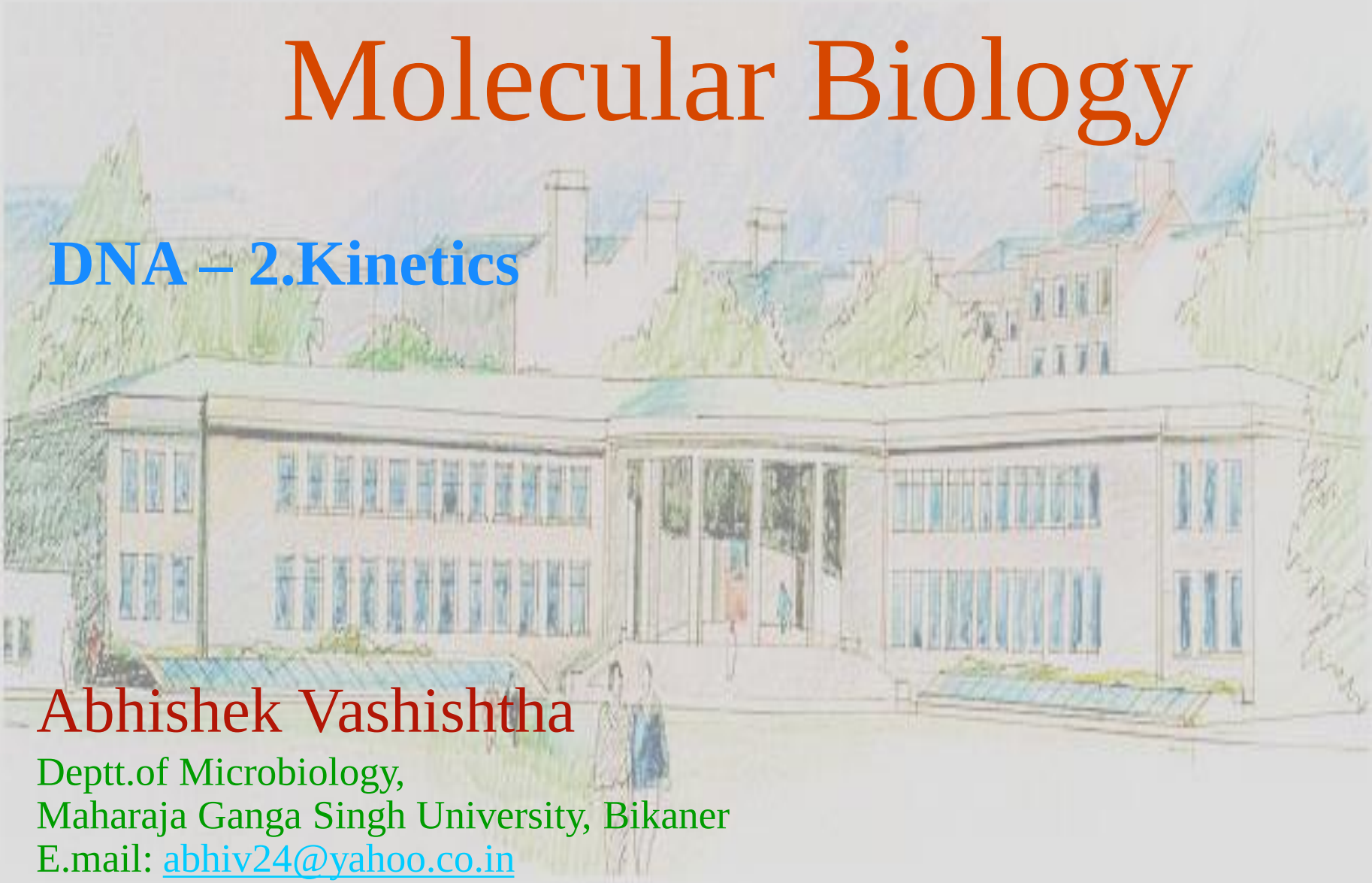
Molecular Biology



DNA – 2.Kinetics

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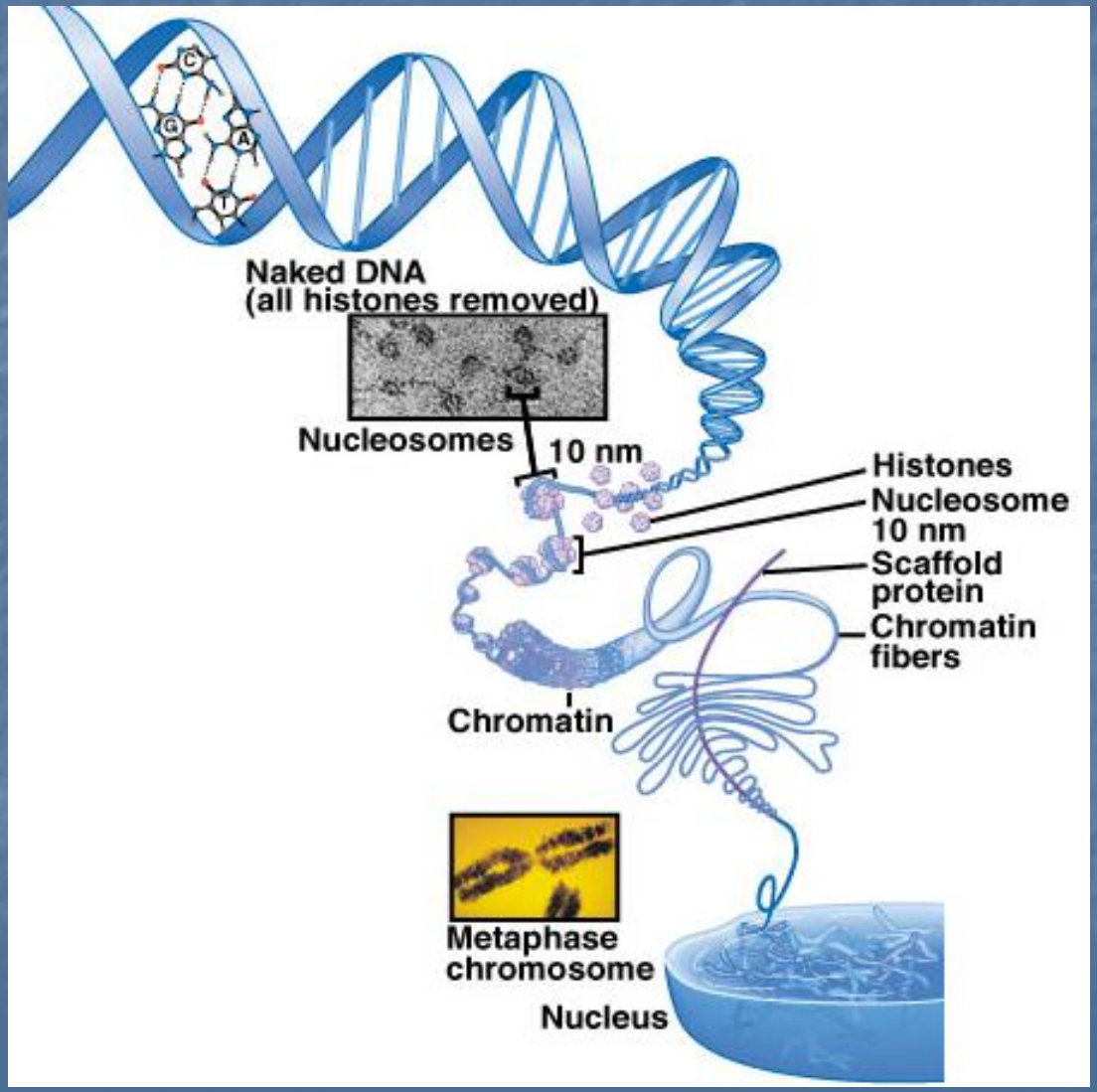
Chromatin = DNA and associated proteins

DNA winds around histone proteins (nucleosomes).

