Carbohydrates

A. Definition
B. Roles
C. Monosaccharides-Chemistry
   1. Chirality
      a. One or more asymmetric carbons
      b. Linear and ring forms
   2. Derivatives: the chemistry of carbohydrates
      a. Oxidation
         i. C1
         ii. C6
      b. Reduction
         i. C1/C2
         ii. Other carbons
      c. Ester formation
      d. Amino sugars
   3. Polymerization
      a. The Glycosidic Bond
      b. Non-covalent bonds in macro-molecular structure
      c. Disaccharides
D. Oligosaccharides
   1. Glycoproteins & glycolipids
   2. O-linked
   3. N-linked
   4. Sequence determination-ABO
E. Polysaccharides
   1. Polymers of glucose
   2. Polymers of disaccharides

Nucleic Acids

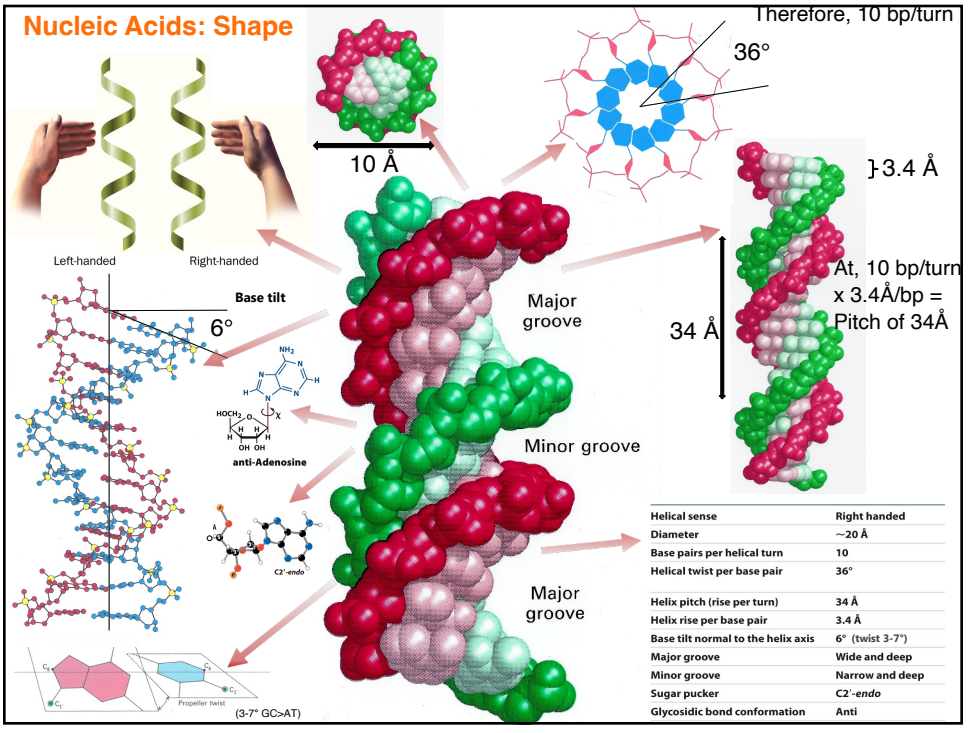
A. Nucleotides
   1. Parts-structure
   2. Nomenclature
   3. Numbering
   4. Properties
B. Nucleic Acids
   1. Polymer: phospho-diester bond
   2. H-bonds
   3. Roles
      a. Nucleotides
      b. Nucleic acids
C. The 4 S's
   1. Size
   2. Solubility
   3. Shape
      a. B-DNA
      b. A-DNA
      c. Z-DNA
      d. Topology
         i. Packaging
         ii. Supercoiling
         iii. Topoisomerases
   4. Stability
      a. Nucleotides: mutagenesis
         i. Tautomers
         ii. Acid/base
      b. Nucleic Acids
         i. Chemistry
         ii. Denaturation
         iii. Stability
         iv. Nucleases
D. Structure of the Information
   1. Exceptions to flow
   2. Structure
   3. Levels of Control
E. Recombinant DNA: Biochemical Basis of Biotechnology
F. Replication

Lecture 25 (11/14/25)

TODAY
- Reading: Ch8; 269-274; Ch24; 881-894
- Homework: #24

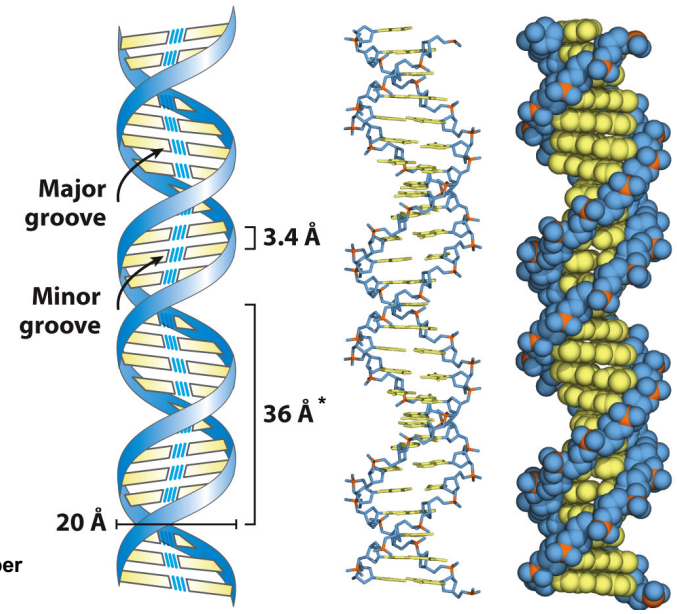
NEXT
- Reading: Ch8; 278-283; Ch24; 895-897
  Ch9; 301-313, 326-327
  Ch8; 283-286
- Homework: #25 & #26

Nucleic Acids: Shape



Helical sense	Right handed
Diameter	~20 Å
Base pairs per helical turn	10
Helical twist per base pair	36°
Helix pitch (rise per turn)	34 Å
Helix rise per base pair	3.4 Å
Base tilt normal to the helix axis	6° (twist 3-7°)
Major groove	Wide and deep
Minor groove	Narrow and deep
Sugar pucker	C2'-*endo*
Glycosidic bond conformation	Anti

