Chapter 8 Notes

Nucleotides and Nucleic Acids

Figure 1 Nucleotides

- Three components
  - Heterocyclic Base (derivative of purine or pyrimidine)
  - β-D-ribofuranose or 2-deoxyribofuranose
  - One or more phosphates
- Nucleotide without phosphate—nucleoside
- Pyrimidine numbered to give nitrogens lowest possible number
- Purine numbered to give nitrogens, bridging carbons lowest possible number

Figure 1 Continued

- Carbons in ribose moiety are numbered with primes to distinguish them from base numbers
- Furanose sugar is in β-configuration

Figure 2: Major Bases of Nucleotides

- Adenine: purine with amino group at carbon 6
- Guanine: purine with oxo group at carbon six, amino group at carbon 2
  - Oxo group on guanine means N-1 must be protonated, single bond to C-6
- Uracil: pyrimidine with oxo groups on carbons 2 and 4
  - N-1, N-3 are both protonated
- Thymidine: uracil with methyl group on carbon 5
- Cytosine: 4-amino-2-oxopyrimidine

Figure 4 Nucleotides and Nucleosides

- Purines connect to sugars at N-9
- Pyrimidines connect at N-1
- Base gives name to nucleoside
  - adenine: adenosine
  - guanine: guanosine
  - uracil: uridine
  - thymine: thymidine
  - cytosine: cytidine
- Nucleotides named as nucleosides with phosphate

Figure 5 Minor Bases

- They exist
- Don't be surprised to see them
- Don't worry about the names
- Do be able to draw a base from its systematic name (i.e. 6-oxopurine)

Figure 6 Other Phosphate Positions

- Anywhere there is a hydroxyl group, a phosphate ester can be formed
- Know that the major location for phosphates is the 5' position

Figure 7 Phosphodiester Linkage in Nucleic Acids

- Phosphate ester formed between 5' OH and phosphate, and between 3' OH and phosphate
  - Two phosphoester bonds on same phosphate makes phosphodiester
- Polymer of nucleotides can extend indefinitely, just like peptides
- 5' end: Nucleotide with 5' OH not connected to another nucleotide
- 3' end: Nucleotide with 3' OH not connected to another nucleotide
  - Analogous to N- and C- termini of peptides

Figure 8: Hydrolysis of Phosphodiester Bonds

- 2' OH participates in reaction
  - DNA not susceptible to this attack
    - More stable
- Forms phosphodiester bond at 2' and 3' carbons of a single nucleotide

Writing Nucleic Acid Sequences

- Conventionally written 5'-3'
- Oligonucleotide: short polymer of nucleotides
- Polynucleotide: longer polymer of nucleotides

Figure 10 Absorptivity of Nucleotides

- Heterocyclic bases generally absorbs UV light
- Maximum wavelength for absorbance is typically at or near 260 nm
  - Contrast with peptide absorbance at 280 nm

Figure 11 Basepairing

- A pairs with U or T, G pairs with C
- Drawing base pairs
  - Orient purine and pyrimidine such that N-3 of pyrimidine and N-1 of purine are pointing toward each other
  - Fill in oxo and amino groups, hydrogen on purine or pyrimidine
  - Amino groups will have one hydrogen pointed toward other base
- GC has three hydrogen bonds, AT has two

Figure 12 DNA Stores Information in Bacteria

- Live virulent bacteria kill mouse
- Dead virulent bacteria don't kill mouse
- Live nonvirulent bacteria don't kill mouse
- Dead virulent bacteria and live nonvirulent bacteria together kill mouse
- DNA from virulent bacteria and live nonvirulent bacteria kills mouse
- Ergo, DNA contains information for virulence

Figure 13 DNA Stores Information in Viruses

- Labeling virus with 32P labels DNA (and RNA) in viruses
- Labeling virus with 35S labels proteins in viruses
- When 32P labeled viruses were incubated with bacteria, label was injected into bacteria
- When 35S labeled viruses were incubated with bacteria, radiolabel was not injected into cells
- DNA is injected to cause viral infection

Chargaff's DNA Composition Observations

- Base composition of DNA varies from one organism to another
- [Adenine] = [Thymine], [Guanine] = [Cytosine] in DNA of all organisms

Figure 14 X-ray Diffraction of DNA

- Indicates a helical structure

Figure 15: Watson-Crick Model for DNA

- Basepairing interactions between adenine and thymine, and between guanine and cytosine
- Bases are stacked on top of each other, sugar phosphate backbone winds around outside forming helix
- Because sugars aren't arranged on opposite sides of helix, two distinct grooves are observed
- Major groove, minor groove

