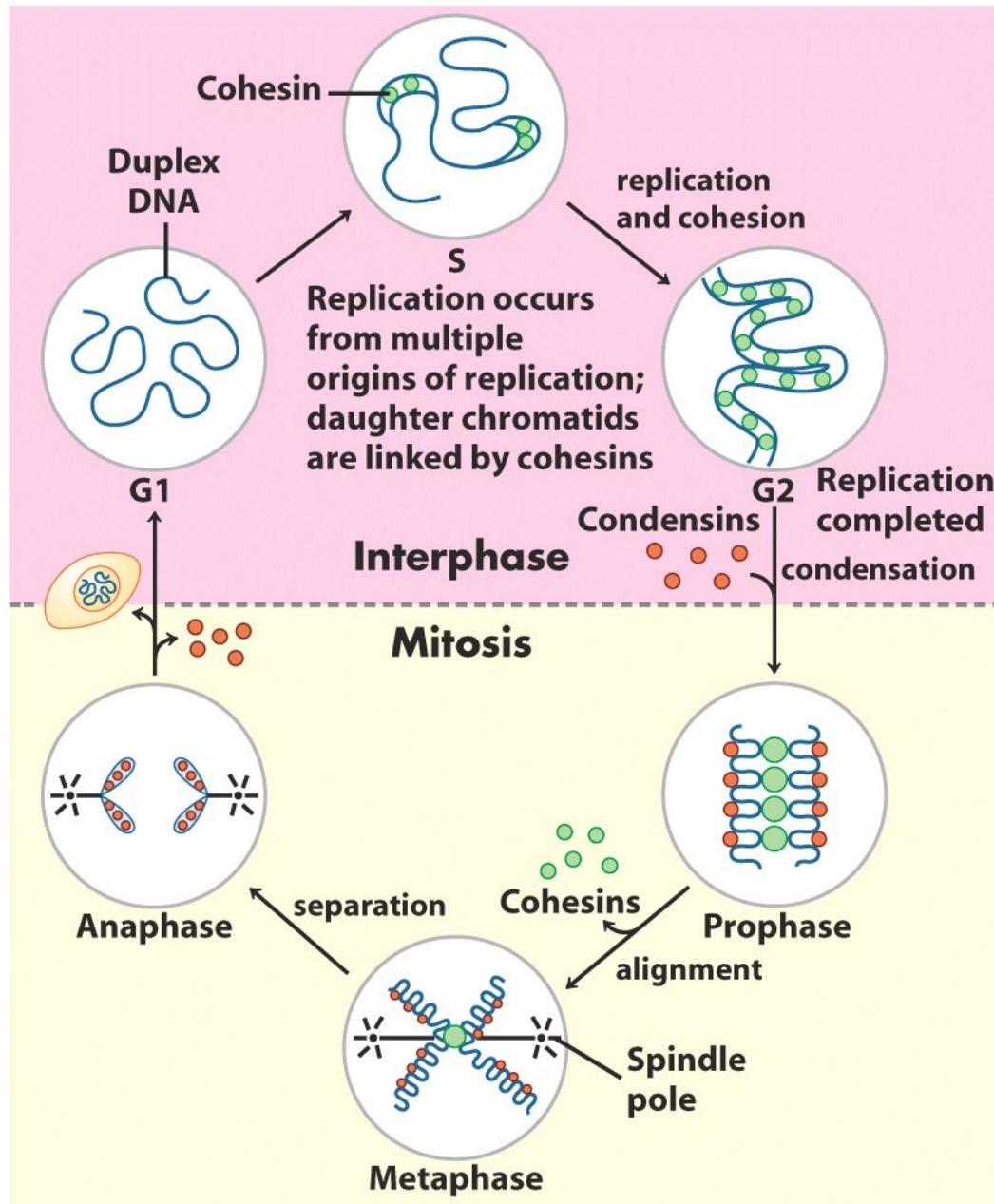


(b)