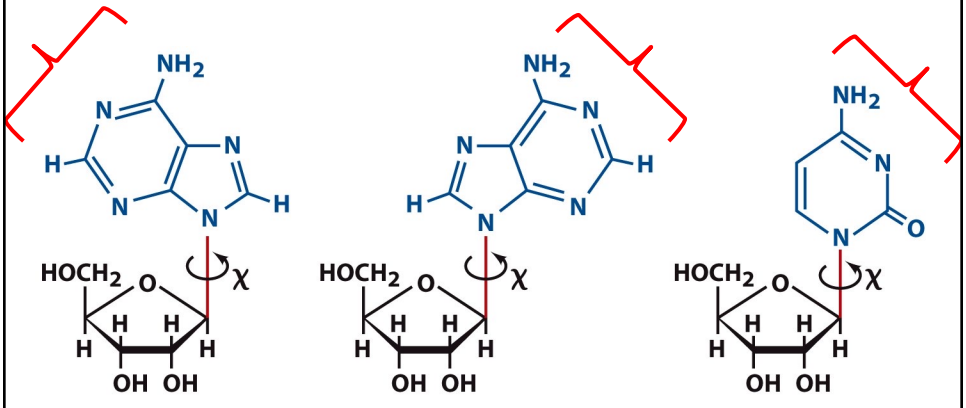
Watson-Crick Model of B-DNA



*(based on 10.5 bases per turn)

Nucleic Acids: Shape

Sterically Allowed Base Orientations



syn-Adenosine | **anti-Adenosine** B-DNA | **anti-Cytidine** B-DNA

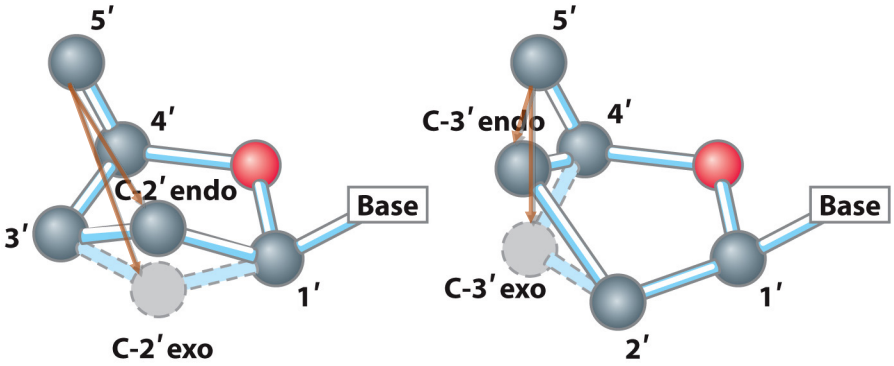
Notice the bp interface

Nucleic Acids: Shape

Nucleotide Sugar Conformations

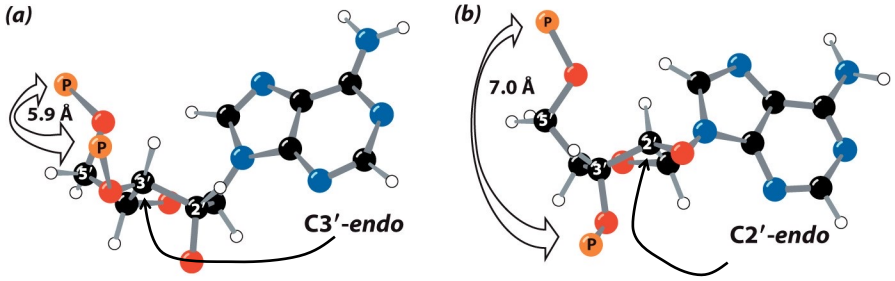
4 possible pucker conformations:

NUCLEIC ACIDS ARE ALWAYS *endo*



Nucleic Acids: Shape

Nucleotide Sugar Conformations

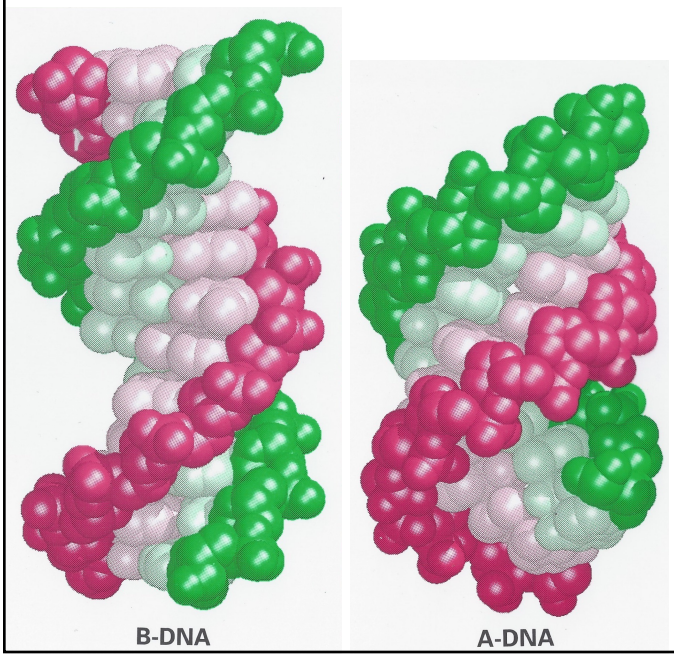


RNA
RNA-DNA hybrid
A-DNA

B-DNA

What is this "A-DNA"?

