Lecture 23 (11/9/20)

- Reading: Ch1; 29-34
  Ch8; 295-299
  Ch9; 319-325, 346

- Problems:
  Ch8 (text); 6,7,8,10
  Ch8 (study-guide: applying); 1,3
  Ch8 (study-guide: facts); 10,11
  Ch9 (text); 1,2,3,4
  Ch9 (study-guide: facts); 1,2,3,4,5
  Ch24 (study-guide: facts); 3,5,6
  Ch26 (text); 3
  Ch26 (study-guide: applying); 2,3
  Ch26 (study-guide: facts); 7
  Ch27 (text); 1,2,3,4

NEXT

- Reading: Ch9; 328-332
  Ch25; 990-995, 1005-1012

- Problems: Ch9 (study-guide: applying); 1,2
  Ch9 (study-guide: facts); 7,8
  Ch25 (text); 1-3,5-7,9,10,13-15
  Ch25 (study-guide: applying); 1,4
  Ch25 (study-guide: facts); 3,4,6

Nucleic Acids

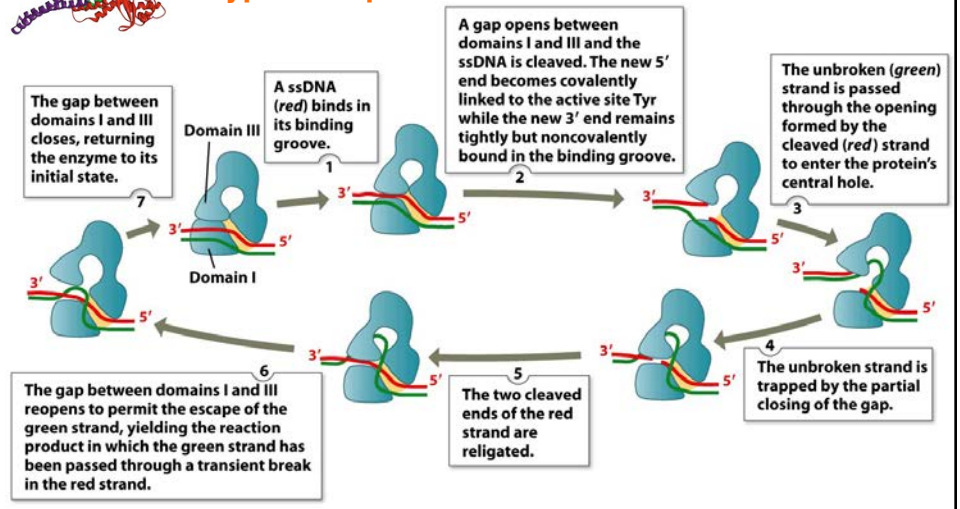
A. The 4 S's
   1. Size
   2. Solubility
   3. Shape
      a. A-DNA
      b. Z-DNA
      c. Topology
         i. Packaging
         ii. Supercoiling
         iii. Topoisomerases
   4. Stability
      a. Nucleotides
         i. Tautomers
         ii. Acid/base
      b. Nucleic Acids
         i. Chemistry
         ii. Denaturation
         iii. Stability
         iv. Nucleases

B. Structure of the Information
   1. Exceptions to flow
   2. Structure
   3. Levels of Control

C. Recombinant DNA: Biochemical Basis of Biotechnology
   1. Restriction enzymes, DNA ligase
   2. Vectors and Inserts to make recombinant DNA (rDNA)
   3. Transformation of hosts
   4. Selection of transformants
   5. Expression
   6. Site-directed mutagenesis

Topoisomerase

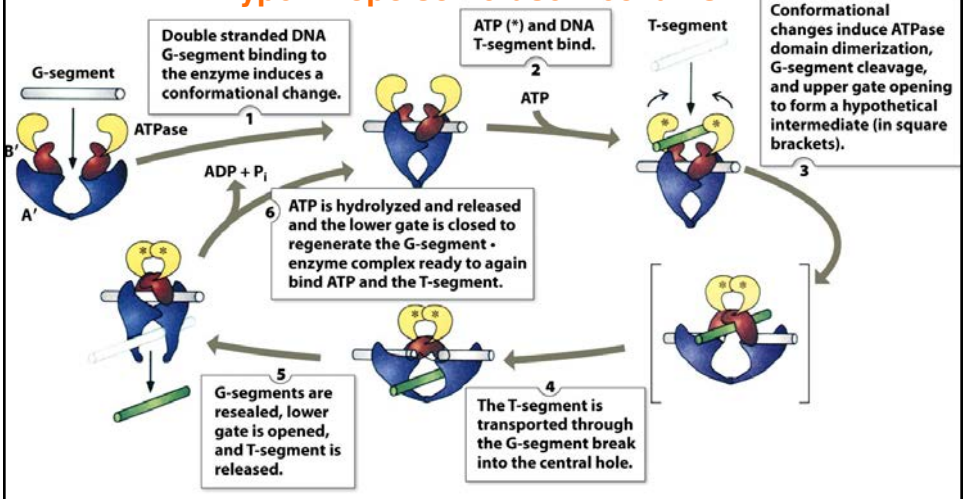
Type IA Topoisomerase Mechanism



How are negative supercoils generated in the first place?