Figure 16: Antiparallel Double Helix

- Two strands of DNA run in opposite directions
- Sequences are complementary to each other
- Example: 5'-CAATAGGCA-3'
- Complement: 5'-TGCCTATTG-5'
- Palindrome: 5'-CAAGCTTG-3'
- Complement is the same sequence

Figure 18: Conformational Flexibility in Nucleic Acid Backbone

- Six bonds between phosphates of adjoining nucleotides
- Rotation around ring bond is restricted
- Compare to peptide backbone
- 3 bonds, 1 restricted (peptide bond)

Figure 19: Three Types of Nucleic Acid Structure

- A-form: More loosely wound, right handed helix
- Occurs in RNA double helices, dehydrated DNA
- B-form: More tightly wound, larger grooves in right-handed helix
- Predominant conformation of DNA in cell (water)
- Z-form DNA: left-handed helix, found in some nucleic acid sequences

Figure 20: Palindromes and Mirror Repeats

- Palindromes have identical sequence on forward and reverse strands
- Mirror repeats have mirrored sequence in the same strand

Figure 21: Hairpin Structure

- Hairpins are possible where palindromic sequences occur
- Nucleic acid forms helix within a strand rather than between two strands

Figure 25: RNA Structure

- RNA exists primarily as single-stranded nucleic acid rather than double-stranded

Figure 26: RNA Secondary Structure

- Helices form within RNA rather than between RNA molecules
- Bulges, loops and single strands can also form
- Compare to protein secondary structure

Figure 27: Secondary Structure in Larger RNA

- Many interactions occur within RNA primary structure
- Some unusual basepairs are allowed (not B-form helices)

Figure 28: RNA Tertiary Structure

- Secondary structure elements are brought together in a folded structure
- Mediated by hydrogen bonds rather than hydrophobic effect
- Function related to tertiary structure
- RNA-dependent enzymes, tRNA, etc.

Figure 29: Denaturation and Annealing

- Denaturation: Separation of two strands of helix
- Disruption of hydrogen bonds
- Annealing: Reforming of helices, hydrogen bonds

Figure 30: Heat Denaturation

- Nucleic acids can be denatured with high temperature
- Denaturation is highly cooperative
- Once hydrogen bonds start breaking, it becomes easier to break the rest
- Temperature at which transition occurs depends on GC content of nucleic acid
- # of hydrogen bonds

Figure 32: Hybridization

- Complementary DNA sequences from different sources can be combined together to form a duplex
- If complementary strands are present in both sources, reannealed DNA will contain both original duplex and hybrid

Figure 36A: Synthesis of DNA

- DNA starts with template and primer
- Primer is complementary to template at given sequence
- DNA polymerase can't start synthesizing nucleic acid without primer
- Next complementary nucleotide triphosphate is added to 3' hydroxyl of extending polynucleotide

Figure 36B Dideoxynucleotides

- Have hydrogens instead of hydroxyls at both 2' and 3' carbons
- Incorporation of dideoxynucleotides terminates DNA synthesis (no 3' hydroxyl to form phosphodiester bond)

Figure 36C: Dideoxy Sequencing

- Radiolabeled primer is added to template DNA in each of 4 tubes
- Four deoxynucleotides are added to each tube
- One dideoxynucleotide is added to each tube (different ddNTP to each tube)
- Extending polynucleotide will have different lengths depending on when ddNTP is incorporated into chain
- Sequence of complementary strand can be read from bottom to top of gel run on oligos

Figure 37: Automated DNA Sequencing

- Just like Sanger method, but dideoxynucleotides have fluorescent tags of different colors
- All four dideoxynucleotides are included in the same reaction
- Polynucleotides with the same color end in the same dideoxynucleotide
- Sequence of complementary strand is read by identifying the colors on gel from bottom to top

Figure 38: Oligonucleotide Synthesis

- Nucleotides can be added from 5' to 3' using chemical techniques
- Length of oligonucleotide is limited by chemical efficiency of synthesis

Figure 39: Nucleotide Phosphates

- NMP: 1 phosphate
- NDP: 2 phosphates
- NTP: 3 phosphates

Figure 40: Phosphoanhydride Bonds

- ATP has two, ADP has one, AMP has none
- Higher energy than ester, but lower energy than mixed or carbonic

Figure 41: Coenzyme Nucleotides

- Coenzyme A, NAD, and FAD all contain adenosine
- Nicotinamide and Flavin are both part of additional nucleotides
  - D stands for dinucleotide

Figure 42: Cyclic Nucleotides

- Contain phosphodiester bonds within the nucleotide (6-membered ring)
- Used as signaling molecules