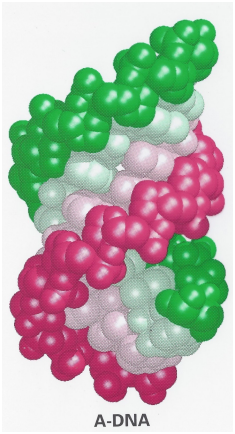
Nucleic Acids: Shape



Nucleic Acids: Shape

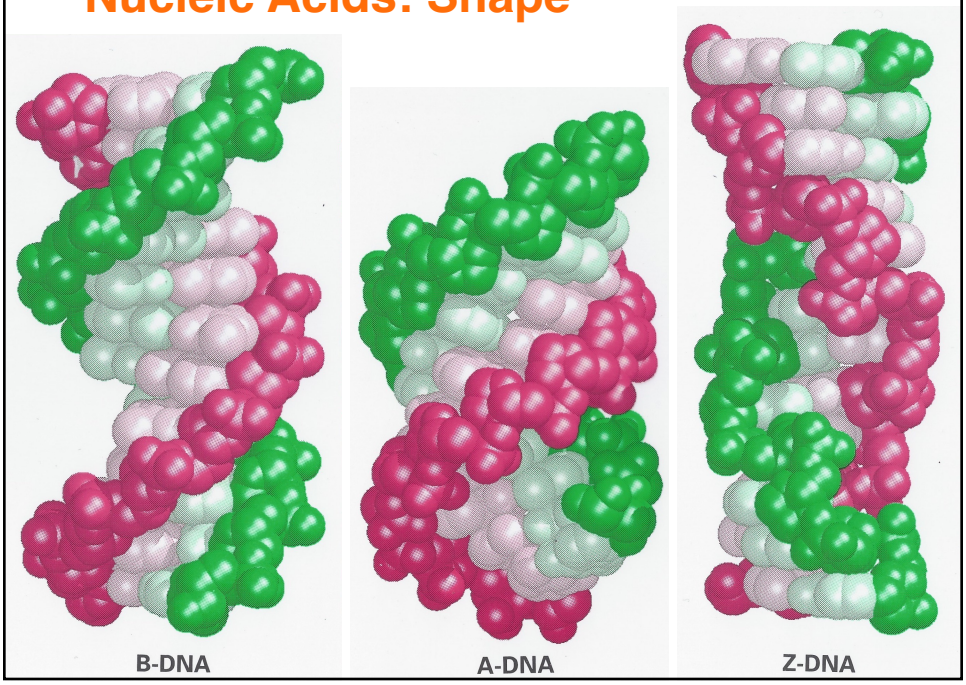
TABLE 24-1 Structural Features of Ideal B- DNA

	B
Helical sense	Right handed
Diameter	~20 Å
Base pairs per helical turn	10
Helical twist per base pair	36°
Helix pitch (rise per turn)	34 Å
Helix rise per base pair	3.4 Å
Base tilt normal to the helix axis	6°
Major groove	Wide and deep
Minor groove	Narrow and deep
Sugar pucker	C2′-*endo*
Glycosidic bond conformation	Anti



Are there other shapes of DNA?

Nucleic Acids: Shape

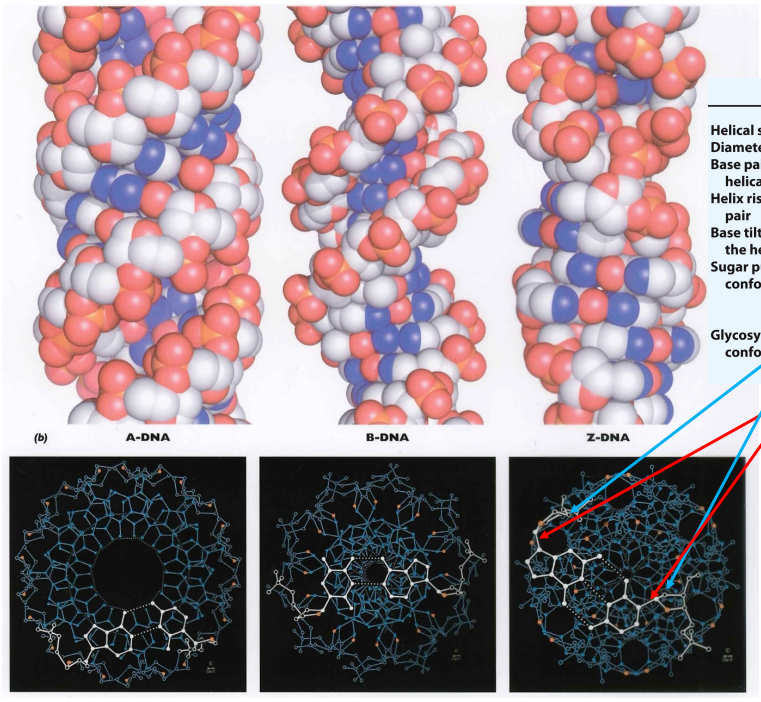


Nucleic Acids: Shape

Structural Features of A-, B-, & Z-DNA

TABLE 24-1 Structural Features of Ideal A-, B-, and Z-DNA

	A	B	Z
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10	12 (6 dimers)
Helical twist per base pair	31°	36°	9° for pyrimidine–purine steps; 51° for purine–pyrimidine steps
Helix pitch (rise per turn)	29 Å	34 Å	44 Å
Helix rise per base pair	2.6 Å	3.4 Å	7.4 Å per dimer
Base tilt normal to the helix axis	20°	6°	7°
Major groove	Narrow and deep	Wide and deep	Flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep
Sugar pucker	C3′-*endo*	C2′-*endo*	C2′-*endo* for pyrimidines; C3′-*endo* for purines
Glycosidic bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines



	Z form
Helical sense	Left handed
Diameter	~18 Å
Base pairs per helical turn	12
Helix rise per base pair	3.7 Å
Base tilt normal to the helix axis	7°
Sugar pucker conformation	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti for pyrimidines; syn for purines

Other Forms of DNA

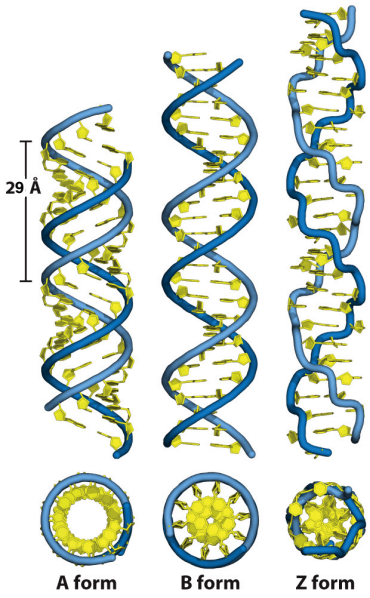


Figure 8-17 part 1

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

Nucleic Acids: Shape

Nucleotide Sugar Conformations

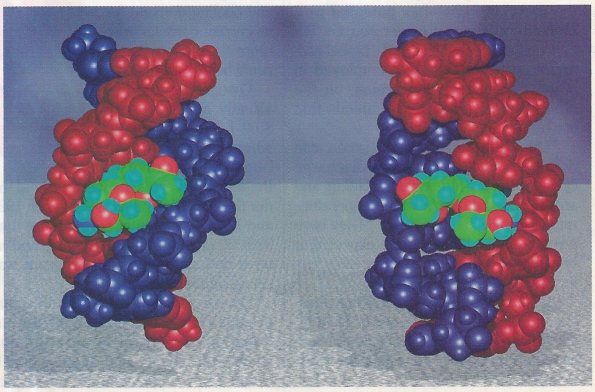


Figure courtesy of John Trent, University of Louisville

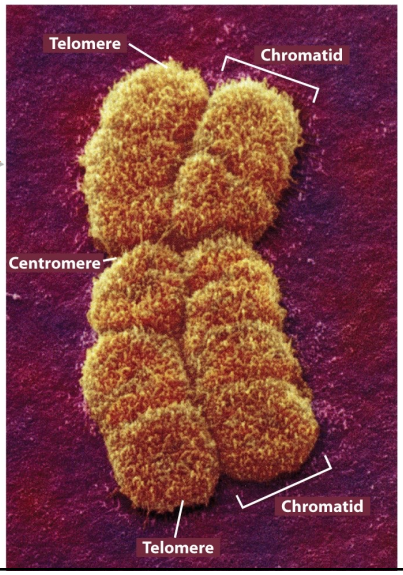
Daunorubicin binds by intercalation with B-DNA (left), whereas its enantiomer, WP900, binds in a similar manner with Z-DNA (right).