Topoisomerase

Type II Topoisomerase Mechanism

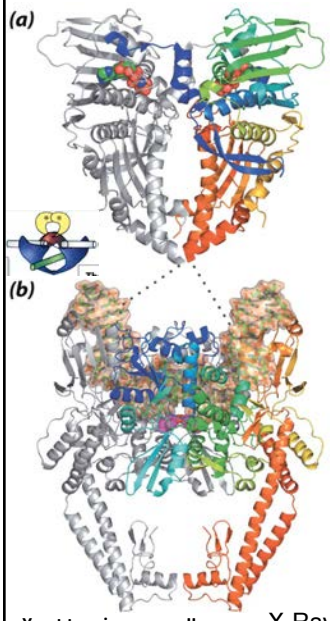


Type II Topoisomerase also called "gyrase"

- 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 ($\Delta L = -2$)

Topoisomerase

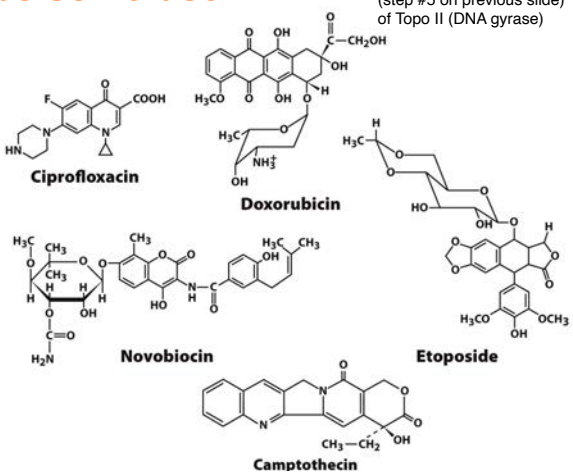
Topoisomerase II



Yeast topoisomerase II
PDBids 1PVG and 2RGR

X-Ray structures of *yeast* topoisomerase II.

Related to Daunorubicin, which inhibits re-joining in (step #5 on previous slide) of Topo II (DNA gyrase)



Topoisomerase Inhibitors as Antibiotics/Anticancer Chemotherapeutics

The 4 S's

Size

Solubility

Shape

Stability

Tautomeric Forms of Bases

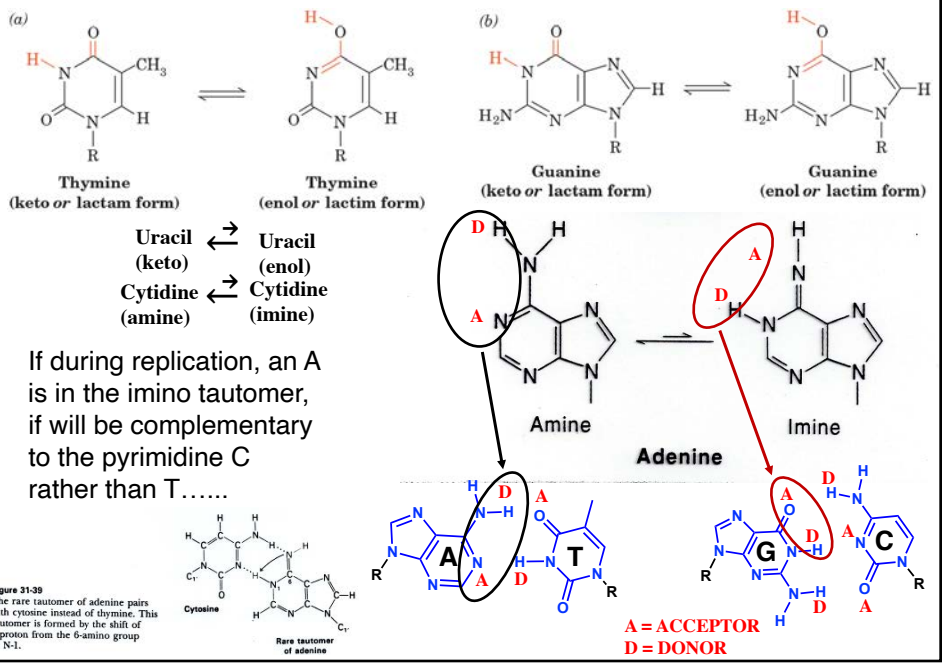
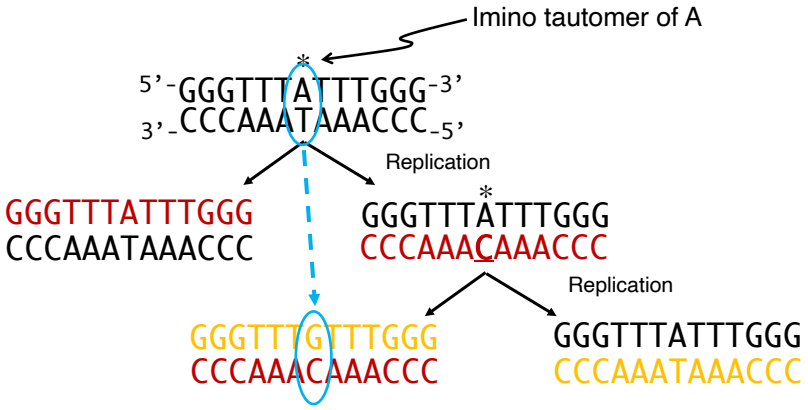


Figure 31-39
The rare tautomer of adenine pairs with cytosine instead of thymine. This tautomer is formed by the shift of a proton from the 6-amino group to N-1.