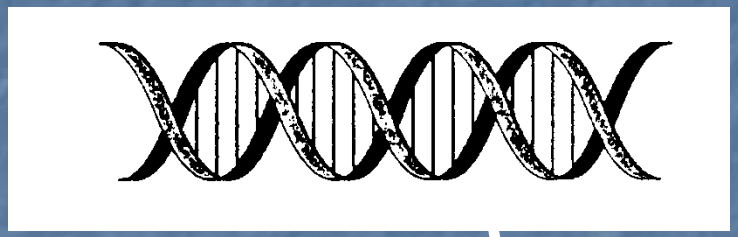
Other proteins wind DNA into more tightly packed form, the chromosome.

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



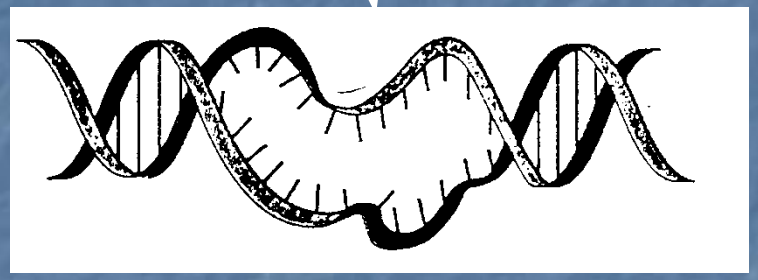
Denaturation of DNA

Double-stranded DNA

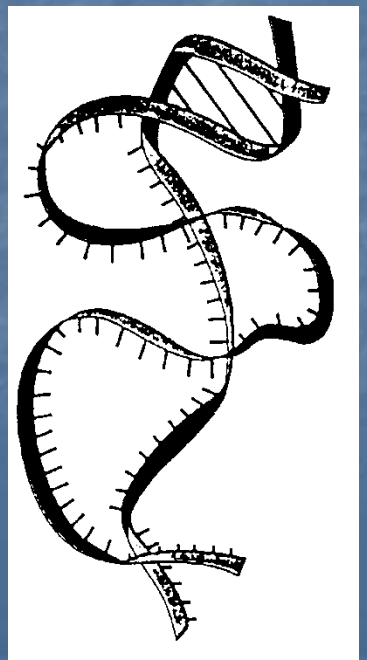


Extremes in pH or high temperature

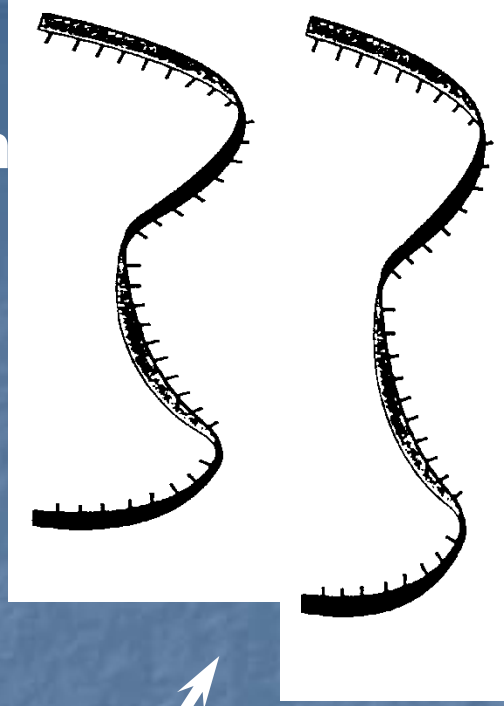
A-T rich regions denature first



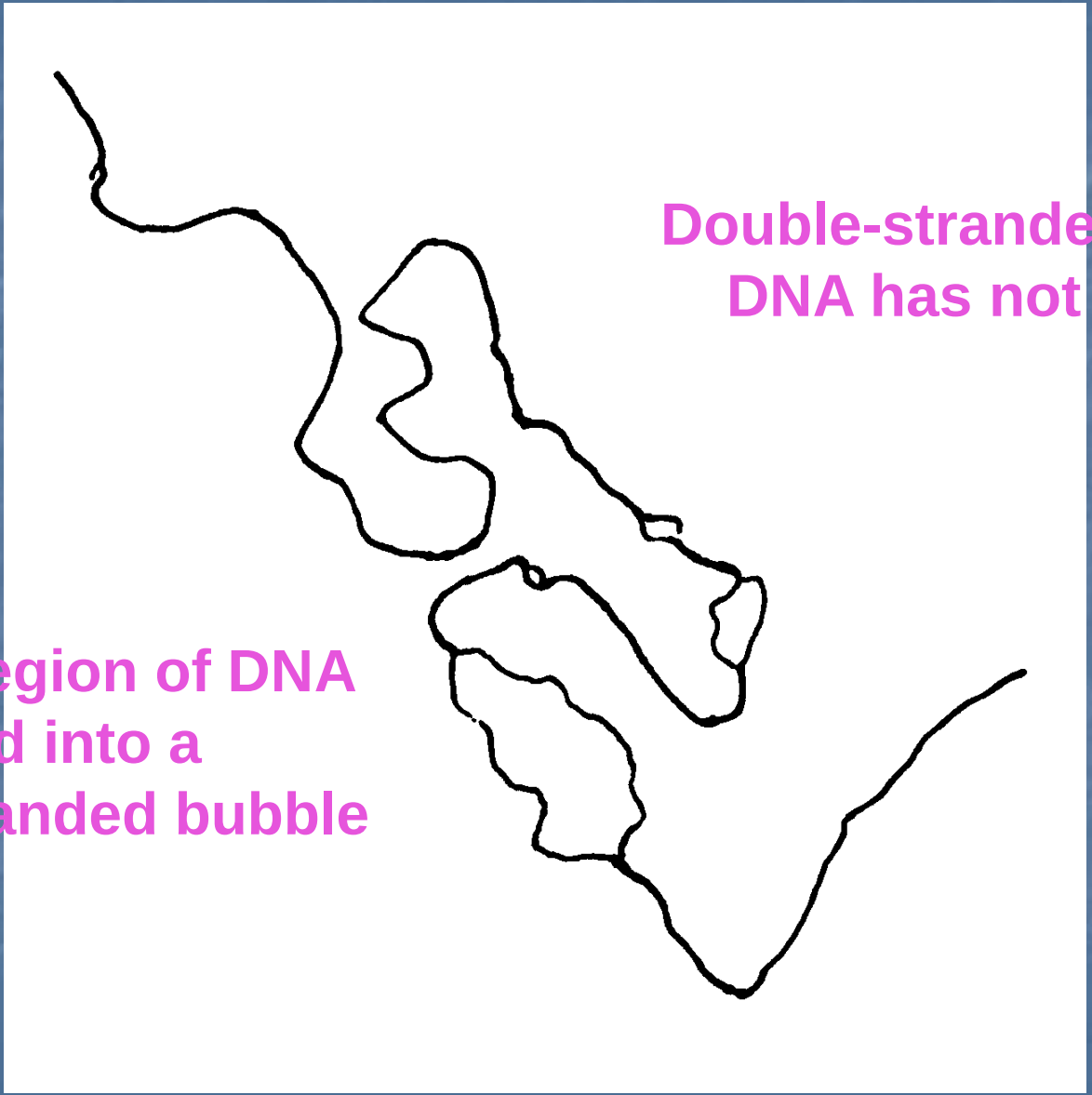
Cooperative unwinding of the DNA strands



