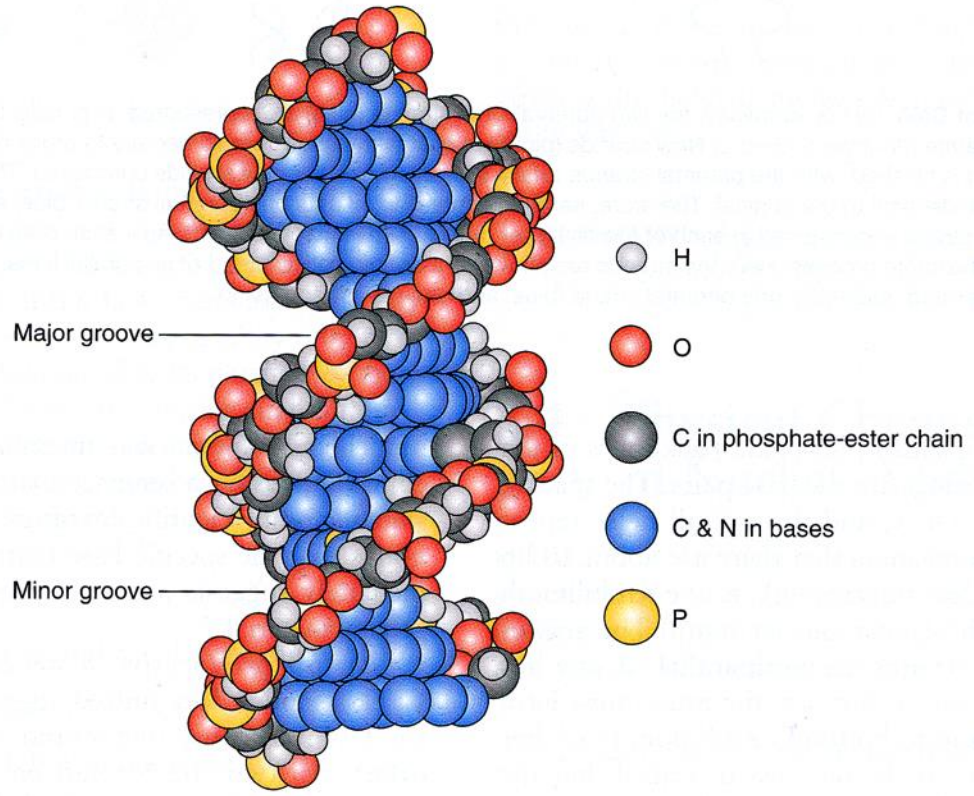
Lecture 2

DNA and RNA structure
DNA topology

Required reading: Chapter 6, Chapter 9 (pp. 304-317)

DNA STRUCTURE



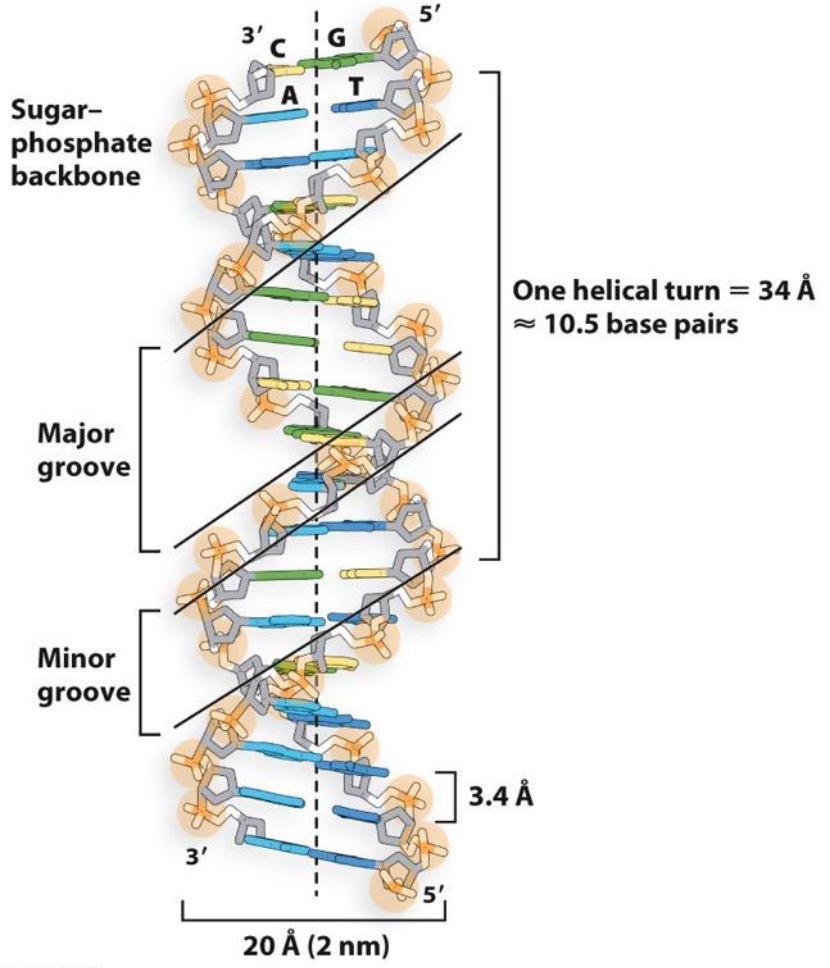


Figure 6-14
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- **Phosphate backbone, major & minor groove**
- **3.4 nm/turn, 0.34 nm between bases, 2 nm diameter**
- **Antiparallel, pyrimidines pairing with purines**

Practice question:

Given the DNA sequence 5’-AGCCTA-3’, which of the following represents the complementary strand of DNA?

A. 5’-ATCCGA-3’
B. 5’-TCGGAT-3’
C. 5’-AGCCTA-3’
D. 5’-TAGGCT-3’

Correct answer to the question on previous slide is D:

Only this sequence is both complementary and antiparallel

5’-AGCCTA-3’ (Given)
3’-TCGGAT-5’ (Antiparallel and complementary)

The complementary strand should have the correct bases for base pairing (if A then T, if T then A, if C then G, if G then C). In addition, the two strands must be ***antiparallel,*** meaning one is oriented from 5’→3’ and the other is oriented from 3’→5’.

Two important themes for today's lecture:

Chemistry
Conditions

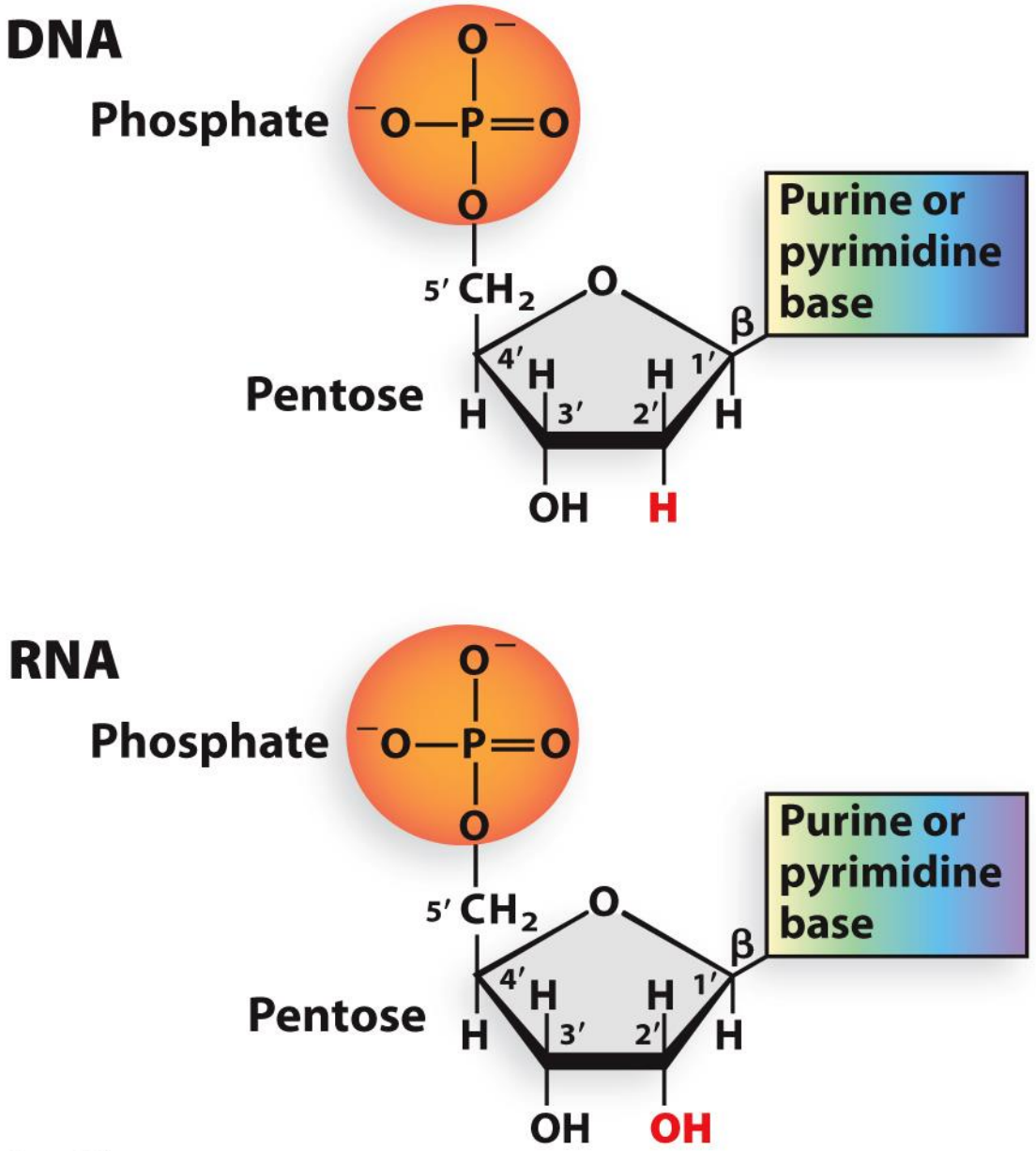

Figure 6-2b
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Pentose (5-carbon) ring structures in nucleic acids

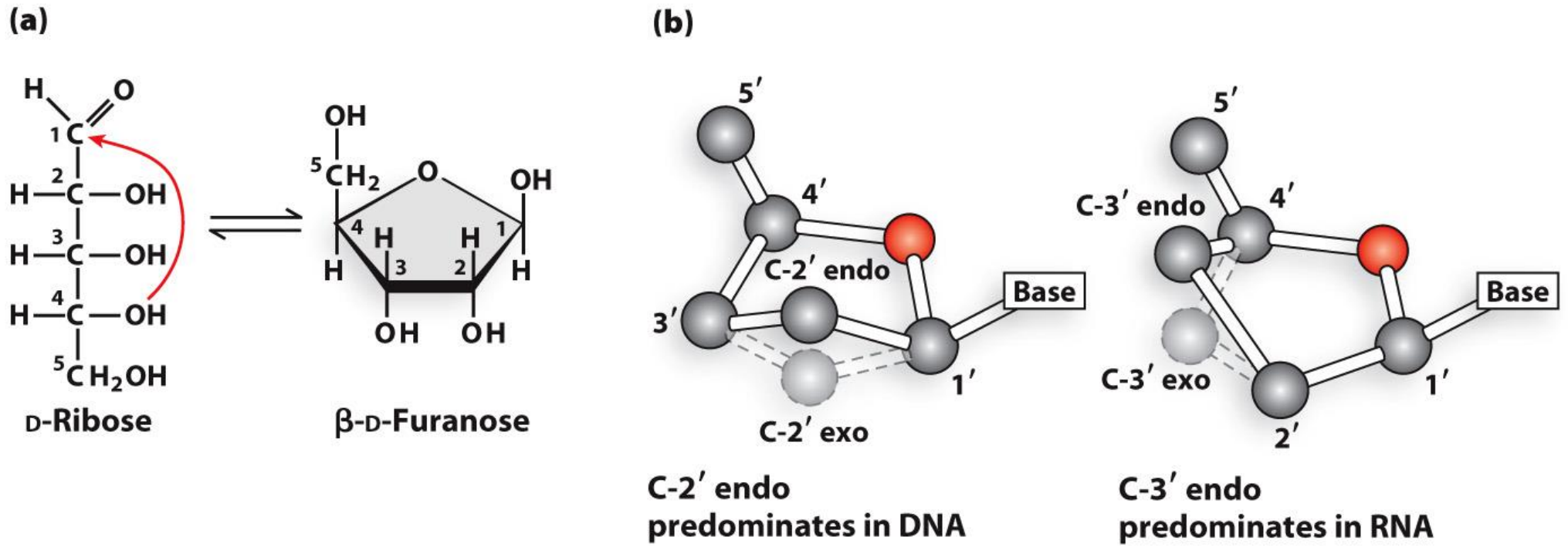

Figure 6-3
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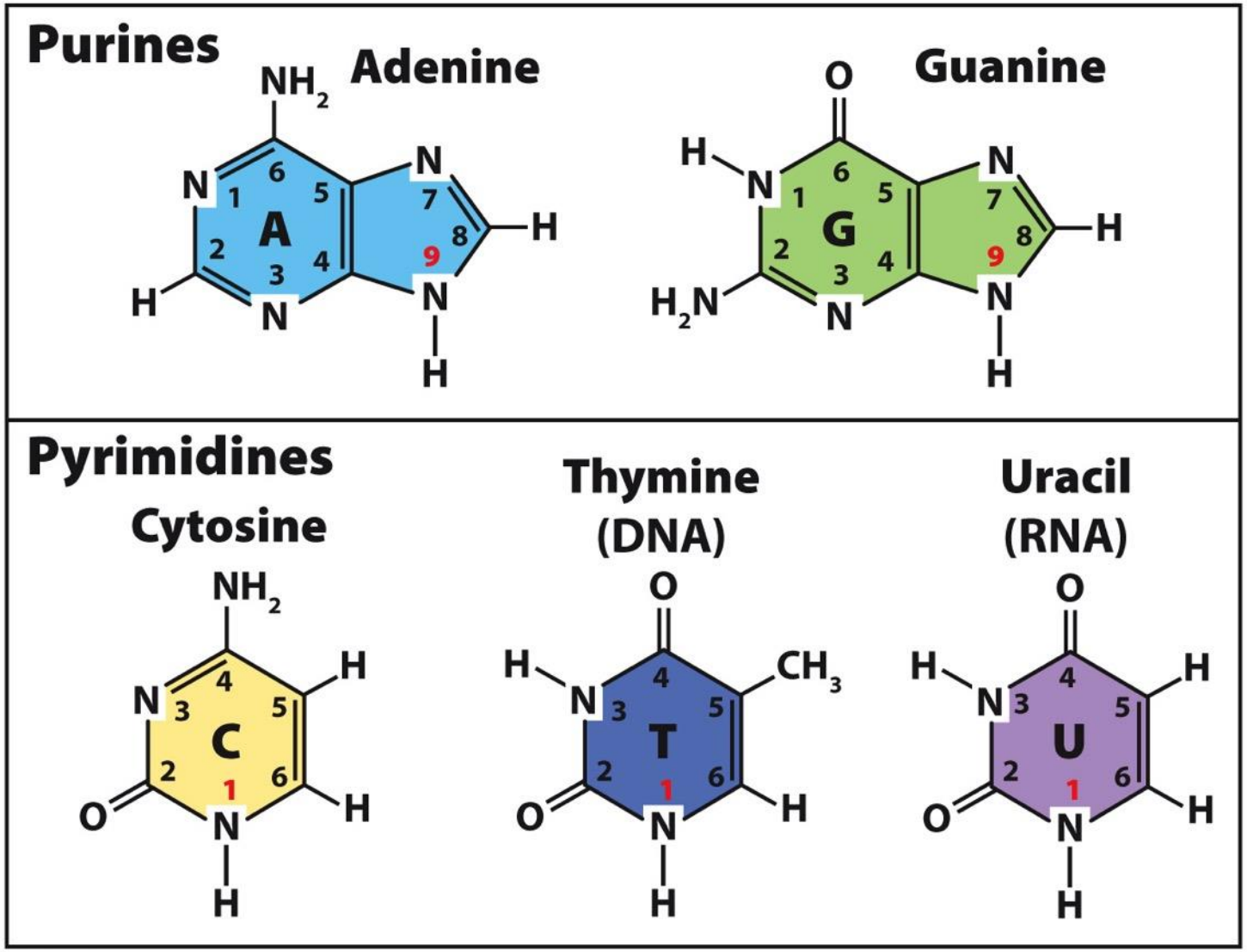

Figure 6-2a
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Note the numbering system of ring atoms.

Where does the pentose attach in each case? By what kind of bond?

These are the dNTPs and rNTPs of nucleic acids.

Cells contain other nucleotides where phosphate groups are on the 2´ and 3´ (e.g., cAMP, cGMP have 3´-P-5´ cyclic linkages)

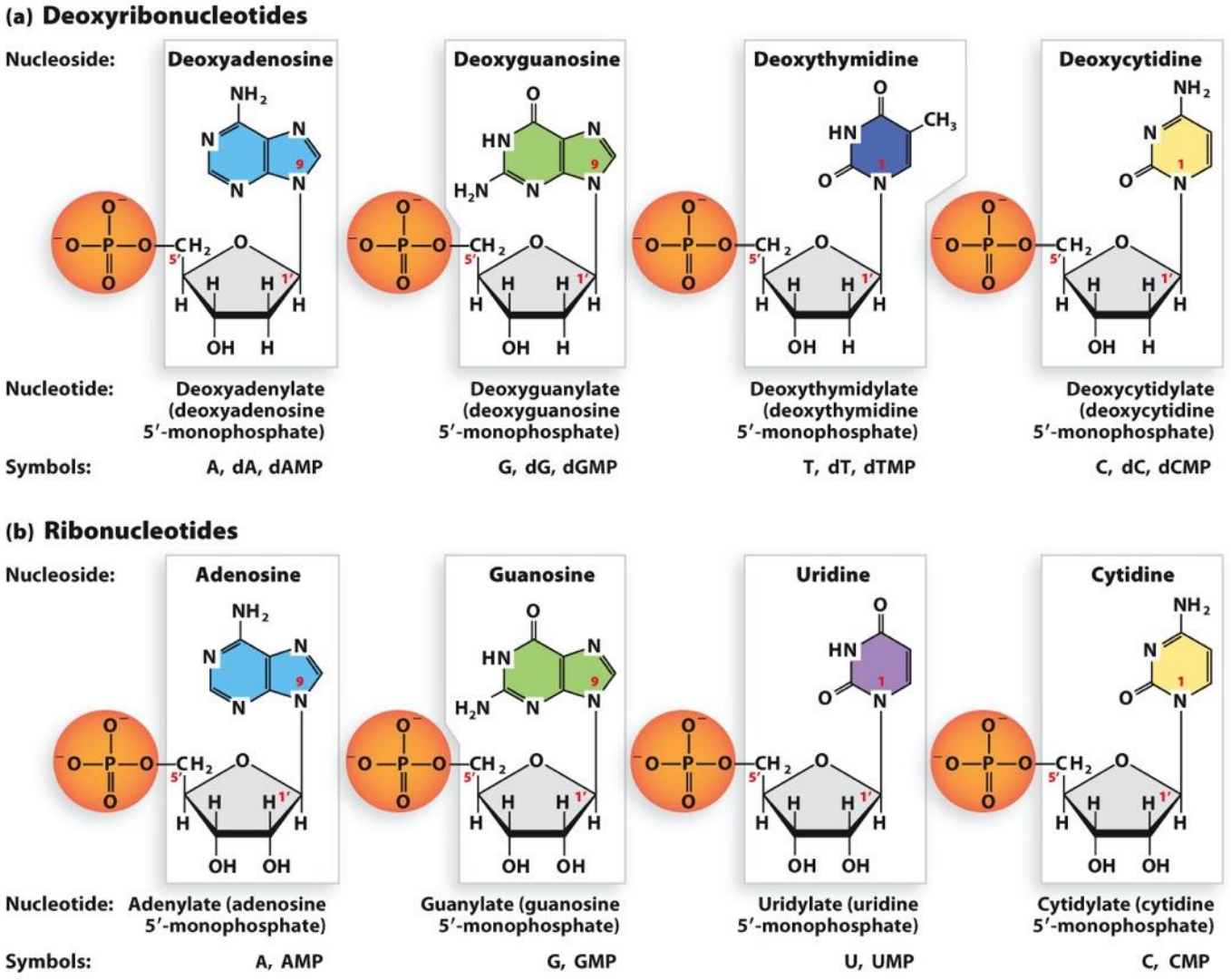

Figure 6-4

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The phosphodiester linkages in nucleic acids

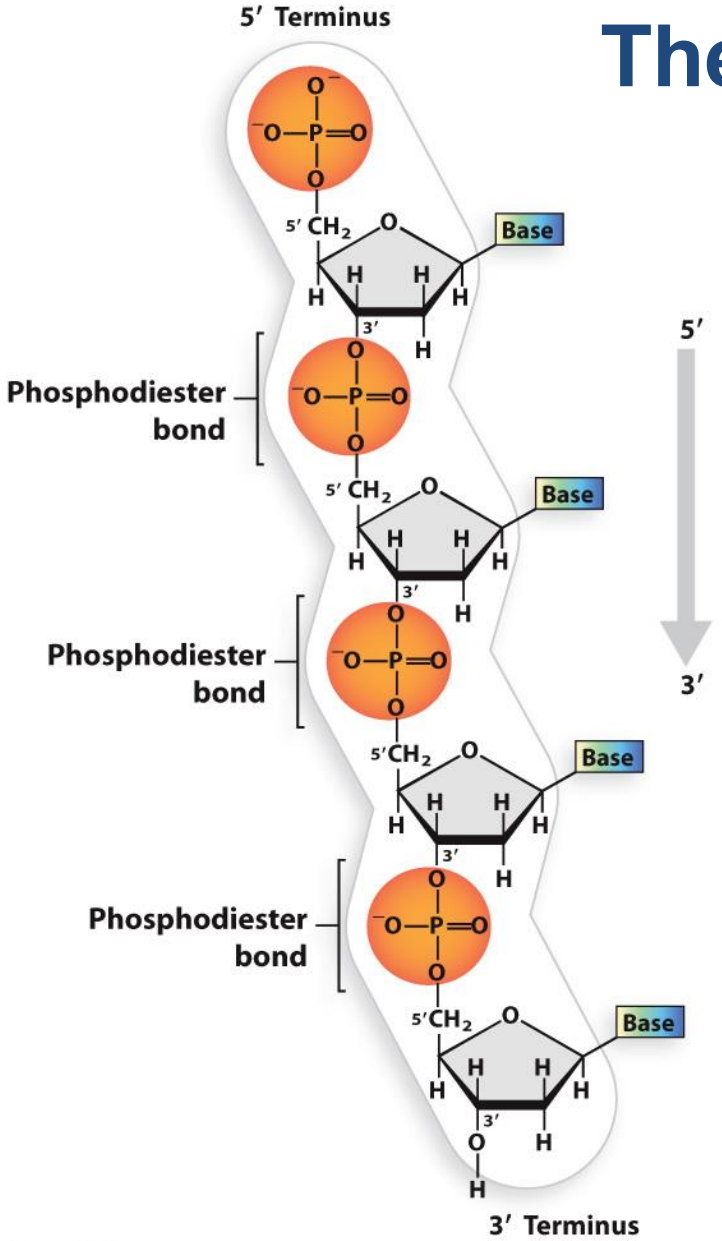

Figure 6-6
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5´ phosphate
3´ OH

Polarity of DNA/RNA strand

Are the phosphodiester linkages covalent, permanent?