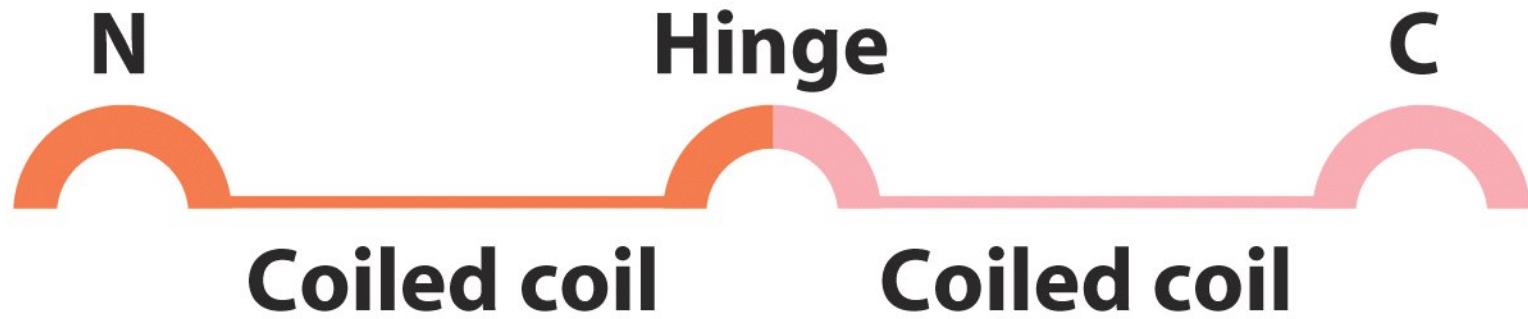


(c)