Strand separation and formation of single-stranded random coils

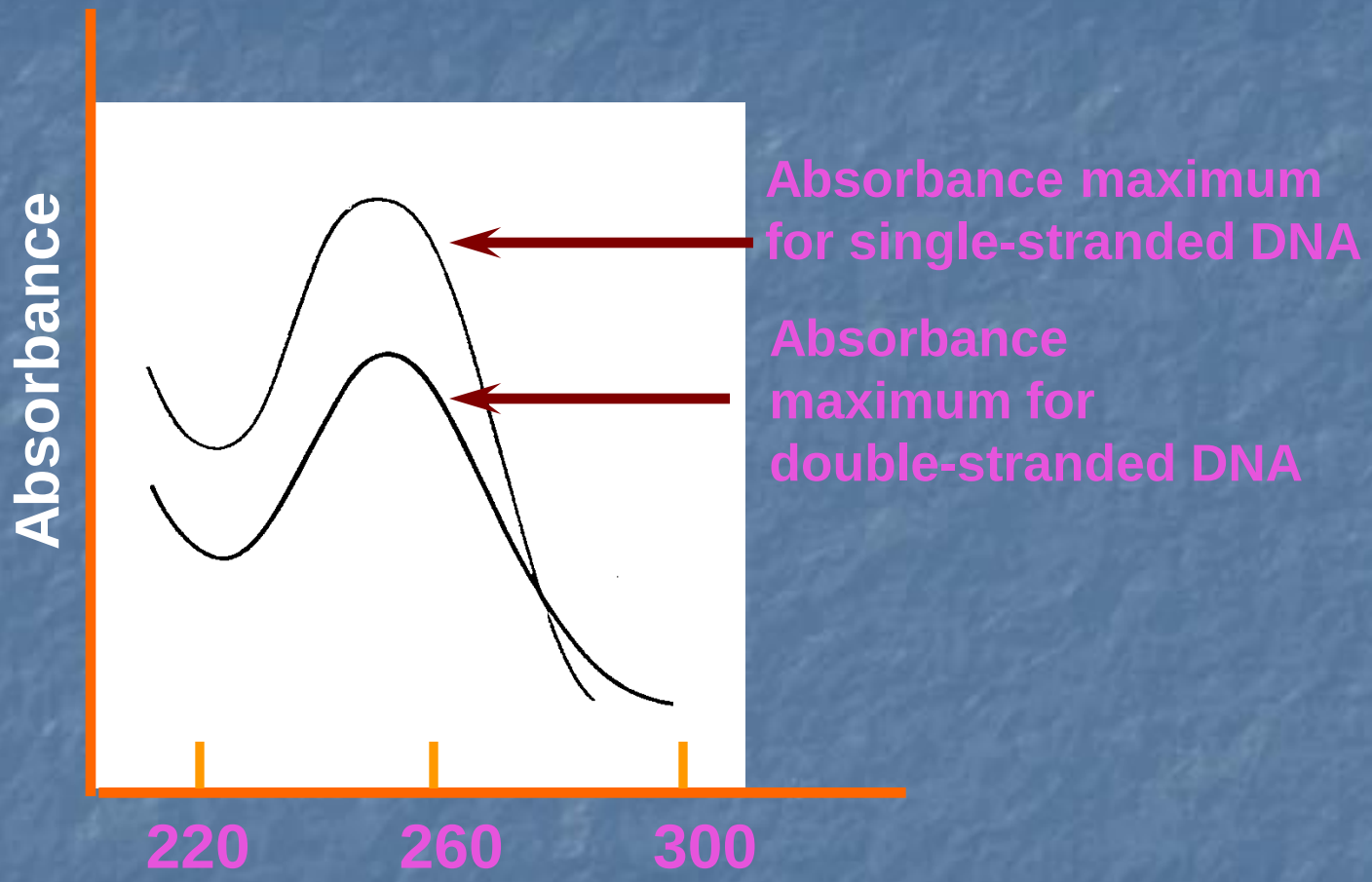


Electron micrograph of partially melted DNA



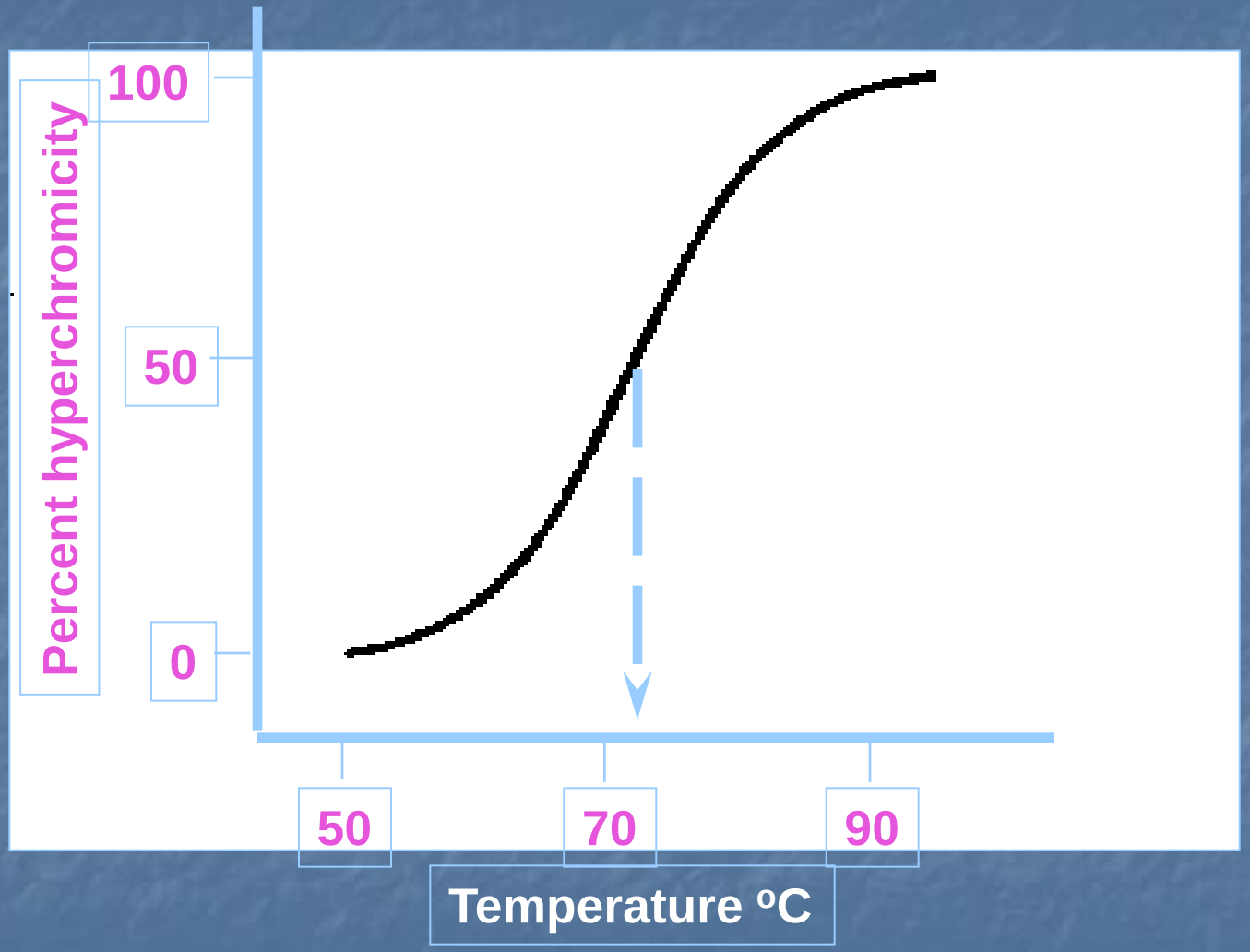
- A-T rich regions melt first, followed by G-C rich regions

