

# Molecular Biology



## DNA – 2.Kinetics

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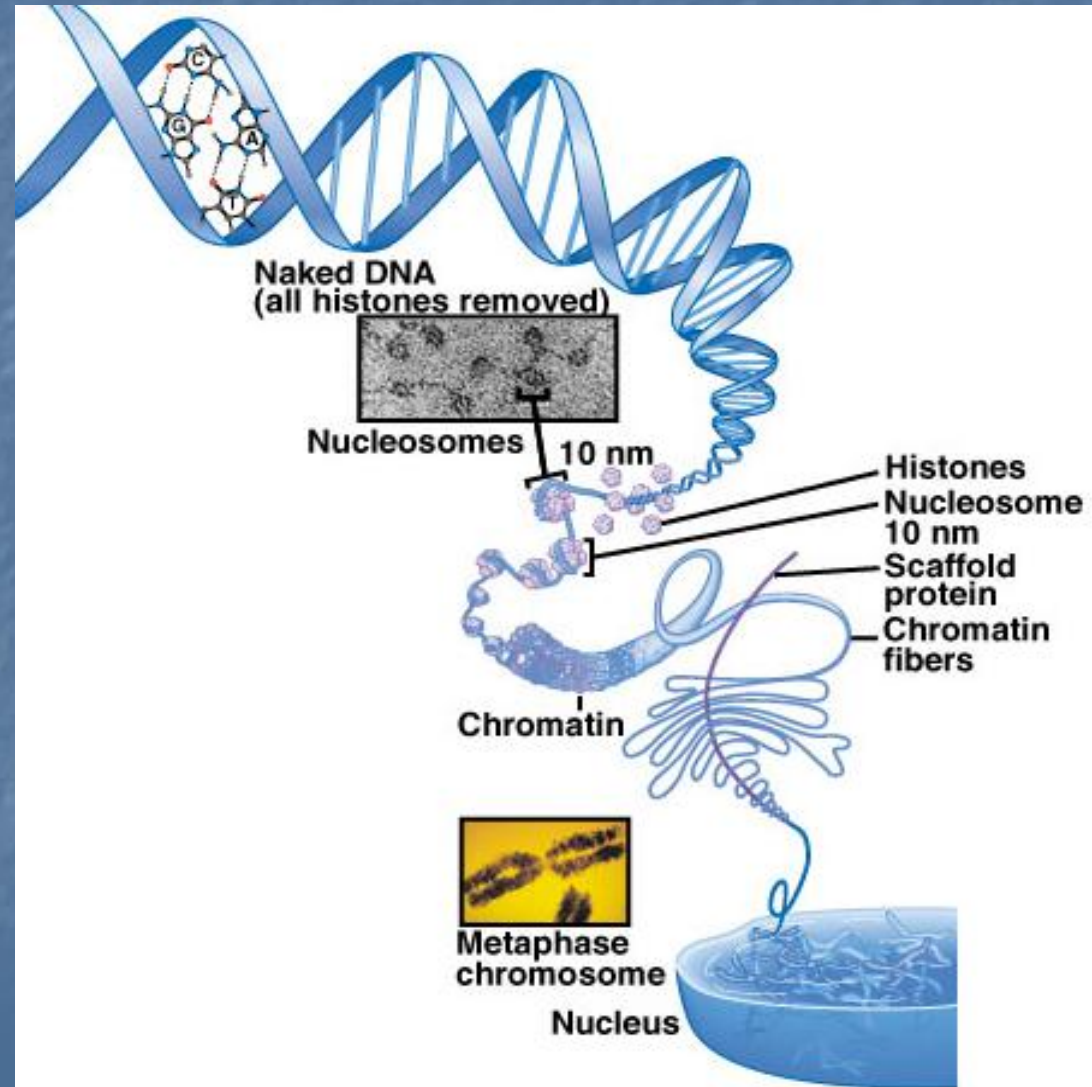
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# Chromatin = DNA and associated proteins

DNA winds around histone proteins (nucleosomes).

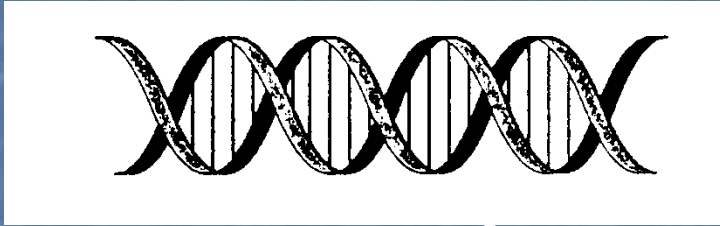
Other proteins wind DNA into more tightly packed form, the chromosome.

Unwinding portions of the chromosome is important for mitosis, replication and making RNA.

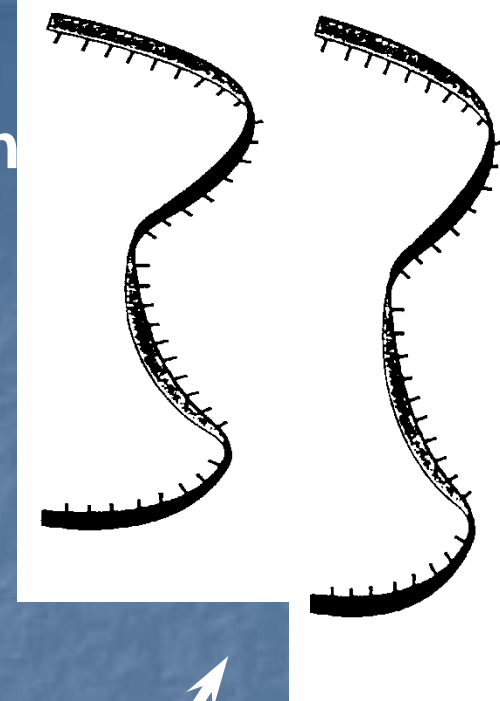


# Denaturation of DNA

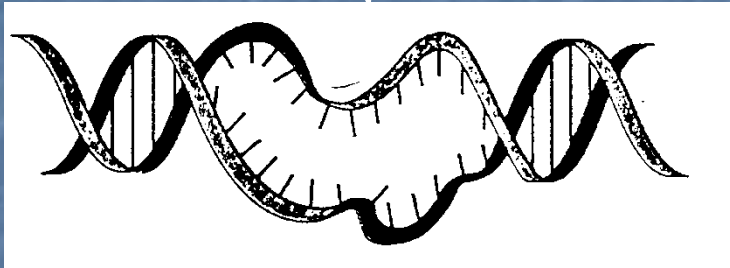
Double-stranded DNA



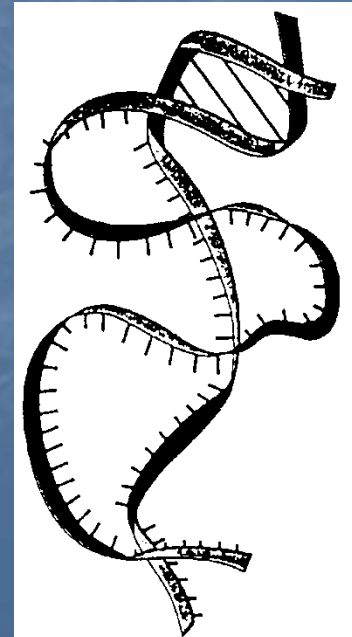
Strand separation and formation of single-stranded random coils