Tautomeric Forms of Bases

Mutagenesis



Tautomeric Forms of Bases

Modified bases can stabilize the rare tautomer

enol tautomer of U

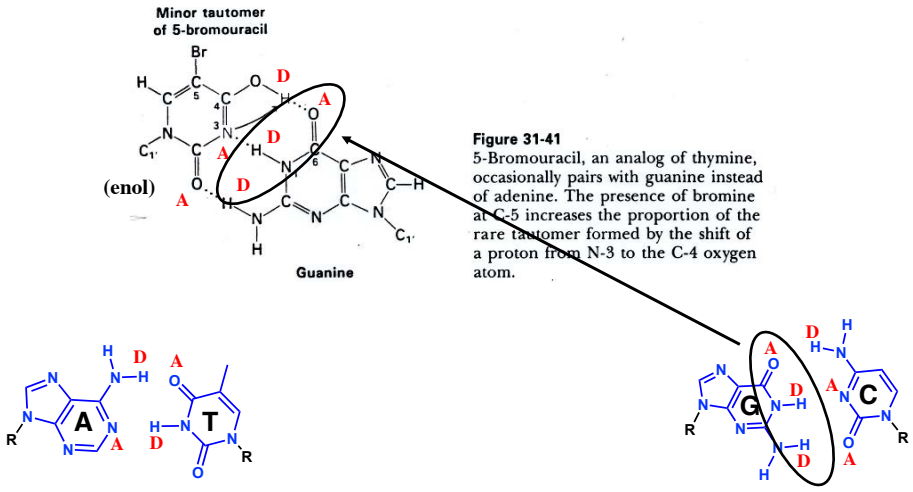


Figure 31-41
5-Bromouracil, an analog of thymine, occasionally pairs with guanine instead of adenine. The presence of bromine at C-5 increases the proportion of the rare tautomer formed by the shift of a proton from N-3 to the C-4 oxygen atom.

Acid/base Properties of Bases

At pH 7, all bases are neutral. They can fit into middle of B-DNA without repulsions
But, acidic or basic treatment will protonate or deprotonate these bases.

At low pH, N7 of guanine, N1 of adenine, and N3 of cytosine become protonated.

At high pH, N1 of Guanine and N3 of Uracil (Thymine) become deprotonated.

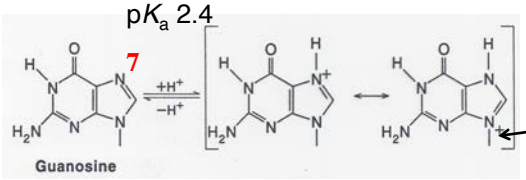
Table 26.2
Ionization Constants of the Ribonucleotides (Presented as p*K* Values)

	Base	Secondary Phosphate	Primary Phosphate
Adenosine-5′-phosphate (AMP)	3.8	6.1	0.9
Uridine-5′-phosphate (UMP)	9.4	6.4	1.0
Cytidine-5′-phosphate (CMP)	4.5	6.3	0.8
Guanosine-5′-phosphate (GMP)	2.4, 9.4	6.1	0.7

Acid/base Properties of Bases

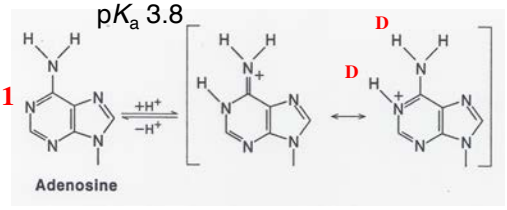
Charged bases can de-stabilize the glycosidic bond and mutagenesis

pK_a 2.4



Destabilizes glycosidic bond, leads to "AP-site" *

pK_a 3.8



Leads to A → G mutations

* "AP-site" refers to a-purinic or a-pyridinic site

Other Chemical Changes of Bases

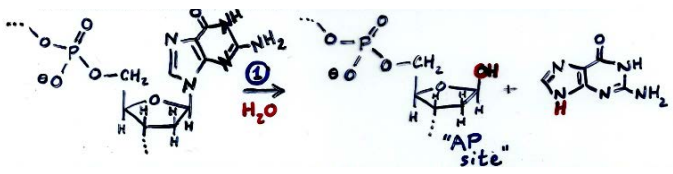
DNA Damage:

- oxidation
- alkylation
- degradation

Other Chemical Changes of Bases

Table I: *Processes which chemically degrade DNA*

Process	Events/cell/day
1. Spontaneous hydrolysis of glycosidic bonds [G ~ A ≈ 20X C,T]	2-10 x 10^3
2. Deamination of cytosine (and 5-MeC) [C ~ 5-MeC ≈ 50X A ≫ G]	100-500
3. Oxidation of guanine to 8-hydroxyguanine	100-500
4. Formation of 3-methyladenine	600
5. Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]	variable



