Slide 1

Chapter 12

Structure of Nucleic Acids

Slide 2

Primary Structure of Nucleic Acids

- Ability to sequence DNA required discovery of restriction enzymes, which could produce homogeneous fragments
  - (pure DNA, being very large, will shear randomly to make a mixture of fragments)
- Sequencing involves labeling nucleotides with a radioactive tag (^{32}P) or a flourescent tag so that small amounts can be detected

Slide 3

Sequencing Strategies

- Enzymatic—uses DNA polymerase and dideoxy nucleosides as chain terminating reagents
  - Developed by F. Sanger—earned him a second Nobel Prize
- Chemical—uses base-specific chemical cleavage reactions developed by Maxam and Gilbert

Slide 4

Sanger Method

- Uses DNA polymerase, which requires a **primer**, a **template**, and all deoxynucleoside triphosphates
  - (See diagram Figure 12.2)
- Inclusion of a small amount of a dideoxynucleoside triphosphate produces random fragments with no free 3'-OH, so chain terminates
- Run four separate incubations each containing one dideoxynucleotide (ddATP, ddGTP, ddCTP or ddTTP).

Slide 5

Sanger Method, con't.

- Because ddNTP concentration is low, most of the time the normal dNTP is incorporated.
- Occasionally a ddNTP is inserted, and the chain terminates.
- A set of "nested fragments" is produced.

Slide 6

Nested Fragments from Chain Termination

- For example, if the DNA sequence were:
  ATCCGGTAGCAATCGA
- Termination at G would produce
  ATCCG
  ATCCGG
  ATCCGGTAG
  ATCCGGTAGCAATCG
- These fragments can be separated by size on electrophoresis (See Figure 12.3)

The fragments must be labeled some way so they can be detected. One technique is to use one of the dNTP's labeled with ^{32}P. Another is to put a flourescent label onto one of the nucleotides, or to attach a flourescent label to the primer oligonucleotide.

Slide 7

Maxam-Gilbert (Chemical Cleavage) Method

- Developed before Sanger's method, but not as widely used now.
- Nested fragments are produced by specific chemical cleavage.
- DNA labeled with ^{32}P, usually at the 5' end
- Reaction conditions set so that on average only one cleavage will occur in a single molecule.

Slide 8

Cleavage Reactions-Purines

- G-specific cleavage by methylation with dimethyl sulfate, followed by treatment with piperidine. (See Figure 12.4 for mechanism)
- Purine specific cleavage by treating with acid prior to methylation and piperidine treatment.
- Electrophoresis of the two reaction mixtures gives G fragments in the first lane, both A and G fragments in the second. A fragments are identified as those in the second but not in the first.

Slide 9

Cleavage Reactions-Pyrimidines

- Pyrimidines react with hydrazine to open the ring, and piperidine treatment then cleaves the sugar as in the purine cleavage. (See Figure 12.5 for mechanism)
- High salt concentration protects cleavage at Thymine, so two reactions run—one in high salt and one normal.
- Electrophoresis of the two reactions separately give a "C" specific lane and a "C + T" lane.

Slide 10

Cleavage Reactions, con't.

- Electrophoresis produces four lanes of nested fragments as in the Sanger method.
- However, reading the lanes is a bit more complicated since the reactions aren't as clean. (See Figure 12.6)
- Either end of the DNA could be labeled to give the sequence in either direction.
- The end base is not identified in this procedure.

While the Sanger method is better for sequencing, the chemical cleavage method is useful for DNA "footprinting" to determine the sites of proteins binding to DNA. Bound proteins protect the DNA bases from chemical cleavage, so sequencing gels would show no fragments at the positions where the protein is bound.

Slide 11

Automated Sequencing

- Automated sequencers use flourescently labeled primers in the Sanger procedure. A different flourescent color is used in each of the four incubations.
- Machines can read the flourescence of fragments as they elute from a gel, and the information can be passed to a computer. (See Figure 12.8)

Slide 12

DNA Secondary Structure

- We have already introduced the double helical "twisted ladder" structure for DNA (See Figure 12.9)
  - Sugar-phosphate backbone on outside
  - Bases inside with AT and GC specific pairings
  - Twisted structure gives base-pair spacing of 0.34 nm

Slide 13

Watson-Crick Base Pairs

- Note the dimensions of the AT and GC base pairs are almost identical. (Figure 12.10).
- Purine-purine pairing would be too big.
- Pyrimidine-pyrimidine pairing would be too small.
- AT pairs have two hydrogen bond; GC pairs have three hydrogen bonds.