Hyperchromicity



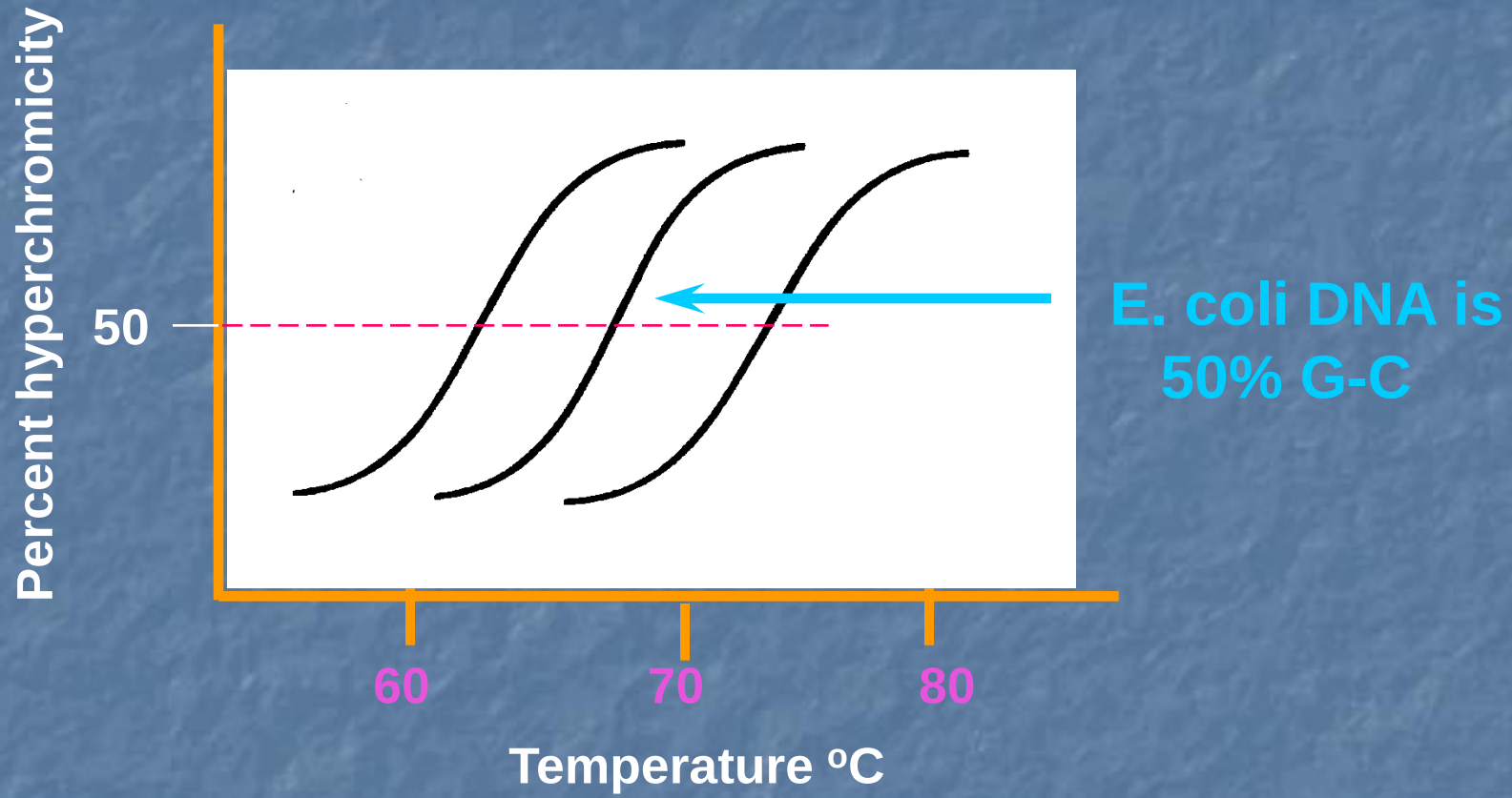
The absorbance at 260 nm of a DNA solution increases when the double helix is melted into single strands.

DNA melting curve



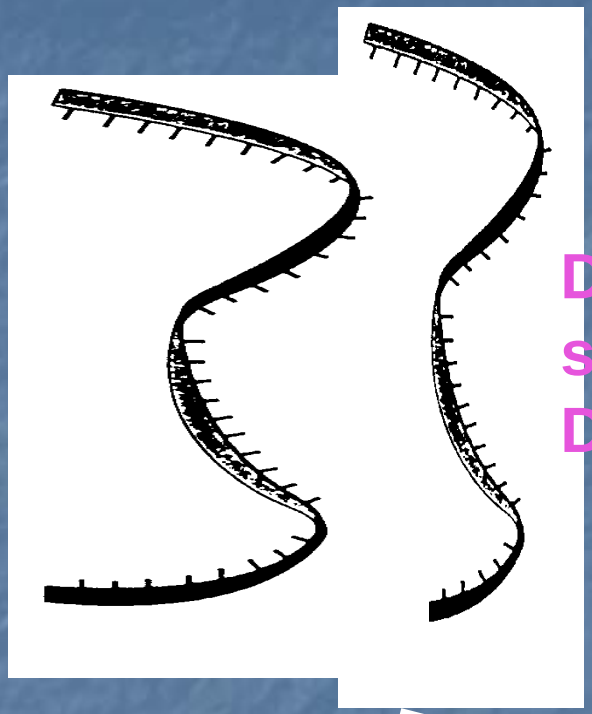
- T_m is the temperature at the midpoint of the transition

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

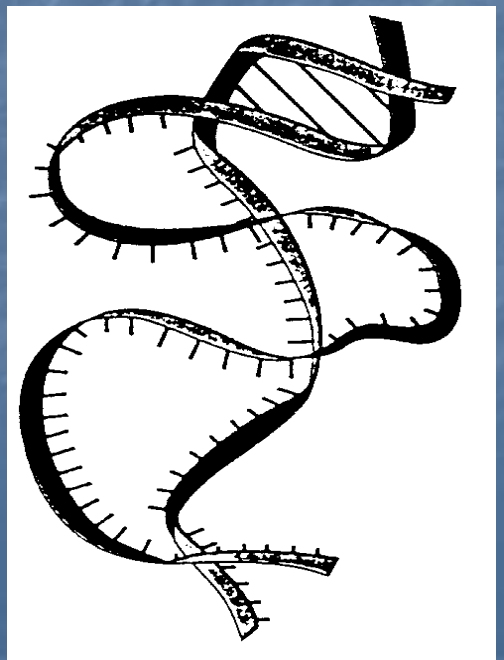
DNA reassociation (renaturation)