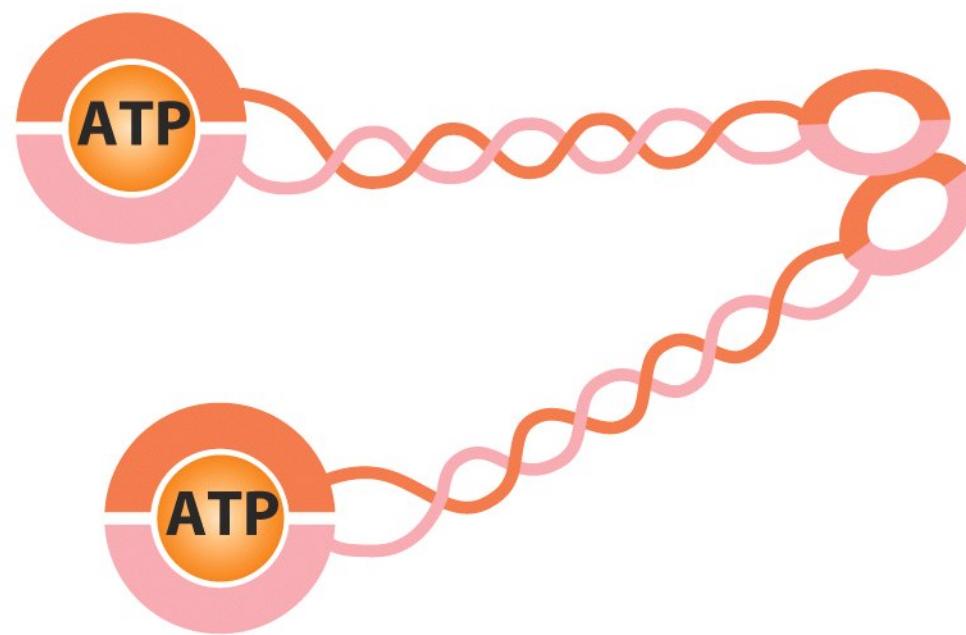


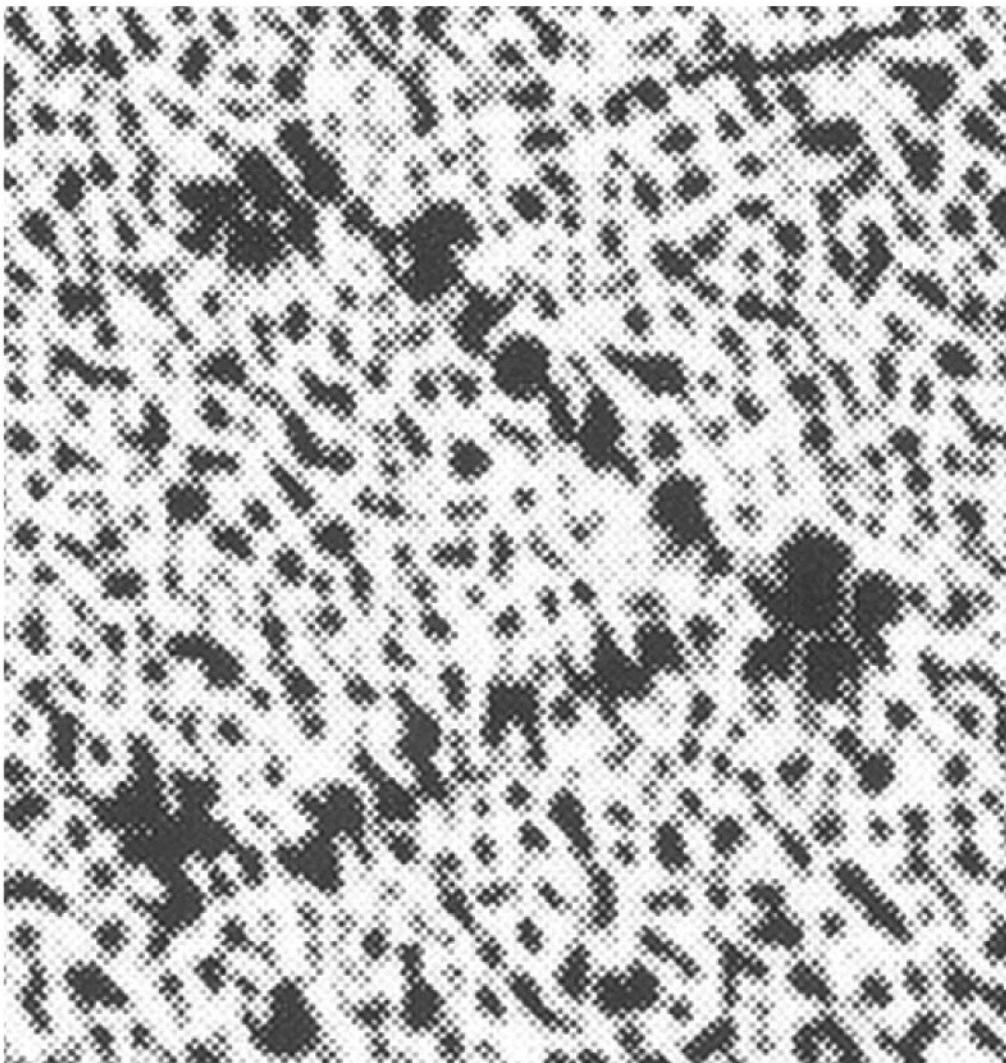


**(a)**

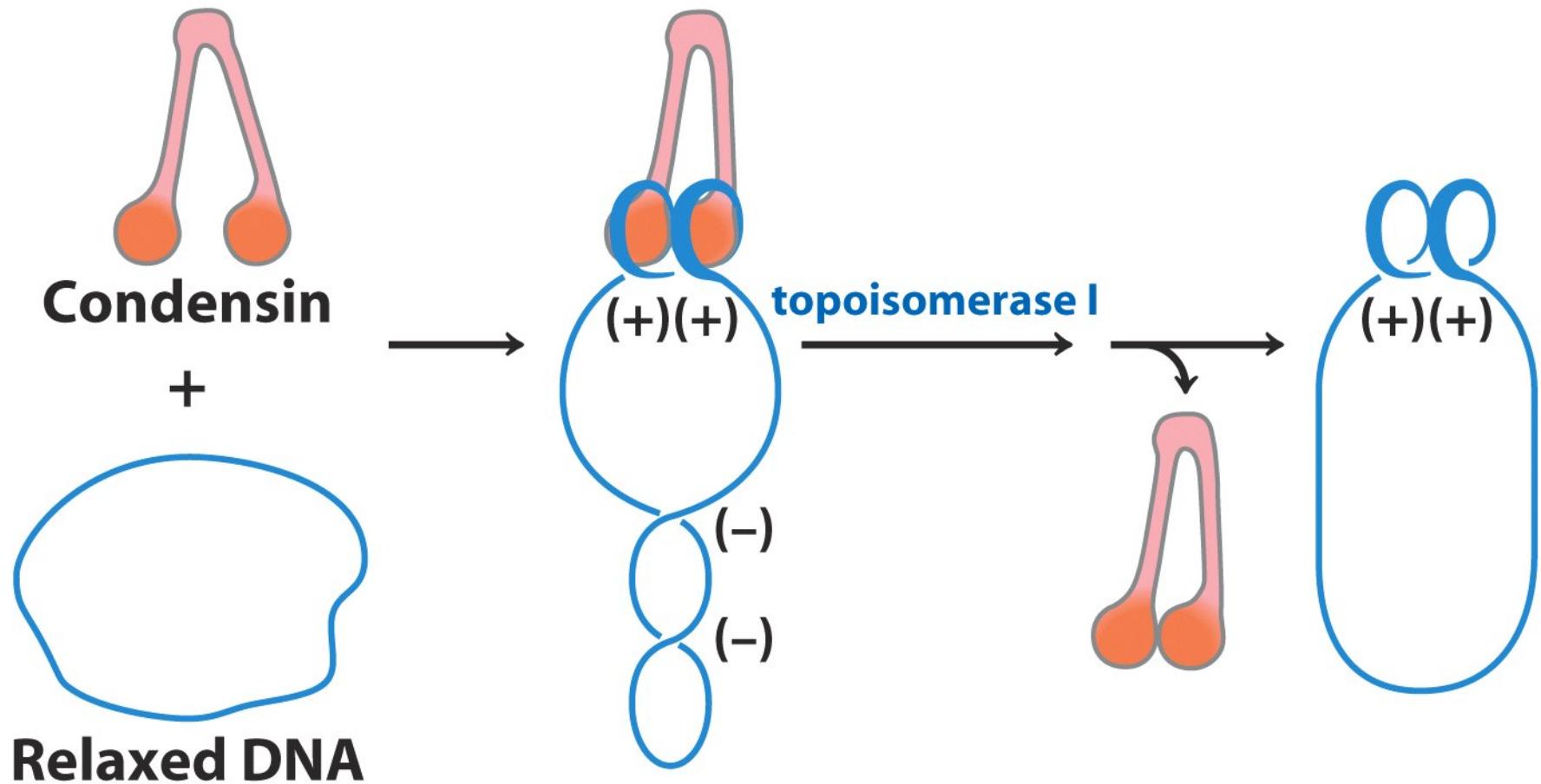


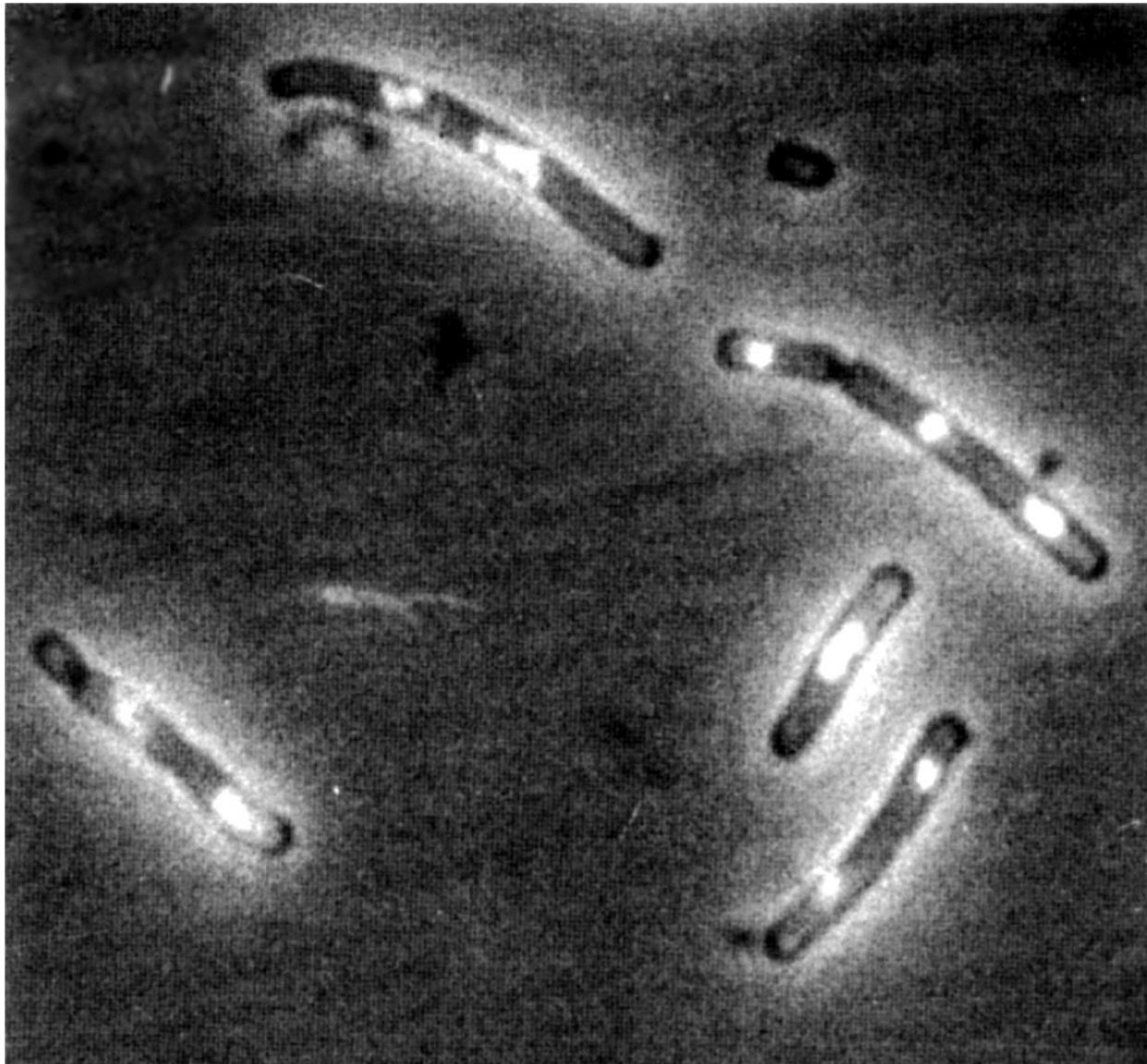
**(b)**



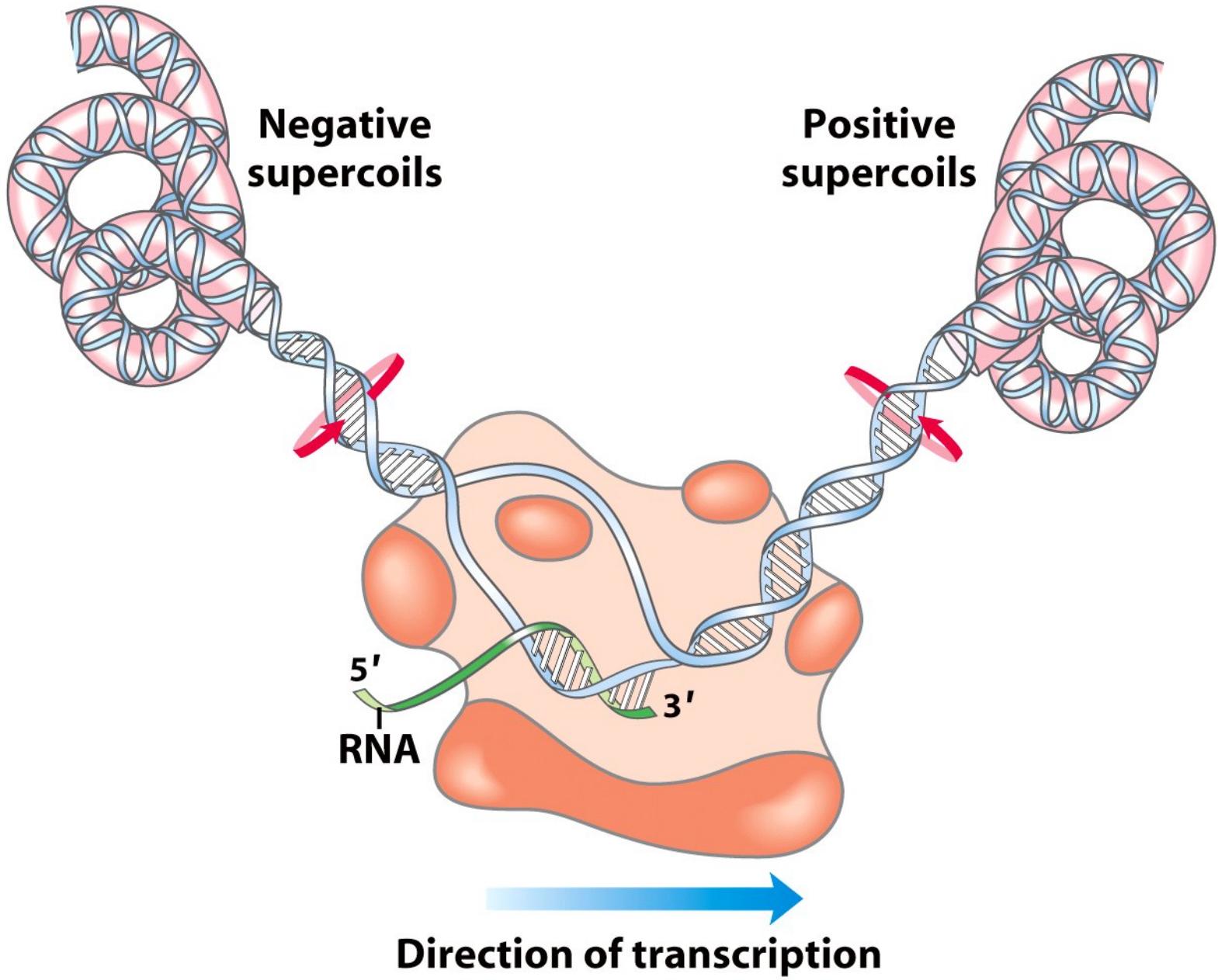


**50 nm**





2  $\mu\text{m}$



# Summary

DNA exists in different topological forms  
in vivo and in vitro

DNA topoisomerases catalyze the  
interconversion of DNA forms