Can they be hydrolyzed as in cut/broken?

The hydrolysis of RNA

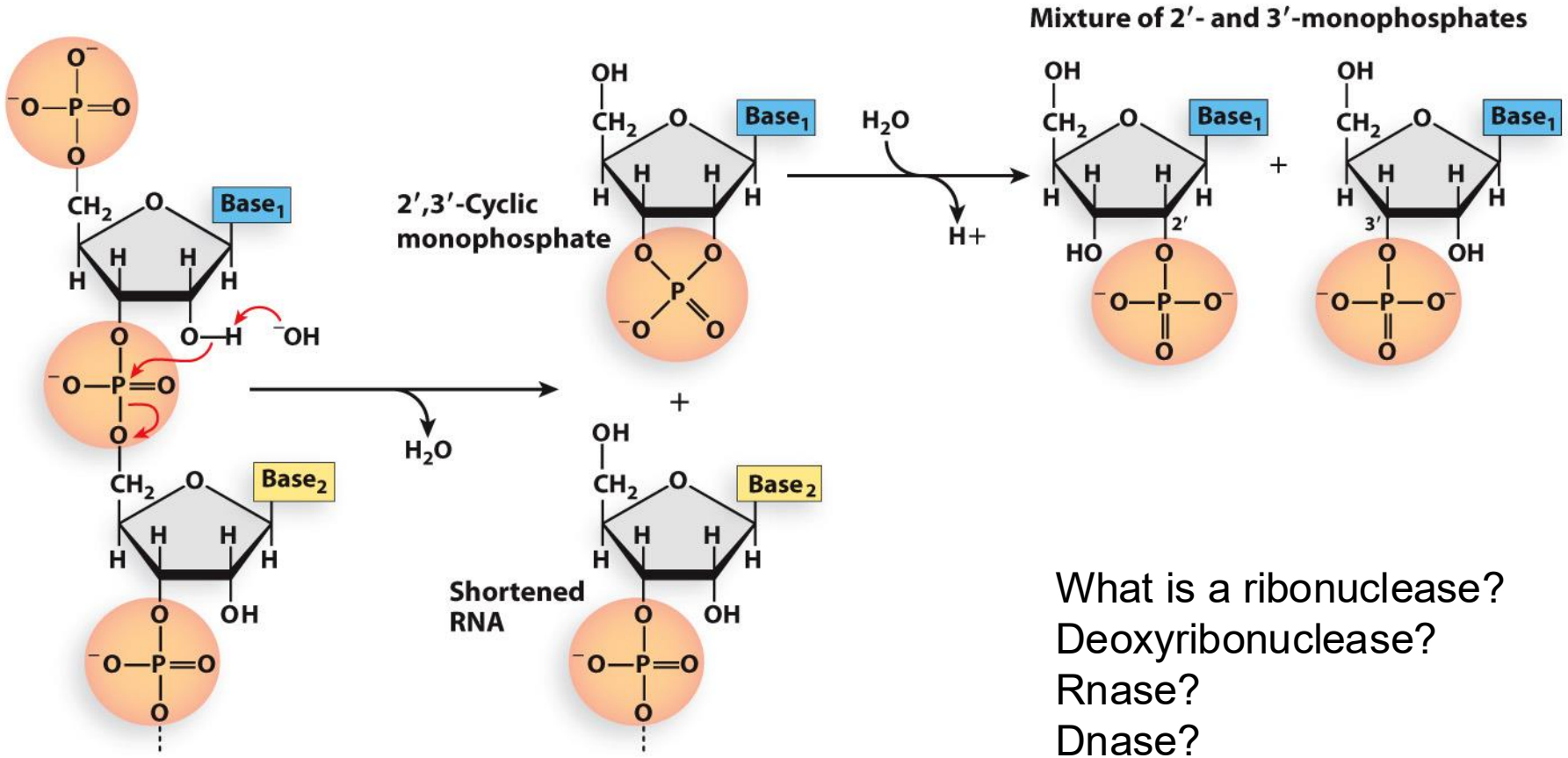

Figure 6-7
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What is a ribonuclease?
Deoxyribonuclease?
Rnase?
Dnase?

Remember: chemistry and conditions

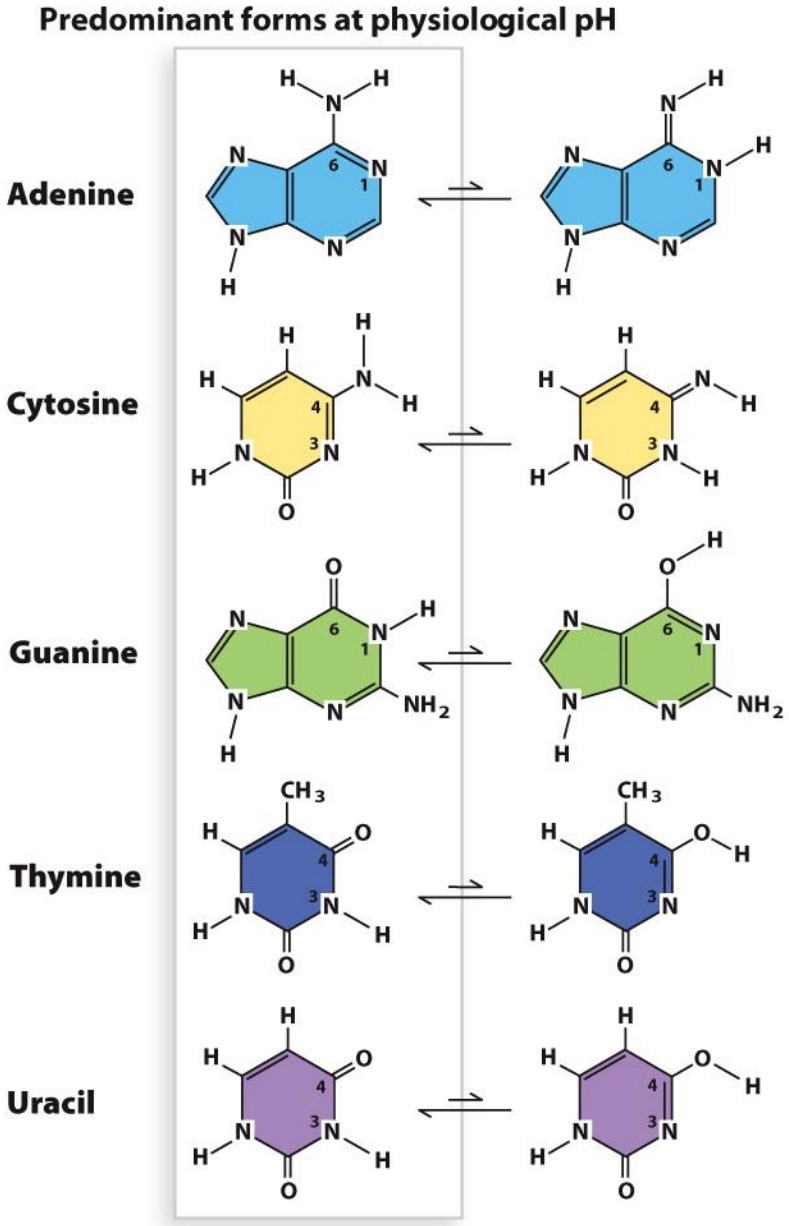

Figure 6-8
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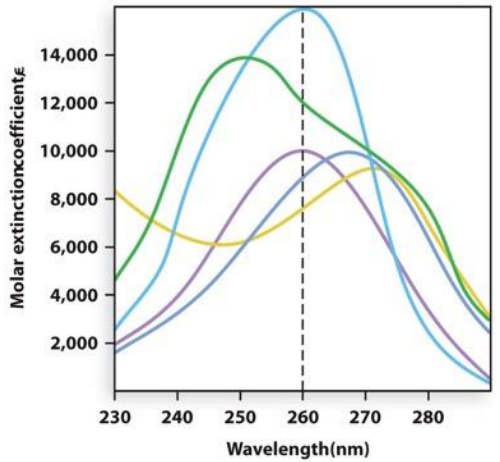
The properties of nucleotide bases affect the 3D structure of nucleic acids

Free Pu and Py can exist in two or more forms called **tautomers**, depending on pH (notice the arrows)

As a result of resonance, delocalized electrons in the conjugated rings are available to absorb UV light at 260 nm

What does this mean and why do we care?



Molar extinction coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)			
AMP	15,400	UMP	9,900
GMP	11,700	dTMP	9,200
CMP	7,500		

Figure 6-9
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Base Stacking and base pairing

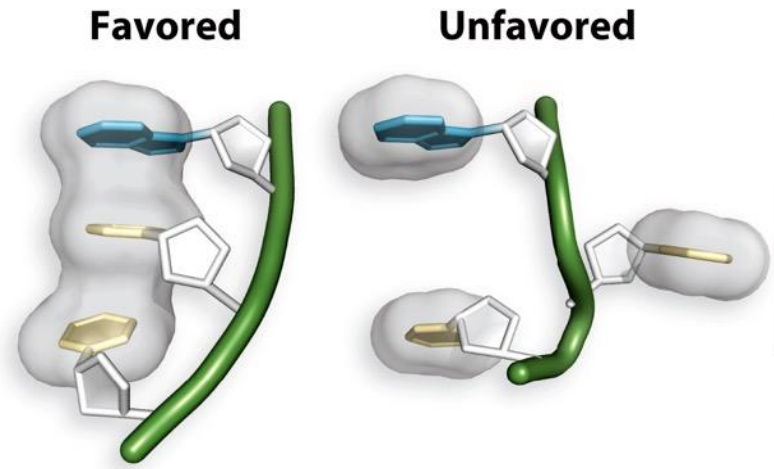

Figure 6-10
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The chemistry of purines and pyrimidines define DNA/RNA structure.

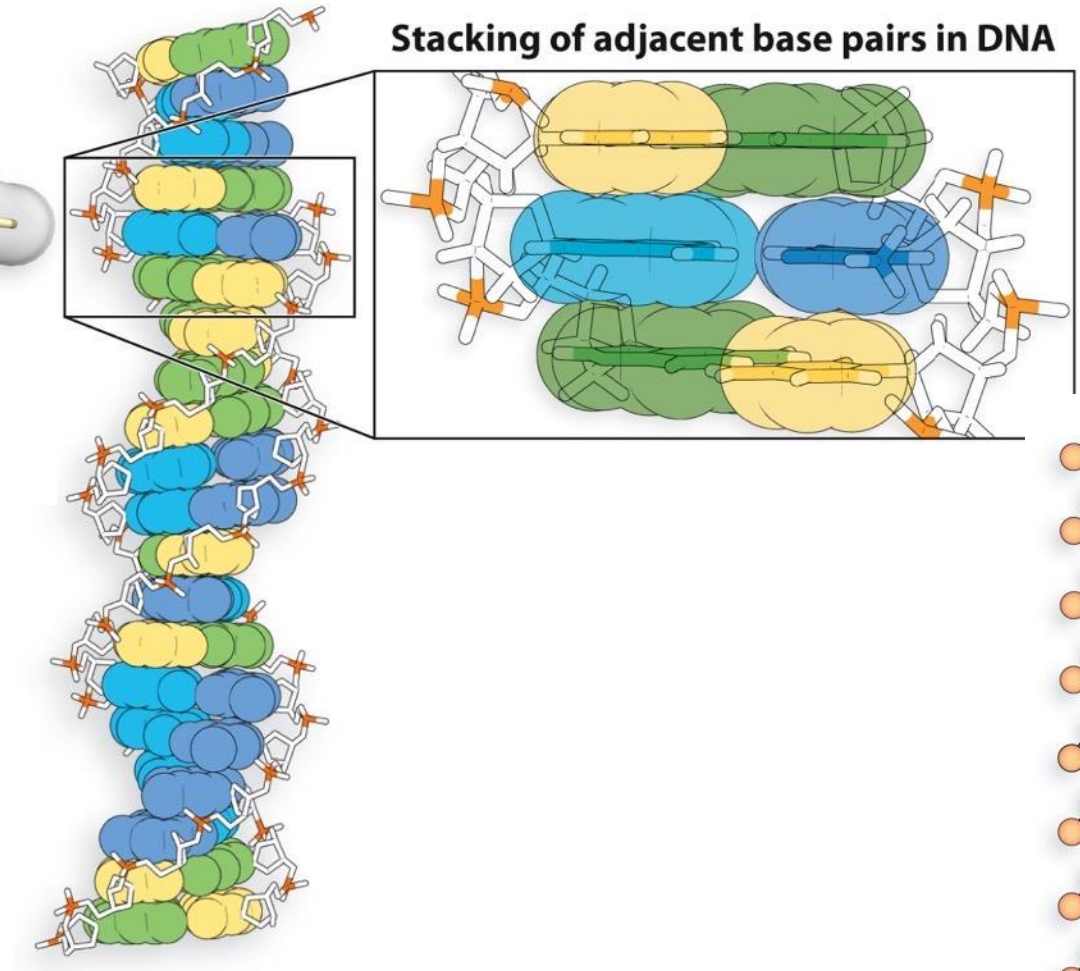

Figure 3-18
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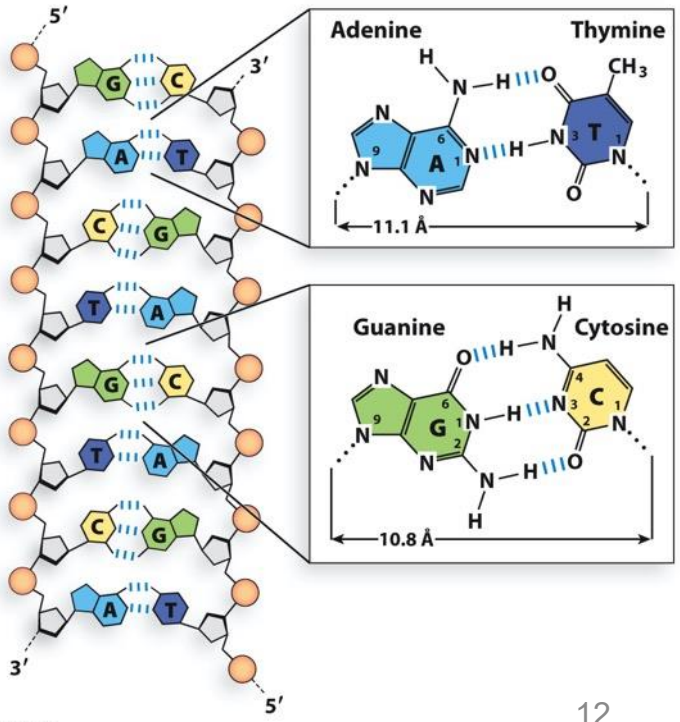

Figure 6-11
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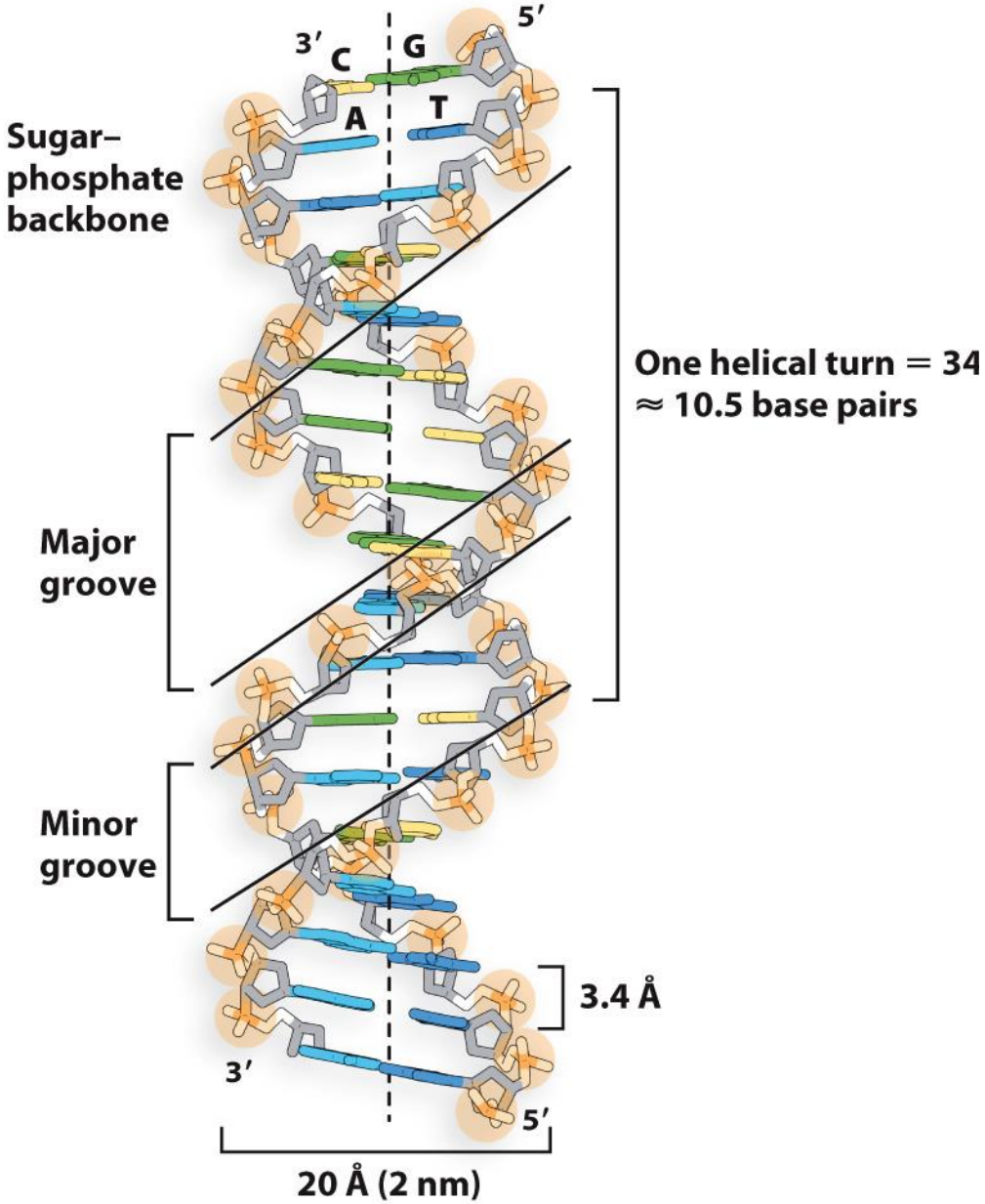