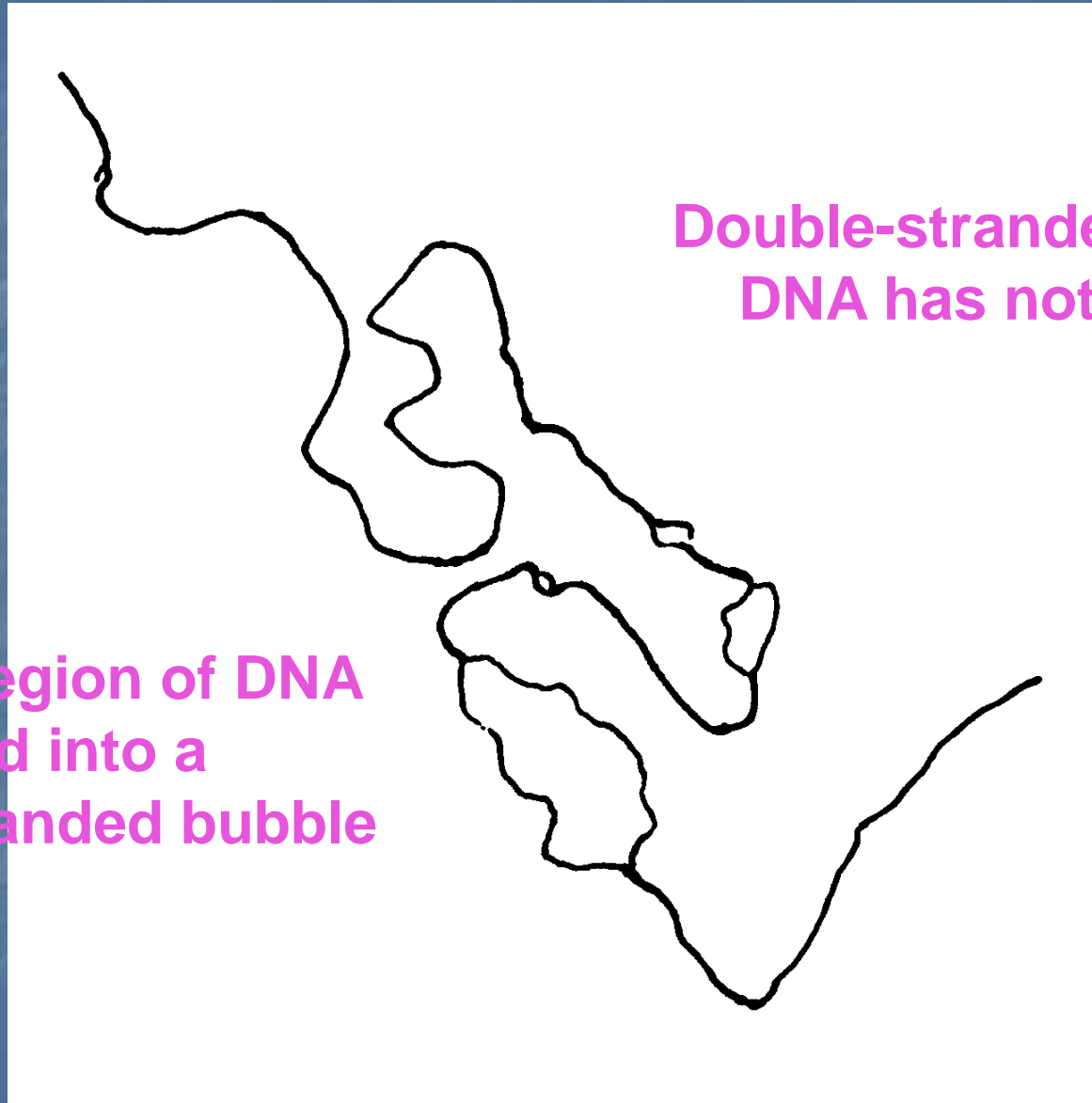
Extremes in pH or high temperature  
A-T rich regions denature first