Nucleic Acids: Global Shape

Metaphase Chromosome

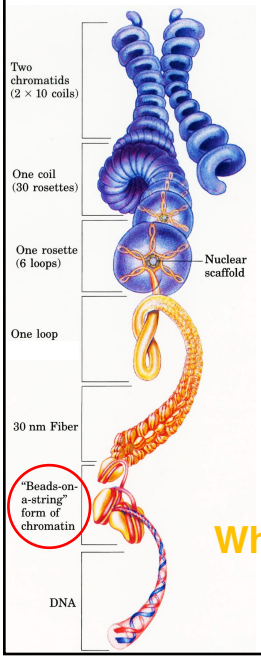
How do we get something that is 2-10 cm long into one of these, which is only 10 μm?

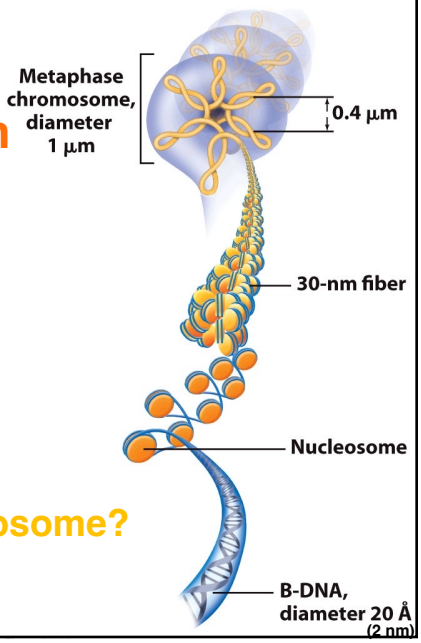
This condensation is 10,000x. Even interphase its 1000x



Nucleic Acids: Global Shape

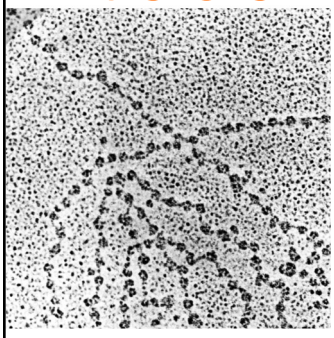
Metaphase Chromatin Organization



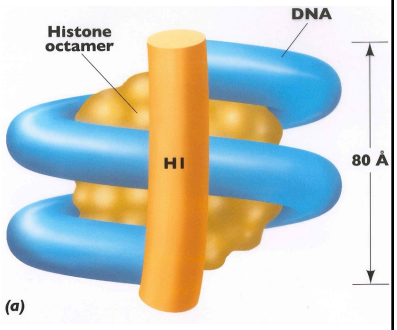


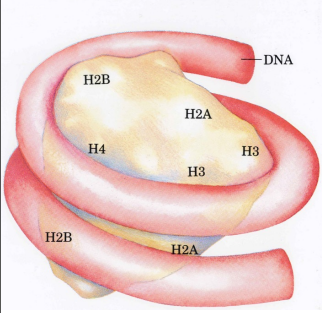
What is this nucleosome?

Nucleic Acids: Global Shape



~240 bp (3.4x240=816Å) is now compacted to 80Å = 10x



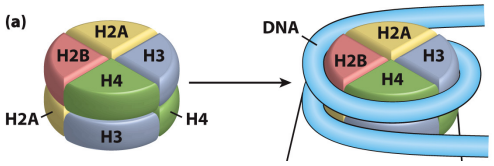


8 Histones make up the core

Nucleic Acids: Global Shape

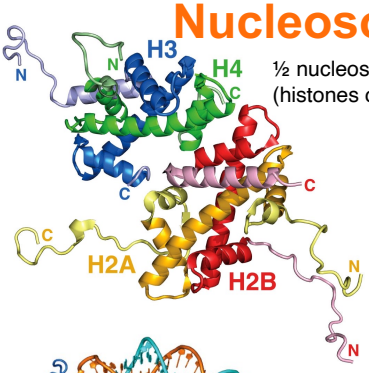
Histones Are Highly Conserved

Histone	Number of Residues	Mass (kD)	% Arg	% Lys	Stoichiometry
H1	215	23.0	1	29	1
H2A	129	14.0	9	11	2
H2B	125	13.8	6	16	2
H3	135	15.3	13	10	2
H4	102	11.3	14	11	2

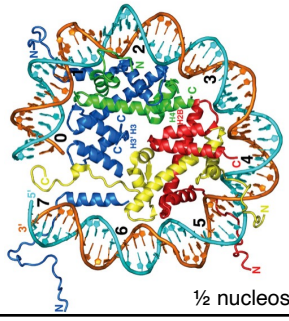


Nucleic Acids: Global Shape

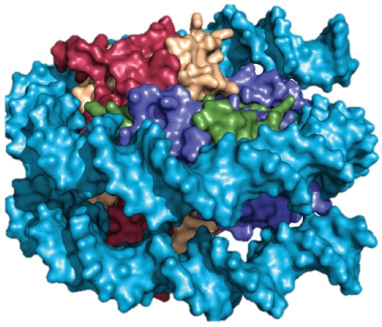
Nucleosome Core Particle



½ nucleosome core (histones only)



½ nucleosome



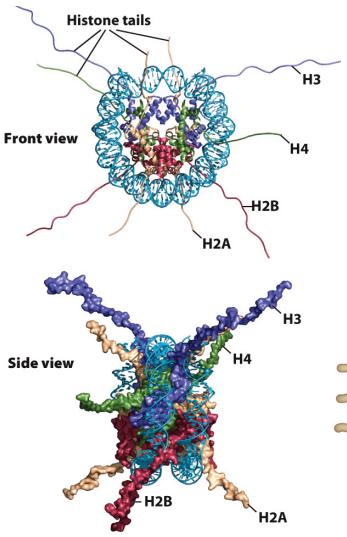
Whole nucleosome (top view)