Figure 6-14
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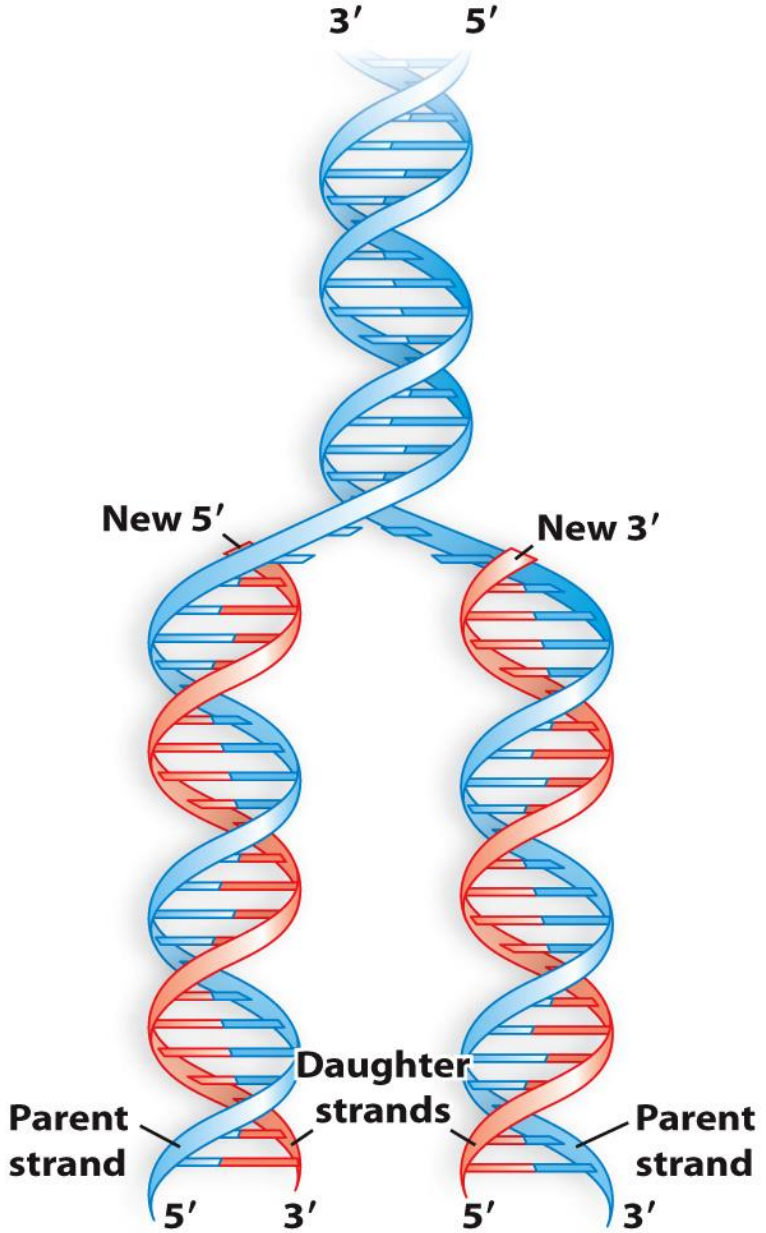

Figure 6-15
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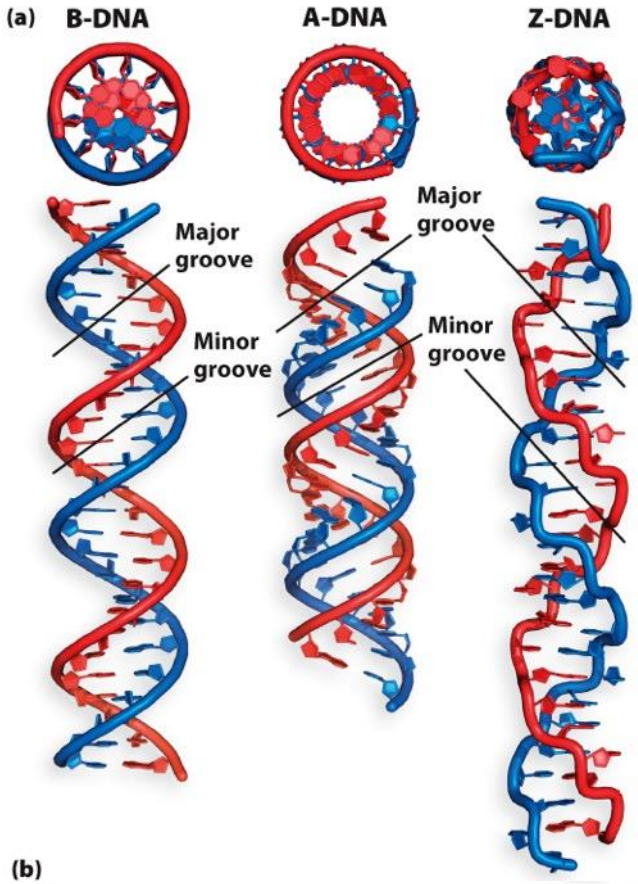
FORMS OF THE DOUBLE HELIX



DNA is usually a right-handed double helix known as **B-form (aka BR)**

Key words to be familiar with:
Major groove
Minor groove
Antiparallel
WC base-pairing

But… DNA does not always have to be right-handed. The phosphate sugar backbone is flexible due to the number of bonds.

(b)

	B-DNA	A-DNA	Z-DNA
Helix sense	Right-handed	Right-handed	Left-handed
Diameter	~20 Å	~23 Å	~18 Å
Base pairs per helical turn	10.5	11	12
Helix rise per base pair	3.4 Å	2.6 Å	3.7 Å
Base tilt in relation to the helix axis	−6°	+20°	−7°
Sugar pucker conformation	C-2′ endo	C-3′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

Figure 6-17
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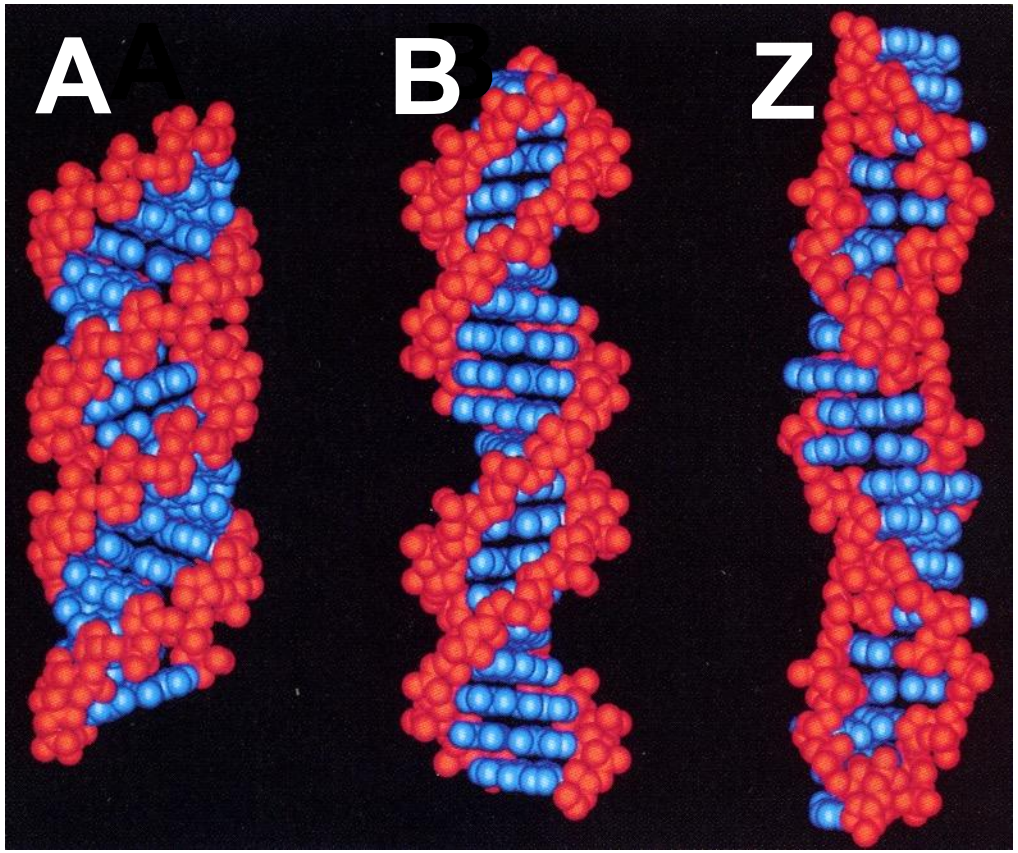
When are other forms of the double helix possible? *chemistry and conditions*

AR: favoured by dehydration
ZL: (CG)n alternating sequences

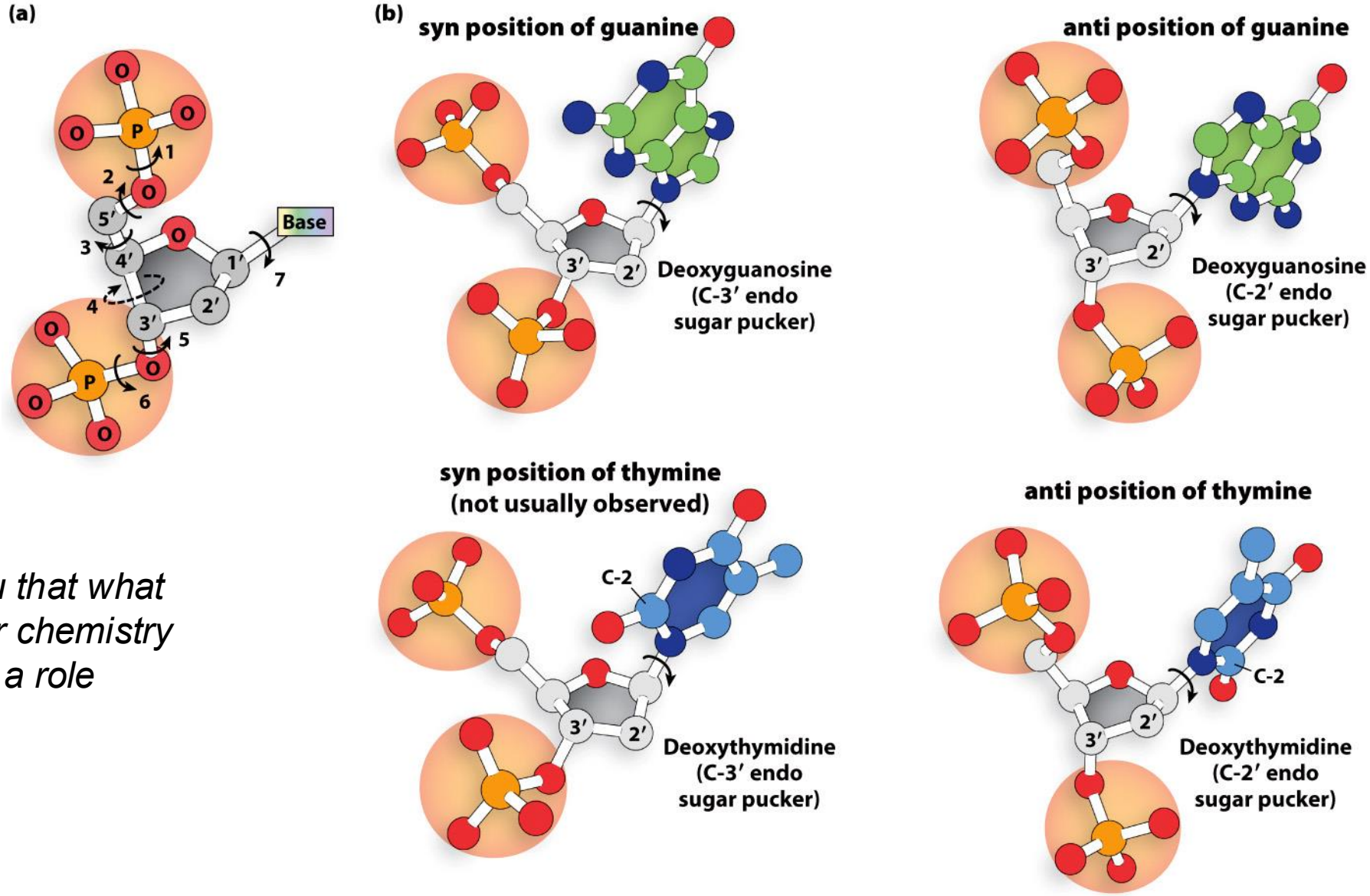


All of these DNA models are depicted with the same number of base pairs, emphasizing the differences in compactness of the three DNA forms.

Form	Pitch Å	Residues per Turn	Inclination of Base Pair from Horizontal (degrees)
A	24.6	10.7	+19
B	33.2	~10	−1.2
Z	45.6	12	−9

Right-handed vs left-handed helix?

Imagine that the DNA is a screw, and the backbone strands are the grooves in the screw. Now, take your imaginary screwdriver and screw the DNA into a piece of imaginary wood. The direction you twist the screwdriver determines the handedness. If you have to twist clockwise, then its a right handed helix. Counterclockwise, is left handed.



Here to remind you that what you learned in your chemistry prerequisites have a role here in MBB 331.

Figure 6-16
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Certain DNA sequences adopt unusual structures (chemistry and conditions)

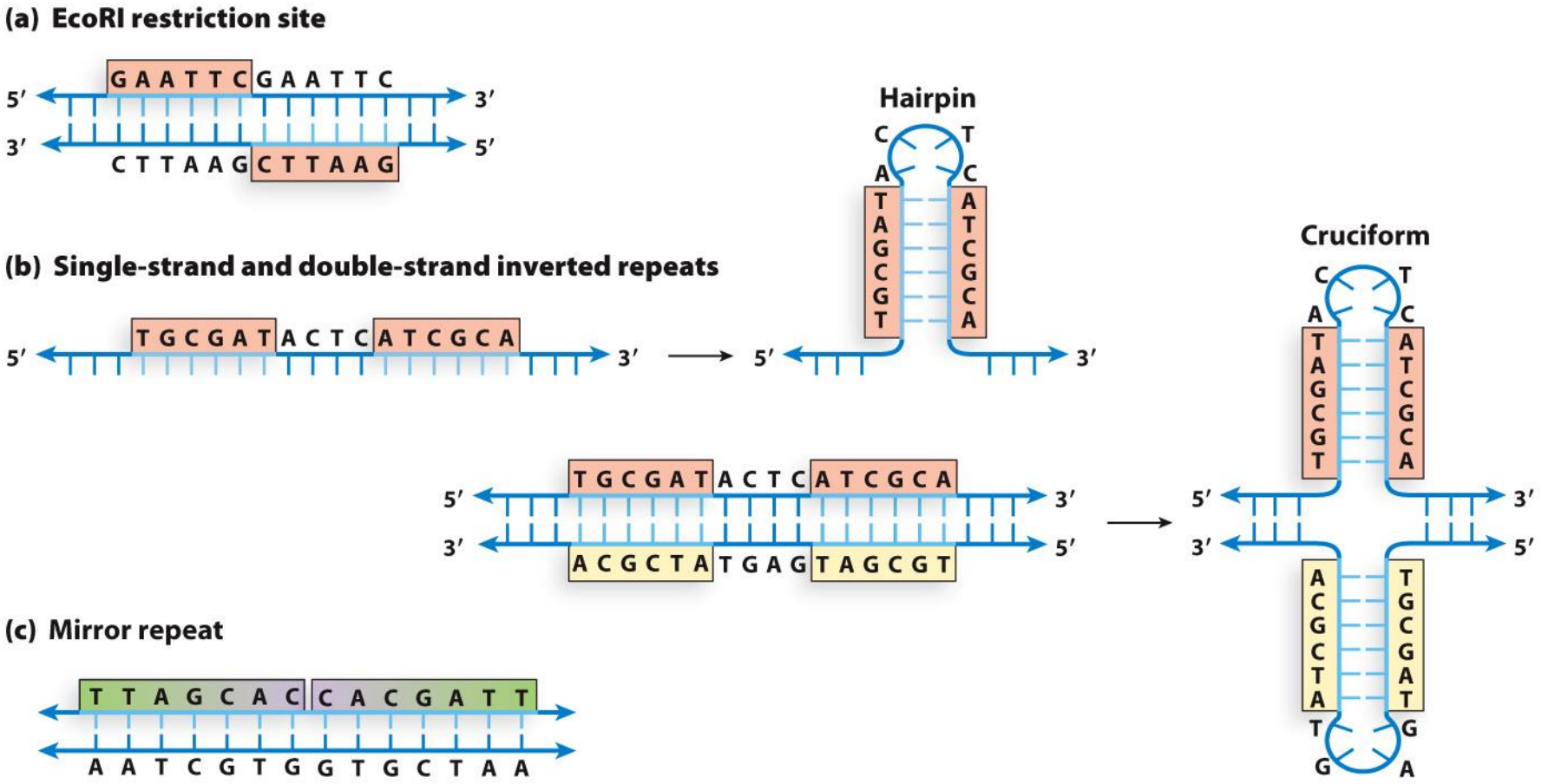

Figure 6-20
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3- and 4-stranded DNA structures also possible.

When and how? (Chemistry and conditions!)

Base triples
(additional hydrogen bonding here called Hoogsteen pairing)

Triplexes are highly sequence specific

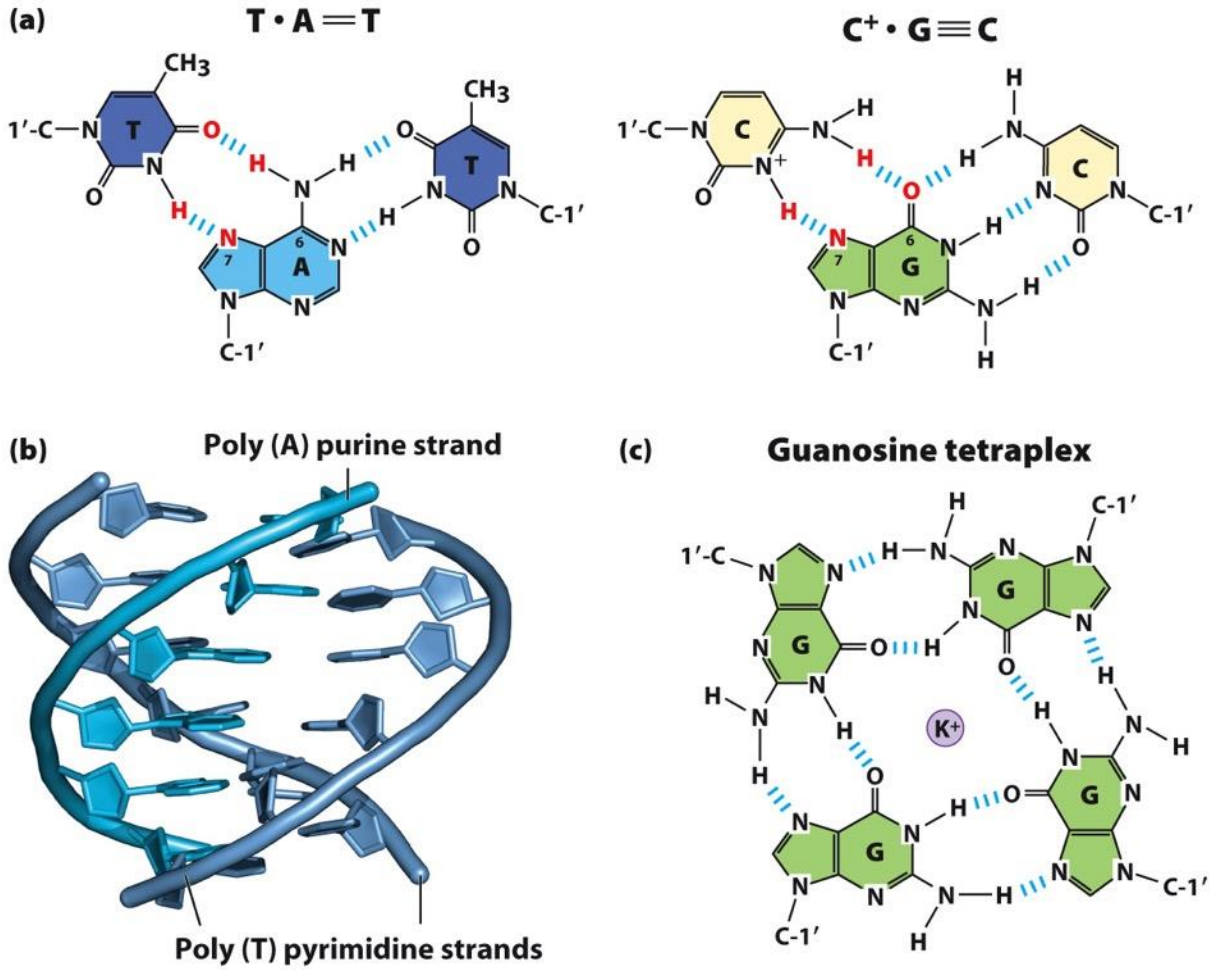

Figure 6-21
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RNAs have helical secondary structures