Cooperative unwinding of the DNA strands



# Electron micrograph of partially melted DNA

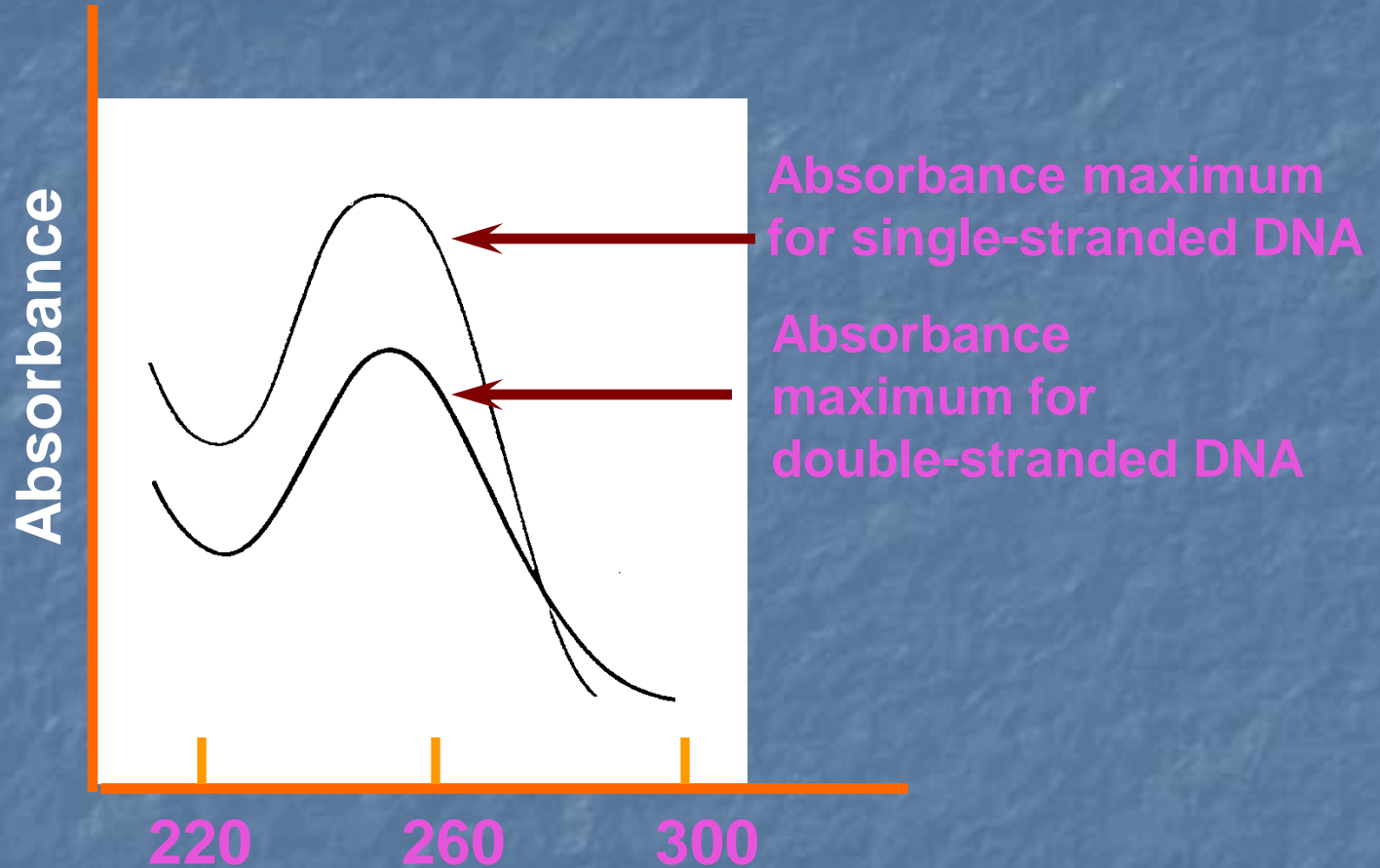


Double-stranded, G-C rich DNA has not yet melted

A-T rich region of DNA has melted into a single-stranded bubble

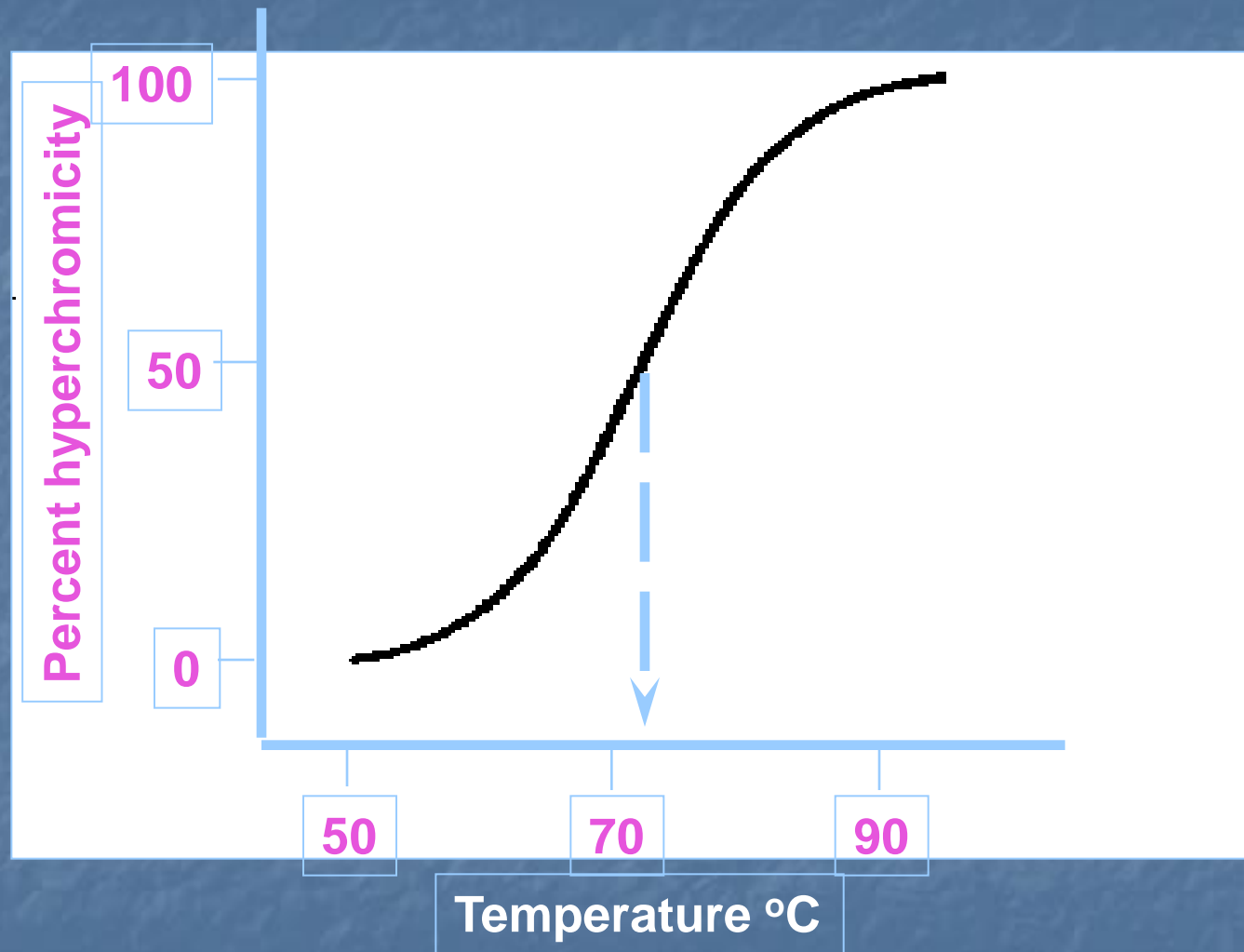
- A-T rich regions melt first, followed by G-C rich regions

# Hyperchromicity



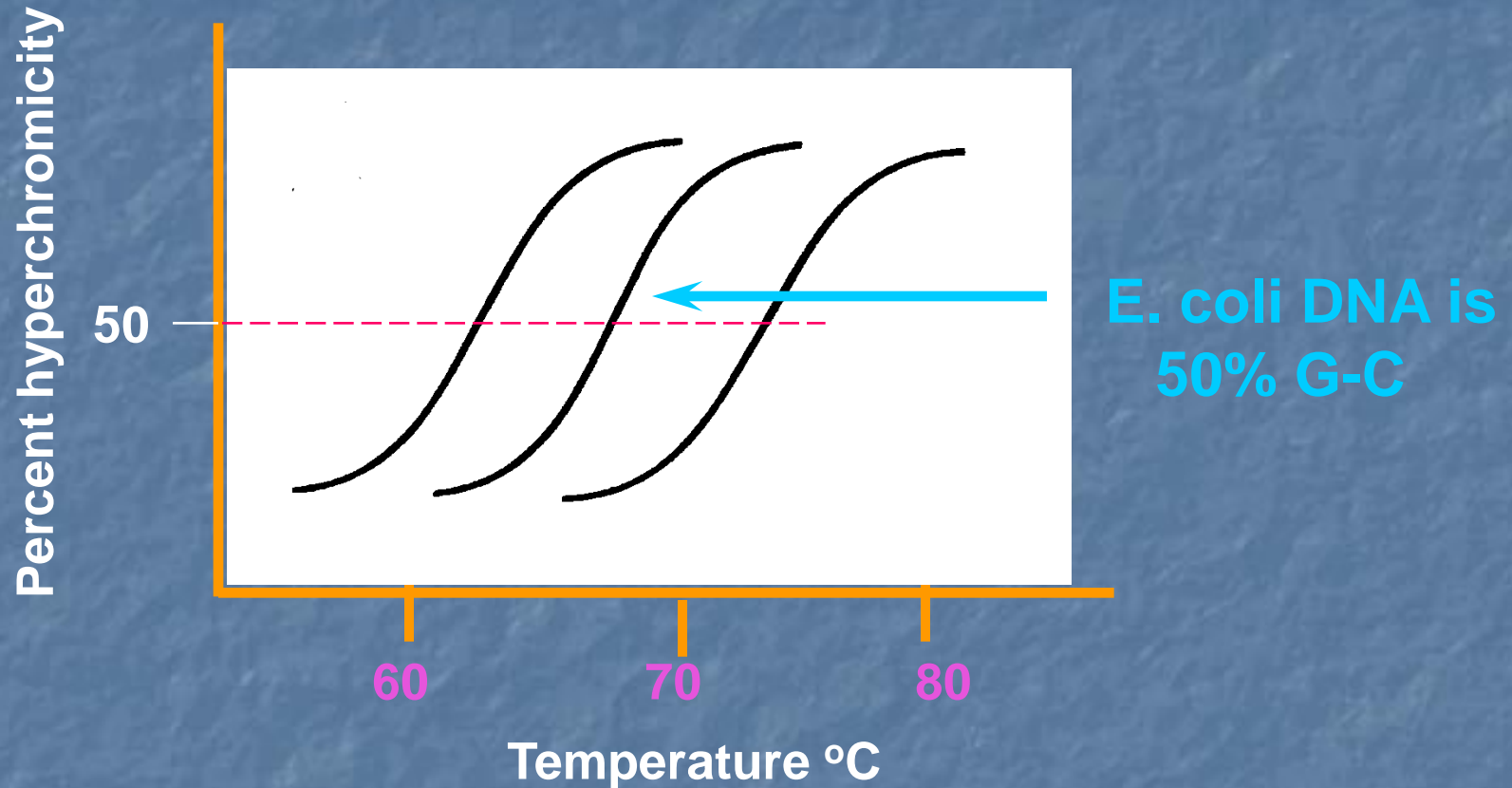
The absorbance at 260 nm of a DNA solution increases when the double helix is melted into single strands.