Other Chemical Changes of Bases

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3. Oxidation of guanine to 8-hydroxyguanine	100-500
4. Formation of 3-methyladenine	600
5. Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]	variable

Deamination of C yields U, which leads to C → T mutations

Other Chemical Changes of Bases

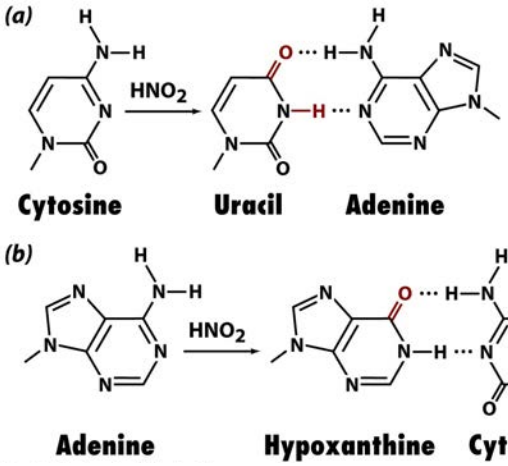
Deamination

	Frequency
Cytosine → Uracil	50
5-Methylcytosine → Thymine	50
Adenine → Hypoxanthine	2
Guanine → Xanthine	1

Figure 8-29a

Other Chemical Changes of Bases

Point-mutation by Oxidation: Nitrous Acid



Deamination of C yields U, which leads to C → T mutations

Deamination of A yields Hyp, which leads to A → G mutations

Other Chemical Changes of Bases

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	Process	Events/cell/day
1.	Spontaneous hydrolysis of glycosidic bonds [G ~ A ≈ 20X C,T]	2-10 x 10^3
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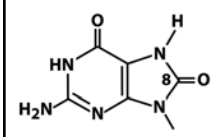
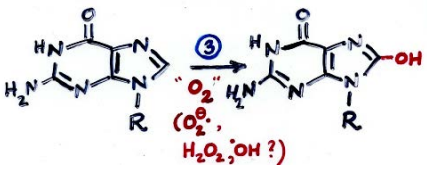
3.	Oxidation of guanine to 8-hydroxyguanine	100-500
4.	Formation of 3-methyladenine	600
5.	Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]	variable



8-Oxoguanine (oxoG)



8-Oxoguanine favors the *syn* conformation, which leads to O^8-G:A bp (G → T mutations)

Other Chemical Changes of Bases

Table I: *Processes which chemically degrade DNA*

Process	Events/cell/day
1. Spontaneous hydrolysis of glycosidic bonds [G ~ A ≈ 20X C,T]	2-10 x 10^3
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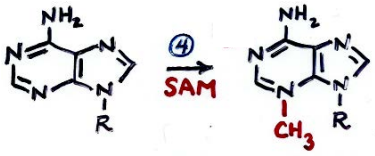
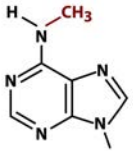
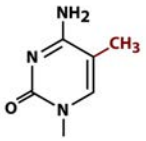
3. Oxidation of guanine to 8-hydroxyguanine	100-500
4. Formation of 3-methyladenine and other bases	600
5. Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]	variable



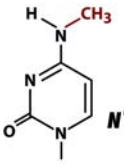
Other Chemical Changes of Bases



N^6-Methyladenine (m^6A) residue

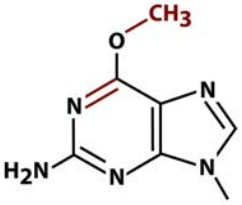


5-Methylcytosine (m^5C) residue



N^4-Methylcytosine (m^4C) residue

DNA Methylation



O^6-Methylguanine residue

Some methylations are damaging.

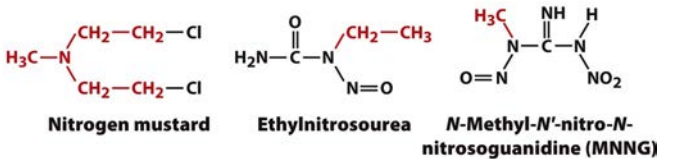
- Methylation of G at O6 can base-pair with C or T, which causes G → A mutations

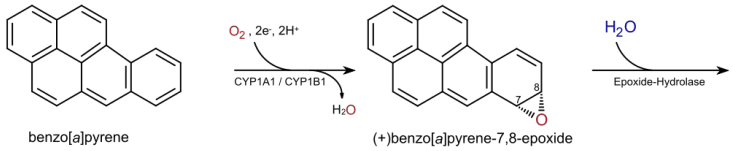
Some methylations are important.

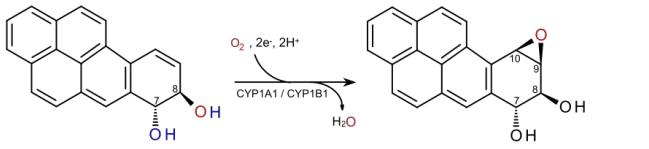
- Methylation for endonuclease restriction recognition or protect DNA from digestion by endonuclease.
- Methyl group lie in the major groove and can be used in the interaction with DNA interaction proteins.
- Importance of DNA methylation in replication: it is used to differentiate between the new and old strand. If there is a mutation, the repairing system will use the methylated strand as the template.
- In mammalian system, the promoter region has regular CpG content. Methylation at these sites can switch off eukaryotic gene expression.