Denatured, single-stranded DNA

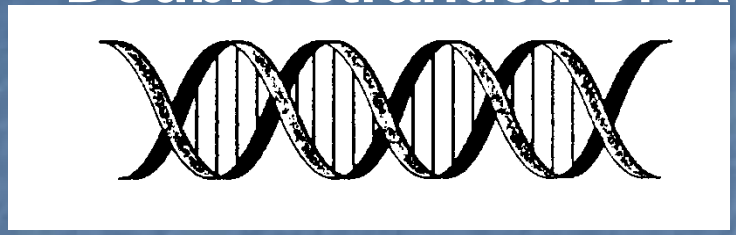
k_2

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

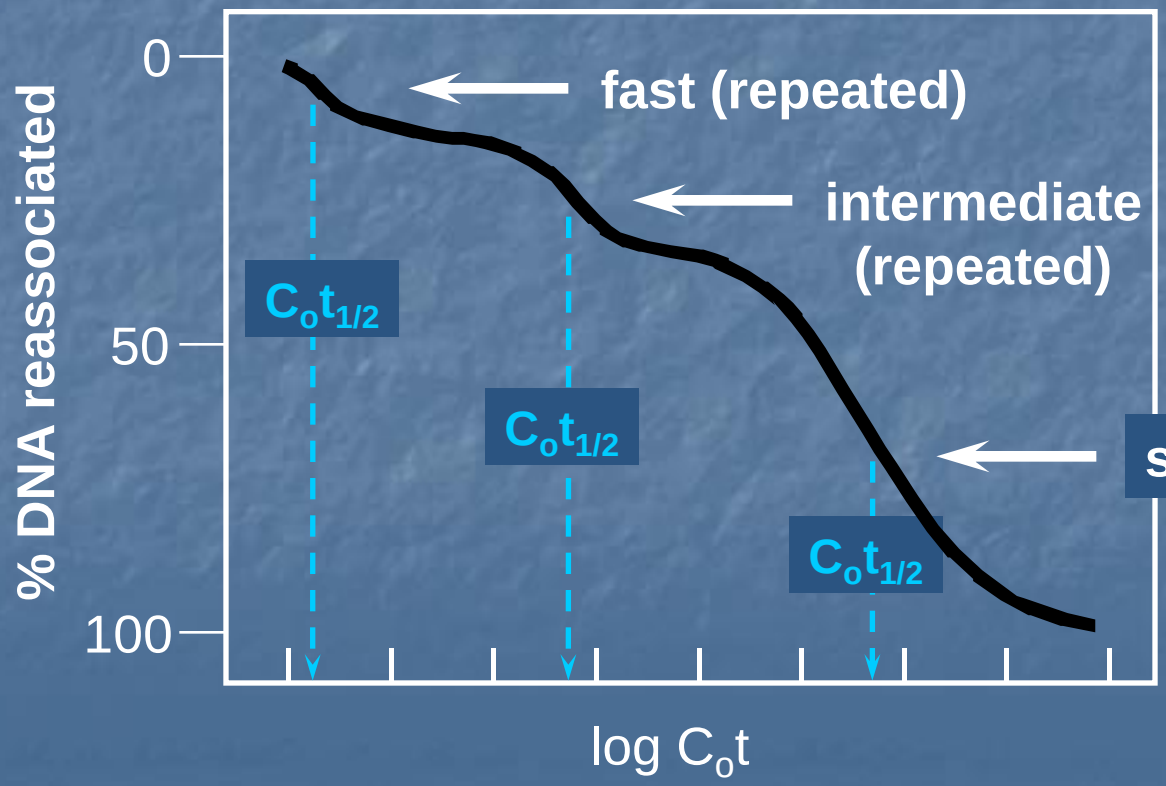
Double-stranded DNA



DNA reassociation kinetics for human genomic DNA

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

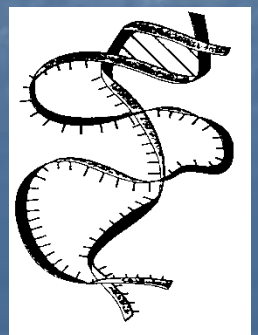
k_2 = second-order rate constant
C_o = DNA concentration (initial)
$t_{1/2}$ = time for half reaction of each component or fraction



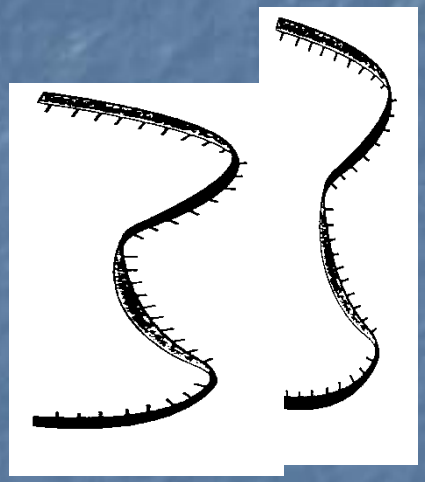
Kinetic fractions:
- fast
- intermediate
- slow

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

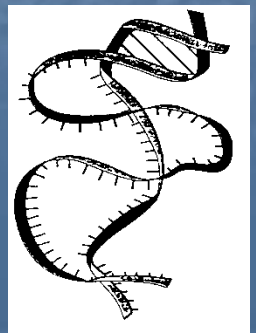
high k_2



1 copy per genome of a "high complexity" sequence of e.g. 300 x 10^6 base pairs



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

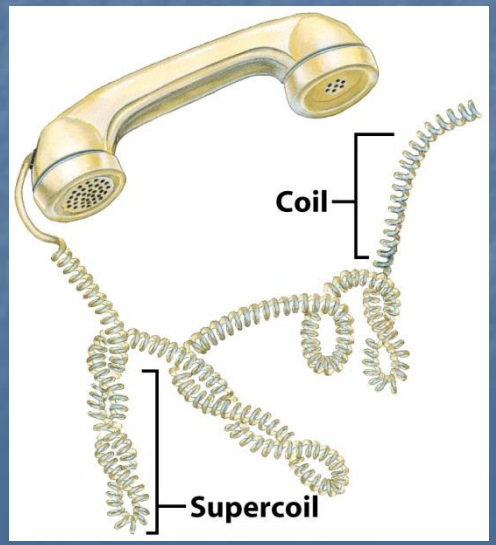
Twists (T or T_w) = number of helical turns

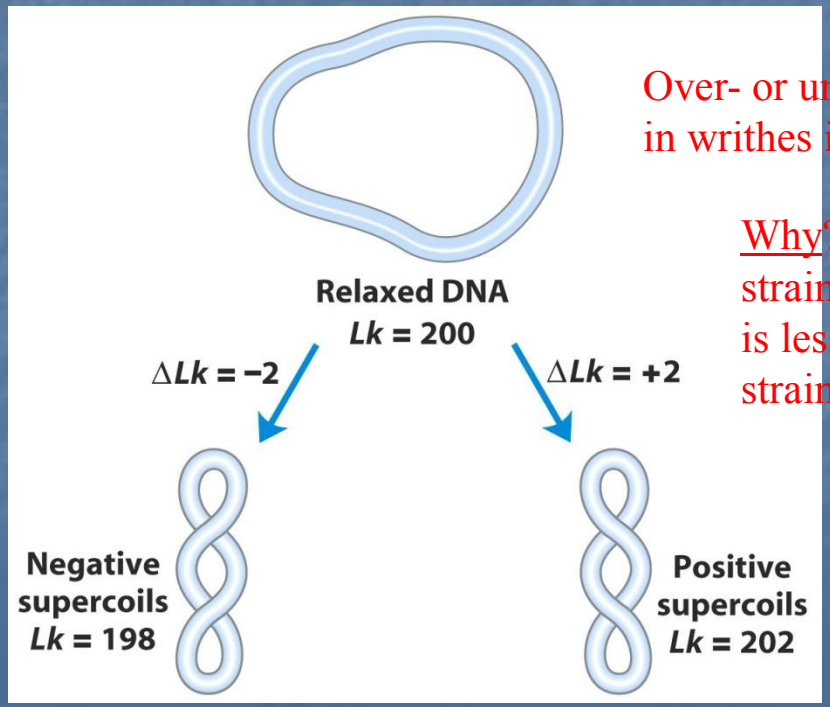
For a 2,000 bp DNA duplex, T = 200 (2,000 bp × 1 turn/10 bp = 200 turns)

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

A **relaxed** DNA molecule has zero writhes. (**∴ 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** (ΔL) 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 $\Delta L = L - L_o$.

ΔL is a measure of the number of writhes

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

The **Superhelica l Density** (σ) is a measure of supercoiling that is independent of length.
The equation is $\sigma = \Delta L / L_o$.

σ is a measure of the ratio of writhes to twists

For a relaxed molecule: $\sigma = 0$

DNA in cells has a σ 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.) ($\Delta L = L - L_o$) ($\sigma = \Delta L / L_o$)

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 (ΔL) after treatment with DNA gyrase?

D. What is the superhelical density (σ) after treatment with DNA gyrase?

A. $L = T + W$; for relaxed molecule $W = 0$ $\therefore$ $L_0 = T$; 5500 bp X 10 bp/turn = 550 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 X 11 bp/turn = 500 helical turns

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

Type I and II Topoisomerases (usually relax supercoiled DNA)

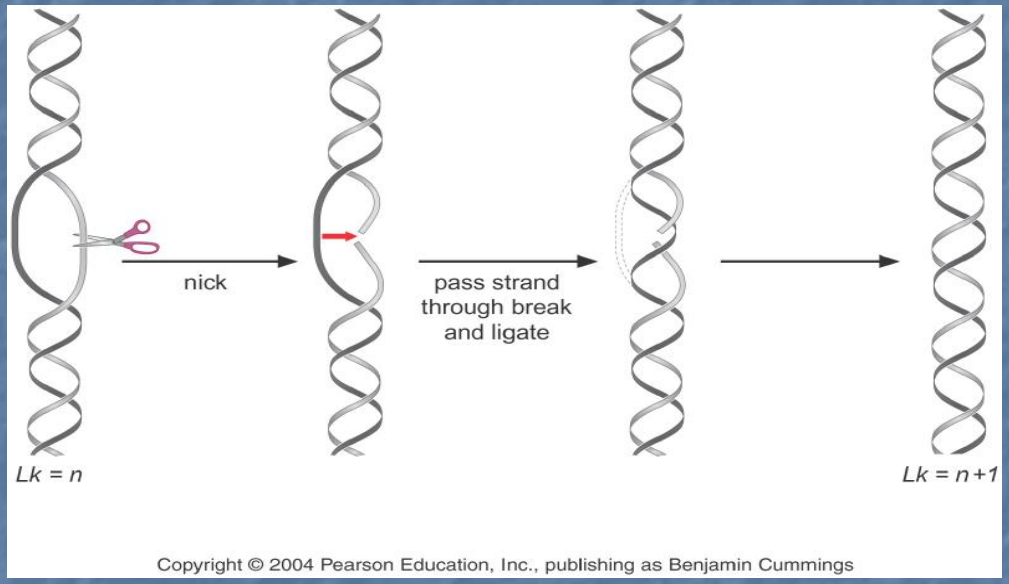
Rule #1: They change the linking number by changing the # of writhes.