Chicken nucleosome core particle
PDBid 1EQZ

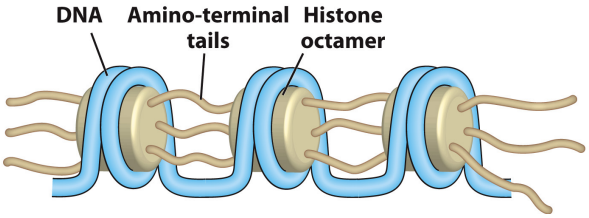
Nucleic Acids: Global Shape

Interactions between Nucleosomes

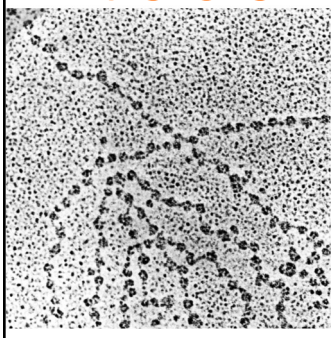
Front and Side Views of Histone Amino-Terminal Tails



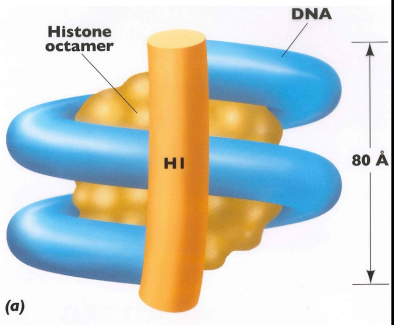
Amino-Terminal Tails

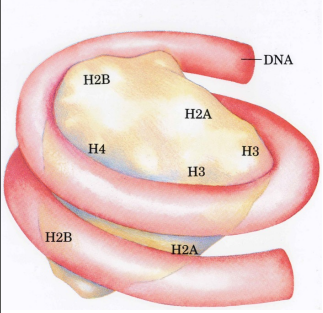


Nucleic Acids: Global Shape



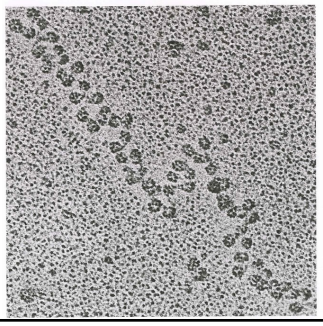
~240 bp (3.4x240=816Å) is now compacted to 80Å = 10x





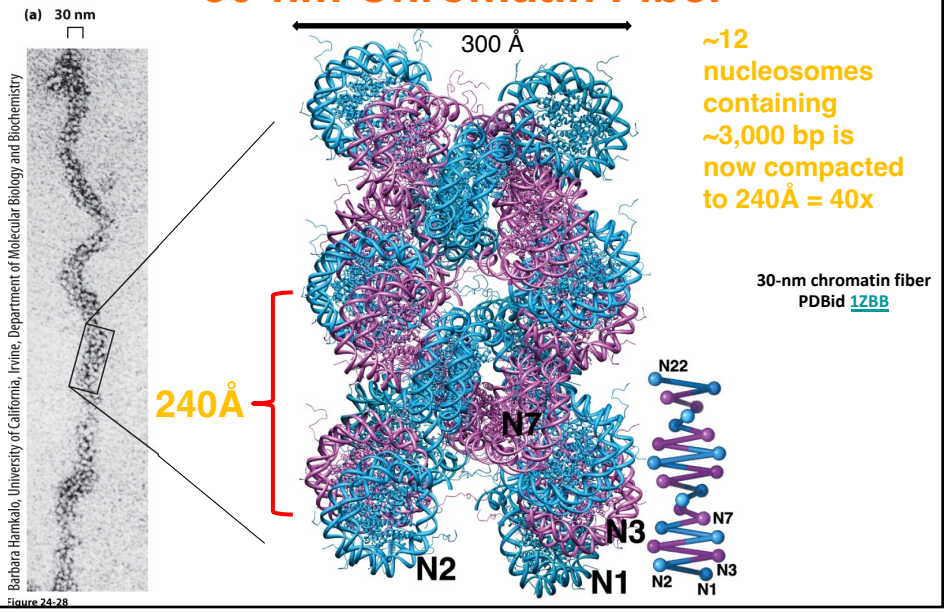
Histone H1 Bound to Nucleosome

8 Histones make up the core



Nucleic Acids: Global Shape

30-nm Chromatin Fiber



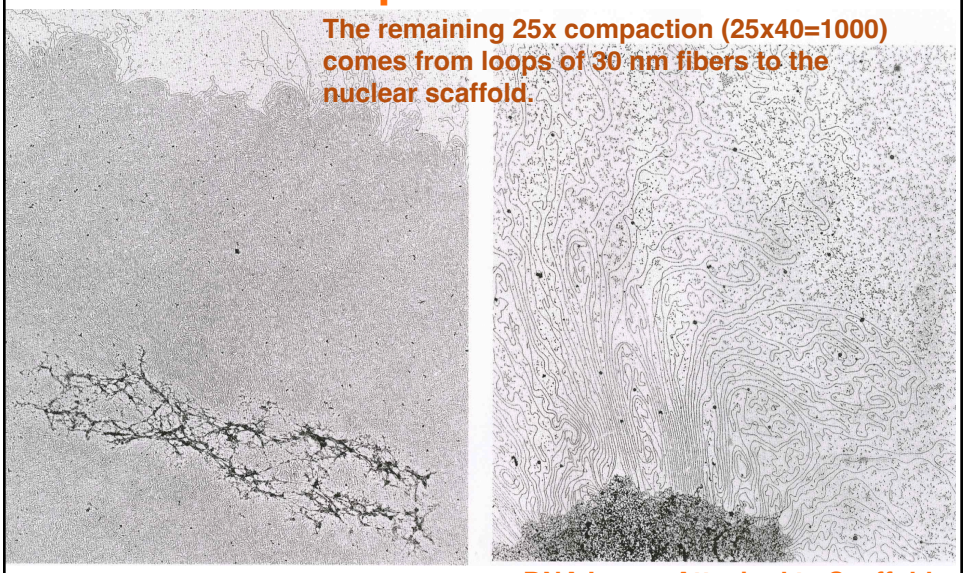
~12 nucleosomes containing ~3,000 bp is now compacted to 240Å = 40x