Other Chemical Changes of Bases

Alkylating Agents







Other Chemical Changes of Bases

Table I: *Processes which chemically degrade DNA*

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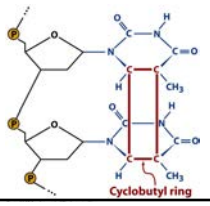
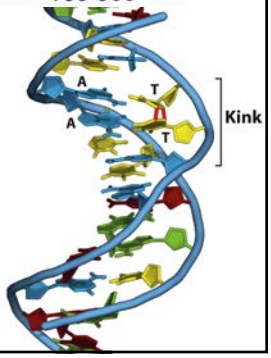
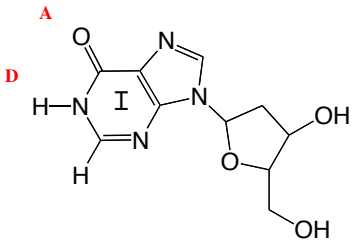
3. Oxidation of guanine to 8-hydroxyguanine	100-500
4. Formation of 3-methyladenine	
5. Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]	

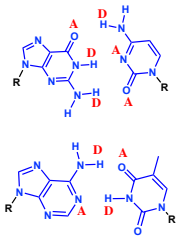


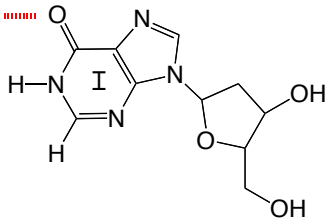


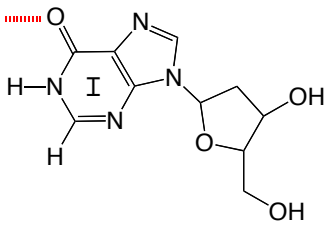
Problem

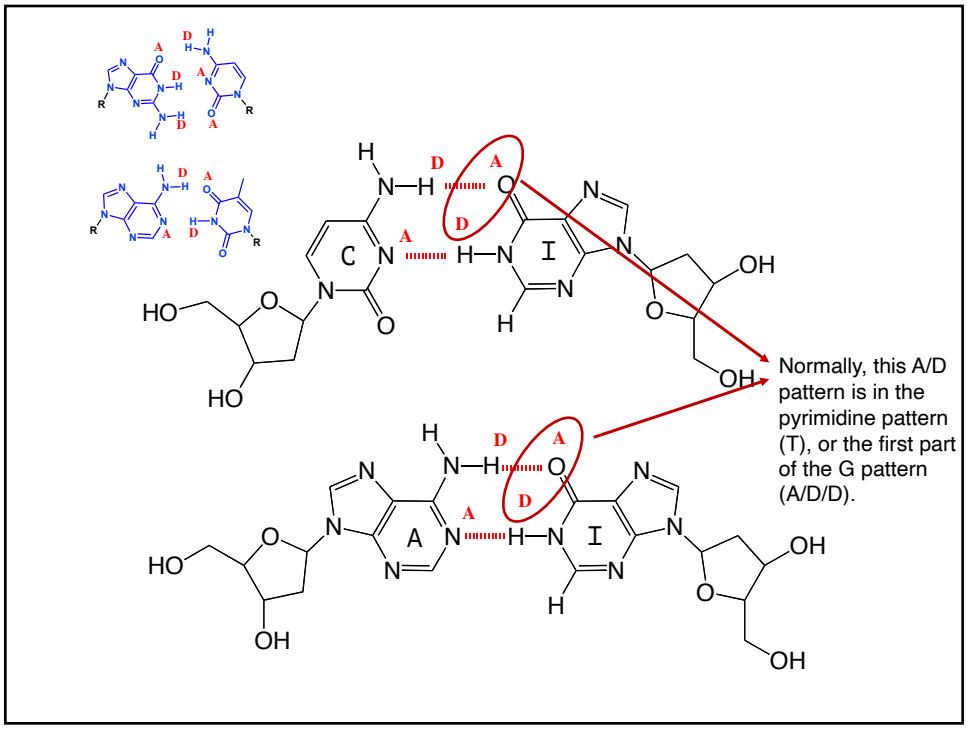
- Hypoxanthine (Hyp) is an oxidized (deaminated) derivative of adenine. As the nucleoside inosine, it can base-pair with both cytidine and adenosine. Draw the structures of these base-pairs.











Stability of the Polymers: Nucleic Acids

Acid/base treatment of DNA

In Base: deprotonation at G (N1) & U (N3) destabilizes the glycosidic bond, which leads to AP-sites.