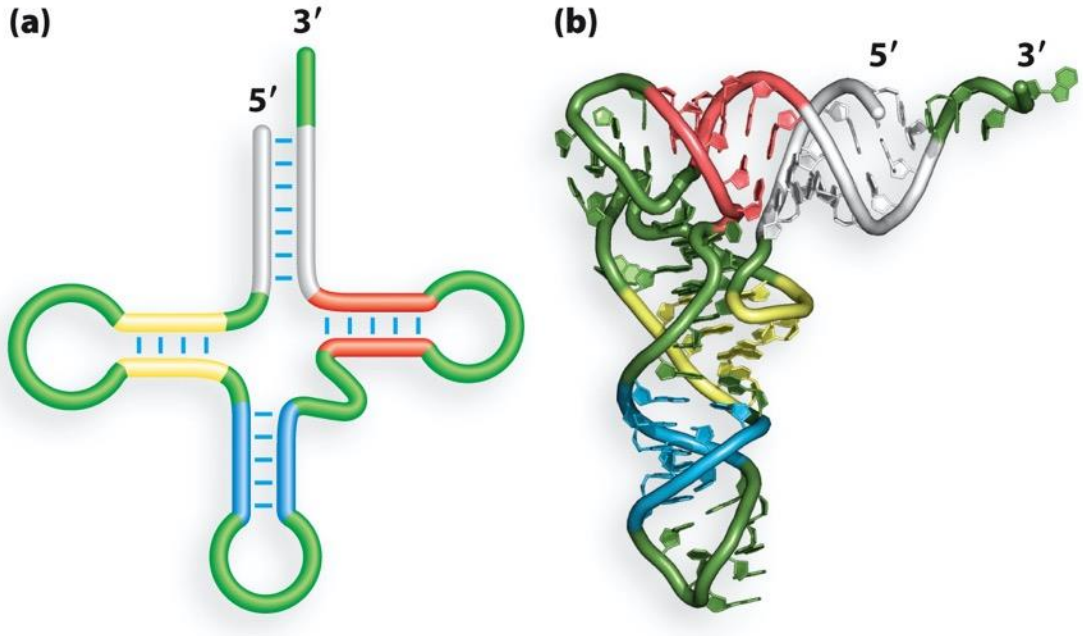

Figure 6-22
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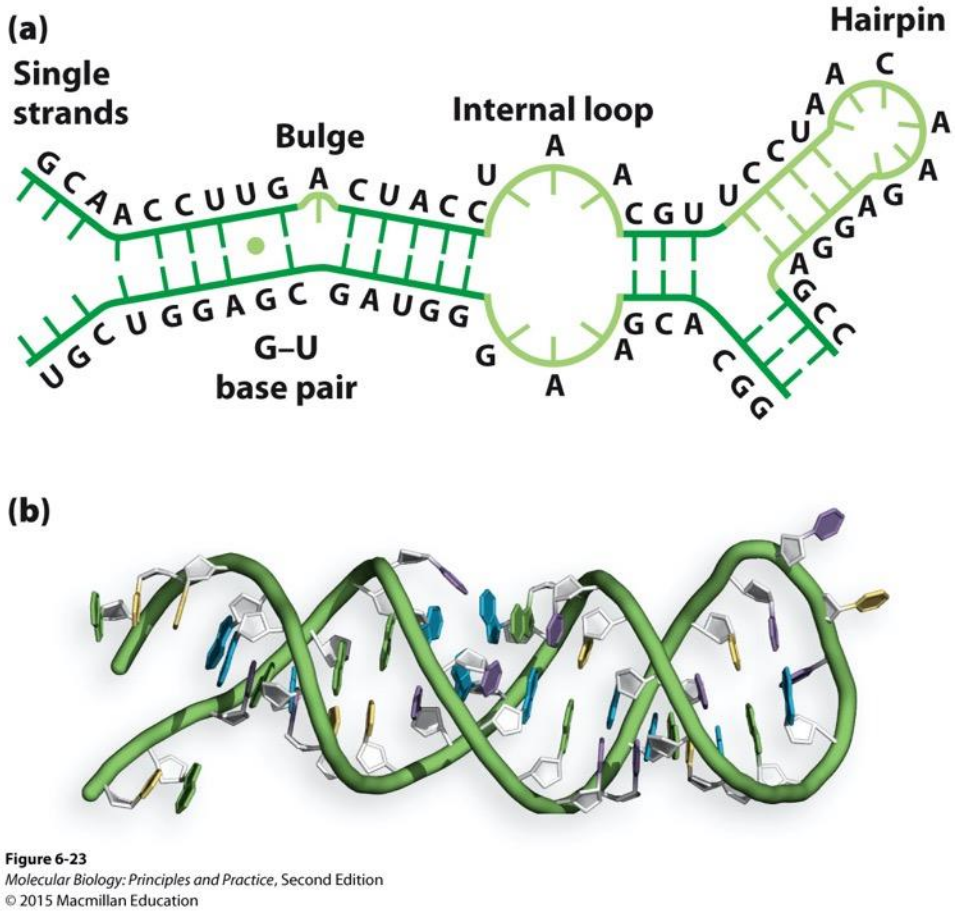

Figure 6-23
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Single stranded RNA folding back on itself.
Note: there are unpaired regions!

Denaturation of DNA

- DNA "Melting" Temp (Tm) can be affected by :
  - GC content
  - [salt]
  - # of bps
- Melting can be monitored by UV light absorption at 260 nm

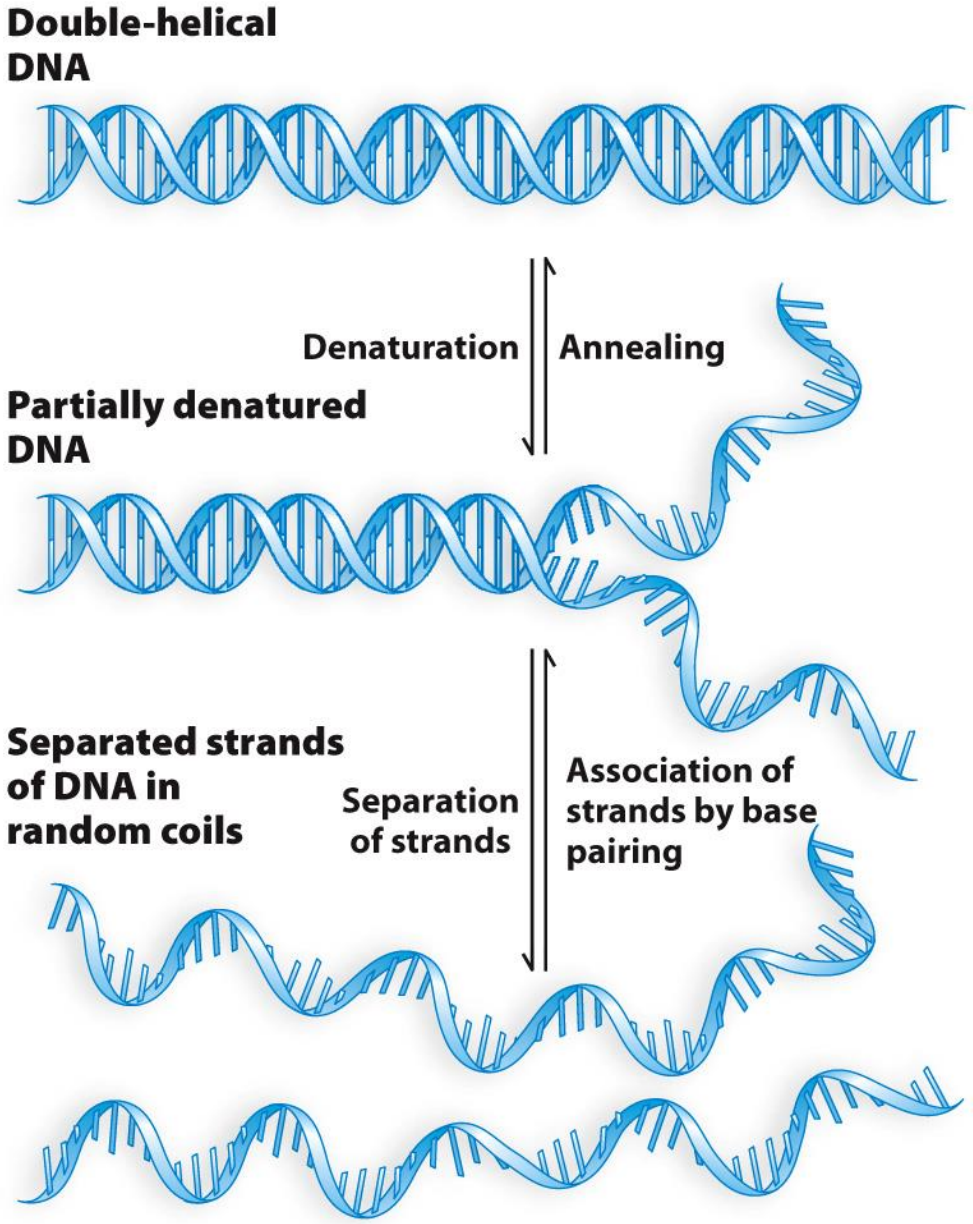

Figure 6-28
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THE PHYSICAL CHEMISTRY OF DNA

Hyperchromic Shift:

The transition from double-stranded DNA to the single-stranded, denatured form can thus be detected by monitoring the increase in absorption of UV light

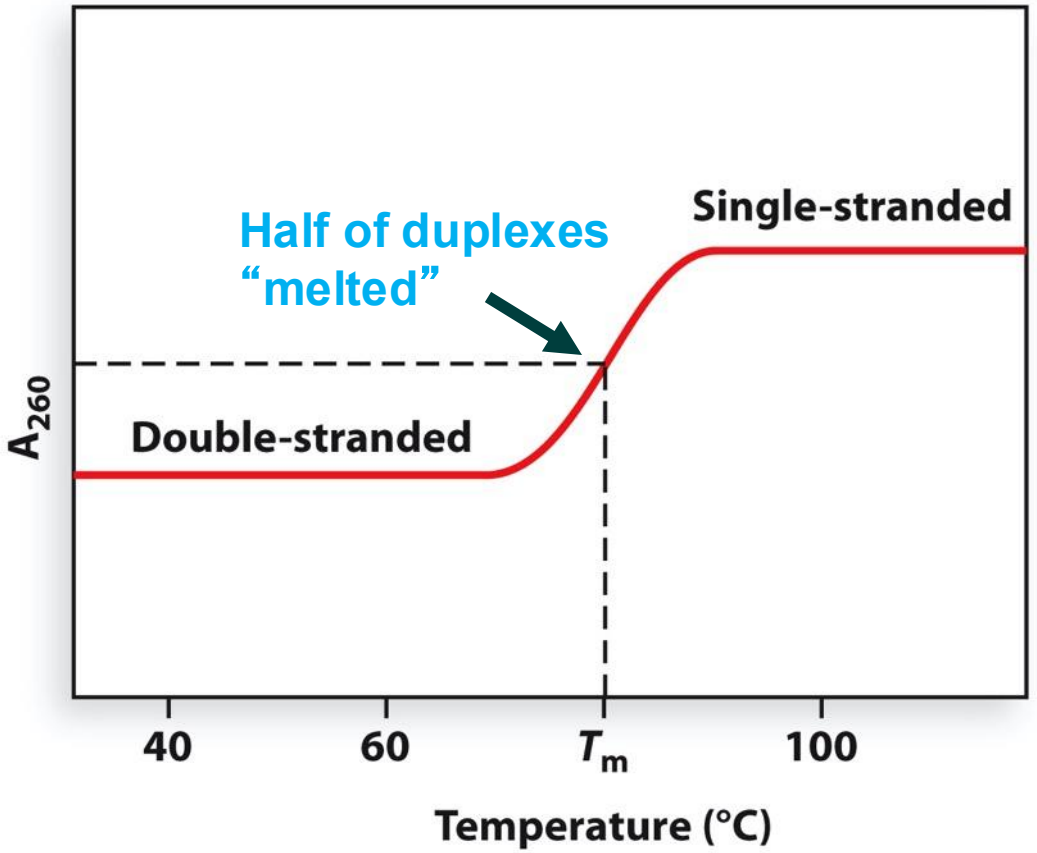

Figure 6-29
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Partially denatured DNA

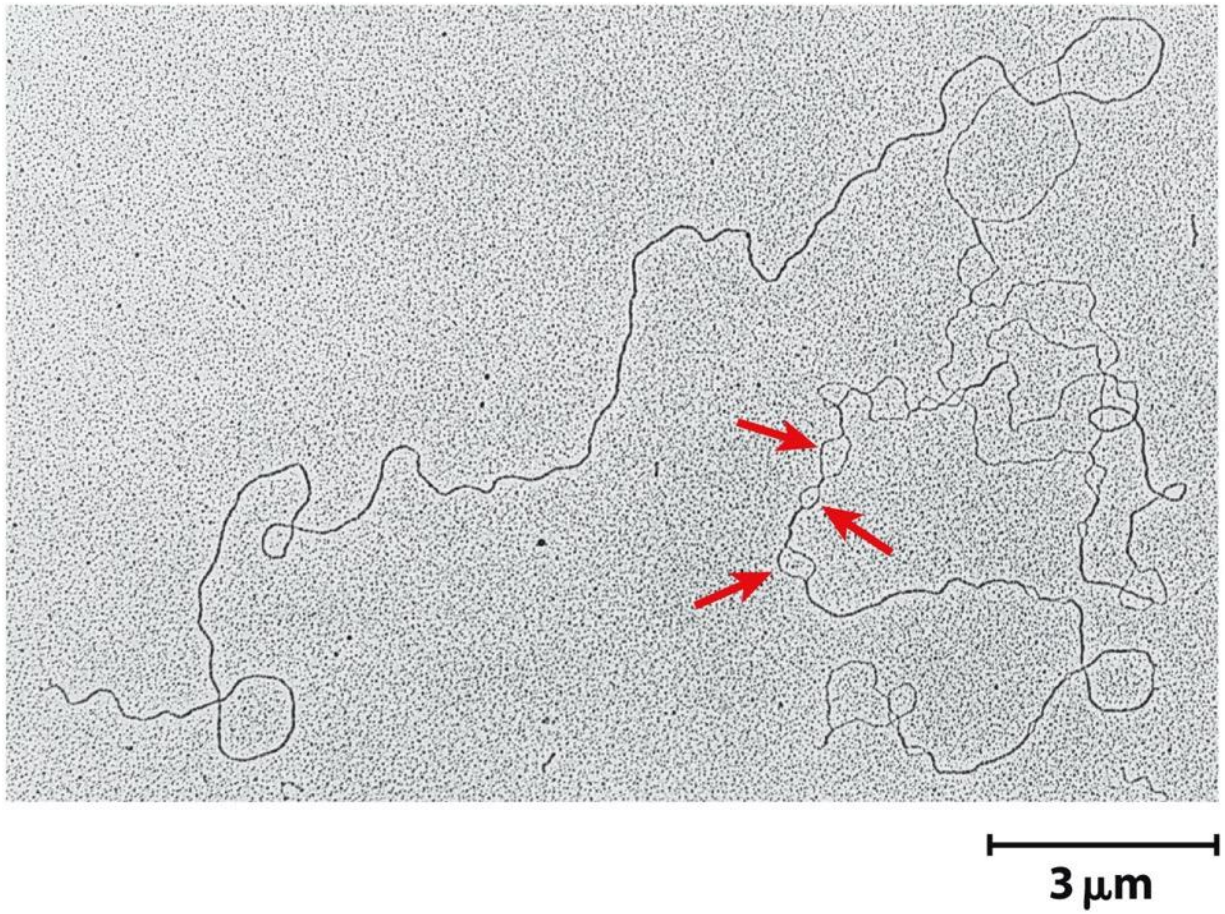

Figure 6-30
Molecular Biology: Principles and Practice, Second Edition
Ross B. Inman, University of Wisconsin–Madison, Department of Molecular Biology

The DNA shown in this EM was partially denatured, then fixed to block renaturation (annealing) during sample preparation.

The arrows point to some ss regions (aka bubbles) where denaturation has occurred.

Would you predict the bubble sequences to be AT rich or GC rich?

Cross-species DNA hybridization

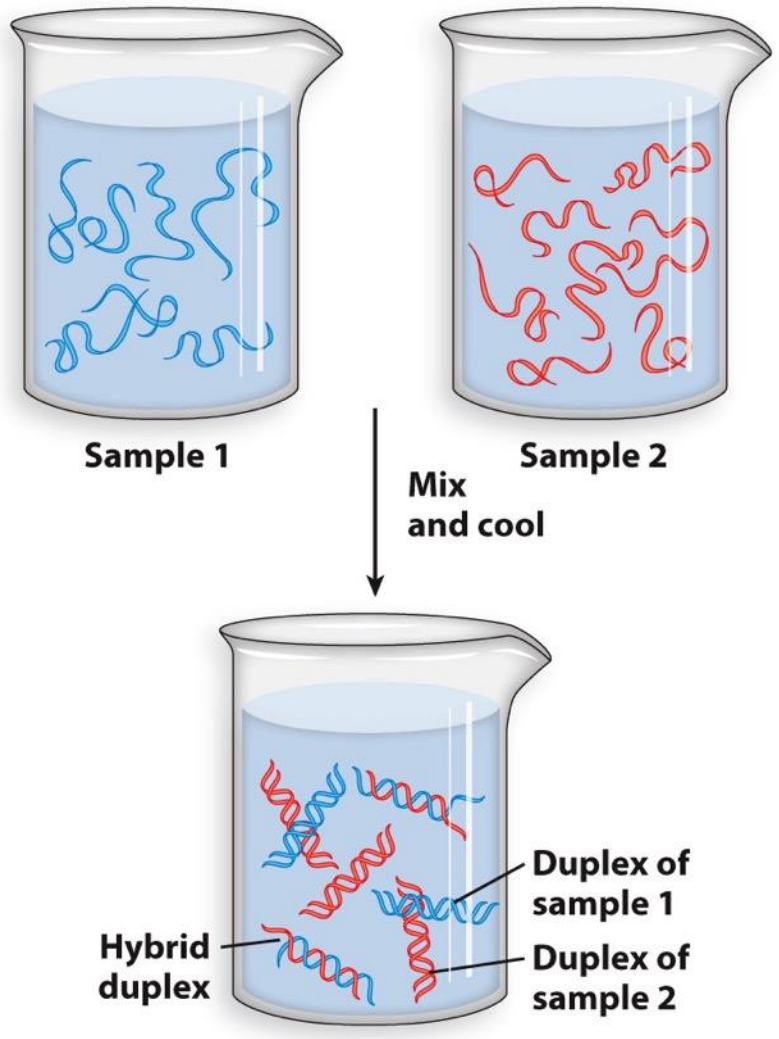

Figure 6-31
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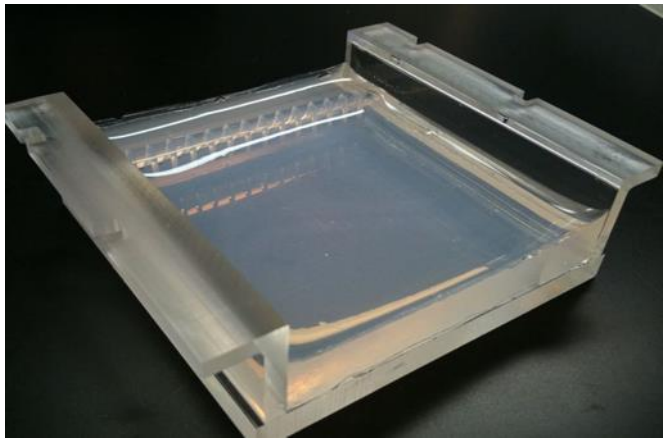
- Very valuable tool to explore evolutionary relationships
- Short stretches of DNA or RNA sequence can be used as probes
- Must be:
  - Single stranded
  - Labeled

Visualizing DNA via gel electrophoresis

Gel is made of **agarose**, a kelp-derived material that does not disrupt nucleic acid base-pairing

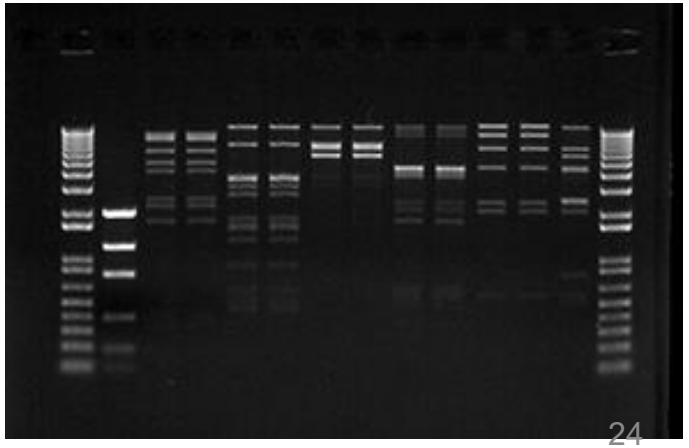
How it's made:

- Powered agarose is added to a gel buffer, mixed and melted by microwaving, then poured into a casting tray when it's molten.
- When set, the gel forms a meshwork. When an electric field is applied to the gel submerge in running buffer, DNA molecules move through the gel toward the anode. *WHY?*
- Agarose gels are typically 0.5% - 2%. *Why the range?*



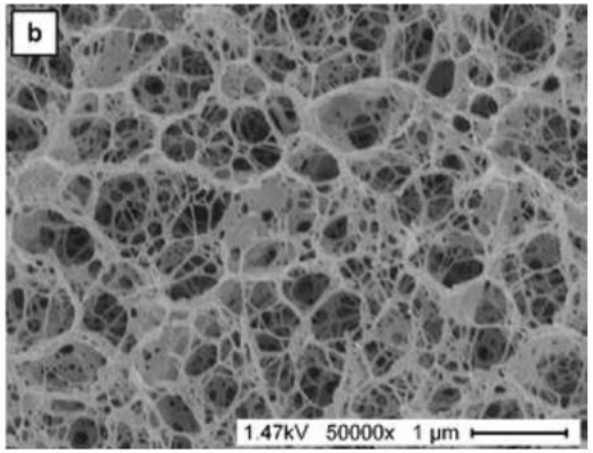
How it's visualized:

- A DNA dye SYBR-Safe (non mutagenic) is added to the gel before pouring into the mold; it binds to DNA and can be visualized using visible light
- Ethidium bromide (mutagenic/carcinogenic) is commonly used in research labs: intercalates into the DNA and fluoresces when exposed to UV light