Negative superhelicity (underwinding)  
helps proteins bind DNA by favoring  
unwinding of the helix.

# Variation d'enlacement

$$\Delta L = L - L^\circ$$

$$L^\circ = L(\text{initial})^\circ$$

$$L = T + W$$

$$\Delta L = (T + W) - (T^\circ + W^\circ)$$

$$W^\circ = 0 \text{ (par définition)}$$

$$\Delta L = (T + W) - T^\circ$$

$$\Delta L = \Delta T + W$$

$L$  est un invariant topologique (pour le changer il faut couper un des brins)

Un plasmide circulaire relaxé a 9090 paires de bases. En variant un paramètre physico-chimique, 60 paires de bases adoptent la forme Z  
Quel sera alors le nombre de supertours du plasmide?

Il n'y a pas de changement du nombre d'enlacement (l'ADN n'a pas été coupé). Donc, comme

$$\Delta L = \Delta T + W \text{ avec } \Delta L = 0$$

$$W = -\Delta T$$

$$\Delta T = T(\text{état avec } Z) - T(\text{initial})$$

$$T(\text{initial}) = 9090/10 \text{ (si 10 pdb/tour)}$$

$$T(\text{état } Z) = (9090-60)/10 - 60/12$$

(12 pdb/tour pour forme gauche Z)

$$\Delta T = -11 \gg W = +11$$

Introduction de 11 supertours positifs

En partant du plasmide relaxé, combien de paires de bases devraient adopter la forme A pour obtenir le même topoisomère?

Soit  $x$  le nombre de paires de bases qui devraient passer en forme A (avec 11 bases par tour)