# DNA melting curve



- $T_m$  is the temperature at the midpoint of the transition

$T_m$  is dependent on the G-C content of the DNA



Average base composition (G-C content) can be determined from the melting temperature of DNA

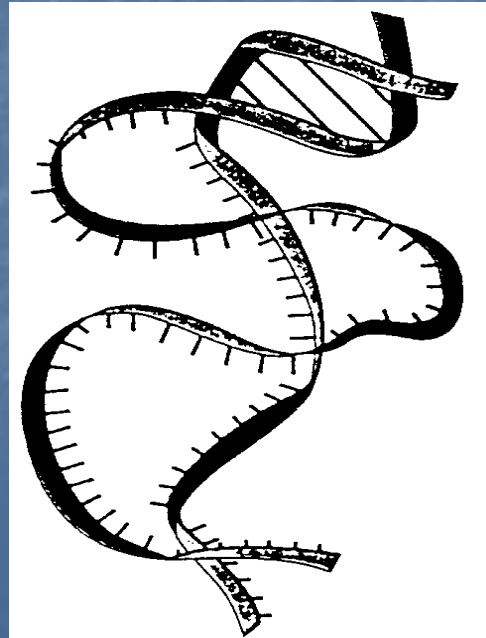
# DNA reassociation (renaturation)



Denatured,  
single-stranded  
DNA

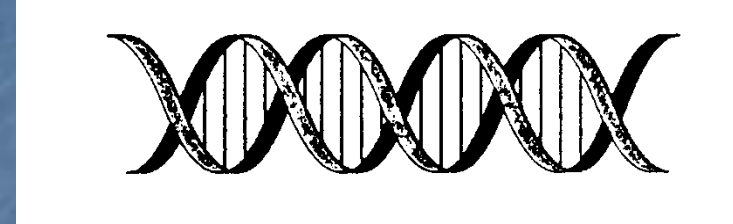
$k_2$

Slower, rate-limiting,  
second-order process of  
finding complementary  
sequences to nucleate  
base-pairing



Faster,  
zippering  
reaction to  
form long  
molecules  
of double-  
stranded  
DNA

Double-stranded DNA





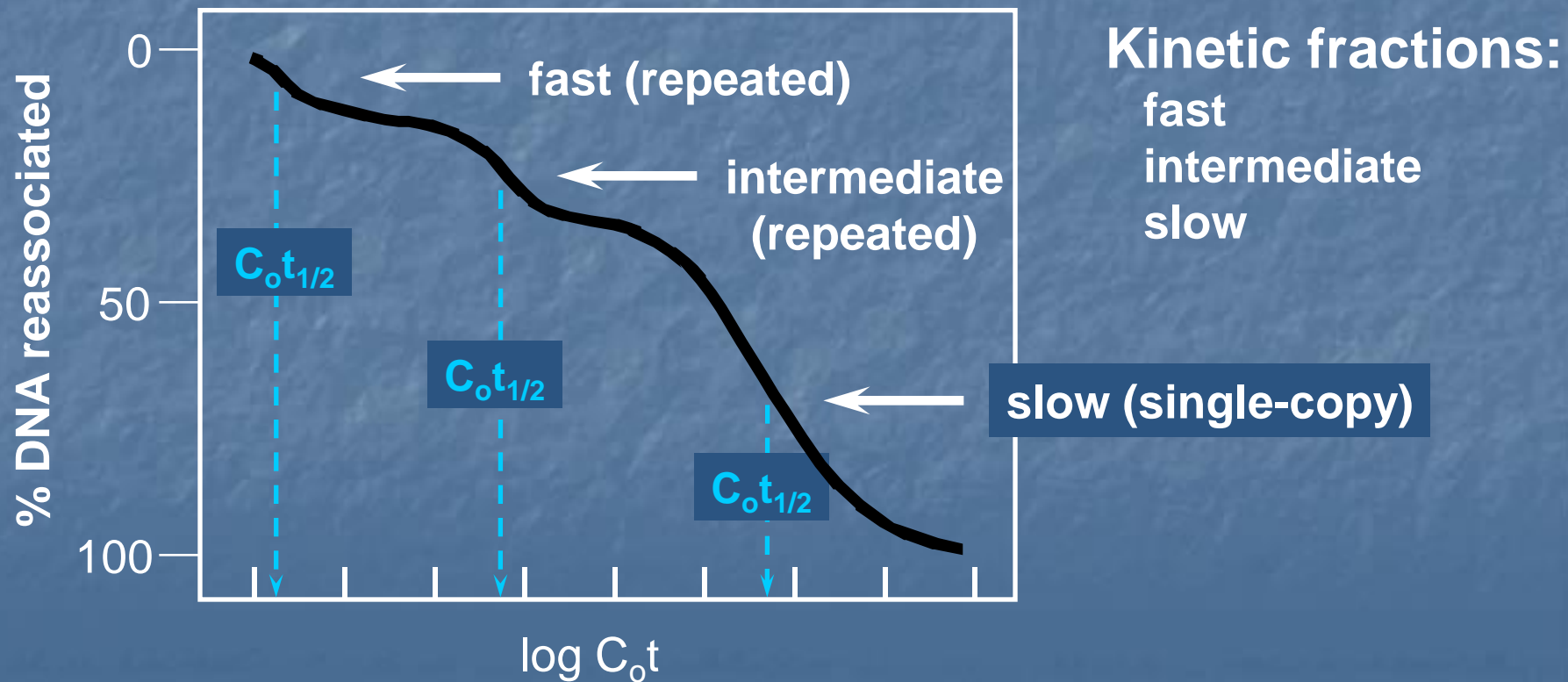
# DNA reassociation kinetics for human genomic DNA

$$C_0 t_{1/2} = 1 / k_2$$

$k_2$  = second-order rate constant

$C_0$  = DNA concentration (initial)

$t_{1/2}$  = time for half reaction of each component or fraction

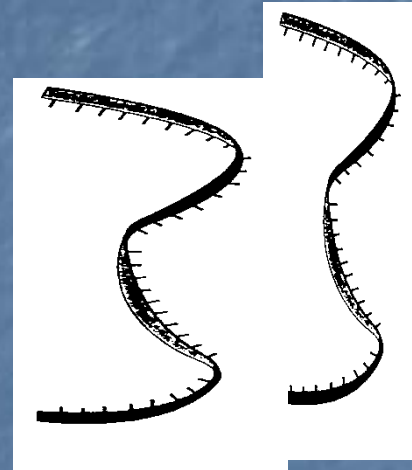
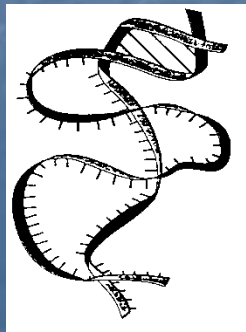


$10^6$  copies per genome of  
a “low complexity” sequence  
of e.g. 300 base pairs

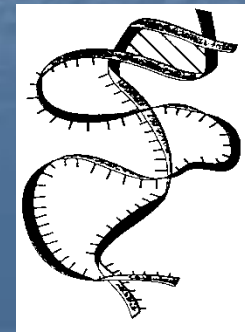
1 copy per genome of  
a “high complexity” sequence  
of e.g.  $300 \times 10^6$  base pairs



high  $k_2$



low  $k_2$



# What is Supercoiling?

- The pitch of B-DNA in solution is approx. 3.4 nm/helical repeat ← 10.5 bp
- In addition to the helical coiling of single strands to form a double helix, the double stranded DNA molecule can also twist upon itself. This is what is known as supertwisting or “**supercoiling.**”

Supercoiling occurs in nearly all chromosomes (**circular or linear**)

## Relaxed vs Supercoiled DNA

Relaxed DNA has no supercoils

Negatively supercoiled DNA is underwound (favors unwinding of the helix)  
(circular DNA isolated from cells is always negatively supercoiled)