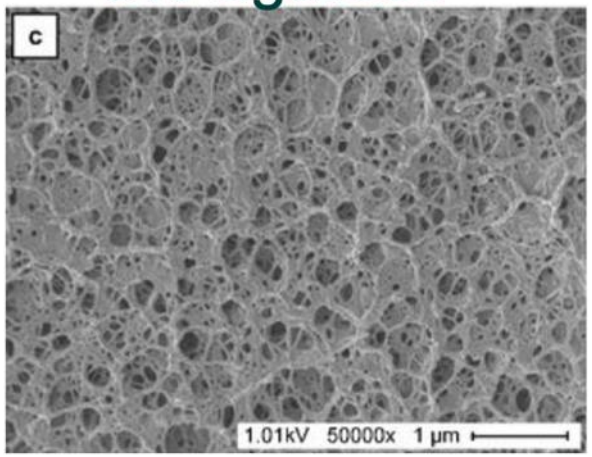


Intro to gel electrophoresis:
Loading and Running an Agarose gel

2% agarose



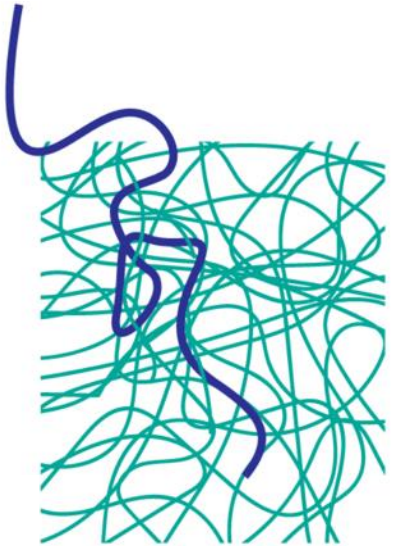
6% agarose

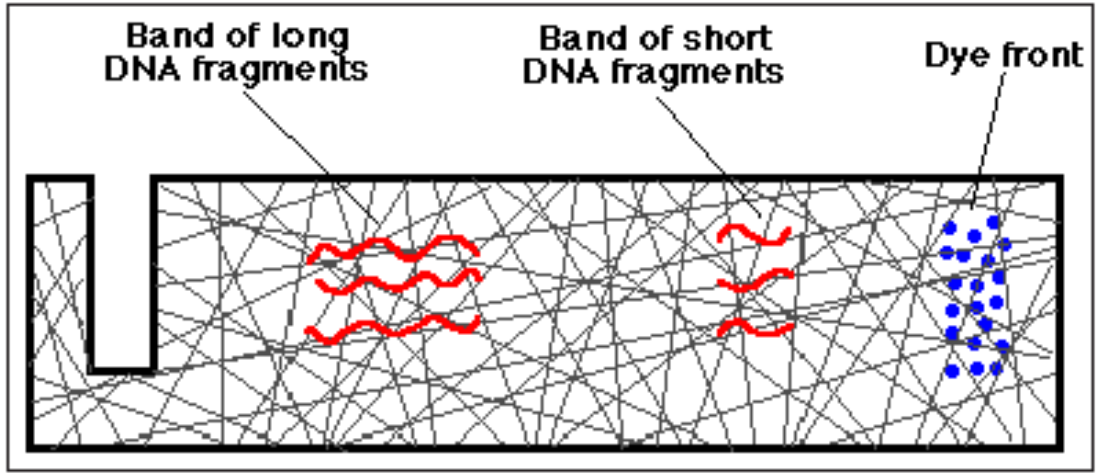


Scanning electron microscope: show different pore sizes

The gel matrix impedes movement of all DNA molecules

- Smaller DNA molecules move more freely than the larger ones and thus move faster through the gel
- Different DNA molecules are separated based on size; separation of certain sized fragments can be optimized by changing the percentage of agarose in the gel

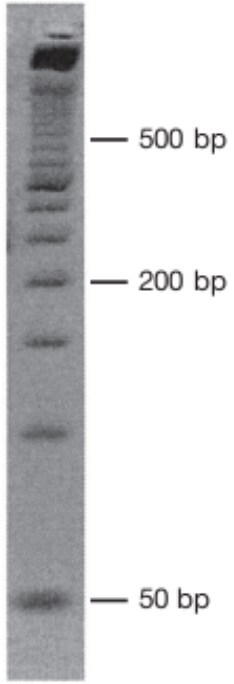


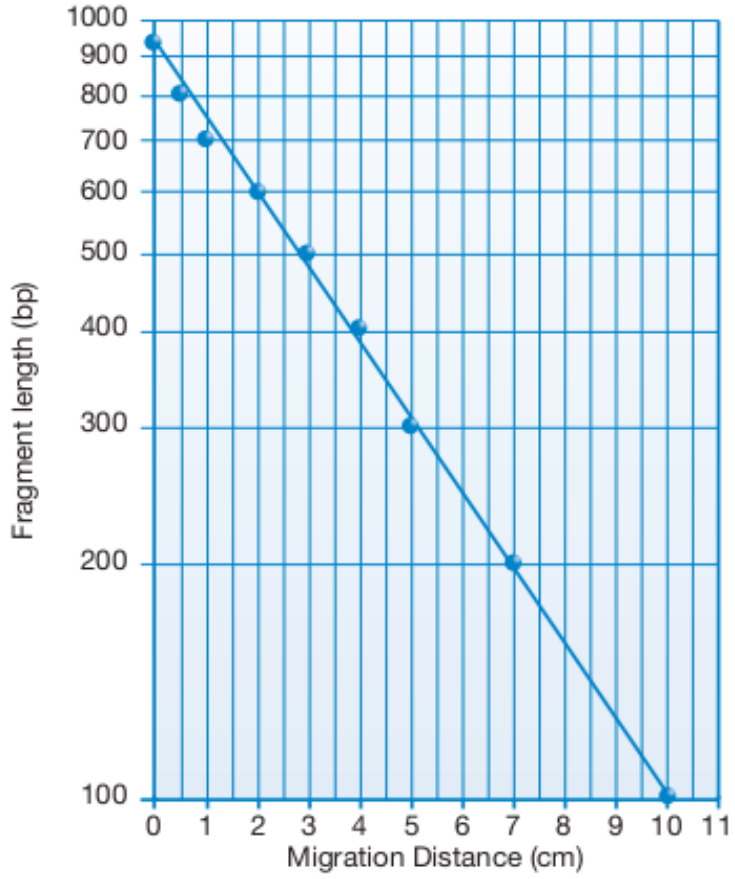


Running and analyzing an Agarose gel

How can you determine the size of the DNA by looking at the bands on the gel?

- dsDNA fragments migrate on a gel with a mobility that is inversely proportional to the $\log_{10}$ of its length
- Always run a DNA ladder (usually lane 1) to size and quantify DNA fragments
- DNA fragments are separated based on size and shape (as we will see





The log of DNA size and the distance have **linear** relationship

Gel electrophoresis for DNA detection

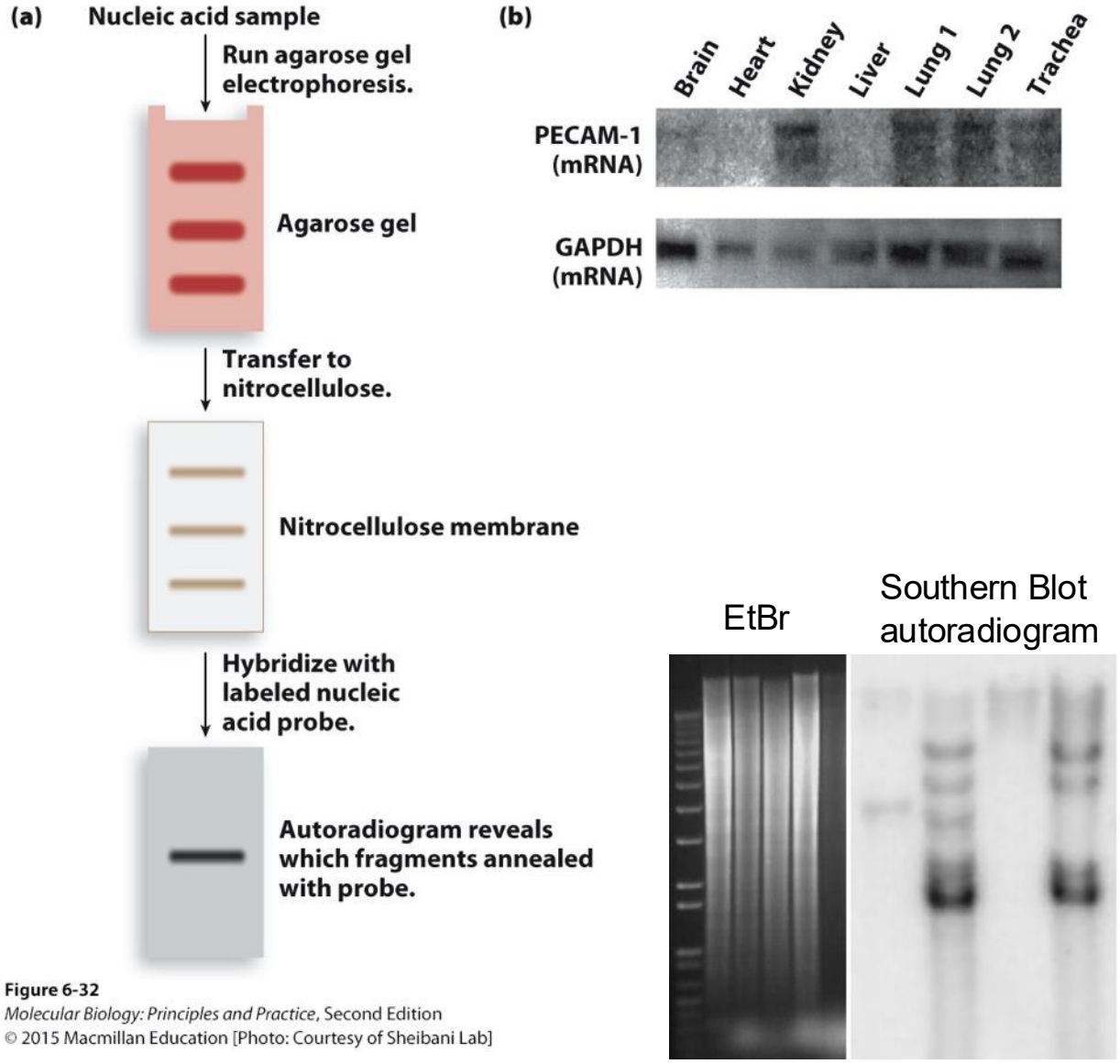


Figure 6-32
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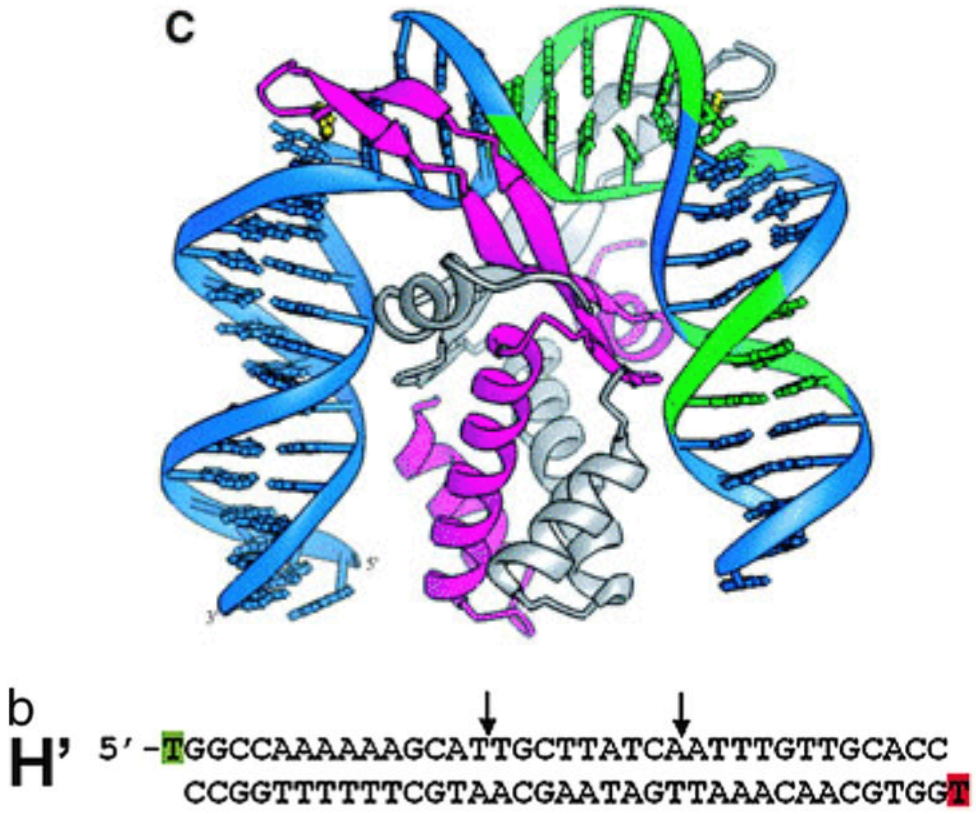
- In some techniques, DNA fragments are transferred to a nitrocellulose membrane so that their position in the gel is preserved.
- Once on the membrane, the nucleic acid can be hybridized with a DNA or RNA probe, labeled so that it can be detected by measuring radioactivity or fluorescence.
- **DNA** detection: **SOUTHERN BLOT**
- **RNA** detection: **NORTHERN BLOT**

DNA twists and bends



Certain DNA sequences adopt unusual structures

DNA is a relatively rigid polymer, but it has three significant degrees of freedom: twisting, bending, and compression, each of which cause particular limitations on what is possible with DNA within a cell.

A closed double-stranded molecule of DNA can be compacted (made smaller) by a process called *supercoiling*

Relaxed DNA → Supercoiled DNA

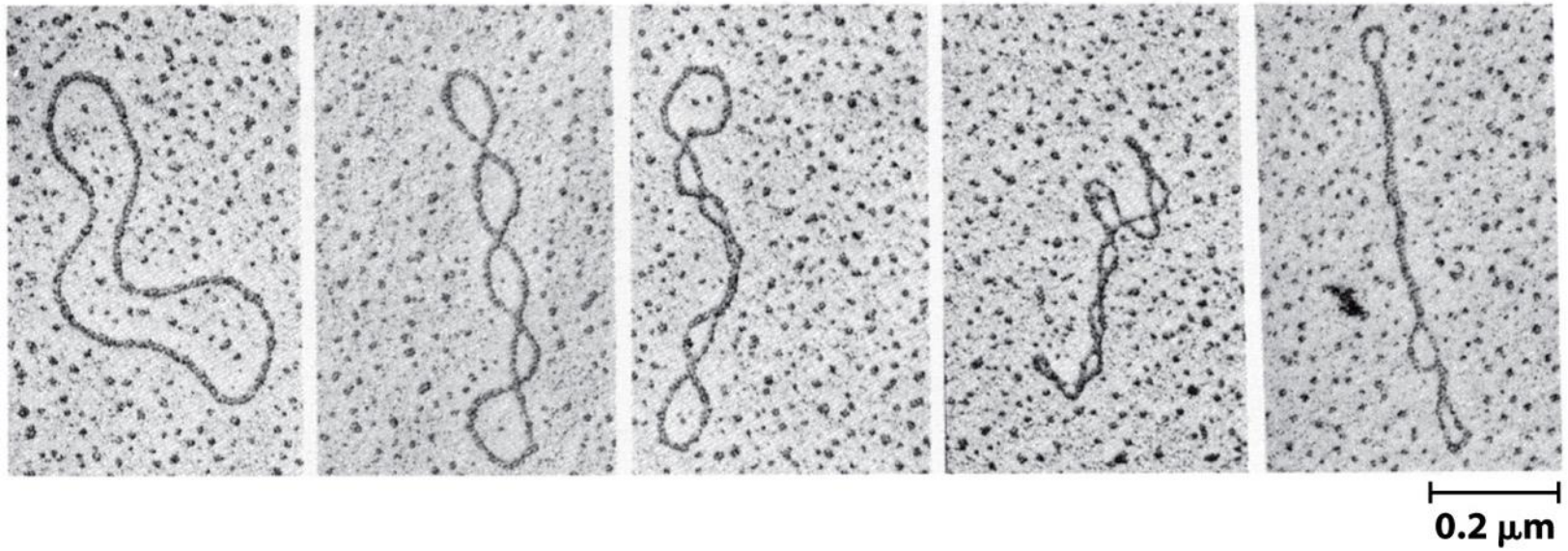


Figure 9-9
Molecular Biology: Principles and Practice, Second Edition
Laurien Polder from Kornberg, A. (1980) *DNA Replication*, p. 29, W.H. Freeman, New York.

Supercoiling can only exist in a DNA molecule where both strands of DNA are closed circles or otherwise fixed at one end. If one strands breaks the DNA rapidly loses its supercoiling (i.e. it relaxes).

DNA molecules in different coiled forms that have the same nucleotide sequence are called **topoisomers**

Large linear DNA *in vivo* is supercoiled

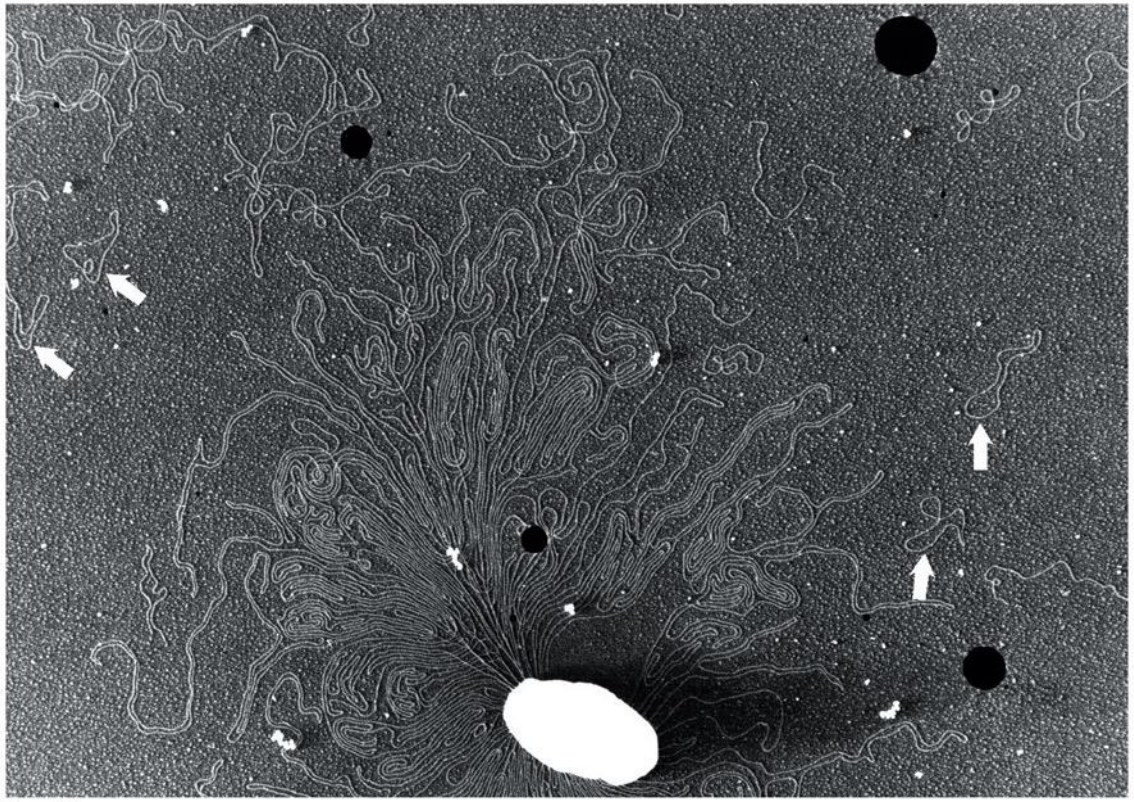


Figure 9-3
Molecular Biology: Principles and Practice, Second Edition
Huntington Potter, University of Colorado, Anschutz Medical Campus and David Dressler, Balliol College, Oxford University.