On a

$$+ 11 = -\Delta T$$

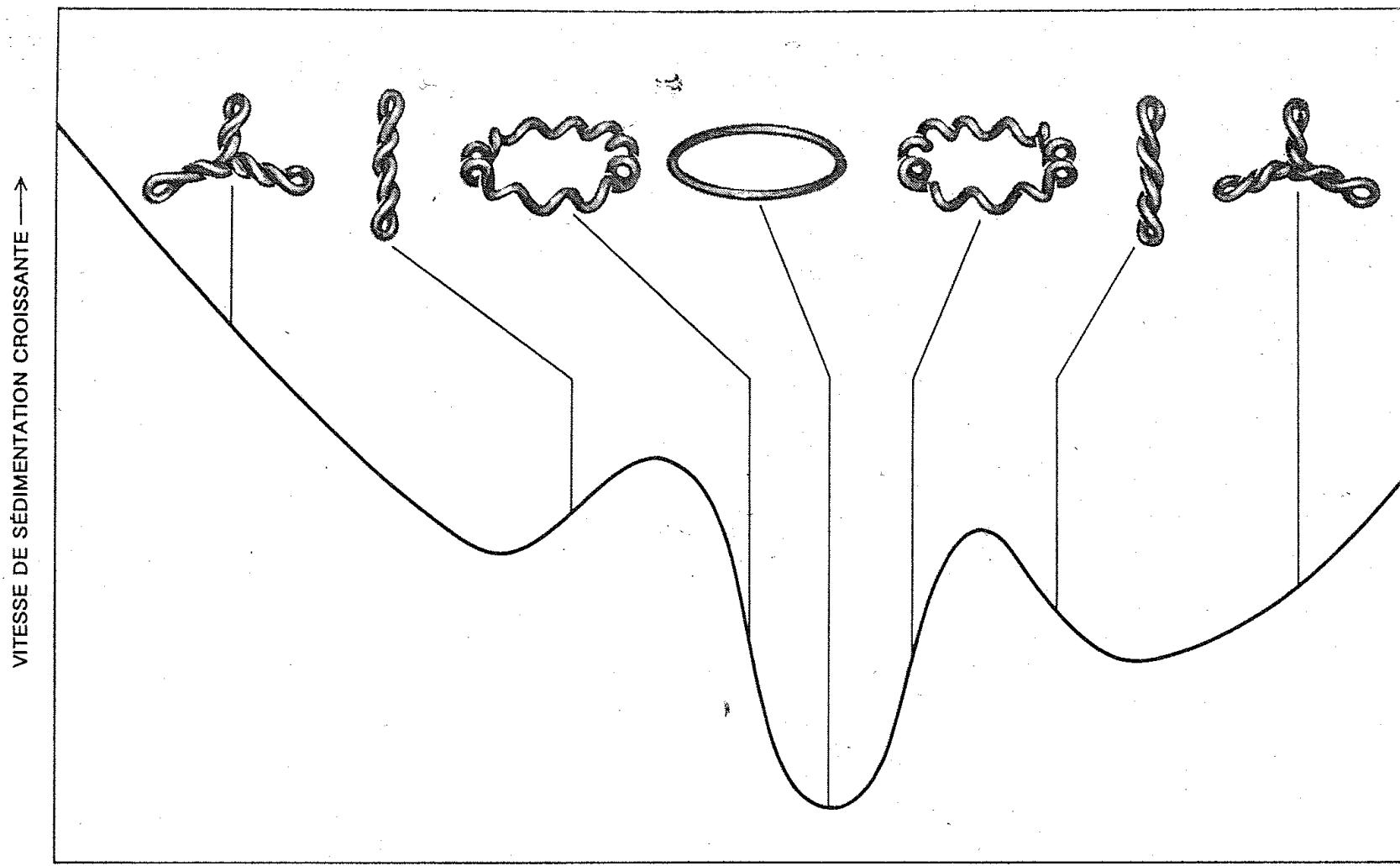
$$\Delta T = T(\text{état A}) - T(\text{état initial})$$