Positively supercoiled DNA is overwound

$$L = T + W$$

**Linking Number (L or  $L_k$ )** = number of times the two strands are intertwined

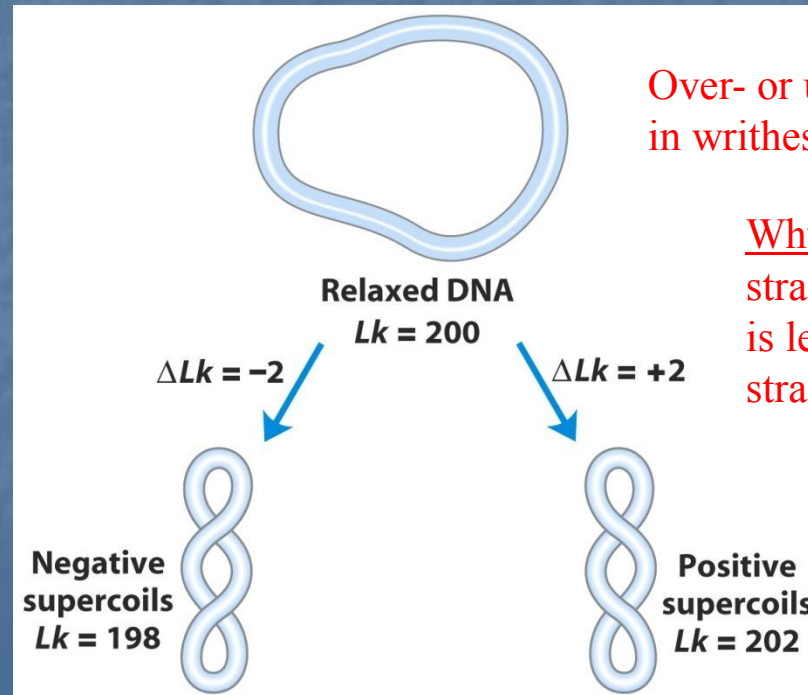
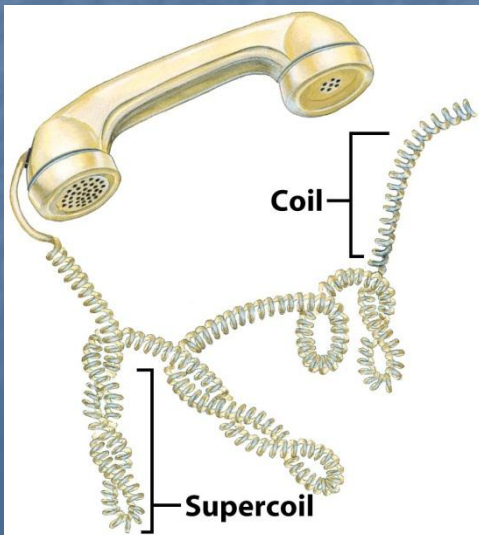
**Twists (T or  $T_w$ )** = number of helical turns

For a 2,000 bp DNA duplex,  $T = 200$  (2,000 bp  $\times$  1 turn/10 bp = 200 turns)

**Writhes (W or  $W_r$ )** = number of times the duplex crosses itself (only topologically constrained DNA molecules can have writhes)

A relaxed DNA molecule has zero writhes. ( $\therefore$  For a relaxed DNA molecule,  $L = T$ )

**Writhes = Supercoils**



**Over- or underwinding results in writhes instead of twists**

Why? Because the strain of writhes is less than the strain of twists

## Additional Terms Used To Describe Topology

The **Linking Number Difference** ( $\Delta L$ ) is the difference between the linking number of a DNA molecule ( $L$ ) and the linking number of its relaxed form ( $L_0$ ). The equation is  $\Delta L = L - L_0$ .

$\Delta L$  is a measure of the number of writhes

For a relaxed molecule:  $\Delta L = 0$

The **Superhelical Density** ( $\sigma$ ) is a measure of supercoiling that is independent of length.

The equation is  $\sigma = \Delta L / L_0$ .

$\sigma$  is a measure of the ratio of writhes to twists

For a relaxed molecule:  $\sigma = 0$

DNA in cells has a  $\sigma$  of  $-0.06$  (for circular molecules purified from bacteria and eukaryotes)

## Sample Linking Number Questions

- 1) You have a relaxed 5,500 bp plasmid DNA molecule, which you treat with DNA gyrase to add 50 negative supercoils. (Assume that B-DNA has 10 bp/turn.) ( $\Delta L = L - L_0$ ) ( $\sigma = \Delta L / L_0$ )

- A. What is the linking number of the molecule (before gyrase treatment)?**
- B. What is the linking number of the molecule after treatment with DNA gyrase?**
- C. What is change in linking number ( $\Delta L$ ) after treatment with DNA gyrase?**
- D. What is the superhelical density ( $\sigma$ ) after treatment with DNA gyrase?**

- A.  $L = T + W$ ; for relaxed molecule  $W = 0 \therefore L_0 = T$ ;  $5500 \text{ bp} \times 10 \text{ bp/turn} = 550 \text{ turns}$
- B.  $L = T + W = 550 + (-50) = 500$
- C.  $\Delta L = L - L_0 = 500 - 550 = -50$
- D.  $\sigma = \Delta L / L_0 = -50 / 550 = -0.09$

2) Instead of treating the relaxed 5,500 bp plasmid DNA molecule above with DNA gyrase, you transfer it from aqueous solution to 50% ethanol. Under these conditions, the structure changes from B-DNA to A-DNA due to the relatively lower water concentration. (A-DNA has 11 bp/turn).

**A. What is the linking number after transfer to 50% ethanol?**

**B. How many helical turns will there be after transfer to 50% ethanol?**

**C. How many writhes will there be after transfer to 50% ethanol?**

A.  $L = 550$  (linking # stays the same because no bonds are broken)

B.  $5500 \times 11 \text{ bp/turn} = 500$  helical turns

C.  $L = T + W$ ;  $550 = 500 + W$ ;  $W = +50$

## Type I and II Topoisomerases (usually relax supercoiled DNA)

Rule #1: They change the linking number by changing the # of writhes.

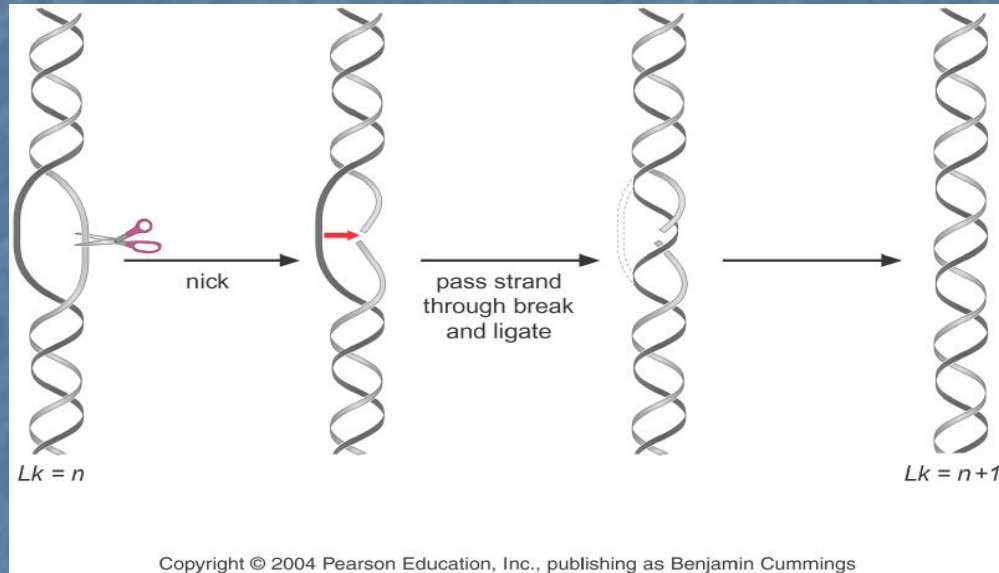
Rule #2: They change the linking number by breaking one or both strands of the DNA molecule, winding them tighter or looser, then rejoining the ends.

Rule #3: They work only on topologically constrained DNA molecules because only topologically constrained DNA molecules can have writhes.