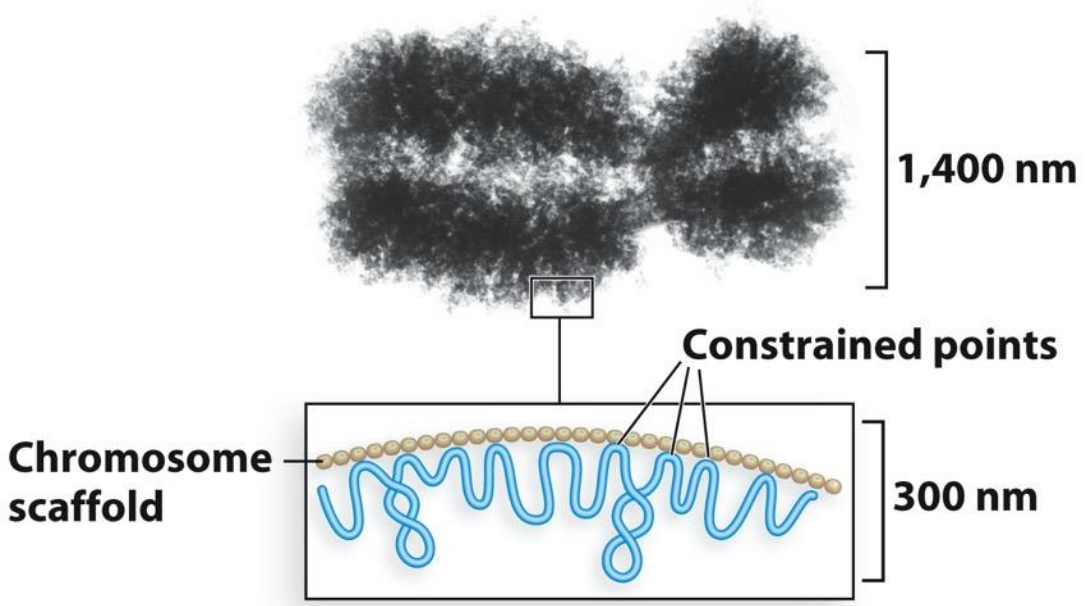
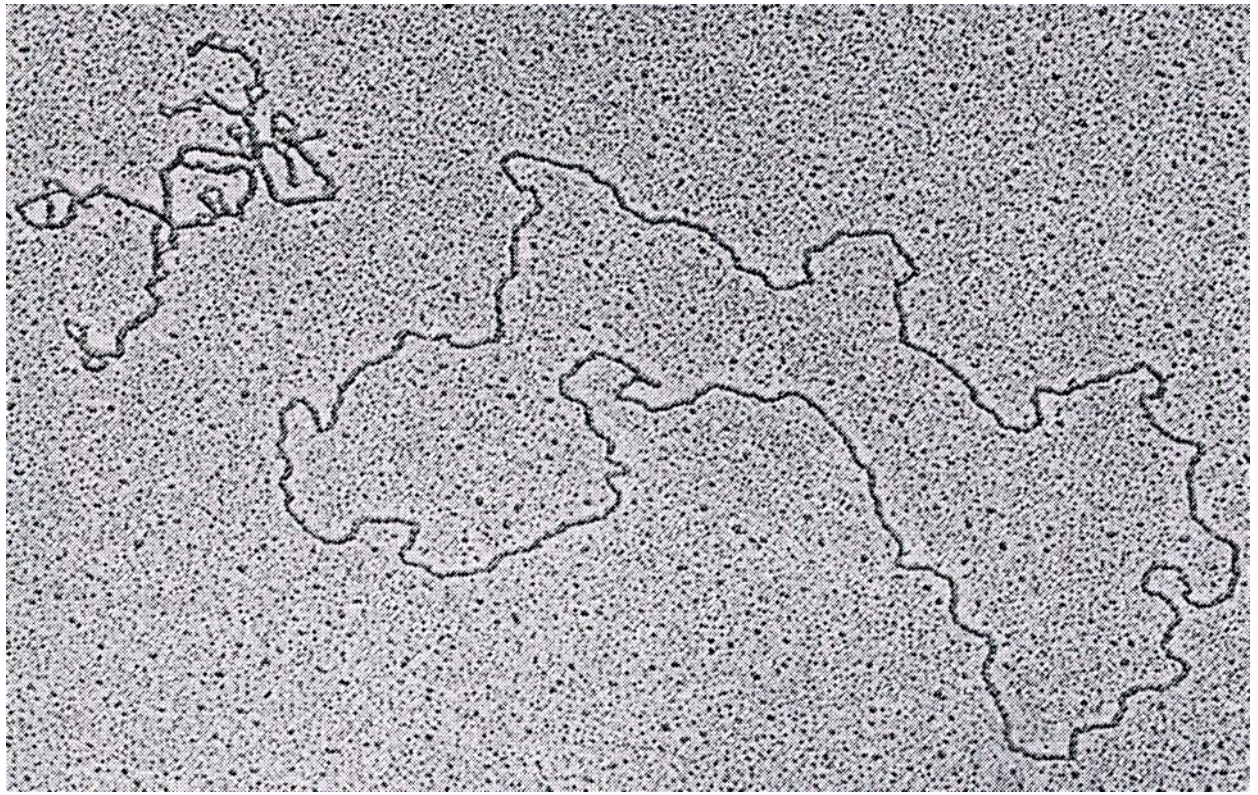


Figure 9-11
Molecular Biology: Principles and Practice, Second Edition

In most bacteria, genomes are circular, i.e. covalently closed dsDNA, organized into anchored loops

Eukaryotic genomes are linear but organized into “closed” loops with extensive supercoiling

WHAT CAUSES SUPERCOILING *in vivo?*
Chemistry, conditions and outside agents



- Physiological conditions (e.g. salt concentration)
- Proteins (e.g. topoisomerases and helicases)

DNA Supercoiling

- Supercoiling simply means the coiling of a coil. Here, the coil is DNA double helix. So supercoiling is the helix coiling upon itself.
- Supercoiled DNA is generally a manifestation of structural strain. When there are no supercoils, the DNA is referred to as **relaxed DNA**
- Supercoiling occurs in all cells (and viruses that have dsDNA genomes!)

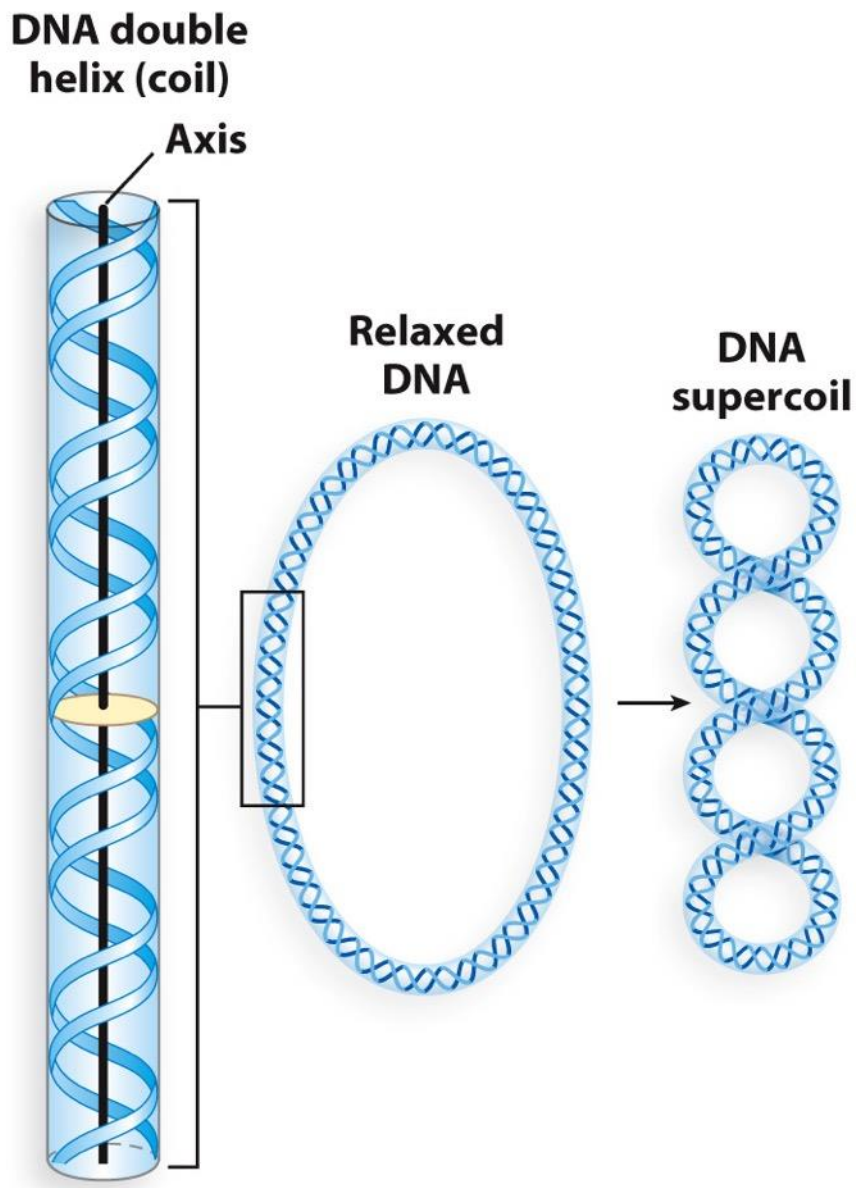


Figure 9-7
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The effects of replication and transcription on DNA supercoiling

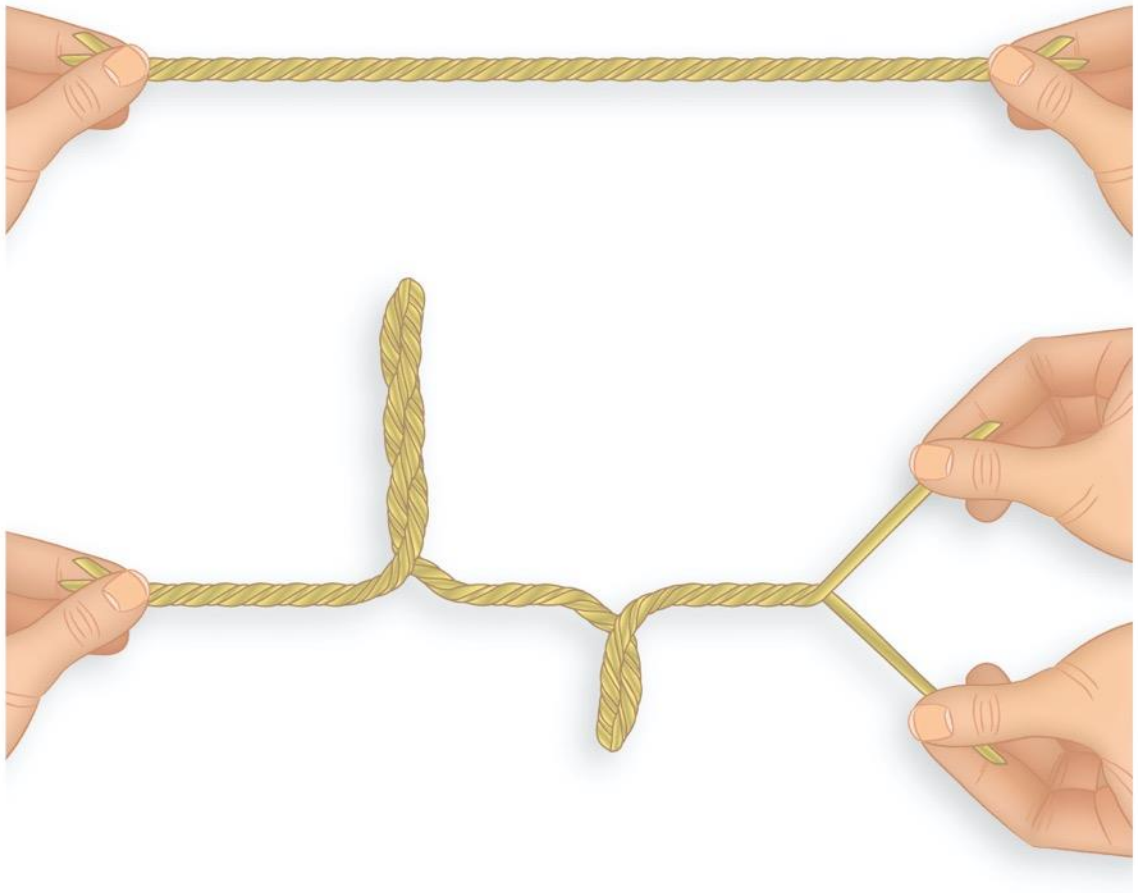

Figure 9-8a
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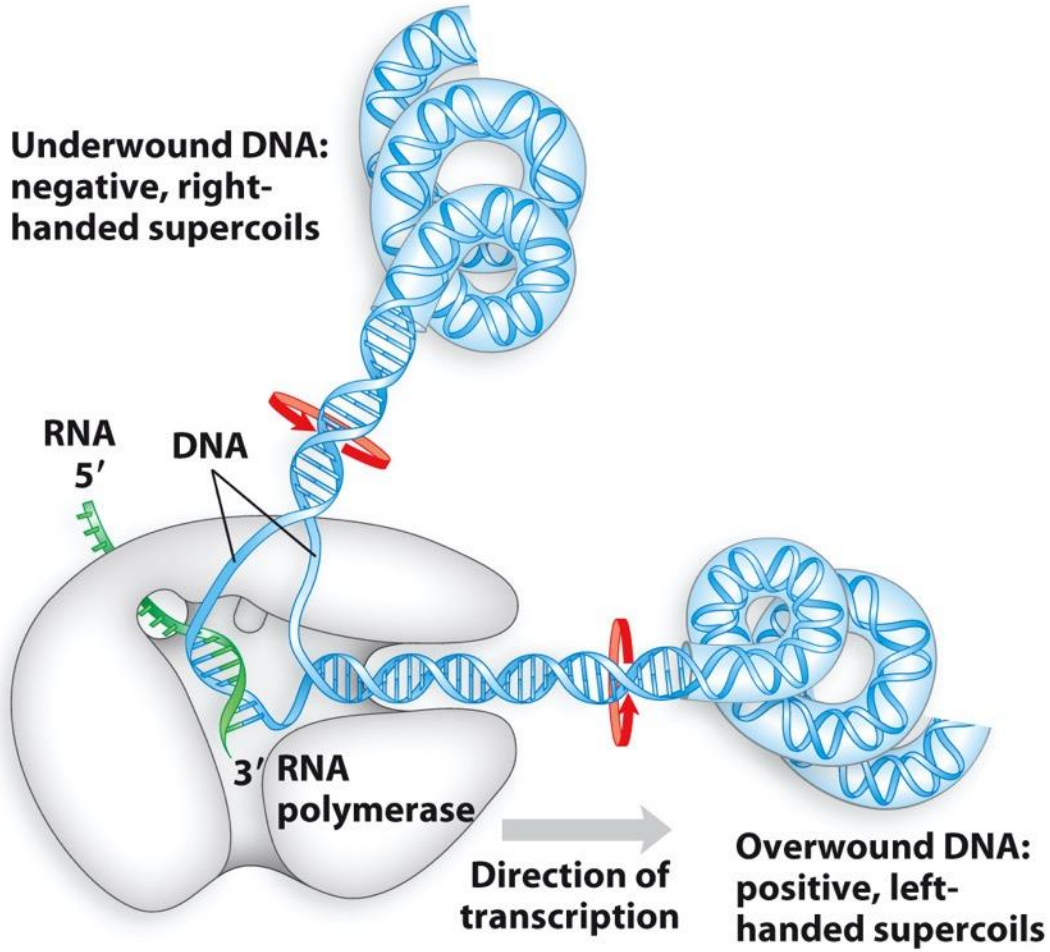

Figure 9-8b
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This will come up again later…

DNA Supercoiling is measurable!

The topology (shape) of dsDNA can be defined in terms of **LINKING NUMBER (Lk)** which is the number of times one strand would have to be passed through the other strand in order for the other strands to be entirely separated from each other

Right handed helix → Lk negative

Left handed helix → Lk positive

For closed-circular DNA, linking number is always an integer!

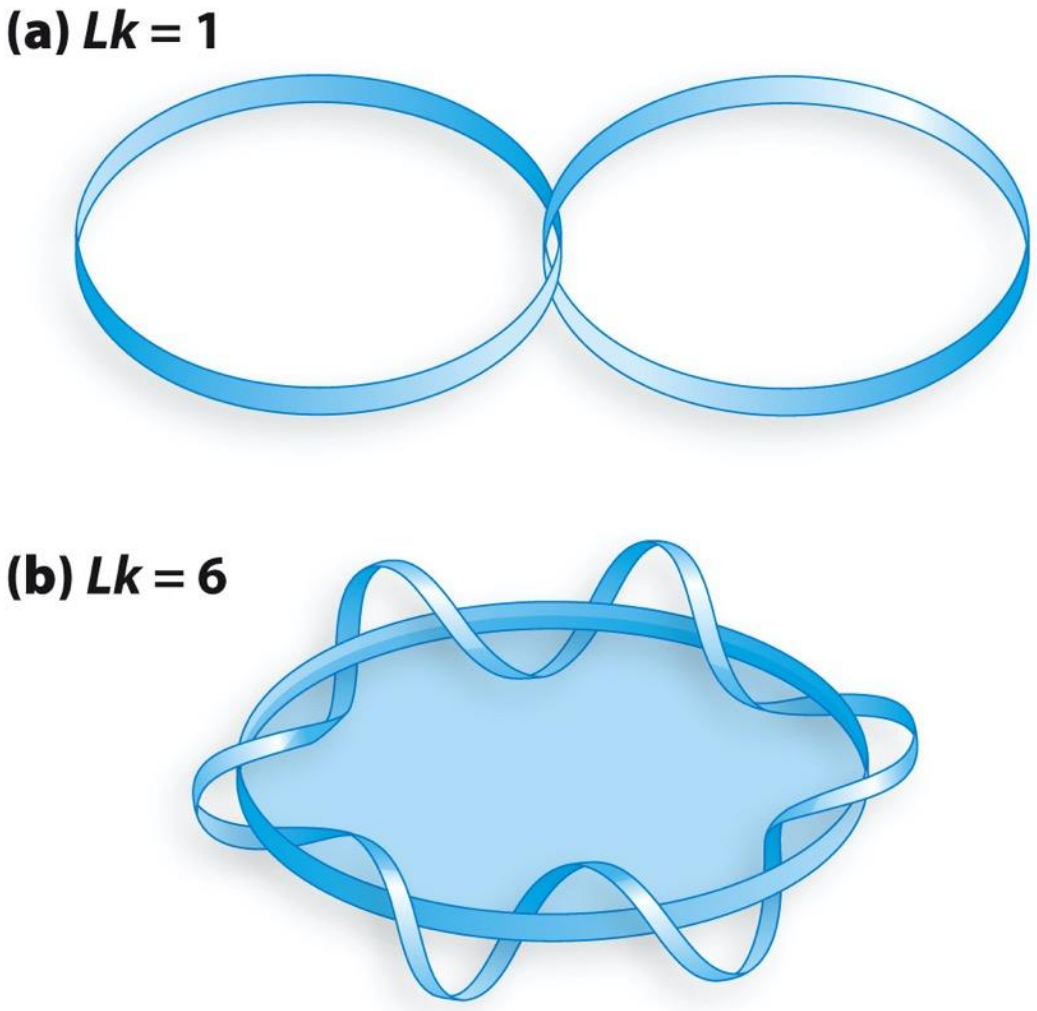

Figure 9-12
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*In this figure we are looking at the individual **strands** of DNA*

Linking number of closed-circular DNA

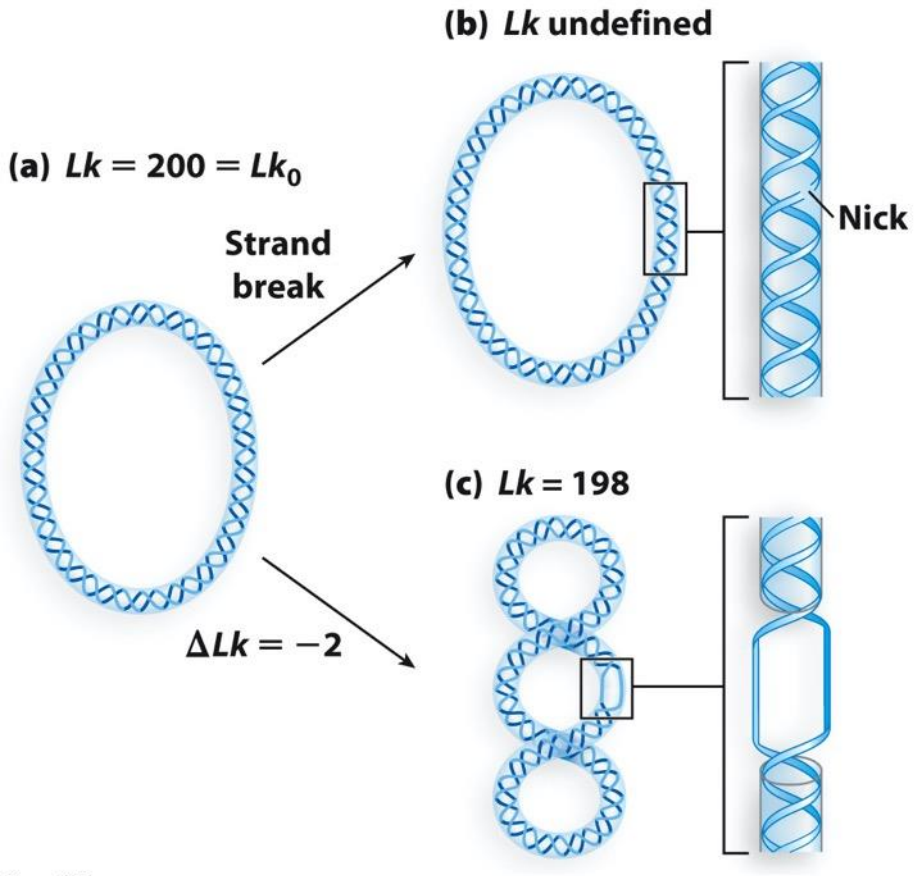

Figure 9-13
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- When ccDNA is relaxed: $\mathbf{Lk = \frac{\text{the total \#bp}}{\text{\# bp per turn}}}$

  ccDNA: 2100 bp

  10.5 bp/turn **Lk = ?**

- If there is a break in either strand, the strands can unravel and separate. In this case Lk would be undefined but would be considered relaxed