Rule #2: The 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 writhe.

Type I Topoisomerases

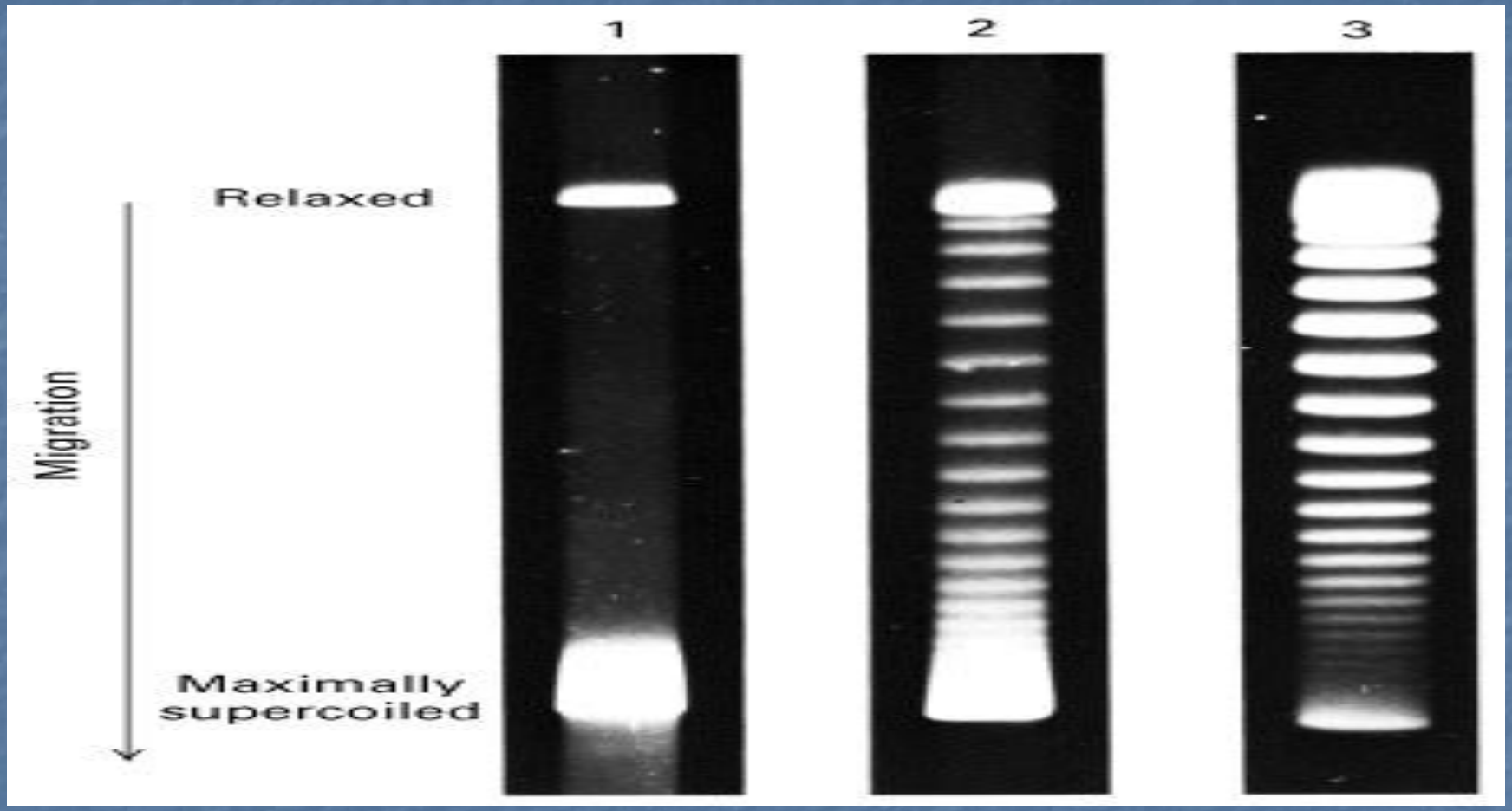
They relax DNA by nicking then closing one strand of duplex DNA. They **cut one strand** of the double helix, pass the other strand through, then rejoin the cut ends. They change the linking number by increments of +1 or –1.