30-nm chromatin fiber
PDBid 1ZBB

Nucleic Acids: Global Shape

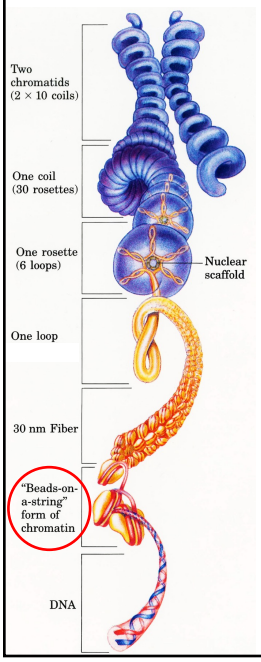
Histone-Depleted Chromosome

The remaining 25x compaction (25x40=1000) comes from loops of 30 nm fibers to the nuclear scaffold.

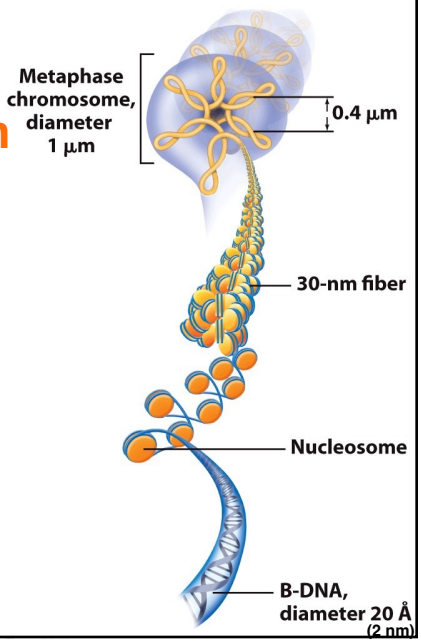


DNA Loops Attached to Scaffold

Nucleic Acids: Global Shape



Metaphase Chromatin Organization



Nucleic Acids: Global Shape

All this looping leads to a supertwist of the DNA…

Nucleosomal DNA Is Underwound

- **Supercoils**

What is Supercoiling: the Coiling of a Coil

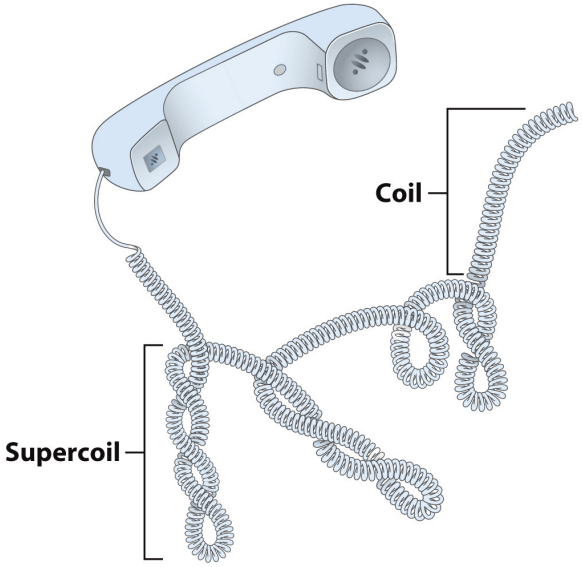
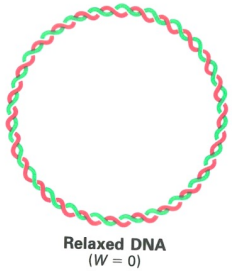


Figure 24-9

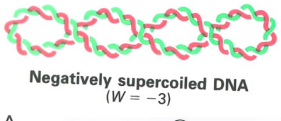
Nucleic Acids: Global Shape

All this looping leads to a supertwist of the DNA…

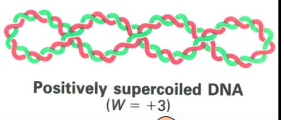


Topology

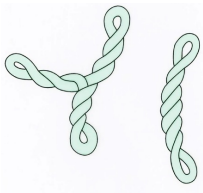
Supercoiling is called W (writhing number)



Relaxed DNA (W = 0)

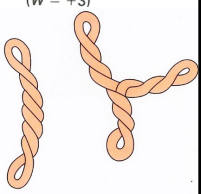


A

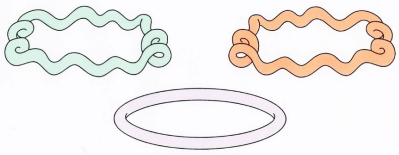


L = T + W

(for B-DNA: T=#bp/10)



Does this occur in biology?



L (Linking)

T (Twist)

W < 0 **W = 0** **W > 0**

Nucleic Acids: Global Shape

Topology

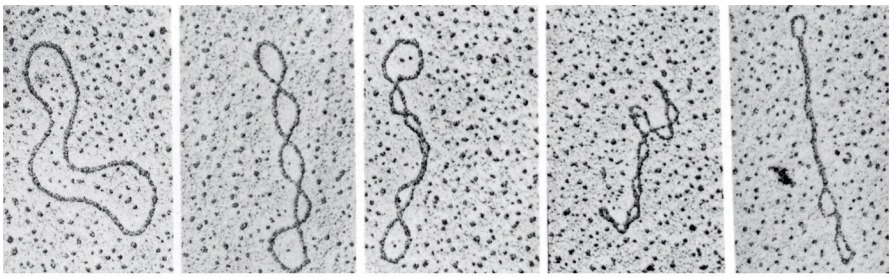


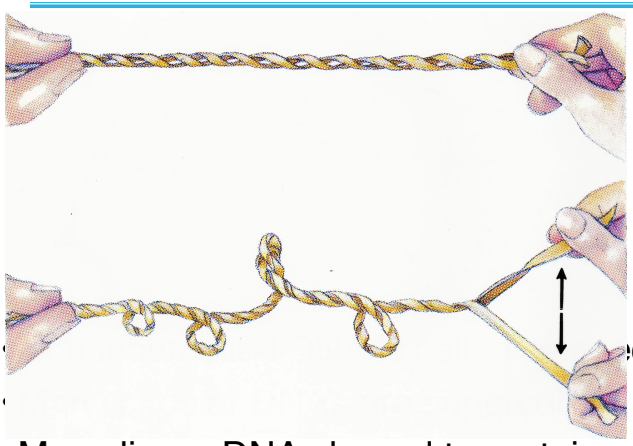
Figure 24-8
Electron micrographs by Laurien Polder. From Kornberg, A. and Baker, T.A., *DNA Replication* (2nd ed.), p. 36, W.H. Freeman (1992). Permission provided courtesy of Roger Kornberg.