- If DNA is unwound, we can describe Lk as **ΔLk**

$$\Delta Lk = Lk - Lk_0$$

Linking Number, Lk

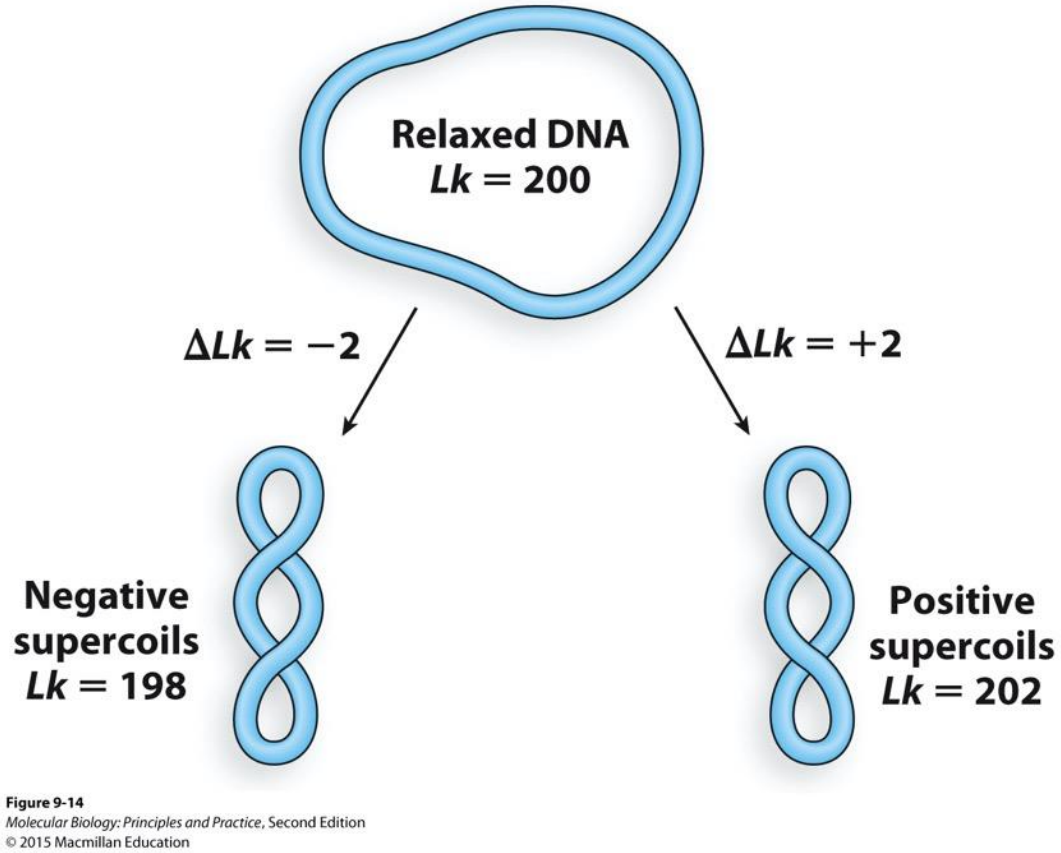

Figure 9-14
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- **Topological property of the helix**
- **LINKING NUMBER (Lk)** is the sum of two geometric components, twist (Tw) and writhe (Wr):

  Lk = Tw + Wr

- **Twist: the turns of the double helix**
- **Writhe: no. times helix turns about itself**
- **Supercoiling induced by underwinding is defined as negative supercoiling** (and vice versa for overwinding, positive)

Remember that the linking number is always an integer!

Lk cannot be changed without strand breakage

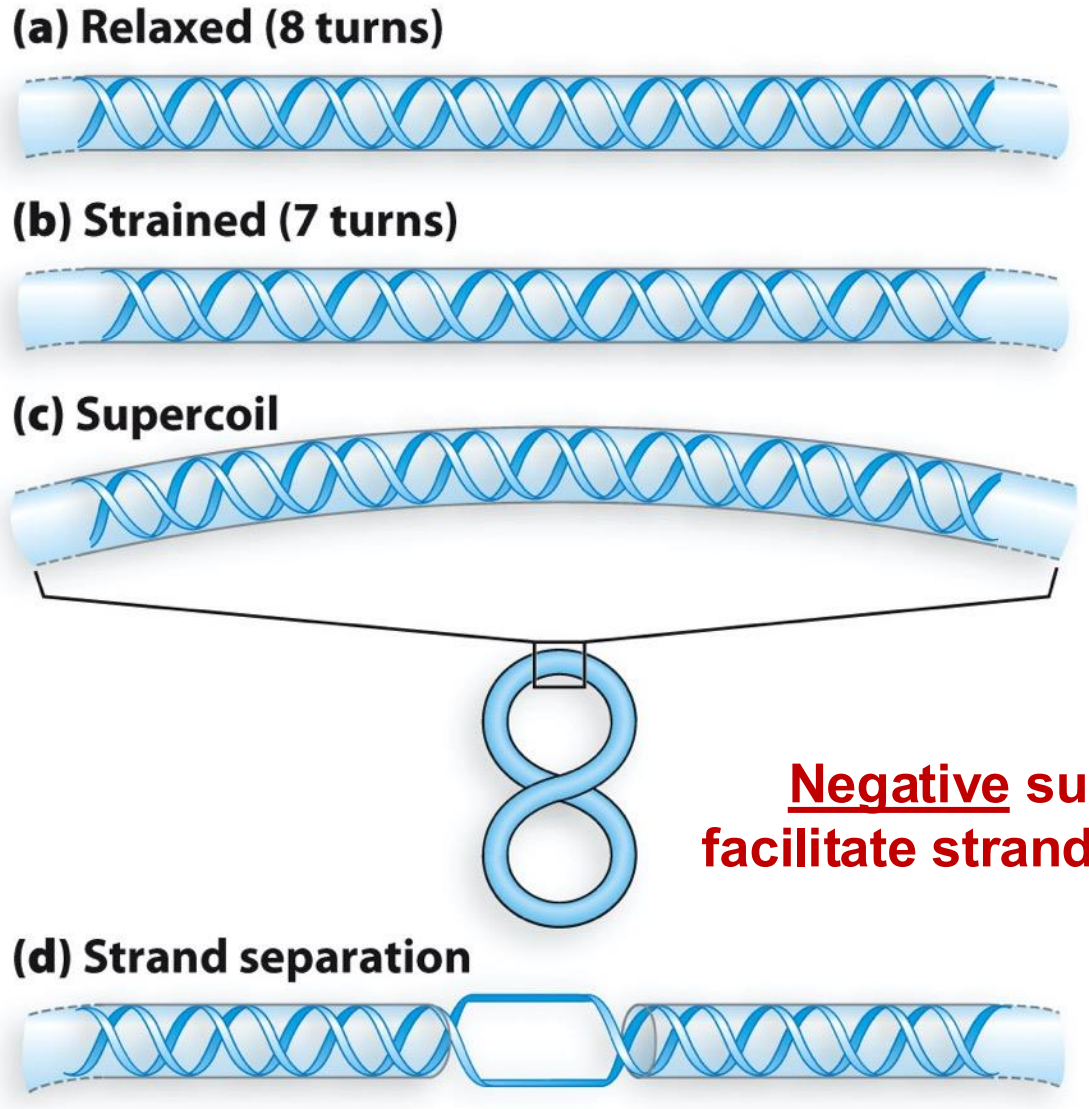
The effects of DNA underwinding

84 bp
84/10.5 = 8 turns

84/12 bp = 7 turns
(deviation from 10.5 bp, DNA is unstable, thermodynamically strained as a result

Much of this strain (the twist) in DNA is compensated for by **coiling the helix on itself, forming a supercoil** (increasing the writhe)

In principle, the strain can also be accommodated by separating the two DNA strands over a distance of about 10 bp, to restore the number of turns to 8.



Negative supercoils facilitate strand separation

Figure 9-10
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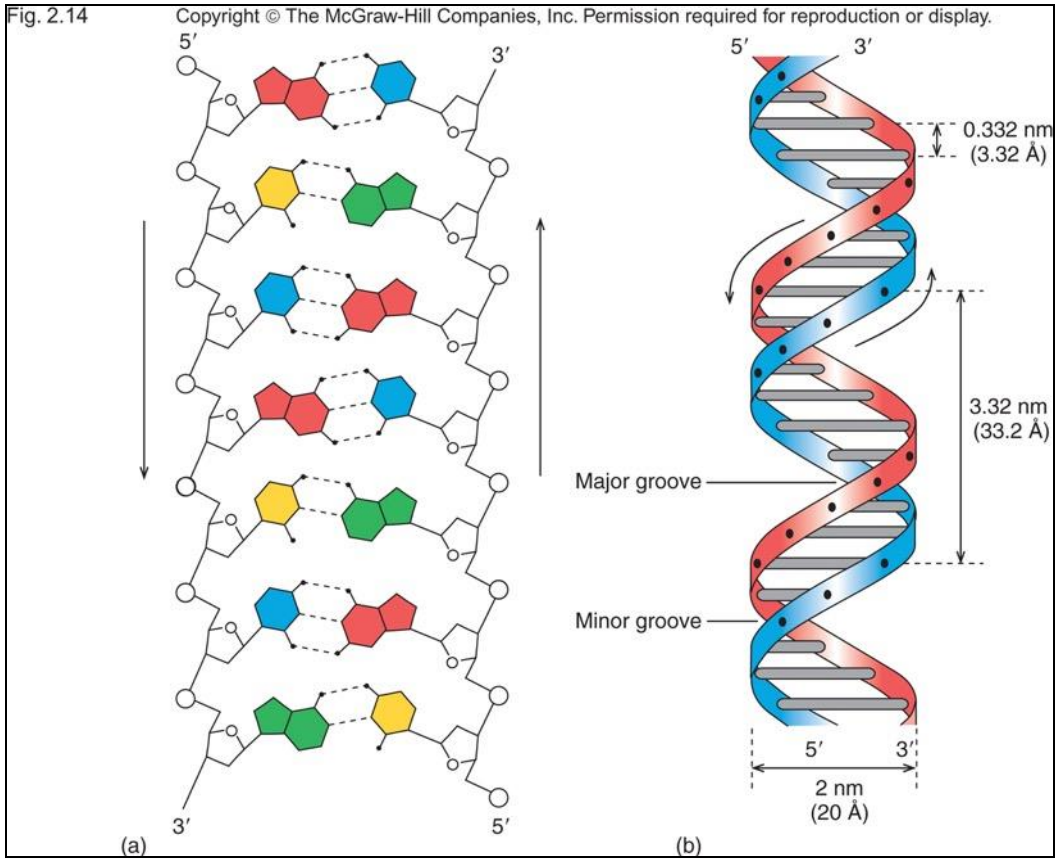
Lk can be broken down into two structural components: Twist (Tw) and Writhe (Wr)

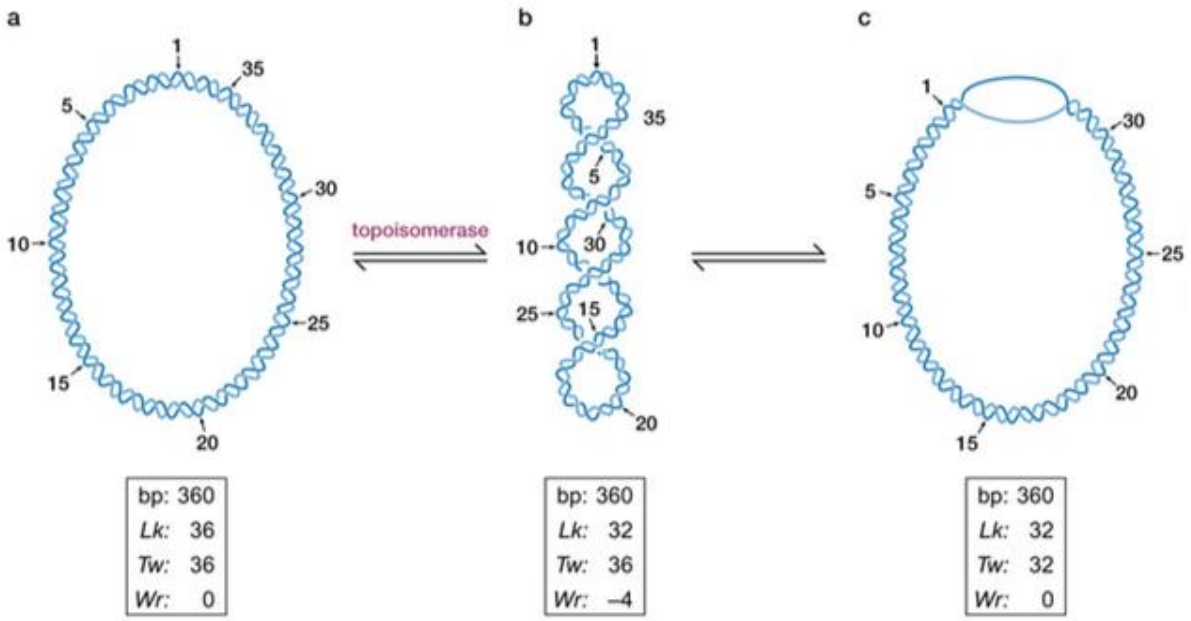


- Tw is a geometrical property of the helix
  - *Describes the turns of the double helix*
- **Tw** does not need to be an integer because, like **Wr**, it is a geometric and not a topological property of DNA double helix
- **Tw** may be changed by deformation of a ccDNA molecule
- **Tw** and **Wr** change in a reciprocal manner*, without altering Lk

Lk = Tw + Wr

*If Lk remains the same, **Tw** will go up as **Wr** goes down

Writhe, Wr: the measure of the degree of supercoiling

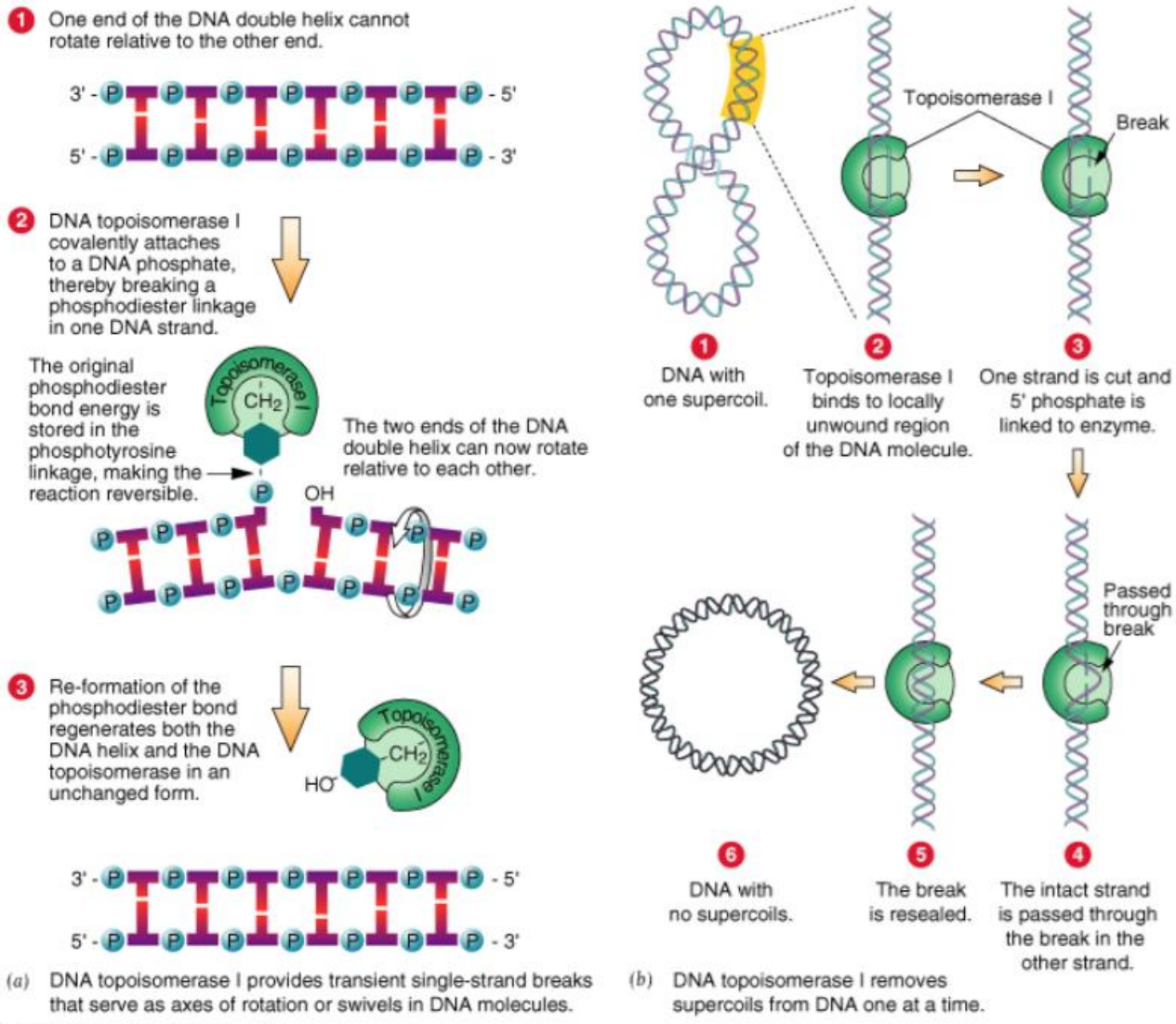


The Fig. above shows conversions of the relaxed DNA in (a) to the negative supercoiled DNA form in (b).

Note that the strain of local disruption of base pairing in a double helix as in (c), may be taken up by supercoiling as in (b) to restore Tw to 36. But **the Lk does not change in these two molecules (b and c). It remains the same.**

- **A measure of the coiling of the double helix**
  - *The number of times double helix crosses itself*
- **Wr** for relaxed dsDNA is 0
- Like **Tw**, **Wr** does not need to be an integer
- Underwinding results in **–ΔLk** (negative supercoiling)
  - easier to separate the strands of the double helix
- Overwinding results in **+ΔLk** (positive supercoiling)
  - more difficult to separate the strands of the double helix

TOPOISOMERASES



The only way to change Lk for a constrained dsDNA molecule is to cut, twist, and rejoin the ends.

Remember that Lk is a topological property of constrained dsDNA - it cannot be changed unless one of the strands is broken.

This can be accomplished in the cell (*in vivo*) by means of enzymes called topoisomerases.

These enzymes play an especially important role in processes such as replication and DNA packaging.

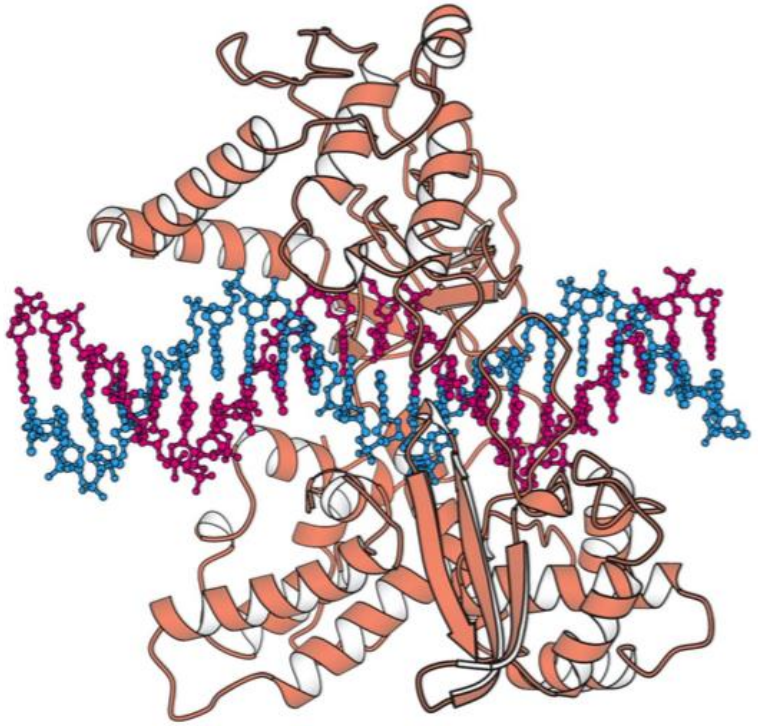
Topoisomerases are enzymes that catalyze the interconversion of topoisomers

i.e., they change the superhelicity!

Topoisomerases **change the linking number** of DNA by catalyzing a three-step process:

1) the cleavage of one or both strands of DNA
2) the passage of a segment of DNA through this break
3) resealing of the DNA breaks

Topoisomerases help relieve torsional stress induced by unwinding.



Structure of a complex between a fragment of human topoisomerase I and DNA

(From Berg, Tymokzko & Stryer, Biochemistry, 5th Ed.)

There are two types of topoisomerses

Topo I

- nicks **one strand** of DNA - the other strand then passes through the ss break, and the broken strand re-seals
- thermodynamically favorable process , driven by release of energy of supercoiling
- **Changes the linking number by increments of 1**
- *E. coli* type I topoisomerases generally relax DNA by removing negative supercoils (**increasing Lk**)

Topo II

- breaks **both strands, pass another duplex through the break,** and then re-seals both strands
- can create high energy supercoils using ATP hydrolysis
- **Changes the linking number by increments of 2**
- *E. coli* type II topoisomerase (known as DNA gyrase) **introduces negative supercoils** (**decrease Lk**)

The degree of supercoiling of DNA is maintained by regulation of the net activity of topoisomerase types I and II

Remember that the linking number is always an integer!