# 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.



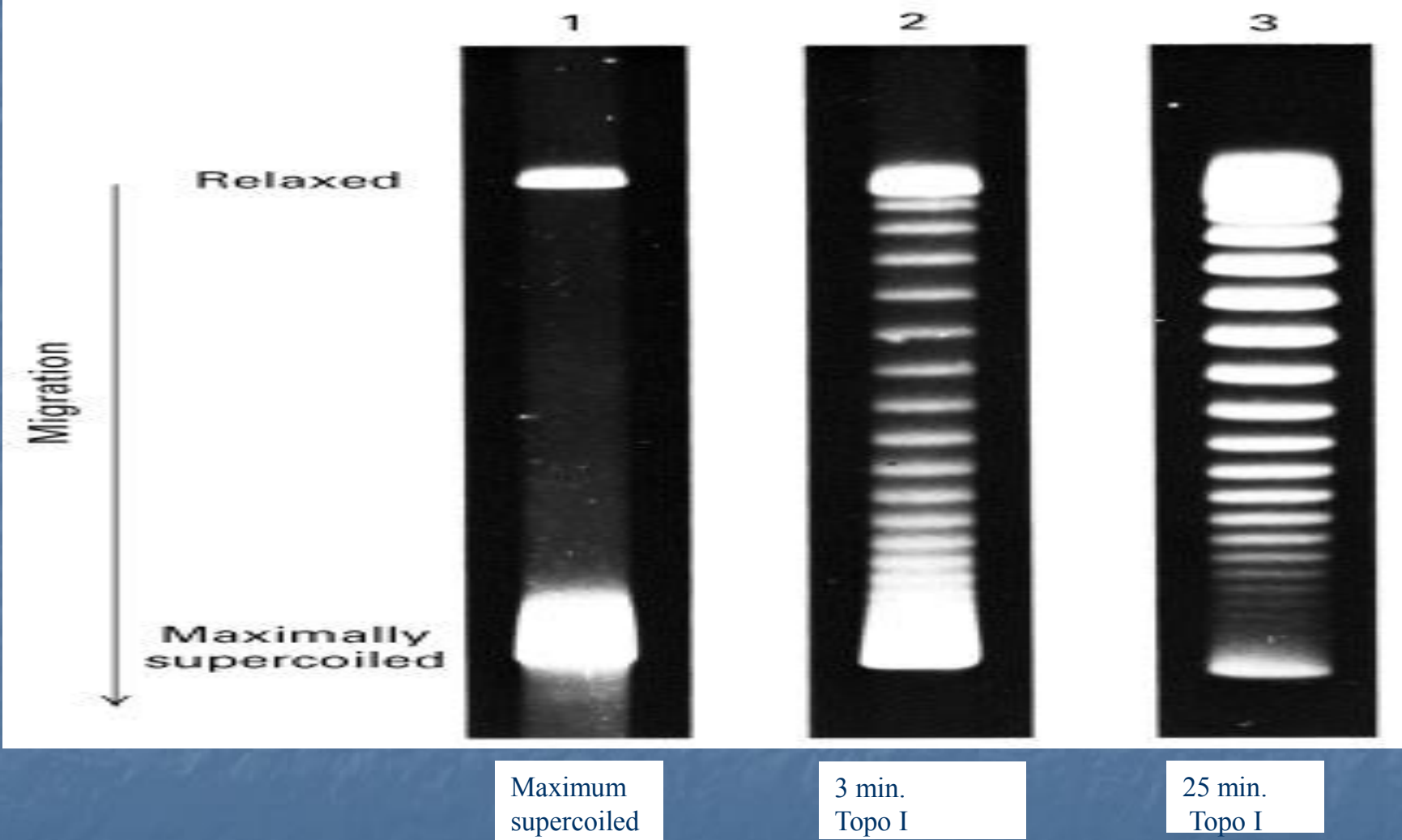
## Topo I of *E. coli*

- 1) acts to relax only negative supercoils
- 2) increases linking number by +1 increments

## Topo I of eukaryotes

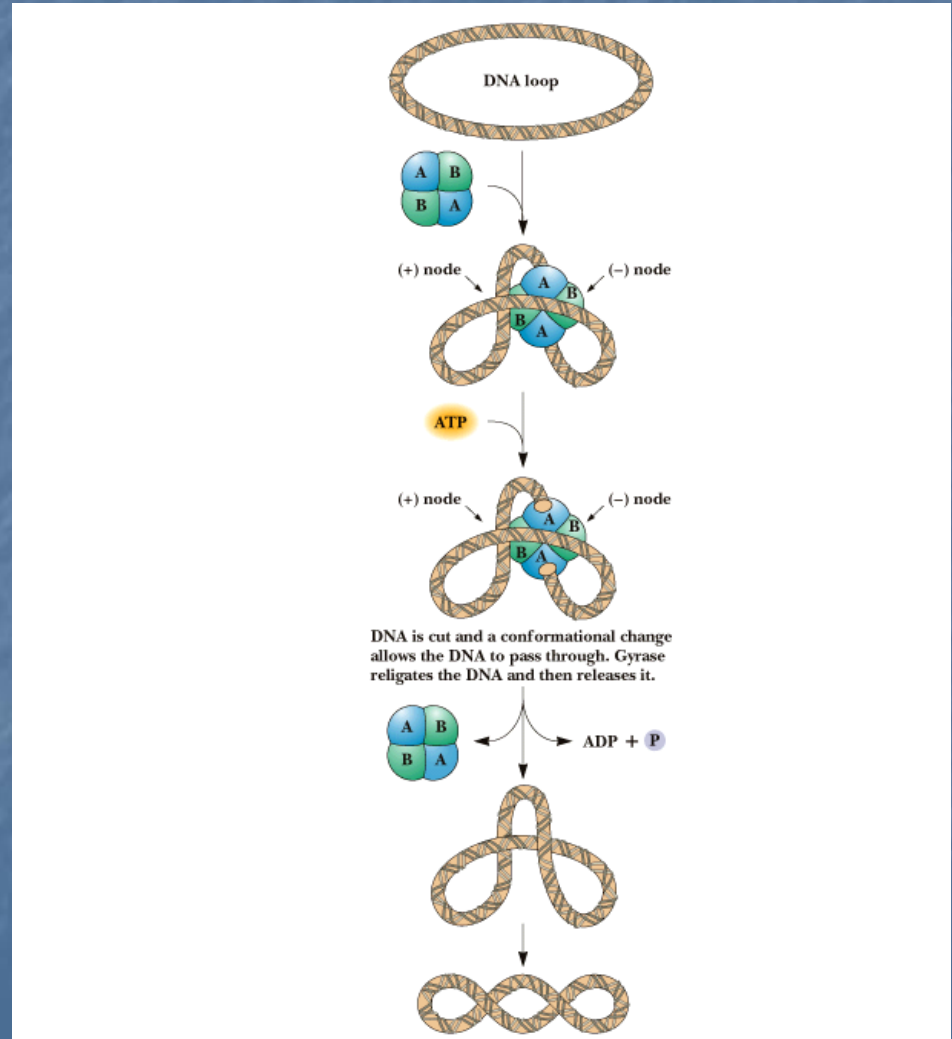
- 1) acts to relax positive or negative supercoils
- 2) changes linking number by -1 or +1 increments