Slide 14

Features of the Helix

- Major and Minor Grooves (See Figure 12.11)
- Helical twist and propeller twist of the bases (See Figure 12.12)
- See Chime tutorial on DNA structure in the Course Links for Chapter 12
  - (Note—the tutorial doesn't work with Internet Explorer, and sometimes gives problems with javascript errors)

Major groove is large enough to accommodate an alpha-helix of a protein. The edges of the bases in the major and minor grooves show a different hydrogen bonding possibility for each base pair, hence proteins can recognize which base pair is which. Many regulatory proteins (as well as the restriction enzymes we discussed earlier) are therefore capable of recognizing specific base sequences.
The propeller twist of the bases increases the hydrophobic overlap of bases in the same strand.

Slide 15

Other DNA Helical Structures

- B-DNA—first one determined
  - Right-handed; 2.37 nm diameter
  - 0.33 nm rise; ~10 bp per turn
- A-DNA—dehydrated fibers (and RNA)
  - Right-handed; 2.55 nm diameter
  - 0.23 nm rise; ~11 bp per turn
- Z-DNA—GC pair sequences
  - Left-handed; 1.84 nm diameter
  - 0.38 nm rise; 12 bp per turn
  - (See Table 12.1)

A-DNA is "short and broad"; B-DNA is a little "longer and thinner"; Z-DNA is "longest, thinnest"

Slide 16

A, B, and Z DNA, con't.

- See Figure 12.13 for side-by-side comparisons of the three helices.
- See also a Chime presentation of A, B, and Z DNA side by side in the Course Links for Chapter 12.

Slide 17

A, B, and Z DNA, con't.

- The G in Z-DNA has the *syn* conformation.
  - (See Figure 12.14)
- Base pair rotated to form left-handed structure (G flips *anti* to *syn*, while the C-ribose flips as a unit).
  - (See Figure 12.15)
- Methylation of C also favors B to Z switch

Slide 18

Denaturation of DNA

- Heating DNA solutions cause strands to unwind.
- Unwinding is accompanied by an increase in absorbance at 260 nm. (See Figure 12.17)
  - This is called the "hyperchromic shift"
- Melting temperature depends on ionic strength and on GC content. (See Figure 12.18)
  - DNA from various sources therefore has different melting temperatures. (Figure 12.17

Stacking of bases in the helix causes an interaction between the pi clouds of the bases, affecting the electronic transitions in the structure that results in decreased absorbance at 260 nm. Unwinding of the strands removes this stacking and the normal absorbance returns.
The effect of GC content on melting temperature is only partly due to the fact that GC forms three hydrogen bonds and AT forms only two. There is also more hydrophobic stacking energy involved in GC pairs than in AT pairs.

Slide 19

Reannealing of DNA

- Reannealing is the term given to the renaturation—the reformation of the helix
- Temperature must be lowered slowly for proper nucleation to occur (See Fig. 12.19)
- Renaturation is a second order process, so the **rate** depends on concentration of complementary base sequences.

Slide 20

Reannealing of DNA, con't.

- Rate of reannealing is used to measure genome complexity. The more a sequence is repeated, the higher its concentration, and the faster it reassociates. (See Figure 12.20)
- Reannealing is used for sequence matching of DNA samples, and RNA with DNA
  - Only complementary strands will renature
  - The process is called **hybridization**

Since the rate of reassociation can vary over very large time-scales, one can arrange for convenient times of annealing by controlling the overall concentration of the sample. To relate experiments run at different concentrations, one can multiply the concentration (C_o) by the half-life of the reassociation ($t_{1/2}$) to give a $\mathbf{C_o t}$ value characteristic of the DNA. The derivation on page 373 shows how the $C_o t$ value is related to the second order rate constant for the process.

Slide 21

Skip Sections 12.4-12.6

- Supercoiling makes more sense in connection with replication and transcription of DNA next term
- Chromosome structure fits better there, too.
- Chemical synthesis of nucleic acids is an important topic, but we don't have time to give it justice. Just as with peptide synthesis, there are now machines available to synthesize reasonably large oligonucleotides.

Slide 22

RNA Secondary and Tertiary Structure

- RNA is single stranded, but there is extensive secondary structure characterized by loops, base pairing, and hydrogen bonding.
- Some of the base pairing is of the Watson-Crick type, but other associations occur as well.

Slide 23

Transfer RNA Structure

- Secondary structure shows a "cloverleaf" pattern. All t-RNA's are similar.
  - (See Figure 12.34)
- All end in CCA-3', with amino acid attached at the 3'-OH
- Lots of unusual modified bases.
  - (See Figure 12.36 for yeast alanine t-RNA)
- Tertiary structure is L-shaped
  - Lots of "noncanonical H-bonding. (Fig. 12.38)

Slide 24

Ribosomal RNA Structure

- A great deal of intra-strand sequence complementarity.
- Computer generated secondary structure very complex, but seems to be highly conserved in evolution.
  - (See Figure 12.39 and 12.40)
- Low resolution x-ray structures of ribosomes are becoming available.