$$-\Delta T = T(\text{état initial}) - T(\text{état A})$$

$$+ 11 = \frac{9090}{10} - \left( \frac{9090-x}{10} + \frac{x}{11} \right)$$

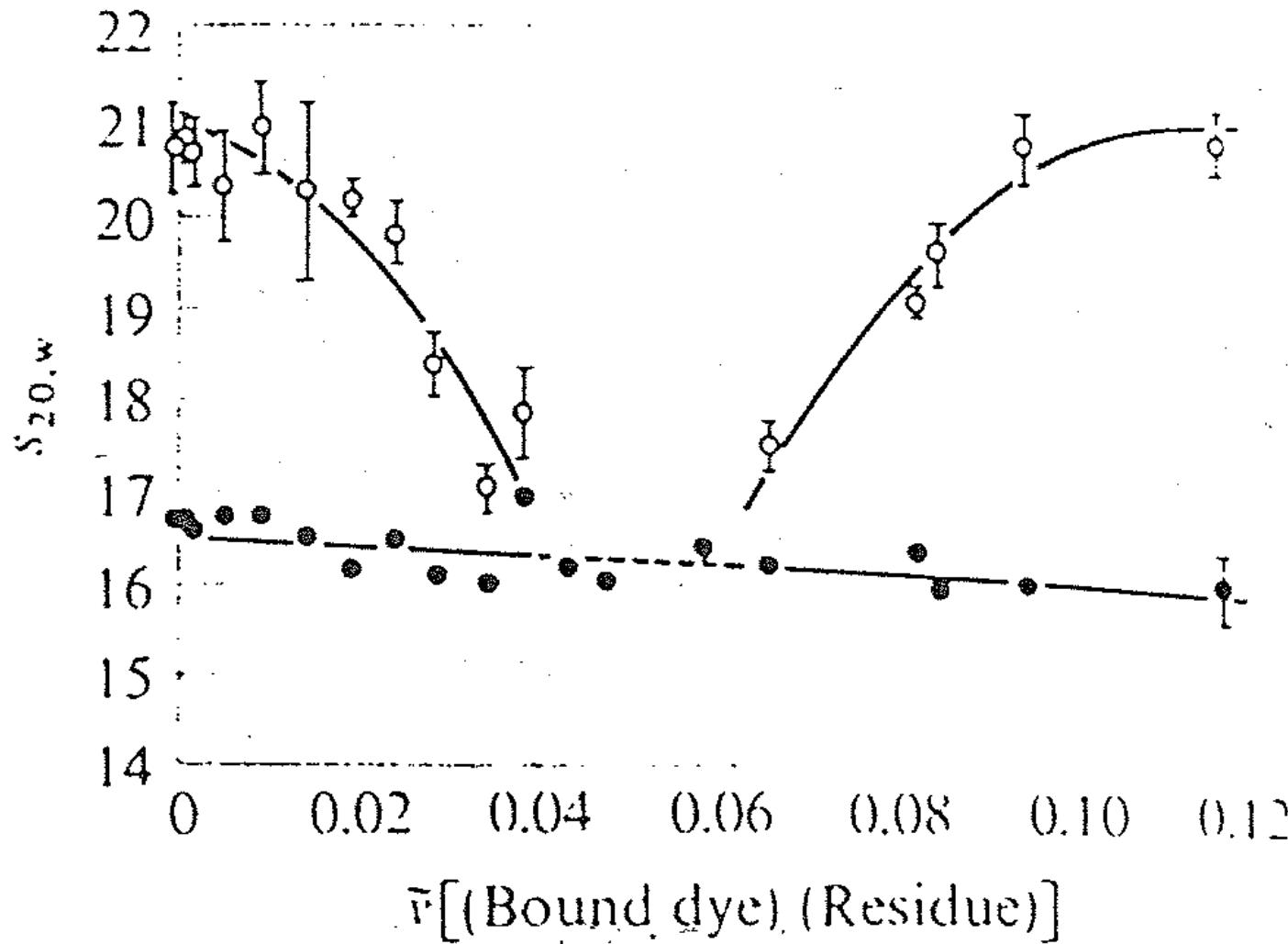
$$+ 11 = \frac{9090}{10} - \frac{9090-x}{10} - \frac{x}{11}$$

$$\gg x = 1210 \text{ bases}$$



**9. LES SUPERHELICES SE RELACHENT** lorsque l'on ajoute du bromure d'éthidium, (un colorant qui a une structure moléculaire plane) à une solution d'ADN surenroulé. Ce phénomène est illustré par cette figure qui représente la variation de la vitesse de sédimentation de l'ADN lorsqu'on ajoute le colorant. La vitesse de sédimentation est la vitesse à laquelle les molécules d'ADN se déplacent dans le solvant lorsque la solution est soumise à un champ de gravitation intense par ultracentrifugation : plus les molécules sont surenroulées, plus elles sont compactes, et plus leur vitesse de sédimentation est élevée. En augmentant la concentration de bromure d'éthidium, on diminue

progressivement le vrillage des molécules d'ADN, non pas en augmentant la valeur du nombre d'enlacements (ce qui ne peut se faire qu'en coupant les chaînes polynucléotidiques) mais en réduisant leur tortilllement. Les molécules de colorant s'intercalent entre les paires de bases de l'ADN, détordant localement la double hélice. Le graphe montre qu'une concentration suffisante de colorant provoque le relâchement total des molécules d'ADN, et que si l'on en ajoute encore plus, l'ADN commence à se surenrouler en sens inverse. Les changements de structure de l'ADN que l'on a représentés ici sont confirmés par la microscopie électronique.



*Sedimentation velocity of SV40 DNA as a function of bound ethidium.* Form I DNA is closed circular duplex (○); form II is nicked circular duplex (●). Measurements were made in 1.0 M NaCl. [After W. Bauer and J. Vinograd, *J. Mol. Biol.* 33:141(1968).]

## Effet d'un intercalant

Si chaque molécule de bromure d'éthidium intercalée détourne la double hélice de  $26^\circ$ , combien faut-il de molécules de bromure d'éthidium pour diminuer le nombre d'enlacement d'une unité?