In Acid: protonates at A (N1), C (N3), & G (N7). For C & G, this also destabilizes the glycosidic bond, which leads to AP-sites

AP-sites can lead to cleavage of the phosphodiester bond

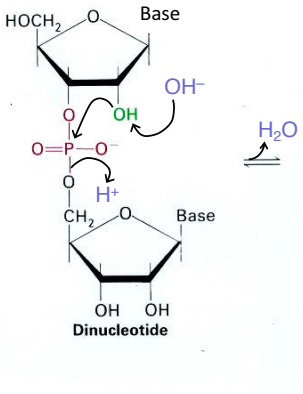
BOTH acid or base lead to ssDNA

Acid/base treatment of RNA

Similar generation of AP sites, except importantly in Base:
complete cleavage of the phosphodiester bonds!

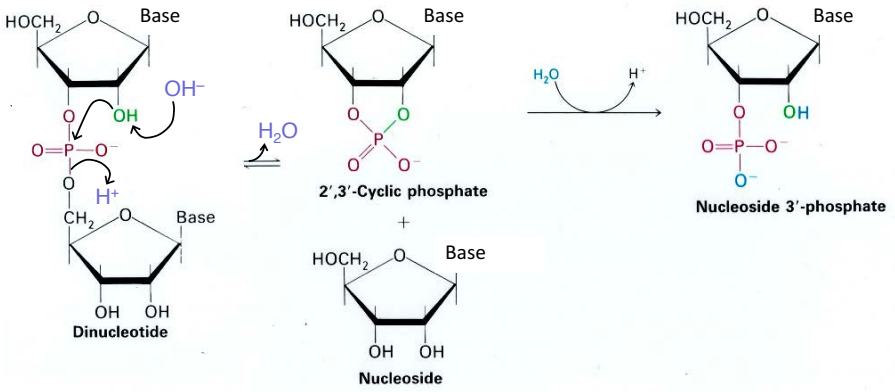
Stability of the Polymers: Nucleic Acids

Base treatment of RNA

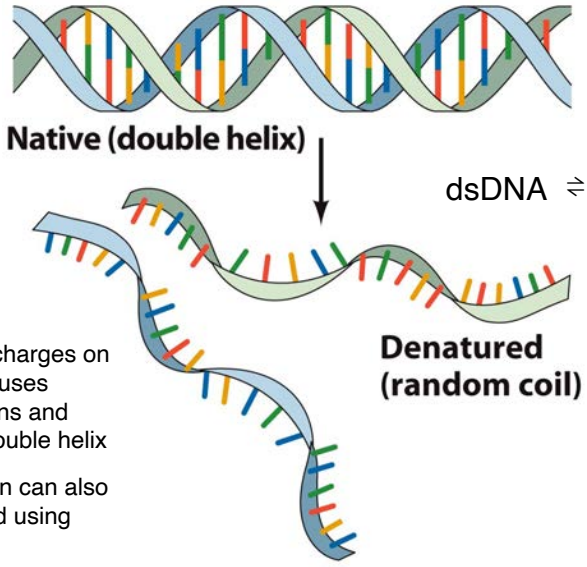


Stability of the Polymers: Nucleic Acids

Base treatment of RNA



Stability of the Polymers: Nucleic Acids
DNA Denaturation

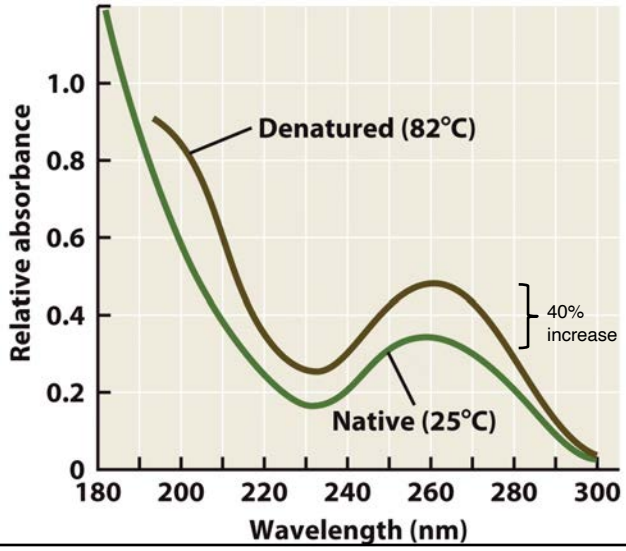


Acid/base puts charges on bases, which causes internal repulsions and breaks up the double helix

This denaturation can also be accomplished using heat.

Stability of the Polymers: Nucleic Acids
DNA Denaturation

UV Spectrum: Native vs. Denatured DNA



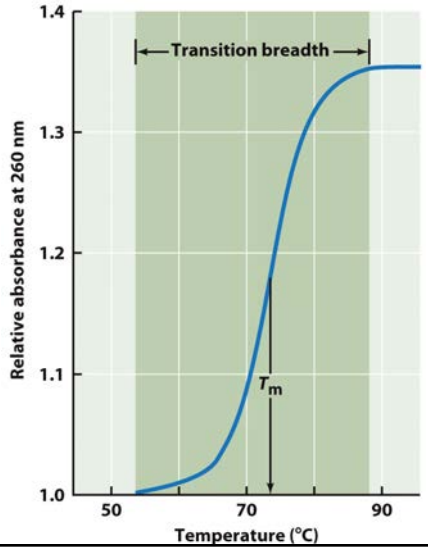
This denaturation causes a hyperchromic shift.