# Relaxation of SV40 DNA by Eukaryotic Topo I



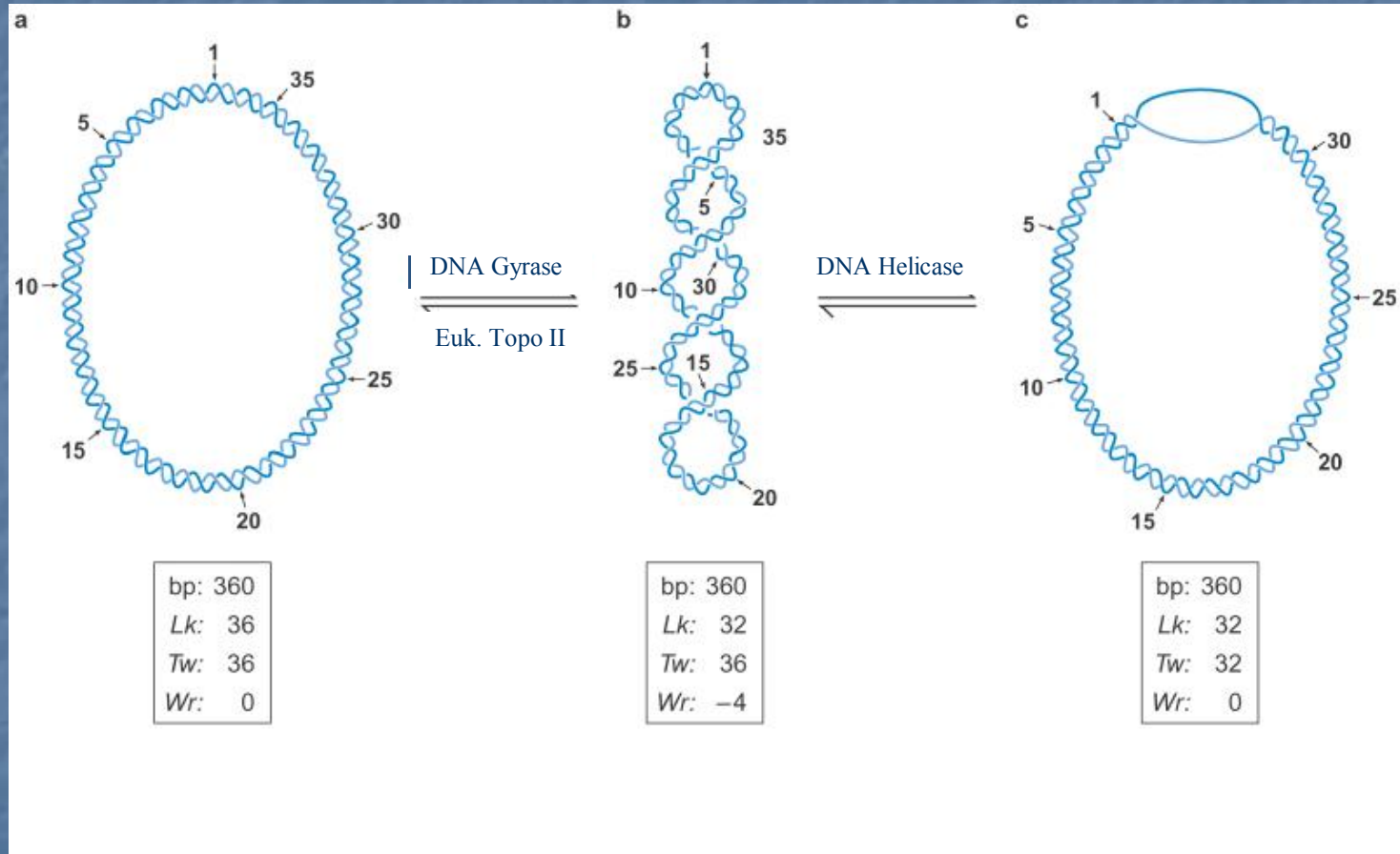
# Type II Topoisomerases

They relax or underwind DNA by cutting both strands then sealing them. They change the linking number by increments of +2 or -2.



# E. Coli vs. Eukaryotic Type II Topoisomerases:

The strain of underwinding DNA is relieved by: Negative supercoils or Local disruption of base pairs



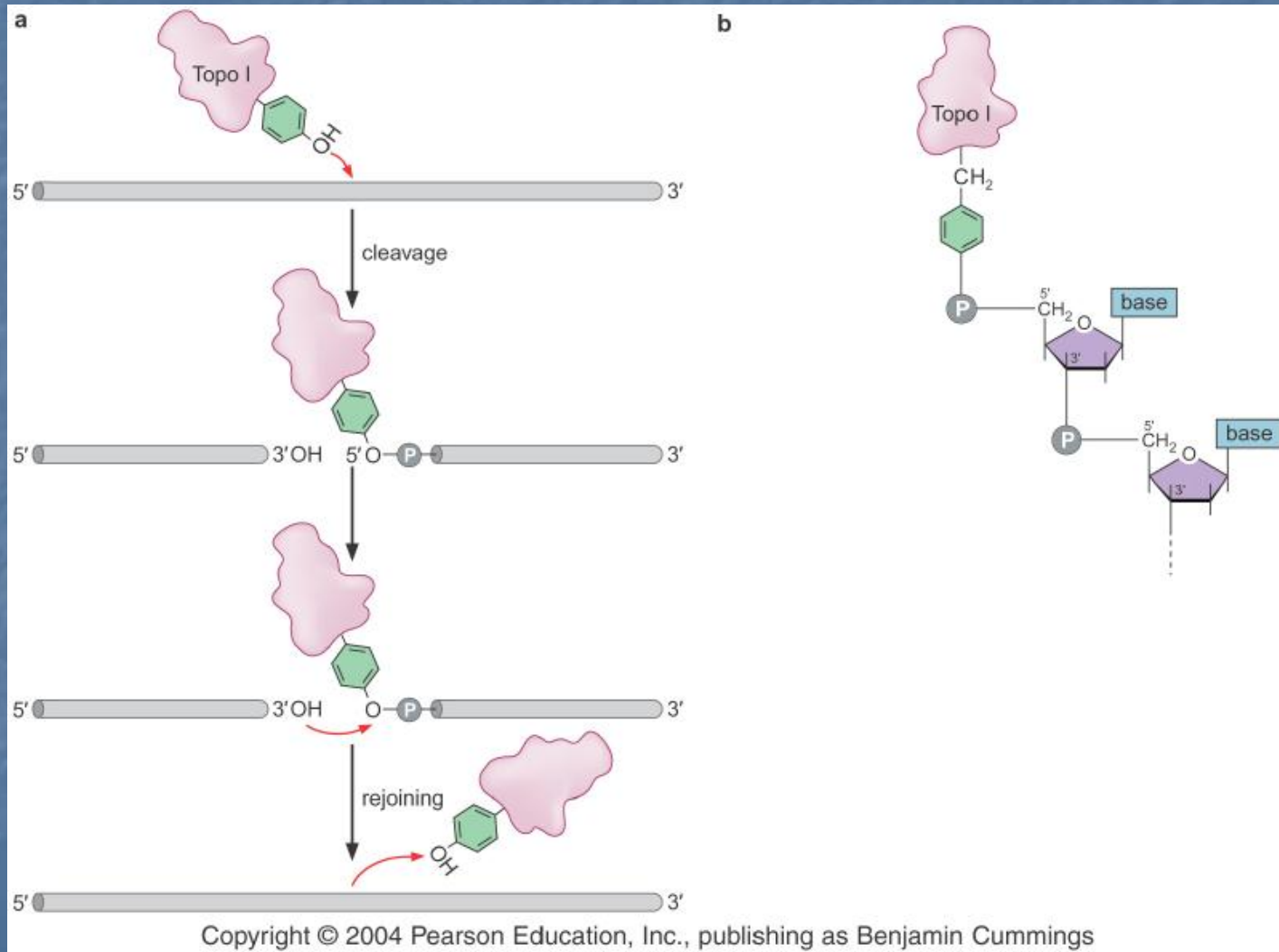
## Topo II of E. coli (DNA Gyrase)

- 1) Acts on both neg. and pos. supercoiled DNA
- 2) Increases the # of neg. supercoils by increments of -2
- 3) Requires ATP

## Topo II of Eukaryotes

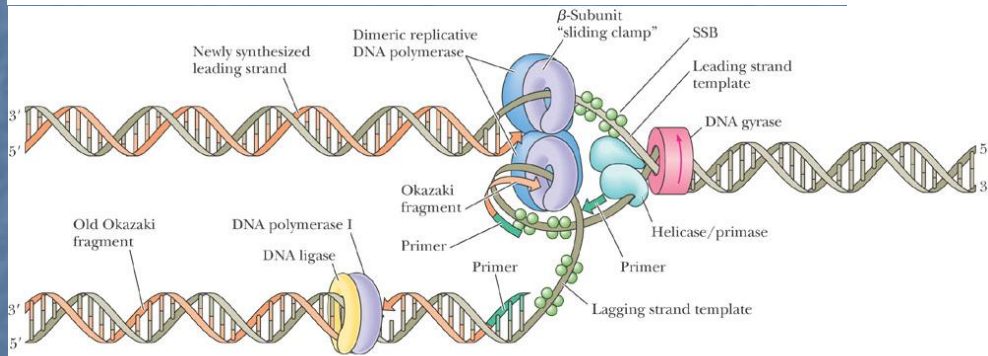
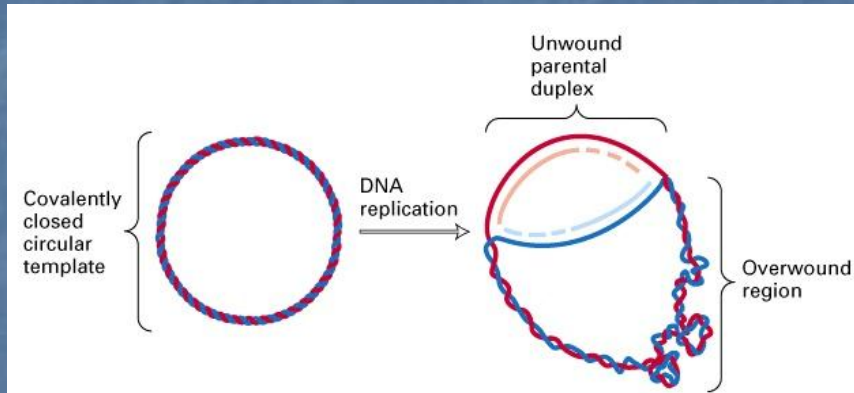
- 1) Relaxes only negatively supercoiled DNA
- 2) Increases the linking number by increments of +2
- 3) Requires ATP