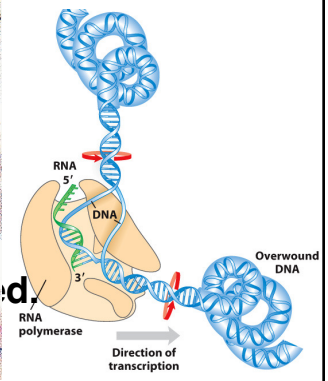
Is supercoiling only possible with circular DNA? Eukaryotic chromosomes are linear…..

Supercoiling of Circular Duplex DNA from the adenovirus, SV40

Nucleic Acids: Global Shape

Topology





- Many linear DNAs bound to proteins are supercoiled.
- Supercoiling has great influence on transcription and replication of DNA.
- Supercoiling can be highly regulated.

Nucleic Acids: Global Shape

Consequences of supercoiling:

1) Required for information retrieval; must be negative
2) All circular extra-chromosomal DNAs are negatively supercoiled
3) Can be used for isolation of these DNAs in the laboratory

Nucleic Acids: Global Shape

All this looping leads to a supertwist of the DNA…

Nucleosomal DNA Is Underwound

- Wrapping DNA around the histone core requires removal of one helical turn.
  - The under-winding occurs without a strand break, so a compensatory (+) supercoil forms.
  - This (+) supercoil is relaxed by a topoisomerase, which puts in 2 negative supercoils, leaving DNA with **net (–1) negative supercoil**.

Nucleic Acids: Global Shape

Techniques to Detect Topology

Separate DNAs with different topology based on density

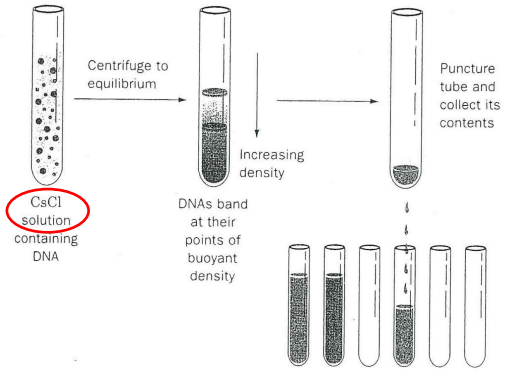


Figure 23-31 The separation of DNAs by equilibrium density gradient ultracentrifugation in CsCl solution. An initially 8 M CsCl solution forms a density gradient that varies linearly from ~1.80 g · cm^{-3} at the bottom of the centrifuge tube to ~1.55 g · cm^{-3} at the top. The sedimentation rates of the DNAs depend on their base composition. The amount of DNA in each fraction is estimated from its UV absorbance, usually at 260 nm.

Nucleic Acids: Global Shape

Techniques to Detect Topology

Modify topology with topoisomerase I

What is Topo I?

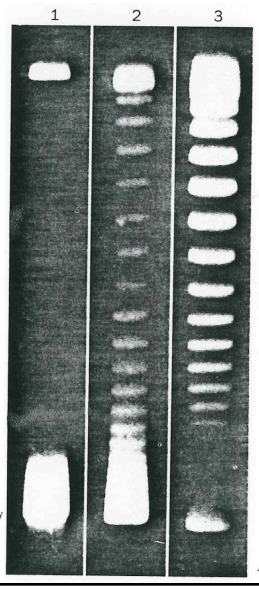
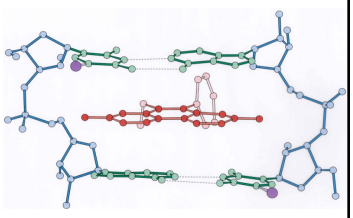
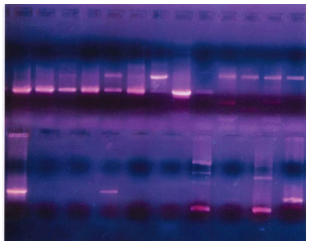


FIGURE 28-40. The agarose gel electrophoresis pattern of SV40 DNA. Lane 1 contains the negatively supercoiled native DNA *(lower band;* the DNA was applied to the top of the gel). In lanes 2 and 3, the DNA has been exposed for 5 and 30 min, respectively, to an enzyme, known as a Type I topoisomerase (Section 28-5C), that relaxes negative supercoils one at a time by increasing the linking number (L). The DNAs in consecutively higher bands of a given gel have successively increasing linking numbers ($\Delta L = +1$). [From Keller, W., *Proc. Natl. Acad. Sci.* **72,** 2553 (1975).]

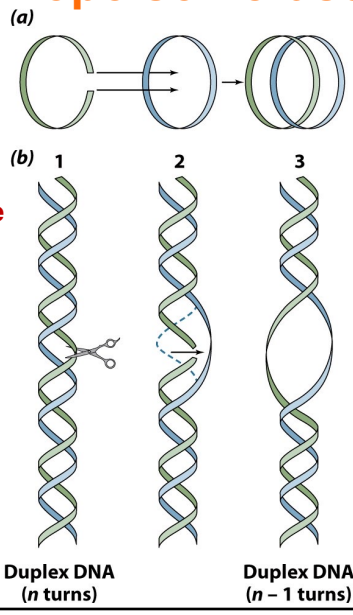
Gel Electrophoresis/Ethidium-Bromide Staining





Nucleic Acids: Global Shape

Type IA Topoisomerase Activity



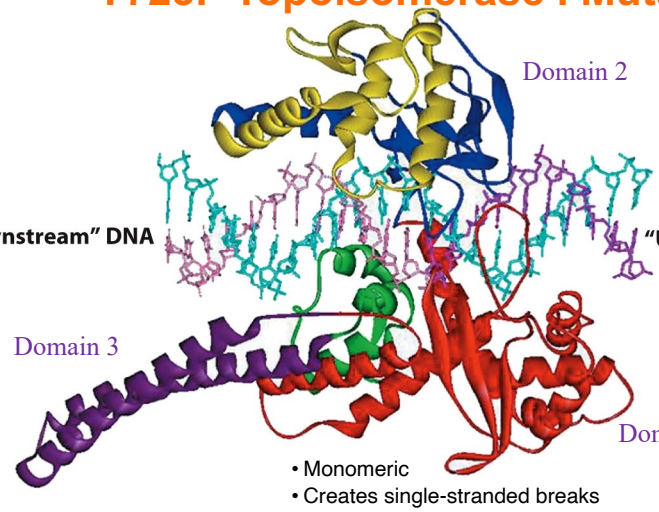
Can generate concatemers of circular ss-DNAs