This makes a nice "observable" (A_{260}) and "perturbable" (heat).

What will such a plot of A_{260} vs. Temp. look like?

Stability of the Polymers: Nucleic Acids DNA Denaturation

DNA Melting Curve



This T_m value is dependent on a number of parameters.

The shape is dependent on the sequence and size.

Like protein denaturation, this is a cooperative process.

Stability of the Polymers: Nucleic Acids DNA Denaturation

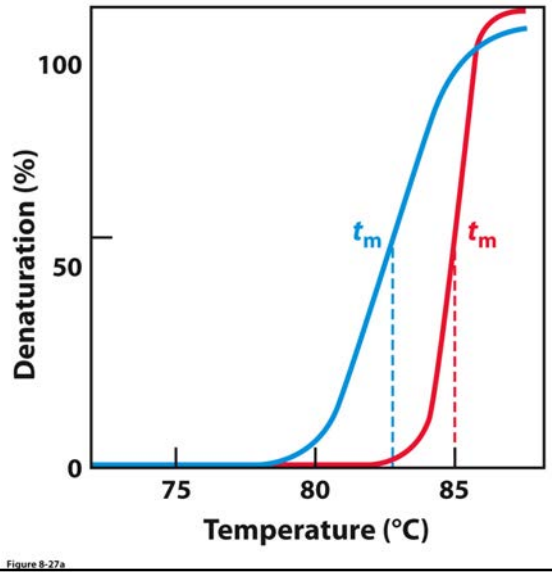


Figure 8-27a

Stability of the Polymers: Nucleic Acids
DNA Denaturation

This T_m value is dependent on:

- [salt]
- solvents (urea, formamide, guanidine salts)
- G:C content of sequence

1x SSC (Salt-Sodium Citrate)

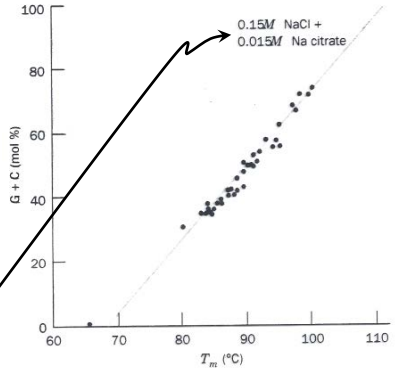
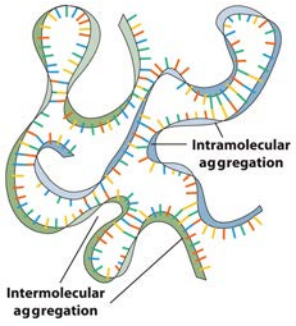


FIGURE 28-16. The variation of the melting temperatures, T_m, of various DNAs with their G + C content. The DNAs were dissolved in a solution containing 0.15*M* NaCl and 0.015*M* Na citrate. [After Marmur, J. and Doty, P., *J. Mol. Biol.* **5**, 113 (1962).]

Stability of the Polymers: Nucleic Acids
DNA ***Renaturation***

ssDNA ⇌ dsDNA

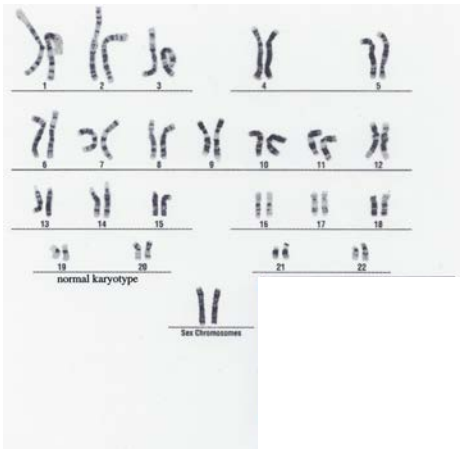
- Also called re-annealing or hybridization
- Depends on conditions (temp, [salt], solvent) that are maintained BELOW the T_m value.
- In addition, the proper formation of the complete, pristine, double helix (completely double-stranded) requires the proper amount of TIME and CONCENTRATION of nucleic acid.
- Plots of this are called $C_o t$ curves, which are much like T_m curves.
- $C_o t$ values are dependent on the complexity of the sequence.
- Not enough time, you get scrambled structures
- Given enough time, very specific annealing occurs….

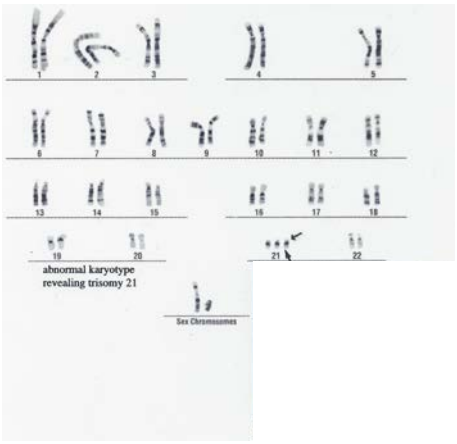


Partially Renatured DNA

Stability of the Polymers: Nucleic Acids
DNA *Renaturation*

EXAMPLE: Karyotyping





Fluorescence *in situ* hybridization (FISH)

FISH using fluorescence probe for gene on chromosome 21.