# All Topoisomerases Cleave DNA Using a Covalent Tyrosine-DNA Intermediate

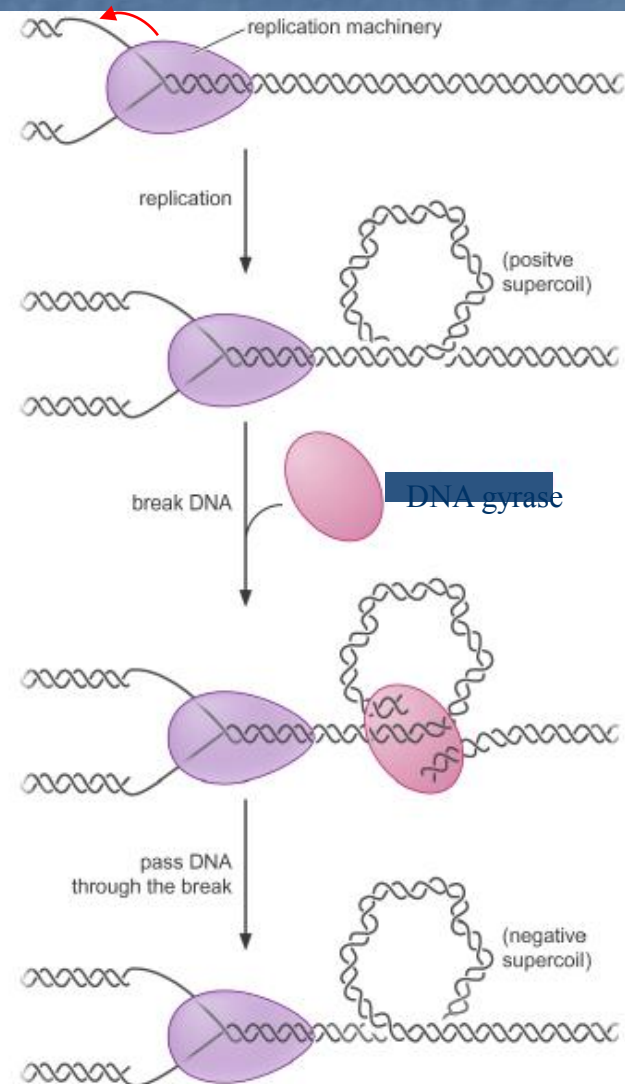


# The Role of Topoisomerases in DNA Replication

1) Topoisomerases remove positive supercoils that normally form ahead of the growing replication fork



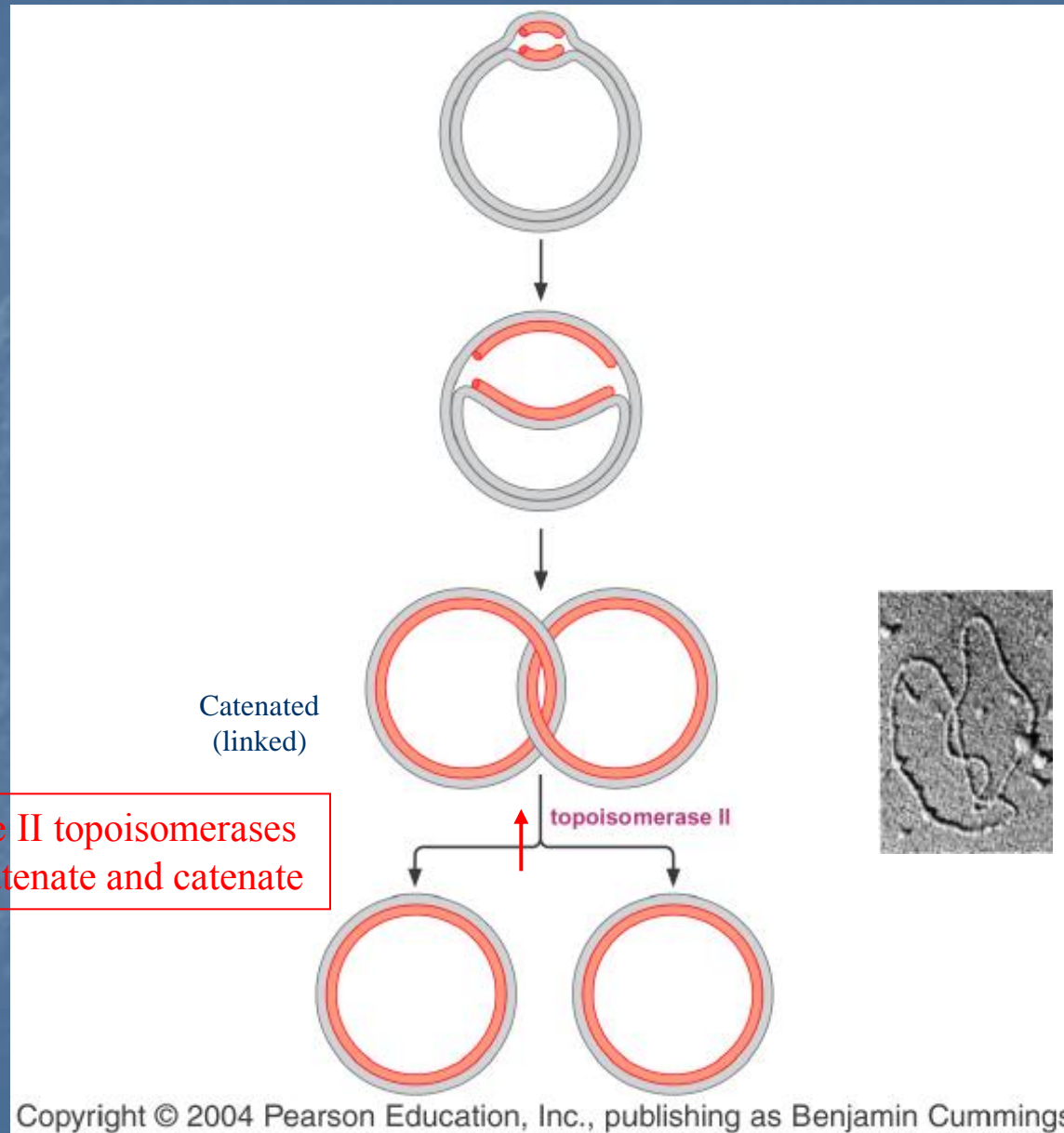
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E. Coli → DNA gyrase (adds neg. supercoils)

Eukaryotes → Topo I (relaxes pos. supercoils)

2) Replicated circular DNA molecules are separated by type II topoisomerases



# A Review of the Different Topoisomerases

Topo Type	E. coli	Eukaryotic
I	Topo I	Topo I
Cleaves 1 strand (nicks) & reseals	Relaxes only – supercoils Changes linking # by +1 Requires no cofactors	Relaxes – and + supercoils Changes linking # by +1 or –1 Requires no cofactors
II	Topo II (DNA Gyrase)	Topo II
Cleaves 2 strands (ds cut) & reseals	Acts on – and + supercoils Changes linking # by increments of -2 Catenates and decatenates DNA Requires ATP Introduces net neg. supercoils	Relaxes only – supercoils Changes linking # by increments of -2 Catenates and decatenates DNA Requires ATP

**Eukaryotic topoisomerases cannot introduce net supercoils,  
Therefore, how can eukaryotic DNA become negatively supercoiled?**