Lecture 23 (11/9/20)

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
   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

• 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

Topoisomerase
Type IA Topoisomerase Mechanism

The gap between domains I and III closes, returning the enzyme to its initial state.

A gap opens between domains I and III and the ssDNA is cleaved. The new 5' end becomes covalently linked to the active site Tyr while the new 3' end remains tightly but noncovalently bound in the binding groove.

The unbroken (green) strand is passed through the opening formed by the cleaved (red) strand to enter the protein’s central hole.

The two cleaved ends of the red strand are religated.

The unbroken strand is trapped by the partial closing of the gap.

How are negative supercoils generated in the first place?
Type II Topoisomerase Mechanism

- Tetrameric (α2β2; 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 (ΔL = –2)

Topoisomerase Inhibitors as Antibiotics/Anticancer Chemotherapeutics

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

X-Ray structures of yeast topoisomerase II.
The 4 S’s
Size
Solubility
Shape
Stability

Tautomeric Forms of Bases

If during replication, an A is in the imino tautomer, it will be complementary to the pyrimidine C rather than T....

A = ACCEPTOR
D = DONOR
Tautomeric Forms of Bases

Mutagenesis

Imino tautomer of A

5'-GGGTATTTGGG-3'
3'-CCCAAATAAACC-5'

Replication

GGGTATTTGGG
CCCAAATAAACC

GGGTATTTGGG
CCCAAATAAACC

Replication

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 $\text{pK}_a$ Values)

<table>
<thead>
<tr>
<th>Base</th>
<th>Base $\text{pK}_a$</th>
<th>Secondary Phosphate $\text{pK}_a$</th>
<th>Primary Phosphate $\text{pK}_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine-5'-monophosphate (AMP)</td>
<td>2.4</td>
<td>6.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Guanine-5'-monophosphate (GMP)</td>
<td>3.8</td>
<td>6.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Cytidine-5'-monophosphate (CMP)</td>
<td>4.5</td>
<td>6.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Guanosine-5'-phosphate (GMP)</td>
<td>2.4 9.4</td>
<td>6.1</td>
<td>0.7</td>
</tr>
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**Acid/base Properties of Bases**

Charged bases can de-stabilize the glycosidic bond and mutagenesis.

Destabilizes glycosidic bond, leads to “AP-site” *

Leads to A $\rightarrow$ G mutations

* “AP-site” refers to a-purinic or a-pyridinic site
Other Chemical Changes of Bases

DNA Damage:

- oxidation
- alkylation
- degradation

Table I: Processes which chemically degrade DNA

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<thead>
<tr>
<th>Process</th>
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<td>5. Photochemical pyrimidine dimer formation [cyclobutane type; 6,4-product; spore photoproduct]</td>
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Diagram: Depiction of DNA damage processes.
Other Chemical Changes of Bases

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

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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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8-Oxoguanine favors the syn conformation, which leads to $O^8$-G:A bp (G → T mutations)
Other Chemical Changes of Bases

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<td>variable</td>
</tr>
<tr>
<td>[cyclobutane type; 6,4-product; spore photoproduckt]</td>
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DNA Methylation

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.

Some methylations are damaging.
- Methylation of G at O6 can base-pair with C or T, which causes G → A mutations
Other Chemical Changes of Bases

Alkylating Agents

Nitrogen mustard

EthylNitrosourea

N-Methyl-N’-nitro-N’

nitrosoguanidine (MNNG)

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  product; spore photoproduct]                                          | 100-500         |
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

\[ \text{Dimucleotide} \]

\[ \text{Nucleoside 3'-phosphate} \]

\[ \text{2',3'-Cyclic phosphate} \]

\[ \text{Nucleoside} \]
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.

\[ \text{dsDNA} \rightleftharpoons \text{ssDNA} \]

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?
Like protein denaturation, this is a cooperative process.

This $T_m$ value is dependent on a number of parameters. The shape is dependent on the sequence and size.
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)

![Graph showing the relationship between $T_m$ and Na citrate concentration](image)

**Stability of the Polymers: Nucleic Acids**

**DNA Renaturation**

\[ \text{ssDNA} \rightleftharpoons \text{dsDNA} \]

- 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 $C_{ot}$ curves, which are much like $T_m$ curves.
- $C_{ot}$ 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)

This FISH probe (a fluorescent DNA) found its complementary sequence among 3,000,000,000 bp in each cell!
Stability of the Polymers: What is responsible for **STABILITY** of dsDNA?

<table>
<thead>
<tr>
<th>Base Pair</th>
<th>$K(M^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Self-Association</strong></td>
<td></td>
</tr>
<tr>
<td>A·A</td>
<td>3.1</td>
</tr>
<tr>
<td>U·U</td>
<td>6.1</td>
</tr>
<tr>
<td>C·C</td>
<td>28</td>
</tr>
<tr>
<td>G·G</td>
<td>$10^3$–$10^4$</td>
</tr>
<tr>
<td><strong>Watson–Crick Base Pairs</strong></td>
<td></td>
</tr>
<tr>
<td>A·U</td>
<td>100</td>
</tr>
<tr>
<td>G·C</td>
<td>$10^4$–$10^5$</td>
</tr>
</tbody>
</table>

* Data measured in deuterohloroform at 25°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 $\rightarrow$ dsDNA reaction is enthalpy driven process; $\therefore$ 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

<table>
<thead>
<tr>
<th>Stacked Dimer</th>
<th>Stacking Energy (kJ · mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - G</td>
<td>-61.0</td>
</tr>
<tr>
<td>G - C</td>
<td>-44.0</td>
</tr>
<tr>
<td>C - G</td>
<td>-41.0</td>
</tr>
<tr>
<td>T - A</td>
<td>-50.5</td>
</tr>
<tr>
<td>G - C</td>
<td>-34.0</td>
</tr>
<tr>
<td>G - C</td>
<td>-28.4</td>
</tr>
<tr>
<td>A - T</td>
<td>-27.5</td>
</tr>
<tr>
<td>T - A</td>
<td>-27.5</td>
</tr>
<tr>
<td>A - T</td>
<td>-22.5</td>
</tr>
<tr>
<td>A - T</td>
<td>-16.0</td>
</tr>
</tbody>
</table>


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)
  • RNAse are very stable, DNAse require Mg²⁺ cofactor
  • Can be inhibited by DEPC or EDTA, respectively