TABLE 9-4

Topoisomerases in Bacteria and Eukaryotes

Topoisomerase	*Class*	*Function*
Bacteria		
Topoisomerase I	Type I	Relaxes negative supercoils
Topoisomerase II (DNA gyrase)	Type II	Introduces negative supercoils
Topoisomerase III	Type I	Has specialized functions in DNA repair and replication
Topoisomerase IV	Type II	Unlinks replicated chromosomes
Eukaryotes		
Topoisomerase I	Type I	Relaxes negative supercoils, especially during DNA replication
Topoisomerase IIα	Type II	Relaxes positive or negative supercoils; functions in chromatin condensation, replication, transcription
Topoisomerase IIβ	Type II	Relaxes positive or negative supercoils; functions in chromatin condensation, replication, transcription
Topoisomerase III	Type I	Has specialized functions in DNA repair and replication

Table 9-4
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In your textbook: Highlight 9-2 Curing disease by inhibiting topoisomerases

Without topoisomerases cells cannot replicate or package their DNA, or express their genes – and they die. Inhibitors of topoisomerases have therefore become important pharmaceutical agents, targeted at infectious organisms and malignant (cancer) cells.

Antibiotics and Chemotherapy

Highlight 9-2 Curing disease by inhibiting topoisomerases

Antitumour drugs impede DNA uncoiling by topoisomerase I

Daniel A. Koster[1], Komaraiah Palle[2], Elisa S. M. Bot[1], Mary-Ann Bjornsti[2] & Nynke H. Dekker[1]

Increasing the ability of chemotherapeutic drugs to kill cancer cells is often hampered by a limited understanding of their mechanism of action. Camptothecins, such as topotecan, induce cell death by poisoning DNA topoisomerase I, an enzyme capable of removing DNA supercoils[1–4]. Topotecan is thought to stabilize a covalent topoisomerase–DNA complex[5–7], rendering it an obstacle to DNA replication forks[2,3,8,9]. Here we use single-molecule nanomanipulation to monitor the dynamics of human topoisomerase I in the presence of topotecan. This allowed us to detect the binding and unbinding of an individual topotecan molecule in real time and to quantify the drug-induced trapping of topoisomerase on DNA. Unexpectedly, our findings also show that topotecan significantly hinders topoisomerase-mediated DNA uncoiling, with a more pronounced effect on the removal of positive (overwound) versus negative supercoils. *In vivo* experiments in the budding yeast verified the resulting prediction that positive supercoils would accumulate during transcription and replication as a consequence of camptothecin poisoning of topoisomerase I. Positive supercoils, however, were not induced by drug treatment of cells expressing a catalytically active, camptothecin-resistant topoisomerase I mutant. This combination of single-molecule and *in vivo* data suggests a cytotoxic mechanism for camptothecins, in which the accumulation of positive supercoils ahead of the replication machinery induces potentially lethal DNA lesions.

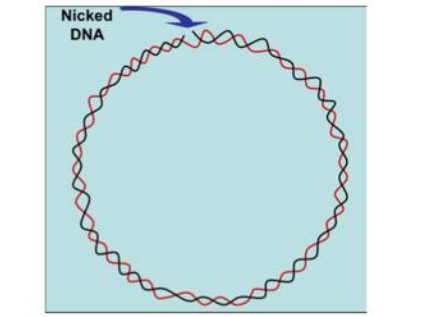
DNA topoisomerases resolve topological problems by means of transient DNA strand breakage and religation[1,4,10]. However, drug-stabilized topoisomerase–DNA complexes may also induce potentially lethal DNA damage. Indeed, eukaryotic DNA topoisomerase IB (TopIB) is the cellular target of the camptothecin class of chemotherapeutics[2,3,11]. The camptothecin analogues topotecan and irinotecan have significant activity against adult and paediatric solid tumours, and have gained US Food and Drug Administration approval for the treatment of ovarian and small-cell lung cancer[12–14]. A detailed understanding of topoisomerase–drug interactions is critical for optimal clinical development of these chemotherapeutics. However, the dynamic interactions underlying this poisoning and their biological ramifications remain largely unknown.

TopIB removes DNA supercoils by first forming a clamp around duplex DNA[15,16]. The active-site tyrosine acts as a nucleophile to cleave a single DNA strand, forming a transient DNA–(3′-phosphotyrosyl)-enzyme 'covalent complex' and a free 5′-OH DNA end. Torsional energy within the DNA then drives uncoiling about the intact DNA strand. Following the removal of a random number of supercoils, a ligation reaction restores the DNA backbone[17,18]. Topotecan intercalates into the nick generated by TopIB, thereby preventing religation and trapping TopIB on the DNA[5–7,19]. During S phase, these reversible ternary topotecan–TopIB–DNA complexes are converted into cytotoxic DNA lesions that cause cell death[2,3,8,9]. Because DNA replication is required for topotecan-induced cell lethality[8,9], it has been proposed

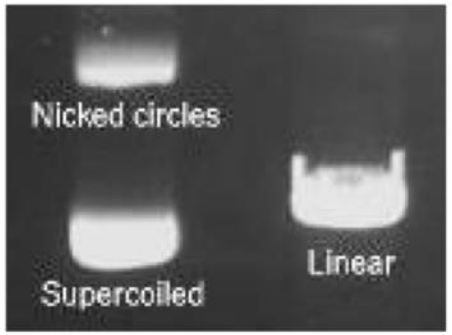
Source: *Nature*, Vol 448, 12 July 2007

How to study DNA topoisomers?
Gel electrophoresis

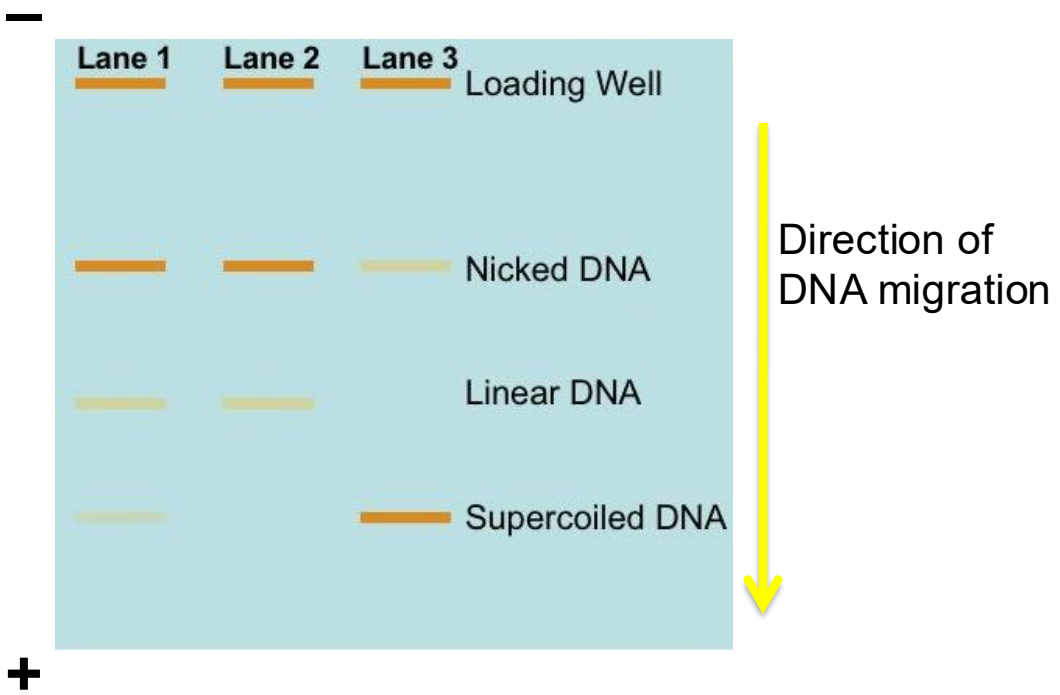
DNA **shape** also affects the migration distance in gels. ***Why?***



DNA supercoil



Agarose gel



BIOCHEMICAL ANALYSIS OF SUPERCOILING

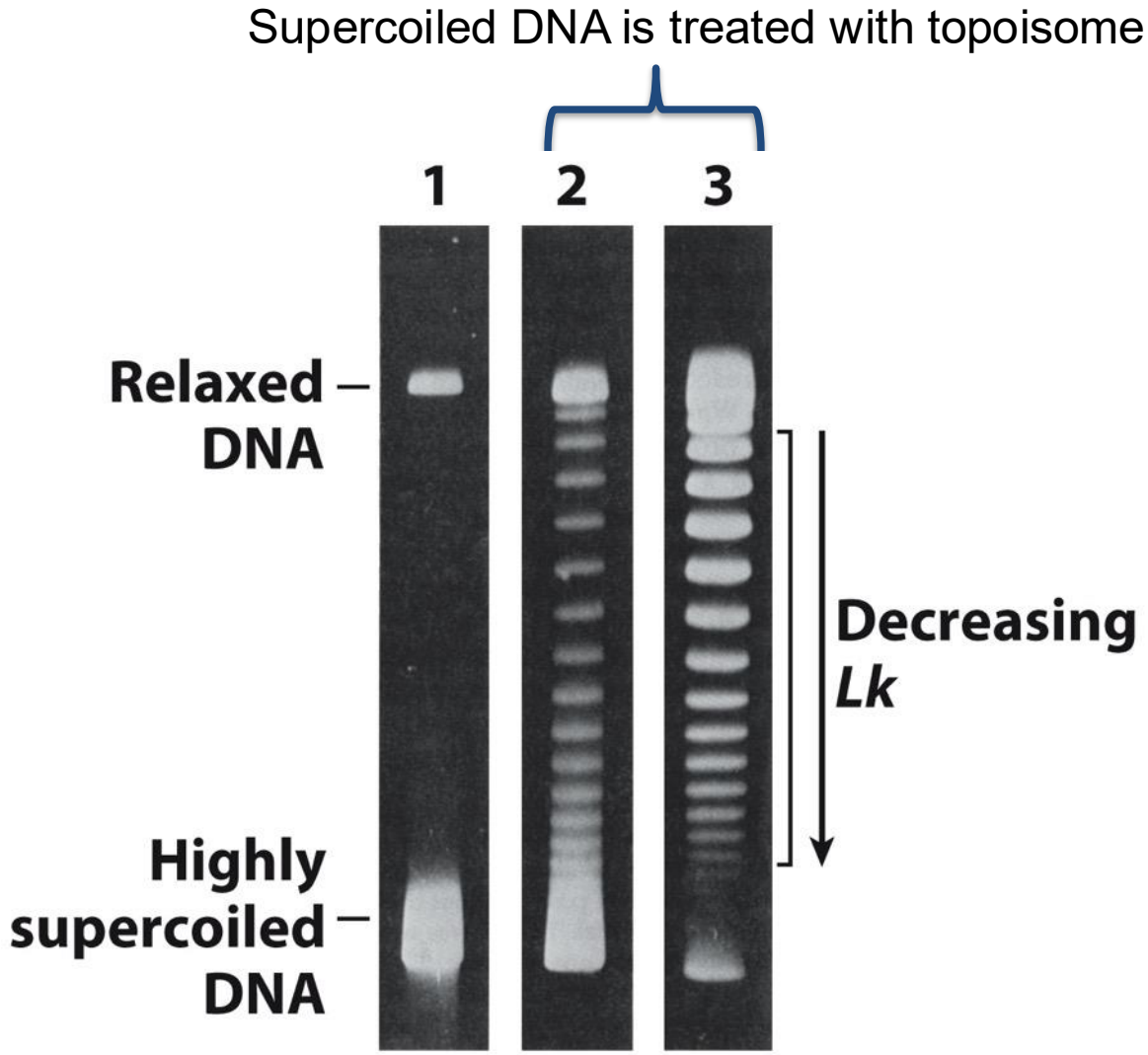
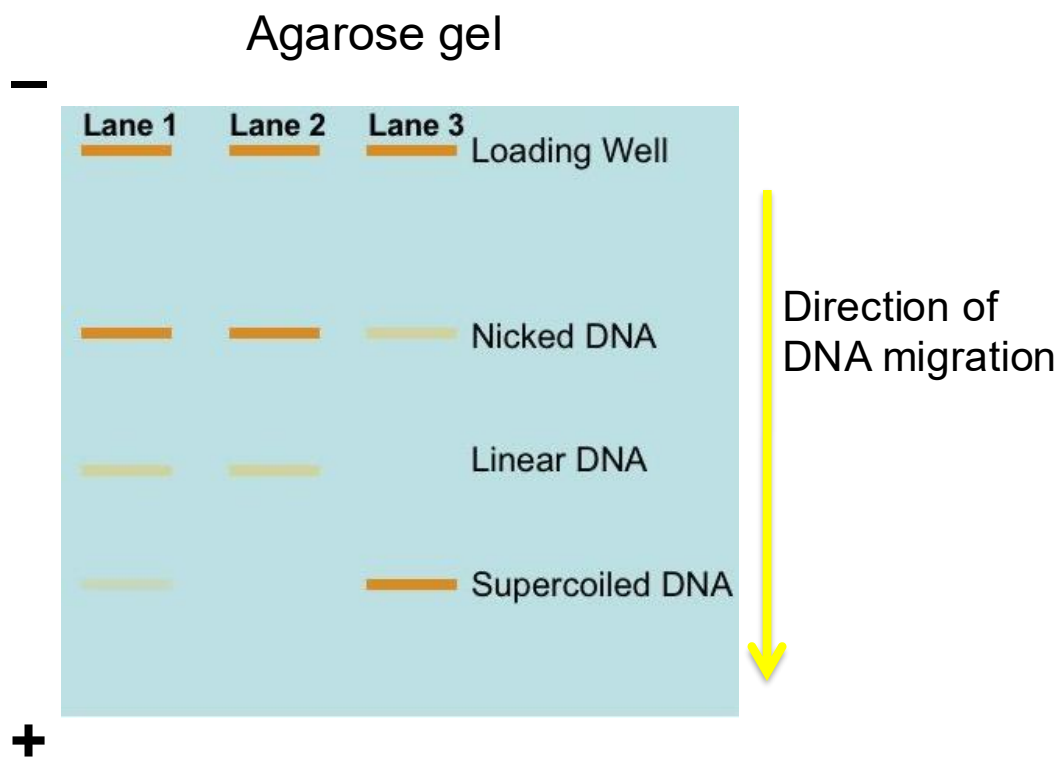


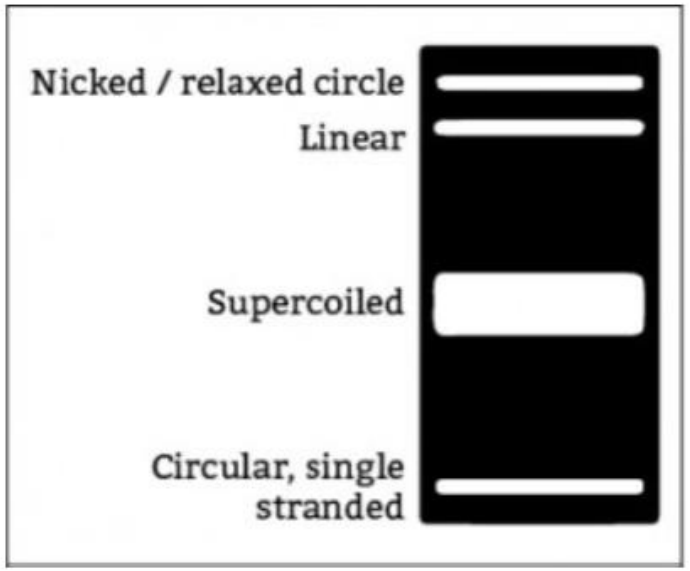
Figure 9-18
Molecular Biology: Principles and Practice, Second Edition
W. Keller, *Proc. Natl. Acad. Sci. USA* 72:2553, 1975. Courtesy Michael M. Cox.

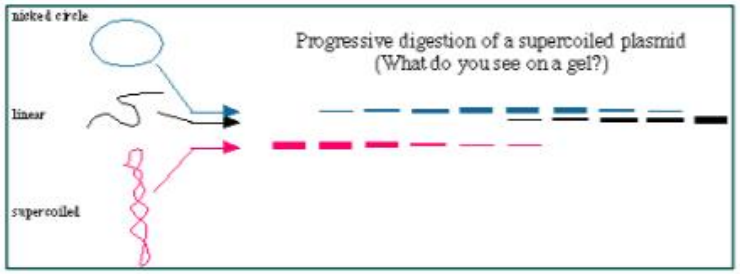
- Supercoiling can be assayed by agarose gel electrophoresis
- Addition of Topo I relaxes supercoils one at a time (~25 bands visible)

Supercoiling markedly changes the overall shape of DNA. A supercoiled DNA molecule is more compact than a relaxed DNA molecule of the same length. Hence, supercoiled DNA moves faster than relaxed DNA when analyzed by centrifugation or gel electrophoresis.

Relaxed DNA can lie flat on a planar surface. Wr = 0. Lk = Tw.
Since the helix crosses itself in a supercoiled molecule, it cannot lie flat on a planar surface. Supercoiling involves a higher order folding of DNA double helix; it is therefore sometimes called the **tertiary structure of DNA**.







Genome packing in viruses: example

What do you notice?

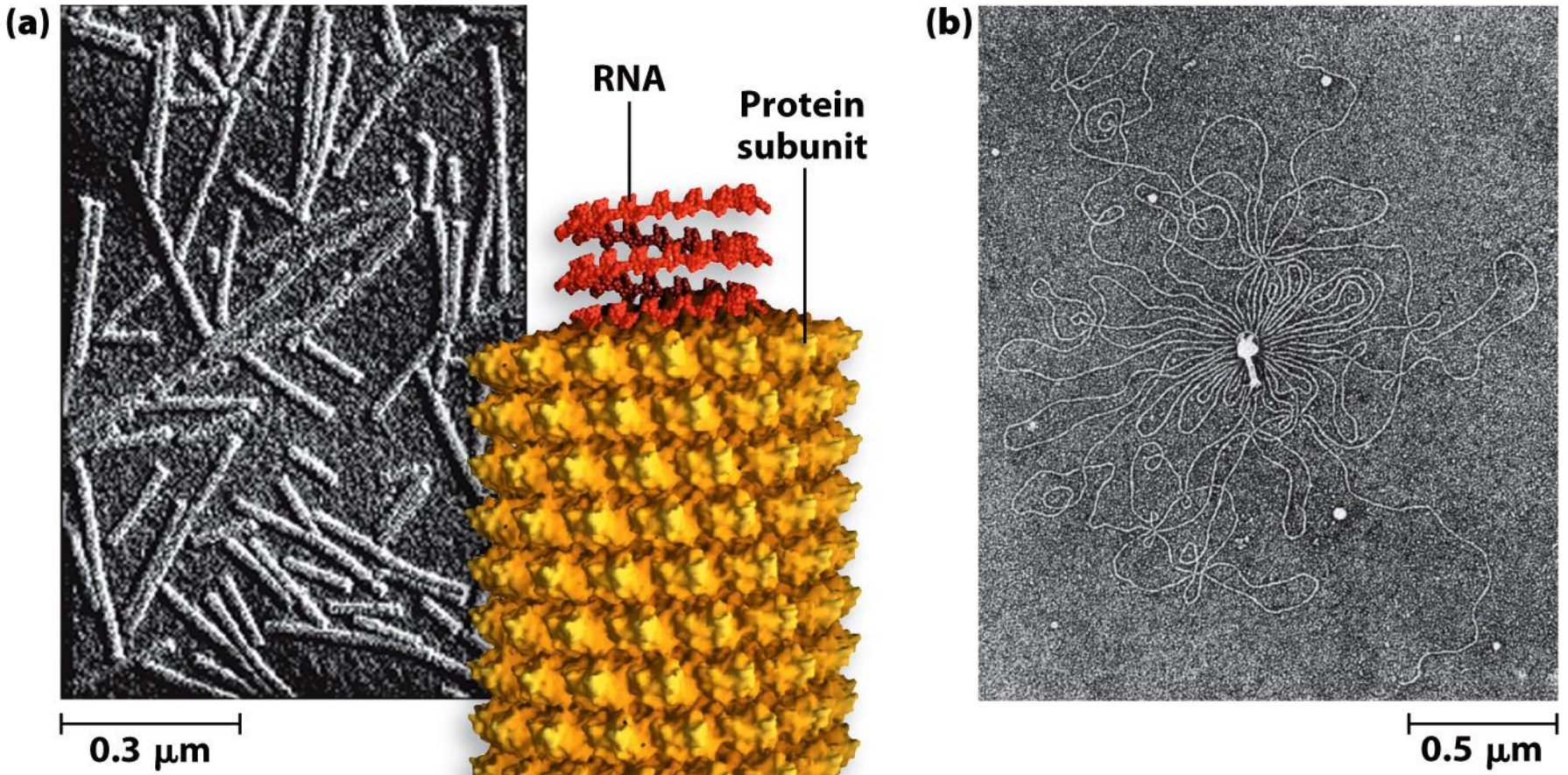


Figure 9-2
Molecular Biology: Principles and Practice, Second Edition
(a) Science Source. (b) Reprinted from Kleinschmidt, A.K., Land, D., Jackerts, D., & Zahn, R.K. (1962) *Biochem. Biophys. Acta* 61, pp. 857–864, with permission from Elsevier.