LECTURE 19

<u>The Genetic Material</u>

A. Experiments that demonstrated DNA or RNA to be the genetic material

1. Avery et al. (1944) in *Diplococcus*
   Hershey & Chase (1952) in bacteriophage T2
   Fraenkel-Conrat et al. (1957) in tobacco mosaic virus (TMV)

B. Structure of DNA: Watson and Crick (1952)

1. Other players included M. Wilkins and R. Franklin

2. Watson and Crick proposed the following model regarding the structure of DNA

   a) DNA is a polymer of nucleotides, where a nucleotide is comprised of

      a 5-carbon sugar (deoxyribose, ribose)
      a phosphate group
      a nitrogenous base (nucleic acid) $\rightarrow$ purines (A, G) & pyrimidines (C, T or U)

   b) The nucleotides are linked through phosphodiester bonds (sugar-phosphate group-sugar-phosphate group on each of two strands and formed into a right-handed, double helical structure.

      (i) evidence for the model was based on data generated by E. Chargaff's that show %A = %T and %G = %C, and from X-ray diffraction patterns generated by M. Wilkins and R. Franklin

   c) The two strands of DNA are complementary to one another, with A=T and G≡C, but are antiparallel (i.e., have opposite chemical polarity). Polarity is "read" through carbon atoms of sugar molecule, i.e. 5'→3' and 3→5'.

   d) The stability of the DNA molecule stems from

      (i) covalent bonds (shared electrons) holding the phosphodiester linkages
      (ii) hydrogen bonds between complementary base pairs
      (iii) hydrophobic bonding (stacking forces) between adjacent base pairs

C. Forms of DNA

1. B-DNA: physiological DNA (low salt, aqueous conditions); right-handed, with 10 bp/turn

2. A-DNA: artificial or rare (high salt, partially dehydrated); right-handed, with 11 bp/turn

   a) Potentially important because DNA:RNA heteroduplexes occur in a similar structure

3. Z-DNA: artificial (?); thought to occur in GC-rich tracts (alternating purine-pyrimidine regions); left-handed, with 12 bp/turn

D. Supercoiling: positive (over-wound) and negative (relaxed) supercoils

1. Physiological DNA is in a negative supercoil: negative supercoiling is required for DNA to be "metabolized" and are generated by a class of enzymes called topoisomerases.

a) The best known topoisomerase is an enzyme called DNA gyrase that is involved in DNA replication. Several anti-microbial (pharmaceutical) agents are targeted towards neutralizing this enzyme.

E. Structure of RNA

1. The structure of RNA is very similar to the structure of DNA; a ribose sugar replaces the deoxyribose sugar, uracil (U) replaces thymine (T), and the molecule normally is single-stranded instead of double-stranded. RNA also has a different function, at least in organisms where DNA is the genetic material.

F. Structure of chromosomes

1. *Prokaryotes*

a) The chromosome is a "naked" DNA molecule (there are a few proteins) that normally exists in a "folded" state with more than 50 loops or domains, each of which is negatively supercoiled. Each loop or domain is relaxed by "nicks" that permit DNA to be metabolized.

2. *Eukaryotes*

a) Basic information

(i) diploid, not haploid
(ii) chromosomal proteins comprise 75-80% of chromosomal material
(iii) there is 2 - 25 times as many genes as prokaryotes, but 10^3 - 10^5 times as much DNA

b) Chromosomal proteins are primarily basic proteins called <u>histones</u>, Histones are present in virtually all cell types except for sperm of some species (where they replaced by another basic class of proteins called protamines).

(i) histones are comprised of 20-30% lysine and arginine residues
(ii) there are five types of histones, in molar ratios H1 (1), H2a (2), H2b (2), H3 (2), and H4 (2)
(iii) histones complexes with DNA to form *nucleosomes* ["beads" on a string]

3. DNA is "unineme" → a continuous molecule of double-stranded DNA throughout a chromosome

4. There are at least three levels of DNA packaging whereby more than 10^4 μm of DNA is packaged into chromosomes that are on the order of 10 μm in length.

   a) <u>Level 1</u>: Nucleosomes

      (i) a nucleosome core of 140-146 nucleotides is wrapped around an octamer of two molecules each of histones H2a, H2b, H3, and H4 – this is the nucleosome or nu-body

      (ii) each nucleosome (nu-body) is separated by 60 or so bases (linker) to which a single molecule of histone H1 is attached

   b) <u>Level 2</u>: Solenoid

      (i) chromatin fibers with diameter of 30 nm – the hypothesis is that each 30 nm fiber is a supercoil of nucleosomes – the structure not well known but probably involves non-histone proteins

   c) <u>Level 3</u>: Scaffold

      (i) the next level up – a "structure" revealed when chromosomes are treated with heavy acid to remove basic (histone) proteins; it may be an artifact

G. Centromeres and telomeres

1. Centromeres (at the molecular level) are best characterized in yeast (*S. cerevisiae*) where the functional centromere region is about 110 - 120 bases in length and contains three essential regions.

   a) Regions I and III are short, conserved sequences at either end of the sequence that serve as binding sites for proteins involved in spindle fiber attachment.

   b) Region II is A:T rich (>90% A:T base pairs); its function is likely related its size and AT richness. The latter may relate to the ease with which strand separation occurs in AT-rich regions.

2. Telomeres are short nucleotide sequences present as tandem repeats. The basic sequence "motif" is 5'-$T_xA_yG_z$-3' and the number of repeats varies with species.

   a) Telomeres have a single-stranded region at the 3' end that forms a "hairpin" loop at the terminus of chromosomes.

   b) Telomere sequences added to the end of chromosome by a special enzyme called a *telomerase*.