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



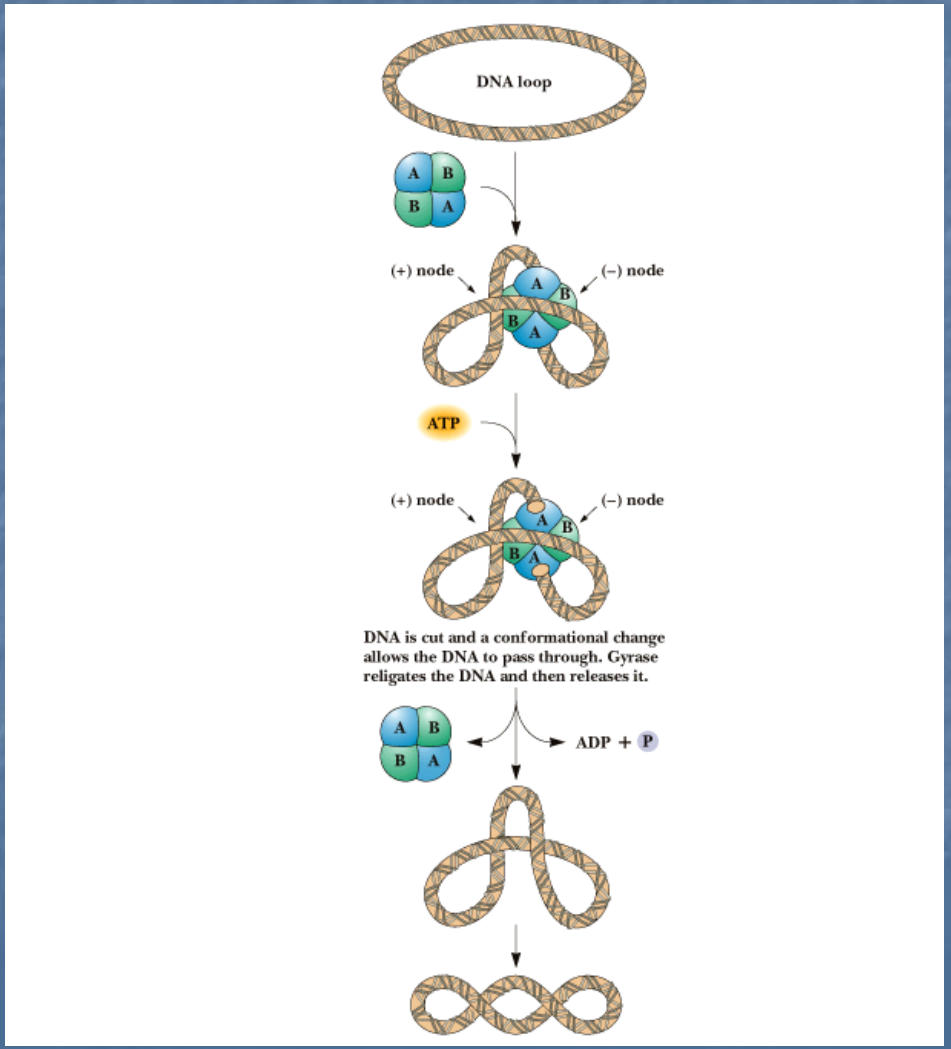
Maximum supercoiled

3 min. Topo I

25 min. Topo I

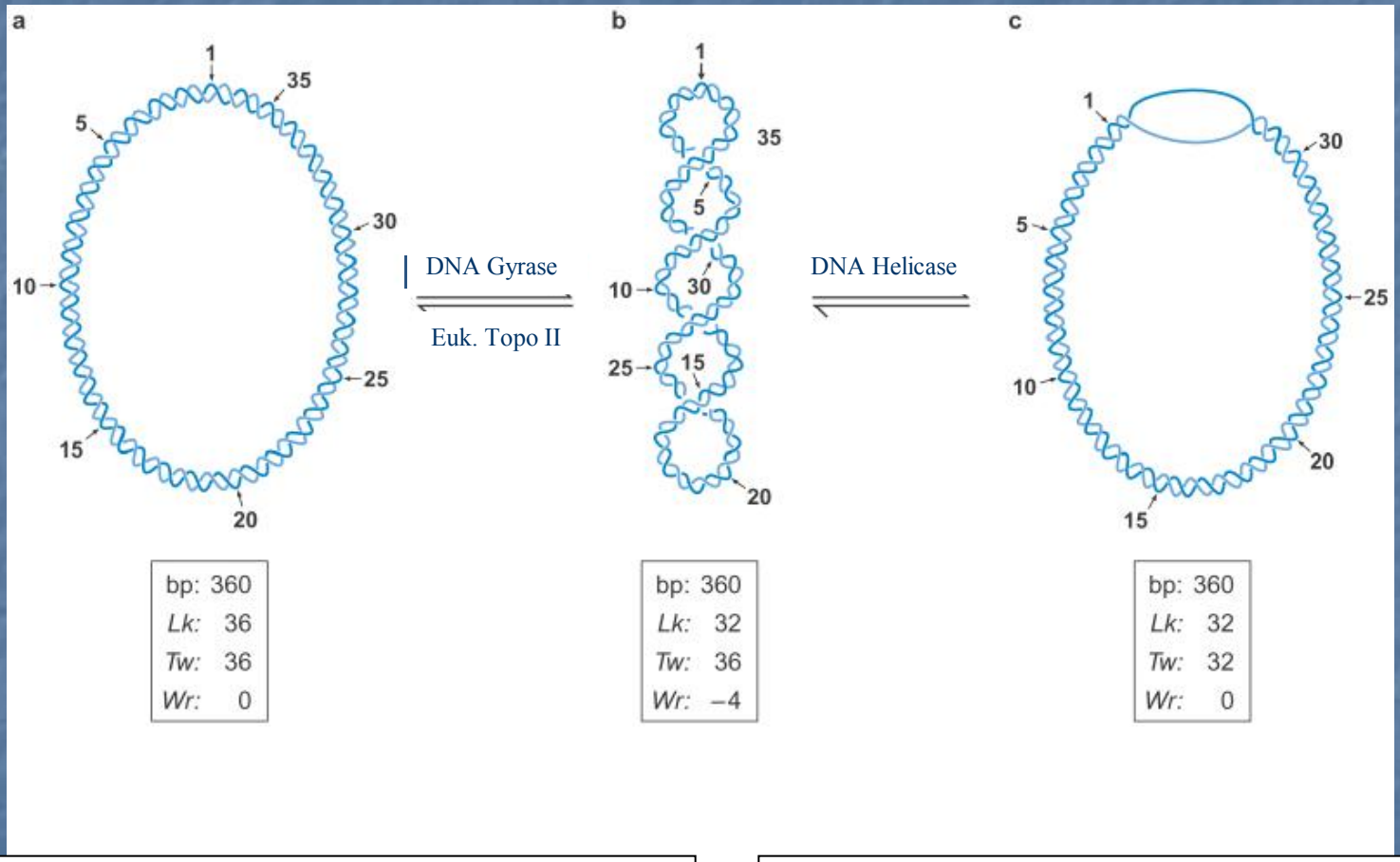
Type II Topoisomerases

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:

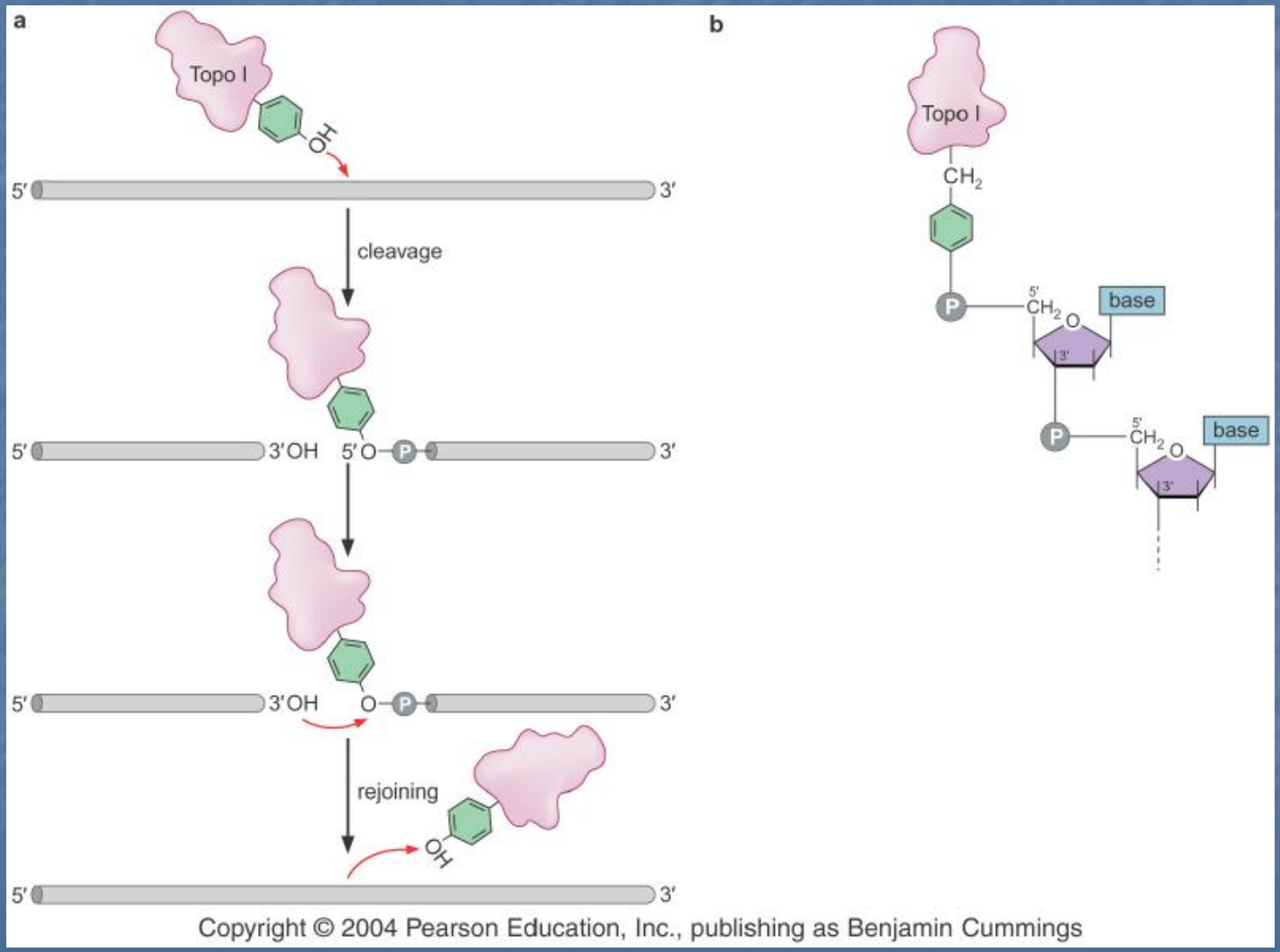
The strain of underwinding DNA is relieved by: Negative supercoils or Local disruption of base pairs



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

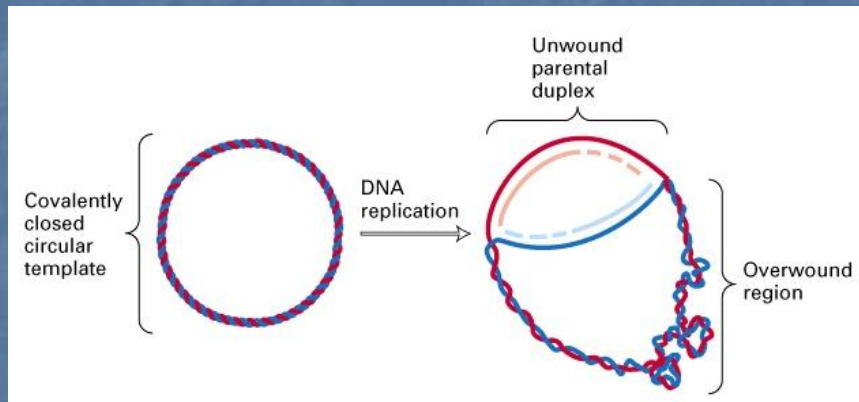
Topo II of Eukaryotes
1) Relaxes only negatively supercoiled DNA
2) Increases the linking number by increments of +2
3) Requires ATP