This FISH probe (a fluorescent DNA) found its complementary sequence among 3,000,000,000 bp in each cell!

Stability of the Polymers: Nucleic Acids
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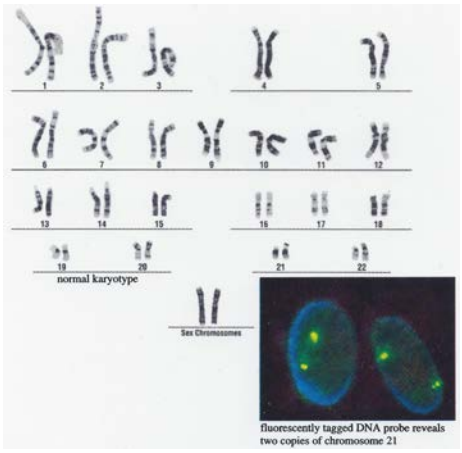

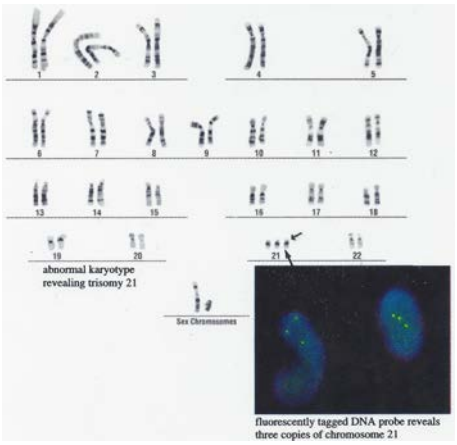

Fluorescence *in situ* hybridization (FISH)

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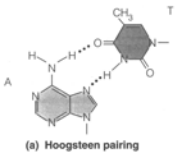
Stability of the Polymers: What is responsible for STABILITY of dsDNA?

Base Pair	$K(M^{-1})^a$
Self-Association	
A·A	3.1
U·U	6.1
C·C	28
G·G	10^3-10^4
Watson–Crick Base Pairs	
A·U	100
G·C	10^4-10^5

[a] Data measured in deuterochloroform at 25°C.

Source: Kyogoku, Y., Lord, R. C., and Rich, A., *Biochim. Biophys. Acta* **179,** 10 (1969).



C:C nearly as stable as A:U

G:G more stable than A:U and as much as G:C

Hoogstein bp is very stable

Stability of the Polymers: What is responsible for STABILITY of dsDNA

What forces operate? If it's not the H-bonds, then what is it?

- Ionic/electrostatics (salt-bridges)?

  It's a poly-anion; so charges actually de-stabilize

- Hydrophobic?

  Unlike proteins, where this is the driving force, experiments show that the ssDNA ⇌ dsDNA reaction is enthalpy driven process; ∴ bonds

- van der Waals?

  Yes! This is the most important. As uniform bp come together due to complementarity, the planer bases "stack" on each other and are close enough (<2 Å) to generate induced dipoles.
  Once started, it "zips" together as long as there are complementary bp being formed.

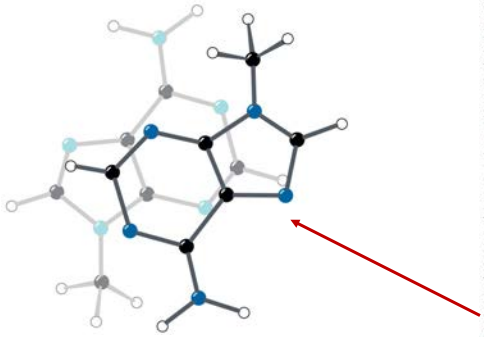
Stacking energy = stability

H–bonds in complementary bp = specificity

Stability of the Polymers: What is responsible for STABILITY of dsDNA

So, if its stacking energy, why are G:C rich sequences more stable than A:T rich sequences?

Stacking Energies in B-DNA



Adenine ring stacking

TABLE 24-2 Stacking Energies for the Ten Possible Dimers in B-DNA

Stacked Dimer	Stacking Energy ($kJ \cdot mol^{-1}$)
C · G G · C	−61.0
C · G A · T	−44.0
C · G T · A	−41.0
G · C C · G	−40.5
G · C G · C	−34.6
G · C A · T	−28.4
T · A A · T	−27.5
G · C T · A	−27.5
A · T A · T	−22.5
A · T T · A	−16.0

Source: Ornstein, R.L., Rein, R., Breen, D.L., and MacElroy, R.D., *Biopolymers* 17, 2356 (1978).

Stability of the Polymers: Biochemical

- Enzymes that catalyze the hydrolysis of the phosphodiester bonds:
- These enzymes are called "Nucleases"
  - Like proteases, if they cleave in the middle, they are called endonucleases (e.g., restriction endonucleases)
  - If they cleave at the ends, they are called exonucleases

    Exonucleases can be specific for either 5'-ends or 3'-ends, or either double-stranded or single-stranded nucleic acids (e.g., S1 nuclease)
- Also specificity for either DNA (DNases) or RNA (RNAses)
  - RNAases are very stable, DNAases require Mg^{+2} cofactor
  - Can be inhibited by DEPC or EDTA, respectively