Can take supercoiled DNA and make it less supercoiled; usually less negative (L = +1)

Type I Topoisomerase also called "nicking-closing" enzyme

Topoisomerase

Y723F Topoisomerase I Mutant

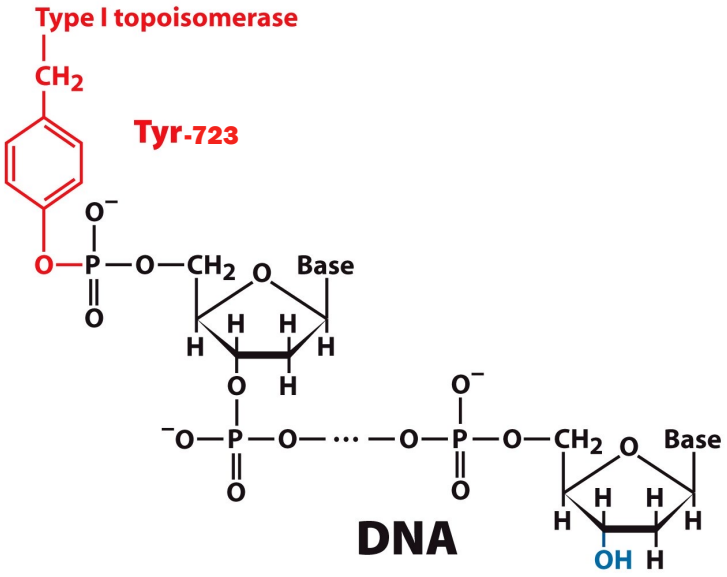


- Monomeric
- Creates single-stranded breaks
- Use of covalent Tyr intermediate
- For negatively supercoiled DNA, it changes L by +1 (if W≥0)
- Uses energy in supercoils (W# ≈ 9 kcal/mole)
- IA & IB types differ in how they relax the cleaved strand

Y723F mutant of human topoisomerase I
PDBid 1A36

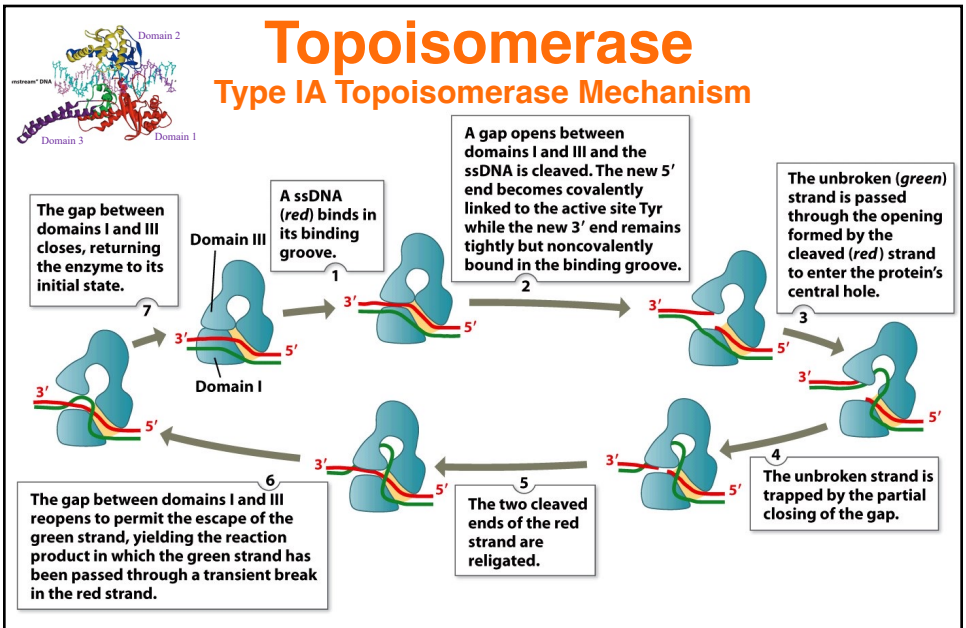
Topoisomerase

Covalent Topoisomerase-DNA Adduct



Topoisomerase

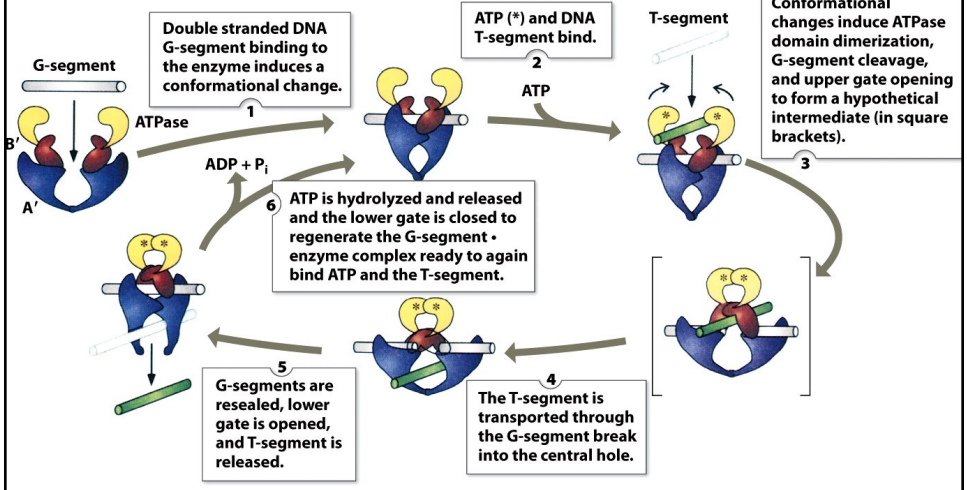
Type IA Topoisomerase Mechanism



How are negative supercoils generated in the first place?

Topoisomerase

Type II Topoisomerase Mechanism



Type II Topoisomerase also called "gyrase"

- Tetrameric ($\alpha_2\beta_2$; α=105 kDa, β=95 kDa (ATPase))
- Creates double-stranded breaks
- Use of covalent Tyr intermediate
- Changes L by –2
- Uses ATP for energy to put in supercoils ($\Delta L = -2$)