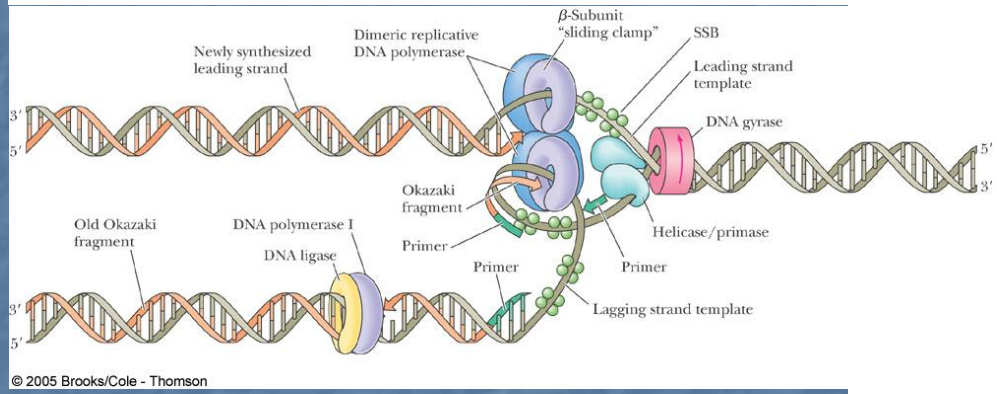
All Topoisomerases Cleave DNA Using a Covalent Tyrosine-DNA Intermediate

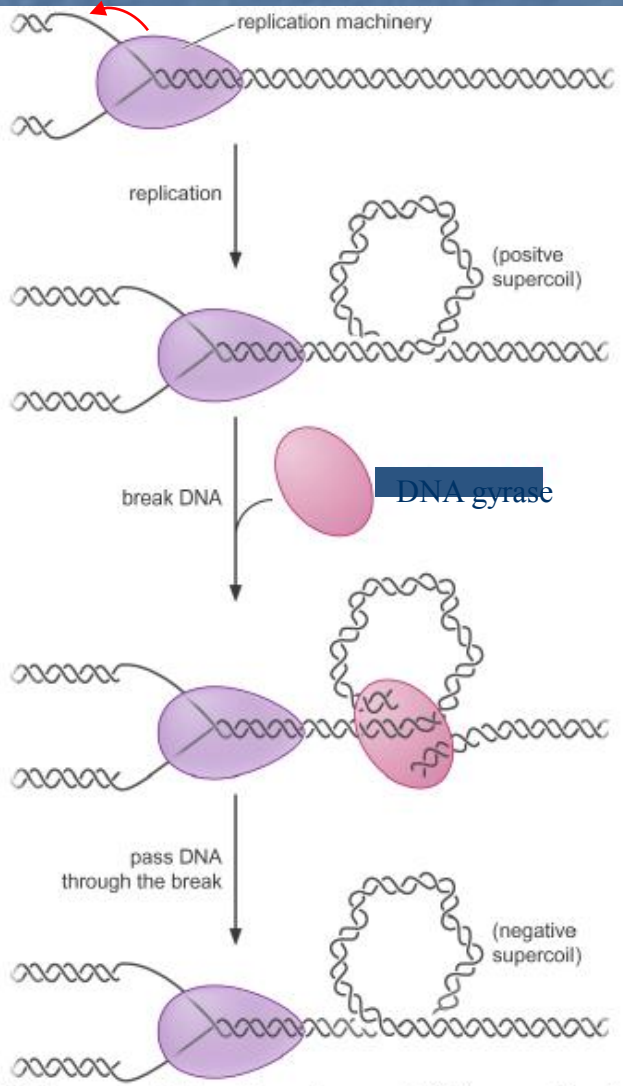


The Role of Topoisomerases in DNA Replication

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



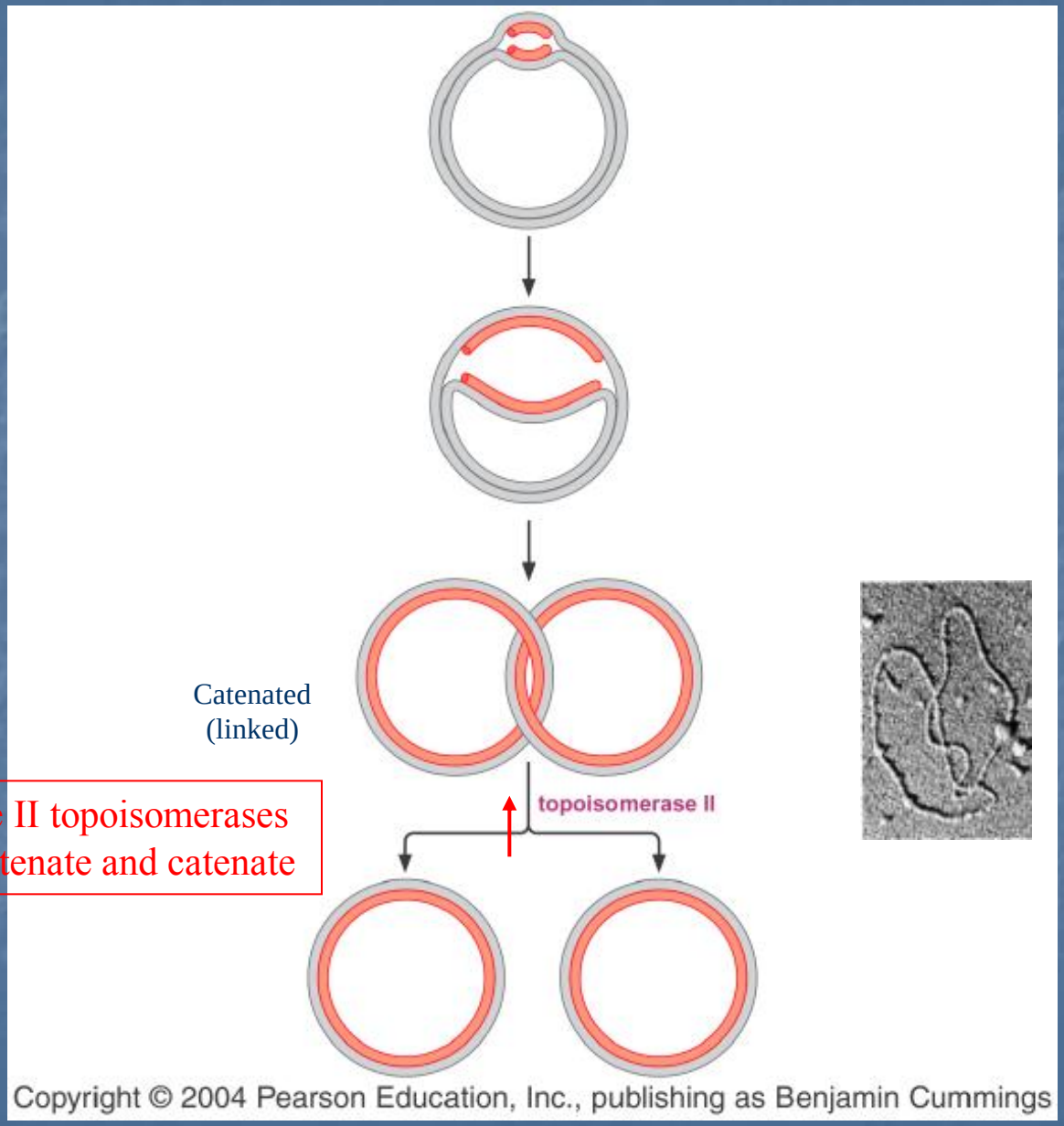




E. Coli → DNA gyrase (adds neg. supercoils)

Eukaryotes → Topo I (relaxes pos. supercoils)

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



Type II topoisomerases decatenate and catenate

A Review of the Different Topoisomerases

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